Human Transcription Release Factor 2 Dissociates RNA Polymerases I and II Stalled at a Cyclobutane Thymine Dimer*

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RNA polymerase II stalled at a lesion in the transcribed strand is thought to constitute a signal for transcription-coupled repair. Transcription factors that act on RNA polymerase in elongation mode potentially influence this mode of repair. Previously, it was shown that transcription elongation factors TFIIIS and Cockayne’s syndrome complementation group B protein did not disrupt the ternary complex of RNA polymerase II stalled at a thymine cyclobutane dimer, nor did they enable RNA polymerase II to bypass the dimer. Here we investigated the effect of the transcription factor 2 on RNA polymerase II and RNA polymerase I stalled at thymine dimers. Transcription factor 2 is known to release transcripts from RNA polymerase II early elongation complex generated by pulse-transcription. We found that factor 2 (which is also called release factor) disrupts the ternary complex of RNA polymerase II at a thymine dimer and surprisingly exerts the same effect on RNA polymerase I. These findings show that in mammalian cells a RNA polymerase I or RNA polymerase II transcript truncated by a lesion in the template strand may be discarded unless repair is accomplished rapidly by a mechanism that does not displace stalled RNA polymerases.

Preferential repair is the repair of certain regions of the genome at a faster rate compared with the bulk of genomic DNA (1–5). The single most significant contributor to preferential repair is transcription. It has been found that pyrimidine dimers in the template strand of transcribed sequences in Escherichia coli and in humans are repaired at a fast rate relative to the nontranscribed strand and the rest of the genome (2, 3). The molecular mechanism of coupling transcription to repair in E. coli is relatively well understood (5). A protein called transcription-repair coupling factor (TRCF)1 displaces stalled RNA polymerase while simultaneously recruiting the excision repair complex to the site of damage and thus accelerates the rate of damage recognition and removal (6). In mammalian cells, transcription-coupled repair depends on the CSA and CSB proteins (7). The CSB protein, like the TRCF of E. coli, has the so-called helicase motifs (8, 9), and initially it was suspected that it may function in a manner similar to that of TRCF in coupling repair to transcription. However, the purified CSB protein, in contrast to TRCF, does not disrupt the ternary complex of stalled RNA polymerase II but instead it appears to function as a transcription elongation factor for RNA polymerase II (10). Furthermore, in a study aimed at uncovering the mechanism of stimulation of repair by transcription, it was found that human RNA polymerase II stalled at a thymine dimer was rapidly dissociated from the template/substrate by human cell-free extract without detectable stimulation of repair (11). This observation raised the possibility that the basic mechanism of coupling repair to transcription in humans may be different from that of E. coli. Hence, we wished to investigate the effect of other factors known to act on stalled RNA polymerase II in order to gain some insight on the parameters that affect the accessibility of transcription-blocking lesions to repair enzymes, the stability of the ternary complex that forms at such lesions, and the fate of the truncated transcript during and after repair.

Factor 2 is a well-characterized transcription factor that acts during elongation. The factor was first identified in Drosophila Kc cells as an activity that suppressed the appearance of incomplete transcripts by unknown mechanism (12). Later factor 2 was identified as one of the negative transcription elongation factors, N-TEF (13, 14), that are responsible for the production of short, prematurely terminated transcripts. Factor 2 has been shown to be an ATP-dependent RNA polymerase II termination factor (14). Its activity resides in a 154-kDa polypeptide with DNA-stimulated ATPase but no helicase activity (14–16). Recently, the human homolog of factor 2 (HuF2) was isolated and characterized (17, 18). It exhibits essentially the same properties as the Drosophila factor 2. Since RNA polymerase stalled at a lesion is a key component for most models for transcription-coupled repair, we wished to investigate the effect of factor 2 on RNA polymerases I and II stalled at thymine cyclobutane dimers. We found that factor 2 releases both polymerases. Thus, any model for transcription-coupled repair in humans must account for the presence of a relatively abundant nuclear factor that disrupts ternary complexes rapidly and efficiently.

MATERIALS AND METHODS

Templates/Substrates—pMLU112 and pPU192 have been described (9, 10). Both constructs have the adenovirus major late promoter. pMLU112 has been constructed such that there is no U in the first 112 nt of the transcripts (“U-less cassette”; Ref. 19), and there is a cleavage site for restriction endonuclease PvuII at 330 bp downstream of the transcription start site. pPU192 has a single thymine-thymine dimer (T–T) in the template strand at nucleotide positions 149–150 downstream of the transcription start site. pHr163-T–T was prepared by hybridization of the 5′-32P-labeled T–T oligonucleotide to the single strand form of pHr163 followed by second strand synthesis by T4 DNA polymerase (Roche Molecular Biochemicals) as described (20). To construct pHr163, pBI25 (International Biotechnologies, Inc.) was modi-
Mfd C-X, the transcription-repair coupling factor, which

Recombinant protein of human homolog of

Tris-HCl, pH 7.9, 108 mM

Pvu

EDTA, 0.5 mM

dithiothreitol, 2.8 mM

dithiothreitol, 20% glycerol) containing 100 mM

Pvu

creatine phosphate, 100

M

7.8 mM

M

GTP, 20

M

MgCl

Tran-

E. coli

2 mM

MgCl

a

b

KCl, 0.33 mM

gene polymerase II release factor (HuF2) was prepared as described (17). Native (RNA polymerase II and TFIIH) and recombinant factors (TBP, IIB, IIE, and HF) for RNA polymerase II transcription reactions were prepared as described previously (11). Purified recombinant CSB protein was prepared as described (9). The partially purified RNA polymerase I fraction was prepared as described elsewhere (23, 25) with some modifications. 200 ng of whole cell extract from yeast cells (Saccharomyces cerevisiae) grown in YPD medium and applied on DEAE-Sepharose column (Sigma), which was washed with 5 column volumes of buffer TM (50 mM Tris-HCl, pH 7.9, 12.5 mM MgCl1, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol) containing 100 mM KCl and step-eluted with 280 mM KCl. This fraction was dialyzed against TM buffer containing 100 mM KCl and loaded onto a 5-ml heparin-agarose column (Sigma); the column was washed with TM buffer containing 100 mM KCl and eluted with 200, 400, and 600 mM KCl in TM buffer. A single fraction, eluted with 400 mM KCl in TM buffer supported transcription from the human ribosomal gene promoter. This fraction was dialyzed against 100 mM KCl in TM buffer and used for transcription experiments as partially purified RNA polymerase I fraction. The mutant E. coli Mfd-C-X, the transcription-repair coupling factor, which lacks amino acids 1–575 but possesses RNA polymerase dissociating activity, was prepared as described previously (27). E. coli RNA polymerase was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden).

**Proteins—Recombinant protein of human homolog of Drosophila melanogaster RNA polymerase II release factor (HuF2) was prepared as described (17). Native (RNA polymerase II and TFIIH) and recombinant factors (TBP, IIB, IIE, and HF) for RNA polymerase II transcription reactions were prepared as described previously (11). Purified recombinant CSB protein was prepared as described (9). The partially purified RNA polymerase I fraction was prepared as described elsewhere (23, 25) with some modifications.**

**Footprinting Experiments by Human RNA Polymerase I—**For footprinting experiments, 50 ng of template/substrate DNA was incubated with 3 μg of partially purified RNA polymerase I fraction in 10 μl of transcription buffer as described above except that 0.05 mM CTP and 2 μCi of [α-32P]CTP were replaced by 0.5 mM CTP. After 45 min of incubation at 30 °C, 30 units of DNase I (Life Technologies) was added directly to the reactions and incubated for 5 min at 30 °C. The DNA was then phenol-extracted, precipitated, and analyzed on an 8% polyacrylamide sequencing gel. Control reactions (Trn−) were performed by omitting CTP from the reaction mixture. To test the release activity, HuF2 was added directly to the reactions after forming stable RNA polymerase I ternary complex, and the reactions were incubated for 30 min at 30 °C before DNase I digestion.

For T4 DNA polymerase 3′-5′ exonuclease protection experiments, pHr163-T<−T was first transcribed with partially purified RNA polymerase I fraction and nonradioactive ribonucleotides at 30 °C for 45 min as described above to form a ternary complex at the T<−T site. The template DNA was labeled by site-specific modification. T<−T were radiolabeled at the 5′-3′ phosphodiester bond 5′-T by Nε-Nα-butyraamine (Toray Industries, Tokyo, Japan) as described by Learned and Tjian (23). To analyze blockage of transcription by a T<−T, we used a linearized template/substrate. The pHr163-T<−T plasmid and the unmodified template/substrate, pHr163, were digested with HindIII restriction endonuclease (Promega, WI), which cuts these plasmids at 331 nucleotides downstream of the transcription start site. 50 ng of linearized pHr163 and pHr163-T<−T were incubated individually with 30 μg of whole cell extract in 10 μl of transcription buffer (25 mM Tris-HCl, pH 7.9, 50 mM KCl, 6.25 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, 100 μM α-aminatin, 10% glycerol, 0.5 mM each ATP, GTP, and UTP, 0.05 mM CTP, and 2 μCi of [α-32P]CTP) for 30 min at 30 °C. The reactions were stopped by adding 100 μl of stop buffer (10 mM EDTA, 300 mM sodium phosphate, 0.2% sodium deoxycholate, and 25 μg of yeast tRNA). The reaction products were then extracted with phenol, precipitated with ethanol, and analyzed on a 5% polyacrylamide sequencing gel.

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FIG. 1. Templates/substrates used. pMLU112 contains U-less cassette sequence such that the first 112 nt of the transcript contains no U. pPU192 and pHr163-T-C-T possess a single T-C-T indicated with an asterisk, located in the template strand downstream from the promoter and were labeled with $^{32}$P at the 13th phosphate 5’ to the dimer as indicated by an angle bracket in the template. Template 1 contains sequence such that 20-nt-long transcript was obtained by including ApU and three of the ribonucleoside triphosphates in the reaction. Start sites for transcription from the adenovirus major late promoter (MLP), human ribosomal gene promoter (HrP), and promoter for E. coli RNA polymerase (T7 A1 promoter) are indicated by bent arrows. The nucleotide positions of P54II and HinP1 restriction sites, thymine cyclobutane dimer, and the upstream and downstream edges of Template 1 are indicated with respect to the transcription start site. The first 20 nucleotide sequences of the coding strand of Template 1 are also indicated.

**RESULTS**

**Release of RNA Polymerase II Stalled by Nucleotide Starvation or Thymine Dimer**—Previous work has shown that two distinct classes of complexes form after transcription initiation with RNA polymerase II. One class undergoes abortive elongation giving rise to short transcripts (28). A factor called N-TEF2 was shown to promote this abortive termination (12). Further studies showed that one of the components of N-TEF2 called factor 2 was responsible for release of prematurely terminated transcripts (14, 17). We wished to know if factor 2 dissociates RNA polymerase II ternary complex stalled because of “nucleotide starvation” at a U-less cassette or because of a physical block in the template strand in the form of a thymine dimer.

To test for the effect of HuF2 on RNA polymerase II, transcription was carried out with U-less cassette template, pMLU112 (Fig. 1), then HuF2 was added to the reaction mixture, and the reaction was supplemented with UTP and incubated further. The products were analyzed on a denaturing polyacrylamide gel (Fig. 2). In the absence of HuF2, nearly 50% of the short transcripts are converted to the full-length transcripts, indicating that at least this fraction of ternary complex remained stable during nucleotide starvation. In contrast, when HuF2 was included in the reaction mixture in sufficient quantities, nearly all of the transcripts remained truncated (lanes 3–5), indicating that all of the ternary complexes were disrupted by HuF2. These results confirm the previous studies on factor 2 using nuclear extracts for transcription (17). By conducting our experiments with highly purified RNA polymerase II and recombinant factor 2, however, we have greatly reduced the possibility of contribution of other proteins to the release of RNA polymerase II by HuF2.

The effect of HuF2 on RNA polymerase II stalled at a thymine dimer was determined by different strategy. pPU192, which contains a single T-C-T at 149–150 bp in the transcribed strand downstream of the transcription start site and radiolabel at the 13th phosphate 5’ to the dimer, was employed for this purpose (Fig. 1). pPU192 was digested with DNase I after transcription with highly purified RNA polymerase II to prove the existence of RNA polymerase on the template/substrate DNA. It must be noted that because of poor template utilization (1–10% of template is transcribed), we could not perform conventional DNase I footprinting. Instead, by having radiolabel in the vicinity of damage, we relied on the protection of the region containing radiolabel from DNase I degradation. RNA polymerase II ternary complex stalled at the dimer on pPU192 protected a 29–46-nucleotide region centered near $^{32}$P at the 13th phosphate 5’ to the dimer (Fig. 3, lane 1), which contains a single T-C-T at 149–150 bp in the transcribed strand downstream from the stall site and the reactions were incubated with increasing amounts of HuF2 as indicated for 15 min. Finally, RNA polymerase II was chased by adding UTP and CTP and incubating for another 15 min. RNA polymerase II was not chased in the control reaction (lane 1). The reactions were extracted and analyzed on a 5% sequencing gel. The 330-nt-long run-off transcription product is indicated. Lane M, DNA markers of the sizes indicated, which have a slightly different mobility from RNA. Schematic representations of the assay are also shown.
complexes at lesion sites as it does for early elongation complex
generated by pulse-transcription (17).

Cyclobutane Pyrimidine Dimer Is an Absolute Block for Hu-
man RNA Polymerase I—HuF2 is known to act on RNA poly-
merase II in elongation mode. However, there were no data on
whether or not HuF2 had affected any other class of RNA
polymerase. We therefore were interested in the effect of HuF2
on RNA polymerase I stalled at a thymine dimer. Toward that
end, we first determined the effect of thymine dimer in the
template strand on RNA polymerase I. A template/substrate
consisting of a plasmid with a ribosomal RNA gene promoter
(HrP) located 163 nucleotides upstream of a cyclobutane thy-
mine dimer, pHr163-T<><T, was used in our experiments (Fig.
1). In order to analyze the effect of T<><T on transcription by
RNA polymerase I, we linearized the plasmid with HindIII
restriction enzyme, which incises the DNA 166 nucleotides
downstream of the dimer in order to obtain transcripts of
defined size resulting from run-off transcription. Fig. 4 shows
the results of transcription experiments carried out with con-
trol and dimer-containing templates. With the control tem-
plate/substrate, run-off transcripts of 310–320 nucleotides in
length are produced as expected (lane 2). With the template
containing the thymine dimer, truncated transcripts of 148–
160 nucleotides are observed exclusively. With this template,
within the resolution of our assay, there is no detectable run-off
transcript. Thus, we conclude that cyclobutane pyrimidine
dimer is an absolute block for human RNA polymerase I as it
was shown to be for RNA polymerase II (29).

Footprint of RNA Polymerase I Stalled at a Thymine Dimer—
Having found that the thymine dimer blocks the progression of
RNA polymerase I, we wished to find out if the polymerase
remained at the lesion site following blockage and, if so, to
what extent the DNA around the lesion was covered by RNA
polymerase. We employed DNase I and exonuclease footprint-
ing methods to answer these questions as in the case of RNA
polymerase II (Fig. 3 and Ref. 11). The pHr163-T<><T tem-
plate/substrate was digested with DNase I or exonuclease after
transcription with partially purified RNA polymerase I. The
results of footprinting experiments with this substrate are
shown in Fig. 5 for RNA polymerase I. A region of 29–43
nucleotides in the damaged strand is protected from DNase I
(Fig. 5A). These data show that the blocked RNA polymerase I
makes a stable complex at the site of the lesion and also
provides an approximate idea about the DNA region covered
by RNA polymerase I. However, because of the nature of the
footprinting strategy, the 3′ and 5′ boundaries of the region
protected by RNA polymerase I cannot be ascertained by this
method.

To determine the footprint boundaries more precisely, we
used the T4 DNA polymerase 3′ to 5′ exonuclease and the
lambda 5′ to 3′ exonuclease. Ternary complexes were formed,
and the DNA was digested with HinFI, which cut the DNA 48
nt 5′ and 65 nt 3′ to the damage to separate the promoter from
the photodimer and thus prevent multiple rounds of transcrip-
tion and also to make the DNA susceptible to exonucleases.
Then, the DNA was digested with the exonucleases individu-
ally. The results are shown in Fig. 5B. Digestion with T4 DNA
polymerase 3′ to 5′ exonuclease in the absence of transcription
generates a fragment of 50 nt, consistent with a previous report
of block of T4 polymerase 3′ to 5′ exonuclease immediately at
the dimer (30). When RNA polymerase I is present under transcrip-
tion conditions, in addition to the 50-mer arising from the
nontranscribed DNA, two specific bands of 73 and 74 nt are
observed (Fig. 5B). Thus, RNA polymerase I stalled at a T<><T
protects the template strand 23–24 nt 3′ to the dimer. Similar
experiments with lambda 5′ to 3′ exonuclease reveal that this
enzyme specifically generates a fragment 86 nt in length,
meaning that stalled RNA polymerase I blocks the exonuclease
19 nt 5′ to the dimer (Fig. 5C). Thus, the RNA polymerase I
forms a 45-bp exonuclease footprint around the dimer (Fig. 5D).
This is in reasonable agreement with the 43-nt maximum
DNase I footprint obtained in Fig. 5A.

Stability of RNA Polymerase I Ternary Complex at DNA

Fig. 3. HuF2 releases RNA polymerase II stalled at a T<><T.
pFU192, radiolabeled at the 13th phosphate 5′ to the dimer, was first
transcribed with human RNA polymerase II for 30 min. The reactions
were incubated with and without 2 nM HuF2 for 15 min and then
digested with DNase I. Reaction products were then extracted, precip-
itated, and resolved on an 8% sequencing gel. The reaction in lane 1
contains 20 μg/ml α-amanitin to inhibit RNA polymerase II transcrip-
tion. Sizes of DNA segments protected from DNase I (bracket) are
indicated. M indicates DNA size markers in nucleotides.

Fig. 4. Thymine cyclobutane dimer is an absolute block for
elongation by RNA polymerase I. The pHr163-T<><T plasmid was
digested with HindIII restriction endonuclease and then transcribed
with RNA polymerase I in the presence of [α-32P]CTP. Elongation block
at the T<><T site generated the transcript marked Blocked (lane 1).
Reaction with the unmodified version of the template DNA (UM),
pHR163, which was linearized with HindIII (H), generated a run-off
transcript indicated as Run-off (lane 2). Schematic representations
of the products are also shown. The transcription start site and the T<><T
are indicated by a bent arrow and a triangle, respectively. The expected
lengths of transcripts are also indicated in nucleotide numbers. DNA
size markers of indicated nucleotide length were run in lane M.
Lesion—Since RNA polymerase stalled at DNA lesions is considered to be a beacon for coupling of transcription to repair (1, 2), we wished to find out if the stalled polymerase made a stable complex at the site of the damage or dissociated rapidly after encountering the roadblock. We performed a transcription reaction with the thymine dimer substrate/template and probed the ternary complex at the damage site by DNase I footprinting as a function of time. Stalled complex was formed, and then template/substrate was cleaved by *Hin*P11 restriction endonuclease that has restriction sites between the transcription start site and the dimer site (Fig. 1). At time intervals after the addition of *Hin*P11 restriction endonuclease, samples were taken and subjected to DNase I digestion. The results are shown in Fig. 6. The complex is quite stable. It dissociates with a biphasic kinetic with the faster species having a half-life of about 6 h and the slower species with a half-life of about 36 h. The samples were also tested for *Hin*P11 digestion of the DNA region between the promoter and the dimer to ensure that no reinitiation occurred during the course of the experiment. More than 90% of the template/substrate was cut within 5 min after the addition of *Hin*P11, excluding the possibility that footprints shown resulted from multiple initiations (data not shown). Thus, clearly the ternary complex that forms at the lesion site is, like that formed with RNA polymerase II (11), quite stable and capable of acting as a signal for the excision repair system.

**Fig. 5.** Characterization of RNA polymerase I elongation complex stalled at T<>T in the transcribed strand. A, footprinting of RNA polymerase I elongation complex stalled at the T<>T in the transcribed strand. *P. hr163-T<>T*, radiolabeled at the 13th phosphodiester bond 5’ to the dimer, was transcribed with partially purified human RNA polymerase I to form ternary complex at the T<>T. The DNA was then digested with DNase I and analyzed on an 8% polyacrylamide sequencing gel. Control reaction (Trn–, lane 2) was performed by omitting CTP from the reaction mixture. The size of DNA fragments protected from DNase I (bracket) are indicated in nucleotide numbers. M, DNA size markers. B and C, high resolution footprinting of RNA polymerase I elongation complex. *P. hr163-T<>T* was first transcribed with partially purified RNA polymerase I (RNAP I) to form ternary complex at the T<>T site. The DNA was then digested with *Hin*P11 restriction endonuclease and either with T4 DNA polymerase 3’ to 5’ exonuclease (T4 Exo) or 5’ to 3’ lambda exonuclease (λ Exo) (C). *Hin*P11 cuts template DNA at the 65th nt 3’ and the 48th nt 5’ to the T<>T, which generates 115-nt-long radiolabeled DNA fragment. Exonuclease activity of T4 DNA polymerase digested this 115 nt fragment from its 3’ end to the upstream edge of the elongation complex, which generates 73- and 74-nt-long DNA fragments (Fig. 5B, lane 4). The lambda exonuclease digested the 115 nt fragment from its 5’ end to the downstream edge of the ternary complex, which generates 86-nt-long DNA fragment (Fig. 5C, lane 4). The 50-nt-long DNA fragment was due to the digestion by T4 DNA polymerase up to but not past the dimer (Fig. 5, B and C, lanes 2–4). Control reactions (Trn–) were performed by omitting CTP from the reactions. DNA ladders were run in lane L, and DNA size markers of the indicated nucleotide length were run in lane M. Schematic representations of the assays are also shown. The ellipse indicates RNA polymerase I elongation complex stalled at the T<>T site (dark triangle). The asterisk indicates the position of radiolabeled phosphodiester bond. Cleavage sites for *Hin*P11 restriction endonuclease, upstream and downstream edges of the ternary complex determined by T4 Exo and λ Exo, are also indicated. D, the template DNA sequence around the T<>T site is shown. The vertical arrows indicate the edges of the DNA polymerase I elongation complex determined by T4 DNA polymerase and lambda exonuclease. The thick bar above the transcribed strand of the template DNA indicates the region protected against DNase I digestion, which was deduced from the footprinting experiments using three DNA nuclease.
CSB Does Not Affect the Transcription Termination Effect of HuF2—Curiously, although CSB appears in vivo to be the functional counterpart of Mfd of E. coli, the biochemical properties of HuF2 are more similar to those of Mfd (27, 31). There are no known HuF2 mutants, and therefore whether or not HuF2 plays any role in transcription-repair coupling is not known. To find out whether there exist cooperative or competitive interactions of CSB (transcription elongation) and HuF2 (transcription termination), we tested their joint effect on stalled RNA polymerase II. Fig. 9 reveals that even at comparatively high concentrations CSB does not overcome the transcription termination effect of HuF2.

DISCUSSION

The molecular mechanism of transcription-repair coupling in mammalian cells is unknown. Although a possible coupling mechanism has been proposed whereby coupling is mainly due to redistribution of TFIIH between transcription initiation and assembly of the excision nuclease complex (32, 33), we favor an active mechanism whereby the excision nuclease is targeted by a "transcription-repair coupling factor" to the site of stalled RNA polymerase. Presently, there is no in vitro system for transcription-coupled repair in eukaryotic cells. We are unable to differentiate between the two models.

A crucial element of the active model is the interaction of CSA and CSB with a stalled elongation complex and the recruitment of the excision repair proteins to the template-blocking lesion. Genetic evidence shows that both CSA and CSB are required for transcription-coupled repair (7), and biochemical data have revealed interactions among CSB and RNA polymerase II, XPA, TFIIH, and XPG (9, 34). However, the addition of cell extract to stalled RNA polymerase II failed to elicit enhanced repair and instead disrupted the ternary com-

![Image](image-url)
complex (11). Similarly, the addition of CSB, or CSB plus CSA to a partially purified transcription and repair system did not induce preferential repair of the template strand. Hence, it is plausible that other factors in addition to CSA and CSB contribute to preferential repair. Thus, it was of interest to test other factors that are known to have an effect on the elongation step of transcription on RNA polymerase stalled at a thymine dimer. Indeed, in a previous study we found that nuclear extracts contained a “release factor,” which disrupted ternary complexes formed by RNA polymerase II (11). In this regard, HuF2 is of special significance because this factor has several similarities to the prokaryotic transcription-repair coupling factor TRCF (27, 31); both are DNA stimulated ATPases, and both release stalled cognate RNA polymerases from transcriptional pause sites. Here we have demonstrated that HuF2 releases RNA polymerase II stalled at a thymine dimer as well.

The present study provides direct evidence of the function of HuF2 as a RNA polymerase release factor by demonstrating the displacement of RNA polymerase from the template DNA. The possible role of HuF2 in transcription machinery is to retrieve RNA polymerase from template DNA after stalling, thus making RNA polymerase available for transcription initiation. It is known that human RNA polymerase I and III transcription termination factors, PTRF (polymerase I and transcript release factor) and La, respectively, have a stimulatory effect on transcription (35, 36).

Unexpectedly, we find that up until now what was thought to be RNA polymerase II-specific transcription termination factor works with comparable efficiency on RNA polymerase I. It is not unprecedented, however, that one transcription elongation factor has the same effect on two different classes of RNA polymerase. TFIIS has been shown to have a stimulatory effect and cause transcript cleavage on both RNA polymerase I and II transcription at elongation mode (37). These data suggest that regulatory mechanisms of transcription elongation are shared among different classes of eukaryotic RNA polymerases. Recently, a factor that induces dissociation of RNA polymerase I ternary complex paused at terminator sequence, PTRF, was identified (35). PTRF does not require ATP but requires a U-run at the 3′-end of transcript to exert its function. HuF2, on the other hand, does not require any specific RNA sequence for its function based on our results that showed that HuF2 dissociates RNA polymerase stalled at unrelated sequences, the end of the U-less cassette and the T<<T site. Thus, HuF2 constitutes an additional termination factor for RNA polymerase I transcription.

In the present study, we have found that a thymine cyclobutane dimer in the template strand constitutes an absolute block for RNA polymerase I and that the stalled polymerase makes a long lived complex at the site of the lesion. These findings raise certain questions regarding the stimulation or inhibition of excision repair by stalled RNA polymerase I.

There is extensive literature on transcription-coupled repair of genes transcribed by RNA polymerase II (1-5, 30). In contrast, there are only a few studies addressing the questions of the effects of lesions in the rRNA genes on RNA polymerase I and the effect of RNA polymerase I transcription on repair of rRNA genes (38-43). This is, in part, due to the fact that rRNA genes are members of a multigene family, and under physiological conditions only about 30% of the family members are transcribed (39, 41, 44). This makes the study of the effect of transcription on repair problematic. Nevertheless, the repair of thymine dimers and of psoralen monoadducts and cross-links in rRNA genes has been investigated in vivo by taking special precautions to separate transcribed from nontranscribed DNA, and it was found that in rRNA genes transcription was not coupled to repair (38, 41). Yet, another study that measured the relative rates of recovery from initial inhibition of rRNA synthesis after UV irradiation found that rRNA synthesis recovered normally in an XP-C cell line but not in other XP cell lines, suggesting that the transcription-inhibiting photoproducts were removed from the transcriptionally active rRNA genes only in XP-C cell (42). Since the same kinetics of RNA synthesis recovery is observed for genes transcribed by RNA polymerase II, this finding suggested that transcription-coupled repair machinery operates on the rRNA gene because XP-C cells are proficient for transcription-coupled repair but defective in genome overall repair (45).

Recently, it was observed that in yeast UV photoproducts are removed from the transcribed strand of rRNA gene in rad7 and rad16 mutants (43), demonstrating the transcription-coupled repair of rRNA genes because both rad7 and rad16 mutants are defective in genome overall repair but proficient in transcription-coupled repair, as is the XP-C mammalian mutant (46). The present study revealed that RNA polymerase I ternary complex formed at the T<<T has the same biochemical features as RNA polymerase II ternary complex, satisfying one of the potential prerequisites of transcription-coupled repair of rRNA genes.

Finally, the results presented here show that although CSB does not release RNA polymerases stalled at a lesion, HuF2 does. Furthermore, HuF2 is a relatively abundant nuclear protein (15, 16). Hence, any model for transcription-repair coupling that includes the step of targeting of repair proteins by CSB to a stalled RNA polymerase complex and eventual repair of the damage without discarding the transcript (47) must take into account that there is an abundant protein in the nucleus.
that rapidly and efficiently dissociates RNA polymerase and discards the transcript.

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