RNA Polymerase Signals UvrAB Landing Sites*

Byungchan Ahn and Lawrence Grossman‡

From the Department of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205

Transcription when coupled to nucleotide excision repair specifies the location in active genes where preferential DNA repair is to take place. During DNA damage-induced recruitment of RNA polymerase (RNAP), there is a physical association of the β subunit of Escherichia coli RNAP and the UvrA component of the repair apparatus (G. C. Lin and L. Grossman, submitted for publication). This molecular affinity is reflected in the ability of the RNAP to increase, in a promoter-dependent manner, DNA supercoiling by the UvrAB complex. In the presence of the RNAP, the UvrAB complex is able to bind to promoter regions and to translocate in a 5′ to 3′ direction along the non-transcribed strand. As a consequence of this helicase-catalyzed translocation, preferential incision of DNA damaged sites occurs downstream on the transcribed strand. Because of the helicase directionality, the initial binding of the UvrAB complex to the transcribed strand would inevitably lead to its collision with the RNAP. These results imply that the RNAP-induced DNA structure in the vicinity of the transcription start site signals a landing or entry site for the UvrAB complex on DNA.

The binding of the UvrA to undamaged DNA is 102- to 103-fold weaker than to damaged sites (6, 19, 20). Hence, the specificity of UvrA for damaged sites is quite limited given that UvrA must be able to recognize a single damaged site per E. coli genome. It seems unlikely that UvrA or UvrAB can locate damage by random passive diffusion, suggesting an alternative scanning mechanism for interaction with damaged substrates (6, 14, 20). The mechanism by which the UvrAB complex recognizes damaged sites during translocation is not yet understood.

A sophisticated requirement of NER is transcription-coupled repair, which quickly targets the repair machinery to genes that are actively transcribed by RNAP. The process is responsible for the rapid removal of possible transcription blocking lesions from the transcribed strand, while the non-transcribed strand is repaired at a slow rate similar to the overall genome (21). Preferential repair was found to be conserved from E. coli (22) to yeast (23) and to mammalian cells (21, 24). The identification of those factors that play a role in transcription-coupled repair is the elucidation of the mechanism of this conserved pathway. In E. coli it has been resolved to a considerable detail. A TRCF was isolated that is necessary and sufficient for transcription-coupled repair in a defined in vitro system (25). TRCF, encoded by the mfd gene, is able to recognize and displace the stalled RNAP complex and to lead the UvrABC complex to the site of the lesion possibly due to its affinity for the damage recognition subunit UvrA (26). One of the astonishing recent discoveries was that a basal transcription factor, TFIIH, contains components that are integral to nucleotide excision repair, thus establishing an important link between transcription and DNA repair in higher organisms (27–31). It is speculated that the preferential targeting of repair proteins to actively transcribed genes in eukaryotes is facilitated by the obligatory loading of repair proteins onto promoter regions during transcription initiation (26).

It is unclear how the translocation of the UvrAB complex is linked to transcription-coupled repair at the molecular level. From reversible cross-linking experiments and resolution of the resultant components of E. coli RNAP with the Uvr proteins during SOS, it is suggested that a physical interaction between RNAP and repair proteins may be required for the transcription-coupled repair. The effect of this physical interaction is the subject of this study in which its effect on damaged DNA was examined.

Here we show that the UvrAB complex preferentially binds to the promoter regions of DNA in an open complex induced by RNAP resulting in enhancing UvrAB induced supercoiling. Further, incision takes place in the strand opposite to which the UvrABC endonuclease binds. The model proposed for the role of RNA polymerase in the site and specificity of nucleotide excision repair is presented.

EXPERIMENTAL PROCEDURES

Materials—E. coli strain (DH5α) was from Life Technologies, Inc. Deoxyribonucleoside triphosphates (ATP and GTP) were purchased from Pharmacia LKB Inc. Ribonucleoside triphosphates (CTP and UTP)

---

* This research was supported by National Institutes of Health Grant GM22846 (to L. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed.

1 The abbreviations used are: NER, nucleotide excision repair; bp, base pair(s); BSA, bovine serum albumin; DTT, dithiothreitol; ERCC, excision repair cross-complementing; λ, bacteriophage λ; mfd, mutation frequency decline; RNAP, RNA polymerase; rNTP, ribonucleoside triphosphate; TRCF, transcription repair coupling factor; TS, transcribed strand; NTS, non-transcribed strand.
were obtained from BRL. Radioactive compounds (dNTP: 3000 Ci/mmole) were purchased from Amersham or ICN. Potassium permanganate was from Aldrich. Urea (Ultra-Pure grade) and acrylamide were from Life Technologies, Inc. DTT, piperidine, and glycerol were from Boehringer Mannheim. Tris base was from Sigma. Iso-mercaptoethanol was from J. T. Baker. Other chemicals were purchased from Sigma, J. T. Baker, Boehringer Mannheim, or Life Technologies, Inc.

The UvrA, UvrB, and UvrC proteins were purified according to published procedures (33). E. coli DNA topoisomerase I was purified from E. coli strain (RB 968) harboring the plasmid (p W 312-Sal) encoding the topoisomerase I isoenzyme (Professor James C. Wang, Harvard). Proteinase K was from Sigma. BSA was purchased from Sigma or Miles. T7 RNA polymerase and Sequenase (United States Biochemical Corporation) were obtained from Boehringer Mannheim. Klenow fragment and restriction enzymes were obtained from New England Biolabs. Calf thymus DNA topoisomerase I was obtained from Life Technologies, Inc. Micrococcus luteus DNA topoisomerase (1 mg/ml) was from Applied Bio-technology Inc. Plasmid DNAs, ptZ18R (Pharmacia Biotech Inc.) and phiE6 (34), were prepared using the Qiagen column protocol.

UV Irradiation—DNA molecules were placed on parafilm floating on ice water and irradiated by ultraviolet (UV) light (germicidal lamp) at 13 cm (UV intensity 2000 mW/cm2). Preparation of DNA Fragments Containing the Aβ, Promoter—Aβ, promoter fragments were prepared by cutting the plasmid phiE6 with restriction enzymes and labeled at their 3' ends using the Klenow fragment or Sequenase. The 461-bp DNA fragments containing the Aβ promoter were separated on a native polyacrylamide gel after restriction digestion with BglII and HpaII and then digested with HpaII and then labeled by filling in with [α-32P]dCTP for the non-transcribed strand (NTS). To label the transcribed strand (TS), BglII-Smal digested fragments were filled in with [α-32P]dCTP and then digested with HaeIII restriction enzyme. The sample was loaded onto a 5% polyacrylamide gel with 1 TBE (44.5 mM Tris, 89 mM glycine, 0.04 cm, 19:1 (w/w); 0.04 cm, 19:1 (w/w); 8 M urea, 1 TBE). For sequencing comparison, Maxam-Gilbert sequencing was carried out (35).

KmO4 Reactions on Transcription Complexes—Transcription complexes were formed as in the footprinting experiment, and treated with 1 μl of 100 mM KMnO4 and incubated for 2 min at 37°C (36). The reactions were terminated by the addition of 2 μl of β-mercaptoethanol and placed on ice, followed by the addition of 23 μl of stop solution. After phenol/chloroform extraction, each sample was precipitated with 100% ethanol and 20 μg of glycogen and then treated with 1 μl piperidine as described by Maxam and Gilbert (35). Sequencing reactions were performed as described by Maxam and Gilbert with the identical substrate used in transcription complexes. After drying in vacuo, all samples were resuspended in loading buffer and electrophoresed through 5% urea-sequencing gels. Gels were dried and exposed to Kodak XAR-5 film using a Dupont Lightning Plus intensifying screen or to a Phosphor imaging plate.

Incision—Incision by the UvrABC endonuclease or M. luteus UVE-endonuclease was conducted in a volume of 20 μl. The final reaction mixtures contained 30 mM Tris-HCl (pH 7.6), 85 mM KCl, 10 mM MgCl2, 1 mM EDTA, 2 mM ATP. After incubation of the UV-irradiated (400 J/m2) DNA substrates with RNAP at 37°C for 10 min, UvrA (0.1–0.2 pmol) and UvrB (0.3–0.6 pmol) protein were added and then incubated for another 10 min. Incision was initiated by the addition of UvrC (0.3 pmol) protein and incubated for 5 min. The reactions were stopped by the addition of 21 μl of stop solution and placed on ice. The samples were extracted with phenol in a ratio of 1 to 1.1 to remove proteins, precipitated with 100% ethanol and then treated with 70% (v/v) ethanol, and dried. Pellets were resuspended in 4 μl of loading solution and heated for 3 min at 90°C. The samples were loaded onto a 5–6% urea-sequencing gel. The M. luteus UVE-endonuclease was carried out in the buffer as described previously (37) or under the same conditions as in the UvrABC incision reaction.

RESULTS

The Effect of RNA Polymerase on UvrAB Supercoiling—RNA polymerase unwinds DNA as it binds to the promoter unwinding DNA continuously as the enzyme extends the growing nascent RNA chain (38). The localized unwinding results in the transient formation of positive supercoils ahead of the RNAP complex and negative supercoils behind it (39–41). Simian virus 40 large tumor antigen (SV40 T antigen) acts similarly as a helicase and is able to unwind duplex DNA producing positive and negative supercoils in an ATP hydrolysis-dependent reaction (42). This process relies on the translocation of proteins along DNA which is accompanied by the unwinding of closed circular duplex DNA. Like SV40 T antigen and RNA polymerase, the UvrAB complex can also unwind DNA generating both positive and negative supercoils in an ATP hydrolysis-dependant reaction (12). It seems that the initial entrance into DNA by helicases such as SV40 T antigen and the UvrAB complex is due to localized unwinding because Dnab and UvrD, which cannot unwind duplex DNA by just binding to it, are unable to generate supercoils. However, the efficiency with which the UvrAB complex unwinds duplex DNA has not yet been determined. RNA transcription-dependent supercoiling requires the specific binding of RNAP to its promoter for translocation along a transcription unit, whereas SV40 T antigen and the UvrAB complex can bind to DNA nonspecifically. It, however, has not yet been determined where the UvrAB complex initiates translocation.

The specific interaction between the β-subunit of RNAP and UvrA provides insights into the potential interaction between transcription and UvrAB catalyzed translocation. Supercoring reactions were performed under non-transcribing conditions to avoid the complication of dealing with the supercoiling introduced by RNA transcription. UvrA and UvrB proteins were

2 H. Koo and B. Ahn, unpublished information.
3 G. C. Lin and L. Grossman, submitted for publication.
incubated with the closed circular relaxed plasmid, pTZ18R, in the presence of ATP and E. coli DNA topoisomerase I protein. The accumulation of highly positively supercoiled species of DNA (marked in (+)) was greatly increased by T7 RNAP (28-fold, lane 6 in Fig. 1A) or E. coli RNAP (10-fold, lane 7 in Fig. 1B). This synergistic effect on supercoiling is clearly RNAP concentration-dependent. Even at equimolar ratios of relaxed plasmid DNA:RNA polymerase (lane 3 in Fig. 1A, lanes 3 and 5 in Fig. 1B) enhancement is evident. One to 0.1 molar ratios of UvrAB to RNAP were sufficient for stimulation of supercoiling (lane 4 in Fig. 1A and lane 5 in Fig. 1B). Saturation was reached at 1:1 ratios (lane 5 in Fig. 1A). At these concentrations little supercoiling occurred by RNAP alone (lane 7 in Fig. 1A, lane 8 in Fig. 1B). Supercoiling stimulation may reflect UvrAB tracking along the template DNA because of the supercoiling reaction under these non-transcribing conditions. Enhancement of supercoiling by the UvrAB complex seems to be due to the binding of RNAP to its promoter (this paper).

Topoisomeric Forms Generated by the UvrAB—Two-dimensional gel electrophoresis was used to determine the nature of
the DNA species formed in the presence of E. coli DNA topoisomerase I, UvrA, UvrB, and RNAP. The first dimension was carried out in TPE buffer, and the second dimension was performed in chloroquine-containing TPE buffer (Fig. 1C). The electrophoretic positions of relaxed DNA (d), nicked form (a), and highly positively supercoiled forms (c) are shown (1 in Fig. 1C). It was reported in previous studies (12) that highly positive supercoils are formed by UvrAB proteins in this supercoiling reaction (2 in Fig. 1C). When the DNA substrate was incubated in the presence of E. coli DNA topoisomerase I, UvrA, UvrB, and T7 RNAP, highly positively supercoiled DNA was produced as the amount of T7 RNAP was increased (3 and 4 in Fig. 1C). These data are in agreement with those in Fig. 1A and show that the UvrAB complex can generate transient positive and negative supercoils in the presence of RNAP under non-transcribing conditions.

The Effect of UV Irradiation of the DNA on Supercoiling—The supercoiling by the UvrAB complex was greatly increased when UV-irradiated relaxed DNA was used in the supercoiling reaction (12). It has been postulated that DNA-damaged sites may provide the UvrAB complex with an anchoring site enhancing the translocation of the UvrAB complex along the DNA. Therefore, the binding of the UvrAB complex to DNA was increased, resulting in increasing the number of plasmid molecules being converted into highly positively supercoiled form. In Fig. 1D, supercoiling by the UvrAB complex is increased (8.3-fold) as a consequence of UV damage (393 J/m²) as reported previously (12, 13). The increase (14-fold) in the formation of highly positive supercoils in the presence of RNAP (lane 5) was greater than that of damaged DNA alone. When UV-damaged DNA and RNAP were included in the supercoiling reaction, supercoiling (19.5-fold, lane 6) was greater than that of undamaged DNA (lane 5) and that in the absence of RNAP (lane 4). The enhancement of supercoiling suggests that RNAP and/or UV-damaged sites provide binding sites for the UvrAB complex. The shift of topoisomers (lane 2) is due to the unwinding of DNA by the presence of photodimers.

Promoter Requirement for Enhancement of Supercoiling—T7 promoterless plasmids were constructed to determine if the specific binding of RNA polymerase to its promoter is required for enhancement of the UvrAB supercoiling. The T7 promoter region of pTZ18R was excised as a 365-bp fragment by PvuII restriction enzyme followed by ligation. It can be seen in Fig. 2 that the positively supercoiled form (+) was generated by the UvrAB complex with the DNA template containing the T7 promoter (lanes 1 and 2). Under conditions of preincubation with T7 RNAP and relaxed plasmid pTZ18R, there was no further effect on the extent of supercoiling (lanes 3–7). However, in the reaction with the T7 “promoterless” plasmid DNA, the extent of supercoiling was not affected with synergistic levels of T7 RNAP. There were some changes within the spectrum of DNA topoisomeric species generated by the T7 RNAP alone (lane 12) without the UvrAB complex, suggesting some nonspecific binding of T7 RNAP under these experimental conditions. The data from these experiments suggest that the specific binding of T7 RNAP to its promoter may be related to the enhancement of DNA supercoiling by the UvrAB complex and that the RNAP may exert its influence through its effect on DNA structure. The location and extent of binding of UvrAB may be enhanced by the specific binding of RNAP to its promoter. It suggests that the local DNA structure around the promoter is a potential site for the strand and site preferential binding of the UvrAB protein complex.

E. coli RNAP Footprinting with ΔPr Promoter—DNase I footprinting was performed to localize the binding of the UvrAB complex to DNA influenced by E. coli RNAP. ΔPr promoter fragments isolated from plasmid pHE6 were incubated with E. coli RNAP and then digested with DNase I. E. coli RNAP bound to the ΔPr promoter fragment protects a region from 48 to +18 on the non-transcribed strand and from −48 to +20 on the transcribed strand (Fig. 3). This cleavage site is similar to that observed by Oppenheim’s group (43). The DNase I footprinting pattern obtained for RNAP at ΔPr is also similar to other strong promoters like those in the lac UV5 and ΔPr promoters. For both promoters, protection by RNAP is extended to about 20 nucleotides downstream beyond the transcription start site. The UV5 promoter was protected upstream of the start site to around −52 (44) and the ΔPr was protected in the promoter to position −57 and to −52 on the transcribed and non-transcribed strands, respectively (45).

In Fig. 3, the DNase I footprinting pattern reflects the location of RNAP, and the UvrAB complex in the presence of ATP. Lanes 6 and 12 are control lanes of free DNA. Lanes 1 and 7 are those regions protected by RNAP. The UvrAB footprints in lanes 4, 5, 10, and 11 show no specific binding by the UvrAB complex under these reaction conditions. When UvrAB and RNAP were incubated together, there is an extended protection region downstream (position a showed 50% protection) beyond the transcription start site at +1 in only the non-transcribed strand (lanes 2 and 3 in Fig. 3B), whereas no like protection is seen in the transcribed strand (lanes 8 and 9). The extended region of protection in the non-transcribed strand is not well defined. It is likely that the UvrAB complex is moving downstream as a consequence of ATP hydrolysis. The extended region of protection in the non-transcribed strand is seen with T7 RNAP as well (data not shown). The direction of the region on the non-transcribed strand of the DNA helix is 5’ to 3’, which is consistent with the directionality of the UvrAB helicase. The UvrAB complex should predominantly bind to this strand to translocate along the strand in a 5’ to 3’ direction.

Potassium Permanganate Reactivity—The thymine residues in the single-stranded region of DNA within the open complex (so called transcription bubble) were sensitive to KMnO₄ reactivity. The oxidized thymine residues are detected by piperi-
The KMnO₄ reactivities, but more reactive sites were on the non-
single-stranded region. Oppenheim’s group observed similar
2 (Fig. 4) may be uniquely melted generating a unique
B). This indicates that the region between RNAP (
form of RNAP because cleavage is not observed in the presence
bypiperidinetreatment. This cleavage is specific for the active
form of RNAP because cleavage is not observed in the presence
of heat-denatured RNAP (data not shown) or in the absence
of RNAP (lane 3). This indicates that the region between −11 and −2 (Fig. 4B) may be uniquely melted generating a unique single-stranded region. Oppenheim’s group observed similar KMnO₄ reactivities, but more reactive sites were on the non-transcribed strand in the ßl promoter (43). Open complexes of RNAP and the lac UV5 promoter (36) and late promoters in phage (48) melt the DNA helix from −10 to +4 (lac UV5), from −11 to +3 or +4 (phase λ), from −11 or −12 to +4 or +5 (phase 82), and from −12 to no more than +5 (phase 21) are melted since thymines in this region are reactive with KMnO₄. Those modified bases are detected by primer extension treatment. In the presence of UvrAB (lanes 6 and 11), the reactivities of these sites were significantly diminished (70%) on both the transcribed and non-transcribed strand. However, the extent of reduction is less decreased in the presence at equal molar concentrations of single-stranded binding protein (data not shown). It is likely, therefore, that the binding of UvrAB occurs at the distorted promoter region generated by RNAP binding and such binding allows the UvrAB complex to specifically translocate along DNA in a 5’ to 3’ direction on only the non-transcribed strand.

Incision Reactions—The DNA fragments used in the DNase
footprinting were irradiated with UV-light (254 nm) and were

![Diagram](image-url)
employed to determine those sites incised in UV-irradiated DNA by the UvrABC endonuclease which occurs on both sides (3' and 5') of the damaged site (1, 2). The incision experiments are shown in Fig. 5A. Lane 3 shows the DNase I protection in the promoter region in the transcribed strand by the E. coli RNAP. Heat-denatured RNAP provides no protection (lane 2). Incision at the 3' side of the damaged site was detected with the 3' end-labeled substrate when catalyzed by the UvrABC endonuclease (lanes 4 and 7). In lane 5, the extent and sites of incision by the UvrABC complex in the presence of heat-denatured RNAP is the same as that in lane 4. In the presence of active RNAP, the two bands (a and b) at the promoter region disappeared, whereas the intensity of incised sites downstream (lane 6 in Fig. 5A) from the transcription start site increased 1.7–3-fold. However, no increase of incision was detected in the case of the non-transcribed strand (lane 8) or one strand of DNA fragment lacking E. coli RNAP promoter (lane 14) in the presence of RNAP. Under transcribing conditions, the increase is about 1.6–2-fold (lanes 11 and 13), which is lower than that in the presence of ATP (lanes 4 and 7 in Fig. 5B). It is likely that the UvrAB complex is able to displace stalled RNAP complexes.

The M. luteus UV-endonuclease recognizes UV-damaged sites and hydrolyzes the phosphodiester bond between sugar moieties of pyrimidine dimers in UV-irradiated DNA (37). Incision of the M. luteus UV-endonuclease incision is unaffected by RNAP. However, under transcribing conditions, preincubation with RNAP decreased incision by the M. luteus UV-endonuclease (lanes 3 and 6 in Fig. 6).

Therefore, the increase in incision of damaged sites on the transcribed strand is specific for UvrABC. It is likely that the preferred binding of the UvrAB complex to the non-transcribed strand in the presence of RNAP led to increased incision downstream from the transcription start site in the transcribed strand, suggesting that the UvrAB complex translocates along the non-transcribed strand and recognizes damaged sites on the complementary (the transcribed strand).

**DISCUSSION**

The findings presented herein suggest that RNAP enhances UvrAB supercoiling as a consequence of the specific binding of RNAP to its promoter. The proposed DNA structure, of an open complex surrounding a transcription start site, provides a potential mechanism for this supercoiling.

Fig. 5. Incision of UV-irradiated DNA by the UvrABC endonuclease. A, E. coli RNAP (0.3 pmol) was preincubated with 3' terminally labeled TS or NTS in the presence of 2.3 μM (bp) unlabeled DNA for 10 min at 37 °C and then UvrA (0.1 pmol) plus UvrB (0.3 pmol) were added to reaction and continued incubation for 10 min. The reactions were treated with UvrC (0.3 pmol) for 5 min. The reactions were terminated by adding stop solution. Lanes 1–6 show experiments with labeled TS. Lanes 7–9 show experiments with labeled NTS. Lanes 10–15 show experiments with labeled DNA (no promoter). Lane 1, DNase I digestion of free DNA; lane 2, DNase I digestion in the presence of heat-denatured RNAP; lane 3, DNase I digestion in the presence of native RNAP; lane 4, UvrABC incision; lane 5, UvrABC incision in the presence of heat-denatured RNAP; lane 6, UvrABC incision in the presence of native RNAP; lane 7, UvrABC incision; lane 8, UvrABC incision in the presence of native RNAP; lane 9, DNA cleavage by M. luteus UV-endonuclease. Lane 10, DNA; lane 11, DNase I digestion of free DNA; lane 12, DNase I digestion in the presence of native RNAP; lane 13, UvrABC incision; lane 14, UvrABC incision in the presence of native RNAP; lane 15, DNA cleavage by M. luteus UV-endonuclease (1 μg). DNA were UV-irradiated (400 J/m²). The positions a and b represent the decreased band in incision of the UvrABC endonuclease. B, comparison of incision between non-transcribing and transcribing conditions. Incision reactions were followed as described in A. Lanes 1–6 show experiments under non-transcribing (0.5 mM ATP) and lanes 7–12 show experiments under transcribing conditions (0.125 mM each GTP, CTP, and UTP with 0.5 mM ATP). Lanes 1 and 8, DNase I digestion of free DNA; lanes 2 and 9, DNase I digestion in the presence of RNAP; lanes 3 and 12, UvrA (0.1 pmol) B (0.3 pmol) C incision; lanes 6 and 10, UvrA (0.05 pmol) B (0.15 pmol) C incision; lanes 4 and 13, UvrA (0.1 pmol) B (0.3 pmol) C incision in the presence of native RNAP (0.3 pmol); lanes 7 and 11, UvrA (0.05 pmol) B (0.15 pmol) C incision in the presence of native RNAP. Lane 5, DNA cleavage with M. luteus UV-endonuclease (1 μg).
endonuclease. The digestion of UV-damaged DNA (TS DNA of non-transcribed strand as a consequence of its 5\'-9\' endonuclease incision after incubation with RNAP for 10 min. In lanes 1–3, 300 ng of M. luteus UV-endonuclease were added; in lanes 4–6, 1.0 \( \mu \)g of M. luteus UV-endonuclease were added.

Fig. 6. The cleavage of UV-damaged DNA by M. luteus UV-endonuclease. The digestion of UV-damaged DNA (TS DNA of \( \lambda l\) promoter fragment) in the presence of RNAP (0.3 pmol) under transcribing conditions. The M. luteus UV-endonuclease was incubated for 5 min. Lanes 1 and 4, M. luteus UV-endonuclease incision; lanes 2 and 5, M. luteus UV-endonuclease incision in the presence of RNAP without preincubation; lanes 3 and 6, M. luteus UV-endonuclease incision after incubation with RNAP for 10 min. In lanes 1–3, 300 ng of M. luteus UV-endonuclease were added; in lanes 4–6, 1.0 \( \mu \) g of M. luteus UV-endonuclease were added.

The UvrAB complex should be free to translocate along the non-transcribed strand as a consequence of its 5\' to 3\' unidirectionality, whereas its binding to the transcribed strand would lead to a collision with the RNAP. Incision of DNA damaged sites on the transcribed strand occurs preferentially as the UvrAB complex translocates along the non-transcribed strand.

There are several explanations for the enhancement of supercoiling. First, negative and positive supercoils generated by the protein tracking along the DNA could spontaneously diffuse along the DNA helical axis. The supercoiling assay was developed to monitor the formation of highly positive supercoils (41), and its sensitivity clearly relies on the action of E. coli DNA topoisomerase I. If the relaxation rate of negative supercoils is slower than the diffusion rate of both supercoils, then positive supercoils may not accumulate. If diffusion of both supercoils slows down, the extent of the resultant supercoiling should increase. Therefore, the binding of RNAP may be one of many factors able to block the diffusion of supercoils. It has been speculated, however, that the frictional torque of the transcription complex, including the nascent mRNA, has to be greater than the DNA supercoiling torque in order to prevent the positively and negatively supercoiled regions from mutual annihilation (39, 41). Thus, the RNAP itself may not be sufficient to block mutual annihilation by diffusion. In this experiment, it seems that the binding of RNAP may not contribute to the blocking of rotational diffusion of both supercoils because the supercoiling reactions in this work were performed in the absence of transcription. Second, the binding of RNAP to its promoter may create DNA topoisomerase I-accessible sites. However, topo I acts randomly on negatively supercoiled DNA regions. Third, the extent of supercoiling was saturated when the supercoiling reaction was incubated for 1 h (12). If the UvrAB complex dissociates from highly positively supercoiled DNA and rebinds to the rest of relaxed DNA, the extent of supercoiling would be expected to be concentration-independent. However, the extent of supercoiling was increased as the concentration of the UvrAB complex was increased even when the reactions were incubated for 1 h (12), implying that the final extent of supercoiling may be proportional to the number of plasmid molecules initially bound by the UvrAB complex. RNAP seems, as a consequence, to increase binding sites for the UvrAB complex in this study.

The binding of UvrA to DNA forms complexes that are very short lived because of nucleotide binding and hydrolysis (6, 49). In solution, UvrA can interact with UvrB forming a UvrAB complex, resulting in the modulation of the UvrA associated ATPase (5). The helicase activity of the UvrAB complex reflects the active UvrAB complex binding to a single-stranded DNA region with the stimulation of its ATPase. At least a 10-nucleotide-long fragment at the 5\' single-stranded flanking region is required for helicase activity (50). Strand displacement is not due to just unwinding of the duplex DNA upon the binding of the UvrAB complex to DNA but does need the directional movement of the UvrAB complex (11). The DNA unwinding efficiency of the UvrAB complex upon binding to DNA is not known. Even though DNA binding of the UvrAB complex is necessary for unwinding, each DNA binding event may not induce concomitant unwinding. For instance, using a helicase substrate, a 20-bp partial duplex consisting of a very long 3\' tail but no overhang at 5\' end, there was no helicase-catalyzed displacement reaction (11). If the UvrAB complex can bind to the duplex region of this substrate and unwind one helical turn and translocate along the DNA in a 5\' to 3\' direction, a 20-bp region with two helical turns can be easily displaced by the UvrAB complex. It seems that the preferential binding of the UvrAB complex to a single-stranded region or 5\'-overhang may render the UvrAB complex competent as a substrate for helicase action.

Promoter regions around nucleotide position (−10) are exposed as a consequence of the binding of the RNAP. For the lac UV5 and tac promoters, cytosines at positions −6, −4, −2, and −1 are sensitive to methylation, indicating that these cytosines are in unpaired regions (51). Using KMnO\(_4\), the single-stranded region of lac UV5 promoter (36) and the late promoters of phages (\( \lambda l\), phage 82, phage 21) (48) in the open complex were monitored. The KMnO\(_4\) reactive sites in the \( \lambda l\) promoter region are located in the region from −11 to −2, which is similar to these other promoters, providing RNAP-induced unpairing of bases is in a specific region of the \( \lambda l\) promoter. The KMnO\(_4\) reactivity of thymines in the promoter region is greatly decreased in the presence of the UvrAB complex, suggesting that the UvrAB complex binds to the sites of KMnO\(_4\) modification. The tight binding of the UvrAB complex to bubble or loop regions with a \( K_d\) of \( 10^9 \) M\(^{-1}\) (59) is consistent with the binding of the UvrAB complex to distorted DNA regions. The half-life of the open complex, hence, may be a critical factor for the binding of the UvrAB complex to single-stranded regions in the transcription open complex.

The productive binding of the UvrAB complex to single-stranded regions of the transcription open complex may be required for the translocation of the UvrAB complex along the
bound strand in a 5’ to 3’ direction. A unique damage recognition domain in the UvrAB complex may dock to the complementary strand. The extent of DNase I protection of the UvrAB complex on the undamaged strand of duplex DNA is greater than that on the UV-damaged strand (19), suggesting asymmetric binding of the UvrAB complex to duplex DNA. The 3-1.6-fold increase (toward the 5’-end) of incision on the downstream of the transcribed strand in Fig. 5 (A and B) is in agreement with the increase of DNA repair in terms of in vitro and in vivo DNA repair synthesis of the transcribed strand (22, 52). That the M. luteus UV-endonuclease neither unwinds DNA nor has helicase activity (37) shows no preferential strand incision in the presence of RNAP. Further, M. luteus UV-endonuclease-catalyzed incision is greatly decreased under transcribing conditions (Fig. 6).

For the following reasons, preferential incision of DNA-damaged sites on the transcribed strand as a consequence of the unique translocation of the UvrAB complex may contribute in part to specific transcription-coupled repair.

First, transcription-coupled repair is responsible for the rapid removal of possible transcription-blocking lesions from the transcribed strand, while the non-transcribed strand is repaired at a slower rate similar to that of the overall genome. TRCF appears to be necessary for transcription-coupled repair in a defined in vitro system. TRCF, encoded by the mfd gene in E. coli, is able to recognize and displace a stalled RNA polymerase leading the UvrABC complex to the lesion presumably via its affinity for the damage recognition subunit of UvrA (26). Parenthetically, however, the stimulation of repair synthesis has not been demonstrated with this coupling factor. In addition, the mfd gene encoding the TRCF null mutant does not render these mutants UV sensitive or sensitive to other damaging agents (26), indicating a minor contribution of transcription-coupled repair to survival of cells after genotoxic treatment. In eukaryotes, the yeast rad26 null mutant is also insensitive to UV when compared to wild-type yeast (53). Repair of the transcribed strand is no more strongly inhibited or incomplete in this mutant. Apparently, yeast has other means to relieve the inhibition of repair caused by a stalled RNA polymerase complex. Although the stability of the stalled RNAP complex has not been determined, the replication machinery can dissociate the blocked RNA polymerase from the DNA (54). Even though the ability of the UvrAB complex to displace the stalled RNAP complex remains to be studied, the data in Fig. 5B indirectly show displacement of the stalled RNAP complex because the incision is increased on the transcribed strand under transcribing conditions.

Second, mammalian genes are transcribed more slowly than genes in E. coli. It would seem inefficient to abort nearly completed transcripts of such genes every time RNA polymerase encounters a lesion (55). The transcription elongation factor SII may provide an alternative mechanism; this factor catalyzes nascent transcript cleavage by RNA polymerase II at natural pause sites, enabling the polymerase to back off and try again without aborting the incomplete transcript (56). However, studies of the displacement of stalled RNA polymerase complex at varying sites have not been undertaken in E. coli.

Third, it has been recently discovered that one of the transcription initiation factors, TFIIH (factor b), contains components that are involved in DNA nucleotide excision repair mechanism, thus establishing an important link between transcription and repair in higher organisms. TFIIH obviously is able to repair DNA damage in cell free extracts of XP-D, XP-B, or the rad3 mutant (27, 29, 31). TFIIH contains XP-B (RAD25) and XP-D (RAD3) subunits of nucleotide excision repair and possesses DNA helicase activity (28, 30). These experiments suggest that the helicase activity of this factor is necessary in DNA repair, even though its exact role as a helicase is not known. A recent study showed that fast repair rates are seen near the transcription initiation sites, and there is a general gradient of repair efficiency of the transcribed strand with faster repair within the 5’-end and diminished repair toward the 3’-end of the gene (57). This study may be explained either by increased local concentrations of DNA repair factors that are associated with TFIIH functioning in transcription initiation (57) or by the loading of TFIIH onto promoter region during transcription initiation providing preferential targeting of repair proteins to actively transcribed genes (32).

Results of this work lead us to propose that the RNA polymerase provides the UvrAB complex with preferred binding sites at transcription bubble region via the physical interaction of RNAP with the UvrAB complex. Once the UvrAB complex binds to the promoter region, a competent nucleoprotein complex for helicase action is formed. The UvrAB complex translocates along the non-transcribed strand in an ATP hydrolysis-dependent reaction. During translocation, the DNA damage recognition domain of the UvrA subunit of the UvrAB complex senses damaged sites on the complementary strand.

Acknowledgments—We thank Professor James C. Wang, Harvard University, for providing the E. coli strain harboring the plasmid containing the E. coli DNA topoisomerase I.

REFERENCES
1. Yeung, A. T., Mattes, W. B., Oh, E. Y., and Grossman, L. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 6157–6161
2. Sancar, A., and Rupp, W. D. (1983) Cell 33, 249–260
3. Van Houten, B. (1990) Microbiol. Rev. 54, 18–51
4. Sancar, A., and Tang, M. S. (1993) Photochem. Photobiol. 57, 905–921
5. Oh, E. Y.; Claassen, L.; Thijagalingam, S.; Mazur, S.; and Grossman, L. (1989)
RNA Polymerase Signals UvrAB Landing Sites
Byungchan Ahn and Lawrence Grossman

J. Biol. Chem. 1996, 271:21453-21461.
doi: 10.1074/jbc.271.35.21453

Access the most updated version of this article at http://www.jbc.org/content/271/35/21453

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 58 references, 31 of which can be accessed free at http://www.jbc.org/content/271/35/21453.full.html#ref-list-1