Transcriptional Analysis of Chromatin Assembled with Purified ACF and dNAP1 Reveals That Acetyl-CoA Is Required for Preinitiation Complex Assembly*  

To investigate the role of chromatin structure in the regulation of transcription by RNA polymerase II, we developed a chromatin transcription system in which periodic nucleosome arrays are assembled with purified recombinant ATP-utilizing chromatin assembly and remodeling factor (ACF), purified recombinant nucleosome assembly protein 1 (dNAP1), purified native core histones, plasmid DNA, and ATP. With this chromatin, we observed robust activation of transcription with three different transcription factor sets (nuclear factor κB p65 + Sp1, estrogen receptor, and Gal4-VP16) added either before or after chromatin assembly. In fact, the efficiency of activated transcription from the ACF + dNAP1-assembled chromatin was observed to be comparable with that from naked DNA templates or chromatin assembled with a crude Drosophila extract (S190). With ACF + dNAP1-assembled chromatin, we found that transcriptional activation is dependent upon acetyl-CoA. This effect was not seen with naked DNA templates or with crude S190-assembled chromatin. We further determined that acetyl-CoA is required at the time of preinitiation complex assembly but not during assembly of the chromatin template. These findings suggest that there is at least one key acetylation event that is needed to assemble a functional transcription preinitiation complex with a chromatin template.

The regulation of gene expression at the level of transcription is a key control point for many cellular processes. In eukaryotes, the transcription of protein-coding genes by RNA polymerase II occurs in the milieu of chromatin and involves the covalent and noncovalent modification of nucleosomes (for reviews, see Refs. 1–13). There are, for instance, enzymes that modify histones by acetylation/deacetylation, phosphorylation, methylation, ubiquitination, or ADP-ribosylation. In addition, ATP-dependent proteins, termed chromatin remodeling factors, alter histone-DNA contacts and catalyze nucleosome mobility. It will thus be important to understand the relation between chromatin structure and the activity of each of the tens of thousands of genes in an organism.

To investigate the role of chromatin in transcriptional regulation, it is possible to use biochemical systems for the analysis of in vitro reconstituted chromatin. To this end, we previously developed a chromatin transcription system that is based on a crude chromatin assembly extract termed the S190 extract, which is derived from Drosophila embryos (14, 15). This S190-based chromatin transcription system has been used for the analysis of many transcription factors.

Although the S190 extract has been a reliable source of chromatin assembly activity, we have recently achieved the ATP-dependent assembly of chromatin with purified, recombinant chromatin assembly factors (16). In these reactions, periodic nucleosome arrays are assembled with purified recombinant ACF,1 purified recombinant dNAP1, purified native core histones, plasmid DNA, and ATP. dNAP1 functions stoichiometrically as a core histone chaperone, whereas ACF acts catalytically in an ATP-dependent manner to mediate the deposition of histones onto DNA as well as to catalyze the formation of periodic nucleosome arrays.

In this work, we have used the purified chromatin assembly factors, instead of the S190 extract, to assemble chromatin for transcriptional analyses. With this new chromatin transcription system, we have found that the presence of acetyl-CoA at the time of transcription preinitiation complex assembly is essential for activation with chromatin templates.

EXPERIMENTAL PROCEDURES

Transcription Factors and DNA Templates—FLAG-tagged human estrogen receptor α (ER), His-tagged NF-κB p65, and His-tagged p300 were expressed in Sf9 cells and purified as described previously (17, 18). Purified Sp1 was obtained from Promega (Madison, WI). Gal4-VP16 was purified as described previously (19). Transcription reactions with NF-κB p65 and Sp1 were carried out with a DNA template containing the IRF-1 promoter region (from –1312 to +39 relative to the RNA start site) (20). Transcription with the ER was performed with pERE, which contains four estrogen response elements upstream of the adenovirus E4 core promoter (17). Transcription with Gal4-VP16 was carried out with pGIE-0, which contains five Gal4 binding sites upstream of the adenovirus E4 core promoter (14).

Chromatin Assembly—The assembly of chromatin with purified recombinant ACF, purified recombinant dNAP1, purified Drosophila core histones, plasmid DNA, and ATP was performed as described by Ito et al. (16), except that the scale of the reactions was typically about five times larger than that used previously. The assembly of chromatin with the Drosophila S190 extract was carried out as described by Bulger and Kadonaga (21). The resulting chromatin samples were subjected to in vitro transcription analysis as well as to micrococcal nuclease digestion analysis to confirm the efficient assembly of periodic nucleosome arrays.

In Vitro Transcription—In vitro transcription reactions were performed with a chromatin template.
human p300 were added after chromatin assembly and prior to the addition of the nuclear transcription extract.

In initial experiments with the Gal4-VP16 activator and the soluble nuclear fraction (a crude, undialyzed nuclear transcription extract derived from Drosophila embryos) (23, 24), we observed comparable levels of transcription with the recombinant ACF + dNAP1-assembled chromatin as with the S190-assembled chromatin (data not shown). These results with recombinant ACF and dNAP1 are similar to those that we had obtained previously with native ACF and dNAP1 in conjunction with Gal4-VP16 and the soluble nuclear fraction (25).

In contrast, when we performed transcription reactions with Gal4-VP16 and a standard HeLa nuclear extract (26) instead of the soluble nuclear fraction, there was robust transcription from the S190-assembled chromatin, but no detectable transcription from the ACF + dNAP1-assembled chromatin. To investigate the basis for the lack of Gal4-VP16-activated transcription with the ACF + dNAP1-assembled chromatin, we performed a series of experiments in which we tested the ability of proteins and protein fractions as well as nonprotein factors to stimulate transcription with the ACF + dNAP1-assembled chromatin and the HeLa extract. These studies led to the identification of acetyl CoA as a key, essential cofactor that is present in the S190 chromatin assembly extract (and presumably the soluble nuclear fraction) and is absent from our preparations of purified ACF and dNAP1.

This acetyl-CoA dependence of transcription was then tested with NF-κB p65 + Sp1 (Fig. 2, top row) and human estrogen receptor α (Fig. 2, middle row) as well as Gal4-VP16 (Fig. 2, bottom row). With ACF + dNAP1-assembled chromatin, we observed a strong enhancement of transcription by acetyl-CoA that varied from 21- to 41-fold with a standard HeLa transcription extract and from 17- to 41-fold with HeLa extract supplemented with purified p300 coactivator (Fig. 2, left panels). In contrast, with S190-assembled chromatin, there was no significant change in transcriptional activity upon addition of acetyl-CoA (ranging from a 0.7- to 1.4-fold effect), presumably due to the presence of acetyl-CoA in the S190 extract (Fig. 2, middle panels). With naked DNA templates, we observed a slight increase in transcription upon addition of acetyl-CoA that varied from 1.2- to 2.5-fold (Fig. 2, right panels). In addition, trichostatin A, an inhibitor of histone deacetylases, did not enhance transcription in the absence of exogenously added acetyl-CoA with the ACF + dNAP1-assembled chromatin (data not shown). Thus, these findings collectively indicate that there is a general chromatin-specific requirement for acetyl-CoA for transcriptional activation.

It is also important to note that the exposure times of the three autoradiograms in each row of Fig. 2 are either identical (i.e. derived from the same autoradiogram) or nearly identical. Therefore, the levels of activated transcription from the ACF + dNAP1-assembled chromatin are comparable with those seen with S190-assembled chromatin or with naked DNA templates. Hence, there is robust transcription from the ACF + dNAP1-assembled chromatin in the presence of acetyl-CoA.

To investigate further the transcriptional properties of the ACF + dNAP1-assembled chromatin, we examined whether or not there is a greater amount of transcription when activators are added to naked DNA templates prior to chromatin assembly than when activators are added to chromatin that is previously assembled. It is possible, for instance, that the purified chromatin assembly system may lack nucleosome remodeling activities that are necessary for activators to bind to chromatin and/or to recruit coactivators and the basal transcriptional machinery. As shown in Fig. 3, we observed essentially identical levels of transcriptional activation by either NF-κB p65 +

![Fig. 1. Assembly of periodic nucleosome arrays with purified recombinant ACF and dNAP1.](http://www.jbc.org/content/3982/2/24015/F1.large.jpg)

The cloning of the Acf1 subunit of ACF led to the development of a purified chromatin assembly system that consists of defined components: purified recombinant ACF, purified recombinant dNAP1, purified native core histones, plasmid DNA, and ATP (16). The purified proteins are shown in Fig. 1. ACF consists of Acf1 and ISWI subunits and catalyzes the ATP-dependent deposition of histones onto DNA as well as the periodic spacing of nucleosomes (16). dNAP1 is a core histone chaperone that is a homomultimer of a polypeptide with a calculated molecular mass of ~43 kDa, although it migrates with a larger apparent mass on an SDS-polyacrylamide gel (22). The assembly of extended, periodic nucleosome arrays with the purified factors is shown in Fig. 1.

With this system, we compared the transcriptional properties of chromatin assembled with purified ACF + dNAP1 to those of chromatin assembled with the crude S190 extract. These experiments were performed as follows. First, plasmid DNA was assembled into chromatin with either purified ACF + dNAP1 or S190 extract, and then a nuclear extract was added as a source of the RNA polymerase II transcriptional machinery. When desired, sequence-specific activators and purified

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**RESULTS**

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Acetyl-CoA is required for transcription of chromatin assembled with purified ACF and dNAP1. The transcriptional properties of chromatin assembled with purified ACF and dNAP1 (left panels), chromatin assembled with crude S190 extract (center panels), or naked DNA (right panels) were tested with purified NF-κB p65 + purified Sp1 (top row), purified estrogen receptor (ER) + 17β-estradiol (middle row), or purified Gal4-VP16 (bottom row). Sequence-specific activators, purified human p300, and/or acetyl-CoA (AcCoA) were added, as indicated, subsequent to chromatin assembly and prior to in vitro transcription with a HeLa nuclear extract. The resulting transcripts were detected by primer extension analysis, and the reverse transcription products are shown. The final concentrations of acetyl-CoA, p300, NF-κB p65, Sp1, estrogen receptor, 17β-estradiol, and Gal4-VP16 in the transcription reaction media were 9 μM, 15 μM, 60 μM, 9 μM, 4.5 μM, 30 μM, and 7.5 μM, respectively. For each row of reactions performed with a particular activator set (NF-κB p65 + Sp1, estrogen receptor, or Gal4-VP16), the time of exposure of the autoradiograms is either identical or nearly identical. Therefore, for each activator, the autoradiograms reflect the relative amounts of transcription from the ACF + dNAP1-assembled chromatin relative to the S190-assembled chromatin and to naked DNA templates.

Sp1 or Gal4-VP16 when the activators were added either before or after chromatin assembly. Similar results were also obtained with the estrogen receptor (data not shown). Therefore, transcriptional activators function effectively with chromatin that is previously assembled with ACF and dNAP1.

In this manner, single round transcription experiments were carried out in which acetyl-CoA was added at different times in the chromatin assembly and transcription processes. The results obtained with NF-κB p65 + Sp1 at the IRF-1 promoter are shown in Fig. 4B. First, when acetyl-CoA was added at the beginning of the chromatin assembly process (time = 0 min), we observed a strong stimulation of transcription, as seen in Fig. 2. Similarly, there was also a strong stimulation of transcription when acetyl-CoA was added after chromatin assembly and prior to PIC assembly (at time = 185 min). These results indicate that acetyl-CoA is not required during chromatin assembly for transcriptional activation. In contrast, when acetyl-CoA was required for transcription of chromatin assembled with purified ACF and dNAP1. The transcriptional activators NF-κB p65 + purified Sp1, estrogen receptor, 17β-estradiol, and Gal4-VP16 in the transcription reaction media were 9 μM, 15 μM, 60 μM, 9 μM, 4.5 μM, 30 μM, and 7.5 μM, respectively. For each row of reactions performed with a particular activator set (NF-κB p65 + Sp1, estrogen receptor, or Gal4-VP16), the time of exposure of the autoradiograms is either identical or nearly identical. Therefore, for each activator, the autoradiograms reflect the relative amounts of transcription from the ACF + dNAP1-assembled chromatin relative to the S190-assembled chromatin and to naked DNA templates.

Transcriptional activators function effectively with chromatin that is previously assembled with ACF and dNAP1. The transcriptional activators NF-κB p65 + Sp1 (left panel) or Gal4-VP16 (right panel) were added either to naked DNA templates that were subsequently assembled into chromatin with purified ACF and dNAP1 (“Added before Assembly”) or to chromatin templates that were previously assembled with purified ACF and dNAP1 (“Added after Assembly”). The resulting templates were then subjected to in vitro transcription analysis with a HeLa nuclear extract in the presence or absence of acetyl-CoA and/or purified p300, as noted. The transcripts were detected by primer extension analysis. The concentrations of reagents are as indicated in the legend to Fig. 2.

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CoA was added subsequent to PIC incubation and prior to transcription initiation (at time = 259 min 50 s), we did not observe transcriptional activation in either the absence or the presence of exogenously added p300. Hence, these findings indicate that acetyl-CoA is required at the time of PIC assembly. We therefore conclude that acetyl-CoA is required at the time of PIC assembly. Under these conditions, we still observed a strong dependence upon acetyl-CoA for transcription of ACF + dNAP1-assembled chromatin (data not shown). We therefore conclude that acetyl-CoA is required at the time of PIC assembly for the efficient transcription of chromatin templates.

**DISCUSSION**

We have used a defined, purified chromatin assembly system to generate periodic nucleosome arrays for the analysis of chromatin structure and transcriptional regulation. In these experiments, we found that acetyl-CoA is essential for transcriptional activation with chromatin templates and that acetyl-CoA is required at the time of PIC assembly.

Given the extensive acetylation of core histones and the chromatin specificity of the acetyl-CoA dependence, it is reasonable to postulate that the potent stimulation by acetyl-CoA (e.g., 41-fold enhancement with the estrogen receptor) is due to histone acetylation. On the other hand, it is possible that the acetylation of a transcription factor will enhance its ability to function with chromatin. In this regard, the acetylation of sequence-specific DNA binding factors (see, for example, Refs. 30–32), the ACTR transcriptional coactivator (33), and the basal transcription factors TFII E and TFII F (34) have been found. Moreover, it has been found that acetyl-CoA can enhance the transcription of naked DNA (greater than 5-fold) as well as the binding of TFII D to chromatin DNA in the absence of histones (35). (Note that we observe a more modest 1.2–2.5-fold increase in transcription by acetyl-CoA with naked DNA templates (Fig. 2, right panels).) Thus, in our experiments, protein acetylation might affect not only the properties of components of chromatin, but also the activity of transcription factors and coactivators that function with chromatin.

For transcription with chromatin templates assembled with recombinant ACF and dNAP1, we observed a strong dependence upon acetyl-CoA with a standard HeLa nuclear extract (26) (Fig. 2, left panels). In contrast, only a 2–3-fold enhancement by transcription by acetyl-CoA was seen with a standard HeLa transcription extract in conjunction with a nucleosomal template consisting of a promoter-containing dinucleosome embedded in an array of nucleosomes in tandem 5 S rDNA repeats (36, 37). In the same series of experiments, however, the magnitude of acetyl-CoA stimulation was increased upon the addition of any one of four different acetyltransferase-containing complexes. Then, in other studies with a chromatin template consisting of a promoter embedded in a tandem 5 S rDNA array, Kundu et al. (38) have described p300-dependent, Gal4-VP16-activated transcription that is stimulated by acetyl-CoA. These results suggest that p300/CBP and/or HAT complexes such as SAGA, NuA3, NuA4, or Ada may contribute to the acetyl-CoA requirement that we have observed. Yet, unlike the chromatin in the tandem 5 S rDNA arrays, the ACF + dNAP1-assembled chromatin exhibits a strong dependence upon acetyl-CoA with a standard HeLa extract that is not supplemented with exogenously added acetyltransferases.

In the future, we hope to use the ACF + dNAP1-based chromatin transcription system to gain a better understanding of the essential acetylation event(s) at the time of PIC assembly. Importantly, the efficiency of transcription from the ACF + dNAP1-assembled chromatin is comparable with that obtained with either S190-assembled chromatin or with naked DNA templates. Thus, the ACF + dNAP1-based chromatin transcription system is likely to be of general utility for the analysis of the function of a broad range of transcriptional regulators at their downstream target genes. These studies should yield new insights into fundamental aspects of the complex and fascinating mechanisms of gene regulation.

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