claims that the isotope separation is present in the uranyl acetate photoreduction either in water or in water containing complex-forming additives (fluoride, carbonate, oxalate, and others). As seen from the patent description, the authors[42] were guided by "general considerations" rather than by experimental data.

According to ref 43, the isotope separation occurs during the photopolymerization of uranyl acrylate in ethanol solution in a strong permanent magnetic field. The precipitated polymer contains the enriched uranium; the content of the ^{235}U isotope may be up to 50%.[43] The content of this isotope in the starting uranyl acrylate has not been reported. We have not been able to reproduce the data in ref 43.[35]

A reproducible MIE on uranium has been found in the reaction of uranyl oxalate decomposition[44] (simultaneously with our study[38] and independently of it). Both the enrichment of the precipitated uranium(IV) oxalate containing ^{235}U and the depletion of the starting uranyl have taken place during the photolysis of acidic solutions of uranyl oxalate having 10% ^{235}U content. Logically, it is the cage reaction product uranium(IV) that has been enriched:[44] reaction 5b between uranoyl and $CO_2^{\bullet-}$ proceeds faster in the case of uranoyl containing the ^{235}U magnetic isotope. The "spoiling" isotope exchange (reaction 10) in solvent bulk is probably retarded due to the presence of uranyl in the form of an oxalate complex. The extent of isotope separation decreases with the reaction conversion.[44] The data of ref 44 have been reproduced.[35]

Conclusions and Perspectives. In a series of elements for which a MIE has been found (^{13}C, ^{17}O, ^{29}Si, ^{33}S)[3-6] the SOC constant increases from $\zeta = 28$ cm^{-1} (for carbon) to 382 cm^{-1} (for sulfur), more than 1 order of magnitude.[2] The MIE scale, however, does not change much in the series.

The separation of uranium isotopes based on the MIE has been a challenging problem during the past 15 years.[31,45] One can suggest that the existence of a uranium heavy atom and overestimation of the SOC role a priori led researchers be pessimistic about success in demonstration of a measurable MIE for uranium systems. A MIE has been determined quite recently in two uranyl photosensitized reactions: the oxidation of phenols and the decomposition of oxalic acid.[38,44] It is to be hoped that the data obtained on the MIE involving heavy isotopes will stimulate progress in the development of RP theory, which accounts for significant SOC and new channels of singlet–triplet evolution.

The uranoyl radical differs considerably from radicals centered on carbon and other elements, for which the MIE was previously discovered, with its magnetic resonance parameters. For better understanding of the MIE, a detailed study of the ESR spectrum and magnetic resonance characteristics of $^{235}UO_2^+$ is required.

It is difficult at present to speculate on the practical exploitation of the uranium enrichment method based on the MIE without examination of pertinent technological and commercial aspects. In favor of the MIE method we shall mention that the coefficient A obtained in this method seems to be higher than the A found in widely used gas diffusion and ultracentrifuge methods.[46] Additionally, uranyl photoreactions can be initiated by solar light. It is probable that in the future, if one employs viscous liquids or micellar solutions, a more significant isotopic separation than that presented above will be found. This improvement may allow the practical use of MIE. In the present paper we have tried to show that to obtain MIE, one should be able to govern the reaction and its physics and chemistry and to provide conditions of spin, molecular, and chemical dynamics favorable for MIE.

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Registry No. UO_2, 16637-16-4; ^{235}U, 15117-96-1.

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Mapping the Path of a Growing Ribonucleic Acid Molecule

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The proteins and nucleic acids responsible for the orderly functioning of biological cells are linear polymers. They are synthesized processively (without stopping) by elaborate assemblies of macromolecules. The synthesis of ribonucleic acid begins with a ribonucleotide monomer whose ribose 3′-OH is phosphorylated by another nucleotide; this process is repeated thousands of times to produce a finished RNA molecule. The sequence of nucleotide bases in the RNA is determined by the pairing of each successive incoming nucleotide to a complementary nucleotide in a DNA molecule that serves as a template. Only those bases that pair with the template (e.g., uracil with adenine,

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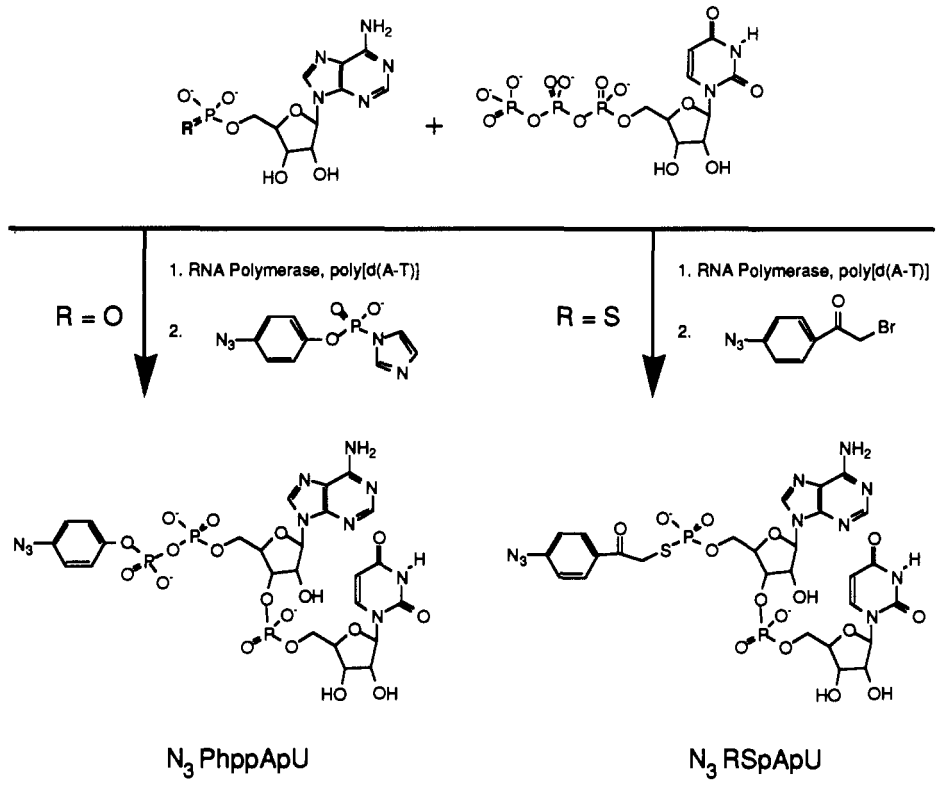

Figure 1. Syntheses of the photoprobes discussed in the text.

cytosine with guanine, etc.) are incorporated into the final RNA product. Thus the information coded in the base sequence of the DNA is transcribed into RNA, rather as the sequence of bits in one magnetic tape is copied onto another.

Besides involving a large DNA molecule (10^3–10^8 nucleotides), the synthesis of RNA requires a highly specialized catalyst: RNA polymerase. In bacteria, RNA polymerase has five protein subunits ($\alpha_2\beta\beta'\sigma$), of which one ($\sigma$) is chosen from a group of related proteins with different DNA-binding specificities. In many preparations, σ is present in substoichiometric amounts. Viruses that attack bacteria can have simpler RNA polymerases; the enzyme from bacteriophage T7 has a single polypeptide chain. In the nucleated cells of higher organisms, at least three types of RNA polymerase are used to carry out the functions performed by one enzyme in *Escherichia coli*. Most of these enzymes are composed of more than five proteins, which bind together noncovalently to form the catalytic assembly, plus other proteins that bind specifically to certain base sequences in DNA.

We are interested in mapping the path that nascent RNA follows through the macromolecular complex that catalyzes its synthesis. Because of the large sizes and relative scarcity of the molecules involved, we have developed a strategy that uses the tools of chemistry and molecular biology to carry this out. The synthesis of RNA proceeds in the 5′ to 3′ direction; the initial residue at the 5′ (leading) end may be as small as a nucleoside or as large as nicotine adenine dinucleotide or flavin adenine dinucleotide.[1-3] This property suggested that chemical or physical probes such as photoaffinity labels could be incorporated at the 5′ end of the nascent RNA molecule by RNA polymerase. These probes could then provide information about their surroundings as the nucleotide chain is elongated. In particular, photoaffinity probes could help in identification of enzyme subunits that make contact with the nascent RNA.

Photoprobes

DeRiemer and Meares[4] synthesized mono- and dinucleotide conjugates containing photoreactive aryl azides. Aryl azides were chosen because they are chemically unreactive until activated by photolysis; then they form nitrenes or other highly reactive species, which react indiscriminately with molecules in their vicinity.[5,6] A dinucleotide probe was prepared because dinucleotides are more efficient at initiating RNA synthesis than mononucleotides.[7] The "abortive initiation" reaction of RNA polymerase provides a convenient, high-yield route for the synthesis of dinucleotides. Abortive initiation is the catalytic production of dinucleotides or oligonucleotides by RNA polymerase in the presence of a DNA template.[8] The dinucleotide photoaffinity probe β-(4-azidophenyl)-adenylyl-(3′–5′)-uridine 5′-diphosphate (N_3PhppApU, Figure 1) was synthesized by reacting pApU with *p*-azidophenyl phosphorimidazolidate.[4]

It was then necessary to demonstrate that these probes could serve as primers (initiators) of RNA synthesis. Enzyme·DNA·RNA complexes containing RNA of known lengths (two, three, and four nucleotides) with

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a photoaffinity probe on the 5′-phosphate and a ^{32}P radiolabel at the 3′ end were prepared in situ by RNA polymerase in the presence of poly[d(A-T)] (a DNA template that contains alternating adenine and thymine bases), N_3PhppApU or N_3PhppA, and [α-^{32}P]ATP or UTP.[9] Both N_3PhppA and N_3PhppApU are *di*phosphates and do not act as substrates for the elongation reaction catalyzed by RNA polymerase. However, they can be incorporated at the 5′ end of the nascent RNA chain.

After verification of the probes' biological activity, photoaffinity experiments could be performed. Enzyme·DNA·RNA complexes were photolyzed, then dissociated by using sodium dodecyl sulfate, and fractionated by gel electrophoresis.[10] To be labeled, an RNA polymerase subunit must have been contacted by a photogenerated reagent located at the 5′ end of a nascent RNA molecule in a transcription complex. With an RNA dinucleotide and a poly[d(A-T)] DNA template, the photolabeled enzyme subunits were β and σ, in a 3.5:1 ratio. Photolysis of the ternary complex containing trinucleotide RNA also resulted in labeling of the β and σ subunits, in a 2:1 ratio. With a tetranucleotide, the β subunit was very heavily labeled, and only a small amount of labeling of the β' and σ subunits was observed. The α subunit was not labeled with any of the probes, while the DNA template could not be conveniently examined. Another protein that is associated with *E. coli* RNA polymerase, ω, was not present in detectable quantities in these preparations; it is not required for transcription.[11]

The photoaffinity labeling pattern changes markedly as the ribonucleotide is elongated from a tri- to a tetranucleotide, with σ labeling much decreased. A possible explanation for this is that, since the probe attached to the 5′ end of RNA must undergo helical displacement as the RNA is elongated, the probe simply moves away from contact with σ. If it has the shape of an ordinary RNA·DNA duplex, the 5′ end of the RNA–DNA helix would be expected to translate 2.6 Å and rotate 33° when a base is added to the 3′ end of the RNA at the active site of RNA polymerase.

Another possibility—which must always be considered in a photoaffinity experiment—is that the probe dissociates from the active site of the transcription complex and then reassociates at other locations on the enzyme. This can be rigorously ruled out for longer RNA probes, as described below. The best evidence that the shorter RNA probes provide a reliable report on their surroundings is the substantial difference in the labeling patterns observed for the tri- and tetranucleotide probes. Very similar behavior was observed for the related RNA polymerase from *Bacillus subtilis* (L. DeRiemer, unpublished observations) with the same probes. The α subunits of RNA polymerase, which are always present in stoichiometric amounts, were never observed to be labeled significantly on either enzyme.

The photoprobe design was later improved by Hanna and Meares.[12,13] A new dinucleotide probe was synthesized, containing an aryl azide connected to the 5′-phosphate by an S–P bond (N_3RSpApU, Figure 1). This linkage can be selectively hydrolyzed at neutral pH and room temperature in the presence of mercurials,[14,15] allowing release of the label from photoaffinity-labeled molecules when desired. SpApU is synthesized by the abortive initiation reaction using UTP and adenosine 5′-*O*-(thio monophosphate). The dinucleotide SpApU is then alkylated with azidophenacyl bromide to give the photoprobe.

Photoaffinity Mapping

The experimental strategy for determining the entire path of the leading end of the nascent RNA through the transcription complex is diagrammed in Figures 2 and 3. For these experiments, a DNA fragment from bacteriophage T7 was used instead of the simple poly[d(A-T)] template. The T7 DNA fragment contains a single strong "promoter" site, at which RNA polymerase binds and begins transcription. The RNA transcript base sequence begins 5′-AUCGAGAGGGACACGGCGAAU.... Since the probe N_3RSpApU provides the first AU in the transcript, UTP is not needed as a substrate until the transcript reaches a length of 20 nucleotides. It was convenient to do the mapping in two stages; one with RNA $\leq$20 bases long, the other with RNA >20.

Using four base-specific terminators (3′-deoxy or 3′-*O*-methyl nucleoside triphosphates) in four parallel reactions, transcription complexes containing radiolabeled RNA chains of various lengths (4–116 nucleotides) were prepared.[13] Then the 5′ end of RNA was covalently attached to the nearby polymerase subunits or DNA by irradiation with UV light; the photolabeled enzyme subunits and DNA were separated by gel electrophoresis using sodium dodecyl sulfate/urea; radiolabeled RNA chains were cleaved from DNA and protein subunits in the presence of phenylmercuric acetate; and the chain lengths released were determined by RNA sequencing gels.

The distribution of label between DNA and polymerase subunits is shown in Figure 4. Most of the photolabeling by RNA lengths 4–11 occurred on the DNA. The RNA 12-mer showed labeling of DNA, β, and β'. On elongation to the RNA 14-mer, the photoaffinity label distribution shifted such that DNA labeling was reduced, with a large increase in β and β' labeling. Thus the 5′ end of the transcript appears to be involved in a DNA·RNA hybrid until the RNA chain length exceeds 12 bases.

Accessibility

Aryl azides can be reduced and thereby inactivated by thiol reagents under very gentle conditions.[16] By using thiols to reduce accessible photoprobes, Bernhard[17] modified the photoaffinity technique so that the contacts between the leading end of the nascent RNA and small molecules in the solution could be determined as a function of RNA length. Thiols were chosen to explore the effects of size, charge, and number of SH groups on azide reduction. It was found that small, neutral dithiothreitol (DTT) molecules can readily

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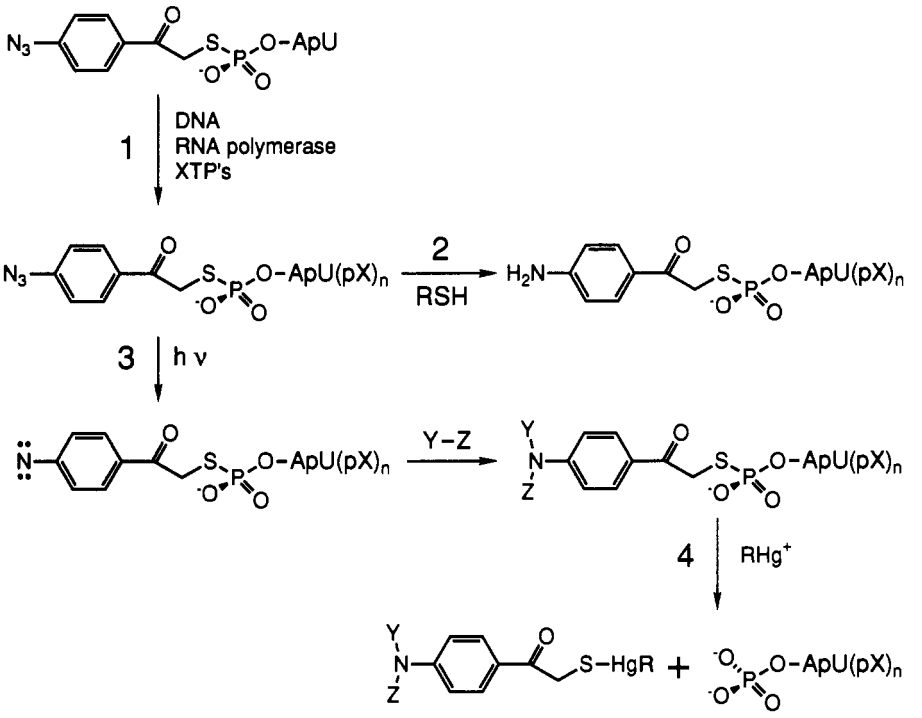

Figure 2. Chemical reactions employed in these experiments. Reaction 1: the dinucleotide probe (upper left) is incorporated into the 5′ end of RNA in a transcription complex. Reaction 2: (optional) accessible azides may be reduced by thiols. Reaction 3: UV irradiation converts azides into nitrenes or other highly reactive species, which nonselectively insert into chemical bonds on neighboring macromolecules. Reaction 4: the P–S bond is hydrolyzed in the presence of a mercurial, releasing RNA from the cross-link. Reprinted with permission from ref 17. Copyright 1986 American Chemical Society.

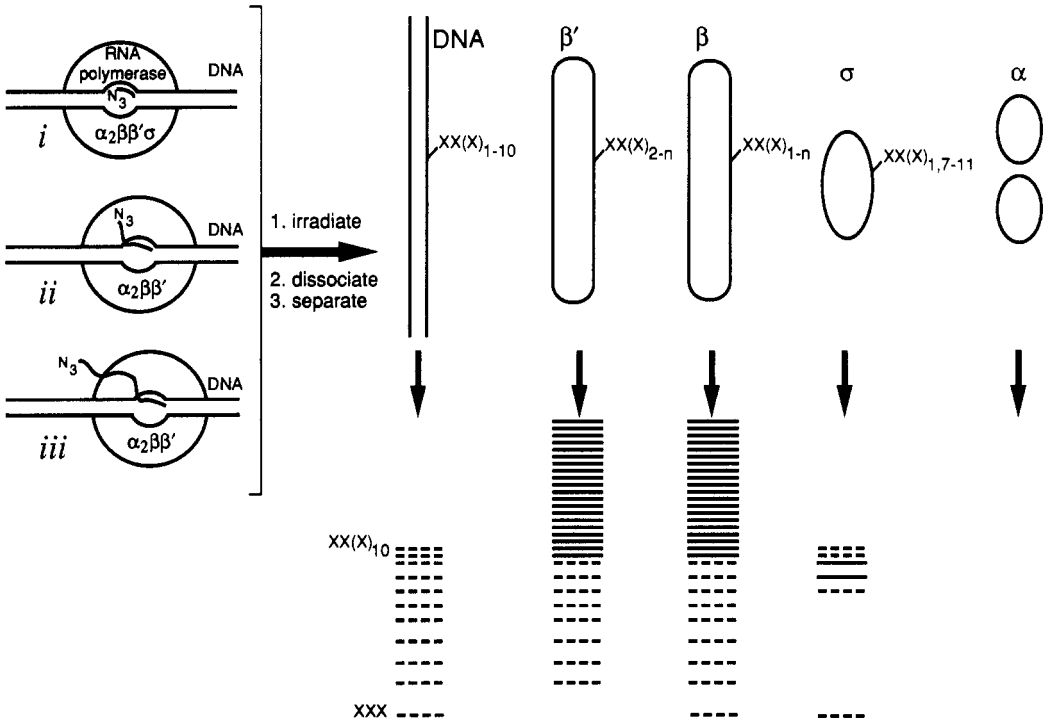

Figure 3. Experimental strategy for determining the path of the leading (5′) end of RNA through the *E. coli* transcription complex. First, transcription complexes containing RNAs of various lengths are formed. The drawing shows chain lengths in which (i) the 5′ end of RNA contacts both the enzyme and DNA or (ii) the 5′ end has diverged from the DNA but still contacts the enzyme or (iii) the 5′ end has left the surface of the enzyme. After irradiation of the complex to attach the RNA covalently to surrounding protein or DNA, the complex is dissociated and the components are separated by gel electrophoresis. The radiolabeled RNA chains are cleaved from the DNA and protein subunits in the presence of phenylmercuric acetate, and the chain lengths of the released RNAs are determined by a second gel electrophoresis (X = a ribonucleotide residue). In practice, four reactions are done in parallel; each contains a base-specific terminator (A, C, G, or U), so that the base sequence of the transcript is verified. Reprinted with permission from ref 13. Copyright 1983 National Academy of Sciences, USA.

penetrate into the restricted spaces on the transcription complex. The cross-linking of RNAs to the enzyme subunits is reduced in the presence of thiols, to a degree that depends on RNA chain length. We examined in

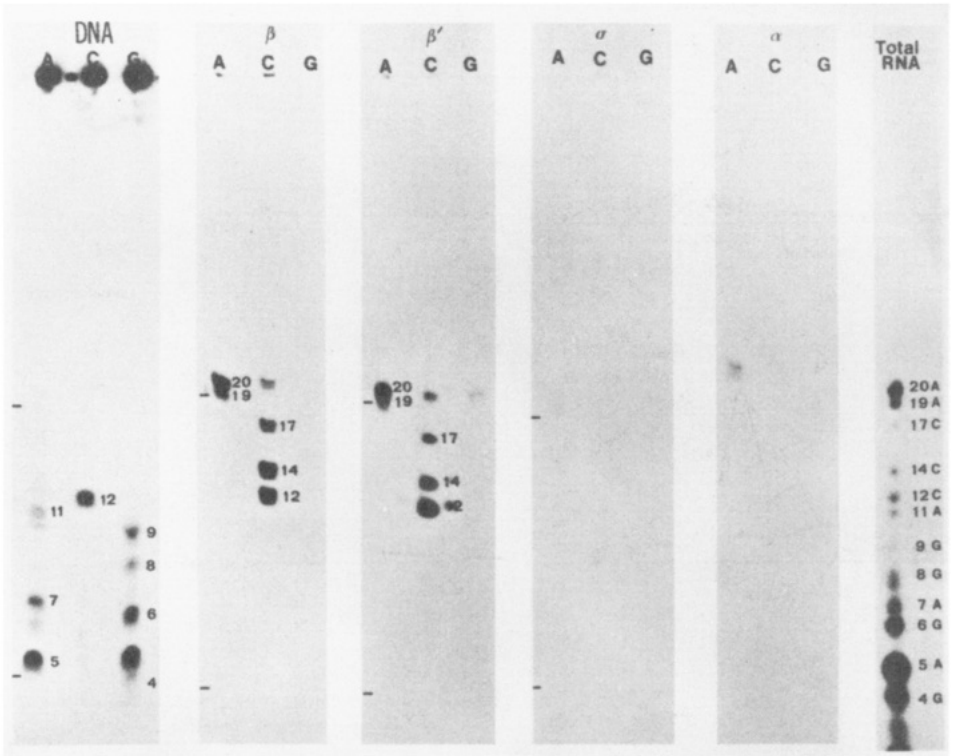

Figure 4. RNA sequencing gels showing the RNA chains containing 4–20 bases that were released from the components of transcription complexes treated as outlined in Figure 3. Labeled components are visualized by autoradiography of ^{32}P-labeled RNA, using X-ray film. The lanes labeled A, C, and G contain RNA chains terminated with 3′-deoxy-ATP, 3′-*O*-methyl-CTP, or 3′-*O*-methyl-GTP. The "Total RNA" lane contained equal amounts from each of these reactions (before photolysis) and shows the distribution of RNA chains present in transcription complexes (or as released abortive initiation products). The numbers identify the RNA chain length of each band, and the letters identify the base at which the chain is terminated. The radioactivity at the top of the DNA panel is due to the DNA itself, which becomes radiolabeled in the presence of RNA polymerase and substrates [9,13]. The bands near 20A in the α panel are artifacts due to comigration of free RNA with the α subunit. Reprinted with permission from ref 13. Copyright 1983 National Academy of Sciences, USA.

detail RNA molecules 11–50 nucleotides long, whose 5′ ends label the β and β′ enzyme subunits with good yield. The thiol's accessibility to the leading end of each transcript was determined by comparing the RNAs cross-linked to ββ′ in thiol-treated samples to controls not treated with thiol. Incubation of T7A1 transcription complexes with 1 mM dithiothreitol for 5 min reduced approximately 36% of the 5′ azides on RNAs 11–13 bases long and approximately 43% on RNAs 28–37 bases long, but practically none on RNAs 40–43 bases long. It is also interesting that the dithiothreitol treatment reduced 34% of the 5′ azides on RNA 12 bases long, but only 14% on RNA 14 bases long; remember that on the T7A1 promoter, the leading end of the transcript appears to move from the DNA template to the interface between β and β′ as the transcript elongates from 12 to 14 bases.

σ–DNA Interactions

The σ subunit of RNA polymerase is responsible for promoter recognition and dissociates from the core polymerase after initiation.[18,19] What is the molecular reason for this intriguing change in affinity? Making the question more complicated, photoaffinity-labeling experiments performed with two different DNA promoters, λ P_R and T7A1, revealed that the σ subunit of RNA polymerase was contacted by the 5′ ends of *different lengths* of nascent RNA.[20] The labeling of ββ′ is quite similar for both promoters while the α subunit was not labeled in either case, suggesting that contacts between RNA and the subunits of the $\alpha_2\beta\beta'$ "core enzyme" are not strongly dependent on the nature of the DNA. On the λ P_R promoter, the σ subunit is labeled by RNA chains 9–13 nucleotides long, whereas on the T7A1 promoter, only the trinucleotide transcript[12] has been shown to contact the σ subunit. Differences in the staging of σ release may be related to differences in transcriptional regulation of the templates. Because of the different base sequences, promoter efficiencies, and other characteristics of different DNAs, it was necessary to make quantitative comparisons of photolabeling yields in order to draw reliable conclusions.

Quantification of Photolabeling

Stackhouse[21–23] developed a way to quantify the photoaffinity labeling produced by aryl azides in transcription complexes. Photoaffinity labeling percent yields were measured as a function of RNA length, by comparing the counts in the electrophoresis bands containing RNA released from protein (or DNA) to

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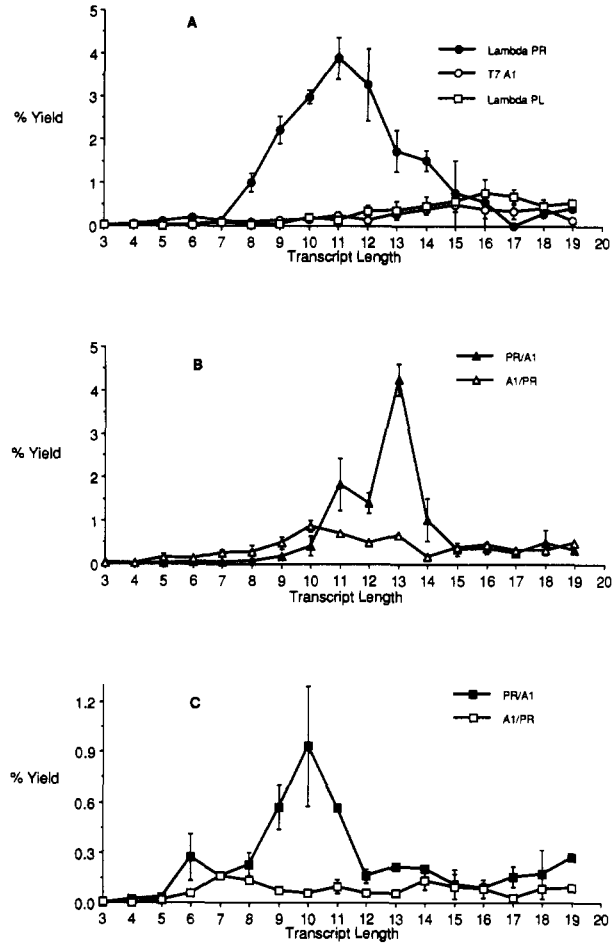

Figure 5. Percent photoaffinity labeling yield on σ as a function of RNA transcript length, analyzed from transcription complexes containing the following DNA: (A) λ P_R (filled circles), T7A1 (open circles), or λ P_L (open squares); (B) P_R/A1 (filled triangles) or A1/P_R (open triangles) DNA. (C) Percent yield of RNA immunoprecipitated with polyclonal antibodies against the σ subunit, analyzed as a function of transcript length from transcription complexes containing the recombinant templates P_R/A1 (filled squares) or A1/P_R (open squares). In each case, the results of two independent experiments are shown (bars) along with their average. Reprinted with permission from ref 22. Copyright 1989 American Chemical Society.

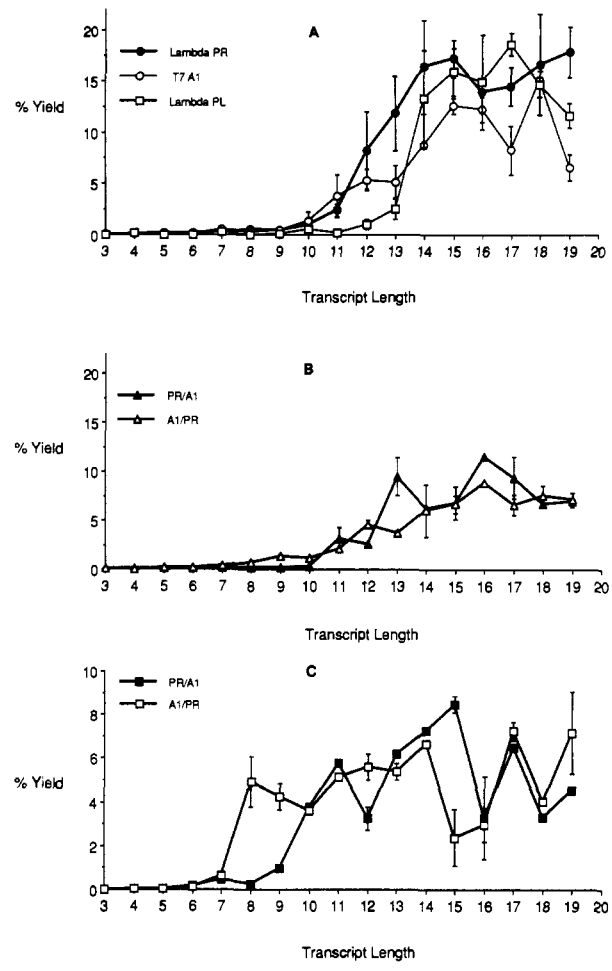

Figure 6. Percent photoaffinity labeling yield on $\beta\beta'$ as a function of RNA transcript length, analyzed from transcription complexes containing the following DNA: (A) λ P_R (filled circles), T7A1 (open circles), or λ P_L (open squares); (B) P_R/A1 (filled triangles) or A1/P_R (open triangles) DNA. (C) Percent yield of RNA immunoprecipitated with polyclonal antibodies against the holoenzyme, analyzed as a function of transcript length from transcription complexes containing the recombinant templates P_R/A1 (filled squares) or A1/P_R (open squares). In each case, the results of two independent experiments are shown (bars) along with their average. Reprinted with permission from ref 22. Copyright 1989 American Chemical Society.

those in bands containing unphotolyzed RNA from the same transcription mixture. Different DNA templates were used. With poly[d(A-T)], the σ subunit is labeled most heavily by transcript lengths of 9–13, with a 15% yield at a length of 10 bases; $\beta\beta'$ together are labeled by 60% of the RNA molecules 14 bases long. When transcribing from the λ P_R template, the σ subunit is labeled by RNAs 8–14 bases long, with a maximum 4% yield at 11 nucleotides (in comparison to 0.7% for either λ P_L or T7A1). Thus photolabeling of σ is unmistakably dependent on the DNA sequence.

It has been pointed out by several researchers that specific interactions between proteins and double-stranded nucleic acids can depend on the spatial distribution of hydrogen-bond donors and acceptors in the major groove of nucleic acid duplex.[24-27] Presumably this donor–acceptor distribution exerts its effect through its affinity for a complementary surface on the protein, containing a matching matrix of hydrogen-bond acceptors and donors.

To determine the nucleic acid region responsible for the different σ interactions, two recombinant DNA templates were constructed. One of these contained the λ P_R promoter and T7A1 transcript, while the other contained the T7A1 promoter and λ P_R transcript. The labeling of transcription complexes containing three natural bacteriophage promoters, λ P_R, λ P_L, and T7A1, and the two recombinant constructs A1/P_R (T7A1 promoter with λ P_R transcript) and P_R/A1 (λ P_R promoter with T7A1 transcript) were compared. Significant labeling of the σ subunit (Figure 5A,B) is observed only on the templates containing the λ P_R promoter region regardless of the transcribed region.

Clear evidence that the labeling occurs within transcription complexes rather than after dissociation and

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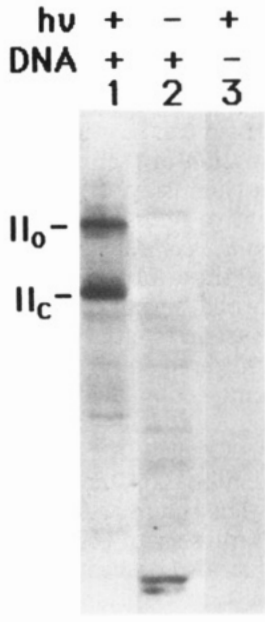

Figure 7. Analysis of photoaffinity-labeled eukaryotic RNA polymerase II subunits by sodium dodecyl sulfate gel electrophoresis, using a 5–17% polyacrylamide linear gradient. Labeled components are visualized by autoradiography of ^{32}P-labeled RNA, using X-ray film. Lane 1 shows the results of a complete experiment (subunits IIo and IIc labeled). The sample in lane 2 was not irradiated, and the sample in lane 3 contained no DNA. Reprinted with permission from ref 28. Copyright 1986 American Society for Biochemistry and Molecular Biology.

reassociation of the RNA photoprobes is provided by a comparison of parts A and B of Figure 5. The RNA photoprobes prepared by using T7A1 DNA in Figure 5A are exactly the same molecules as those prepared by using P_R/A1 DNA in Figure 5B, but only the latter probes label the σ subunit significantly. Likewise, the RNA photoprobes prepared by using λ P_R DNA in Figure 5A are exactly the same molecules as those prepared by using A1/P_R DNA in Figure 5B, but only the *former* probes label the σ subunit significantly.

Further studies on transcription complexes[22] containing A1/P_R and P_R/A1 were performed by using polyclonal antibodies against σ or holo RNA polymerase. Transcripts precipitated by a brief treatment with anti σ are shown in Figure 5C. These results show that less than 0.3% of any given length of RNA synthesized from A1/P_R was precipitated by anti σ, while 1.1% was precipitated in the case of P_R/A1. Labeling of the $\beta\beta'$ subunits occurs similarly on all templates (Figure 6), with yields reaching 15% for transcripts of length >13 bases. These data indicate that the major interactions leading to the release of σ from ternary transcription complexes involve the promoter sequence of the template DNA, "upstream" from the transcribed region.

These experiments have allowed identification of a region of DNA that plays a major role in controlling the release of σ during early stages of transcription. Using similar techniques in the future we can proceed to identify the nucleotide(s) responsible and examine the role of other transcription factors responsible for elongation and termination. We might also be able to identify specific amino acids on the σ, β, and β' subunits that are photolabeled.

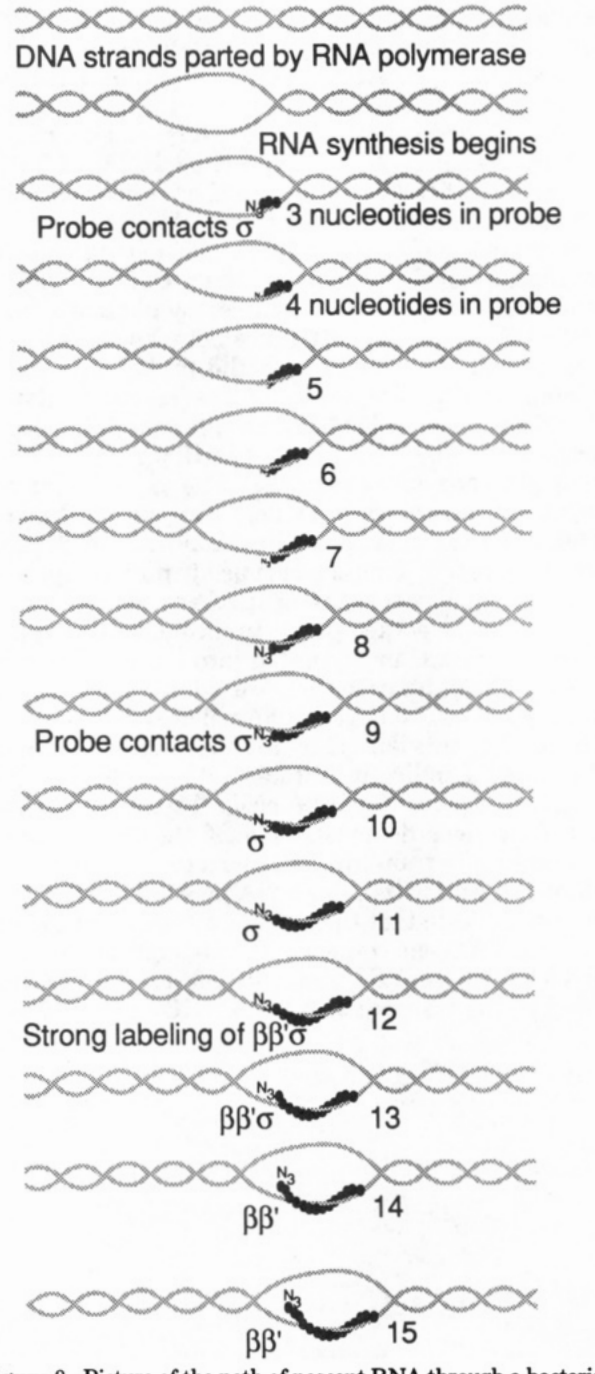

Figure 8. Picture of the path of nascent RNA through a bacterial transcription complex, suggested by photoaffinity mapping (see Figures 4–6). The DNA double helix (top) is partially unwound when the enzyme binds to it. The figures below represent transcription complexes containing RNA 3–15 nucleotides long, with the trailing (3′) end of RNA at the active site of the enzyme and an azide (N_3) at the leading (5′) end. Contact of the azide with the σ subunit, observed for transcription from the λ P_R promoter, is indicated for RNAs ≈9–13 nucleotides long. The heavy labeling of the β and β' subunits, which becomes evident for RNAs longer than ≈12 nucleotides, is also noted.

Human RNA Polymerases

Photoaffinity-labeling techniques have also been utilized by Bartholomew[28,29] to study transcription by eukaryotic RNA polymerase II and III. RNA polym-

(28) Bartholomew, B.; Dahmus, M. E.; Meares, C. F. *J. Biol. Chem.* **1986**, *261*, 14226–14231.
(29) Bartholomew, B.; Meares, C. F.; Dahmus, M. E. *J. Biol. Chem.* **1990**, *265*, 3731–3737.

erases found in eukaryotic (nucleated) cells may be divided into three classes, each with a distinct subunit structure and transcription function. RNA polymerase I catalyzes synthesis of ribosomal RNAs, polymerase II the synthesis of mRNA precursors, and polymerase III the synthesis of tRNA and 5S RNA. Multiple subspecies of RNA polymerase II have been described in a variety of eukaryotic cells. These enzymes are designated as IIO, IIA, and IIB. Bartholomew and coworkers studied the path of nascent RNA through the eukaryotic transcription complex by photoaffinity labeling.[28] A primary difference between these experiments and those done with the prokaryotic *E. coli* system is the complexity of the reaction mixture. Multiple transcription factors are required for selective transcription by RNA polymerase II, whereas in *E. coli* only pure enzyme is required. The experiments with eukaryotic enzymes were performed in fractionated HeLa cell extracts containing hundreds of different proteins, rather than the chemically pure components used for the *E. coli* experiments. Because the 5′-tagged azide primers worked poorly in the eukaryotic system, 4-thiouracil was incorporated into the transcript and used as the photoprobe. On the adenovirus major late promoter, the two largest RNA polymerase II subunits, IIo and IIc, were labeled by RNA transcripts containing 4-thiouracil, indicating interaction between these subunits and the growing RNA chain (Figure 7). This was the first clear demonstration of the importance of subunit IIo in transcription. Bartholomew carried out similar experiments using RNA polymerase III;[29] these results indicate that the nascent transcript of the adenovirus VAI gene contacts the two largest subunits of RNA polymerase III until the transcription complex reaches the transcription factor IIIC binding site, at which point the transcript appears to contact transcription factor IIIC.

Concluding Remarks

As we have seen, the molecular mechanisms by which genes are transcribed can be studied with high resolution by a combination of techniques that all share a common basis in chemistry. The cartoon in Figure 8 provides a qualitative summary of the path of nascent RNA through the *E. coli* transcription complex, based on the results of these experiments. The use of a photochemical probe permits lengthy and elaborate preparation of a system for study before the key chemical reaction is triggered to form cross-links between probe and macromolecule. The ability to dissect and identify the product formed is essential, as is the availability of specific chain terminators that permit the synthesis of terminated RNA molecules covering a large range of lengths. The choice of a dinucleotide substrate analogue which serves as an efficient, specific primer of transcription has also proved important. The great stability of most transcription complexes makes it possible to carry out the experiments without elaborate equipment and analyze the results unambiguously. These natural properties and elements of experimental design have led to information about RNA synthesis that would have been difficult for us to imagine otherwise. They suggest a route to further discoveries.

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