The structure of the Saccharomyces cerevisiae RNA polymerase III transcription complex on the SUP4 tRNA\(^{\text{yr}}\) gene was probed at distances of ~10 to ~23 Å from the C-5 methyl of thymidine in the major groove of DNA using photoreactive aryl azides attached to deoxyuridine by variable chain lengths. The nucleotide analogs contained an azidobenzoyl group attached with chain lengths that were incrementally increased by 2 Å by inserting 1-3 glycine residues into the chain. Another photoreactive deoxyuridine analog was made that contained a butyl chain (ABU-dUMP) to assess the effect of the chain’s hydrophobicity on its ability to photoaffinity label the transcription complex. These nucleotide analogs were incorporated at base pairs (bp) with DNA containing a 4-azidobenzoylglycylglycine; NHS-ABG 3, 4-azidobenzoyl(glycyl)2glycine; NHS-ABG 2, 4-azido-2-aminoethyl]dCMP; NHS-ABG, 4-azidobenzoylglycine; NHS-ABG, 4-azidobenzoyl[glycyl]glycine; ABU, 4-(azidophenyl)butyric acid; 5-aa-dUTP, 5-[N-(p-azido- benzoyl)-3-aminoallyl]-dUMP (AB-dUMP), or deoxycytidine analog, 4-[N-(p-azidobenzoyl)-2-aminoethyl]dCMP (AB-dCMP), were constructed into specific sites in DNA in close proximity to radioactive nucleotides using an immobilized single-stranded DNA template annealed to specific oligonucleotides.

The 27-kDa subunit of TFIIIB or the TATA box binding protein was photoaffinity labeled at bp ~26/–21 with nucleotide analogs containing a ~19– or ~23-Å chain and not with shorter chains of ~10 to ~15 Å in length. The B\(^*\) subunit of TFIIIB (M\(_{r}\) = 90 kDa) was photoaffinity labeled at bps ~26/–21 with DNA containing a ~14-Å chain and not with shorter or longer chains. Cross-linking of the B\(^*\) subunit was inhibited by binding of RNA polymerase III (Pol III) to the TFIIIB-DNA complex and suggested that Pol III binding causes a conformational change in the TFIIIB-DNA complex resulting in the displacement of the 90-kDa subunit at bps ~26/–21. Next, the chain length dependence of photoaffinity labeling the 34-kDa subunit of Pol III at bps ~17 and ~3/–2 indicated that the 34-kDa subunit of Pol III is slightly removed from the major groove at bp ~17 in the initiation complex and makes closer contact at bps ~3/–2 in a stalled elongation complex.

Transcription of yeast tRNA genes requires RNA polymerase III (Pol III)\(^*\) with 16 different subunits, transcription factor (TF) IIC with 5–6 different subunits, and TFIIIB with 3 different subunits (1–4). TFIIIC can be completely reconstituted from recombinant proteins and has as one of its subunits the universal transcription factor TBP or the TATA box binding protein (5–15). TFIIIB is recruited to a site on DNA upstream of the start site of transcription through interactions with TFIIIC bound downstream to two internal promoter elements called box A and box B (16). Once TFIIIB is bound to DNA it binds extremely tightly as evident by it being insensitive to high salt concentrations or polyanions, and alone is responsible for the binding of Pol III to the start site. The N terminus of the Brf subunit (67 kDa) of TFIIIB has sequence homology with TFIIIE, an RNA polymerase II (Pol II) transcription factor, and Brf has been shown by the two hybrid system to interact with the 34-kDa subunit of Pol III (17, 18).

In order to study the mechanism of promoter recognition by Pol III, we and others have used DNA photoaffinity labeling to map the positions of transcription factors and Pol III subunits to specific sites in DNA in initiation and elongation complexes (19–24). The photoreactive deoxyuridine analog, 5-[N-(p-azidobenzoyl)-3-aminoallyl]-dUMP (AB-dUMP), or deoxycytidine analog, 4-[N-(p-azidobenzoyl)-2-aminoethyl]dCMP (AB-dCMP), were constructed into specific sites in DNA in close proximity to radioactive nucleotides using an immobilized single-stranded DNA template and site-specific oligonucleotides. The photoreactive DNA was used to radioactively tag protein(s) associated near specific sites on DNA using either highly purified proteins or crude protein extracts. The Brf subunit of TFIIIB has been shown to interact extensively with the central and 3’ end of the TFIIIB binding region or UBR and the B\(^*\) subunit (M\(_{r}\) ~ 90 kDa) to be associated with the 5’ end of the UBR by DNA photoaffinity labeling and DNase I footprinting (21, 24). The 34- and 128-kDa subunits of Pol III has been shown to be in close contact with TFIIIB by being photoaffinity labeled at base pairs within the UBR (22).

We have expanded the information garnered from DNA photoaffinity labeling by redesigning the photoreactive deoxyuridine nucleotide to have different linker arms connecting the photoreactive aryl azide to the nucleotide base to probe at various distances from the major groove of DNA. In this report we show that this series of deoxyuridine analogs could be effectively incorporated into DNA at specific sites using the exonuclease-free Klenow fragment of DNA polymerase I and that these DNA probes were able to reveal additional details of the topography of the Pol III transcription complex. The 27-kDa subunit (or the TATA box binding protein) of TFIIIB was mapped to bps ~26/–21 and ~19 to ~23 Å from the major groove of DNA. The proximity of the other two subunits of TFIIIB and several subunits of Pol III were examined at bps ~26/–21, ~17, and ~3/–2, with +1 being the start site of transcription.

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\(^1\) The abbreviations used are: Pol III, RNA polymerase III; TF, transcription factor; TBP, TATA box binding protein; AB-dUMP, 5-[N-(p-azidobenzoyl)-3-aminoallyl]-dUMP; AB-dCMP, 4-[N-(p-azidobenzoyl)-2-aminoethyl]dCMP; NHS-ABG, 4-azidobenzoylglycine; NHS-ABG, 4-azidobenzoylglycine; NHS-ABG, 4-azidobenzoylglycine; ABU, 4-(azidophenyl)butyric acid; 5-aa-dUTP, 5-[N-(3-aminoallyl)]deoxouridine triphosphate; bp, base pair(s); PAGE, polyacrylamide gel electrophoresis.
EXPERIMENTAL PROCEDURES

Synthesis of the N-Hydroxysuccinimide Esters of 4-Azidobenzoylglycine (NHS-ABG), 4-Azidobenzoyglycine (NHS-ABG), and 4-Azidobenzoylglycine triphosphate (NHS-ABG3) were obtained from Aldrich Chemical. All reactions were done under limited and indirect lighting using a 60 watt incandescent lamp. Para-azidobenzoyl (ABU)-dUTP at 250 nm, and 26 mM 

After the photoaffinity labeling reaction was incubated at 25°C for 30 min, divided into 4–50-μl aliquots, and irradiated for 2 min. The samples were pooled together and digested with DNase I and S1 nuclease as described previously, except for the omission of washing the DNA beads with 0.1 M Tris-HCl, pH 8.0 (5% Triton X-100). In the same manner DNA photoaffinity probes containing photoreactive deoxyuridine analogs were made at bps −17 and −3/−2, except that the modified nucleotide was incorporated first and unincorporated nucleotide removed by washing the immobilized DNA before the incorporation of [32P]dATP or [32P]dCTP. The concentrations of photoreactive deoxyuridine nucleotides were the same for the DNA probes at bps −17 as that for bps −26/−21, but for the synthesis of DNA probes at bps −3/−2 the final concentration was 0.5 μM for all five nucleotides.

RNA polymerase III transcription complexes were formed and photoaffinity labeled as described using protein extracts made from Saccharomyces cerevisiae strains BJ926 and NZ90 (19). The NZ9 strain was kindly provided by Nick Zecherle and Benjamin Hall’s (University of Washington) and has the second largest subunit of RNA polymerase III epitope tagged at the N terminus with four copies of the octapeptide FLAG sequence (IBI/Kodak). Unless otherwise indicated all experiments were done using proteins derived from the BJ9326 strain. After photocross-linking, the samples were treated with DNase I and S1 nuclease, and analyzed on a 4–20% gradient SDS-polyacrylamide gel. The dried gel was exposed to XAR-5 film. In some experiments highly purified TFIIIB and TFIIIC were used instead of the 500 mM KCl fraction from Bio-Rex 70 chromatography of the S-100 extract and, in addition to photoaffinity labeling, were examined by gel-shift assay as performed previously (22). Highly purified Pol III was obtained using a strain with the second largest subunit of Pol III histidine tagged and purified by Ni-NTA chromatography (19). The appropriate amount of Pol III to be added was determined by gel shift assay using a fixed amount of TFIIIB and TFIIIC.

Transcription assays with the modified DNAs were conducted to examine the effect of the modification on the ability of the DNA to form competent transcription complexes and has been described previously (19).

Immunoprecipitation of Photoaffinity Labeled TBP—A 200-μl photoaffinity labeling reaction was incubated at 25°C for 30 min, divided into 4–50-μl aliquots, and irradiated for 2 min. The samples were pooled together and digested with DNase I and S1 nuclease as described. After adjusting the pH to 7 by the addition of 0.5 M Tris base, the concentration of NaCl was adjusted to 0.2 M, and then 3 volumes (750 μl) of buffer A (20 mM NaHEPES, pH 7.8, 10% glycerol, 300 mM NaCl, 0.1% Nonidet P40, 10 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin and pepstatin) was added. Affinity purified rabbit anti-TBP antibody (1.64 μg) was added and the reaction incubated at 4°C overnight. The antibody-antigen complex was precipitated by the addition of 5 μl of a suspension of 1 mg/ml of Sepharose 4B (6 mg/ml) equilibrated in buffer A and gently mixed for 1 h at 4°C. The Sepharose 4B resin was washed 3 times with 500 μl of buffer A, making sure to leave 20–30 μl of buffer behind each time. Eluted immunoprecipitated proteins from resin by the addition of one-fourth of the volume of the resin suspension of buffer B (10% sodium dodecyl sulfate, 350 mM Tris-HCl, pH 6.8, 25% [v/v] 2-mercaptoethanol, 0.25% bromphenol blue) and heating at 90°C for 5 min. Samples were analyzed by SDS-PAGE and autoradiography.

Molecular Modeling of B-DNA containing Single Photoreactive Deoxyuridine Analogs—Molecular modeling of the photoreactive deoxyuridine analogs incorporated into B-DNA was performed using the TOM/FRODO program on a Silicon Graphics System. Possible conformations of the modified nucleotide were examined by adjusting the dihedral angles of the bonds from the methylene carbon atoms. The amide and allyl bonds were fixed in their planar conformation. The atomic coordinates of the DNA were taken from the crystal structure of the doxycar DggCgAAATTCggcG (Protein Data Bank code 1BNA) originally characterized by Dickerson and colleagues (30).

RESULTS

Synthesis of DNA Photoaffinity Probes Containing an Aryl Azide Attached to DNA by Variable Chain Lengths—Deoxyuridine analogs having a photoreactive aryl azide attached by variable chain lengths were synthesized and incorporated into DNA to cross-link protein bound to DNA at different distances from the major groove of DNA in order to probe the structure of yeast RNA polymerase III transcription complexes. The nucleotide analogs all contained the initial linker consisting of an amino acid group attached to the C-5 carbon of deoxyuridine. The amino group was used to attach on several aryl azido
compounds containing one, two, three, or no glycine residues to create a ~4.3-Å incremental increase in the tether length used to attach the photoactive moiety to deoxyuridine (Fig. 1). A more hydrophobic linker was also tested by making a nucleotide analog with a butyl group (ABU-dUTP) instead of glycine (ABG-dUTP), both with about the same overall chain length. The modified nucleotides were purified by anion exchange chromatography and analyzed by PEI-cellulose thin layer chromatography (TLC).

The relative purity and uniformity of the deoxyuridine analogs were indicated by the detection of a single UV quenching spot from TLC analysis and the detection of single primer extension products at equivalent concentrations. The DNA probe with the shortest chain length of DNA polymerase I (exonuclease-free). At higher concentrations the modified nucleotide read-through in the primer extension assay was detected, but this was also observed for fast protein liquid chromatography purified deoxyuridine triphosphate (dTTP) at equivalent concentrations. The approximate distance in angstroms from the carbon attached to the C-5 of uridine to the azido group is indicated in brackets for each nucleotide analog.

The 27-kDa subunit of Pol III (67 kDa) and the second largest subunit of Pol III (128 kDa). Photoaffinity labeling of these two polypeptides was shown to be TFIIIC-dependent by DNA competition with plasmid DNA containing an up mutant of the SUP4 gene (result not shown). The 67-kDa protein was labeled in the presence of heparin (100 μg/ml); whereas labeling of the 120/128-kDa protein was eliminated, consistent with the 67-kDa protein being a subunit of TFIIIB (lane 6). The Brf subunit of TFIIIB was more efficiently cross-linked with photoactive nucleotides containing longer chain lengths ≥~14 Å and a new protein with an apparent molecular mass of about 27 kDa was detected with analogs that have chain lengths of ~19 and ~23 Å (Fig. 3A, lanes 1–5). Photoaffinity labeling of the 27-kDa protein was not eliminated by the addition of heparin (100 μg/ml) or ATP, CTP, and UTP (100 μM) (Fig. 3A, lanes 8, 9, 13, and 14) and was shown to be TFIIIC-dependent (results not shown). The heparin-resistant photoaffinity labeling of the 27-kDa polypeptide suggested that this is a subunit of TFIIIB and by apparent electrophoretic mobility to be the TATA box binding protein (TBP) subunit of TFIIIB. The photoaffinity labeled 27-kDa protein was shown to be TBP by the highly selective immunoprecipitation of the 27-kDa protein using rabbit anti-TBP antibody (Fig. 3B).

Photoaffinity Labeling of the B' Subunit of TFIIIB (90 kDa) Required a ~14 Å Chain Length and Was Inhibited by the Binding of Pol III—A 90-kDa protein was photoaffinity labeled in transcription complexes by photoactive deoxyuridine analogs with ~14-Å chain length at bps −26/−21 that have heparin or ATP, CTP, and UTP added and photoaffinity labeled consistently less in the initiation complex (Fig. 3A, lane 2 compared to lanes 7 and 12). Photoaffinity labeling of the 90-kDa subunit has a specific tether or chain requirement, as it is not readily labeled by analogs containing chain lengths of −10 or −19–23 Å (compare lanes 7 and 12 with lanes 8, 9, 13, and 14) nor by analogs containing approximately the same chain length but a more hydrophobic chain, i.e. ABU-dUMP (compare lanes 7 and 12 with lanes 10 and 15). The conditions under which the 90-kDa subunit of TFIIIB was cross-linked suggested that when Pol III initially binds to the TFIIIB-DNA complex, it causes a conformational change or displacement of the 90-kDa subunit at a position −14 Å from base pairs −26/−21. The dislocation of the 90-kDa subunit by Pol III appears to be reversed by either stripping Pol III from the complex by the addition of heparin or by initiating transcription resulting in the downstream transposition of Pol III.
Fig. 2. Incorporation of AB-dUMP, ABG₃-dUMP, and ABU-dUMP into regions of DNA upstream of the start site of transcription of the SUP4 tRNA gene. A, photoaffinity probes. The DNA sequence from the SUP4 tRNA gene from bp −42 to +13 with +1 being the start site of transcription is shown with the modification sites highlighted above for the three DNA photoaffinity probes 1 and 2. The photoactive deoxuridine is indicated by U and the radioactive nucleotide is marked by an asterisk. The shaded boxes below the sequence indicate the DNase I footprint of TFIIIB (bp −42 to −5) and the extreme 5′ end of the footprint attributable to Pol III in the initiation and elongation complexes. In the footprint of TFIIIB-Pol III initiation complex, the individual contributions cannot be assessed unequivocally on the basis of DNase I footprinting alone and is therefore symbolized by a diagonal boundary. B, site-directed incorporation of photoactive deoxuridine. Samples from the initial step in the synthesis of DNA photoaffinity probes were analyzed by polyacrylamide gel electrophoresis and autoradiography as described under “Experimental Procedures.” The photoactive deoxuridine nucleotides were incorporated at bps −26, −24, −22, −21 (lanes A-E); −17 (lanes F-J); and −3, −2 (lanes K-O) along with [α-32P]dGMP at bps −25 and −23 (lanes A-E) or [α-32P]dAMP at −18 (lanes F-J) or [α-32P]dATP at −1 (lanes K-O) with +1 being the start site of transcription. Samples in lanes A, F, and K had AB-dUMP; lanes B, G, and L had ABG₃-dUMP; lanes C, H, and M had ABG₃-dUMP; lanes D, I, and N had ABG₃-dUMP; and lanes E, J, and O had ABU-dUMP incorporated into DNA.

Highly purified TFIIIIC, TFIIIIB, and Pol III were used in photoaffinity labeling experiments with probes containing ABG₃-dUMP (−14 Å chain length) at bps −26/−21 in order to monitor the extent of complex formation of Pol III-TFIIIIC-TFIIIIB-DNA by gel mobility shift assay (Fig. 4). No significant protein cross-linking was detected with TFIIIIC alone, but upon addition of TFIIIIB, 90- and 67-kDa subunits of TFIIIIB could readily be detected (lanes 2 and 3). If Pol III was added the photoaffinity labeling efficiency of the 90-kDa protein was reduced, whereas the labeling efficiency of the 67-kDa subunit remained constant (lane 4). Gel shift analysis showed that the majority of complexes formed in the presence of TFIIIIC, TFIIIIB, and Pol III were the fully assembled initiation complexes, but that some TFIIIIC-TFIIIIB-DNA complexes were still present (result not shown). It might be that the residual amount of photoaffinity labeling of the 90-kDa subunit observed after the addition of Pol III or that in the BR500 extract is due to a fraction of the complexes containing TFIIIIC and TFIIIIB only.

Proximity of the 34-kDa Subunit of Pol III and the 67-kDa Subunit of TFIIIIB to DNA at bp −17—The 34-, 67-, and 120/128-kDa polypeptides were more efficiently photoaffinity labeled at bp −17 by DNAs containing AB-dUMP and ABG₃-dUMP with −14- and −19-Å chain lengths than with DNAs containing AB-dUMP and ABG₃-dUMP with −10- and −23-Å chain lengths (Fig. 5, lanes 1–4). Photoaffinity labeling of the 34-kDa protein was eliminated by the addition of ATP, UTP, and CTP (lanes 5–8), consistent with this protein being the 34-kDa subunit of Pol III and therefore translocated on DNA upon elongation. The 34- and 120/128-kDa proteins were not photoaffinity labeled with the addition of heparin to the initiation complex (lanes 9–12) or to the elongation complex (lanes 13–16). The persistent labeling of the 67-kDa protein in initiation or elongation complexes and in the presence of heparin is consistent with it corresponding to the Brf subunit of TFIIIIB. The greatly enhanced labeling efficiency of both the 34-kDa subunit of Pol III and the 67-kDa subunit of TFIIIIB with
The ability
of photoreactive DNA to form active Pol III transcription complexes was examined by measuring the transcriptional efficiency of the SUP4 tRNA⁷⁵ gene containing modified deoxyuridine versus unmodified DNA. DNA containing four modified deoxyuridines incorporated at bps −26, −24, −22, and −21 was found to be an effective DNA template for Pol III transcription with the amount of RNA transcripts obtained ranging from 75 to 100% of that obtained with plasmid DNA containing the SUP4 tRNA⁷⁵ gene. The transcription assays were repeated multiple times and in duplicates to verify the reproducibility of these transcription assays. Transcription assays with modified DNAs containing either one or two photoreactive deoxyuridines at bps −17 or −3 and −2 gave similar transcriptional activities to that of the DNA photolabile at bps −26/−21. We concluded that DNA modifications with varied chain length had no significant effect on the formation of the transcription complex or catalysis of RNA. Any difference in photoaffinity labeling with varied chain length should therefore be due to the placement of the photoreactive group and not its interference with the formation of the transcription complex. Previously the incorporation of ABU-dUMP at these same and other positions had been shown not to interfere with transcription complex formation by gel-shift and DNA competition assays using highly purified Pol III, TFIIC, and TFIIB (20–22).

DISCUSSION

In order to more fully map the topography of the yeast RNA polymerase III transcription complex, we have synthesized deoxyuridine analogs with a photoreactive aryl azide attached to the C-5 carbon of uridine tethered with chain lengths of approximately 10, 14, 15, 19, and 23 Å. The chain lengths were incrementally increased by 4.3 Å by inserting 1–3 glycine residues into the chain to carefully monitor the effect of chain length on protein-DNA cross-linking. A second photoreactive deoxyuridine was made that contained a more hydrophobic chain (ABU-dUMP) to assess the efficiency of the chain’s hydrophobicity on its ability to photoaffinity label the transcription complex. These nucleotide analogs were efficiently incorporated into DNA by primer extension using the Kleneq fragment of DNA polymerase I. The incorporation of the photoreactive nucleotide was targeted to specific sites in DNA (bps −26/−21, −17, or −3/−2 of the SUP4 tRNA⁷⁵ gene) along with a radioactive label using immobilized single-stranded DNA and several specific oligonucleotides.

By increasing the chain length of the photoreactive deoxyuridine it was possible to photoaffinity label protein(s) associated with the protein-DNA complex that do not make close contact with the major groove of DNA. Photoaffinity labeling at bps −26/−21 in the SUP4 tRNA⁷⁵ gene revealed that the 27- and 90-kDa subunits of TFIIB were cross-linked only with those DNA probes containing longer chain lengths.

The 27-kDa subunit of TFIIB or the TATA box binding protein (TBP) was photoaffinity labeled by DNAs containing either ABG₂-dUMP (−19 Å) or ABG₃-dUMP (−23 Å), and not by DNAs containing AB-dUMP (−10 Å), ABG-dUMP (−14 Å), or ABU-dUMP (−15 Å). Although the SUP4 tRNA⁷⁵ gene has been extensively studied by DNA photoaffinity labeling, the TBP subunit of TFIIB had not been cross-linked before in the fully assembled initiation complex; although it was known by several lines of evidence that TBP was a component of TFIIB (5, 6, 14, 15, 31, 32). TBP had been photoaffinity labeled using AB-dUMP on the Pol II transcribed adenosine major late promoter and in the context of the Pol I transcription factor TIF-1B (33, 34). Our data shows that TBP is located at a greater distance from the major groove of DNA than that of the assembled Pol II transcription complex on the adenosine major late promoter or Pol I transcription complex.

The positioning of TBP from 21 to 26 bps upstream of the start site of transcription is reminiscent of the position of TBP when bound to the TATA box of the U6 snRNA gene (35, 36). TFIIB can bind to the U6 snRNA gene without TFIIC and is recruited to the complex through TBP-TATA box interactions. In other experiments with the SUP4 tRNA gene, the assembly of TFIIB onto DNA is shown to be a concerted effort of TFIIC bound downstream and TBP binding to AT-rich sequences located 22–30 bps upstream of the start site of transcription (37). Although the requirement of AT-rich sequences indicates the requirement of TBP binding to DNA in the assembly of TFIIB onto DNA, it was unclear whether TBP stays associated with DNA in the final complex. TBP was suggested to bind transiently to DNA and then be subsequently displaced by the B′ subunit of TFIIB. Furthermore, using a less purified system, the requirement for the upstream AT-rich sequence was minimal in contrast to that with highly purified components. It is therefore significant that the cross-linking data demonstrates the location of TBP at bps −26 to −21 in the fully assembled complex using the less purified system.

Molecular modeling of some of the possible conformations of the modified nucleotides incorporated into DNA demonstrated that ABG₂-dUMP and ABG₃-dUMP have sufficient length for the photoreactive group to contact the minor groove, whereas the analogs with shorter chain lengths would not. The tethered photoreactive group of AB-dUMP and ABG₂-dUMP were modeled extending from the center of the major groove and wrapping over the phosphate backbone with the shortest path possible to the minor groove.

Next, photoaffinity labeling of the 90-kDa subunit of TFIIB at bps −26/−21 not only had a fairly strict chain length requirement of −14 Å (ABU-dUMP), but also was most efficiently labeled in those complexes which had Pol III removed from TFIIB-DNA complexes by the addition of heparin to the initi-
The 34-kDa subunit of Pol III was most efficiently photoaffinity labeled using the longer chains, the efficiency of photoaffinity labeling varied a great deal with chain length and helped to suggest the relative proximity of the protein to the major groove of DNA. The 34-kDa subunit of Pol III was much more efficiently labeled with ABG-dUMP (-14 Å) or ABG2-dUMP (-19 Å) at bp -17 than with the shorter chain length of AB-dUMP (-10 Å) in the initiation complex, suggesting that the 34-kDa subunit is slightly removed from the major groove of DNA. However, at bp -3/-2 in the stalled elongation complex, the 34-kDa subunit of Pol III was most efficiently photoaffinity labeled with DNA containing AB-dUMP; the implication is that the 34-kDa subunit is in close contact with the major groove. The 67-kDa subunit of TFIIIB also appears to be slightly removed from DNA at bp -17 in the initiation complex and the 128-kDa subunit of Pol III to make fairly close contact with DNA at bps -3/-2 in the stalled elongation complex.

The composition of the initiation complex appears to be potentially important as evident when comparing photoaffinity labeling with DNA probes containing ABG-dUMP versus ABU-dUMP at bps -26/-21 and to a lesser degree at -3/-2. The 90-kDa subunit of TFIIIB was cross-linked by DNA containing ABG-dUMP and not with DNA containing ABU-dUMP at bps -26/-21. It is unlikely that the absence of cross-linking is due to the -0.5-Å additional chain length of ABU-dUMP, but is probably due to a possible repulsion of the hydrophobic chain by a nearby charged surface of the protein. DNA containing ABU-dUMP at bps -3/-2 had the opposite effect and more efficiently photoaffinity labeled the 90-kDa subunit of TFIIIB than DNA containing ABG-dUMP, indicating that ABU-dUMP is not less phoreactive. Finally the use of longer alkyl chains, as often found in available photochemical reagents, may have some inherent problems due to their hydrophobicity. Photoactive deoxyuridine nucleotides with hexyl linkers have significant amounts of nonspecific cross-linking, apparently due to hydrophobic interactions with proteins not bound to specific sites on DNA.

Previously with a set chain length in the photoreactive nucleotide, DNA photoaffinity labeling gave a two-dimensional view of the transcription complex by correlating the position of a particular protein with a specific site on DNA. The added dimension afforded by systematically probing at different distances from DNA has given a more three-dimensional perspective of the transcriptional complex, and has allowed for the positioning of some previously unmapped proteins in the complex as well as giving information about proximity to DNA. Although this information is of particular use to those interested in the structure of the yeast Pol III transcription complex, the approach will be of potential use for studying other protein-DNA complexes as well.

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Fig. 7. Model for the displacement of the 90-kDa subunit of TFIIIB at bps -26/-21 by the association of Pol III. The three subunits of TFIIIB are represented as filled-in shapes at three different stages of transcription: 1) binding of TFIIIB to DNA upstream of the start site of transcription; 2) the subsequent binding of Pol III; and 3) translocation of Pol III as a result of transcriptional elongation. The distance or placement of TFIIIB subunits relative to DNA is suggested by its proximity to DNA. RNA polymerase III is represented as an open circle and is not necessarily in scale with the subunits of TFIIIB.

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Mapping Protein-DNA Contacts at Various Distances from DNA

Mapping the Contacts of Yeast TFIIIB and RNA Polymerase III at Various Distances from the Major Groove of DNA by DNA Photoaffinity Labeling

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