The C-terminal domain of mammalian RNA polymerase subunit IIa consists of 52-tandem repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser. This C-terminal domain is essentially unmodified in RNA polymerase IIA and extensively phosphorylated in RNA polymerase IIO. A monoclonal antibody directed against the C-terminal domain was shown by kinetic enzyme-linked immunosorbent assay to have a 10-fold higher reactivity with RNA polymerase IIA than with RNA polymerase IIO. The ability of increasing concentrations of this monoclonal antibody to inhibit the initiation and elongation phase of transcription was determined. Although both phases of the transcription reaction were inhibited, a 10-fold higher concentration of antibody was required to inhibit elongation than was required to inhibit initiation. These results support the hypothesis that RNA polymerase IIA, containing an unphosphorylated C-terminal domain, is involved in the formation of an initiated complex, whereas elongation is catalyzed by RNA polymerase IIO, containing a phosphorylated C-terminal domain. Further indication that the C-terminal domain undergoes a structural change during the transcription cycle results from the observation that this domain is 3-fold more sensitive to clostripain cleavage in the elongation enzyme than in the free enzyme.

RNA polymerase II is a multiaubunit enzyme composed of two large subunits, with molecular weights in excess of 100,000, and a complex array of small subunits (for review see Sentenac, 1985). Analysis of the largest subunit gene from a variety of eukaryotes has led to the discovery of an unusual C-terminal domain consisting of tandem repeats of the consensus sequence Tyr-Ser-Pro-Thr-Ser-Pro-Ser (Corden et al., 1985 and Allison et al., 1985). This sequence is repeated 52 times at the C-terminus of the largest subunit of mammalian RNA polymerase II (Corden et al., 1985; Ahearn et al., 1987; Allison et al., 1988), 42-44 times in Drosophila (Allison et al., 1988; Zehring et al., 1988), and 26 times in yeast (Allison et al., 1985). Analysis of mutations within this domain establishes that a minimal number of repeats are essential for in vivo function (Nonet et al., 1987; Allison et al., 1988; Zehring et al., 1988).

Mammalian cells contain two forms of RNA polymerase II, designated IIO and IIA, that differ in the extent of phosphorylation within the C-terminal domain of the largest subunit (Kim and Dahmus, 1986; Cadena and Dahmus, 1987). RNA polymerase IIO is heavily phosphorylated relative to RNA polymerase IIA (Dahmus, 1981; Cadena and Dahmus, 1987). Photoaffinity labeling of RNA polymerase II in cell-free transcription systems, utilizing the major late promoter of adenovirus-2 (Bartholomew et al., 1986), and in isolated HeLa nuclei (Cadena and Dahmus, 1987) indicates that elongation is catalyzed primarily by RNA polymerase IIO.

The conservation of the C-terminal domain of subunit IIa from yeast to mammals and the lethal effect of mutations contained in the exon encoding this domain provide strong evidence that the C-terminal domain is required for the transcription of at least some essential cellular genes. The mechanism by which the C-terminal domain functions in transcription is, however, unknown. One possibility is that the C-terminal domain interacts with another component of the transcription apparatus, via protein-protein interactions, to help direct and orient RNA polymerase II to the start site of transcription (Corden et al., 1985; Allison et al., 1988; Sigler, 1988). Such an interaction could be mediated, at least in part, by phosphorylation of the C-terminal domain (Cadena and Dahmus, 1987). In these studies, a monoclonal antibody directed against the consensus repeat of the C-terminal domain has been used in an effort to define at what step in the transcription reaction the C-terminal domain is involved and as a probe to detect structural changes during transcription. Results suggest that the extent of phosphorylation of the C-terminal domain changes during the transcription cycle and that extensive phosphorylation of the C-terminal domain may be involved in the transition of enzyme from the initiation to the elongation complex.

**EXPERIMENTAL PROCEDURES**

**Materials**

High performance liquid chromatography-purified nucleotides were purchased from ICN. Radiolabeled nucleotides were obtained from Amersham. Sarkosyl (N-lauroylsarcosine, sodium salt), heparin, and clostripain were purchased from Sigma.

A stock clostripain solution of 1 mg/ml was prepared by dissolving lyophilized enzyme in 50 mM Pipes, pH 6.8, 1 mM CaCl₂, 5 mM 2-mercaptoethanol and incubating on ice for 2 h. Mouse myeloma IgM was purchased from Sigma.

The abbreviations used are: Pipes, piperazine-N,N-bis(2-ethanesulfonic acid); EGTA, ethyleneglycol-bis(2-aminoethyl ether)-N,N,N’,N’-tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; BSA, bovine serum albumin.

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was obtained from Litton Bionetics, Inc. Synthetic peptide containing three copies of the consensus repeat, Tyr-Ser-Pro-Thr-Ser-Pro-Ser, coupled to BSA was a generous gift from J. Corden (The Johns Hopkins University).

**Methods**

**Preparation of Monoclonal Antibody**—Monoclonal antibody G7A5 directed against calf thymus RNA polymerase II was prepared as described by Christmann and Dahmus (1981). Monoclonal antibody E3E9 directed against the M talented subunit of calf thymus RNA polymerase II was prepared by in vitro immunization as described by Dahmus et al. (1988). The polymerase II IgM (G7A5 and E3E9) and the stimulatory factor (E100) were purified by DEAE-cellulose gradient centrifugation and dialyzed against 50 mM Tris-HCl, pH 7.9, 5 mM MgCl₂, 0.5 mM dithiothreitol, and 20% (v/v) glycerol (buffer A) containing 100 mM KC{l} as described by Dahmus and Kedinger (1983). IgM concentration was determined by A{sub}280, assuming an extinction coefficient of 13.

**Determination of Relative Affinity**—Affinity for RNA Polymerases IIO, IIA, and IIB—RNA polymerases IIO, IIA, and IIB were purified from calf thymus as described by Kim and Dahmus (1988). Aliquots (100 {mu}l) containing 1, 2, 3, 4, 5, 10, 20, 30, and 50 ng of purified species were coated onto round microtiter plates (Nunc) by incubation at 37 °C for 1 h. The relative affinity of monoclonal antibodies G7A5 and E3E9 for RNA polymerases IIO, IIA, and IIB was determined by kinetic ELISA (Tsang et al., 1983; Dahmus et al., 1988).

**Preparation of Transcription Extract**—S100 extract was prepared from HeLa cells as described by Wray et al. (1979) and modified by Dahmus and Kedinger (1983). The S100 extract (15 {mu}l) was applied to a 15 ml heparin-Sepharose column equilibrated with buffer A containing 100 mM KC{l} (Davison et al., 1983). The column was washed with 2 column volumes of the same buffer, followed by 1 column volume of buffer A containing 0.24 mM KC{l}. RNA polymerase II and transcription factors were eluted with 2 column volumes of buffer A containing 0.6 mM KC{l}. The flow-through fractions contain a stimulatory factor that was further purified by DEAE-cellulose (DE22) chromatography as described by Egly et al. (1984). Peak fractions were identified by promoter-dependent assay and stored in liquid nitrogen. A mixture of 5 {mu}l of the 0.6 mM KC{l} heparin-Sepharose peak fraction and 1 {mu}l of DE22-purified flow-through peak fraction was used in subsequent transcription reactions.

**Preparation of DNA Templates**—The adenovirus-2 7401 DNA fragment containing the major late promoter (positions -260 to +405) (designated template 1) was prepared by the method of Wray et al. (1979) and modified by Dahmus and Kedinger (1983). This DNA fragment was digested with HindII to obtain a fragment from positions -260 to +405 (designated template 2).

**Transcription Reactions**—Transcription reactions were carried out in three successive steps. Reaction schemes are shown diagrammatically in Fig. 1. The first two steps involved incubation with antibody and the formation of an initiated complex. Initiation assays refer to reactions in which monoclonal antibody was incubated with extract prior to the formation of an initiated complex, whereas elongation assays refer to reactions in which antibody was added after the formation of an initiated complex. The third step was elongation and was initiated by the addition of remaining nucleotides.

**For initiation assays, monoclonal antibody (see figure legend for concentrations) was preincubated with 6 {mu}l of transcription extract in a 12-{mu}l reaction containing 50 mM Tris-HCl, pH 7.9, 5 mM MgCl₂, 100 mM KC{l}, 0.6 mM dithiothreitol, and 20% (v/v) glycerol at 30 °C for 60 min. A 6 {mu}l aliquot containing 25 ng of DNA template 1 (560-mer duplex), 2.5 mM ATP, and 50 {mu}M [α-32P]CTP (5.6 Ci/mmol) was added, and the incubation continued for an additional 60 min at 30 °C. At this point, a third aliquot (20 {mu}l) containing 0.2% Sarkosyl, 25 ng of DNA template 2 (405-nucleotide runoff), 6 {mu}l of transcription extract, 35.3 mM Tris-HCl, pH 7.9, 10% (v/v) glycerol, 67 mM KC{l}, 3.3 mM MgCl₂, 0.4 mM dithiothreitol, and 10{mu}M [α-32P]CTP (2 Ci/mmol), 0.6 {mu}M concentration each of ATP, GTP, and UTP, 0.08% Sarkosyl, 12 {mu}l of transcription extract, and variable amounts of monoclonal antibody as indicated in the figure legend.

For elongation assays, transcription extract (6 {mu}l) was preincubated with 25 ng of DNA template 1 in a 12-{mu}l reaction containing 25 mM Tris-HCl, pH 7.9, 12.9 mM MgCl₂, 50 mM KC{l}, 0.25 mM dithiothreitol, 0.05% glycerol, 1.25 mM ATP, and 25 {mu}M [α-32P]CTP for 60 min at 30 °C. A 6 {mu}l aliquot of monoclonal antibody (see figure legend for concentrations) was then added, and the reaction was incubated for 60 min at 30 °C. The reaction was then stopped by the addition of 1.5 {mu}l of 200 mM EDTA, and the concentration of components was identical in both the initiation and elongation reactions. A third aliquot (20 {mu}l) and fourth aliquot (12 {mu}l) identical in composition to those described above in the initiation assay were added, and the incubation continued for 60 min at 30 °C.

**Results**

**Monoclonal Antibody G7A5 Reacts with the Consensus Repeat of the C-terminal Domain**—Monoclonal antibody G7A5 was produced using calf thymus RNA polymerase II as immunogen and shown to react with subunits IIO and IIB, but not IIB (Christmann and Dahmus, 1981; Cadena and Dahmus, 1987). Subunits IIO and IIB are products of the same gene and appear to differ only by the absence of the C-terminal domain from subunit IIB (Corden et al., 1985; Kim and Dahmus, 1988). This result implies the epitope recognized by this monoclonal antibody resides in the C-terminal domain. This was confirmed by determining the reactivity of monoclonal antibody G7A5 with a synthetic peptide containing three copies of the consensus repeat, Tyr-Ser-Pro-Thr-Ser-Pro-Ser. This peptide was coupled to BSA, and immunoreactivity was determined by ELISA and protein blotting. The relative affinity as determined by kinetic ELISA (Tsang et al., 1983; Dahmus et al., 1988) for the synthetic peptide was one-half that determined for RNA polymerase IIA (data not shown). This monoclonal antibody also reacted with the BSA-coupled synthetic peptide when transferred to nitrocellulose, but did not react with control BSA (data not shown).
Relative Affinity of Monoclonal Antibody G7A5 for RNA Polymerases II0, IIa, and IIB—RNA polymerases II0, IIa, and IIB were purified from calf thymus, and the relative reactivity of monoclonal antibody G7A5 with each subspecies was determined by kinetic ELISA as described under "Experimental Procedures." The concentration of RNA polymerase subtypes was determined by enzymatic assay, assuming a specific activity of 400 units/mg (Kim and Dahmus, 1988). To confirm that wells were coated with equimolar amounts of each subspecies, equivalent units of activity were subjected to SDS-PAGE and stained with Coomassie Blue, and the densitometer scans of RNA polymerases II0, IIa, and IIB were compared. To verify that the retention of RNA polymerases II0, IIa, and IIB was comparable, identically coated wells were also reacted with monoclonal antibody E3E9 directed against the M0 = 34,000 subunit. The results summarized in Table I show that the relative affinity of G7A5 for RNA polymerases II0 is less than one-tenth that of RNA polymerase IIa. The fact that phosphorylation of the C-terminal domain reduces antibody by greater than 10-fold suggests that either most of the repeats are phosphorylated or that the phosphorylation of a limited number of repeats results in a major conformational change. Removal of the C-terminal domain (RNA polymerase IIB) reduces the relative affinity of G7A5 for RNA polymerase II an additional 10-fold.

The relative reactivity of monoclonal antibody G7A5 for RNA polymerases II0, IIa, and IIB was also confirmed by immunoblotting (data not shown). Equimolar amounts of each subspecies were electrophoresed on SDS-polyacrylamide gels, transferred, and reacted with monoclonal antibody G7A5 or E3E9 as previously described (Dahmus et al., 1988). Under these conditions, G7A5 reacts strongly with subunit IIa, but not to an appreciable extent with either subunit II0 or IIB. Monoclonal antibody E3E9 reacts comparably with the M0 = 34,000 subunit of RNA polymerases II0, IIa, and IIB.

Effect of Monoclonal Antibody on the Initiation and Elongation Phase of Transcription—One approach to defining the role of the C-terminal domain in transcription is to identify the step(s) in transcription in which it is involved. Since monoclonal antibody G7A5 is known to inhibit promoter-dependent transcription (Dahmus and Kedinger, 1983) and to react with the consensus repeat, this antibody provides a useful probe in the analysis of C-terminal domain function. In order to measure the effect of monoclonal antibody G7A5 on initiation, antibody was added in the reaction sequence prior to the formation of an initiated complex, and the effect on transcription was ascertained. The effect of monoclonal antibody on elongation was determined by the addition of antibody after the formation of an initiated complex. "Initiated complex" refers to transcription complexes in which the first phosphodiester bond has been formed. For the adenovirus-2 major late promoter, this requires ATP and CTP. Sarkosyl was added to limit initiation to a single round (Hawley and Roeder, 1987). The second DNA template, truncated to give a runoff transcript of 405, serves as a control to ensure that Sarkosyl effectively inhibited any new initiation. Reaction sequences are shown schematically in Fig. 1.

In order to determine the effect of this monoclonal antibody on the formation of an initiated complex, transcription extract containing RNA polymerase II was preincubated with a constant amount of monoclonal antibody containing increasing proportions of polymerase specific antibody. Formation of an initiated complex occurred during the second phase of the reaction following the addition of ATP, CTP, and DNA containing the major late promoter of adenovirus-2, cut to give a runoff transcript of 560 nucleotides. Following the formation of an initiated complex, Sarkosyl was added along with GTP, UTP, and a second DNA template containing the same promoter but truncated to give a runoff transcript of 405 nucleotides. The amount of transcript produced at the end of the final 60 min incubation was determined as described under "Experimental Procedures." Fig. 2, lane 2, shows that in the absence of Sarkosyl, both DNA templates were transcribed. The fact that the addition of Sarkosyl, after preincubation with template 1, but before the addition of template 2, abolished the formation of the 405-nucleotide transcript but not the 560-nucleotide transcript indicates that this level of Sarkosyl effectively inhibits initiation (Fig. 2, lane 3). In this system, we found that 0.08% Sarkosyl completely inhibited new initiation and had only a slight effect on transcription by previously initiated complexes (results not shown).

Reactions in lanes 4–7 contained a constant amount of monoclonal antibody with varying proportions of control and RNA polymerase II antibody. The addition of non-polymerase II antibody had no effect on the level of transcription (compare lanes 3 and 4). In contrast, the addition of RNA polymerase II antibody showed a concentration-dependent inhibition of transcription when added prior to initiation (lanes 5–7). Quantitation of several such experiments shows that a concentration of about 300 µg/ml is required for 50% inhibition of the initiation phase of the reaction (Fig. 3). This corresponds closely to the sensitivity found previously (Dahmus and Kedinger, 1983) which indicates that the presence of Sarkosyl does not have an appreciable effect on antibody binding.

In a similar series of reactions, varying concentrations of monoclonal antibody were added after the formation of an initiated complex, and the effect on transcription was ascertained (Fig. 2, lanes 6–12). Similar to results presented above, the absence of a 405-nucleotide transcript confirms that transcription was limited to a single round. Significant inhibition of transcription was observed only at the highest concentra-

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**Table I**

Relative affinity of monoclonal antibodies for RNA polymerases II0, IIa, and IIB

| Monoclonal Ab (epitope) | Ligand (RNA polymerase) | Relative affinity |
|-------------------------|------------------------|------------------|
| G7A5 (Tyr-Ser-Pro-Thr-Ser-Pro-Ser) | II0 | 0.07 |
|                         | IIa | 1.00 |
|                         | IIB | 2.17 |
| E3E9 (M0 = 34,000 subunit) | II0 | 1.03 |
|                         | IIa | 1.00 |
|                         | IIB | 0.01 |

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**Fig. 1.** Reaction sequence for determining the effect of monoclonal antibody on initiation and elongation. DNA (560) and DNA (405) refer to DNA templates truncated to give runoff transcripts of 560 (template 1) and 405 (template 2) nucleotides, respectively. Factors and RNA polymerase are both contained in the HeLa cell transcription extract prepared as described under "Experimental Procedures."
Fig. 2. Effect of monoclonal antibody on initiation and elongation. Reaction conditions are as specified under “Experimental Procedures” and schematically represented in Fig. 1. Lane 2 is a control reaction in which Sarkosyl was not added. Lanes 3 and 8 contain Sarkosyl, but no antibody. Lanes 4–7 and 9–12 all contained 1270 μg/ml antibody made up of varying proportions of non-polymerase and RNA polymerase II monoclonal antibody G7A5. Lanes 4 and 9 contain only non-polymerase II antibody. Lanes 5 and 10, 6 and 11, and 7 and 12 contain 127 μg/ml, 423 μg/ml, and 1270 μg/ml RNA polymerase II monoclonal antibody, respectively. In reactions contained in lanes 4–7, antibody was added prior to the formation of an initiated complex (initiation assay). In reactions contained in lanes 9–12, antibody was added after the formation of an initiated complex (elongation assay). Lanes 1 and 13 contain MspI cut and 32P-end-labeled pBR322 DNA. The length in nucleotides of the four largest DNA fragments is indicated in the right margin. The position of runoff transcripts from DNA template 1 and 2 is indicated in the left margin.

Fig. 3. Quantitation of the effect of monoclonal antibody on initiation and elongation. The amount of the 560-nucleotide transcript was quantitated as described under “Experimental Procedures.” The percent activity remaining is equal to the amount produced in the presence of control antibody only. The percent activity remaining for initiation phase (■) and elongation phase (■) assays was plotted as a function of the log of RNA polymerase II monoclonal antibody concentration.

Fig. 4. Schematic representation of the procedure for determining relative sensitivity of the C-terminal domain to clostripain cleavage in the free and elongating enzyme. The conditions for labeling free and elongating enzyme, the purification of the photoaffinity-labeled transcription complex, and the clostripain digestion were as described under “Experimental Procedures.”

The percent activity remaining is equal to the amount produced in the presence of control antibody only. The percent activity remaining for initiation phase (■) and elongation phase (■) assays was plotted as a function of the log of RNA polymerase II monoclonal antibody concentration.

Free Enzyme

Elongating Enzyme

Fig. 3. Quantitation of the effect of monoclonal antibody on initiation and elongation. The amount of the 560-nucleotide transcript was quantitated as described under “Experimental Procedures.” The percent activity remaining is equal to the amount produced in the presence of control antibody only. The percent activity remaining for initiation phase (■) and elongation phase (■) assays was plotted as a function of the log of RNA polymerase II monoclonal antibody concentration.

A T P  C E L L E X T R A C T  C L O S T R I P A I N

Schematic representation of the procedure for determining relative sensitivity of the C-terminal domain to clostripain cleavage in the free and elongating enzyme. The conditions for labeling free and elongating enzyme, the purification of the photoaffinity-labeled transcription complex, and the clostripain digestion were as described under “Experimental Procedures.”

The percent activity remaining is equal to the amount produced in the presence of control antibody only. The percent activity remaining for initiation phase (■) and elongation phase (■) assays was plotted as a function of the log of RNA polymerase II monoclonal antibody concentration. The experimental protocol is shown diagrammatically in Fig. 4.

Under limiting conditions, clostripain cleaves the C-terminal domain from subunit IIo leaving the remainder of the subunits and the C-terminal domain otherwise intact. The rate of cleavage of the C-terminal domain was estimated by following the rate of disappearance of radiolabeled subunit IIo.

Subunit IIo, in the free enzyme, was labeled by phosphorylation with casein kinase I in the presence of [γ-32P]ATP (Dahmus, 1981; Cadena and Dahmus, 1987). Under these conditions, casein kinase I selectively phosphorylates the C-terminal domain of subunit IIo (Cadena and Dahmus, 1987). 32P-Labeled RNA polymerase II was digested with increasing concentrations of clostripain in the presence of the transcription extract and analyzed by SDS-polyacrylamide gel electrophoresis. It is apparent from Fig. 5 (lanes 5–9) that increasing concentrations of clostripain resulted in a decrease in the amount of subunit IIo. Quantitation of these results from scans of such autoradiographs is shown in Fig. 6.

Since only a small fraction of the RNA polymerase II in the extract is transcriptionally active under the conditions of the assay (Dahmus and Kedinger, 1983), the clostripain sensitivity of an elongating enzyme can only be established after selectively labeling the transcriptionally active enzyme. The transcribing enzyme was photoaffinity-labeled as described by Bartholomew et al. (1986). Transcription was carried out

J. Hamaguchi, personal communication.
in the presence of 4-thio-UTP and [$\alpha$-32P]CTP and the nascent transcript cross-linked to RNA polymerase II by irradiation with near UV light. This results in the specific labeling of the two largest subunits, namely I0 and I1c (see Fig. 5, lane 10, and Bartholomew et al., 1986). Transcription complexes containing photoaffinity-labeled RNA polymerase II were purified by gel filtration and digested with increasing concentrations of clostripain as described under "Experimental Procedures." Results presented in Fig. 5 show that incubation in the presence of relatively low concentrations of clostripain results in the disappearance of subunit I0 (lanes 11–14) and the appearance of label in the region of subunit I1b and the free C-terminal domain (lanes 13–16). The concomitant appearance of subunit I1b and free C-terminal domain with the disappearance of subunit I0 verifies that this range of clostripain concentrations results in the selective cleavage of the C-terminal domain. Quantitation of the disappearance of subunit I0 is shown in Fig. 6. Similar results were obtained when RNA polymerase I0 was photoaffinity-labeled in isolated HeLa nuclei (Cadena and Dahmus, 1987) and digested with increasing concentrations of clostripain.

**DISCUSSION**

The observation that monoclonal antibody G7A5 reacts with a synthetic peptide containing three copies of the C-terminal domain consensus repeat and reacts with RNA polymerases I0 and IIA but not I1b (Christmann and Dahmus, 1981; Cadena and Dahmus, 1987) establishes that G7A5 recognizes a determinant in the C-terminal domain of subunit IIA. The relative affinity of this monoclonal antibody for RNA polymerases I0 and IIA, as determined by kinetic ELISA, suggests that the epitope recognized is the unmodified repeat. Phosphorylation of the C-terminal domain decreases monoclonal antibody reactivity by at least 10-fold.

The monoclonal antibody G7A5 inhibits the initiation phase of transcription at a 10-fold lower concentration than is required to inhibit elongation. The observation that higher concentrations of antibody also inhibit elongation suggests that binding of monoclonal antibody to the C-terminal domain can inhibit both the initiation and elongation phase of transcription. The differential sensitivity of the initiation and elongation phase to inhibition by monoclonal antibody G7A5 is likely the result of differential immunoreactivity brought about by phosphorylation of the C-terminal domain during the transcription cycle. Photoaffinity labeling of RNA polymerase II, in a cell-free transcription system (Bartholomew et al., 1986) and in isolated HeLa nuclei (Cadena and Dahmus, 1987), establishes that elongation of most class II genes is catalyzed by RNA polymerase I0. These studies do not, however, preclude the possibility that another form of RNA polymerase II is involved in initiation. Since the relative affinity of monoclonal antibody G7A5 is 10-fold higher for RNA polymerase IIA than for RNA polymerase I0, the increased sensitivity of the initiation phase is consistent with the idea that RNA polymerase IIA is involved in the formation of an initiation complex. These results are in agreement with the model recently proposed in which phosphorylation of the C-terminal domain is thought to lead to the release of enzyme from the initiated complex (Sigler, 1988; Dahmus et al., 1988; Kim and Dahmus, 1989).

The possibility that the phosphorylation of RNA polymerase II may be associated with a conformational change within the C-terminal domain was examined by determining the relative sensitivity of this domain to cleavage in the free and elongating enzyme. Results of such experiments indicate that the C-terminal domain of subunit I0 in the elongating complex is approximately 3 times more accessible to cleavage by clostripain than in the nontranscribing enzyme. This suggests that the C-terminal domain of subunit I0 in the elongating enzyme may be in a more extended conformation, relative to its conformation prior to initiation. This is also supported by the observation that the sedimentation constant of RNA polymerase I0 is slightly less than that of RNA polymerase IIA (Dahmus, 1981). It is not possible to conclude from these studies, however, whether or not the conformational change observed results directly from the phosphorylation of the C-terminal domain.

An alternate interpretation of the increased resistance of clostripain cleavage of the C-terminal domain of RNA polymerase II.}

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2 M. E. Dahmus, unpublished results.
elongation to inhibition by monoclonal antibody is that conformational changes, or the association of other factors with the enzyme, mask the determinants recognized by monoclonal antibody G7A5. Although such possibilities cannot be eliminated at this time, the observation that the C-terminal domain of RNA polymerase IIO appears to be in a more extended conformation suggests that this domain is accessible.

In addition to the studies presented here, the idea that RNA polymerase IIA is involved in the formation of an initiation complex and that phosphorylation of the C-terminal domain is involved in the transition to an elongating complex is supported by the following observations. In vitro transcription of the adenovirus-2 major late promoter with purified RNA polymerases IIO and IIA indicates that the rate of transcription catalyzed by RNA polymerase IIA is greater than that of RNA polymerase IIO (Kim and Dahmus, 1989). The subspecies refer to the nature of the input enzyme. The decreased transcriptional activity of RNA polymerase IIO may result from the need to dephosphorylate the C-terminal domain prior to initiation. Secondly, transcription extracts prepared from HeLa cells according to the procedures of Weil et al. (1979), Davison et al. (1983), and Moncollin et al. (1986) contain an activity that catalyzes the conversion of RNA polymerase IIA to IIO. Finally, it is important to note that an ATP requirement has previously been demonstrated for an early step in promoter-dependent transcription catalyzed by RNA polymerase II (Sawadogo and Roeder, 1984; Conaway and Conaway, 1988).

According to the model proposed above, activation of transcription is dependent on phosphorylation of the C-terminal domain of subunit IIa. Consequently, the protein kinase(s) that catalyze this conversion would be an essential transcription factor(s). A critical test of this model requires an identification of the factor(s) responsible for the phosphorylation of the C-terminal domain and a demonstration that such a factor(s) is essential for promoter-dependent transcription. A characterization of the state of phosphorylation of the RNA polymerase II as a function of its position in the transcription cycle will also be essential to more precisely define the functional significance of the C-terminal domain of subunit IIa.

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