Research Article

Sequence-Specific Antirepression of Histone H1-Mediated Inhibition of Basal RNA Polymerase II Transcription

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To understand the principles of control and selectivity in gene expression, the biochemical mechanisms by which promoter- and enhancer-binding factors regulate transcription by RNA polymerase II were analyzed. A general observed repressor of transcription was purified and identified as histone H1. Since many aspects of H1 binding to naked DNA resemble its interaction with chromatin, purified H1 bound to naked DNA was used as a model for the repressed state of the DNA template. Three sequence-specific transcription factors, Sp1, GAL4-VP16, and GAGA factor, were shown to counteract H1-mediated repression (antirepression). In addition, Sp1 and GAL4-VP16, but not the GAGA factor, activated transcription in the absence of H1. Therefore, true activation and antirepression appear to be distinct activities of sequence-specific factors. Furthermore, transcription antirepression by GAL4-VP16 was sustained for several rounds of transcription. These findings, together with previous studies on H1, suggest that H1 participates in repression of the genome in the ground state and that sequence-specific transcription factors induce selected genes by a combination of true activation and release of basal repression that is mediated at least in part by H1.

IN EUKARYOTES, THE SPATIAL AND TEMPORAL PATTERNS OF gene expression are directed by a complex information network. An important step at which the diverse protein coding genes are regulated is initiation of RNA polymerase II transcription. RNA polymerase II and several auxiliary (or "general") factors, which are collectively referred to as the RNA polymerase II transcriptional machinery, are required for basal transcription (*1*). The activity of the general transcriptional machinery is controlled by sequence-specific DNA binding factors that interact with promoter and enhancer elements (*2*). Despite the progress toward understanding both the general and sequence-specific factors, the mechanisms by which the promoter- and enhancer-binding factors modulate the activity of the transcriptional machinery have yet to be clarified.

The analysis of RNA polymerase II transcription regulation requires characterization of the entire transcription process, which comprises the general and sequence-specific factors as well as the packaging of the template DNA. This system is complex, yet it is possible to unravel particular aspects of the entire process with the eventual goal of assembling the array of information into a coherent scheme. Many problems are encountered, however, in the biochemical analysis of RNA polymerase II transcription, including the failure of in vitro transcription systems to reproduce phenomena observed in vivo (*1, 2*). Moreover, transcription in vitro also tends to be indiscriminate, and it is therefore difficult to observe specific regulation of genes due to a high basal level of RNA synthesis (*1, 2*). These difficulties may reflect specific defects in existing in vitro transcription systems, and resolution of these weaknesses may provide new insight into the mechanism of the transcription process.

In vitro transcription reactions are usually performed with either crude or partially purified nuclear extracts with naked DNA templates (*1*), and an important step toward the improvement of these systems has been the use of chromatin templates. These studies have shown that in vitro reconstitution of chromatin represses basal RNA polymerase II transcription (*3*). In addition, a number of results suggest that basal transcription is also repressed by histone H1 (*4*), a protein that interacts with the linker DNA between nucleosomal cores and with the nucleosome dyad (*5, 6*).

We had previously examined transcriptional activation of the *Drosophila Krüppel* gene by a sequence-specific factor known as the GAGA factor (*7*). The GAGA factor was originally identified as a sequence-specific transcriptional activator that binds to several GA-rich sites in the proximal promoter of the *Drosophila Ultrabithorax* gene (*8*). With the *Krüppel* gene, we found that purified GAGA factor binds to several sites in the promoter region, but activation by the GAGA factor varied depending on the method of preparation of the nuclear extract used for in vitro transcription. Initial characterization of the different nuclear extracts led to the hypothesis that the GAGA factor counteracted basal repression that was mediated by a global transcriptional repressor. Furthermore, this repression did not involve assembly of nucleosomes. We refer to ability of sequence-specific factors to counteract repression of basal transcription as antirepression. Transcriptional antirepression is distinct from true activation, which is acceleration of the inherent transcription process.

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To clarify the mechanism of antirepression, we sought to purify the repressor. We previously observed (7) that, when a crude *Drosophila* nuclear extract is precipitated with 2.26 M ammonium sulfate, the general RNA polymerase II factors were in the pellet, whereas the repressor activity was present in the supernatant. Hence, we began the purification with the supernatant fraction and used the ammonium sulfate–precipitated transcription factors as a source of general transcriptional activity that was depleted of the basal repression activity. To monitor the activity of the DNA binding repressor, we used a commitment assay in which a first template DNA was incubated with the repressor and subsequently a second template was added with transcription factors and ribonucleoside triphosphates (NTP's) to initiate transcription (Fig. 1). If transcription from the first template was selectively inhibited, then the repression was probably caused by a nonspecific DNA binding factor rather than a soluble species such as ribonuclease. We then subjected the basal repressor in the ammonium sulfate supernatant fraction to chromatography on phenyl-Sepharose and S-Sepharose resins (*9*) (Fig. 1). The template committing repressor activity copurified with a 39-kilodalton polypeptide. We confirmed that the 39-kD protein was the repressor by eluting the polypeptide from an SDS gel and renaturing the protein (Fig. 2). After S-Sepharose chromatography, the repressor was more than 95 percent homogeneous. We then examined the generality of repressor function and found that the purified repressor was capable of transcription inhibition from several different template DNA's, including the *Drosophila jockey*, alcohol dehydrogenase (proximal), and *Ultrabithorax* promoters as well as the adenovirus major late and E4 promoters (*10*). Hence, the basal repressor appeared to be a nonspecific DNA binding protein.

Identity of the basal repressor with histone H1. Amino acid sequence analysis of the purified repressor revealed that it was identical to histone H1 (Fig. 3). Although the identification of H1 as a basal transcriptional repressor was consistent with previous findings (*4, 11*), we observed repression with purified H1 bound to naked DNA in the absence of nucleosomes. Although this result might at first appear to be surprising, many aspects of H1 binding to naked DNA resemble its interaction with DNA packaged into chromatin (*12*). These results can be summarized as follows: (i) H1 in chromatin appears to interact primarily with the linker DNA rather than with the core histones; (ii) the interactions between H1 and DNA are similar whether or not the DNA is packaged into nucleosomes; (iii) the spatial relations between adjacent H1 molecules are similar in both H1 bound to naked DNA and H1-containing chromatin; and (iv) the amount of H1 bound to naked DNA that is required for basal repression in our experiments (1 molecule per 30 to 45 bp of DNA) is roughly the same as the physiological amount of H1 bound to accessible linker DNA in chromatin (1 molecule per 35 to 45 bp, excluding the DNA occupied by the core histone octamers). Furthermore, we have also found that transcription repression is not a general property of

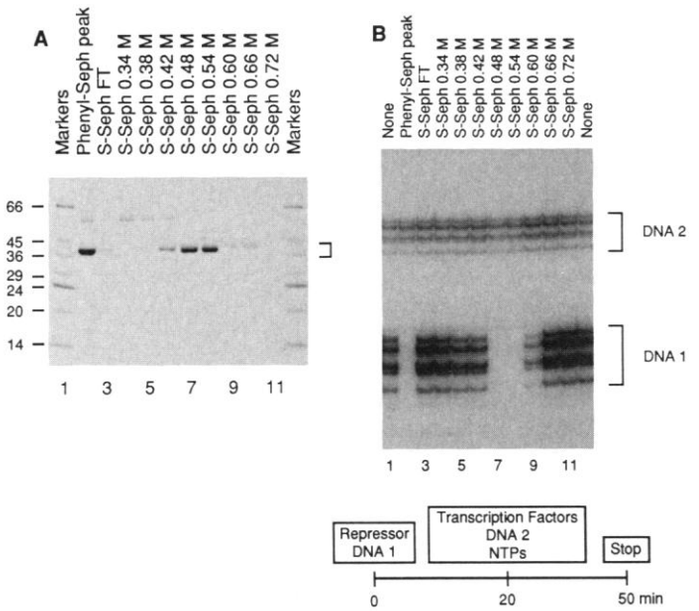

Fig. 1. Purification of the basal transcriptional repressor. The peak fraction from a phenyl-Sepharose column and selected column fractions from an S-Sepharose column are shown. The salt concentrations of the S-Sepharose fractions are indicated. (**A**) Analysis of the repressor by 12 percent polyacrylamide–SDS gel electrophoresis. The protein was visualized with Coomassie blue staining. (**B**) Template commitment assay of DNA binding repressor activity. Standard transcription reactions (*34*) were carried out with 200 ng each of template DNA1 (pKr) and template DNA2 (pKr-mut) as indicated. These templates contain two variants of the *Krüppel* promoter (*35*). The *Krüppel* gene has several RNA start sites clustered over ten nucleotides (7). The reverse transcription products are indicated by brackets.

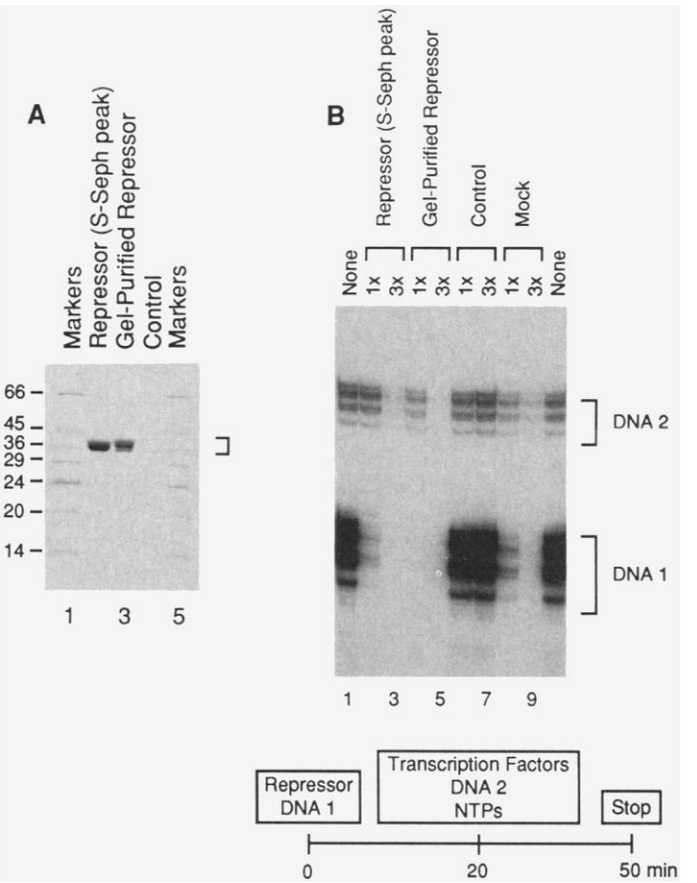

Fig. 2. Identification of the 39-kD polypeptide as the basal repressor. The S-Sepharose–purified repressor was subjected to 10 percent polyacrylamide–SDS gel electrophoresis, and the 39-kD polypeptide was eluted from the gel and renatured (*36*). As a control, a segment of the SDS gel that did not contain the 39-kD polypeptide was subjected to elution and renaturation (see lanes designated "control"). In addition, a sample of repressor was treated in the same way that the 39-kD polypeptide was treated except that it was not subjected to gel electrophoresis (mock). (**A**) Analysis of the polypeptides by 12 percent polyacrylamide–SDS gel electrophoresis. The protein was visualized with Coomassie blue staining, and the sizes of the molecular mass standards are given in kilodaltons. (**B**) Template commitment assay of DNA binding repressor activity. Standard transcription reactions (*34*) were carried out with 100 ng each of template DNA1 (pKr) and template DNA2 (pKr-mut) as indicated. Lanes 3, 5, 7, and 9 contain three times the quantity of repressor as lanes 2, 4, 6, and 8, respectively. The reverse transcription products are indicated by brackets.

nonspecific DNA binding proteins because preparations of high mobility group (HMG) proteins HMG 1/2 and HMG 14/17 did not inhibit in vitro transcription by RNA polymerase II (*13*). It thus appeared that H1 bound to naked DNA would be a reasonable model for transcriptionally repressed chromatin, and we therefore proceeded to characterize the properties of sequence-specific transcription factors with purified H1 as a repressor of basal transcription.

Antirepression of H1-mediated inhibition of RNA polymerase II transcription by sequence-specific factors. We then examined the ability of the GAGA factor to counteract repression mediated by purified H1 (Fig. 4A). In the absence of H1, the GAGA factor mildly repressed transcription when bound to its three downstream sites in the *Drosophila Krüppel* gene (Fig. 4A, lanes 11 and 12). As increasing amounts of H1 were included in the reactions, however, GAGA factor that was first bound to the template prevented transcriptional inhibition in a binding site-dependent manner (Fig. 4A, lanes 19 and 20). Thus, the activation of templates bound with GAGA factor in comparison to unbound templates results from antirepression. In the absence of GAGA factor, there was a progressive decrease in transcription when H1 was added to the reactions (Fig. 4A, lanes 11, 13, 15, 17, 19), whereas in the presence of DNA-bound GAGA factor, H1 had little effect upon the efficiency of transcription (Fig. 4A, lanes 12, 14, 16, 18, 20). Hence, the GAGA factor functions as an antirepressor under conditions of H1-mediated repression of basal transcription.

Since the GAGA factor was an antirepressor, we explored the possibility that other sequence-specific transcription factors also function as antirepressors. In these experiments, we tested transcription factor Sp1 (*2*) and a GAL4-VP16 fusion protein (*14, 15*), both of which have been characterized in vitro and in vivo as strong transcriptional activators. In the absence of H1, the basal level of transcription was relatively high, and consequently, the activation by Sp1 was typically two- to threefold (Fig. 4B, lanes 11 and 12). When H1 was included in the reactions, however, the degree of Sp1-mediated activation increased dramatically to levels that are similar to those observed in vivo (Fig. 4B, lanes 17 and 18). This effect was due to the ability of Sp1 to counteract H1-mediated repression. Experiments with the GAL4-VP16 hybrid protein yielded results similar to those obtained with Sp1 (Fig. 4C). The GAGA factor was studied with a template that contained three downstream sites, whereas the Sp1 and GAL4-VP16 experiments were performed with templates that contained five binding sites upstream of the TATA box. Consequently, antirepression can occur with factors bound either upstream or downstream of the RNA start sites.

To examine the generality of antirepression, we also purified H1 from HeLa cells and calf thymus by using the same procedure that was developed for the isolation of *Drosophila* H1 (*9*). Both the HeLa

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1
S D S A V A T S A S P V A A P P A T V E K K V V Q K K A S G

31
S A G T K A K K A S A T P S H P P T Q Q M V D A S I K N L K

61
E R G G S S L L A I K K Y I T A T Y K C D A Q K L A P F I K

91
K Y L K S A V V N G K L I Q T K G K G A S G S F K L S A S A

121
K K E K D P K A K S K V L S A E K K V Q S K K V A S K K I G

151
V S S K K T A V G A A D K K P K A K K A V A T K K T A E N K

181
K T E K A K A K D A K K T G I I K S K P A A T K A K V T A A

211
K P K A V V A K A S K A K P A V S A K P K K T V K K A S V S

241
A T A K K P K A K T T A A K K
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Fig. 3. The basal repressor is H1. The amino terminus of the protein was blocked. The purified protein was digested with either trypsin or *Staphylococcus aureus* V8 protease, and peptides were purified by high-performance liquid chromatography. These peptides were subjected to automated Edman degradation (Applied Biosystems 473A) and to plasma desorption mass spectrometry (Bio-Ion 20K). The amino acid sequence of *Drosophila* H1, as deduced from the DNA sequence of an H1 gene (*37*), is shown. The thick underlined portions of the amino acid sequence designate regions of the protein that were sequenced by Edman degradation, and the thin underlined segments indicate regions confirmed by mass spectrometry. Single-letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

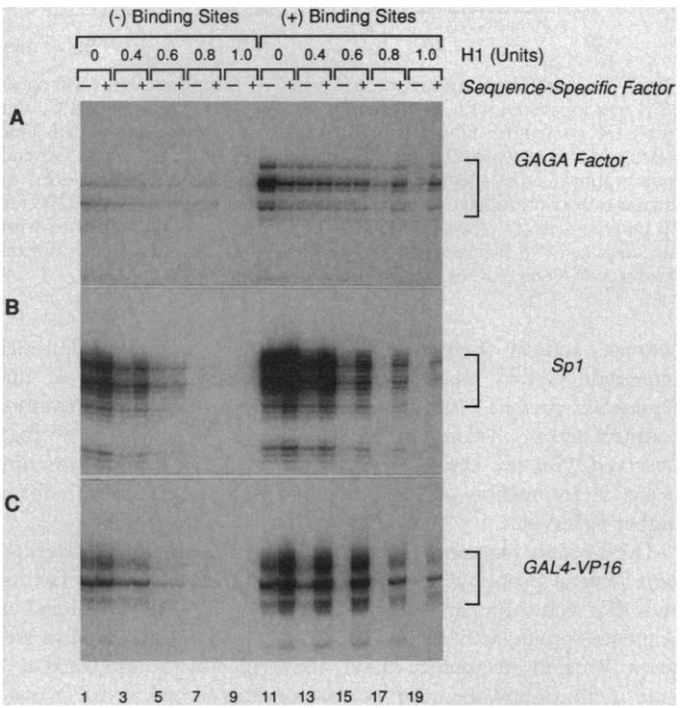

Fig. 4. Transcriptional antirepression by sequence-specific factors. The transcription reactions (*34*) were performed as follows. Template DNA (100 ng) was incubated with the designated sequence-specific factor (or buffer as a control) for 20 minutes at 4°C, and a mixture of general transcription factors and the indicated amount of H1 was added. Ribonucleoside triphosphates were then added to initiate transcription, and the reaction mixture was incubated at 21°C for 30 minutes. The reverse transcription products of the transcripts are indicated by brackets. (**A**) The GAGA factor. Where indicated, reactions contained 35 ng of affinity-purified GAGA factor from *Drosophila* embryos (*7*), which corresponds to a 2.6 times molar ratio of GAGA factor monomers to binding sites on the pKr5′−26 template. The templates used were pKr−31/+13, which does not contain any GAGA binding sites, and a pKr5′−26, which contains three downstream GAGA binding sites (*35*). (**B**) Transcription factor Sp1. Where indicated, reactions contained 30 ng of affinity-purified Sp1 from HeLa cells (*38*), which corresponds to a 1.3 times molar ratio of Sp1 monomers to binding sites on the pSV-Kr template. The templates used were pKr−31/+13, which does not contain any Sp1 binding sites, and pSV-Kr, which contains five upstream Sp1 binding sites (*35*). (**C**) GAL4-VP16. Where indicated, reactions contained 100 ng of bacterially synthesized GAL4-VP16 protein of approximately 80 percent purity (*14*), which corresponds to an eight times molar ratio of GAL4-VP16 dimers per binding site in pG_5E4. The templates used were pG_0E4, which does not contain any GAL4 binding sites, and pG_5E4, which contains five upstream GAL4 binding sites (*35*).

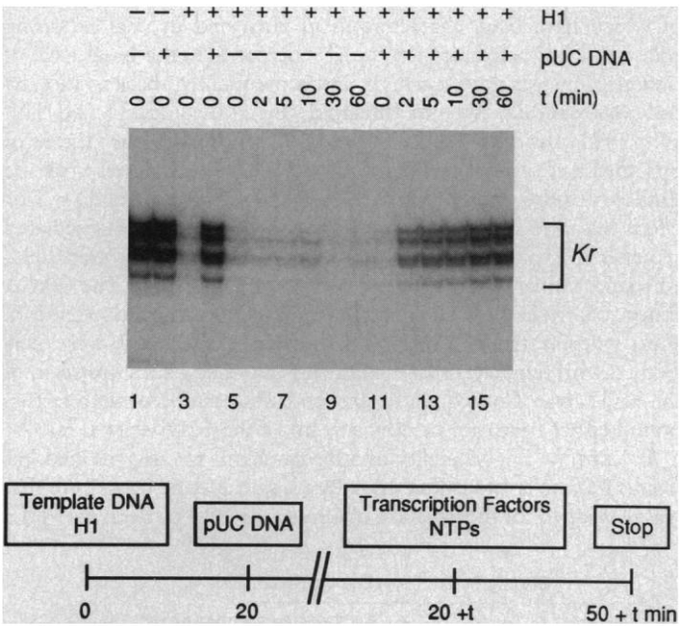

Fig. 5. Reversibility of transcription repression. Template DNA (100 ng of pKr) was incubated with purified H1 (1 unit) for 20 minutes at 4°C, and then pUC competitor DNA (1 μg) or buffer (as a control) was added. This mixture was allowed to incubate at 4°C for the indicated time, *t*. General transcription factors and ribonucleoside triphosphates were then added to initiate transcription, and the reaction mixtures were incubated at 21°C for 30 minutes (*34*). In lane 4, the pUC competitor DNA was combined with the template DNA before addition of the repressor. The reverse transcription products of *Krüppel* RNA are shown.

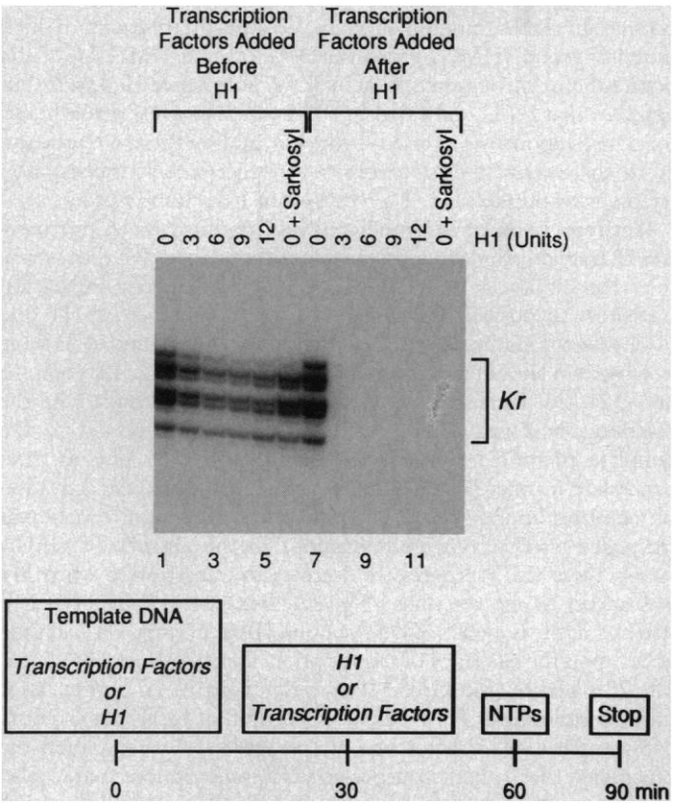

Fig. 6. Preassembled transcription initiation complexes are resistant to the repressor. Transcription reactions (*34*) were carried out with pKr template DNA (300 ng) at 21°C as indicated. In lanes 1 to 6, transcription factors were added to the template DNA before H1 (or Sarkosyl). In lanes 7 to 12, H1 (or Sarkosyl) was added to the template DNA before the transcription factors. Sarkosyl was added to 0.2 percent (w/v) final concentration either 10 seconds after the ribonucleoside triphosphates (lane 6) or before addition of the transcription factors (lane 12). The reverse transcription products of *Krüppel* RNA are shown. Similar results were obtained with lower concentrations of H1 (0.4 to 0.8 U per reaction; see Fig. 7).

(human) and calf thymus H1 preparations showed transcriptional repression activity that was indistinguishable from that of the *Drosophila* protein (*10*). In addition, sequence-specific activators counteracted inhibition by HeLa H1 in a manner identical to that observed with the *Drosophila* H1 (*10*). Hence, in vitro transcriptional antirepression appears to occur generally with H1 from higher eukaryotes.

These results suggest that transcriptional antirepression is likely to be a general property of promoter- and enhancer-binding factors and that activation and antirepression are distinct functions of sequence-specific activators. Whereas all three factors acted as antirepressors, in the absence of H1, the GAGA factor also acted as a mild inhibitor and the other two factors functioned as true activators. The magnitude of transcription activation by the sequence-specific factors (that is, the relative levels of transcription in the presence or absence of the factors) increased by a factor of about 10 when H1 was included in the reactions (Fig. 4). In a typical series of reactions with GAL4-VP16 (as in Fig. 4C), the extent of activation (as determined by scintillation counting of the gel slices containing reverse transcription products of the reactions) was twofold in the absence of H1 and slightly more than 20-fold in the presence of H1 (*10*). Also, Sp1 and GAL4-VP16 do not completely counteract repression by H1 (Fig. 4). This phenomenon may be due either to an excess of the inhibitor or to the lack of additional factors that may be required for complete antirepression. For instance, a stable complex formed between factors found to proximal promoter and enhancer elements may be strongly resistant to transcriptional repression, whereas the factors bound only to the proximal promoter may function only weakly as antirepressors.

To explore the dynamics of the H1-DNA interaction, we examined the reversibility of transcription repression. It is known that incubation of purified H1 with DNA can lead to the formation of various protein-DNA aggregates (*16*), and we therefore examined the possibility that transcriptionally incompetent H1-DNA aggregates might be irreversibly formed under the standard conditions of our transcription experiments. Either pUC plasmid DNA or buffer (as a control) was added to an incubated mixture of H1 and template DNA. Then, after a variable period of time (during which H1 could dissociate from the template DNA and bind to the excess pUC DNA), transcription factors and ribonucleoside triphosphates were added to initiate RNA synthesis. In these experiments, there was a gradual dissociation of H1 from the template DNA in the presence of pUC (Fig. 5, lanes 11 to 16), but not the buffer control (Fig. 5, lanes 5 to 10). As a control, if pUC was added to the template DNA before H1, then transcription occurred at the same level observed if no H1 was added (Fig. 5, compare lanes 1 and 4). In this case, H1 bound nonspecifically to the excess pUC DNA, and the template DNA was accessible to the transcription factors. The reversibility of repression indicates that the interaction of H1 with DNA is dynamic, rather than static. These findings are consistent with previous data indicating that H1 can rapidly exchange between chromatin fragments at physiological salt concentrations (*17*). Yet, although the binding of H1 to DNA is reversible in a simple mixture of purified protein and DNA, H1 is committed to the template in the presence of a nuclear extract. For example, H1 remains committed to template DNA1 for multiple rounds of transcription (Fig. 1B) (*10*). In those experiments, the nuclear extracts may have either stabilized the interaction of H1 with DNA1 or prevented the binding of H1 to DNA2.

Resistance of preassembled transcription complexes to H1-mediated repression. To characterize the interaction of H1 with the general RNA polymerase II transcriptional machinery in the absence of sequence-specific factors, we first examined the resistance of preformed initiation complexes to repression. In the course of a transcription reaction, the template DNA and general transcription factors slowly assemble into an initiation complex. This process follows apparent first-order kinetics with a half-time of assembly of 3.2 ± 1.3 minutes. Then, in a step requiring ribonucleoside triphosphates, initiation and elongation of transcription rapidly occur (*18*). Since transcription initiation complexes are stable for at least 30 minutes (*18*), it was possible that they would be resistant to H1. Preassembled initiation complexes were resistant to repression (Fig. 6, lanes 2 to 5), and initiation complexes could not be assembled on template DNA that had been incubated with H1 (Fig. 6, lanes 8 to 11). Furthermore, when the preformed initiation complexes were treated with H1, transcription was limited to a single round, which was determined by comparison with reactions performed in the presence of Sarkosyl under conditions that prevented reinitiation of transcription (*18, 19*) (Fig. 6, compare lanes 2 and 6 with lane 1). These data indicate that transcription initiation complexes are resistant to H1, but after the first round of transcription, the initiation complex disassembles and becomes sensitive to repression. In addition, these findings are consistent with the previous observation that there is complete assembly and disassembly of the initiation complex during every round of transcription (*18*). Thus, in the presence of H1 and absence of sequence-specific factors, preassembled initiation complexes can undergo a single round of transcription, but cannot sustain multiple rounds of transcription.

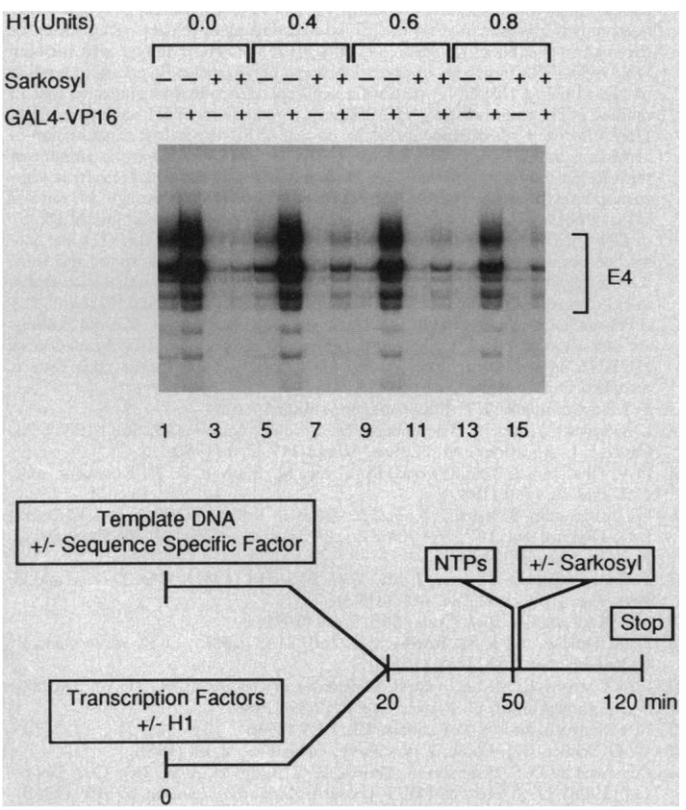

Fig. 7. Antirepression by GAL4-VP16 occurs for several rounds of transcription. Transcription reactions (*34*) were carried out as indicated. Template DNA (pG_5E4; 100 ng) (*35*) was incubated in the presence or absence of bacterially synthesized GAL4-VP16 (100 ng) (*15*) for 20 minutes at 4°C, and a mixture of transcription factors and H1 (as indicated) was added. After incubation at 21°C for 30 minutes, ribonucleoside triphosphates were added to initiate transcription, which was allowed to proceed for 70 minutes. In reactions containing Sarkosyl, the detergent was added to 0.2 percent (w/v) final concentration 10 seconds after addition of the ribonucleoside triphosphates. The reverse transcription products of adenovirus E4 RNA are shown.

To investigate the possible mechanisms by which a transcriptionally active state can be maintained for multiple rounds of RNA synthesis, we then examined reinitiation of transcription from templates in which repression was counteracted with the sequence-specific factor, GAL4-VP16. We carried out a set of reactions with or without the GAL4-VP16 activator in the presence or absence of Sarkosyl (Fig. 7). This set of reactions was repeated, but with increasing amounts of H1. By comparison of identical reactions in the presence or absence of Sarkosyl, we determined the number of rounds of transcription that occurred in each reaction condition. In the absence of H1, multiple rounds of transcription occur with or without the GAL4-VP16 activator (Fig. 7, lanes 1 to 4). As H1 was added, several rounds of transcription were still observed with the GAL4-VP16 activator (Fig. 7, compare lane 14 with lane 16), whereas in the absence of activator, transcription was limited to one round (Fig. 7, lane 13 compared to lane 15). These results indicate that sequence-specific activators can maintain an activated state of the promoter through multiple rounds of transcription. Thus, these data suggest that antirepression by sequence-specific factors may be an important mechanism for the establishment and maintenance of an activated state of a gene.

The role of nucleosomes and H1 in repression of transcription. In previous studies on sequence-specific DNA binding factors, antirepression of H1 may have occurred in situations where activation was observed. For example, we have found that the well-characterized factors, Sp1 and GAL4-VP16, are both activators and antirepressors, whereas the GAGA factor appeared to function as an antirepressor and as a mild transcriptional inhibitor. It is important to note that in vitro transcription extracts prepared by salt extraction of HeLa nuclei by procedures related to that described by Dignam *et al.* (*20*) contain high levels of H1. The ionic strength of nuclear extraction used in the preparation of such extracts is greater than that at which H1 dissociates from chromatin (*21*), and we have purified roughly 1 mg of H1 from a standard nuclear extract (*20*) from a 36-liter culture of HeLa cells (*9, 10*). Similarly, nuclear extracts from *Drosophila* embryos (*22, 23*) also contain moderate levels of H1. Consequently, reactions that were carried out with such extracts included significant quantities of H1, and therefore, sequence-specific factors that had been previously observed to activate transcription in vitro may have been functioning at least in part as antirepressors.

A vast body of data indicates that both nucleosomal cores and histone H1 are involved in repression of transcription (*4, 24*). Since at least 146 bp of DNA per nucleosome repeat is in contact with the core histone octamer, it is reasonable to expect that nucleosomes repress transcription in vivo. Consistent with this notion, the conversion of genes from a repressed state to a transcriptionally active or competent state is often accompanied by changes in specific positioning of nucleosomes in vivo (*25*). In addition, alterations in the synthesis of core histones in vivo in yeast have been found to cause increased expression of a subset of genes (*26*). Moreover, nucleosomes reconstituted in vitro have been observed to repress transcription by RNA polymerase II (*3*).

H1 is present at roughly one molecule per nucleosome repeat (*27*), and various experiments have led to the proposal that H1 is also involved in transcription repression (*4*). In studies on RNA polymerase III transcription in *Xenopus*, Schlissel and Brown (*28*) and Wolffe (*28*) demonstrated that the normally inactive oocyte 5*S* RNA gene can be transcribed in H1-depleted somatic cell chroma-

tin. They found that addition of H1 to the H1-depleted chromatin repressed transcription of the oocyte 5*S* RNA genes more effectively than that of the somatic 5*S* RNA genes, which contained stable transcription complexes. Shimamura *et al.* (*11*) have also directly demonstrated H1-mediated repression of RNA polymerase III transcription by analysis of in vitro–reconstituted chromatin to which varying amounts of purified H1 were added. Kamakaka and Thomas (*29*) have shown by protein-DNA cross-linking experiments that H1 and H5 were depleted from active and potentially active (competent) class II genes, and Tazi and Bird (*30*) have found that, in nonmethylated CpG-rich islands, which appear to represent active chromatin, more than 90 percent of H1 is depleted and that hyperacetylated histones H3 and H4 as well as nucleosome-free areas are present. Nacheva *et al.* (*31*) have demonstrated by protein-DNA cross-linking studies with *Drosophila* nuclei that both core histones and H1 are depleted or reconfigured at the promoter region, but not the coding region, of the *hsp70* gene. Weintraub (*32*) has also observed that H1-dependent higher order structures have a looser, more open conformation in active class II genes. In apparent contrast to the above findings, Ericsson *et al.* (*33*) detected H1 on actively transcribing Balbiani ring genes, but in that study, the presence of H1 on promoter regions, in which H1 would be expected to be depleted, was not addressed. Thus, the available data strongly suggest that either H1 is depleted or H1 binding is altered at promoter regions of active genes and are consistent with the possibility that sequence-specific factors counteract transcriptional repression by H1 in vivo.

We have used purified H1 bound to DNA as a model for the repressed state of chromatin, and it appears that sequence-specific transcription factors are able to counteract H1-mediated repression of transcription by RNA polymerase II. At this stage, it is worthwhile to address potential weaknesses in the interpretation of the experiments described as well as to consider further studies that might lead to a better understanding of antirepression. As mentioned previously, it is possible that antirepression may occur by a mechanism in which sequence-specific factors prevent the formation of insoluble H1-DNA aggregates that are refractory to transcription. Although we cannot unequivocally disprove this hypothesis, several findings indicate that antirepression is probably not due to inhibition of irreversible aggregation of H1-DNA complexes. First, antirepression was observed with three different sequence-specific factors and was dependent on binding of the factors to the promoter region (Fig. 4). Thus, repression is not inhibited by a trivial interaction between H1-DNA complexes and transcription factors. Second, H1-mediated repression can be counteracted by addition of excess nonspecific pUC DNA (Fig. 5), which indicates that H1-mediated repression does not involve formation of irreversibly precipitated aggregates. Third, if GAL4-VP16 is added to H1-DNA complexes that have already formed, antirepression that is indistinguishable from the data presented in Fig. 4C is observed (*10*). Hence, the promoter regions of the repressed H1-DNA complexes are accessible to binding by GAL4-VP16. It is important to examine H1-mediated repression with templates that have been reconstituted with nucleosomes. Such experiments would make it possible to analyze the function of H1 in the natural context of chromatin, and the results should provide additional insight into the antirepression phenomenon.

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9. Purification of histone H1. One unit of repression activity was defined to be the amount of protein that is required to repress >95 percent of the transcription from 100 ng of pKr template DNA (*35*). Purified *Drosophila* H1 has a specific activity of 7140 U/mg. To prevent loss of H1 as a result of nonspecific adsorption to plastic and glass, it is important to include 0.01 percent (v/v) Nonidet P-40 in buffers in which H1 is diluted. (i) For *Drosophila* H1, nuclear extracts were prepared from *Drosophila* embryos and precipitated with 2.26 M ammonium sulfate (*23*). The ammonium sulfate supernatant was then subjected to chromatography on a phenyl-Sepharose column, and protein was eluted with a 2.1 to 0.1 M ammonium sulfate gradient in HEMG buffer (25 mM Hepes, K^+, pH 7.6, containing 12.5 mM $MgCl_2$, 0.1 mM EDTA, 10 percent (v/v) glycerol, and 1 mM dithiothreitol). The peak fractions were dialyzed into HEG buffer (HEG is identical to HEMG except that it does not contain $MgCl_2$) containing 0.1 M KCl and then applied to a S-Sepharose column. Protein was eluted with a 0.1 to 1.0 M KCl gradient in HEG buffer. (ii) For HeLa (human) H1, nuclear extracts were prepared according to the procedure of Dignam *et al.* (*20*), except that 0.42 M KCl was used instead of 0.42 M NaCl in the nuclei extraction buffer. The extract was precipitated with 2.26 M ammonium sulfate, and the supernatant was subjected to chromatography as described for the *Drosophila* H1.
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12. The interaction of H1 with chromatin is believed to be primarily with the linker DNA and the nucleosome dyad where it may simultaneously contact two or three strands of DNA (*5, 6*).. In chromatin, H5 (an analogue of H1) molecules in adjacent nucleosomes contact one another in a head-to-tail fashion (*39*), and an H1 homopolymer is obtained when H1 is cross-linked on either naked DNA or chromatin (*16*). These results suggest that there are similar interactions between DNA-bound H1 molecules in naked DNA and in chromatin. In addition, binding of H1 to naked DNA (*40*) displays a salt dependence that is similar to that of binding of H1 to chromatin (*21*). Moreover, cooperativity of H1 binding to naked DNA (*16*) has a salt dependence similar to that of H1-dependent condensation of chromatin into higher order structures (*5*). Our data show that the amount of purified H1 required to repress >95 percent of the transcriptional activity in vitro corresponds to one molecule of H1 per 30 to 45 bp DNA. Although this ratio of H1 to DNA is higher than the one molecule per nucleosome repeat [about 185 bp of DNA in *Drosophila* embryos (*41*)] that is observed in chromatin (*27*), the core histone octamer normally occupies 146 bp of DNA per nucleosome, and thus, there is approximately 39 bp of linker DNA per nucleosome repeat that is accessible to H1 in chromatin. One potential problem with the use of purified H1 with DNA is H1-mediated aggregation of DNA (*16*). We believe, however, that our findings are not consistent with transcription repression that is caused by formation of H1-DNA aggregates that are inaccessible to transcription factors (this issue is discussed further in the text).
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34. Transcription reactions and primer extension analysis of the RNA were carried out as described (*7, 18*). Transcription reactions were performed with a partially purified preparation of general RNA polymerase II transcription factors that were depleted of H1 (7). Briefly, a *Drosophila* nuclear extract was prepared (*22*) by extraction with 0.45 M KCl. The general transcription factors were precipitated with 2 M ammonium sulfate, and the factors were dialyzed into HEMG buffer (*9*) containing 100 mM KCl. Alternatively, a soluble nuclear fraction can be prepared that is active for RNA polymerase II transcription, but is deficient in H1 (*42*). A simple indicator of the presence of H1 in an extract is activation of transcription by addition of a nonspecific DNA, such as pUC (the H1 binds to the competitor DNA and thus enables the general transcription factors to interact with the of template DNA). H1 is likely to be present in an extract if the addition of 100 to 200 ng of pUC DNA stimulates transcription in a standard reaction with 25 ng of template DNA. The preparations of general transcription factors that we used in this study did not contain detectable H1-mediated repression activity (Fig. 4, lane 4).
35. The plasmid DNA's were as follows. The plasmid pKr contains the promoter region (−861 to +426 relative to the major upstream RNA start site) of the *Drosophila Krüppel* gene, and pKr-mut is identical to pKr except that it has 29 bp inserted between the positions corresponding to +18 and +19 of the *Krüppel* transcript (*18*). The *Krüppel* gene has several RNA start sites clustered over ten nucleotides (7). pKr−31/+13 contains the *Krüppel* minimal promoter from −31 to +13 relative to the major upstream RNA start site and does not possess any GAGA or Sp1 binding sites. pKr5′−26 contains a *Krüppel* promoter fragment from −26 to +426 relative to the start site and has three downstream GAGA binding sites (7). pSV-Kr contains the three 21-bp repeat elements of the SV40 early promoter fused upstream of the TATA box of the *Krüppel* minimal promoter in pKr−31/+13 (*10*). pSV-Kr contains six potential Sp1 binding sites to which only five Sp1 protomers can be bound simultaneously (*43*). pG_0E4 (equivalent to pE4Δ−38) and pG_5E4 both contain the adenovirus E4 promoter from −38 to +250 relative to the major RNA start site (*44*). pG_0E4 does not have any GAL4 binding sites, whereas pG_5E4 possesses five GAL4 binding sites upstream of the E4 TATA box.
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45. We thank R. Kamakaka, P. Laybourn, and D. Clark for invaluable advice on H1 and chromatin; G. Binns for sequencing of H1; and R. Tjian, J. Newport, M. Dasso, M. DePamphilis, P. Geiduschek, C. Murre, K. Jones, P. Laybourn, R. Kamakaka, and S. Yoshinaga for improving the quality of this manuscript. Supported in part by NIH grants (J.T.K. and D.R.M.), a National Down Syndrome Society scholarship (D.R.M.), an NSF predoctoral fellowship (G.E.C.), and the Lucille P. Markey Charitable Trust (J.K.M.).

24 October 1990; accepted 4 January 1991

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