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# FACT, a Factor that Facilitates Transcript Elongation through Nucleosomes

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## Summary

**The requirements for transcriptional activation by RNA polymerase II were examined using chromatin templates assembled in vitro and a transcription system composed of the human general transcription factors and RNA polymerase II. Activator-induced, energy-dependent chromatin remodeling promoted efficient preinitiation complex formation and transcription initiation, but was not sufficient for productive transcription. Polymerases that initiated transcription on remodeled chromatin templates encountered a block to transcription proximal to the promoter. Entry into productive transcription required an accessory factor present in HeLa cell nuclear extract, FACT (*fa*cilitates *c*hromatin *t*ranscription), which we have purified. FACT acts subsequent to transcription initiation to release RNA polymerase II from a nucleosome-induced block to productive transcription. The biochemical properties and polypeptide composition of FACT suggest that it is a novel protein factor that facilitates transcript elongation through nucleosomes.**

## Introduction

The minimal protein apparatus required for transcription of class II genes in vitro consists of the general transcription factors (GTFs) IIB, IID, IIE, IIF, IIH, and RNA polymerase II (RNAP II) (reviewed in Orphanides et al., 1996; Roeder, 1996). These factors support efficient transcription of naked DNA in vitro but cannot respond to transcriptional activators without additional cofactors (reviewed in Guarente, 1995). The assays employed to identify the GTFs and cofactors used naked DNA as the template, but in vivo transcription occurs from chromatin templates. Packaging DNA into chromatin inhibits RNAP II transcription in vitro (reviewed in Owen-Hughes and Workman, 1994). Thus, transcription of class II genes in vivo must require other components in addition to RNAP II, the GTFs, and cofactors.

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One aspect in which RNAP II transcription of chromatin templates in vitro differs from transcription of naked DNA is the absolute requirement for a transcriptional activator. In vitro, activators generally bind poorly when their binding sites are in a nucleosome (reviewed in Owen-Hughes and Workman, 1994). By contrast, activators bind their sites efficiently in vivo and, moreover, induce changes in chromatin structure that make the surrounding DNA more sensitive to nucleases (Gross and Garrard, 1988). Therefore, some of the accessory factors required for RNAP II transcription in vivo are chromatin remodeling activities that assist activators to bind to their sites and promote structural alterations in the local chromatin environment.

Activator and ATP-dependent chromatin remodeling can be reproduced in vitro using protein extracts from *Drosophila* embryos (Pazin et al., 1994; Tsukiyama et al., 1994; Varga-Weisz et al., 1995). Remodeling complexes from these extracts that have been characterized include NURF (Tsukiyama and Wu, 1995; Tsukiyama et al., 1995), ACF (Ito et al., 1997), and CHRAC (Varga-Weisz et al., 1997). Functionally similar complexes have also been described from other organisms, i.e., SWI/SNF from yeast (Cairns et al., 1994; Cote et al., 1994) and human (Kwon et al., 1994; Wang et al., 1996), and yeast RSC (Cairns et al., 1996). Genetic evidence suggests a role for the yeast SWI/SNF complex in transcriptional activation at certain promoters (reviewed in Wolffe, 1994; Peterson, 1996). It is important to determine whether this class of chromatin remodeling complexes is the only type of accessory factor required for transcription of chromatin templates or whether other activities are involved.

We have examined the factor requirements for transcription from chromatin templates using a purified transcription system consisting of only RNAP II, GTFs, and plasmid chromatin assembled in the *Drosophila* S-190 extract (Kamakaka et al., 1993). We report that a purified human transcription system is unable to transcribe these chromatin templates productively. Chromatin remodeling at the promoter is sufficient for transcription initiation. However, transcript elongation requires an additional accessory factor. We have purified this factor and have named it FACT (*fa*cilitates *c*hromatin *t*ranscription). FACT acts subsequent to transcription initiation to release RNAP II from a nucleosome-induced block to productive transcription. FACT activity coelutes with two polypeptides of 140 and 80 kDa that appear to form a novel heterodimeric complex.

## Results

### Assembly, Purification, and Characterization of Chromatin Transcription Templates

Several different approaches have been used to assemble nucleosomes onto promoter-containing DNA for in vitro transcription studies. We have primarily employed the *Drosophila* embryo S-190 extract (Kamakaka et al.,

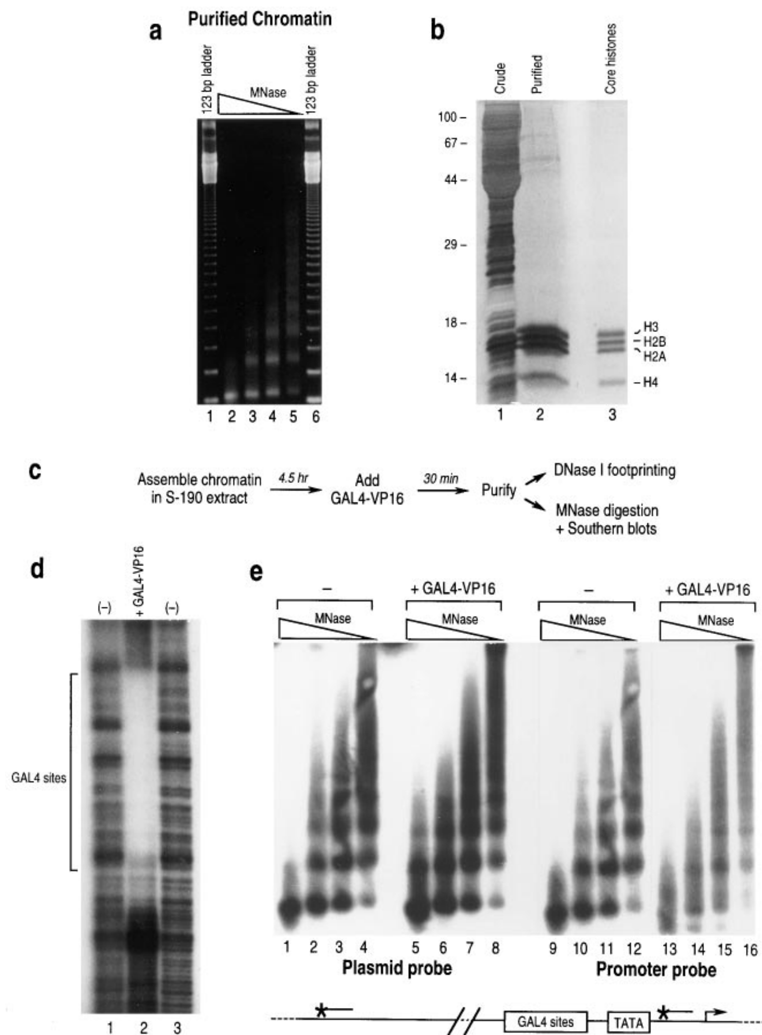


Figure 1. Assembly, Purification, and Characterization of Plasmid Chromatin Templates

(a) MNase digestion pattern of purified chromatin templates. Purified chromatin templates were digested with increasing amounts of MNase and the DNA fragments analyzed by agarose gel electrophoresis. The size of the DNA fragments was estimated by using a 123 bp DNA ladder (GIBCO-BRL).

(b) Analysis of purified chromatin templates by silver staining. Crude chromatin (10 μl, lane 1), purified chromatin (70 μl, lane 2), and HeLa cell core histones (80 ng, lane 3) were analyzed by 18% polyacrylamide–SDS gel electrophoresis followed by silver staining. The position of protein molecular-weight markers is indicated.

(c) Scheme used to assemble, remodel, and analyze chromatin templates.

(d) DNase I footprinting of purified chromatin templates. Purified chromatin lacking (lanes 1 and 3) or containing (lane 2) bound GAL4–VP16 (40 μl) was digested with DNase I. Positions of DNase I cleavage were visualized by thermal-cycle primer extension using a radioactively labeled oligonucleotide.

(e) Purified chromatin containing GAL4–VP16 is specifically remodeled in the vicinity of the promoter. Purified chromatin templates lacking (lanes 1–4 and 9–12) or containing (lanes 5–8 and 13–16) GAL4–VP16 were digested with MNase and analyzed by DNA Southern blot hybridization using oligonucleotides corresponding to promoter sequences (promoter probe, lanes 9–16) or sequences ~1000 bp upstream of the promoter (plasmid probe, lanes 1–8).

1993), which efficiently assembles regularly and physiologically spaced nucleosomes onto plasmid DNA in an ATP-dependent reaction. Our plasmid template contains five GAL4 DNA-binding sites upstream of the adenovirus major late promoter and a 390 bp G-less transcription cassette (Merino et al., 1993). Incubation of this plasmid with S-190 extract, purified HeLa cell core histones, and ATP resulted in the assembly of long arrays of physiologically spaced nucleosomes. The repeat length was 165–170 bp as assayed by micrococcal nuclease (MNase) digestion (data not shown).

Purification of the nucleosome-assembled plasmids by gel filtration chromatography removed most of the proteins from the S-190 extract as well as nonassembled or partially assembled plasmid templates (data not shown). Digestion of the purified chromatin templates with MNase revealed that the regular nucleosome spacing was not perturbed (Figure 1a). HeLa cell core histones were the predominant proteins in the chromatin fraction (Figure 1b, lane 2). We conclude that the purified chromatin templates are completely assembled, greater than 95% homogeneous, and exhibit physiological nucleosome spacing.

The S-190 assembly extract contains components that use ATP hydrolysis to alter chromatin structure ("chromatin remodeling"; Pazin et al., 1994). This remodeling can be directed to specific locations on the template by the binding of regulatory factors. To obtain promoter-proximal remodeled chromatin templates, we used either the model transcription activator GAL4–VP16 (Sadowski et al., 1988) or a protein consisting of only the DNA-binding region of the GAL4 protein (GAL4 [1–94]). Both GAL4(1–94) and GAL4–VP16 can efficiently induce chromatin remodeling in the S-190 extract (Pazin et al., 1994; for brevity, we shall henceforth refer to "promoter-proximal chromatin remodeling" as chromatin remodeling). The procedure we used to remodel the templates is based on a published method and is outlined in Figure 1c. After chromatin assembly, GAL4–VP16 or GAL4(1–94) was added and the reaction was incubated for 30 min to allow DNA binding and chromatin remodeling. The remodeled templates were then purified by gel filtration. Primer extension–mediated deoxyribonuclease I (DNase I) footprinting (Gralla, 1985) established that the GAL4–VP16 (Figure 1d) and GAL4

(1–94) (data not shown) sites were fully occupied after chromatin purification.

To determine whether the purified templates were remodeled, the DNA was digested with MNase and transferred to a nitrocellulose membrane. The membrane was first hybridized with a probe for sequences ~1000 bp upstream of the transcription start site (plasmid probe). Periodic nucleosome arrays were apparent both in the absence and the presence of the activator with the GAL4–VP16 (Figure 1e) and GAL4(1–94) (data not shown) templates. Reprobing the same membrane with an oligonucleotide from the promoter region revealed a general smearing of the DNA when the original reaction contained activator (Figure 1e, lanes 13–16), as expected for remodeled chromatin (see, e.g., Tsukiyama et al., 1994). More DNA was present between bands corresponding to nucleosomes, and small, subnucleosomal fragments could be seen (Figure 1e, compare lanes 9–12 with 13–16). These data establish that the purified chromatin templates are remodeled specifically in the vicinity of the promoter.

**Transcription of Purified Chromatin Templates**

Most previous studies have used crude extracts or partially purified transcription factors to transcribe chromatin templates. This work has provided valuable details concerning the mechanism of activation in a chromatin environment and the role of specific activators. However, these experiments were largely unable to examine whether factors in addition to RNAP II and its GTFs are required to transcribe templates packaged into nucleosomes. In the present study, we have used two different human transcription systems: (1) a purified, activator-responsive, reconstituted system composed of bacterially expressed or highly purified GTFs, pure RNAP II, and the coactivators PC4 (Ge and Roeder, 1994; Kretzschmar et al., 1994) and TFIIA (Ma et al., 1996); and (2) HeLa cell nuclear extract.

Each of these transcription systems could efficiently produce a 390 nt long transcript from the naked DNA templates (Figures 2a and 2b, lane 1). The addition of GAL4–VP16 to reactions with naked DNA resulted in a PC4-dependent, 16-fold stimulation with the purified activation system (Figure 2b, lanes 2 and 3). The addition of GAL4–VP16 gave a 5-fold stimulation with HeLa nuclear extract on naked DNA (Figure 2a, lane 3), while GAL4(1–94) was without effect (Figure 2a, lane 2).

The nonremodeled chromatin template could not support 390 nt RNA synthesis with either transcription system (Figures 2a, 2b, lane 4). HeLa nuclear extract transcribed the GAL4–VP16-containing remodeled chromatin template (Figure 2a, lane 6), but not the GAL4(1–94)-containing remodeled template (Figure 2a, lane 5). With the GAL4–VP16 template, the efficiency of transcription was 59% compared with naked DNA and 11.3% compared with naked DNA in the presence of activator. Surprisingly, the reconstituted system could not transcribe the GAL4–VP16-containing remodeled chromatin template (Figure 2b, lane 6) even in the presence of the coactivator PC4 (Figure 2b, lane 5).

These data show that promoter-proximal chromatin remodeling is not sufficient for transcription using highly purified transcription factors. In contrast, HeLa nuclear extract can efficiently transcribe remodeled, activator-containing chromatin templates. Thus, the nuclear extract must contain a factor (or factors), separate from RNAP II and the GTFs, that is required for transcription of remodeled templates.

**Can the Purified Transcription System Form a Preinitiation Complex and Initiate Transcription on Remodeled Chromatin Templates?**

Having established that the reconstituted transcription system was incapable of productive RNA synthesis from remodeled chromatin templates, we sought to determine whether a preinitiation complex could form and initiate transcription under these conditions. We used an abortive initiation assay (McClure et al., 1978) in which the preinitiation complex was supplied with nucleotides sufficient for the formation of only the first phosphodiester bond. GTFs and RNAP II were incubated with naked DNA or chromatin templates before the addition of ATP and radiolabeled CTP (the nucleotides required for formation of the first bond on the adenovirus major late promoter; Figure 2c), and initiation was monitored by detecting the dinucleotide product formed.

The reconstituted system initiated transcription efficiently on naked DNA (Figure 2d, lane 1). The addition of GAL4(1–94) or GAL4–VP16 did not stimulate initiation in this case (Figure 2d, lanes 2 and 4). However, the addition of the coactivator PC4 resulted in a 4-fold stimulation with GAL4–VP16 but was without effect with GAL4(1–94) (Figure 2d, lanes 3 and 5). The purified system did not initiate transcription on the nonremodeled chromatin template or on the GAL4(1–94)-containing remodeled chromatin template (Figure 2d, lanes 6 and 7). However, this system was able to initiate transcription on the GAL4–VP16-containing remodeled template to a level approximately 30% of that obtained with naked DNA (Figure 2d, lane 9). PC4 stimulated transcription a further 3.2-fold with GAL4–VP16 but was without effect on the GAL4(1–94) template (Figure 2d, lanes 8 and 10). Initiation on remodeled templates was promoter-specific since it depended on the addition of TFIID and template (data not shown). This rules out the possibility that TFIID from the S-190 extract contaminated the purified chromatin.

These results establish that the reconstituted transcription system can efficiently initiate RNA synthesis on GAL4–VP16-containing remodeled chromatin templates. Initiation requires both chromatin remodeling and an activation domain. We conclude that activator-induced promoter remodeling facilitates transcription initiation by purified GTFs and RNAP II but is not sufficient for productive transcription. Together with the results of Figures 2a and 2b, these data imply that on chromatin templates a block to transcription exists after transcription initiation and a factor(s) present in HeLa nuclear extract is required to overcome this block.

We next wished to determine whether both chromatin remodeling and an activation domain are required for

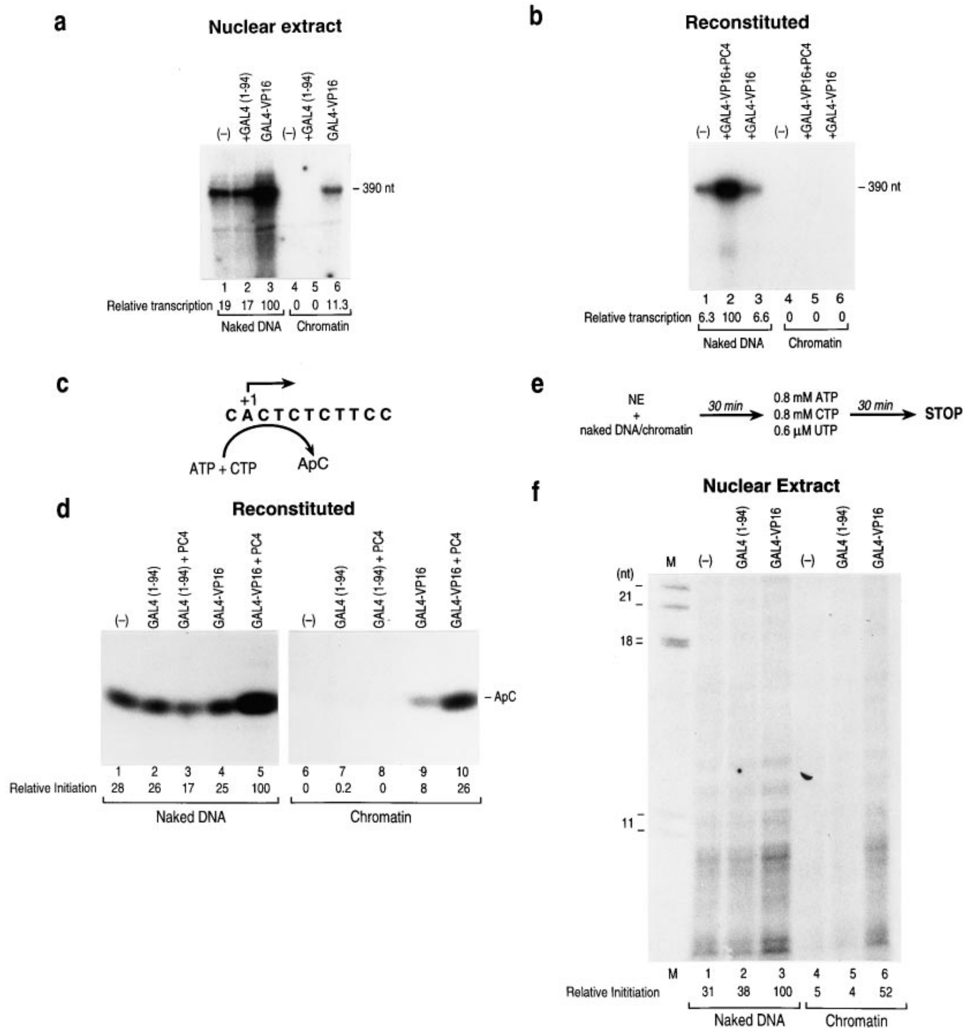


Figure 2. A Purified Transcription System Cannot Synthesize a 390 nt Transcript from Remodeled Chromatin Templates, but Can Initiate Transcription

(a and b) Productive transcription. Naked DNA, naked DNA containing GAL4–VP16 or GAL4(1–94), chromatin, or remodeled chromatin containing GAL4–VP16 or GAL4(1–94) was used as the template in transcription assays using HeLa cell nuclear extract (a) or a reconstituted transcription system (b). Where indicated, the coactivator PC4 was included in reactions using the reconstituted system (b, lanes 2 and 5). GAL4–VP16 or GAL4(1–94) was included in naked DNA reactions as indicated. The position of the full-length, 390 nt RNA product is indicated. Levels of transcription, relative to other lanes in the same panel, are given below each lane.

(c and d) The reconstituted transcription system can initiate transcription efficiently from remodeled chromatin templates. (c) ATP and CTP are sufficient for formation of the first phosphodiester bond on the adenovirus major late promoter. (d) Abortive initiation reactions using the reconstituted system. Naked DNA, naked DNA containing GAL4–VP16 or GAL4(1–94), chromatin, or remodeled chromatin containing GAL4–VP16 or GAL4(1–94) was used as the template in a dinucleotide synthesis assay using the reconstituted transcription system. GAL4–VP16, GAL4(1–94), and the coactivator PC4 were included as indicated. The position of the dinucleotide product ApC is indicated. Levels of initiation, relative to other lanes in the same panel, are given below each lane.

(e) Scheme used to measure transcription initiation using HeLa cell nuclear extract.

(f) Transcription initiation using HeLa cell nuclear extract requires an activation domain. Naked DNA, naked DNA containing GAL4–VP16 or GAL4(1–94), chromatin, or remodeled chromatin containing GAL4–VP16 or GAL4(1–94) was incubated with nuclear extract for 30 min. An excess of ATP, CTP, and a limiting amount of radiolabeled UTP were then added and initiation was allowed to proceed for 30 min. The positions of DNA size markers are indicated. Levels of initiation, relative to other lanes in the same panel, are given below each lane.

transcription initiation in HeLa cell nuclear extract. We performed a pulse labeling experiment that allows visualization of short RNA products (Figure 2e). The single bond abortive initiation assay cannot be used in nuclear extracts because the inevitable NTP contamination leads to transcript elongation (see Luse et al., 1987). Nuclear extract was incubated with naked DNA or chromatin templates for 30 min to allow formation of preinitiation complexes. ATP, CTP, and limiting radiolabeled UTP were then added and transcription was allowed to occur for 30 min. These conditions permit the formation of only short RNAs because complexes stall due to UTP

starvation. Relative levels of initiation were determined by quantitating the formation of short RNA products (Figure 2f). Initiation on naked DNA was stimulated 3.2-fold by GAL4–VP16 but not by GAL4(1–94) (Figure 2f, lanes 1–3). We did not detect initiation on the unremodeled chromatin template or on the GAL4(1–94)-containing remodeled chromatin template (Figure 2f, lanes 4 and 5). However, the nuclear extract could initiate transcription on the GAL4–VP16-containing remodeled template (Figure 2f, lane 6) at 52% of the level seen on naked DNA in the presence of GAL4–VP16 (Figure 2f, compare lanes 3 and 6). We conclude that transcription initiation in crude nuclear extract requires both chromatin remodeling and an activation domain, consistent with the results obtained with the reconstituted transcription system.

### Is There a Block to Transcript Elongation with the Reconstituted System?

Our data suggest that the GTFs and RNAP II cannot extend transcripts initiated on chromatin templates. To determine where this block to transcript elongation occurred, we used a modified pulse-chase protocol that allows short RNA molecules from blocked transcription complexes to be detected (Figure 3a). Naked DNA or remodeled, GAL4–VP16-containing chromatin was incubated with GTFs, RNAP II, and the coactivator PC4 for 30 min to allow formation of preinitiation complexes. ATP, UTP, and a limiting amount of radiolabeled CTP were added and the incubation continued for 1.5 min (in these conditions, RNAP II transcribes only 10–20 nucleotides (nt) before it stalls due to CTP starvation; data not shown). An excess of CTP was then added and transcripts were elongated for a further 20 min. The resulting RNAs were labeled at their 5′ ends, allowing short transcripts to be detected.

Naked DNA templates supported efficient synthesis of 390 nt RNAs (Figure 3b, lane 1). In contrast, polymerase molecules that initiated on remodeled chromatin templates stalled close to the beginning of the transcription cassette (Figure 3b, lane 2). A small proportion of polymerases synthesized RNAs longer than 50 nt, while the majority stalled before they had synthesized RNAs of 40 nt. Stalled polymerases failed to significantly extend their transcripts upon further incubation (data not shown).

To determine whether the short RNA molecules were present in transcription-competent ternary complexes, we added sarkosyl (0.5%) during the chase at a concentration sufficient to remove histones from the DNA (Izban and Luse, 1991). This resulted in extension of most transcripts to full-size, 390 nt RNAs (Figure 3b, lane 4). We conclude that the majority of the complexes remained competent for transcription and were stalled due to the presence of a nucleosome.

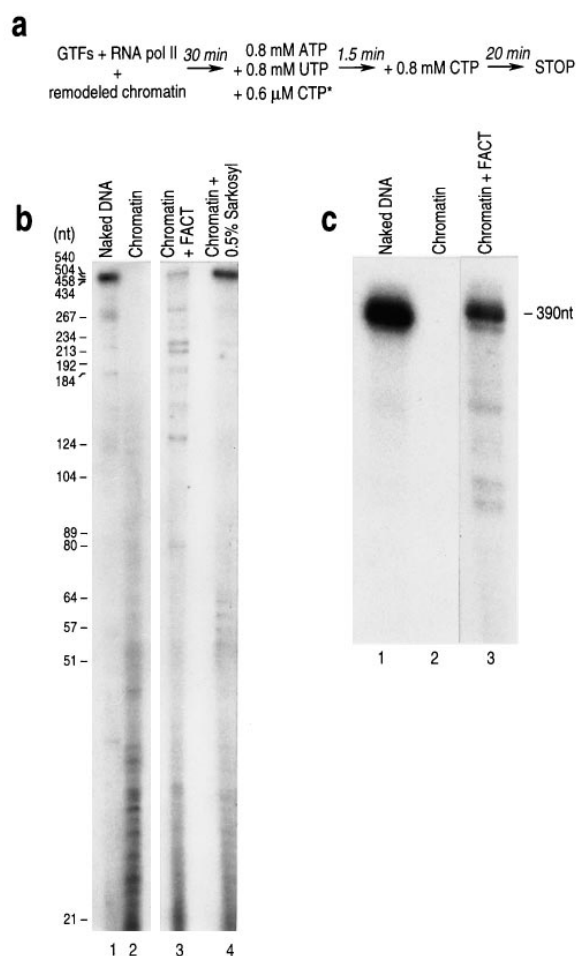


Figure 3. RNA Polymerase II Stalls Proximal to the Promoter on Remodeled Chromatin Templates: Identification of a Factor that Can Relieve Nucleosomal Stalling

(a) Pulse-chase protocol used to detect short, blocked RNAs.

(b) Pulse-chase transcription assay. Remodeled chromatin was incubated with GTFs and RNAP II for 30 min to allow preinitiation complex formation. ATP, UTP, and limiting radiolabeled CTP were added to allow synthesis of short, 5′ end-labeled RNAs. Excess unlabeled CTP was then added and reactions incubated for a further 20 min. Naked DNA was used as the template in lane 1. Reactions containing chromatin templates were scaled up 3-fold to aid comparison with the reaction containing naked DNA. FACT (Phenyl Superose fraction; lane 3) and 0.5% sarkosyl (lane 4) were added 4 min after the addition of excess CTP. The positions of size markers are indicated (nt).

(c) FACT facilitates transcript elongation with the reconstituted system. Naked DNA containing GAL4–VP16 (lane 1) or remodeled chromatin containing GAL4–VP16 (lanes 2 and 3) was used as the template in transcription assays using a reconstituted transcription system containing the coactivator PC4. Where indicated (lane 3) FACT (Mono Q SMART input fraction) was added to the reaction. The position of the full-length, 390 nt RNA product is indicated.

### Identification of a Factor that Can Overcome Chromatin-Induced Transcription Stalling

We attempted to purify a factor from HeLa cell nuclear extract that could counteract the chromatin-mediated block to elongation. Extract proteins were fractionated on phosphocellulose and an activity was observed in the 0.3–0.5 M KCl eluate that could overcome the chromatin block (see below). We have termed this activity FACT.

Figure 3c shows the effect of adding FACT purified through four chromatographic steps (Phenyl Superose fraction, see below) to a reaction using the reconstituted transcription system and remodeled, GAL4–VP16-containing chromatin templates. In this experiment, FACT

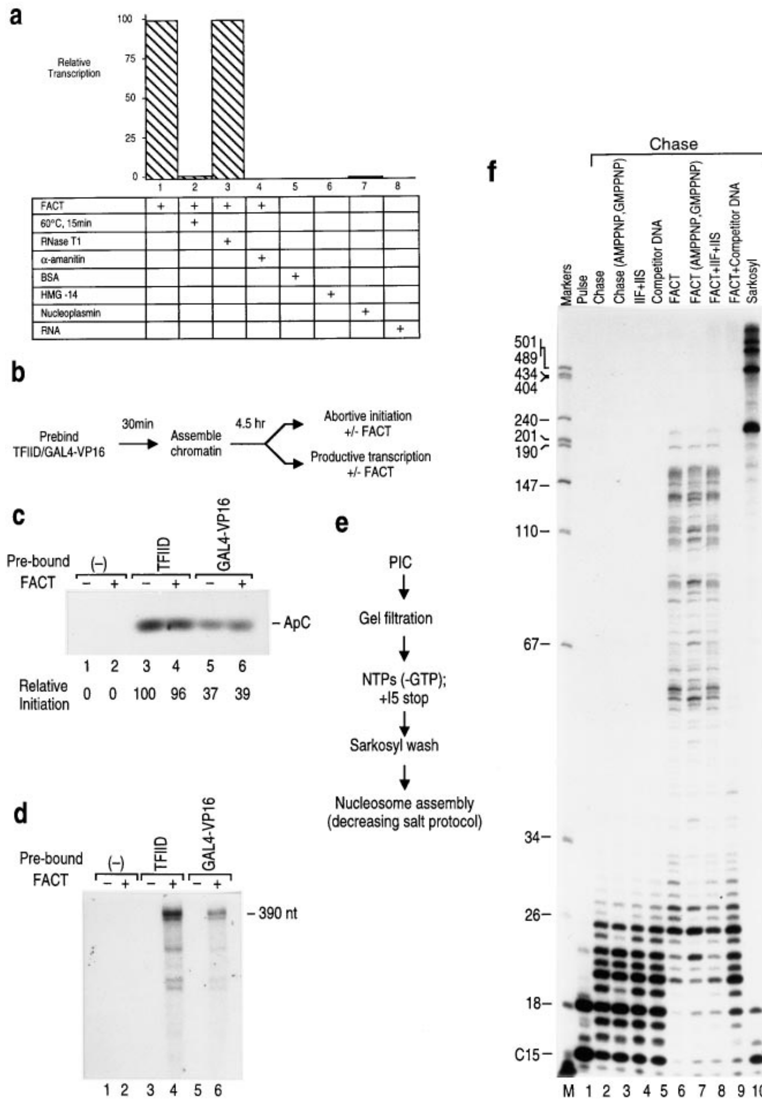


Figure 4. Properties of FACT

(a) Properties of FACT activity. FACT was treated with heat (lane 2) or RNase T1 (lane 3) as indicated in the figure and described in the Experimental Procedures. Where indicated, bovine serum albumin (BSA, 0.1–5 μg, lane 5), HMG-14 (5–500 ng, lane, 6), nucleoplasmin (5–500 ng, lane 7), or purified whole RNA (0.1–4 μg, lane 8) was substituted for FACT as described in the Experimental Procedures. Where indicated α-amanitin (2 μg/ml, lane 4) was included in a reaction containing FACT.

(b) Diagram showing the experimental scheme used to examine whether FACT activity requires an activation domain.

(c and d) FACT activity does not require an activation domain. Purified chromatin (lanes 1 and 2), purified chromatin containing prebound TFIID (lanes 3 and 4), or prebound GAL4–VP16 (lanes 5 and 6) was used as templates for dinucleotide synthesis (c) and productive transcription (d) assays. The positions of the dinucleotide ApC and the full-length, 390 nt RNA product are indicated. Relative initiation values are given below each lane in (c).

(e) Diagram showing the experimental scheme used in the experiment shown in (f).

(f) FACT activity does not require hydrolyzable ATP or GTP and is not synergistic with TFIIF and TFIIS. RNAP II was stalled at +15/+18 downstream of the Ad 2 ML promoter on plasmid pML5–4NR, sarkosyl rinsed, and then assembled into chromatin with purified core histones using a decreasing salt reconstitution method. Transcript elongation assays were performed at 37°C and 8 mM $MgCl_2$ for 30 min with addition of either 1 mM NTPs or 1 mM AMPPNP, GMPPNP, CTP, and GTP as indicated. The presence of FACT (2 μg Phenyl Superose fraction), the elongation factors TFIIF and TFIIS, superhelical pUC18 DNA in a 5-fold excess to template DNA, or the detergent sarkosyl (1%) are all indicated. RNA from the initial +15/+18 complex is shown in lane 1 and DNA size markers are in lane M.

promoted the production of full-length, 390 nt RNAs (Figure 3c, lane 3). Although elongation on chromatin templates in the presence of FACT was not as efficient as elongation on naked DNA, most complexes generated full-length, 390 nt transcripts (Figure 3c, compare lanes 1 and 3). FACT promoted synthesis of long transcripts when it was added during the chase phase of a pulse-chase experiment (Figure 3b, compare lane 2 with lane 3). FACT also facilitated elongation on chromatin templates with a longer nucleosomal spacing (i.e., 180–190 bp) and on templates that contained histone H1 (data not shown).

## FACT Activity Is Not Due to RNA, HMG-14, or Nucleoplasmin

Previous studies have identified molecules that can relieve the repressive effects of nucleosomes or histone H1 on transcription. Croston et al. (1992) reported that a *Drosophila* embryo nuclear fraction could act as a co-antirepressor to allow transcription from DNA templates repressed by histone H1. The co-antirepressor was sensitive to treatment with RNase A, was insensitive to heat treatment at 60°C for 15 min, and could be partially substituted by purified total RNA. In contrast, FACT was inactivated by heat treatment at 60°C for 15 min (Figure 4a, compare columns 1 and 2) and was insensitive to treatment with RNase T1 (Figure 4a, compare columns 1 and 3). In addition, FACT activity could not be substituted by purified total RNA (Figure 4a, column 8). The high mobility group protein HMG-14 has been reported to stimulate transcript elongation in vitro on nucleosomal templates (Ding et al., 1994). However, HMG-14 did not exhibit FACT activity (Figure 4a, column 6). We consider it unlikely that FACT is a histone-binding protein, because nucleoplasmin (Laskey et al., 1978) did not significantly stimulate elongation in our system (Figure 4a, column 7). FACT could not simply be substituted by bulk protein, since the addition of bovine serum albumin was without effect (Figure 4a, column 5). Finally, transcription in the presence of FACT was completely

sensitive to the RNA polymerase II inhibitor α-amanitin (Figure 4a, column 4), ruling out the possibility that FACT activity was due to RNA polymerases I or III.

### FACT Activity Does Not Require an Activation Domain

Several studies have determined that activation domains can stimulate RNAP II transcript elongation in vivo (Yankulov et al., 1994) and in vitro (Brown et al., 1996). We therefore tested whether the stimulation of elongation through nucleosomes by FACT requires an activation domain. To facilitate transcription initiation in the absence of an activator, we prebound TFIID before chromatin assembly (Workman and Roeder, 1987). The nucleosome-assembled template containing TFIID was then purified by gel filtration and used in assays measuring abortive initiation and productive transcription in the absence or presence of FACT (Figure 4b).

We did not detect initiation (Figure 4c, lane 1) or productive transcription (Figure 4d, lane 1) using a template that did not contain prebound TFIID. The addition of FACT to reactions containing this template was without effect (Figures 4c and 4d, lane 2). In contrast, the template on which TFIID was prebound supported efficient initiation (Figure 4c, lane 3) but did not support productive transcription (i.e., elongation; Figure 4d, lane 3). The addition of FACT to reactions containing prebound TFIID did not stimulate transcription initiation (Figure 4c, lane 4) but facilitated productive transcription (Figure 4d, lane 4). A template that contained prebound GAL4–VP16 produced similar results, but both initiation (Figure 4c, lanes 5 and 6) and productive transcription (Figure 4d, lanes 5 and 6) were reduced by 3-fold relative to the TFIID-prebound template. These data demonstrate that relief of the nucleosomal block by FACT does not require an activation domain. However, FACT can only function if transcription initiation is possible, since FACT cannot facilitate transcription initiation.

### FACT Can Function in a Highly Defined System, Does Not Require ATP Hydrolysis, and Does Not Synergize with RNAP II Elongation Factors

The S-190 chromatin assembly extract may have introduced nonhistone protein contaminants into our chromatin templates, which could function in conjunction with FACT. To investigate this possibility, we examined the effect of FACT in an assay that measures the ability of RNAP II to elongate through nucleosomes reconstituted without assembly extracts (Chang and Luse, 1997). Briefly, pure histones and plasmid DNAs bearing purified RNAP II transcription complexes were assembled into nucleosomes using transient exposure to high salt followed by dilution and dialysis. The complexes were then challenged to resume elongation by chasing with NTPs (Figure 4e).

RNAP II complexes bearing 15 or 18 nt RNAs (Figure 4f, lane 1) were used for chromatin assembly. Control experiments with transcription complexes mock-reconstituted in the absence of histones gave the expected rate of elongation (data not shown; see Chang and Luse, 1997). On nucleosomal templates, incubation with NTPs for 30 min resulted in the extension of transcripts by about 10 nt (Figure 4f, lane 2). Dissociation of nucleosomes with sarkosyl restored efficient transcript elongation, indicating that the stalled RNAP II complexes remained active (Figure 4f, lane 10). In the presence of FACT, the majority of complexes were able to extend their transcripts significantly, in some cases synthesizing RNAs of 200 nt (Figure 4f, lane 6). Since FACT stimulated elongation through nucleosomes in this highly defined system, we conclude that FACT can function without any additional factors. The RNAP II elongation factors TFIIF and TFIIS did not stimulate elongation through nucleosomes in the absence (Figure 4f, lane 4) or presence (Figure 4f, lane 8) of FACT. This result suggests that FACT is not a conventional RNAP II elongation factor and does not synergize with known RNAP II elongation factors.

In the defined system, transcripts produced in the presence of FACT are not as long as those synthesized with templates assembled into chromatin in the *Drosophila* S-190 extract (compare panels d and f of Figure 4). This may reflect the different nucleosome densities obtained with the two reconstitution methods. Use of the S-190 extract gives regularly spaced nucleosomes with 20–25 bp linkers, while the decreasing salt method results in close-packed nucleosome arrays that directly abut the stalled transcription complex (see Chang and Luse, 1997).

Substitution of ATP and GTP with their nonhydrolyzable analogs AMPPNP and GMPPNP had no effect on FACT activity (Figure 4f, lane 7). Thus, FACT is not a conventional chromatin remodeling activity, since these complexes require ATP hydrolysis to function (for review, see Kingston et al., 1996). FACT activity was partially inhibited by the addition of excess competitor DNA (Figure 4f, lane 9), suggesting that FACT has the ability to bind DNA.

### FACT Activity Coelutes with Two Polypeptides of 140 and 80 kDa

FACT was purified through a total of six chromatographic steps (Figure 5a) using the two assays described above (see Figures 3c and 4f). Figure 5b depicts a silver-stained SDS-PAGE gel containing active fractions from each step of FACT purification. The elution profile of FACT activity in the 5th and 6th steps of purification (Figures 5d, 5f, and 5g) correlated with that of specific polypeptides visualized by SDS-PAGE and silver-staining (Figures 5c and 5e). FACT activity consistently coeluted with 2 polypeptides of 140 and 80 kDa (designated p140 and p80; Figures 5c and 5e) that were enriched throughout purification (Figure 5b). The p140 subunit appears to be sensitive to proteolytic digestion, as smaller polypeptides appeared upon repeated freeze-thaw cycles (e.g., see Figure 5e, fraction 7).

A third polypeptide of approximately 40 kDa is present in the most purified preparations of FACT (see Figures 5c and 5e), but its elution profile does not correlate with FACT activity. For example, during phosphocellulose chromatography FACT activity elutes in a sharp peak that correlates with the presence of the p140 and p80 polypeptides (Figures 5c and 5d). On this column, the

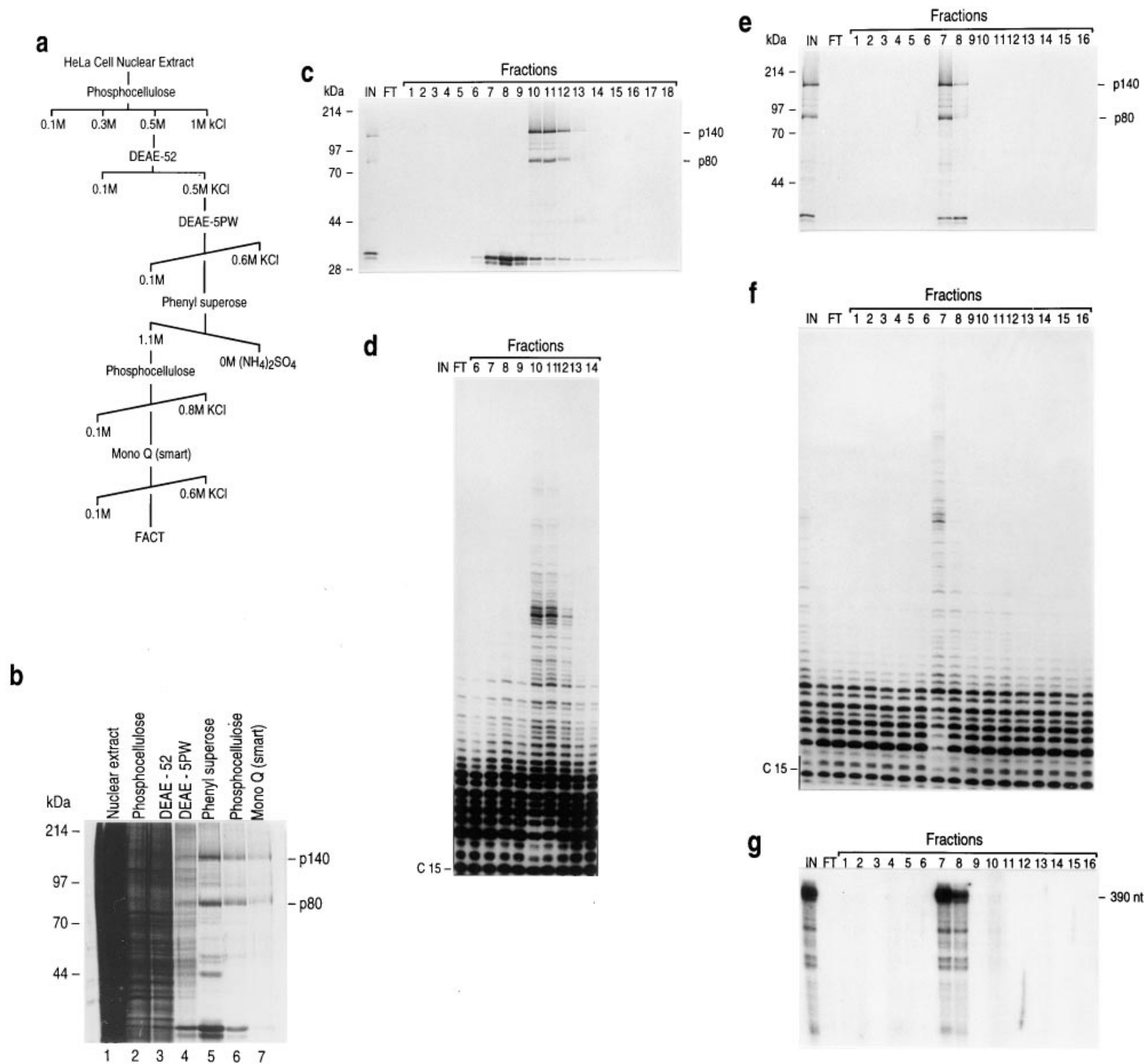


Figure 5. Purification of FACT

(a) Chromatographic scheme used to purify FACT.

(b) Protein composition of active fractions from each step of FACT purification. Fractions containing approximately equal amounts of FACT activity were resolved on a 10% SDS–PAGE gel and stained with silver. The positions of the p140 and p80 subunits of FACT and of protein molecular weight markers are indicated.

(c and e) SDS–PAGE and silver staining of protein fractions from the 5th (c, phosphocellulose) and 6th (e, Mono Q SMART) steps of FACT purification. The positions of the p140 and p80 subunits of FACT and of protein molecular weight markers are indicated.

(d, f, and g) Transcription analysis of protein fractions from the 5th (d, phosphocellulose) and 6th (f and g, Mono Q SMART) steps of FACT purification. Fractions were assayed using the experimental scheme shown in Figure 4e (d and f) or using the reconstituted system and GAL4–VP16-containing, remodeled chromatin templates (g).

peak of the 40 kDa polypeptide elutes in earlier fractions and trails into fractions that contain FACT activity. We cannot conclusively determine whether the 40 kDa polypeptide is required for FACT activity because we have been unable to separate it completely from the p140 and p80 polypeptides.

FACT elutes with a native molecular mass of ~230 kDa by gel filtration chromatography (data not shown), suggesting that the p140 and p80 subunits form a heterodimeric complex. Since we are unable to measure accurately the amount of the p140 and p80 polypeptides present during the final stages of FACT purification, we cannot determine the stoichiometry of FACT to nucleosomes required for maximal stimulation of elongation. However, we can roughly calculate, based on silver-staining intensity, that maximal stimulation of elongation through nucleosomes requires an equimolar stoichiometry of FACT to nucleosomes.

## Discussion

### Chromatin Remodeling Is Not Sufficient for Productive Transcription: Identification of FACT

We report here that chromatin remodeling facilitates the formation of a preinitiation complex and transcription initiation, but is not sufficient for productive transcription

(i.e., synthesis of a 390 nt RNA). Transcription initiation on chromatin templates requires both promoter remodeling and an activation domain. Polymerases that initiate on remodeled templates encounter a block to transcript elongation a short distance from the promoter due to the presence of a nucleosome. We have identified and purified an activity from HeLa nuclear extract that overcomes the block to elongation. This activity, which we have called FACT, appears to be a heterodimeric complex of 140 and 80 kDa subunits.

RNAP II elongation factors can be separated into two classes (for review, see Aso et al., 1995): those that stimulate the rate of elongation (TFIIF, ELL, and the elongin complex) and those that allow RNAP II to overcome intrinsic arrest sites in the DNA (TFIIS). We believe that FACT is not a known RNAP II elongation factor, because TFIIF and TFIIS were without effect in conditions in which FACT stimulated elongation (see Figure 4f). Furthermore, fractions containing FACT activity did not stimulate elongation on naked DNA (data not shown). FACT activity does not require RNA and cannot be substituted by HMG-14 or the histone-binding protein nucleoplasmin.

Brown et al. (1996) have shown that the human SWI/SNF complex can enhance RNAP II elongation on the human *hsp70* gene when the latter is assembled into nucleosomes. Numerous lines of evidence suggest that FACT is not a remodeling complex. First, and most importantly, FACT activity does not require ATP hydrolysis. Second, incubation of purified chromatin templates with FACT did not cause nucleosome loss or any change in the micrococcal nuclease digestion pattern (data not shown). We did not detect any SWI/SNF subunits or human SNF2L protein in FACT fractions (human SNF2L protein is a homolog of the *Drosophila* ISWI protein, which is a subunit of the NURF complex [Tsukiyama et al., 1995]), and a purified SWI/SNF complex from HeLa cells did not exhibit FACT activity (data not shown). In addition, the polypeptide composition of FACT does not match that of any known elongation or chromatin remodeling factor. Finally, we note that a human RNAP II holoenzyme preparation is unable to elongate through nucleosomes (data not shown), suggesting that it does not contain FACT.

We do not yet understand the mechanism by which FACT relieves the block to elongation on chromatin templates. It is interesting that elongation stimulation by FACT is inhibited by DNA (Figure 4f). Thus, we may speculate that FACT binding to the nucleosomal templates displaces histone–DNA interactions and thereby assists the RNA polymerase in accessing the template. While FACT can relieve the nearly absolute block to transcript elongation imposed by nucleosomes, elongation rates on our chromatin templates in the presence of FACT are much lower than those in the nucleus (20–25 nt/s; e.g., see Ucker and Yamamoto, 1984). Thus, it is likely that additional factors work in conjunction with FACT to achieve physiological transcription rates.

## Accessory Factors Are Required at Two Different Stages

We propose that productive transcription on chromatin templates requires the action of factors distinct from RNAP II and its GTFs at two different stages (Figure 6). The first set of factors remodel the promoter region in response to the binding of activators, which makes core promoter sequences accessible to the GTFs and RNAP II. This group of factors may include multisubunit complexes that use the energy of ATP hydrolysis to alter chromatin structure (reviewed in Kingston et al., 1996). Acetylation of core histones may also play a role in facilitating transcription factor access to the promoter (reviewed in Wade and Wolffe, 1997). The second set of factors acts after transcription initiation to overcome a nucleosomal block to elongation. FACT appears to be a member of the second, as-yet-uncharacterized factor group.

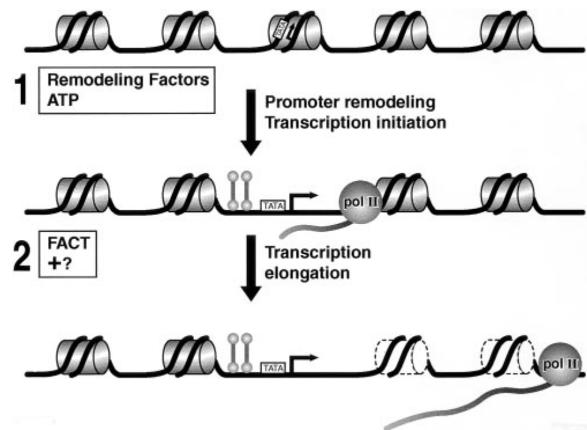


Figure 6. Transcription on Chromatin Templates Requires the Action of Factors Distinct from RNAP II and the GTFs at Two Different Stages

See Discussion for details.

## Reconstituting Transcription Activation in Chromatin

In this study, we have begun to dissect the mechanisms that lead to transcription activation in chromatin using defined components in vitro. We have determined that nucleosomes inhibit transcription at two different stages: transcription initiation and promoter-proximal transcriptional elongation. We have been able to overcome the inhibition of transcription initiation by using remodeled templates. In addition, inhibition of transcript elongation can be counteracted by a factor we call FACT.

A goal of this work is the complete reconstitution of transcriptional activation on chromatin templates. This will require a much more extensive characterization of the chromatin remodeling activities and their roles, along with histone acetyltransferases and transcriptional activators, in establishing active promoters in a chromatin environment. In addition, as we have shown here, factors which work primarily at the transcript elongation level must be characterized and purified.

## Experimental Procedures

### Purification of Proteins

GAL4(1–94) and GAL4(1–94)-VP16 were expressed in *E. coli* and purified according to the method of Reece et al. (1993). TFIIA, TFIIB, TBP, TFIIE, and TFIIF were expressed in *E. coli* and purified as

described by Maldonado et al. (1996). TFIIH (Phenyl Superose fraction) was purified as described previously (Maldonado et al., 1996). TFIID was affinity-purified from HeLa cells expressing Hemagglutin-tagged TBP using a modification of the method described by Zhou et al. (1992). PC4 was expressed in *E. coli* and purified as described previously (Ge and Roeder, 1994). RNAP II was purified by affinity chromatography using the anti-RNAP II antibody 8WG16 (Promega) as described (Maldonado et al., 1996). HeLa cell core histones were purified using a modification of a published procedure (Wolffe and Hayes, 1993).

**Chromatin Assembly**

The S-190 assembly extract was prepared from *Drosophila* embryos as described previously (Kamakaka et al., 1993; Bulger and Kadonaga, 1994). Chromatin assembly was performed as described previously (Kamakaka et al., 1993; Bulger and Kadonaga, 1994) using 200 μl of S-190 extract (2.5–3 mg protein), 2 μg HeLa cell core histones, and 3 μg supercoiled plasmid in a volume of 400 μl. Where indicated, GAL4(1–94) or GAL4–VP16 (200 nM) were added after 4.5 hr of assembly to allow DNA binding and chromatin remodeling.

**Purification and Analysis of Chromatin Templates**

For chromatin purification, a 400 μl chromatin assembly reaction was applied to a 0.5 × 20 cm column containing 4 ml of CL-4B resin (Pharmacia) preequilibrated in 10 mM HEPES (pH 7.5), 10 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, and 10% (v/v) glycerol at 25°C at a flow-rate of ~100 μl/min. Fractions of 300 μl were collected in siliconized tubes (Denville Scientific). Chromatin-assembled plasmid eluted at a volume of ~1.3 ml. The DNA concentration of purified chromatin was estimated by coelectrophoresis of aliquots with DNA standards of known concentration and by measuring A260. The protein content of the purified chromatin templates was examined using 18% polyacrylamide–SDS gel electrophoresis and silver staining.

Partial digestion of chromatin with MNase and Southern hybridization were performed as described previously (Bulger and Kadonaga, 1994). The sequences of the oligonucleotides used for Southern hybridization were as follows: promoter, 5′-GGGGCTATAAAAGGGG GTGGGGGCGCGTTC-3′; plasmid, 5′-CTCCGATCGTTGTCAGAAGT AAGTTGGGCG-3′.

**DNase I Footprinting**

Primer extension–mediated DNase I footprinting was performed on purified chromatin as described previously (Gralla, 1985), except that positions of DNase I cleavage were visualized using thermal-cycle primer-extension with 0.5 U Vent (exo-) DNA polymerase (New England Biolabs) using a primer with the sequence 5′-TAATGAGGA AAGGAGAGTAGGGTGGTATAG−3′ according to the manufacturer's instructions for 20 cycles of 95°C, 1 min; 65°C, 1 min; and 72°C, 1 min.

**In Vitro Transcription Assays**

The transcription template used in this study contained five GAL4 DNA-binding sites, the adenovirus major late promoter, and a 390 nt G-less cassette (~3.2 kbp). Experiments that compared chromatin templates with naked DNA templates contained the same amount of DNA (40 ng).

***Productive Transcription***

This was performed as described previously (Flores et al., 1992). Reactions (40 μl) contained naked DNA or purified chromatin (containing 40 ng DNA), 160 ng TFIIA, 10 ng TFIIB, 50 ng TFIIE, 10 ng TFIIF, 150 ng TFIIH (Phenyl Superose), 100 ng RNAP II, 12 U rRNAsin (Promega), 40 ng TFIID, and 3 ng PC4, 10 mM HEPES (pH 7.9), 50 mM KCl, 5 mM $MgCl_2$, 10% v/v glycerol, 5 mM β-mercaptoethanol, 0.1 mM PMSF, 0.1 mM EDTA, 2.6% polyethylene glycol (average MW 8000 Da), and 3.75 mM $(NH_4)_2SO_4$. Purified RNA products were deproteinized, precipitated with ethanol, and analyzed on 6% acrylamide denaturing gels. Relative transcription levels were quantitated using a Molecular Dynamics phosphorimager.

***Pulse-Chase Protocol***

Reactions were assembled and incubated for 30 min at 30°C as described for productive transcription before the addition of 0.8 mM ATP, 0.8 mM UTP (FPLC-pure; Pharmacia), and 0.6 μm [α-$^{32}$P]-CTP. Reactions were incubated for 1.5 min at 30°C to allow short, labeled RNAs to be synthesized before the addition of 0.8 mM CTP (FPLC-pure; Pharmacia). Transcript elongation was allowed to occur for 30 min at 30°C. In some experiments, 0.5% sarkosyl or FACT (Phenyl Superose fraction, 8 μg) was added 4 min into the elongation phase of the reaction, which is sufficient time for the formation of full-length, 390 nt transcripts on naked DNA (data not shown). The Phenyl Superose FACT fraction, which was dilute, was concentrated by step elution from a Mono Q column for use in transcription assays. Purified RNA products were resolved on 10% polyacrylamide denaturing gels and visualized using X-ray film or a Molecular Dynamics phosphorimager.

***Dinucleotide Synthesis***

Reactions were assembled and incubated for 30 min at 30°C as described for productive transcription. Dinucleotide synthesis was initiated by the addition of 1 mM ATP, 1 μM CTP (FPLC-pure; Pharmacia), and 0.3 μM [α-$^{32}$P]-CTP (NEN). Reactions were incubated at 30°C for 30 min, stopped by heating at 65°C for 5 min, and treated with 8 U alkaline phosphatase (Boehringer Mannheim) for 1 hr at 37°C. Dinucleotide products were analyzed on 15% polyacrylamide denaturing gels and quantitated using a Molecular Dynamics phosphorimager.

***Transcription Initiation in HeLa Nuclear Extract***

Reactions were assembled as described above using 80 μg nuclear extract in place of purified transcription factors. Following incubation at 30°C for 30 min, 0.8 mM ATP, 0.8 mM CTP, and 0.6 μM [α-$^{32}$P]-UTP (NEN) were added and the reactions were incubated for a further 30 min at 30°C. Purified RNA products were resolved on a 20% acrylamide denaturing gel and quantitated using a Molecular Dynamics phosphorimager.

**Heat and RNase T1 Treatment of FACT**

To determine whether FACT was resistant to heat treatment, 2 μg of FACT (Phenyl Superose fraction) was heated at 60°C for 15 min and was allowed to cool before being added to transcription reactions. To determine whether FACT was sensitive to a ribonuclease, 2 μg of FACT (Phenyl Superose fraction) was incubated with 10 U RNase T1 (Boheringer Mannheim) for 20 min at 30°C (this amount of RNase T1 completely digests 4 μg of total purified RNA in the same conditions [data not shown]). The treated protein was then added to transcription reactions. The presence of RNase T1 in transcription reactions was tolerable because the transcript being synthesized lacked Gs and was therefore resistant to the RNase.

**Elongation Assays Using Stalled Elongation Complexes and Nucleosomes Assembled Using a Decreasing Salt Reconstitution Protocol**

This approach is described in detail elsewhere (Chang and Luse, 1997). Briefly, RNAP II preinitiation complexes were assembled onto the pML5–4NR plasmid by incubation in HeLa cell nuclear extracts. Transcription complexes were advanced to positions +15/+18 and purified by the addition of sarkosyl followed by gel filtration. Histones were acid extracted from HeLa cell nuclear pellets and purified by chromatography on Mono S (Pharmacia). Chromatin was assembled by mixing histones (at a mass ratio of 2:1, histones:DNA) with sarkosyl-rinsed transcription complexes at 1 M NaCl, followed by a series of dilutions and dialysis into the 62.5 mM KCl transcription buffer. Each reaction contained 25 ng of template in a final volume of 25 μl. In some cases, TFIIF (1.5 ng/μl), TFIIS (1.5 ng/μl), or a 5-fold molar excess of supercoiled pUC18 competitor DNA was added with the chase. FACT (either 2 μg of concentrated Phenyl Superose fraction, in Figure 4, or 10 μl of the relevant column fraction in Figure 5) was added as indicated in the figures. Nascent RNAs were extended at 37°C with 1 mM NTPs for 30 min.

**Purification of FACT**

HeLa cell nuclear extract (3000 mg protein, 400 ml) prepared by standard procedures (Dignam et al., 1983) was applied to a column containing 500 ml of phosphocellulose resin (Sigma) equilibrated in BC100. BC buffers contain 20 mM Tris-HCl (pH 7.9), 0.2 mM EDTA, 10 mM β-mercaptoethanol, 0.2 mM PMSF, 20% v/v glycerol; the number following BC denotes the concentration (mM) of KCl. The column was washed with BC100 until no protein eluted and then sequentially with BC300, BC500, and BC1000. FACT activity was in

the BC500 eluate. The BC500 eluate (218 mg, 650 ml) was dialyzed against BC100 and loaded onto an 80 ml column of DEAE-52 resin (Whatman). The column was washed with BC100, and bound proteins were eluted with BC500. FACT activity was found in the BC500 eluate. The eluate (135 mg, 200 ml) was dialyzed against BC100 and loaded onto a 38 ml DEAE-5PW column (TosoHaas). The column was washed with BC100 and protein was eluted with a 300 ml linear gradient of BC100-BC600. FACT activity eluted at a KCl concentration of 250 mM. Fractions containing FACT (26 mg, 21 ml) were pooled, dialyzed against BC buffer containing 1.1 M $(NH_4)_2SO_4$, and loaded onto an 8 ml Phenyl Superose FPLC column (Pharmacia). The column was washed with BC buffer containing 1.1 M $(NH_4)_2SO_4$, and proteins were eluted with a 160 ml linear gradient of 1.1 M to 0 M $(NH_4)_2SO_4$ in BC buffer. FACT was found in the flow-through (1.1 M $(NH_4)_2SO_4$ fraction). This fraction (1.1 mg, 21 ml) was dialyzed against BC100 and applied to a 1 ml phosphocellulose column (Sigma). The column was washed with BC100 and proteins were eluted with a 15 ml linear gradient of BC100-BC800. The large majority of FACT activity eluted at a KCl concentration of ~400 mM (in some preparations we have seen a very minor shoulder of activity eluting earlier [see Figure 5d, lane 8]). Fractions containing FACT (~0.3 mg, 2.1 ml) were pooled, dialyzed against BC100, and applied to a 0.1 ml Mono Q column (Pharmacia, SMART) equilibrated in BC100. The column was washed with BC100 and proteins were eluted with a 1 ml linear gradient of BC100-BC600. Fractions containing FACT were pooled, dialyzed against BC100, and frozen at −80°C.

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