Drosophila factor 2, an RNA polymerase II transcript release factor, exhibits a DNA-dependent ATPase activity (Xie, Z., and Price D. H. (1997) J. Biol. Chem. 272, 31902–31907). We examined the nucleic acid requirement and found that only double-stranded DNA (dsDNA) effectively activated the ATPase. Single-stranded DNA (ssDNA) not only failed to activate the ATPase, but suppressed the dsDNA-dependent ATPase. Gel mobility shift assays showed that factor 2 formed stable complexes with dsDNA or ssDNA in the absence of ATP. However, in the presence of ATP, the interaction of factor 2 with dsDNA was destabilized, while the ssDNA-factor 2 complexes were not affected. The interaction of factor 2 with dsDNA was sensitive to increasing salt concentrations and was competed by ssDNA. In both cases, loss of binding of factor 2 to dsDNA was mirrored by a decrease in ATPase and transcript release activity, suggesting that the interaction of factor 2 with dsDNA is important in coupling the ATPase with the transcript release activity. Although the properties of factor 2 suggested that it might have helicase activity, we were unable to detect any DNA unwinding activity associated with factor 2.

A common mechanism employed to control the potential of RNA polymerase II to synthesize full-length transcripts is through an early elongation block. This process, referred to as abortive elongation or premature termination has been observed in various transcription systems (1–5), as well as in our study of Drosophila RNA polymerase II transcription (6, 7). We observed that two distinct classes of complexes were formed after initiation. The predominant class undergoes abortive elongation which only gives rise to short transcripts, whereas the second class overcomes early blocks and carries out productive elongation. Based on our results, a model has been proposed for the control of elongation by RNA polymerase II which highlights the function of both negative and positive factors (6, 7). According to the model, the action of negative transcription elongation factors (N-TEF) was responsible for the abortive elongation. The transition from abortive elongation to productive elongation was mediated by the action of positive transcription elongation factors (P-TEF). P-TEFb, one of the components of P-TEF, has recently been purified and identified as a 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole-sensitive kinase that can phosphorylate the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (8, 9) and is required for Tat-transactivation of transcription from the HIV-LTR (10, 11).

One of the components of N-TEF, factor 2, was originally identified due to its ability to suppress the appearance of shorter than full-length transcripts during transcription in vitro (12). Factor 2 is a 154-kDa protein that associates stably with early elongation complexes under low salt conditions, but dissociates in 1 M KCl (13). Factor 2 causes the release of transcripts by RNA polymerase II in an ATP-dependent manner and results in premature termination (13). Further characterization of the transcript release activity of factor 2 revealed that the process requires ATP hydrolysis. Non-hydrolyzable analogs of ATP did not support factor 2 mediated transcript release (14). The intrinsic ATPase activity of factor 2 was activated by dsDNA and did not require other protein cofactors (14).

All information gathered on factor 2 function suggests that ATP hydrolysis is a key reaction for the transcript release activity of factor 2, but how it associates with elongation complexes and how the ATP hydrolysis is coupled to the transcript release event is not clear. Escherichia coli termination factor rho has an RNA-dependent ATPase activity that allows the translocation of rho along nascent transcripts to track elongation complexes and unwind RNA-DNA hybrids in transcription bubbles (15–17). RNA polymerase III termination factor La also has an ATPase activity that is dependent on RNA binding (18). Like rho, La causes the release of nascent transcripts by unwinding DNA-RNA hybrid using energy from ATP hydrolysis (18, 19). On the other hand, E. coli transcription repair coupling factor (TRCF) that is able to release nascent transcripts from stalled RNA polymerase lacks a helicase activity (20, 21). Instead, TRCF interacts with DNA and may associate with elongation complexes through its interaction with DNA and RNA polymerase and cause the release of RNA polymerase and nascent transcripts by hydrolyzing ATP (20, 21). Though it is not clear how factor 2 associates with elongation complexes, the finding that dsDNA is able to activate the ATPase activity of factor 2 (14) and the observation that factor 2 is able to bind DNA affinity column suggest that factor 2 may bind DNA and associate with elongation complexes through the interaction with DNA template. To understand the molecular mechanism of factor 2 function, in this study we further characterized the effects of various nucleic acids on the ATPase activity of factor 2. In addition, we examined the interaction of factor 2 with DNA and its significance for the ATPase and transcript release.

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‡ Present address: Howard Hughes Medical Institute, Vanderbilt University, Nashville, TN, 37232.

§ To whom correspondence should be addressed. Tel.: 319-335-7910; Fax: 319-335-9570; E-mail: david-price@uiowa.edu.

1 The abbreviations used are: N-TEF, negative transcription elongation factors; dsDNA, double-stranded DNA; ssDNA, single-strand DNA; P-TEF, positive transcription elongation factors; HIV, human immunodeficiency virus; TRCF, transcription repair coupling factor; bp, base pairs(s); ATPγS, adenosine 5’-O-(thiotriphosphate).

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activity of factor 2. Furthermore, we explored the potential helicase activity of factor 2.

**EXPERIMENTAL PROCEDURES**

*ATPase Assay*—The ATPase activity of *Drosophila* factor 2 was assayed by measuring the release of inorganic phosphate from [γ-32P]ATP as described (14). Immobilized dsDNA was used in the ATPase assay to examine the effect of ionic strength on the interaction of factor 2 with dsDNA. The immobilized dsDNA was generated by coupling biotinylated DNA with streptavidin-conjugated paramagnetic Dynabeads as described earlier (7). Factor 2 was first incubated with immobilized dsDNA in the presence of 20 mM HEPES (pH 7.6), 5 mM MgCl₂, 0.2 mg/ml bovine serum albumin, and 55 mM KCl for 5 min at 25 °C. The mixture was then aliquoted, concentrated magnetically, and washed three times with HMBK buffer that contained 20 mM HEPES, 5 mM MgCl₂, 0.2 mg/ml of BSA, and KCl of indicated concentration. The resulting complexes were washed another two times with 55 mM HMBK buffer, resuspended in 55 mM HMBK buffer, aliquoted to individual reaction tubes (6 μl each tube), and then examined for their ability to hydrolyze ATP. The ATPase assay was initiated by the addition of 2 μl of 4 × label mixture (14) and incubated at 25 °C for 20 min. The ATPase reactions were terminated by the addition of 1 μl of 0.5 M EDTA. The supernatant fractions containing the released γ-32P[ATP were isolated by magnetic concentration and analyzed by thin layer chromatography.

**Gel Mobility Shift Assay**—The dsDNA substrate for gel mobility shift assay was a 238-bp polymerase chain reaction product amplified from PET-21a plasmid (Novagen, Inc.). The single-stranded DNA (ssDNA) substrate was a synthetic oligo (dT)₆₀ (kindly provided by Dr. M. Wold, University of Iowa). Both the 238-bp duplex DNA and oligo (dT)₆₀ were 5’ end-labeled and electrophoretically purified. The binding of factor 2 with the labeled DNA was carried out in 20 mM HEPES (pH 7.6), 5 mM MgCl₂, 0.2 mg/ml bovine serum albumin, 1 μM dithiothreitol, and 50 mM KCl in the presence or absence of 0.6 mM ATP at 25 °C for 20 min. The reactions were products were analyzed by electrophoresis on a 4.5% polyacrylamide gel in 0.5 × TAE buffer (40 mM Tris-acetate, pH 8.5, 2 mM EDTA) at 100–200 V.

**Helicase Assay**—Two kinds of helicase substrates were generated to examine the helicase activity of factor 2. One was a partial duplex DNA as described (22). 5.2 ng of 17-mer universal primer was annealed to 2 μl of ssM13 mp18 DNA by incubation in 40 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol and 50 mM NaCl at 95 °C in 5 min followed by 20 min at 65 °C and 20 min at 23 °C. The primer was extended by DNA polymerase I-Klenow fragment (10 units) in the presence of 10 μCi of [γ-32P]dCTP, 50 μM dATP and dGTP, 40 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM dithiothreitol, and 50 mM NaCl at 23 °C for 20 min, then the reaction mixture was supplemented with dCTP with a final concentration of 50 μM and incubated for another 20 min. The reaction was stopped by EDTA and the free nucleotides were removed by gel filtration on a NICKT₄₄ column (Pharmacia Biotech Inc.). The second type of substrate was a 40-bp duplex DNA made by annealing two complementary oligonucleotides, one of which was labeled by γ-32P[ATP at the 5’ end.

The DNA helicase assay (10 μl) contained 20 mM HEPES, 5 mM MgCl₂, 1 mM dithiothreitol, 200 μg/ml bovine serum albumin, 2 mM ATP, DNA substrate, and factor 2. Reactions were incubated at 25 or 37 °C as indicated for 1 h, and stopped by addition of 2 μl of 0.1 mM EDTA, 3% SDS, 0.1% bromphenol blue, 50% (v/v) glycerol. The reaction products were analyzed by electrophoresis on 6% or 12% polyacrylamide gels as indicated in 45 mM Tris-borate, pH 8, 1 mM EDTA, at 100–200 V.

**RESULTS**

The ATPase Activity of Factor 2 Requires dsDNA and Is Inhibited by ssDNA—Factor 2 exhibited an ATPase activity that was dramatically stimulated by DNA (14). To gain further understanding of the nucleic acid requirement, a variety of DNA and RNA molecules were examined for their effect on the ATPase activity of factor 2. A standard ATPase assay that measures the release of free phosphate from [γ-32P]ATP was performed in the presence of a constant amount of different nucleic acids. The percent hydrolysis for each reaction was normalized to the low level of activity exhibited by factor 2 in the absence of any nucleic acid and is presented in Table I. Up to 100-fold stimulation was seen for dsDNA molecules of natural sequences. Superoicoed DNAs stimulated slightly more than linear molecules. When the ability of supercoiled and linear forms of either Bluescript or A2 DNA to stimulate the ATPase activity were compared, we found that supercoiled DNA increased the ATPase from 20 to 30% compared with the linear form (data not shown). Synthetic homopolymeric dsDNA, such as poly(dA:dT), poly(dG:dC), and poly(dA-dG)(dT-dC), also stimulated the ATPase activity of factor 2, although with somewhat lower efficiency than dsDNA with natural sequences. ssDNA or dsRNA did not activate the ATPase. Partial duplex DNA poly(dC), poly(dG)₁₂₋₁₅ or a DNA-RNA hybrid poly(I)-poly(C) both failed to activate the ATPase. Thus, the ATPase activity of factor 2 was efficiently stimulated only by dsDNA.

We were surprised that partial duplex DNA failed to support the ATPase activity and wondered if ssDNA might be inhibitory to the ATPase activity of factor 2. When an equal amount of ssDNA was added with dsDNA we found that the ATPase activity was suppressed. Both linear ssDNA (oligo(dT)₁₀₀₀-poly(C)) and circular ssM13 DNA effectively suppressed the ATPase activity of factor 2 in the presence of linear dsDNA (Fig. 1A) or supercoiled DNA (data not shown). ssRNA (poly(A)) on the other hand, did not have any effect on the dsDNA-dependent ATPase activity even when added in 4-fold excess (Fig. 1B). Since the ATPase activity of factor 2 exhibited no specific sequence requirement for the dsDNA, the conformation and structure of the DNA may be important. Supporting this idea, ethidium bromide that extends the double helices by untwisting DNA (23, 24) inhibited the ATPase activity of factor 2, on the other hand, did not have any effect on the dsDNA-dependent ATPase activity even when added in 4-fold excess (Fig. 1B). Since the ATPase activity of factor 2 exhibited no specific sequence requirement for the dsDNA, the conformation and structure of the DNA may be important. Supporting this idea, ethidium bromide that extends the double helices by untwisting DNA (23, 24) inhibited the ATPase activity of factor 2, on the other hand, did not have any effect on the dsDNA-dependent ATPase activity even when added in 4-fold excess (Fig. 1B).

**Factor 2 Forms a Stable Complex with dsDNA That Is Unstable in the Presence of ATP or ssDNA**. Since dsDNA was required for the ATPase activity of factor 2, we examined the interaction between factor 2 and dsDNA with a gel mobility shift assay (Fig. 2). A 5’-end labeled 238-base pair dsDNA was used as the DNA substrate. Factor 2 formed stable complexes with the dsDNA in the absence of ATP (Fig. 2). Two complexes were observed, a major complex and a lower mobility minor complex. Results with DNAs of different length suggest that the two complexes may be different because of the number of factor 2 molecules bound (data not shown). The binding of

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**Table I**

<table>
<thead>
<tr>
<th>dsDNA</th>
<th>Relative ATPase activity</th>
</tr>
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<tbody>
<tr>
<td>No DNA</td>
<td>1</td>
</tr>
<tr>
<td>Supercoiled dsDNA</td>
<td>100</td>
</tr>
<tr>
<td>Bluescript</td>
<td>100</td>
</tr>
<tr>
<td>pSP73</td>
<td>100</td>
</tr>
<tr>
<td>Linear ds-DNA</td>
<td>85</td>
</tr>
<tr>
<td>Ac5C template</td>
<td>48</td>
</tr>
<tr>
<td>poly(dG)·poly(dC)</td>
<td>27</td>
</tr>
<tr>
<td>DNA-RNA hybrid</td>
<td>7</td>
</tr>
<tr>
<td>poly(dT)·poly(1)</td>
<td>1</td>
</tr>
<tr>
<td>poly(dC)</td>
<td>1</td>
</tr>
<tr>
<td>Poly(dT)₁₂₋₁₅</td>
<td>3</td>
</tr>
<tr>
<td>Poly(dG)</td>
<td>2</td>
</tr>
<tr>
<td>M13mp18</td>
<td>0</td>
</tr>
<tr>
<td>Poly(dC)</td>
<td>0</td>
</tr>
<tr>
<td>ssRNA</td>
<td>1</td>
</tr>
<tr>
<td>Poly(A)</td>
<td>1</td>
</tr>
</tbody>
</table>
factor 2 was not bound to both double and ssDNA at the same time. Although significant inhibition of binding was seen at a 10-fold excess of ssDNA, a 100-fold excess was required to compete most of the dsDNA binding. This is different from the earlier finding that the ATPase activity of factor 2 was dramatically inhibited when the amount of ssDNA in the reaction was the same as that of dsDNA (Fig. 1B). This apparent inconsistency was likely caused by the effect of ATP, which destabilizes the interaction of factor 2 with dsDNA (Fig. 2) but not with ssDNA (Fig. 3A). Therefore, in the presence of ATP, factor 2 may become trapped by ssDNA more easily than in the absence of ATP.

DNA Binding Properties of Factor 2 Correlate with Transcript Release Activity—Since the preceding results showed that ssDNA was able to compete for dsDNA binding by factor 2 and suppress the dsDNA-dependent ATPase activity of factor 2, we tested the effect of ssDNA on the transcript release activity of factor 2. If transcript release mediated by factor 2 is coupled to the dsDNA-activated ATPase, ssDNA should effectively inhibit the transcript release activity of factor 2. High salt-washed early elongation complexes were generated on an immobilized DNA template, and the transcript release reaction was carried out as diagrammed above Fig. 4. Released transcripts were separated from transcripts in early elongation complexes by magnetic concentration of the template. The high salt-washed early elongation complexes lacked factor 2 (10), thus almost all of the transcripts remained associated with early elongation complexes when only ATP was added to the reaction. As was found before, transcripts were released in the presence of both factor 2 and ATP (Fig. 4A, lane 2). However, when factor 2 was first preincubated with 15 μg/ml of ssDNA for 5 min and then incubated with the early elongation complexes in the presence of ATP, more than 70% of the transcripts remained associated with early elongation complexes (Fig. 4A). Almost no transcript release by factor 2 was observed when factor 2 was preincubated with 45 μg/ml of ssDNA (Fig. 4A). These results demonstrate that ssDNA was able to suppress the transcript release activity and suggest that the ATPase activity of factor 2 is essential for the transcript release activity of factor 2.
A somewhat different result was obtained when the factor 2 was preincubated with the early elongation complexes before the addition of ssDNA. Under these conditions, ssDNA was less efficient in suppressing the transcript release (Fig. 4B). High salt-washed early elongation complexes were first incubated with factor 2 and then washed to remove unbound factor. When these complexes were treated with ATP, 85% of the associated transcripts were released, and further supplementation of factor 2 had little effect (Fig. 4B). No suppression of transcript release was observed when 15 μg/ml of ssDNA or dsDNA was added, evidenced by the lack of increase in the number of transcripts associated with the early elongation complexes.

Transcript release was partially suppressed by high levels of ssDNA but not by high levels of dsDNA (Fig. 4B). Although the transcript release activity was partially suppressed by ssDNA, the extent of suppression was less than that seen when the ssDNA was preincubated with factor 2. These results demonstrate that prebinding factor 2 to the elongation complex lessens the inhibitory effect of ssDNA. Possible explanations are discussed later.

Previous studies showed that 250 mM KCl or 0.3% Sarkosyl was able to relieve early elongation blocks and prevent premature termination of early elongation complexes (6, 7). The effect of high salt or detergent on elongation could be due in part to a...
were generated by pulse labeling, followed by 1 M KCl washing, and concentration of KCl. High salt-washed early elongation complexes transcript release activity of factor 2 in the presence of increasing concentrations of KCl. After washing back to 50 mM KCl, the ATPase activity remaining was measured. Previous studies showed that the ATPase activity was proportional to the amount of factor 2 at a fixed amount of dsDNA (14). Consistent with the result from gel mobility shift assays (Fig. 2), a substantial amount of factor 2 remained bound to the immobilized template at 50 mM KCl. In contrast to the effects on the ATPase and transcript release activities, 60% of the factor 2 remained bound even when washed with 200 mM KCl. Elimination of the electrostatic interaction of factor 2 with dsDNA was accomplished only after washing with 1 M KCl. These results indicate that 200 mM KCl can suppress the ATPase and transcript release activities of factor 2 without significantly disrupting the interaction of the factor with the DNA.

Factor 2 Has No Apparent DNA Helicase Activity—Since factor 2 was able to bind dsDNA and hydrolyze ATP, we investigated whether the factor was able to utilize the energy from ATP hydrolysis to unwind duplex DNA. We first used a conventional helicase assay that utilized a labeled 24-nucleotide oligonucleotide annealed to M13 DNA. In the presence of ATP, the 24-mer was readily released by a bona fide DNA helicase, SV40 large T antigen, but not by factor 2 (Fig. 6A). To eliminate the possibility that the potential helicase activity was inhibited by ssDNA, we also examined the ability of factor 2 to first bind and then unwind a 40-bp DNA duplex. A gel mobility shift assay indicated that factor 2 was able to bind the 40-bp duplex (Fig. 6B). As was found previously, the binding was destabilized by ATP, suggesting that the short DNA was able to activate the ATPase activity of factor 2 (Fig. 6B). Indeed, the ATPase assay demonstrated this directly (data not shown).

Even though factor 2 bound to the DNA fragment and exhibited ATPase activity, it failed to unwind the dsDNA either at 25 °C (Fig. 6C) or 37 °C (data not shown). These results suggest that factor 2 does not have a DNA helicase activity.

DISCUSSION

Factor 2 was identified as a component of N-TEF that was able to block transcription elongation by promoting the release of nascent transcripts in an ATP-dependent manner (13). Further study revealed that factor 2 had a DNA-dependent ATPase activity (14). In this study, we characterized the ATPase activity with respect to nucleic acid requirement and explored the possibility of associated helicase activities. In addition, a correlation was made between the ATPase and the transcript release activity of the factor. The results indicate that factor 2 possesses a unique collection of properties.

Characterization of the effect of nucleic acids on the ATPase activity of factor 2 distinguished it from almost all other proteins. Only dsDNA effectively supported the ATPase activity, with supercoiled DNA having a slightly higher efficiency compared with linearized DNA. ssDNA not only failed to activate but actually suppressed the dsDNA-dependent ATPase activity of factor 2. The strict requirement of dsDNA for ATPase activity has only been observed for RuvB protein from eubacteria Thermus thermophilus and Thermotoga martima (25). Studies of T. thermophilus RuvB suggests that is involved in recombination processes (25). The involvement of factor 2 in these processes has not been determined. The inhibitory effect of ssDNA on the dsDNA-dependent ATPase activity of factor 2 has not been observed for other proteins; however, uncovering the significance of this property to factor 2 function will require further investigation.

The strict requirement of dsDNA for the ATPase activity of factor 2 suggested that double helical structure of DNA is an
Transcript Release Factor Binds dsDNA

Fig. 6. Helicase activity of factor 2. A, factor 2 was incubated with a 32P-labeled 24-mer annealed to a single-stranded M13 mp18 DNA in the presence of ATP at 25 °C for 1 h. 100 ng of SV40 large T antigen (T-Ag, a positive control), was incubated with the helicase substrate in the presence or absence of ATP at 25 or 37 °C for 1 h. Reactions were analyzed with 6% polyacrylamide gel in 0.5 X Tris-borate EDTA buffer at 100–200 V. boiled and boiled* represent that the helicase substrate was boiled for 5 min and incubated at 25 °C for 1 h with no addition. *x of factor 2 represents approximately 60 ng of factor 2. B, interaction of factor 2 with 40-bp duplex DNA by gel mobility shift assay. Factor 2 was incubated with 32P-labeled 40-bp duplex DNA in the presence or absence of ATP at 25 °C for 1 h. The free probe was separated from the DNA/factor 2 complexes by electrophoresis on a 6% polyacrylamide gel in 0.5 X TBE buffer at 100–200 V. C, the 32P-labeled 40-bp duplex was incubated with or without 240 ng of factor 2 in the presence of ATP at 25 °C or 37 °C for 1 h. The helicase substrate and the displaced DNA (32P-labeled 40-mer) was separated on a 12% polyacrylamide gel in 0.5 X TBE buffer at 100–200 V. boiled, helicase substrate boiled for 5 min and incubated at 25 °C for 1 h.

The binding of factor 2 to dsDNA seems to be a primary requirement for the transcript release activity of factor 2, and we propose that factor 2 initially associates with the elongation complex through interaction with the template. When the interaction between factor 2 and dsDNA was blocked by ssDNA, the transcript release was also inhibited (Fig. 4). ssRNA, however, did not stimulate or suppress the ATPase activity (Fig. 1B), suggesting that factor 2 does not bind to RNA and may not associate with the elongation complex through nascent RNA. This assumption was also reached from our previous finding that factor 2 was able to release very short transcripts which are sequestered within the ternary complexes and not accessible to factor 2. Recent characterization of factor 2 function using a dC-tailed template suggested that the dsDNA upstream of the elongation complexes was required for the transcript release activity of factor 2 (14). It is possible that not only the binding of factor 2 with dsDNA but also its appropriate orientation to elongation complexes is necessary to effectively couple the dsDNA-dependent ATPase activity of factor 2 with its transcript release activity.

The effect of ssDNA on the properties of factor 2 can be used in understanding some aspects of factor 2 function. Direct binding of factor 2 to dsDNA in the absence of ATP as measured with a gel mobility shift assay was efficiently competed only with a 100-fold excess of ssDNA. However the ATPase activity of factor 2 was about 100 times more sensitive to ssDNA. Since ATP weakens the association of factor 2 with dsDNA but not with ssDNA, it is likely that ssDNA traps factor 2 when both dsDNA and ATP are present. The effect of ssDNA on transcript release also supported this notion. ssDNA had a greater inhibitory effect on transcript release when it was preincubated with factor 2, presumably because the factor 2 complexed with ssDNA was unable to bind to the template. Although ssDNA was able to inhibit transcript release when the factor 2 was prebound to the template, it was required in about a 10-fold excess over the dsDNA. This is a significantly higher single strand/double strand ratio than was required in the ATPase assay even though both assays contained ATP. There are several explanations for the relative resistance to inhibition by ssDNA of transcript release compared with the ATPase activity. One possibility for the lower efficiency of ssDNA inhibition in transcript release is that factor 2 may interact not only with dsDNA but also with other protein factors in the elongation complexes. Therefore, even in the presence of ATP, more ssDNA may be required to dissociate factor 2 from elongation complexes and suppress its transcript release
activity. Another interesting possibility is that factor 2 may exhibit an ATP-dependent translocation along dsDNA. In this way, ATP could cause factor 2 to release dsDNA by causing it to run off the end of the fragment. Translocation along the template would be impeded by proteins including RNA polymerase II ternary complexes. Perhaps the mechanism of termination involves the translocation of factor 2 into the trailing edge of the polymerase. If the translocation model is correct, ssDNA would be less effective at inhibiting transcript release because factor 2 is not released from the template until after it has caused transcript release.

An alternative model for the effect of ATP is that it may cause direct release of factor 2 from the template. Since factor 2 associated tightly with dsDNA in the absence of ATP, destabilization of the dsDNA-factor 2 complexes by ATP may be necessary to reduce the nonspecific retention of factor 2 on dsDNA and facilitate its search for stalled RNA polymerase. However, this model does not explain the relative resistance of transcript release to ssDNA. In addition, factor 2 would be quite inefficient in localizing non-processive elongating polymerase and mediating transcript release. Perhaps more likely is an intermediate model in which factor 2 scans the template for a period of time and then dissociates from the template. Resassociation and resuming of scanning would be the normal pathway for transcript release and this process would be inhibited by ssDNA. The intermediate inhibition by ssDNA of transcript release could mirror the balance between processive scanning and transient interaction. More detailed kinetic experiments will be required to verify or differentiate between the models.

The effect of elevated salt on the transcript release activity of factor 2 correlated with the effect on the ATPase activity but did not correlate with release of factor 2 from dsDNA. At 200 mM KCl, factor 2 remained associated with the template but was not active in transcript release, presumably because the ATPase activity was inhibited. Factor 2 may be responsible for our previous unpublished results which indicated that early elongation complexes would lengthen transcripts in the presence of 250 mM KCl but would stop if the salt was lowered. Evidently, at intermediate salt concentrations, factor 2 remains bound to the template but does not function. This accessible state, in which factor 2 is bound but not active, suggests that a mechanism for regulation of factor 2 transcript release activity may exist. Perhaps other protein factors can interact with factor 2 and cause it to adopt the inactive bound state. Such interactions may be important in regulating the selectivity of factor 2 so that the action of factor 2 is restricted to non-productive elongation complexes.

Biochemical characterization of factor 2 shows that it shares multiple properties with the TRCF. Like E. coli TRCF protein Mfd (20, 21) and two potential eucaryotic TRCF, human ERCC6 (28) and its yeast homologue Rad26 (29), factor 2 has an associated ATPase and ATP-dependent transcript release activity, binds to dsDNA, and lacks an apparent helicase activity. However, ERCC6 lacks one of the major features of E. coli TRCF, the ability to dissociate the stalled RNA polymerase II complexes. It has been argued that RNA polymerase II stalled by DNA lesion may not necessarily be dissociated by eucaryotic TRCF but may be removed from damaged sites through the action of SII or other unknown factors (28, 30).

Though the multiple biochemical properties shared between factor 2 and E. coli TRCF suggests that factor 2 may be a homologue of eucaryotic TRCF, more in vitro and in vivo functional study of factor 2 will be required to justify its cellular function.

In summary, characterization of the enzymatic activity of factor 2 and its interaction with DNA provided further insights into the mechanism of factor 2 action. Compared with other known transcription termination factors, factor 2 may utilize a novel mechanism for transcript release. Unlike E. coli termination factor rho (15–17) or RNA polymerase III termination factor La (18, 19) or termination of vaccinia virus polymerase mediated by VTF (virus-encoded termination factor) (31, 32), no specific signal in nascent RNA or binding to the nascent RNA is required for the transcript release mediated by factor 2. Instead, our results indicate that factor 2 is likely recruited to the elongation complex through interactions with the template DNA. Although a strong correlation between the ATPase activity and transcript release activity of factor has been made, the mechanism of transcript release remains to be determined.

Acknowledgment—We thank Marc Wold (University of Iowa) for kindly providing SV40 large T antigen and the 40-bp dsDNA used for one of the helicase assays.

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Unusual Nucleic Acid Binding Properties of Factor 2, an RNA Polymerase II Transcript Release Factor
Zhi Xie and David H. Price

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