Wheat Germ and Yeast RNA Polymerase II: Photoaffinity Labeling by 4-Thiouracil 5′-Monophosphate Positioned Uniquely at the 3′ End of an Enzyme-Bound [^{32}P]-Containing Transcript

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ABSTRACT: A stable ternary transcription complex was formed with either wheat germ or yeast RNA polymerase II using a ribotrinucleotide primer (GpCpG) to initiate transcription on a short synthetic single-strand DNA template. The template was designed to limit the incorporation of a photoprobe S^4-UMP (4-thio-UMP) to a *unique* position at the 3′ terminus of the transcript. The resulting stable ternary transcription complex was photolyzed to cross-link the bound transcript ([^{32}P]-labeled by the incorporation of [α-^{32}P]CMP) with the protein domain at or near the active site. Separation of the protein components by electrophoresis in polyacrylamide gel containing SDS and analysis by autoradiography and silver staining revealed that for either enzyme only the largest subunit was [^{32}P] labeled.

The multiple eucaryotic DNA-dependent RNA polymerases (EC 2.7.7.6) have been segregated into three classes on the basis of the function of their transcripts. Ribosomal RNAs are produced by polymerase I (or A), messenger RNAs by polymerase II (or B), and transfer RNAs and 5S RNAs by polymerase III (or C). Cell-free preparations of RNA polymerase II (B) from various sources have been described and characterized in a comprehensive review (Sawadogo & Sentenac, 1990).

Preparations of RNA polymerase II from either yeast (Dezelee & Sentenac, 1972) or wheat germ (Jendrisak & Guilfoyle, 1978; Jendrisak & Burgess, 1977) contain multiple subunits ranging in molecular mass from 10 to 240 kDa. The yeast polymerase II enzyme contains a 220-kDa subunit as its largest component; however, this subunit is often replaced by a subunit form which is either ~240 kDa in mass (due to multiple phosphorylations) or ~200 kDa in mass (due to proteolysis of a fragment from the C-terminal end of the subunit). The wheat germ polymerase II enzyme contains a 180-kDa subunit as its largest component. The second largest subunit (140–150 kDa) is stable and present in all preparations of RNA polymerase II. The sequence homology and functional homology between the largest subunits (β'_{155} and β_{150}) of the procaryotic RNA polymerase and the corresponding two largest subunits of the eucaryotic RNA polymerase II have been collected and reviewed (Sawadogo & Sentenas, 1990).

Several different affinity labeling techniques have been used to identify the catalytic domain and/or subunit(s) of various polymerases. These different techniques can be differentiated by noting where the probe is positioned with respect to the transcript as follows: (1) The probe is positioned uniquely at the 5′ end of the transcript in a "catalytic competence" designed experiment (Ruetsch & Dennis, 1985; Riva et al., 1987; Grachev et al., 1987) and therefore reacts with the catalytic domain located in close proximity to the initiation site which may or may not be coincident with the elongation site. (2) The probe is located at multiple positions along the nascent transcript of a transcribing complex (Bartholomew et al., 1986; Hanna & Meares, 1983a,b; Roberge & Bradbury, 1988) and therefore reacts with multiple domains over a large area, most of which are not at the catalytic domain. (3) The probe is positioned uniquely at the 3′ end of the nascent transcript of a transcribing complex and therefore reacts with the catalytic domain located in close proximity to the elongation site (Kelly et al., 1990; Dissinger & Hanna, 1990; Panka & Dennis, 1985).

(*1*) *The first technique* (catalytic competence) was used to derivatize the "B_{140}" subunit of the wheat germ RNA polymerase II (Grachev et al., 1986). The first technique was also used to label the "B_{150}" subunit of the yeast RNA polymerase II, where the subunit labeling was shown to depend on the chain length of the transcript (i.e., the largest subunit and second largest subunit were both derivatized) (Riva et al., 1987).

(*2*) *The second technique* was used to label both the "B_{240}" and "B_{150}" subunits of the HeLa RNA polymerase II transcription complex where the photoprobe S^4-UMP was positioned at multiple nonterminal positions of a [^{32}P]-labeled nascent transcript using adenovirus-2 DNA as a template (Bartholomew et al., 1986). In this study of HeLa RNA polymerase II, the subunit labeling pattern was also observed to be related to the transcript length.

(*3*) *The third technique* reported in this paper employs single-stranded templates which are primed by a 5′ triribonucleotide to initiate transcription at a discrete position on the template. The base sequence of the bound transcript (radiolabeled by [α-^{32}P]CMP at several positions within the transcript) contains only one UMP[1] (or S^4-UMP as a photoaffinity probe) located at the 3′ terminus. The stable transcription complex containing the photoprobe was irradiated, and the catalytic subunit was labeled.

In separate experiments in which the bound transcript was of length 16–19, only the largest subunit was derivatized for either the yeast or wheat germ polymerase II enzyme.

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[1] Abbreviations: S^4-UMP, S^4-UDP, and S^4-UTP, 4-thiouracil 5′-mono-, di-, or triphosphate; NTP, nucleoside triphosphate. Notations of the form "15-mer" denote 3′→5′ oligoribonucleotides which are 15 bases in length.

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MATERIALS AND METHODS

Materials

Yeast RNA polymerase II was kindly supplied by Dr. Hiraku Saito (Oriental Yeast Co., Osaka, Japan). RNA polymerase II isolated from wheat germ was a gift from Dr. N. E. Thompson (University of Wisconsin, Madison, WI). Polynucleotide phosphorylase (*Micrococcus luteus*, primer-dependent) and alkaline phosphatase (*Escherichia coli*) were purchased from Pharmacia. Single-stranded synthetic DNA templates were the kind gift of Dr. L. Ivanoff (SmithKline Beecham Pharmaceuticals). HPLC-pure ATP, UTP, and CTP were purchased from ICN Biochemicals. GDP and snake venom phosphodiesterase were purchased from Boehringer Mannheim. [α-^{32}P]CTP, [α-^{32}P]UTP, and [γ-^{32}P]ATP (3000 Ci/mmol) were purchased from New England Nuclear. All reagents for gel electrophoresis were of electrophoresis grade and were obtained from Bio-Rad. T4 polynucleotide kinase was purchased from Promega. Protease A (subtilisin Carlsberg; subtilopeptidase A), ribonuclease A (bovine pancreatic), 4-thiouridine 5′-diphosphate, nucleoside diphosphate kinase, GpC, and all other reagents were from Sigma.

Methods

Preparation of GpCpG. The oligoribonucleotide primer GpCpG was synthesized enzymatically from GpC and GDP using polynucleotide phosphorylase. The reaction mixture was separated on a DE-52 column, and the products were analyzed for purity using HPLC (Ruetsch & Dennis, 1987). The yield of GpCpG was 10%.

The primer GpCpG was converted to 5′-[α-^{32}P]pGpCpG using [γ-^{32}P]ATP and T4 polynucleotide kinase. This labeled primer with a specific activity of approximately 100 Ci/mmol was used in qualitative product studies.

Preparation of 4-Thio-UTP. The photoaffinity probe 4-thio-UTP was prepared from 4-thio-UDP enzymatically using nucleoside diphosphate kinase (Bartholomew et al., 1986). A yield of 70% was obtained. An extinction coefficient at 330 nm (pH 7.0) of 21 200 M^{-1} cm^{-1} was used to determine the concentration of 4-thio-UTP solutions.

Synthetic DNA Templates. The synthetic single-stranded DNA templates were synthesized by a standard automated solid-phase method (ABI User Bulletin, 1984). The sequences of the four templates used (designated T_{16}, T_{17}, T_{18}, and T_{19}) and the expected RNA transcripts when initiation is directed by the triribonucleotide GpCpG are as follows:

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T16          3' d[CCCCCCGCTTGTGGTGTTTTA]  5'
Transcript:  5'       GCGAACACCACAAAAU    3'

T17          3' d[CCCCCCGCTTGTGGTGTTTTTA]  5'
Transcript:  5'       GCGAACACCACAAAAAU    3'

T18          3' d[CCCCCCGCTTGTGGTGTTTTTTA]  5'
Transcript:  5'       GCGAACACCACAAAAAAU    3'

T19          3' d[CCCCCCGCTTGTGGTGTTTTTTTA]  5'
Transcript:  5'       GCGAACACCACAAAAAAAU    3'
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The underlined residue is the first nucleotide in transcripts primed with GpCpG. The synthetic DNA templates were purified by electrophoresis in 20% polyacrylamide gel containing 7 M urea before use.

Standard Reaction Protocol. Standard yeast polymerase II transcription reactions were 50 mM Tris-HCl (pH 7.9), 80 mM $(NH_4)_2SO_4$, 1.6 mM $MgCl_2$, 9 mM 2-mercaptoethanol, 5 μM GCG, 5 μM DNA template, 0.25 μM enzyme, 160 μM unlabeled nucleoside triphosphate substrate, and 6.6 μM [α-^{32}P]-labeled substrate (300 Ci/mmol). The yeast or wheat germ polymerase II molarity was calculated on the assumption that the isolated multisubunit molecular mass is 600 000 Da.

Standard wheat germ polymerase II transcription reactions were 12.5 mM Tris-HCl (pH 7.9), 12.5 mM $(NH_4)_2SO_4$, 4 mM $MgCl_2$, 5 μM GCG, 5 μM DNA template, 0.25 μM enzyme, 160 μM unlabeled nucleoside triphosphate substrates, and 6.6 μM [α-^{32}P]-labeled substrate (300 Ci/mmol).

Standard assay reactions were incubated for 30 min at 30 °C and terminated by the addition of EDTA to a final concentration of 20 mM. Analysis of transcripts produced was conducted by autoradiography of 5-μL aliquots separated by electrophoresis in 20% polyacrylamide gel containing 7 M urea.

After separation of the transcripts, gel slices containing full-length transcript were excised using the exposed autoradiogram as a template. The isolated gel slice from each lane was placed in an microfuge tube, washed with water for 5 min to remove urea, and immersed in 500 μL of distilled water for approximately 14 h at room temperature. The solution containing transcript was removed from each tube and dried using a Savant Speedvac. The amount of transcript recovered was determined from Cerenkov counting. The isolated RNA transcripts were stored at −20 °C.

Snake Venom Phosphodiesterase Digestions. The enzyme was treated with acetic acid to remove 5′-nucleotidase activity in commercial preparations of snake venom phosphodiesterase (Sulkowski & Laskowski, 1971).

For partial snake venom phosphodiesterase digestion of RNA a 10-μL reaction volume contained the following: 20 mM Tris-HCl (pH 8.9), 20 mM $MgCl_2$, and 6000 cpm of RNA transcripts containing either [^{32}P]CMP or [^{32}P]UMP and variable amounts of snake venom phosphodiesterase (0–1.0 μg). After a 2-min incubation at 37 °C, the reaction was stopped by the addition of 5 μL of 0.15 M EDTA and dried using a Savant Speedvac. The partially digested RNA was analyzed by electrophoresis in 20% polyacrylamide gel containing 7 M urea.

Transcription Product vs Time Study. For the time course study, a total reaction volume of 60 μL was used. The transcription reaction was similar to that described above with the following modifications. The primer GpCpG, synthetic DNA template, and 9 μg of RNA polymerase II were incubated at 30 °C for 5 min to form a preinitiation complex. The nucleotides were then added to a final concentration of 160 μM ATP and S^4-UTP and 6.6 μM [α-^{32}P]CTP (300 Ci/mmol) to initiate the reaction. After incubation at 30 °C for the indicated time, a 5-μL aliquot was withdrawn and terminated by adding 10 μL of a solution containing 25 mM EDTA, 99% formamide, and 0.01% bromophenol blue. The transcription products were separated on a 20% polyacrylamide 7 M urea gel and visualized by autoradiography. The gel slices containing full-length transcripts were excised for Cerenkov counting.

Electrophoretic Analysis of Transcription Products. RNA products from transcription were analyzed by a 20% poly-

acrylamide–urea gel unless otherwise indicated. A 10-μL solution containing 99% formamide and 0.01% bromophenol blue was added to each sample. The sample mixtures were loaded onto a 20% polyacrylamide gel (16 × 16 cm × 0.5 mm) containing 7 M urea. The gel was electrophoresed at 500 V for 30–45 min prior to loading the sample. The gel was then electrophoresed at 500 V until the bromophenol blue dye was approximately 3 cm from the bottom of the gel (about 2.5 h).

Autoradiography was performed on the wet gel at room temperature. The exposed autoradiogram was used as a template to excise gel slices containing radioactive products for quantitative Cerenkov counting.

Photoaffinity Labeling. The standard reaction mixture (40 μL) was prepared for both the wheat germ and yeast polymerase enzymes in combination with each of the four templates T_{16}–T_{19}. Each reaction was assembled in an individual well of a microtiter plate, incubated for 25 min at 30 °C, and irradiated from the top for 15 min at room temperature with a 366-nm UV light (Model UVGL-58, UVP, Inc.) at a distance of 4 cm. After irradiation, a 5-μL aliquot of each reaction was removed and analyzed for nucleic acid on a 20% polyacrylamide–urea gel. The components in the remaining reaction volume (35 μL) were denatured in Laemmli buffer by heating at 100 °C for 5 min and analyzed for protein by electrophoresis in 10% polyacrylamide gel containing SDS according to the method of Laemmli (1970).

Protease or RNase Digestion. A solution of subtilopeptidase A (4 mg/mL) or bovine pancreatic ribonuclease A (4 mg/mL) was prepared in 10 mM Tris-HCl (pH 7.9) and 50 mM NaCl. After irradiation of a photoaffinity labeling reaction mixture, protease or RNase was added to a final concentration of 0.36 mg/mL and incubation was continued at 37 °C for 1 h. The reaction was then stopped by heating at 100 °C for 5 min in Laemmli buffer (Laemmli, 1970) and analyzed as described above.

RESULTS

Transcription. Wheat germ polymerase II and yeast RNA polymerase II efficiently utilize a single-stranded synthetic oligodeoxyribonucleotide as a template in transcription reactions primed with a 5′ triribonucleotide primer. The profiles of the transcripts produced from each of four different length synthetic DNA templates (T_{16}, T_{17}, T_{18}, T_{19}) are shown in Figure 1, Y and W, where [α-^{32}P]UMP is incorporated into the product. The major product ("*n*") for each template was 16–19 bases long for the respective templates T_{16-19}. For each template a small amount of n_{+1} and n_{-1} length transcript was produced when UTP was used as a substrate. A ladder of shorter products can be seen for the T_{19} template when [α-^{32}P]CMP is incorporated (at positions 4, 5, 7, and 10, according to the template). The ladder of shorter products for this reaction provides a precise marker for the location of the corresponding lengths of n = 16, 17, and 18 for the T_{16-18} template directed transcripts. When the ribotrinucleotide primer (GpCpG) was omitted from the reactions (data not shown), no product was detected.

Characterization of the Transcripts. In order to examine the fidelity of substrate incorporation by these polymerase II enzymes, a combination of nucleoside triphosphates was provided in addition to a 5′-[α-^{32}P]-labeled primer (^{32}pGpCpG). The radioactive transcripts produced are shown in Figure 2 for the yeast enzyme using T_{17} as template. Similar results were obtained for the wheat germ enzyme (data not shown). The addition of ATP elongated the primer to a 5-mer as predicted by the template. The addition of both ATP and

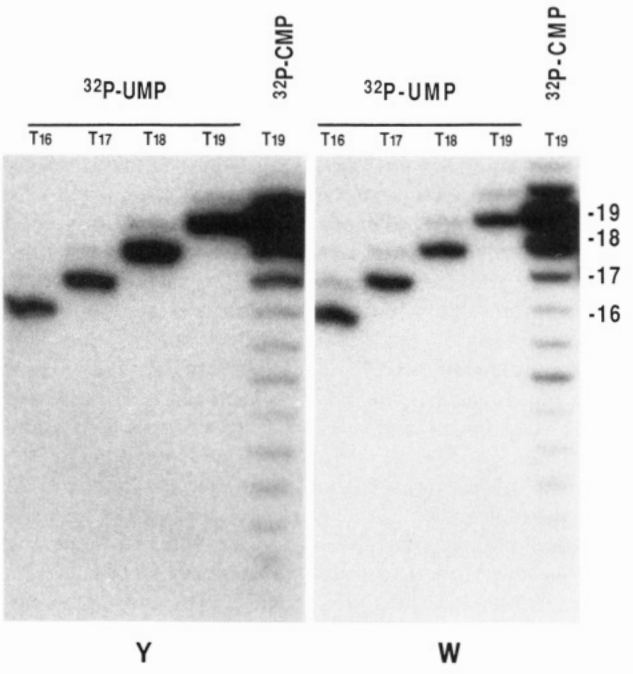

FIGURE 1: Transcription products from short synthetic single-stranded templates T_{16-19} using [α-^{32}P]UTP (U*) *or* [α-^{32}P]CTP (C*) as the labeling substrate and GpCpG as the primer. See methods for template sequences. Yeast enzyme = Y; wheat germ = W.

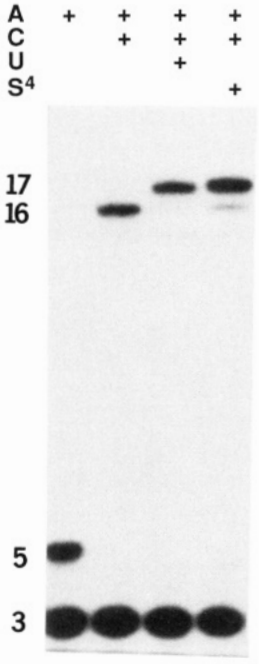

FIGURE 2: Yeast enzyme transcription products from T_{17} template using 5′-[α-^{32}P]-labeled primer (^{32}pGpCpG) and various combinations of nucleoside triphosphate as substrates. The photoprobe 4-thio-UTP was substituted for UTP where indicated.

CTP produced a 16-mer (n_{-1}) product. The addition of ATP, CTP, and either UTP or S^4-UTP produced the 17-mer full-length product (n). Thus UMP or S^4-UMP is located at the 3′ end of the transcript at position 17 when T_{17} is used as a template.

In order to show that UMP is incorporated uniquely at the 3′ end of each of the four different transcripts, *n*-length transcripts were produced and isolated which contained either [α-^{32}P]CMP *or* [α-^{32}P]UMP. The transcripts were subjected to partial hydrolysis using snake venom phosphodiesterase, separated by electrophoresis on a polyacrylamide gel, and

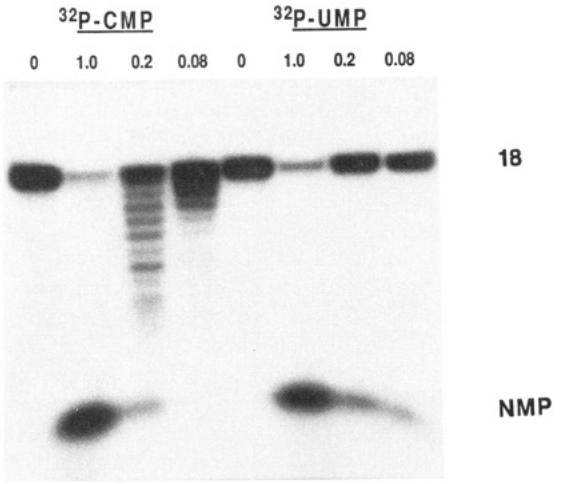

FIGURE 3: Partially hydrolyzed yeast enzyme transcripts from T_{18} template using the primer (GpCpG) and either [α-^{32}P]UTP *or* [α-^{32}P]-CTP as radioactive substrate. The hydrolysis experiments were conducted in parallel for 2 min using the relative amounts of snake venom phosphodiesterase as indicated (1 = 1.0 μg/10 μ).

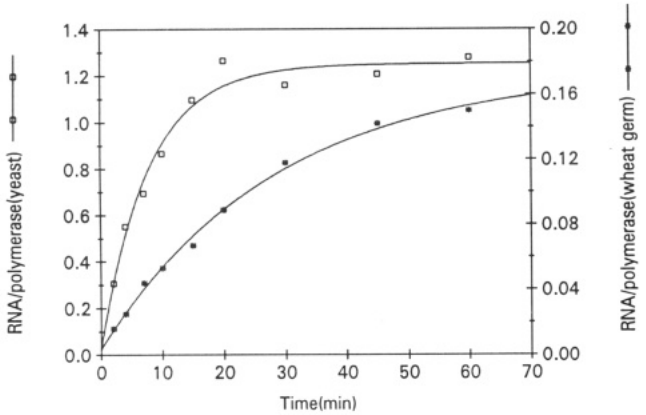

FIGURE 4: Time-dependent production of the T_{18} transcript by the yeast and wheat germ enzymes using the primer (GpCpG) and [α^{32}-P]CTP as radioactive substrate. The principal full-length transcript was separated by PAGE, excised, and assayed for radioactivity. The calculated ratio of transcript/enzyme was plotted vs time for the yeast (⊡) and the wheat germ enzyme (*).

autoradiographed. The results are shown in Figure 3 for the yeast enzyme using T_{18} as template. The progressive loss of the parent transcript (n = 18 as control) can be seen for each labeled series ("U" and "C") as the 3′ nuclease concentration increases. A concomitant increase in the limit product, nucleoside monophosphate, was also observed. The critical difference in the two series is that a series of labeled hydrolysis products $n, n_{-1}, \ldots$, CMP was observed for transcripts containing a "C" label, whereas no such intermediates between n and UMP were observed for the "U"-labeled series. This evidence allows the conclusion that the transcript from the T_{18} template contains a UMP at, and only at, the 3′ terminus of the transcript. Similar results were obtained for all four transcripts with both the yeast and wheat germ enzyme.

Formation of Stable Ternary Complex. The time course for the production of the 18-mer from the T_{18} template (where a large molar excess of template, primer, and substrates is present) using either the yeast RNA or wheat germ polymerase II is shown in Figure 4. The production of the "n" transcript for the yeast enzyme reaches a plateau at 20 min and remains constant for an additional 50 min at a mole ratio of n-mer/enzyme = 1.19. This observation is consistent with (but does not prove) the suggestion that a stable transcription complex is formed between the enzyme, the template, and the n transcript such that the 3′ terminal component of the n transcript (S^4-UMP) is poised at the catalytic site of the enzyme. The production of all other shorter transcripts reaches a plateau value of less than 2% of the major (n) transcript, which may represent the accumulated population of aborted transcripts produced during the course of the formation of the stable ternary complex containing an n-length transcript.

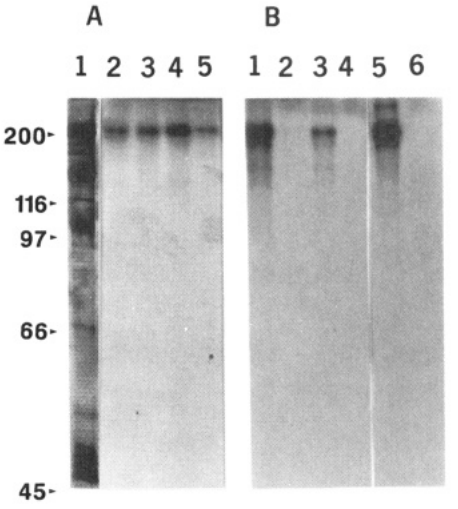

FIGURE 5: SDS–PAGE analysis of the photoaffinity-labeled subunits for the wheat germs enzyme using templates T_{16}–T_{19}. Panel A: (lane 1) native enzyme, silver stained; (lanes 2–5) radioactive subnunit derivatized using T_{16}–T_{19}, respectively, as template. Panel B: control reactions: (lane 1) complete T_{16} template reaction; (lane 2) no light; (lane 3) UTP/S^3-UTP mixed; (lane 4) no S^4-UTP; (lane 5) treated with EDTA prior to photolysis; and (lane 6) treated with SDS at 100 °C just prior to photolysis.

The production of the n-mer transcript for the wheat germ enzyme reaches a plateau at about 80 min at a mole ratio of n-mer/enzyme $\cong$ 0.16. This could mean that only 16% of the enzyme is active and capable of forming a stable transcription complex.

Analysis of Photoaffinity Labeling of the Catalytic Subunit. In order to identify the polymerase subunit(s) which is (are) labeled by the photoprobe contained in a bound transcript, a ternary complex was assembled which contained an [α-^{32}P]-CMP-labeled transcript with an unlabeled S^4-UMP photoprobe positioned at the 3′ terminus. Such stable ternary complexes were prepared (using both the yeast and wheat germ polymerase II enzymes), photolyzed, separated into protein components by electrophoresis in a 10% polyacrylamide gel containing 7.5% SDS, assayed by autoradiography, and visualized for protein by a silver staining technique. The autoradiogram of the separated protein components of a series of photolyzed reaction mixtures containing the wheat germ enzyme is shown in Figure 5. In panel A, lane 1, the silver-stained pattern of the wheat germ enzyme is shown with appropriate molecular weight markers indicated at the left margin. Lanes 2–5 are complete reactions containing different synthetic templates which produce transcripts of length 16–19 bases, respectively. Reactions containing these templates yield the same photolabeling pattern in that only the largest protein subunit (220 kDa) is radioactively labeled.

In Figure 5, panel B, a series of control reactions were examined using the T_{18} template (lane 1). If photolysis is omitted (lane 2), no radioactive proteins are detected. If the S^4-UTP photoprobe is partially replaced with competing UTP (lane 3) or completely replaced by UTP (lane 4), the resultant radiolabeled protein band at 220 kDa is either decreased or eliminated respectively. In lane 5 the reaction sample was

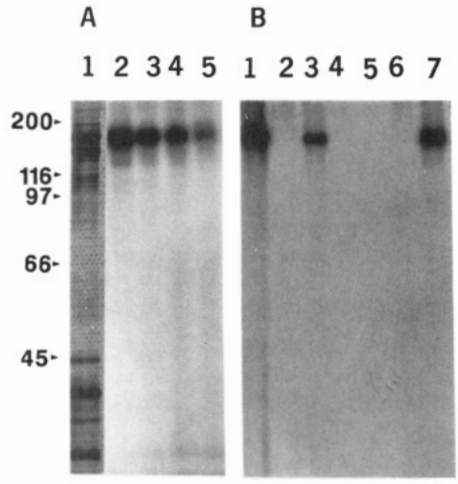

FIGURE 6: SDS–PAGE analysis of photoaffinity-labeled subunits for the yeast enzyme using templates T_{16}–T_{19}. Panel A: (lane 1) native enzyme, silver stained: (lanes 2–5) radioactive subunit derivatized using T_{16}–T_{19}, respectively, as template. Panel B: control reactions: (lane 1) complete T_{16} template reaction; (lane 2) no light; (lane 3) UTP/S^4-UTP mixed; (lane 4) no S^4-UTP; (lane 5) treated with subtilopeptidase after photolysis; (lane 6) treated with RNase after photolysis; and (lane 7) treated with EDTA just prior to photolysis.

treated with 7 mM EDTA just before photolysis in order to ensure that additional synthesis of transcripts could not occur. The radioactive band at 220 kDa was present. In lane 6 the reaction sample was treated with SDS at 100 °C just before photolysis in order to disrupt the ternary complex and dissociate the transcript from the enzyme complex. This treatment resulted in the elimination of the radioactive 220-kDa protein band. Treatment with Pronase or RNase after photolysis also eliminated the radiolabeled 220-kDa band (data not shown). The gel area located at the 220-kDa band for each lane was excised and quantitatively assayed for radioactivity. The total number of femtomoles of 220-kDa labeled subunits was calculated from the specific activity (corrected for the number of [α-^{32}P]-labeled CMP's per transcript) and assuming that only one transcript was bound per transcription complex. The calculated values in femtomoles for Figure 5, panel A, are as follows: 8.1, 7.9, 11, and 6.3 for lanes 2–5, respectively. The calculated values in femtomoles for Figure 5, panel B, are as follows: 7.2, 0.66, 1.78, 0.32, 8.2, and 0.44 for lanes 1–6, respectively. The minimum average percent efficiency of photolysis was calculated from the femtomole ratio of photolabeled 220-kDa subunit to the total amount of ternary complex formed (8.33/896 = 0.93%). The value for femtomoles of ternary complex formed was assumed to be equal to the value for femtomoles of transcript formed; therefore, the value for percent efficiency of photolabeling is a minimum value.

Similar results using the yeast enzyme are shown in Figure 6. In panel A, lane 1, the silver-stained pattern of the native yeast enzyme is shown with appropriate molecular weight marker indicated at the left margin. Lanes 2–5 are complete reaction mixtures containing different synthetic templates which produce transcripts of length 16–19 bases, respectively. Reactions containing these templates yield the same photolabeling pattern as in Figure 5 in that only the largest subunit (180 kDa) is radioactively labeled.

In Figure 6, panel B, a series of control reactions were examined using the T_{16} template (lane 1). If photolysis is omitted (lane 2), no radiolabeled protein bands are detected. If the S^4-UTP photoprobe is partially replaced with competing UTP (lane 3) or completely replaced by UTP (lane 4), the resultant radiolabeled protein band at 180 kDa is either decreased or eliminated, respectively. Treatment of the photolyzed samples with either subtilopeptidase A (lane 5) or RNase (lane 6) eliminated the radioactive protein band at 180 kDa. Treatment with 7 mM EDTA just prior to photolysis (lane 7) did not eliminate the radioactive band at 180 kDa.

The gel area located at the 180-kDa band for each lane was excised and quantitatively assayed for radioactivity. The total number of femtomoles of 180-kDa labeled subunits was calculated from the specific activity (corrected for the number of [α-^{32}P]-labeled CMP's per transcript) and assuming that only one transcript was bound per transcription complex. The calculated values in femtomoles for Figure 6, panel A, as follows: 38.5, 21, 18.5, and 6.2 for lanes 2–5, respectively. The calculated values in femtomoles for Figure 6, panel B, are as follows: 17.2, 0.50, 3.0, 0.44, 0.38, 0.38, and 7.8 for lanes 1–7, respectively. The minimum average percent efficiency of photolysis was calculated from the femtomole ratio of photolabeled 180-kDa subunit to the total amount of ternary complex formed (21/1200 = 1.75%). The value for femtomoles of ternary complex formed was assumed to be equal to the value for femtomoles of transcript formed; therefore, the value for percent efficiency of photolabeling is a minimum value.

DISCUSSION

The catalytic subunit of both wheat germ and yeast RNA polymerase II has been determined during the elongation phase of the reaction. The compound 4-thiouridine 5′-triphosphate was employed as a photoaffinity probe positioned uniquely at the 3′ terminus of a transcript bound in a stable transcription complex. The transcription complex was assembled around a synthetic single-stranded DNA template such that precise initiation occurred at the primer (GpCpG) and elongation continued to produce an exact length of bound transcript with the photoprobe positioned at the 3′ end of the transcript. Radioactive labeling with ^{32}P was introduced either at the 5′ end of the primer or as incorporated substrate monomers between the primer and the 3′ terminal photoprobe.

Four different synthetic templates (T_{16-19}) were shown to produce a single major transcript and to possess a unique photolabel S^4-UMP positioned at the 3′ terminus of the "*n*"-length transcript. Transcripts initiated by primers and elongated to the length "*n*" are suggested to exist as a stable ternary transcription complex. These complexes were photolyzed to derivatize the catalytic subunit.

The largest subunit was exclusively photoaffinity labeled in the case of the yeast (220 kDa) enzyme at each of four different transcript lengths. This result is in contrast with previous results of others which labeled the largest and the second largest subunit for the yeast enzyme (220 and 150 kDa) (Riva et al., 1987) and observed that the labeling ratios of subunits were dependent on the length of the transcript involved in the transcription complex which was derivatized (Grachev et al., 1986). An explanation for this difference might be that the area accessible to the probe during initiation is different from the area accessible during elongation. The synthetic templates employed in our studies do not contain a promoter and might occupy a catalytic domain which is different from that occupied by a natural promoter containing template engaged in a transcription complex. The largest subunit in any case appears to contain the catalytic subunit employed during elongation.

The wheat germ enzyme in these studies was exclusively photoaffinity labeled in the largest subunit (180 kDa) at any

of four different transcript lengths. This result is in contrast with previous results (Grachev et al., 1986) which uniquely labeled the second largest subunit (140 kDa) during initiation. Again, the difference in results could be due to the fact that the initiation area is different from that which is accessible during elongation.

REFERENCES

Bartholemew, B., Dahmus, M. E., & Meares, C. F. (1986) *J. Biol. Chem. 261*, 14226–14231.

Dezelee, S., Sentenac, A., & Fromageot, P. (1972) *FEBS Lett. 21*, 1–6.

Dissinger, S., & Hanna, M. M. (1990) *J. Biol. Chem. 265*, 7662–7668.

Grachev, M. A., Hartmann, G. R., Maximova, T. G., Mustaev, A. A., Schäffner, A. R., Sieber, H., & Zaychikov, E. F. (1986) *FEBS Lett. 200*, 287–290.

Grachev, M. A., Kolocheva, T., Lukhtanov, E., & Mustaev, A. A. (1987) *Eur. J. Biochem. 163*, 113–121.

Hanna, M. M., & Meares, C. F. (1983a) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4238–4242.

Hanna, M. M., & Meares, C. F. (1983b) *Biochemistry 22*, 3546–3551.

Jendrisak, J. J., & Burgess, R. R. (1977) *Biochemistry 16*, 1959–1964.

Jendrisak, J. J., & Guilfoyle, T. J. (1978) *Biochemistry 17*, 1322–1327.

Kelly, S., Sheng, N., & Dennis, D. (1990) *J. Biol. Chem. 265*, 7787–7792.

Laemmli, U. K. (1970) *Nature 227*, 680–685.

Panka, D., & Dennis, D. (1985) *J. Biol. Chem. 260*, 1427–1431.

Riva, M., Schäffner, A. R., Sentenac, A., Hartmann, G. R., Mustaev, A. A., Zaychikov, E. F., & Grachev, M. A. (1987) *J. Biol. Chem. 262*, 14377–14380.

Roberge, M., & Bradbury, E. M. (1988) *J. Biol. Chem. 263*, 18553–18557.

Ruetsch, N., & Dennis, D. (1987) *J. Biol. Chem. 262*, 1674–1679.

Sawadogo, M., & Sentenac, A. (1990) *Annu. Rev. Biochem. 59*, 711–754.

Sulkowski, E., & Laskowski, M. (1971) *Biochim. Biophys. Acta 240*, 443–447.