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Orientation and Topography of RNA Polymerase III in Transcription Complexes

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A photo-cross-linking method has been used to map the subunits of *Saccharomyces cerevisiae* RNA polymerase (Pol) III with respect to DNA in binary (preinitiation) and ternary (RNA-elongating) transcription complexes. Transcription factor- and Pol III-containing complexes have been assembled on *S. cerevisiae SUP4* $tRNA^{Tyr}$ gene probes containing the photoactive nucleotide 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl]-dUMP in different specified positions. Covalent DNA-protein linkages form upon irradiation of these complexes, and the Pol III subunits that are cross-linked to individual positions in the *SUP4* tRNA gene have been identified. RNA Pol III cross-linking has been shown to require the box B downstream promoter element of the tRNA gene and the presence of transcription factor TFIIIB. Further proof of specificity has been provided by demonstrating that particular Pol III subunits move out of the range of upstream-placed photoactive nucleotides, and that others move into the range of downstream-placed photoactive nucleotides, as a consequence of initiating and elongating RNA chains. Binding and specific placement of Pol III have also been shown to require both the B′ and the B″ components of TFIIIB. Nine Pol III subunits are cross-linked from different positions of the *SUP4* tRNA gene's nontranscribed strand. In binary transcription complexes, the two largest Pol III subunits are accessible to photo-cross-linking over the entire stretch of the DNase I footprint. The 27- and 34-kDa Pol III subunits are also relatively extended along DNA; its upstream projection makes the 34-kDa subunit a candidate for interaction with TFIIIB, while the 27-kDa subunit is accessible to photo-cross-linking from the leading edge of the Pol III binding site. Several subunits, including the 82- and 53-kDa subunits in binary transcription complexes, are relatively localized in their accessibility to cross-linking. Multiple Pol III subunits are accessible to specific cross-linking from a single photoactive nucleotide in the middle of the transcription bubble of an arrested ternary transcription complex. It is suggested that this precisely placed transcription complex comprises a dynamic ensemble of structural states rather than a single perfectly constrained entity.

To engage in transcription, a tRNA gene must form a remarkably complex protein assembly that involves more than 20 polypeptides and occupies a 120- to 200-bp stretch of DNA (reviewed in references 7, 8, and 19). The assembly process starts with transcription factor TFIIIC, which is composed of at least five and probably six different subunits and has a molecular weight of 500,000 to 650,000 (1, 4, 23). The orientation of TFIIIC on DNA is determined by the two transcription unit-internal promoter elements that serve as its binding sites. One TFIIIC subunit projects upstream of the transcriptional start site and probably plays the key role in recruiting TFIIIB, which is composed of at least three subunits: two proteins with approximate molecular masses of 70 and 90 kDa (2, 14) together with the 27-kDa TATA-binding protein (also called TFIID) (5, 16a, 23). Once it has been deposited on its 40-bp DNA site, TFIIIB is able, on its own, to position the 15-subunit RNA polymerase III (RNA Pol III) (7) over the transcriptional start site. Although TFIIIB-directed Pol III binding occurs at 0°C, higher temperatures are required to open the promoter so that RNA synthesis can be initiated (15).

We have used a photochemical cross-linking method to map the disposition of proteins within Pol III transcription complexes, focusing first on TFIIIC and subsequently on TFIIIB (1, 2). In this method, a photoactive nucleotide, 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl]-dUMP (N_3RdUMP), is built into a specific position in a selected strand of double-stranded DNA, in close proximity to a radioactive nucleotide. After transcription complexes of various kinds have been assembled on this DNA, they are briefly irradiated with UV light. In the subsequent photochemical reaction, a protein that is located in the vicinity of the photoactive nucleotide can become covalently linked to DNA. {The distance of the photoactive function from the pyrimidine ring in N_3RdUMP is 9 to 10 Å (0.9 to 1 nm). The 5-[*N*-(*p*-azidobenzoyl)-3-aminoallyl] side chain projects from the 5 position of the pyrimidine ring into the major groove and its surrounding space. Thus, a protein should not have to project into the DNA major groove, or be engaged in base pair-specific binding, in order to be cross-linked.} In this work, we extend the analysis of the topography of transcription complexes by examining the disposition of its subunits when Pol III binds at the start site of transcription of a tRNA gene and when it elongates an RNA chain, forming a ternary transcription complex.

MATERIALS AND METHODS

Transcription proteins and plasmids. RNA Pol III and transcription factors TFIIIC and TFIIIB were purified as described previously (14). M13 DNA and plasmid DNA containing *SUP4* $tRNA^{Tyr}$ genes with promoter-up and

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promoter-down mutations have been described previously (1).

Synthesis of DNA photoaffinity probes. Probes were synthesized as described previously (2), with the following modifications. Oligonucleotides (500 pmol) were 5′ phosphorylated with 60 U of T4 polynucleotide kinase (New England Biolabs) and 0.2 mM [γ-^{32}P]ATP (low-level radioactively tagged at approximately 5 Ci/mmol) in 40 μl of 50 mM Tris-HCl (pH 7.5)–10 mM $MgCl_2$–5 mM dithiothreitol–0.2 mM EDTA–0.1 mM spermidine–100 μg of bovine serum albumin (BSA) per ml at 37°C for 30 min. The reaction was stopped by adding EDTA to 14 mM, and the oligonucleotide was isolated on a proprietary matrix (Qiagen tip 5 column) as instructed by the manufacturer. The extent of 5′ phosphorylation was monitored by incorporating a single ^{32}P-labeled deoxynucleotide (3,000 to 6,000 Ci/mmol) at the 3′ end of the oligonucleotide, annealed to M13 DNA. The relative proportions of primer-extended ^{32}P-labeled oligonucleotide with and without 5′-phosphate were determined on 8 or 10% denaturing polyacrylamide gels. DNA probe synthesis using phosphorylated oligonucleotide primers proceeded as previously described, except for the ligation of nicks immediately before *Bam*HI digestion, with 1 U of T4 DNA ligase per 30-μl reaction volume and 0.3 mM ATP at 37°C for 60 min. T4 DNA ligase was inactivated by adding KCl to 100 mM and heating at 65°C for 15 min. Next, *Bam*HI was added for an additional 60 min at 37°C. The ligated, *Bam*HI-cleaved ~270-bp DNA probe was purified on a 4% nondenaturing polyacrylamide gel as described previously (2). The efficiency of ligation of probes made on our standard *SUP4* tRNA gene (with a promoter-up mutation) varied between 85 and 95%, except for probe +16 (Fig. 1), which was 68% ligated. Probes made on a box B promoter-down mutant *SUP4* gene were used as controls for nonspecific binding of proteins. These latter probes (75 to 95% ligation) were closely matched to the ligation efficiency of the corresponding standard gene probe or, in one case, less efficiently ligated.

DNA photoaffinity probes were also made with the biotinylated nucleotide Bio-11-dUTP (Sigma Chemical Co.) incorporated at the 5′ end of the synthesized strand. The site-specific oligonucleotide was annealed to single-stranded M13 DNA and primer extended with N_3RdUTP and ^{32}P-labeled deoxynucleoside triphosphate as described previously (1), using exonuclease-free Klenow fragment DNA polymerase I (U.S. Biochemical). Free deoxynucleotides were removed on Sephacryl S-400 (Pharmacia) 200-μl spun columns, and DNA synthesis continued with the addition of all four deoxynucleoside triphosphates (to 200 μM), BSA (to 100 μg/ml), and 1 U of exonuclease-free Klenow fragment DNA polymerase. After 5 min at 37°C, the reaction was stopped by adding 0.2 to 0.4% sodium dodecyl sulfate (SDS) and heating at 65°C for 10 min. The SDS, nucleotides, and inactivated DNA polymerase were removed by gel filtration on a Sephacryl S-400 spun column. The second oligonucleotide primer, 5′-CTCTAGAGGATCCTTTAG-3′ (3 pmol of oligonucleotide per pmol of M13 DNA; BSA added to 100 μg/ml), was annealed to its upstream site (the *Bam*HI cleavage site of the multiple cloning site) for 30 min at 37°C. DNA synthesis was continued with 1 to 5 U of T4 DNA polymerase (GIBCO/BRL) per 25-μl reaction, 25 μM Bio-11-dUTP, 50 μM dCTP, and 100 μM dATP at 37°C for 10 min, allowing incorporation of seven Bio-11-dUMP residues per DNA template downstream of the second oligonucleotide primer. Finally, all four deoxynucleotides were added to final concentrations of 500 μM each, and DNA synthesis continued at 37°C for 10 min. The DNA product was ligated with T4 DNA ligase and cleaved with *Bam*HI, and the restriction fragment was purified as described above.

Photoaffinity labeling of DNA-protein complexes. Complexes of TFIIIB and TFIIIC with the DNA photoaffinity probes were formed as described previously (2). Preinitiation transcription complexes were made by prior incubation of TFIIIC and TFIIIB with DNA for 30 min at 21°C, followed by the addition of 7 fmol of active RNA Pol III (determined by specific transcription assay) (17) to 2 fmol of DNA template in a final volume of 25 μl for 30 min. Arrested ternary transcription complexes were formed from preinitiation complexes by adding ATP, CTP, and UTP to 100 μM each and incubating the mixture at 21°C for 15 min. RNA Pol III in nontranscribing or inactive preinitiation complexes was removed by adding heparin to 100 μg/ml. Conditions for photo-cross-linking and nuclease treatment using DNase I and S1 nuclease have been described elsewhere (2). Formation of protein-DNA complexes was analyzed by electrophoresis in nondenaturing 4% polyacrylamide gels (1).

Immobilized transcription complexes were made by binding TFIIIC and TFIIIB to DNA photoaffinity probes containing Bio-11-dUMP for 30 min at 21°C. The concentration of NaCl was adjusted to 0.5 M to remove TFIIIC from DNA, and the solution was gently mixed for 30 min with streptavidin-coated paramagnetic particles (nucleic acid qualified; Promega) previously washed four times with 200 μl of buffer C (7 mM $MgCl_2$, 200 μg of BSA per ml, 40 mM Tris-HCl [pH 8.0], 0.5 mM phenylmethylsulfonyl fluoride, 1 μg of pepstatin per ml, 1 μg of leupeptin per ml) with 100 mM NaCl and were resuspended to 1 mg/ml in the same buffer. For a typical reaction mixture of 150 μl, 25 μl of the 1-mg/ml suspension of streptavidin-coated particles was added. Binding efficiency of the DNA photoaffinity probe to streptavidin was near 90%. The magnetic particles were washed three times with 200 μl of buffer C with 500 mM NaCl and three times with 200 μl of buffer C with 100 mM NaCl. The particles were resuspended in the original 150-μl reaction volume of buffer C with 100 mM NaCl and pGEM1 or pLNG56 DNA (900 ng/25-μl reaction mixture). The suspended reaction mixture was distributed as 23-μl aliquots, RNA Pol III or transcription factors were added, and the mixture was incubated at 21°C for 30 min. These samples were then photo-cross-linked and processed as described previously (1, 2).

RESULTS

The upstream end of RNA Pol III. Our analysis of the topography of RNA polymerase specifically positioned at the *SUP4* promoter spans a stretch of 50 bp, starting 20 bp upstream of the transcriptional start (Fig. 1). Previous work has shown that the DNase I footprint of TFIIIB extends, at its promoter-proximal end, to bp −8 and −4 on the transcribed and nontranscribed strands, respectively. In docking on the TFIIIB-DNA complex, Pol III extends the footprint, apparently seamlessly, to bp +21 on the nontranscribed strand. When Pol III initiates transcription and makes a 17-mer RNA chain, the DNase I footprint separates into two parts (16, 17). In view of these considerations, and assuming that the downstream 21-bp segment of the uninitiated (binary) complex is due to Pol III, it also seems likely that Pol III does not occupy more than ~40 bp of DNA and that the DNA segment located 21 to 12 bp upstream of the transcriptional start would be in the proximity of the upstream side of Pol III and of its site of interaction with TFIIIB.

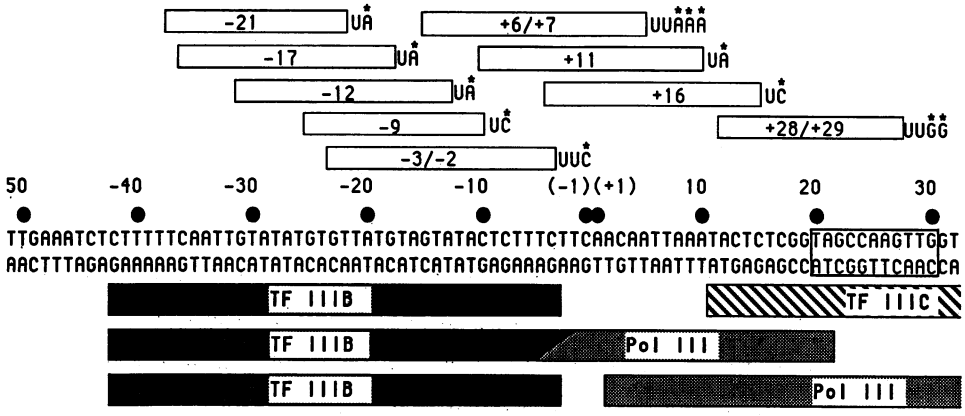

FIG. 1. Photoaffinity probes. The sequence of the *SUP4* tRNA^{Tyr} gene extending from 51 bp upstream to 31 bp downstream of the transcriptional start site (designated bp +1) is shown. The open boxes above the DNA sequence represent oligonucleotides of the nontranscribed strand that were used to synthesize DNA photoaffinity probes. The letters show nucleotides that were incorporated in the labeling step, with U representing N_3RdUMP and asterisks indicating α-^{32}P labeling. Each photoaffinity probe is referred to by a number or pair of numbers indicating the location(s) of an N_3RdUMP residue(s). The shaded boxes below the sequence indicate the DNase I footprint of TFIIIB (bp −42 to −5), the extreme upstream end of the DNase I footprint of TFIIIC, and the footprint extension attributable to RNA Pol III in TFIIIB-Pol III binary initiation complexes as well as elongating ternary transcription complexes arrested at bp 17 (bp 2 to 40 [16]). In the footprint of the TFIIIB-Pol III binary initiation complex, the individual contributions cannot be assigned unequivocally on the basis of DNase I footprinting alone; this is symbolized by the diagonal boundary.

Four probes of this upstream DNA segment were constructed (Fig. 1 and Materials and Methods) to place single N_3RdUMP residues on the nontranscribed strand at bp −21, −17, −12, and −9 (referred to as probes −21, −17, −12, and −9, respectively). Previous photo-cross-linking analysis of the *SUP4* gene has been done with DNA bearing a nick at the 5′ end of the primer (the left-hand end in Fig. 1) that is used for the specific placement of the photoactive nucleotide by primer extension (1, 2). For the experiments described below, we used 5′-phosphorylated primers and sealed the in vitro-synthesized DNA with DNA ligase. All probes were tested for the ability to form specific complexes with transcription proteins by gel retardation. Figure 2A shows the results for probes −21, −17, and −12. Complexes with TFIIIC and TFIIIB (lanes 1 to 3) had gel retardation properties that were previously demonstrated for other photo-cross-linking probes and for normal DNA (1, 2). RNA Pol III reacted with these TFIII(C+B)-DNA complexes (lanes 5, 7, 8, and 10), but two probes constructed on a box B promoter-down mutant gene (designated −21↓ and −17↓) were unable to bind TFIIIC and consequently also unable to form specific complexes with TFIIIB or Pol III (lanes 4 and 6). TFIIIB was required for proper Pol III binding, although minor proportions of ill-defined and heterogeneously migrating complexes were noted in its absence (data not shown, but see below).

These complexes were irradiated and analyzed for proteins cross-linking to DNA. The TFIII(C+B) complexes formed with probes −21, −17, and −12 showed, as expected, cross-linking to the 135-kDa subunit of TFIIIC, to the 70-kDa subunit of TFIIIB, and only very weakly to the 90-kDa subunit of TFIIIB, consistent with prior results (Fig. 2B, lanes 1 to 3) (2). Addition of Pol III brought more proteins within reach of these photoactive probes. The specificity of this cross-linking was indicated by its absence from reaction mixtures with the corresponding box B promoter-mutant probes, −21↓ and −17↓, which do not bind TFIIIC and therefore cannot form TFIIIB- and Pol III-containing transcription initiation complexes (lanes 4 and 6). Upon addition of Pol III, the −21 probe was weakly cross-linked to a protein whose mobility corresponded to a molecular mass of ca. 35 kDa (lane 5), while photoaffinity labeling of the 70-kDa subunit of TFIIIB was enhanced. (A separate experiment showed that only the 70-kDa component of TFIIIB was cross-linked and that the somewhat more slowly migrating 82-kDa subunit of Pol III was not.) At the resolution of Fig. 2B, the 128-kDa subunit 2 of Pol III and the nominally 135-kDa subunit of TFIIIC (1, 8) were barely separated, and further analysis was required to demonstrate that the 128-kDa subunit of Pol III was not photo-cross-linked from bp −21 (see below).

The Pol III subunit giving rise to the ca. 35-kDa band of labeled protein was identified by coelectrophoresis of photo-cross-linked products made with probes −17 and −9 with marker proteins (Fig. 3, lanes 1, 2, 6, and 7). Coelectrophoresis of Pol I, II, and III (lanes 3 to 5) identified the common 27-, 23-, and 14.5-kDa subunits of these enzymes (subunits 5, 6, and 8 of Pol II and subunits 9, 11, and 13 of Pol III, respectively), the common 40-kDa subunits of Pol I and III (subunits 5 of both enzymes), and, by straightforward extension, the Pol III subunits with apparent molecular masses of 34, 31, and 25 kDa (subunits 7, 8, and 10, respectively). The cross-linked protein was slightly retarded relative to the 34-kDa subunit 7, presumably as a result of its attached oligonucleotide, identifying subunit 7 as the protein that photo-cross-linked to probe −21. Further examination of Fig. 3 posed a problem of identification; a band migrating more slowly than the 40-kDa subunit of Pol III, and not invariably resolved from it, apparently replaced the anticipated, more rapidly migrating protein assigned an apparent molecular mass of 37 kDa (7, 24). Comparisons with Pol III generously provided by M. Riva and A. Sentenac (CEN, Saclay) and with a Pol I sample containing approximately 10% Pol III, generously provided by M. Nomura and J. Dodd, showed these Pol III preparations to be identical with regard to the 40-kDa band and its slightly more slowly migrating neighbor. We understand that in the hands of others, the relative mobility of the "37-kDa" band is vari-

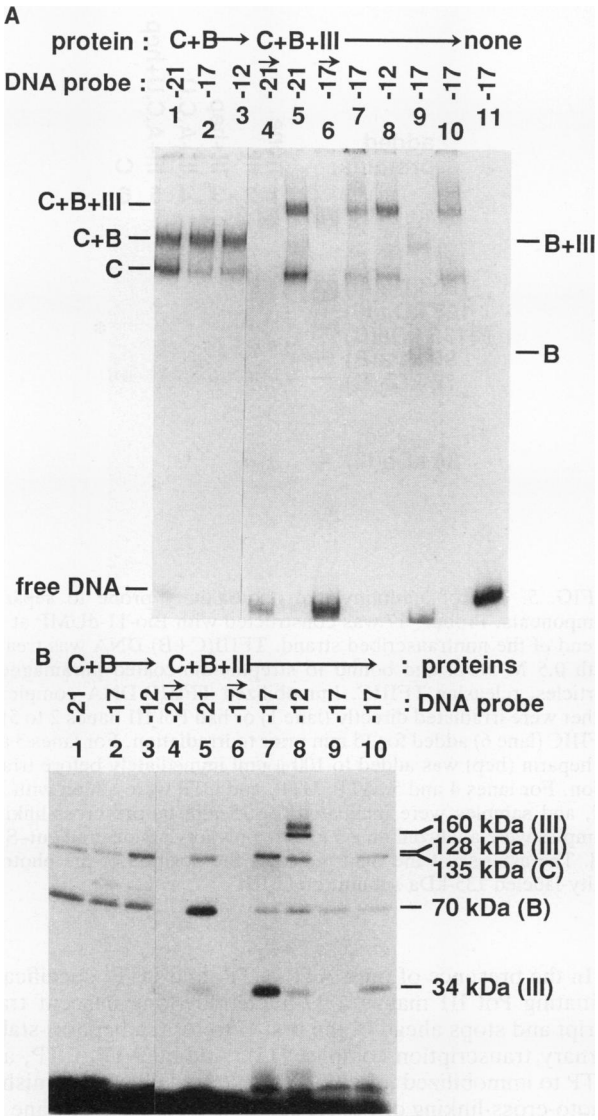

FIG. 2. Evidence that the 34- and 160-kDa subunits of RNA Pol III are located in close proximity to TFIIIB. Photoaffinity probes are identified above the lanes; ↓ further specifies that the probe is constructed with a box B promoter-down mutant *SUP4* gene (lanes 4 and 6). Reaction mixtures, assembled as described in Materials and Methods, were analyzed by gel retardation (A) and photoaffinity labeling (B). Samples for lanes 1 to 10 contained transcription factors TFIIIC and TFIIIB; RNA Pol III was also added for lanes 4 to 10. For lanes 9 and 10, ATP, CTP, and UTP (100 μM each) were added to the reaction mixture; for lane 9, heparin was added to 100 μg/ml 15 min after addition of ribonucleotides. (A) Samples were analyzed on a 4% nondenaturing polyacrylamide gel. The locations of different complexes on the gel are indicated at the margins. (B) Samples were analyzed on a 5 to 15% polyacrylamide gradient–SDS gel after extensive nuclease treatment (as described in Materials and Methods). Autoradiograms of the dried gels are shown. The relative mobilities of photoaffinity-labeled proteins allowed them to be identified as subunits of TFIIIC (C), TFIIIB (B), and RNA polymerase (III), as indicated at the right.

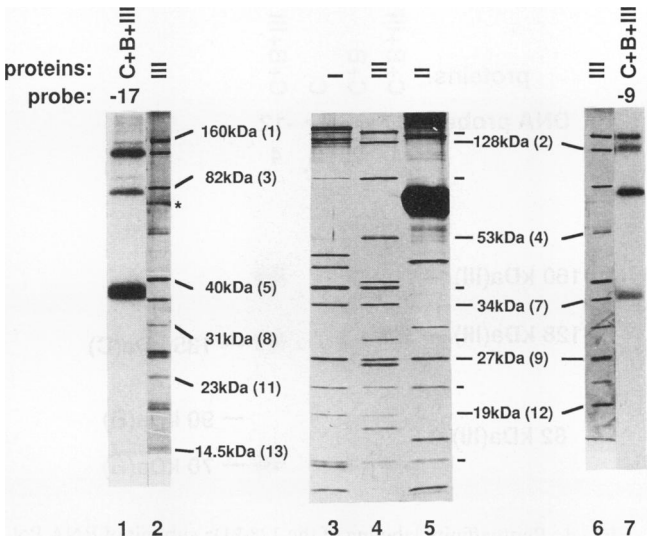

FIG. 3. Identification of RNA polymerase subunits. The flanking pairs of lanes at the left and right are photo-cross-linking analyses coelectrophoresed with Pol III and silver stained on 12% polyacrylamide gels. Lanes 3 to 5 show yeast RNA Pol I (also containing some Pol III, as stated in the text; from J. Dodd and M. Nomura), II (from A. Edwards and R. Kornberg), and III, respectively, analyzed together on a 14% polyacrylamide gel; a large quantity of BSA had been added as carrier to the Pol II. The alignment of the common Pol I-III 40- and 19-kDa subunits and of the common Pol I-II-III 27-, 23-, and 14.5-kDa subunits is to be noted. Other Pol III subunits are also identified and numbered beside lanes 3 and 5 (except for the 37- and 25-kDa subunits, whose genes have not yet been cloned) (7). Lanes 1 and 7 show photo-cross-linking of probes −17 and −9, respectively, to TFIII(C+B)-Pol III-DNA complexes alongside coelectrophoresed and silver-stained Pol III (lanes 2 and 6, respectively). The photo-cross-linked product migrating ahead of the 82-kDa subunit of Pol III in lanes 1 and 7 is the 70-kDa subunit of TFIIIB. The asterisk beside lane 2 marks contaminating BSA.

able and that it is not always resolved, although migration slower than that of the 40-kDa subunit is not the common experience (24a). It should be noted that the gene for the "37-kDa" subunit of Pol III has not yet been cloned. The 27- and 25-kDa bands were frequently resolved as a relatively closely spaced doublet; we verified that our Pol III and the sample from CEN, Saclay, also behaved identically with regard to these components in our gel system.

In TFIII(C+B)-Pol III-DNA complexes, the −17 photoaffinity probe also yielded a band of cross-linked protein that was barely retarded relative to the 135-kDa subunit 2 of TFIIIC (Fig. 2B, lane 7; Fig. 3, lane 1). Electrophoresis in a 5% gel (Fig. 4) showed a shift of cross-linking from TFIIIC subunit 2 in TFIII(C+B) complexes (lane 2) to Pol III subunit 2 upon addition of RNA polymerase (lane 1). It should be noted that the nominally 135-kDa TFIIIC subunit 2 (which has been assigned an apparent molecular mass of 131 kDa by others [7, 20]) migrated on these gels ahead of the nominally 128-kDa subunit of Pol III (the molecular mass of the conceptually translated protein is 129.4 kDa according to James et al. [13]).

The identification of cross-linkable Pol III subunits was also pursued by analyzing immobilized transcription complexes that had been stripped of TFIIIC (Fig. 5). A biotinylated version of probe −17 was converted to TFIII(C+B) complexes, stripped with 0.5 M NaCl, bound to streptavidin-coated magnetic beads, and then washed to remove released TFIIIC and lower the NaCl concentration to 100 mM. Only the 70-kDa component of this immobilized TFIIIB-DNA complex and, much less efficiently, the 90-kDa component were photo-cross-linked to bp −17 (Fig. 5, lane 1). When

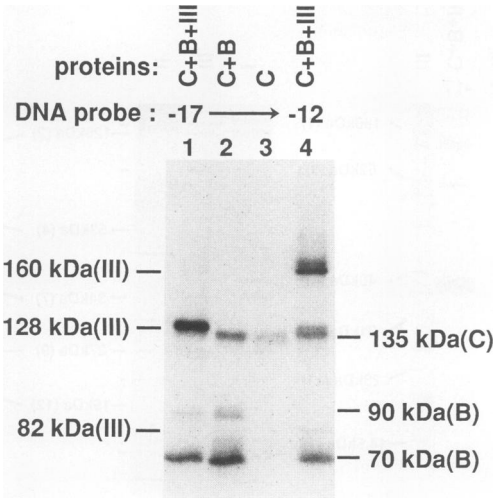

FIG. 4. Photoaffinity labeling of the 128-kDa subunit of RNA Pol III from bp −17 in the initiation complex containing TFIIIC, TFIIIB, and RNA Pol III. Probes and proteins are specified above the lanes. Samples were analyzed on a 5% polyacrylamide–SDS gel alongside purified RNA Pol III and TFIIIC. The gel was silver stained, dried, and subjected to autoradiography. The relative mobilities of Pol III subunits are indicated at the left; the relative mobilities of the 135-kDa subunit of TFIIIC and of the photoaffinity-labeled 70- and 90-kDa components of TFIIIB are indicated at the right.

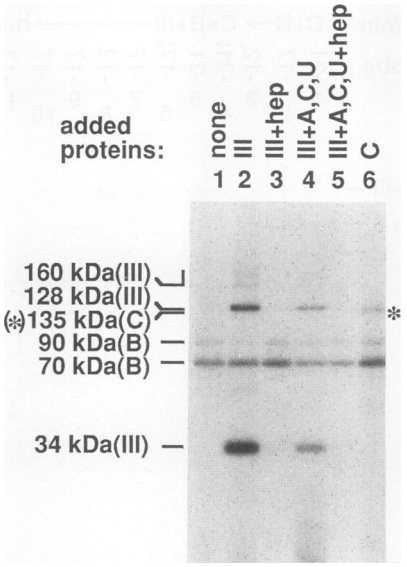

FIG. 5. Use of a biotinylated photoaffinity probe to separate components. Probe −17 was constructed with Bio-11-dUMP at the 5′ end of the nontranscribed strand. TFIII(C+B)-DNA was treated with 0.5 M NaCl and bound to streptavidin-coated paramagnetic particles, releasing TFIIIC. Immobilized TFIIIB-DNA complexes either were irradiated directly (lane 1) or had Pol III (lanes 2 to 5) or TFIIIC (lane 6) added for 15 min prior to irradiation. For lanes 3 and 5, heparin (hep) was added to 100 μg/ml immediately before irradiation. For lanes 4 and 5, ATP, UTP, and CTP were added with Pol III, and samples were incubated for 15 min before cross-linking. Samples were analyzed on a 5 to 15% polyacrylamide gradient–SDS gel. The asterisk at the right indicates the position of the photoaffinity-labeled 135-kDa subunit of TFIIIC.

TFIIIC reattached to this TFIIIB-DNA complex, its 135-kDa subunit 2 could be cross-linked, as expected (lane 6). Pol III bound specifically to the TFIIIB-DNA complex (lane 2), allowing the 128- and 34-kDa subunits to be photoaffinity labeled (photo-cross-linking of the 160-kDa subunit was detectable but very weak). Heparin stripped Pol III from these binary transcription initiation complexes, leaving only TFIIIB attached to DNA (lane 3) as has previously been shown (15, 16). In a similar experiment with biotinylated probe −21, the 34-kDa Pol III subunit 7 was weakly cross-linked, but the 160-kDa subunit 1 and the 128-kDa subunit 2 were not cross-linked.

In contrast to probe −17, probe −12 was more weakly cross-linked to the 34-kDa Pol III subunit 7 (Fig. 2B, lane 8). The strong cross-linking by this probe to the 160-kDa subunit 1 of Pol III yielded two bands in this experiment (Fig. 2B, lane 8; Fig. 4, lane 4) but a single band in some experiments. Electrophoresis at higher resolution (Fig. 4, lane 4) showed that the 135-kDa TFIIIC subunit and the 160-kDa subunit of Pol III were preferentially labeled in these TFIII(C+B)-Pol III complexes and that the 128-kDa Pol III subunit was barely labeled. The preferential labeling of the 160-kDa subunit of Pol III over the 128-kDa subunit from bp −12 was also noted in complexes from which TFIIIC had been removed (data not shown). Probe −12 is shown cross-linking very weakly to the 82-kDa subunit of Pol III in Fig. 4 (lane 4). However, this cross-linking was not significantly and reproducibly above the background of control experiments in which promoter-down mutant probes were used or TFIIIB was omitted.

Probe −9 preferentially and specifically cross-linked to the 160-kDa Pol III subunit and also to the 34-kDa subunit (Fig. 3, lane 7). In TFIIIC-containing complexes, the 135-kDa subunit of TFIIIC was effectively cross-linked regardless of the presence of Pol III (data not shown, but see below).

In the presence of pure ATP, CTP, and UTP, specifically initiating Pol III makes a 17-nucleotide-long nascent transcript and stops ahead of the first G to form a heparin-stable ternary transcription complex (15). Adding ATP, CTP, and UTP to immobilized initiation complexes (Fig. 5) diminished photo-cross-linking of Pol III subunits from bp −17 (lane 4). Treatment of these ternary transcription complexes with heparin eliminated the residual cross-linking of the 128- and 34-kDa subunits 2 and 7 of Pol III to bp −17, showing that it was due to enzyme that had failed to elongate RNA chains (lane 5; also shown by comparison of lanes 9 and 10 in Fig. 2B). The Pol III of the ternary transcription complexes was not detached from DNA by heparin (Fig. 2A, lane 9). (A similar experiment, in which 0.5 M NaCl was used in place of heparin to dissociate TFIIIC from DNA, gave a similar result.) We concluded that when Pol III moved downstream so that its catalytic site for internucleotide bond formation was positioned over bp +17 or +18, its 128- and 34-kDa subunits moved out of range of photoactive nucleotide at bp −17, consistent with the previously observed downstream shift of the DNase I footprint that is summarized in Fig. 1 (17). Similar results were seen with probes −9 and −12 (data not shown).

Summarizing the principal results of the analysis to this point, subunits 1, 2, and 7 (160, 128, and 34 kDa) were shown to be located at, or to extend to, the upstream, trailing end of Pol III. All three of these subunits moved out of the reach of a photoactive nucleotide placed in the nontranscribed strand at bp −17, −12, and −9 when Pol III formed an elongating transcription complex that advanced to bp +17.

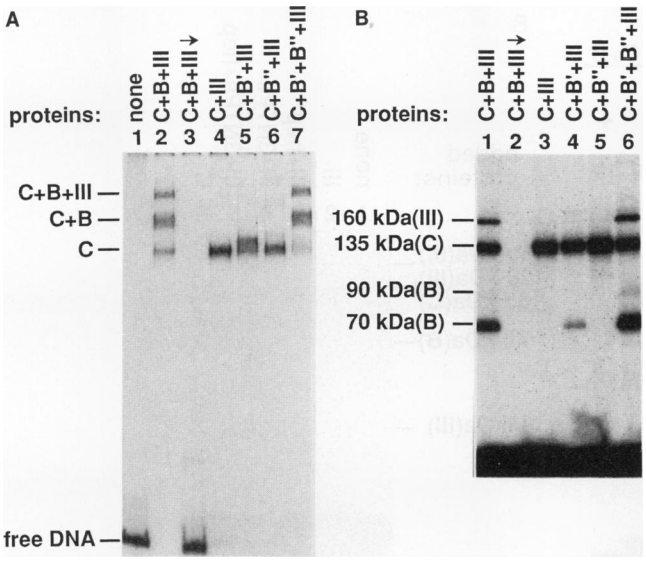

FIG. 6. Evidence that recruitment of Pol III to the initiation complex requires both the B′ and B″ components of TFIIIB. (A) Gel retardation analysis. Probe −9 and its box B promoter-down mutant counterpart, −9↓, were incubated with TFIIIC and TFIIIB (lanes 2 and 3), TFIIIC only (lane 4), or TFIIIB components B′ (contributing the 70-kDa protein) and B″ (contributing the 90-kDa protein) (lanes 5 to 7) for 30 min at 21°C prior to addition of Pol III for another 15 min. Samples were analyzed on a 4% native polyacrylamide gel. Lane 1, no protein added. The positions of TFIIIC-DNA (C), TFIII(C+B)-DNA (C+B), and TFIII(C+B)-Pol III-DNA (C+B+III) complexes are indicated at the left. (B) Photo-cross-linking. Corresponding samples were irradiated, digested with nucleases, and analyzed on an 8% polyacrylamide–SDS gel for examination of large subunits of Pol III. (The cross-linked 34-kDa Pol III subunit 7 was not resolved from the solvent front in this gel.)

TFIIIB′ and TFIIIB″ are required to accurately position Pol III. It has recently been shown that TFIIIB can be separated into two components, B′ and B″. Component B′ contributes the 70-kDa subunit, while B″ contributes the 90-kDa subunit to TFIIIB-DNA complexes. Component B′ interacts with TFIIIC-DNA, while B″ only binds to TFIII(C+B′)-DNA complexes. Both B′ and B″ are required to confer TFIIIB transcription factor activity (14). Probe −9 was used to examine the photo-cross-linking of transcription complexes formed with separated TFIII B′ and B″ fractions. Figure 6A shows the resolution of TFIIIC-DNA, TFIII(C+B)-DNA, and TFIII(C+B)-Pol III-DNA complexes by gel retardation in lanes 2 and 7. No complexes formed on the box B mutant probe, −9↓ (lane 3). Component B′ bound to and slightly retarded TFIIIC-DNA and B″ did not (lanes 5 and 6, respectively), as shown previously, and Pol III was unable to bind unless both components of TFIIIB were present (lanes 4 to 7). Probe −9 cross-linked to the 135-kDa subunit of TFIIIC (Fig. 6B, lane 3) and to the 70-kDa subunit of TFIIIB that is present in component B′ (lanes 1, 4, and 6). The efficiency of cross-linking of the 70-kDa subunit was greatly increased by the presence of B″ (lanes 4 and 6), and only the combination of B′ and B″ allowed the 160-kDa Pol III subunit 1 to be brought within the reach of the photoactive site in probe −9 (compare lanes 1 and 6). A very faint band in Fig. 6B, lane 6 (also detectable in the original in lane 1 and not seen at all at the exposure and sensitivity of Fig. 3, lane 7), was shown separately to be due to the 90-kDa subunit of TFIIIB rather than the 82-kDa subunit 3 of Pol III.

The start site and the transcription bubble. Interactions in the vicinity of the transcriptional start site were analyzed with a probe that placed N_3RdUMP in the nontranscribed strand at bp −3 and −2 (Fig. 7). The DNA, which was fully saturated with TFIIIC and partly saturated with TFIIIB, yielded the TFIIIB-DNA complex and free DNA upon stripping with heparin (data not shown; compare with Fig. 4 of reference 2). When the TFIII(C+B)-DNA complex on probe −3/−2 was irradiated, the 70- and 90-kDa subunits of TFIIIB and the 135-kDa subunit of TFIIIC were cross-linked, as expected (2), but a 16- to 17-kDa protein was also strongly labeled (Fig. 7A, lane 9). The 70-kDa subunit was cross-linked in the heparin-treated complex, but the 16- to 17-kDa protein was not (lane 10). The specificity of transcription factor-DNA complex formation and of the labeling of these components was verified by showing that the same −3/−2 probe made on the box B-down mutant *SUP4* gene failed to form the appropriate protein-DNA complexes (confirmed by gel retardation; data not shown) and failed to photoaffinity label the 135-, 90-, 70-, and 17-kDa proteins (lanes 5 and 6). A band corresponding to an ~20-kDa protein was apparently not specific to transcription complexes since it cross-linked to the promoter-down mutant probe (lanes 5 and 6).

The labeling of a 17-kDa protein in TFIII(C+B)-DNA complexes was unanticipated. Cross-linking of this protein was largely (but not totally) dependent on TFIIIC binding (lane 5) and absolutely required the presence of TFIIIB (lane 7 and data not shown), and thus was probably contributed by the TFIIIB fraction. However, this small protein bound to TFIIIC-DNA much less tightly than did the 70- and 90-kDa subunits of TFIIIB, since (i) its retention in DNA complexes, as judged by cross-linking, was abolished by heparin (lane 4) and (ii) it exchanged a TFIII(C+B)-DNA complex for competing unlabeled TFIIIC-DNA complexes at low ionic strength (data not shown).

Upon addition of Pol III, the major part of this −3/−2 DNA probe was converted to Pol III- and TFIII(C+B)-containing complexes, with minor proportions of TFIIIC-DNA and barely any TFIII(C+B)-DNA remaining (determined by gel retardation; data not shown). When this material was irradiated, the 160-kDa subunit of Pol III in addition to the 135-kDa subunit of TFIIIC and/or the 128-kDa subunit of Pol III were cross-linked, and the 34-kDa subunit of Pol III was weakly cross-linked (Fig. 7A, lane 3). Heparin stripped Pol III and TFIIIC from these binary transcription initiation complexes, as expected (confirmed by gel retardation; data not shown), and also abolished cross-linking to the 160-, 135- or 128-, 34-, and 17-kDa proteins (lane 4). When Pol III advanced to bp +17 in the presence of ATP, CTP, and UTP, the cross-linking pattern of probe −3/−2 changed drastically (lane 1). Photoaffinity labeling of the 160-kDa protein became only barely detectable (that is, detectable in the original only); labeling of the 135/128-kDa band diminished also, as did labeling of the 70- and 17-kDa proteins, while labeling of the 34-kDa subunit of Pol III increased and weak labeling of the 31-kDa subunit became detectable. Stripping with heparin removed TFIIIC, leaving TFIIIB- and Pol III-containing ternary transcription complexes and a proportion of TFIIIB-DNA complexes (data not shown), the latter of which might have originated from TFIII(B+C)-DNA complexes that bound to transcriptionally inactive enzyme. In these complexes, three subunits of Pol III were photoaffinity labeled: the 31-kDa subunit barely, the 128-kDa subunit clearly, and the 34-kDa subunit most heavily (lane 2).

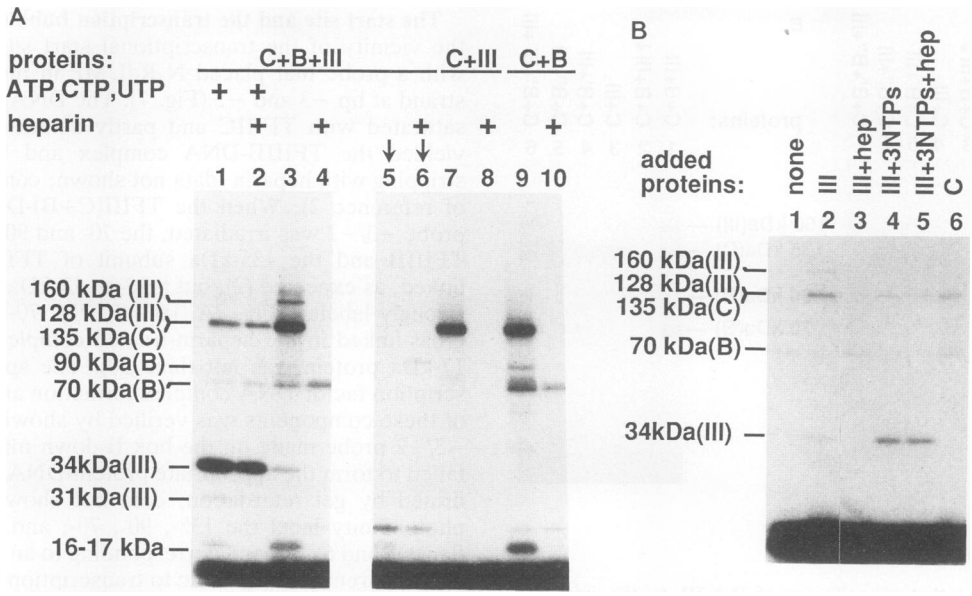

FIG. 7. Evidence that photoaffinity labeling of the 34-kDa subunit of Pol III from bp −3 and −2 is greatly enhanced upon elongation of RNA chains. (A) Samples were irradiated, digested with nucleases, and analyzed on a 5 to 15% gradient polyacrylamide–SDS gel. Proteins, the presence of ribonucleoside triphosphates (NTPs), and heparin (hep) are indicated above each lane; ↓ indicates the box B promoter-down probe. Bands corresponding to photoaffinity-labeled subunits of TFIIIC, TFIIIB, and Pol III are indicated at the side. The 16- to 17-kDa protein band (lanes 3 and 9) is discussed in the text. (B) Biotinylated probe −3/−2 was used to immobilize the TFIIIB-DNA complexes, which either were irradiated directly (lane 1) or had Pol III (lanes 2 to 5) or TFIIIC (lane 6) added for 15 min prior to irradiation. For lanes 3 and 5, heparin was added to 100 μg/ml immediately before irradiation. For lanes 4 and 5, ATP, UTP, and CTP were added with Pol III for 15 min before irradiation. Samples were analyzed on a 5 to 15% polyacrylamide gradient–SDS gel.

Parallel experiments with biotin-tagged DNA complexes immobilized on streptavidin-coated magnetic particles clarified the labeling of the 135- and 128-kDa proteins (Fig. 7B). When TFIII(C+B) complexes were formed, stripped with 0.5 M NaCl, immobilized on streptavidin-coated particles, and washed to remove TFIIIC, only the 70-kDa subunit of TFIIIB was detectably labeled (at the lower sensitivity of this experiment; Fig. 7B, lane 1). Readdition of TFIIIC yielded labeling of its 135-kDa subunit (lane 6). Addition of Pol III in the absence of TFIIIC yielded photoaffinity labeling of its 128-kDa subunit and weak labeling of the 160- and 34-kDa subunits (lane 2), all sensitive to stripping by heparin (lane 3). When Pol III advanced to bp 17 in the presence of ATP, CTP, and UTP (lane 4), the efficiency of photo-cross-linking of the 128-kDa subunit diminished (compare lanes 4 and 2) and that of the 34-kDa subunit increased; photo-cross-linking of the 31-kDa subunit was not detectable at the sensitivity of this experiment. Labeling of these ternary complexes was stable to addition of heparin (lane 5).

Probes with photoactive nucleotides on the nontranscribed strand at bp 6, 7, and 11 were used to explore the downstream end of the transcription bubble in binary transcription initiation complexes and the upstream and central segments of the transcription bubble in elongating transcription complexes arrested at bp 17. Figure 8 shows the results of an analysis of transcription complexes that were formed on a biotin-tagged probe with N_3RdUMP on the nontranscribed strand at bp 6 and 7. Addition of Pol III to the immobilized TFIIIB-DNA complex allowed the 160-, 128-, and, much less efficiently, the 34- and 27-kDa subunits to be photoaffinity labeled (lane 2). In the absence of TFIIIB, there was only a low background of Pol III binding (gel retardation data not shown) and cross-linking (lane 8). Heparin stripped Pol III from TFIIIB-Pol III-DNA complexes (lane 3). When TFIIIC rebound to immobilized TFIIIB-DNA, its 135- and 95-kDa subunits were relatively efficiently cross-linked (lane 6). The 135-kDa subunit of TFIIIC and the 128-kDa subunit of Pol III were barely resolved on this gel (compare lanes 5 and 6).

When elongating transcription complexes were arrested at bp 17 on probe +6/+7 and stripped with heparin to remove inactive Pol III, a distinctive pattern of photo-cross-linking was seen (lane 5): the 160-, 128-, and 34-kDa subunits were still labeled and the 31-kDa subunit was now labeled, whereas the 27-kDa subunit was not. The 82-kDa subunit was very faintly but reproducibly labeled. The establishment of the ternary transcription complex absolutely required TFIIIB, and that requirement was reflected in the photo-cross-linking (compare lanes 5 and 10).

The higher-molecular-mass proteins that cross-linked to probe +11 included the TFIIIC 135- and 95-kDa subunits (the former strongly and the latter only weakly) (Fig. 9A, lane 1). Formation of a binary TFIII(B+C)-DNA-Pol III complex brought the 160-, 128-, and 82-kDa subunits of Pol III within reach of the photoactive nucleotide; the 128-kDa subunit of Pol III and the 135-kDa subunit of TFIIIC were resolved on a 5% gel (lane 2). Among the smaller Pol III subunits (Fig. 9B), the 53- and 27-kDa proteins were also cross-linked (lane 4). In the elongating transcription complex arrested at bp 17 and treated with heparin, the 160-, 128-, and 34-kDa subunits were labeled more strongly than the 82-, 27-, and 40- or 37-kDa subunits (Fig. 9A, lane 5; Fig. 9B, lane 7). The specificity of this remarkably diverse photo-cross-linking was confirmed by demonstrating a dependence on box B and TFIIIB (Fig. 9A, lanes 8 and 7; Fig. 9B, lanes 1 and 9), and in other experiments by specific competition with an unlabeled *SUP4* tRNA gene, and by the dependence of heparin resistance on the presence of three ribonucleoside

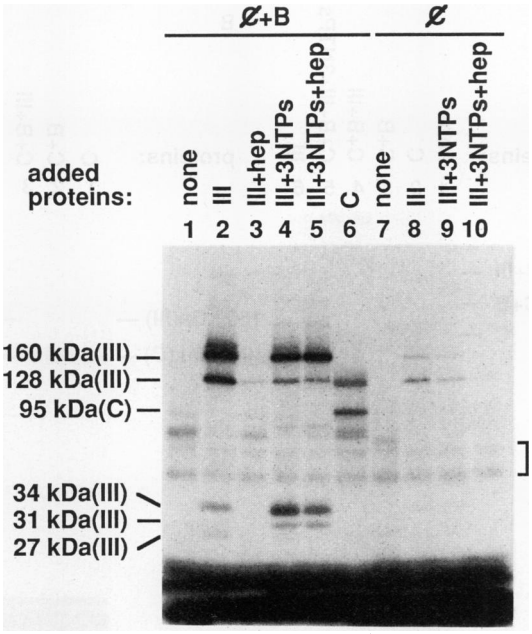

FIG. 8. Subunits of Pol III photoaffinity labeled from bp 6 and 7 in binary and ternary transcription complexes. Biotinylated probe +6/+7 was immobilized on streptavidin-coated paramagnetic particles and washed to removed TFIIIC from TFIII(C+B)-DNA (lanes 1 to 6) or TFIIIC-DNA (lanes 7 to 10) complexes prior to the addition of Pol III as described in the legend to Fig. 5. Samples shown in lanes 1 and 7 contained no Pol III. For lane 6, TFIIIC was added in place of Pol III. For lanes 4, 5, 9, and 10, ATP, CTP, and UTP were added with Pol III and incubated for 15 min at 21°C. For lanes 3, 5, and 10, heparin (hep) was added immediately before irradiation. Samples were processed and analyzed on a 5 to 15% polyacrylamide gradient–SDS gel. The bracket at the right marks a region of low-level nonspecific cross-linking that is evident when no TFIIIB is present and TFIIIC has been removed (lanes 7 to 10). (The bracket also marks the location of the very large quantity of BSA that was used as a carrier in these experiments.)

triphosphates (data not shown). The ability to access at least five and possibly seven different proteins from a single location in the transcription bubble is striking. The separation of the azido function in N_3RdUMP from the pyrimidine ring might allow a single N_3RdUMP residue to access protein-associated cross-linking targets over a wider band (perhaps as many as 4 or 5 bp) along the side of a DNA helix. Nevertheless, putting such a short DNA segment in close proximity to five or more different proteins stretches the imagination. This consideration makes the arrested ternary transcription complex the most interesting object of the analysis at this position, because other proteins and nonspecifically bound or inactive Pol III are removed from the vicinity of bp 11 by heparin; at least five and possibly seven Pol III subunits in ternary transcription complexes on probe +11 remained within the reach of its single photoactive nucleotide.

Base pair 16 lies downstream of the transcription bubble of the binary transcription initiation complex and close to the front of the transcription bubble in the elongating transcription complex arrested at bp 17. The 95-kDa subunit of TFIIIC was efficiently labeled from this position. Addition of Pol III brought the 160-, 128-, and 27-kDa subunits of Pol III into cross-linking range; cross-linking of the 82- and 53-kDa subunits was also detected but was weaker (Fig. 10, lane 3). Upon formation of the arrested and heparin-stripped elongating transcription complexes (lanes 1 and 2), the photocross-linking patterns simplified even further: only the 128-kDa subunit was strongly labeled, and the 160-kDa subunit was labeled much more weakly in the stripped complex (lane 2). In comparing the properties of stripped ternary complexes on probes +16 and +11, it is possible to see the protein cross-linking pattern changing significantly over a span of only 5 bp.

The downstream end of RNA Pol III. The DNase I footprints of a Pol III binary complex and of the elongating transcription complex arrested at bp 17 extend downstream

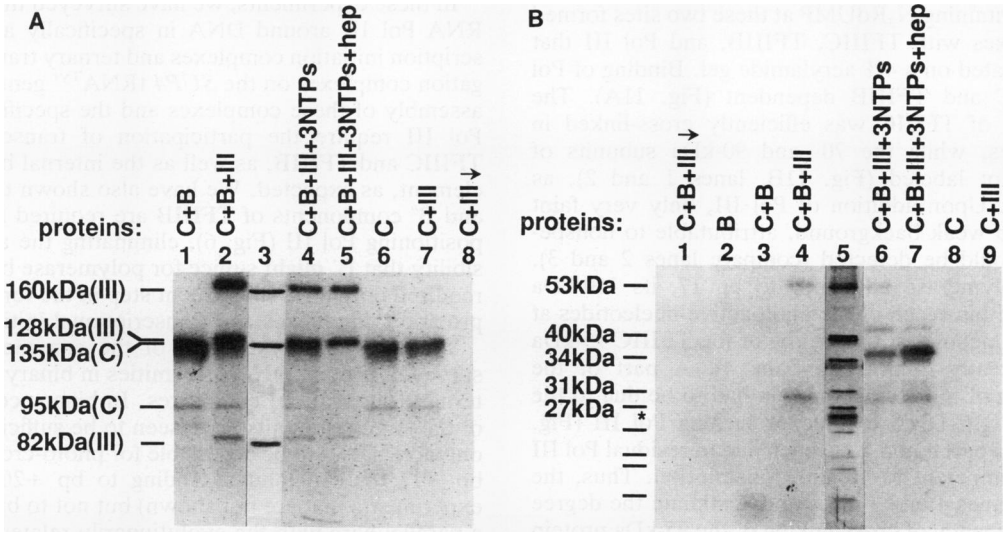

FIG. 9. Cross-linking of probe +11 to multiple subunits of Pol III. Probe +11 and the promoter-down mutant probe +11 ↓ were used to form protein complexes that are indicated above the lanes. Samples were processed and analyzed on a 5% (A) or 12% (B) polyacrylamide–SDS gel; only the bottom half of the latter is shown. Lane 3 of panel A and lane 5 of panel B show coelectrophoresed and silver-stained Pol III subunits, which are identified at the left; the 135- and 95-kDa subunits of TFIIIC are also identified in panel A. The doublet band identified as 40 kDa at the left of panel B may represent both the 40- and 37-kDa subunits, as discussed in the text. The heavy, diffusely stained band in panel B marked with an asterisk was not generally observed.

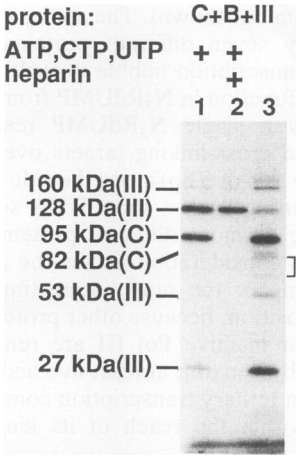

FIG. 10. Cross-linking of the 128-kDa subunit of Pol III by a probe placed close to the 3′ end of the transcription bubble. The elongating transcription complex arrested at bp 17 was formed on probe +16 by adding ATP, CTP, and UTP (lanes 1 and 2) to the binary transcription initiation complex containing TFIIIC, TFIIIB, and Pol III (lane 3). For the sample shown in lane 2, heparin was added to strip TFIIIC and nontranscribing or inactive Pol III from DNA prior to irradiation. Samples were analyzed on a 5 to 15% polyacrylamide gradient–SDS gel. The bracket at the right marks a low level of nonspecific background, associated with the leading and lagging edges of a zone containing a large quantity of BSA, as also noted in the legend to Fig. 8.

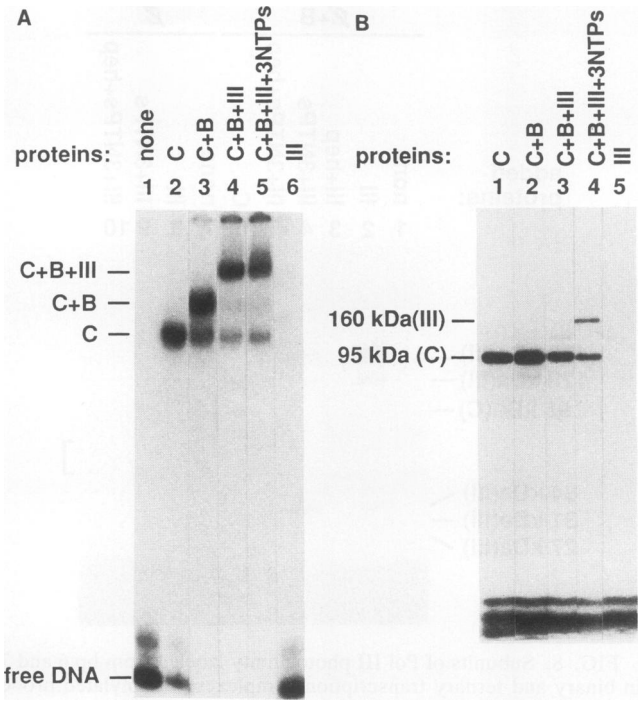

FIG. 11. Evidence that movement of the transcription complex into the region of box A reduces photoaffinity labeling of the 95-kDa subunit by TFIIIC and substitutes labeling of the 160-kDa subunit of Pol III. Probe +28/+29 was used to assemble transcription complexes as indicated above the lanes. (A) Analysis of unirradiated samples on a native 4% polyacrylamide gel. The positions of various complexes and of free DNA are indicated at the left. (B) Photoaffinity labeling. Samples were analyzed on an 8 to 17.5% polyacrylamide gradient–SDS gel. The positions of the 160-kDa subunit of Pol III and the 95-kDa subunit of TFIIIC are shown at the left.

on the nontranscribed strand to bp 21 and 40, respectively (16). The extent of the transcription bubble has previously been defined in terms of the reactivity of T with permanganate (15). For the ternary transcription complex arrested at bp 17, the downstream edge of the transcription bubble is located between bp 17 and 20. Thus, the downstream edges of initiating and transcribing Pol III complexes can be probed from the nontranscribed strand at bp 28 and 29.

The probe containing N_3RdUMP at these two sites formed normal complexes with TFIIIC, TFIIIB, and Pol III that were well separated on a 4% acrylamide gel. Binding of Pol III was TFIIIC and TFIIIB dependent (Fig. 11A). The 95-kDa subunit of TFIIIC was efficiently cross-linked in these complexes, while the 70- and 90-kDa subunits of TFIIIB were not labeled (Fig. 11B, lanes 1 and 2), as expected (1, 2). Upon addition of Pol III, only very faint labeling above a weak background, attributable to nonspecific binding, could be detected (compare lanes 2 and 3). When RNA polymerase advanced to bp 17, its 160-kDa subunit came within reach of the photoactive nucleotides at bp 28 and 29; concomitantly, labeling of the TFIIIC 95-kDa subunit was greatly diminished (lane 4). A part of the residual labeling of the 95-kDa protein had to be due to the presence of TFIIIC-DNA complexes lacking Pol III (Fig. 11A, lane 4), and part could have been due to residual Pol III that was not competent to initiate transcription. Thus, the comparison of lanes 4 and 3 must underestimate the degree to which the efficiency of cross-linking of the 95-kDa protein from the downstream edge of box A was reduced by advancing RNA polymerase. This interpretation is consistent with the change of the DNase I footprint that occurs when Pol III advances into the vicinity of the box A binding site of TFIIIC (16) and extends protection quite far downstream.

DISCUSSION

In these experiments, we have surveyed the disposition of RNA Pol III around DNA in specifically assembled transcription initiation complexes and ternary transcription elongation complexes on the *SUP4* $tRNA^{Tyr}$ gene (Fig. 12). The assembly of these complexes and the specific placement of Pol III require the participation of transcription factors TFIIIC and TFIIIB, as well as the internal box B promoter element, as expected. We have also shown that both the B′ and B″ components of TFIIIB are required for binding and positioning Pol III (Fig. 6), eliminating the alternative possibility that B′ might suffice for polymerase binding, with B″ required only for a subsequent step in the formation of open promoter complexes and transcriptional initiation.

The two largest subunits of the enzyme dominate our survey of protein-DNA proximities in binary (initiation) and ternary (elongation) complexes. In binary complexes, both of the largest subunits were seen to be sufficiently stretched out along DNA to be accessible for photo-cross-linking from bp −17 to +16 (and extending to bp +20, according to experiments that are not shown) but not to bp +28. There is a parallel here with the evolutionarily related eubacterial β′ and β subunits, which are also thought to be elongated and to have their long axes aligned parallel to DNA. It is the 128-kDa subunit (the homolog of β and the subunit that cross-links to a nucleoside triphosphate analog placed in the initiator nucleotide substrate site [22]) that was found to

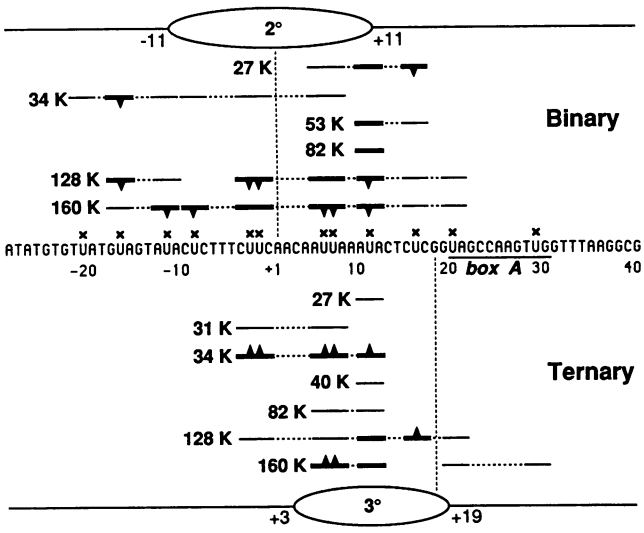

FIG. 12. Summary of the photo-cross-linking of Pol III subunits in binary and ternary transcription complexes. Dotted lines link the sites at which photo-cross-linking of individual Pol III subunits was observed. The thin and thick line segments and arrowheads indicate weak, moderate, and strong relative photo-cross-linking efficiency, respectively. Results for binary complexes are shown above the sequence, and ternary complexes halted at bp 17 are shown below the sequence. The maximum extents of unwound DNA in the binary and ternary transcription complexes (15) are shown at the top and bottom. The dashed vertical lines indicate the principal positions of the catalytic sites in the binary and ternary complexes. The locations of photoactive nucleotides of the different probes (see Fig. 1) are designated by $\overset{x}{U}$.

project further upstream, being cross-linked from bp −17 in binary complexes. The 160-kDa subunit 1 was cross-linked from the furthest downstream point examined (probe +28/+29 in elongating transcription complexes).

The 34-kDa subunit was seen to extend the furthest upstream, with binary complexes very weakly cross-linked from bp −21 and strongly from bp −17. The 34-kDa subunit also appeared to be quite stretched out along DNA, being accessible to cross-linking all the way forward to bp +6 and +7. Relatively efficient cross-linking from several probes, including −17, −9, and −3/−2, is noteworthy. Its position at the back end of Pol III makes the 34-kDa subunit a candidate for interaction with TFIIIB. No homology of the 34-kDa subunit with any Pol II subunit has been discovered (referred to in reference 7).

The 27-kDa Pol III subunit 9 was cross-linked from the front end (probes +11 and +16) but not the rear or center of binary transcription complexes. Four other subunits were quite restricted in their labeling patterns: the Pol III-specific 82-, 53-, and 31-kDa proteins (subunits 3, 4, and 8) and either the 40- or the 37-kDa subunit (subunit 5 or 6). The 40- and 19-kDa subunits that are shared between Pol I and III have some homology with a short N-proximal segment of the eubacterial α subunit (6). A variety of evidence places the two α subunits at the upstream end of *Escherichia coli* RNA polymerase and involves them in interactions with positive transcriptional regulators, such as CRP and FNR (9–11, 26). It would have been interesting to have seen the 40- and 19-kDa subunits 5 and 12 in a comparable location, but that proved not to be the case.

The pattern of cross-linking at and near the front of the transcription bubble was remarkably complex. Five proteins were tagged in binary transcription complexes from bp 11 and 16, and as many as seven proteins were cross-linked in ternary transcription complexes (arrested at bp 17) from bp 11. The photoactive azido function of N_3RdUMP is tethered to the pyrimidine ring by a relatively long, though stiff, linkage that could allow the photoactive function to probe for protein proximity over perhaps as many as 4 to 5 bp. Nevertheless, it is difficult to imagine that so many different subunits can project into so confined a space. We suggest, instead, that this multiplicity of cross-linking targets reflects a multiplicity of concurrently represented structural states. We suppose that in the case of the binary complex, these states are generated as RNA polymerase scans the transcription bubble for its preferred initiation site. It is known that such scanning must take place, because the site of the transcriptional start of Pol III, as of other RNA polymerases, can be pushed around over a span of several base pairs by obliging the enzyme to use different dinucleotides at the 5′ initiating ends of RNA chains. In the arrested ternary transcription complex, a multiplicity of states is generated depending on whether the 3′ end of the nascent RNA chain is in the substrate or primer site (see reference 3 for a related discussion of *E. coli* RNA polymerase; 18) and whether the enzyme turns over its 3′ ends of transcription complexes by nucleolytic degradation and resynthesis (12, 13a, 21, 25). It is readily seen that cross-linking from bp 11 to some of its target proteins is very weak. (We have been at pains to verify that this low-efficiency cross-linking is specific.) The less frequent cross-linking may be associated with the existence of concurrently present minor structural states that are intrinsic to the dynamics of precisely placed binary and ternary transcription complexes.

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