Transcription-coupled DNA repair is dedicated to the removal of DNA lesions from transcribed strands of expressed genes. RNA polymerase arrest at a lesion has been proposed as a sensitive signal for recruitment of repair enzymes to the lesion site. To understand how initiation of transcription-coupled repair may occur, we have characterized the properties of the transcription complex when it encounters a lesion in its path. Here we have compared the effect of cisplatin-induced intrastrand cross-links on transcription elongation by T7 RNA polymerase and mammalian RNA polymerase II. We found that a single cisplatin 1,2-d(GG) intrastrand cross-link or a single cisplatin 1,3-d(GTG) intrastrand cross-link is a strong block to both polymerases. Furthermore, the efficiency of the block at a cisplatin 1,2-d(GG) intrastrand cross-link was similar in several different nucleotide sequence contexts. Interestingly, some blockage was also observed when the single cisplatin 1,3-d(GTG) intrastrand cross-link was located in the non-transcribed strand. Transcription complexes arrested at the cisplatin adducts were substrates for the transcript cleavage reaction mediated by the elongation factor TFIIS, indicating that the RNA polymerase II complexes arrested at these lesions are not released from template DNA. Addition of TFIIS yielded a population of transcripts up to 30 nucleotides shorter than those arrested at the lesion. In the presence of nucleoside triphosphates, these shortened transcripts could be 3'-elongated up to the site of the lesion, indicating that the arrested complexes are stable and competent to resume elongation. These results show that cisplatin-induced lesions in the transcribed DNA strand constitute a strong physical barrier to RNA polymerase progression, and they support current models of transcription arrest and initiation of transcription-coupled repair.

Several lines of evidence indicate that an RNA polymerase in the elongating mode is required to initiate TCR. Induction of the lac operon of *Escherichia coli* is necessary to observe preferential repair of cyclobutane pyrimidine dimers (CPD) in the transcribed strand (1). Treatment of mammalian cells with α-aminopterin to specifically inhibit RNA polymerase (RNAP) II elongation abolishes the preferential repair of CPDs in expressed genes (2, 3). In yeast with temperature-sensitive mutations in the gene encoding a subunit of RNAPII, a loss of TCR is observed at the non-permissive temperature (4). Mammalian ribosomal genes, transcribed by RNA polymerase I, are not preferentially repaired (5–7), although more recent studies suggest that in yeast there is TCR of ribosomal genes (8). Genes transcribed by RNA polymerase III are also not subject to TCR (9).

A current model for TCR proposes that RNA polymerase arrested at a lesion in DNA constitutes a signal for the repair proteins to initiate repair. This model assumes that the polymerase must be removed from the damaged site to provide access for the repair complex to the lesion (10). In *E. coli*, the *mfd* gene product participates in this process (11). The Mfd protein can promote the release of the RNA polymerase and the incomplete transcript from the DNA template and then target components of nucleotide excision repair to the site of transcription blockage (11, 12). In human cells, the CSB gene product is implicated in this process. However, it remains unclear whether the polymerase is released or translocated away from the site of damage without dissociating from the template DNA (13–15).

As a first step in elucidating how initiation of TCR occurs, we have characterized the properties of the transcription complex when it encounters a lesion. The analysis of different types of arrested complexes should help us understand how an RNA polymerase arrested at a lesion signals the repair proteins to initiate a repair event. Previously, we have shown that a CPD, located in the transcribed strand of template DNA in different sequence contexts, is an absolute block to transcription elongation by mammalian RNAPII (16–19). The arrested complexes are stable (16, 20) and competent to resume elongation after reversal of the lesion by the repair enzyme photolyase (18).

Here we describe the effect of cisplatin-induced intrastrand cross-links on transcription elongation by T7 RNA polymerase (T7 RNAP) and mammalian RNAPII from rat liver. cis-Diaminedichloroplatinum (II) (cisplatin) preferentially reacts with purine bases in the DNA in vitro and in vivo to form the cis-[Pt(NH₃)₂(dGpG)-N(7)(1),N(7)(2)] (cis-1,2-d(GG)), with a frequency of 65%, the cis-[Pt(NH₃)₂(dApG)-N(7)(1),N(7)(2)]; cis-[Pt(NH₃)₂(dGpTpG)-N(7)(1),N(7)(3)]; nt, nucleotide.

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‡ The abbreviations used are: TCR, transcription-coupled repair; CPD, cyclobutane pyrimidine dimer; cisplatin, cis-diaminedichloroplatinum(II); RNAP, RNA polymerase; T7RNAP, T7RNA polymerase; RNAPII, RNA polymerase II; AdMLP, adenovirus major late promoter; BPDE, benzo[a]pyrene diol epoxide; cis 1,2-d(GG), cis-[Pt-(NH₃)₂(dGpG)-N(7)(1),N(7)(2)]; cis 1,3-d(GTG), cis-[Pt-(NH₃)₂(dGpTpG)-N(7)(1),N(7)(3)]; nt, nucleotide.
The genomic nucleotide excision repair and by TCR (22–24). These adducts may impose a more serious problem for an elongating RNA polymerase compared with a CPD, because they cause substantial unwinding and bending of the DNA helix (reviewed in Ref. 21). These lesions have been shown to block transcription by T3, E. coli, and wheat germ RNAP (25–27). The cis-1,3-d(GTG) intrastrand cross-link also blocks RNAPII transcription in extracts of human cells. In addition, the presence of cisplatin-induced lesions in plasmids transfected into human or hamster cells almost completely inhibits RNAPII transcription of a reporter gene (28).

To study the effect of a single cis 1,2-d(GG) or a single cis 1,3-d(GTG) on transcription, we have developed an in vitro transcription system consisting of DNA substrates containing a single cis 1,2-d(GG) in two different sequence contexts and a single cis 1,3-d(GTG) located in the transcribed strand downstream of the T7 promoter or the adenovirus major late promoter (AdMLP), with purified T7 RNAP or rat liver RNAPII and initiation factors, respectively. We show that a single cis 1,2-d(GG) or a single cis 1,3-d(GTG) located in the transcribed strand is a strong block to both T7 RNAP and RNAPII. Furthermore, the efficiency of the block at a cis 1,2-d(GG) is not affected by the sequence context around the lesion. Interestingly, we also observed partial blockage when a single cis 1,3-d(GTG) was located in the non-transcribed strand. The arrested RNAPII complex was stable, as indicated by the ability of elongation factor TFIIS to induce transcript cleavage, producing a population of transcripts up to 30 nts shorter than those arrested at the lesion, which could then be re-elongated up to the lesion when the nucleoside triphosphate precursors were added.

**EXPERIMENTAL PROCEDURES**

**Proteins and Reagents—**T7 RNAP was purchased from Promega. RNAPII, transcription initiation factors, and elongation factor TFIIS, purified from rat liver or recombinant sources as described previously (29), were obtained from Dr. Daniel Reines (Emory University, Atlanta, GA). T4 polynucleotide kinase and T4 DNA ligase were obtained from Invitrogen. E. coli strain MV1184 was a gift of Dr. Joachim Messing (Rutgers University, Piscataway, NJ). D44 IgG anti-RNA antibodies (30) were purified from rodent ascites fluid as described previously (31). Highly purified NTPs and radiolabeled nucleotides were purchased from Amersham Biosciences. Formalin-fixed Staphylococcus aureus was obtained from Calbiochem. Custom-made DNA oligonucleotides were obtained from Qiagen (Chatsworth, CA) or Integrated DNA Technologies (Coralville, IA).

**Preparation of DNA Oligonucleotides Containing a Single Flatinated Adduct—**DNA oligonucleotides of sequence 5'-TCTTCTTCTGTG-
in the non-transcribed strand, respectively; 5′–DNA templates were transcribed
GTG are indicated by strand. transcript is indicated by RO
Apa templates digested with acrylamide gel.

Apa plasmid DNA or a 1160-bp
CACTCTTCTCTTCTTCTAGGCCTTCTCT3
/H11032

a n d 5 mM polyethyleneglycol-8000. The DNA was ligated overnight at
7.5° and 3 mM NaCl for 16 h at 37 °C.

purified by electrophoresis on an 8% denaturing polyacrylamide gel. To

in formamide dye. The single-stranded 159-nt DNA fragments were

with a 3:1 ratio of cisplatin to GG or GTG sites in 1 mM NaHPO4 (pH

and 5′/H11032-TCTTCTTCTAGGCCTTCTCTG-3

were labeled with 32P as described in the text. Elongation was allowed

100 µl reaction mixture containing 10 mM

Covalently closed circular DNA containing a single GTG or a single GG on either the transcribed or the non-transcribed strand was generated by priming 10 µg of plus strand of pUCGTG-TS, pUCGG1-TS, or pUCGG2-TS, or they were ligated to a BamHI fragment of pUCGTGNTS (34) to yield pUCGG-TS, pUCGG1-TS, or pUCGG2-TS, or they were ligated to a BamHI fragment of pUCGTGNTS (34) to yield pUCGTGNTS, pUCGG1-NTS, or pUCGG2-NTS. These plasmids were transformed into the F− E. coli strain MV1184 to produce single-stranded DNA for primer extension, as described (18).

To construct plasmids to receive cisplatin-adducted oligonucleotides,

To construct plasmids to receive cisplatin-adducted oligonucleotides,
at which time the first UTP was required for incorporation. Heparin was added to prevent further initiation, and then 100 \( \mu \)M each of CTP, UTP, and GTP were added to allow elongation to continue, typically for 30 min. Reactions were stopped with SDS and protease K, and nucleic acids were precipitated with ethanol. Samples were resuspended in formamide loading dye, heat-denatured, and electrophoresed through an 8% polyacrylamide gel in TBE (89 mM Tris, 89 mM boric acid, 1 mM EDTA, pH 8.0) containing 8.3 M urea. Gels were dried and autoradiographed using intensifying screens. Transcripts were quantified by using a Bio-Rad GS-363 phosphorimaging device. All transcripts were labeled up to nucleotide 6, making quantitation independent of their subsequent length and G content.

**RNAII Transcription Reactions**—DNA templates were incubated for 30 min at 28 °C with rat liver protein fractions D (2 \( \mu \)g, containing TFIHD and TFIHH) and rat liver RNAII (0.5 \( \mu \)g) in a 20-\( \mu \)l mixture containing 20 mM Hepes-NaOH, pH 7.9, 20 mM Tris-HCl, pH 7.9, 2.2% polyvinyl alcohol, 212 units of RNasin, 0.5 mg/ml acetylated bovine serum albumin, 150 mM KCl, 2 mM dithiothreitol, and 3% glycerol. After incubation, 33 \( \mu \)l of a solution containing fraction B (1 \( \mu \)g, containing TFIHP and TFIE) and recombinant rat TFIIH (3 ng) in the same buffer without KCl were added, and incubation continued for 20 min. Reactions were stopped with SDS and proteinase K, and nucleic acids were precipitated with ethanol. Samples were resuspended in formamide loading dye, heat-denatured, and electrophoresed through a 6% polyacrylamide gel in TBE (89 mM Tris, 89 mM boric acid, 1 mM EDTA, pH 8.0) containing 8.3 M urea. Gels were dried and autoradiographed using intensifying screens. Transcripts were quantified by using a Bio-Rad GS-363 phosphorimaging device. All transcripts were labeled up to nucleotide 6, making quantitation independent of their subsequent length and G content.

**Effect of a Single Cisplatin Intrastrand Cross-link in the Transcribed or Non-transcribed Strand of Template DNA on Transcription Elongation by T7 RNAP and Mammalian RNAII**—DNA substrates containing a single platination adduct in the transcribed or non-transcribed strand downstream of the T7 promoter or the AdMLP were constructed as described previously (34). The presence of the lesion in either strand was confirmed by resistance to cleavage with restriction enzymes HaeIII, StuI, or ApaLI that cleave the DNA substrates at the site of the lesion (data not shown). T7 RNAP or RNAII was stalled downstream of the T7 promoter or AdMLP, respectively, after synthesis of a short \( ^{32} \)P-labeled RNA, followed by the addition of heparin to prevent further initiation. As a result, the transcription products represented a single promoter-dependent elongation event (35). All 4 NTPs were then added to allow elongation to continue. In this transcription system, repair of the lesion cannot occur because of a lack of repair proteins. The effect of either lesion on transcription was then monitored as formation of transcripts shorter than those observed with the undamaged template. We found that when a cis 1,3-d(GTG) was located in the transcribed strand, 70% of transcripts produced after T7 RNAP transcription were shorter than the full-length RNA present in the control (Fig. 2, lane 3). Comparison of the size of these transcripts with those obtained from an undamaged template digested at the site of the lesion with ApaLI indicated that these RNAs were extended up to the site of the cis 1,3-d(GTG) (Fig. 2, lane 7). To rule out the possibility that the full-length RNA originated from some undamaged template contaminating the DNA preparation, an

![Fig. 4. Effect of a single cis 1,3-d(GTG) on transcription by RNAII](image-url)
However, a cis 1,2-d(GG) in the non-transcribed strand was completely bypassed (data not shown).

TFIIS-mediated Transcript Cleavage of RNAPII Complexes Arrested at Cisplatin-induced Intrastrand Cross-links—Transcription elongation factor TFIIS facilitates RNAPII readthrough past various impediments encountered during the normal process of transcription (37). TFIIS induces cleavage of a short RNA from the 3’ end of the nascent transcript positioning the new 3’ end of the RNA into the catalytic site of the polymerase so that transcription can resume. To determine whether the RNAPII ternary complex arrested at a cisplatin-induced adduct was subject to the TFIIS-mediated transcript cleavage reaction, immunopurified complexes were incubated with elongation factor TFIIS and magnesium, followed by separation of the resulting transcription products on polyacrylamide gels. We found that TFIIS-induced cleavage of transcripts arrested at a cis 1,3-d(GTG) (Fig. 7A) or a cis 1,2-d(GG) (Fig. 7B) in the sequence contexts 5’-CTGGCC-3’ or 5’-TAGGCC-3’ produced transcripts of discrete lengths, shortened from 10 to 30 nt, with detectable back-up positions observed at 10, 15, 20, and 30 nt. In the presence of nucleoside triphosphates, these transcripts could be re-elongated up to the site of the lesion (Fig. 7A, lane 6; Fig. 7B, lane 11).

DISCUSSION

We studied the effect of a single cis 1,2-d(GG) or a single cis 1,3-d(GTG) intrastrand cross-link on transcription elongation by both T7 RNAP and mammalian RNAPII from rat liver using a reconstituted in vitro transcription system with purified proteins. We found that when these lesions were located in the transcribed strand downstream of the T7 promoter or the AdMLP, they efficiently blocked transcription by either polymerase. Furthermore, the extent of blockage was not affected by the sequence context surrounding a cis 1,2-d(GG). Interestingly, a single cis 1,3-d(GTG) located in the non-transcribed strand slightly inhibited transcription by either polymerase.

The bulky nature of cisplatin-induced intrastrand cross-links and the DNA structural changes induced by their presence in the double helix may help to understand why these adducts cause RNAP arrest. Both lesions unwind the DNA, the
Effect of Cisplatin Adducts on Transcription

Fig. 7. Effect of elongation factor TFIIH-mediated transcript cleavage of RNAPII transcription complexes arrested at cisplatin adducts. A, complexes arrested at a cis 1,3-d(GTG) were incubated without (lane 1) or with (lanes 2-5) increasing amounts of elongation factor TFIIH (from 0.5 to 5 ng) and MgCl$_2$ for 1 h at 28°C. Lane 6, same as lane 5, except that NTPs were added, followed by incubation for 15 min at 28°C. Run-off RNA (RO) and RNA resulting from transcription arrest at a cis 1,3-d(GTG) are marked with an arrow. M, 10-bp ladder. B, complexes arrested at a cis 1,2-d(GG) were incubated without (lane 1 and 6) or with (lanes 2-5 and 7-10) increasing amounts of elongation factor TFIIH and MgCl$_2$ for 1 h at 28°C. Lane 11, same as lane 10, except that NTPs were added, followed by incubation for 15 min at 28°C. Run-off RNA and RNA resulting from transcription arrest at a cis 1,2-d(GG) (GG) are marked with an arrow.

cis 1,2-d(GG) by 13–25° and the cis 1,3-d(GTG) by 19–23° (38). They also cause the DNA to bend toward the major groove, the cis 1,2-d(GG), by 32–78° (39) and the cis 1,3-d(GTG) adduct by 25–35° (39, 40). In addition, NMR analysis of oligonucleotides containing a single cis 1,3-d(GTG) adduct has shown that the overall structure of the DNA is more distorted than that of DNA containing a single cis 1,2-d(GG) (41). This might explain why the cis 1,3-d(GTG) adduct also has an effect on transcription when located in the non-transcribed strand, whereas the cis 1,2-d(GG) does not. Furthermore, the base pairing is lost at the 5′ platinated guanine as well as in the central T:A base pair, and the central thymine is extruded in the minor groove (41).

Several natural transcription arrest sites are characterized by DNA helix distortions (42). It is likely that the structural changes induced by the cis 1,2-d(GG) and the cis 1,3-d(GTG) would affect the formation and/or the stability of the RNA-DNA hybrid, an essential component of the elongation complex (43). A weak RNA-DNA hybrid has been proposed as a primary determinant of the arrest modality, as it promotes backward translocation of RNAP along the DNA template. This in turn can result in the displacement of the 3′ end of the RNA from the catalytic site, leading to polymerase arrest (44, 45). Similar to the effect of cisplatin-intrastrand cross-links, transcription arrest caused by several bulky lesions including CPDs (16), adducts formed by the potent carcinogen N-2-acetylaminofluorene (46), psoralen intra- and interstrand cross-links (47), and benzo[a]pyrene diol epoxide (BPDE)-induced lesions (48), which also cause significant helix distortion in the DNA, has been attributed to weakening of the RNA-DNA hybrid when the lesion is present at the ternary complex.

The sequence context around a cis 1,2-d(GG) did not have a significant effect on the extent of RNAP blockage at the site of the lesion. This result suggests that the distortion induced by the cis 1,2-d(GG) is the major factor in causing transcription arrest at this lesion. Similar to our results, Corda et al. (25) reported that a cis 1,2-d(GG) caused complete blockage of wheat germ RNA polymerase when located in the sequence context 5′-CTGGCC-3′. However, Cullinan et al. (27) found that a cis 1,2-d(GG) in the sequence context 5′-TAGGCC-3′ was not a block to RNAPII transcription in HeLa cell extracts, suggesting that differences in transcription systems and/or in the source of RNA polymerase might play a role in determining the extent of arrest at this lesion.

When a cis 1,3-d(GTG) was located in the transcribed strand, the extent of RNAPII blockage at this lesion was more pronounced for RNAPII than for T7 RNAP. Similarly, T7 RNAP can bypass several bulky lesions including CPD (19, 49), acetylaminofluorene (50), psoralen adducts (33), and BPDE adducts (51) more readily than RNAPII. Perlow et al. (48) have proposed that the ability of T7 RNAP to readthrough an anti-BPDE DNA adduct is due to the more open structure of the catalytic site of the T7 enzyme compared with that of the eukaryotic RNAPII, as revealed from the crystal structures of these proteins (52–54). They have confirmed that, indeed, this is the case by molecular modeling analysis of the active site of T7 RNAP and of RNAPII containing an anti-BPDE DNA. It is likely that, similar to the results with an anti-BPDE DNA adduct, a bulky lesion like the cisplatin-intrastrand cross-link could more easily occupy the catalytic site of T7 RNAP than that of RNAPII and, as a result, could represent a weaker block to T7 RNAP than to RNAPII transcription.

Transcription complexes arrested at a cis 1,2-d(GG) or a cis 1,3-d(GTG) lesion were subject to the transcript cleavage reaction mediated by elongation factor TFIIH. TFIIH activates a cryptic endonucleolytic activity intrinsic to RNAPII that...
cleaves a short oligonucleotide from the 3' end of the RNA, repositioning the 3' end of the transcript into the catalytic site (37). As a result, transcription can resume from this newly formed 3' end. Addition of TFIIS to transcription complexes arrested at a cisplatin intranstrand cross-link produced a population of transcripts up to 30 nt shorter than those arrested at this lesion, and these could be re-elongated up to the damaged site. This result indicates that RNAPII can be displaced from a cisplatin intranstrand cross-link without being released from template DNA. Similar to the results with the cisplatin cross-links, TFIIS induced transcript cleavage when RNAPII was arrested at a CPD in the transcribed strand (16, 18). After TFIIS addition, a population of transcripts up to 35 nt shorter than those arrested at a CPD was produced. However, the fine structure for the population of back-up complexes in the case of cisplatin intranstrand cross-links was dissimilar to that seen when a CPD was the arresting lesion. This pattern could be reflecting the nature and/or the extent of the helix distortion by the respective lesion. Based on our footprinting analysis of RNAPII complexes arrested at a CPD (18) that covers 10 nt downstream and 25 nt upstream of the lesion, we predict that those transcription complexes that had backed up from a cisplatin intranstrand cross-link 20 nt or more had cleared sufficient distance from the lesion to render it accessible for repair.

Our findings that cisplatin intranstrand cross-links cause RNAPII arrest and that transcription complexes arrested at these lesions are subject to the transcript cleavage reaction mediated by elongation factor TFIIS correlate with TCR of that lesion, and these could be re-elongated up to the damaged site. This result indicates that RNAPII can be displaced from a lesion, and these could be re-elongated up to the damaged site. Based on our footprinting analysis of RNAPII complexes arrested at a CPD (18) that covers the respective lesion. Based on our footprinting analysis of RNAPII complexes arrested at a CPD (18) that covers 10 nt downstream and 25 nt upstream of the lesion, we predict that those transcription complexes that had backed up from a cisplatin intranstrand cross-link 20 nt or more had cleared sufficient distance from the lesion to render it accessible for repair.

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