Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6191–6195, October 1982
Biochemistry

Specific interactions of *Saccharomyces cerevisiae* proteins with a promoter region of eukaryotic tRNA genes

(RNA polymerase III genes/DNA-binding proteins/footprint competition/internal promoters)

ROMAN KLEMENZ*, DAVID J. STILLMAN, AND E. PETER GEIDUSCHEK

Department of Biology, University of California at San Diego, La Jolla, California 92093

Contributed by E. Peter Geiduschek, July 19, 1982

ABSTRACT The specific binding of one or several *Saccharomyces cerevisiae* proteins to a segment of genes that code for different yeast tRNAs has been demonstrated with the use of the DNase I-protection "footprint" assay of Galas and Schmitz. The analyzed binding occurs near the 3' ends of the genes and is centered on an 11-base-pair DNA sequence that has been well conserved among eukaryotic tRNA genes. Others have shown the involvement of this sequence in initiating the transcription of tRNA genes by RNA polymerase III. The adenovirus gene that codes for VA_I RNA also contains this conserved sequence element, and we detect binding of yeast protein(s) to this gene. Competition experiments show that a common set of proteins binds to different tRNA genes. The DNA–protein complex is quite stable at 20°C and low ionic strength.

The nature of RNA polymerase III promoters has been the focus of much attention and a lot is now known about them. DNA sequences that act as signals for initiating transcription of the genes encoding 5S rRNA of *Xenopus laevis* and *Xenopus borealis* are part of the transcription unit itself, spanning the region ≈55–80 base pairs downstream from the transcriptional start site (1, 2). The same stretch of DNA binds a 37,000-dalton protein called TFIIIA, which is required for transcription (ref. 3; for review, see ref. 4). Other genes that are transcribed by RNA polymerase III also have internal control regions. These genes include the adenovirus gene encoding VA_I RNA (5, 6), the interspersed repetitive elements of the *Alu* family (7), and tRNA genes of *Xenopus laevis* (8–11), *Drosophila melanogaster* (12, 13), *Bombyx mori* (14), *Caenorhabditis elegans* (15, 16), and *Saccharomyces cerevisiae* (17). Flanking sequences undoubtedly also can influence transcription rate. For example, a *Bombyx mori* $tRNA^{Ala}$ gene's 5' flanking sequences are required for transcription by a homologous extract (18).

Further dissection pinpointed two separable regions of *Xenopus laevis* tRNA genes that are indispensable for transcription (10, 11). Both the anterior and the posterior stretches of DNA contain sequences that are exceptionally well conserved among all eukaryotic tRNA genes during evolution (5, 11, 16). The posterior element also shows homology to sequences in *Alu* family genes (7) and in the VA_I gene (5, 6) that are required for transcription.

A sequence that is necessary for transcription might function as the recognition site for an RNA polymerase or for an accessory transcription factor. Here we demonstrate the existence of one or several proteins in a yeast extract that specifically bind to the three analyzed yeast tRNA genes and to the VA_I gene. The binding sites that we have analyzed are centered on the conserved posterior sequence element.

METHODS

Plasmids. Four plasmids containing yeast tRNA genes were used in these studies: YEp13 ($tRNA_3^{Leu}$) (19), pGT23 ($tRNA^{Gln}$) (20), 18U ($tRNA^{Arg-Asp}$) (21), and Δ13 ($tRNA^{Tyr}$,*SUP6*) (P. Johnson, personal communication). These four plasmids were kindly provided by P. Johnson, R. Ogden, and G. Tschumper. DNA containing the adenovirus VA_I and VA_{II} genes (6) was provided by S. Fuhrman. Plasmids were purified as described by Fuhrman *et al.* (22).

Preparation of Extracts. *Saccharomyces cerevisiae* strain 20B-12 (pep 4-3) (23) was grown in 1% Bacto yeast extract/2% Bacto peptone/3% glucose/0.1% ammonium sulfate in a fermenter to 10^8 cells per ml, collected by centrifugation, washed with distilled water, resuspended in breakage buffer (0.2 M Tris·HCl, pH 8.0/0.3 M ammonium sulfate/0.5 mM EDTA/10% glycerol/0.5 mM phenylmethylsulfonyl fluoride), and frozen in liquid nitrogen. Cells were broken in an Eaton press. The following procedures were performed at 0–4°C. Four volumes of breakage buffer containing 0.67 M ammonium sulfate and additional protease inhibitors (0.2 mM tosyl-L-lysine chloroethyl ketone, 0.2 mM tosyl-L-phenylalanyl chloromethyl ketone) were added to the broken material. After 2 min of sonication, cell debris was removed by centrifugation for 1 hr at 30,000 × g, and ammonium sulfate was added to the supernatant to 3 M. The precipitate was collected by centrifugation at 10,000 × g for 1 hr, resuspended in buffer A (20 mM Tris·HCl, pH 8.0/0.5 mM EDTA/0.5 mM dithiothreitol/10% glycerol) and dialyzed against buffer A50 (buffer A containing 50 mM ammonium sulfate). This extract was frozen in a dry ice/ethanol bath and stored at −70°C. One milliliter of extract was derived from 0.5 g of cells (wet weight). The extract (200 ml) was applied to a 1-liter DEAE-cellulose column (DE52, Whatman). After extensive washing of the column with buffer A50, proteins were eluted in the same buffer containing 0.3 M ammonium sulfate and concentrated by precipitation in 3 M ammonium sulfate. The collected precipitate was dialyzed against buffer A50, insoluble material was removed by centrifugation for 1 hr at 13,000 × g, and the supernatant was loaded onto a 60-ml phosphocellulose column (P11, Whatman). Proteins were eluted in three steps with buffer A containing 0.1, 0.2, and 0.6 M ammonium sulfate, successively. The proteins in each fraction were precipitated with 3 M ammonium sulfate. Each precipitate was resuspended in 2 ml of 20 mM Tris·HCl, pH 8.0/0.5 mM EDTA/0.5 mM dithiothreitol/5 mM $MgCl_2$/25% glycerol and dialyzed against the same buffer, also containing 50 mM ammonium sulfate. These extracts were frozen in portions and stored at −20°C; their protein concentrations ranged from 3 to 10 mg/ml.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

* Present address: Biozentrum, University of Basel, CH-4056 Basel, Switzerland.

Labeling and Isolation of DNA Fragments. Each plasmid containing a gene to be analyzed was digested with a restriction enzyme (New England BioLabs; Bethesda Research Laboratories) that cuts in close proximity to one end of the gene (see Fig. 1). Terminal phosphates were removed with calf intestinal alkaline phosphatase (Boehringer Mannheim) in the restriction buffer supplemented with 0.1 vol of 1 M Tris·HCl (pH 9.5). After phenol extraction and ethanol precipitation, the DNA was either labeled at the 5′ end with T4 polynucleotide kinase (gift of S. Brennan) and [γ-^{32}P]ATP (maximal specific activity, ICN) as a substrate or at the 3′ end with the large fragment of *Escherichia coli* DNA polymerase (Bethesda Research Laboratories) or with T4 DNA polymerase (Bethesda Research Laboratories) and [α-^{32}P]deoxyribonucleoside triphosphates (3,000 Ci/mmol, Amersham; 1 Ci = 3.7×10^{10} becquerels) as substrate. *E. coli* DNA polymerase was used to label those ends that were created by restriction enzymes that leave 5′ overhangs. Blunt 3′ ends of double-stranded DNA were labeled with T4 DNA polymerase, essentially according to O'Farrell *et al.* (24). All end-labeling reactions were terminated by heat inactivation of the enzymes at 70°C for 10 min. Restriction digestion at the other (unlabeled) ends of the genes was done by adding enzymes to the same reaction mixtures. Labeled restriction fragments were separated on polyacrylamide gels; bands were cut out of the gels, and the DNA was eluted by diffusion.

Footprint Assay. End-labeled DNA (5,000–10,000 cpm, Čerenkov) was incubated on ice for 5 min in 20 μl of buffer Z (20 mM Tris·HCl, pH 7.5/25 mM ammonium sulfate/7 mM $MgCl_2$/1.25 mM dithiothreitol/6% glycerol/50 μg of bovine serum albumin per ml) with 1–5 μl of yeast extract. Ten to 40 ng of DNase I (Miles), which had been diluted shortly before use from a stock solution (1 mg/ml) with buffer Z containing 250 μg of bovine serum albumin per ml and 0.1 mM $CaCl_2$, was added, and the digestion was allowed to proceed at 20°C for 2 min. The reaction was stopped by the addition of 50 μl of 10 mM Tris·HCl, pH 8.0/20 mM EDTA/0.2% $NaDodSO_4$/40 μg of sheared calf thymus DNA per ml and boiled for 2 min. Proteins were removed by phenol extraction, and the DNA was precipitated with ethanol. DNA fragments were separated on 5%, 6%, or 8% polyacrylamide gels containing 8 M urea (25). Gels were exposed wet to Cronex film at −70°C (DuPont) using intensifier screens. The A+G sequencing reaction was done by the method of Maxam and Gilbert (26).

RESULTS

Footprints with the $tRNA_3^{Leu}$ Gene. The interaction of proteins with specific regions of genes that are transcribed by RNA polymerase III was searched for by using the DNase digestion pattern assay developed by Galas and Schmitz (27, 28). The appropriate end-labeled restriction fragment was incubated with a yeast protein fraction. During the subsequent partial DNase I digestion, the segments of DNA that specifically interacted with proteins were protected from endonucleolytic cleavage. This protection depletes the digestion products of those DNA strands that extend from the labeled end to the protected DNA segment and creates the more-or-less pronounced "hole" in the banding pattern that is characteristic of the footprint. Binding of protein to the DNA also may enhance the susceptibility of certain adjacent internucleotide linkages to enzymatic cleavage, creating characteristically pronounced bands. The digestion products were resolved on polyacrylamide gels side-by-side with control DNA that had been digested with DNase I without the cell extract. The yeast protein fractions that were tested came from an S-30 extract that had been depleted of most of its nucleic acids on DEAE-cellulose and fractionated on phosphocellulose. A restriction fragment containing the yeast $tRNA_3^{Leu}$ gene (Fig. 1, line 1) was incubated with the different phosphocellulose fractions and assayed for protection from DNase I digestion. One of the fractions did render a region of this DNA resistant to DNase I (Fig. 2*A*, compare lanes 1 and 2). The same region of the $tRNA_3^{Leu}$ gene was protected in other restriction fragments containing this gene (not shown). However, incubation of various fragments of pBR322 DNA with the same phosphocellulose fraction did not appreciably change their DNase I digestion patterns (not shown). The extract did decrease the general sensitivity of DNA to DNase I but without changing the pattern of susceptibility that generates the control pattern of bands. The nonspecific effects of the extract could be compensated by varying the amount of DNase I. The fraction that contained protein specifically interacting with the $tRNA_3^{Leu}$ gene was eluted from the phosphocellulose column between 0.1 and 0.2 M ammonium sulfate. It was used throughout the rest of this study.

The footprint pattern in Fig. 2*A* showed a signal in the 3′ part of the $tRNA_3^{Leu}$ gene (marked with a vertical bar) and also in the 5′ portion of the gene. In what follows, we shall focus the analysis almost entirely on the posterior portion of the gene. In order to more precisely locate the binding site in that part of the gene, we ran a long gel (Fig. 2*B*, lanes 3 and 4) in parallel with a reaction determining the purine sequence of the same DNA fragment as the marker (Fig. 2*B*, lane 5). To see whether binding also protected the complementary DNA strand and to increase the resolution of the footprint, we also used the identical DNA fragment labeled at the same end in the complementary strand (Fig. 2*B*, lanes 1 and 2). The minimum extent of protection (indicated at the side in Fig. 2*B*) was estimated from the outermost DNA bands, whose intensities were clearly reduced by the extract. The maximum extent of protection was judged on the basis of the locations of the proximal DNA bands, on either side, whose intensities were unaltered or even enhanced by the extract. The conserved posterior DNA sequence element of tRNA genes (10, 11) is shown in Fig. 2 by a black box. The center of the protected region in the $tRNA_3^{Leu}$ gene (Fig. 2*B*) falls within this conserved sequence.

Footprints with Other tRNA Genes. If the conserved posterior sequence element actually is the recognition site for the protein(s) that protect the $tRNA_3^{Leu}$ gene from DNase I digestion, we should expect other tRNA genes to interact with the same protein(s). Therefore, footprints of two additional genes coding for tRNAs were made. The DNA probe containing the $tRNA^{Gln}$ gene was labeled in either strand at the *Alu* I site (Fig. 1, line 2). The *Hin*cII–*Eco*RI fragment from plasmid 18U (Fig. 1, line 3) contains the regions coding for $tRNA^{Arg}$ and $tRNA^{Asp}$. Its transcription *in vitro* from a single initiation site yields the dimeric $tRNA^{Arg-Asp}$ precursor (21). These DNA fragments were subjected to DNase digestion pattern analysis (Fig. 2 *C* and *D*).

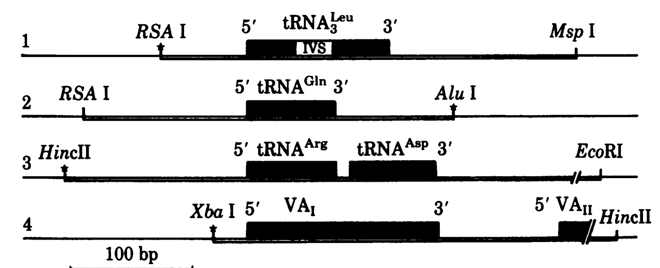

FIG. 1. DNA fragments used to probe protection from DNase. Restriction sites that were radioactively labeled are indicated by asterisks. The DNA fragments used as probes are represented by double lines. IVS, intervening sequence.

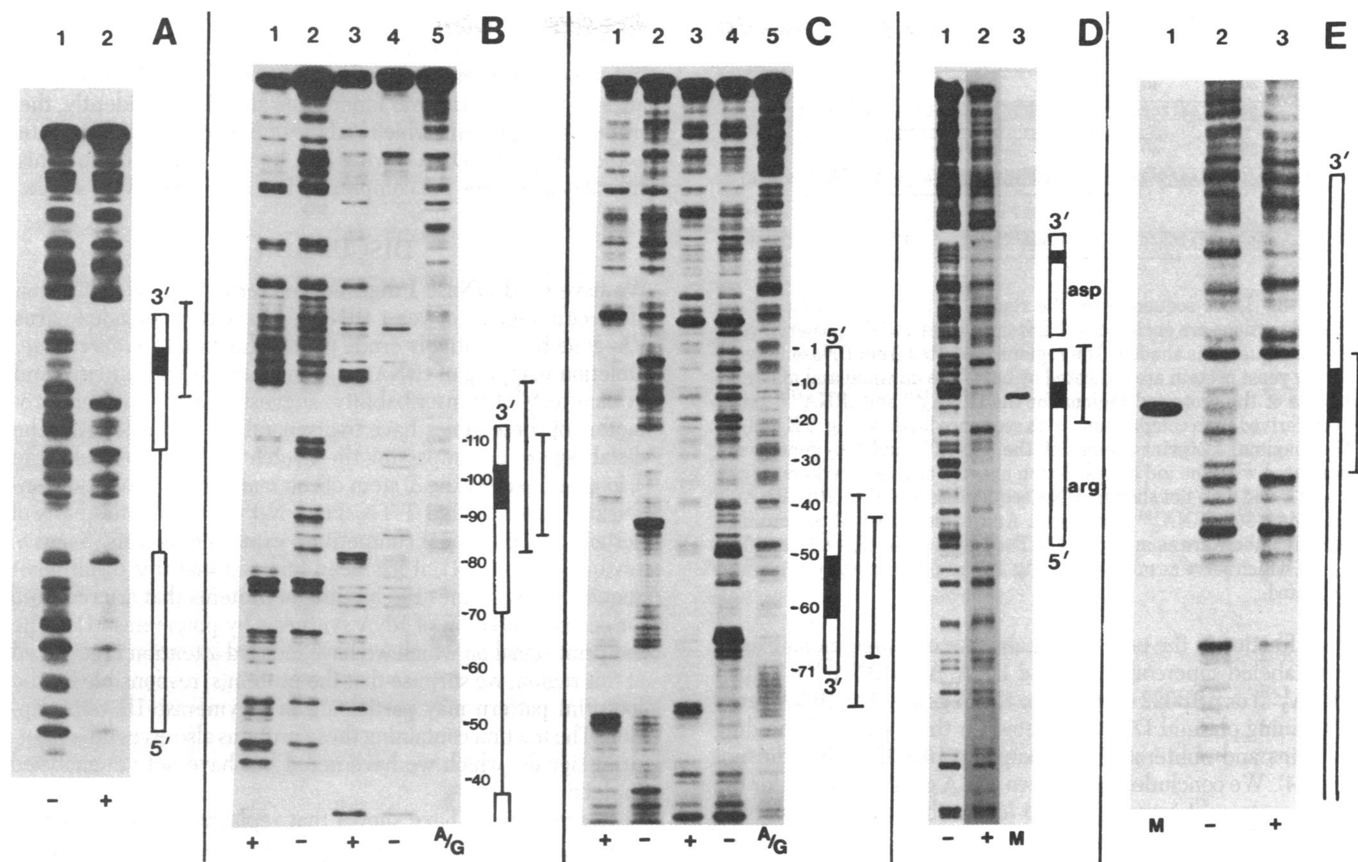

FIG. 2. Digestion patterns with tRNA genes and the adenovirus 2 *VA$_I$* gene without (−) and with (+) added yeast protein fractions. (*A*) tRNA$_3^{Leu}$ gene, labeled at the 5′ end. (*B*) tRNA$_3^{Leu}$ gene. Lanes: 1 and 2, 3′ end-labeled probe; 3 and 4, 5′ end-labeled probe; 5, purine sequence determination reaction (A/G) with 5′ labeled probe. (*C*) tRNA^{Gln} gene. Lanes: 1 and 2, 3′ labeled probe; 3 and 4, 5′ labeled probe; 5, purine sequence determination reaction with 5′ labeled probe. (*D*) tRNA^{Arg}–tRNA^{Asp} genes, labeled at the 5′ end. Lane 3 shows the probe digested with *Taq* I; the *Taq* I site is located within the conserved posterior sequence of the tRNA^{Arg} gene, and the resulting end-labeled fragment (M) is 202 nucleotides long. The photographic exposure times for lanes 2 and 3 are not identical. (*E*) Adenovirus *VA$_I$* gene, labeled at the 3′ end. Lane 1 shows the probe digested with *Taq* I; the *Taq* I site is located within the conserved posterior sequence, and the resulting end-labeled fragment (M) is 94 nucleotides long. Diagrams adjacent to autoradiograms show the genes with 5′ and 3′ ends of mature RNA marked. The thin line in Fig. 2 *A* and *B* indicates the intervening sequence in the tRNA^{Leu} gene (29). The posterior consensus sequence that is referred to in the text is marked by black filled boxes. Numbers refer to the distance in nucleotides from the mature 5′ ends of RNA. The maximal and minimal extents of DNA protected from DNase I digestion by bound protein are indicated by bars in Fig. 2 *B* and *C*; in Fig. 2 *A*, *D*, and *E*, only the maximal extents of protection are indicated.

The regions of tRNA^{Gln} and tRNA^{Arg} DNA that were protected from DNase I digestion by the extract were again centered around the posterior conserved sequence element. Protection of the posterior portion of the tRNA^{Asp} gene also can be seen in Fig. 2*D*. Not having mapped the protected region sufficiently precisely, we are uncertain whether the centering on the posterior sequence element is the same as it is for the other genes.

Additional changes in the DNase I digestion pattern were also recognizable in other regions of other genes, including the proximal segments of the tRNA^{Gln} and tRNA^{Arg} genes (Fig. 2 *C* and *D*). It might well be that different proteins that are present in this rather crude fraction specifically bind to these other DNA sequences, and it is interesting to pursue that possibility further. The point we would like to stress here is that when we incubated tRNA genes with the protein fraction, we found protection of the DNA around the conserved posterior sequence element.

Footprint with the Adenovirus *VA$_I$* Gene. It is interesting that the binding site of yeast proteins to several yeast tRNA genes includes the highly conserved posterior sequence. However, the significance of the conservation for protein–DNA interaction, *per se,* is ambiguous, simply because the requirements of tRNA function alone may well dictate this conservation. Therefore, we wished to examine protein interactions with a non-tRNA gene containing this sequence. The function of VA$_I$ RNA is not clear. It evidently folds into a structure that is globally very different from that of tRNA (30), although it is capable of forming a stem and loop involving the conserved posterior sequence, as tRNA does. In the adenovirus 2 *VA$_I$* gene, nucleotides 59–69 show a perfect match (Fig. 3) to the consensus posterior sequence element of tRNA genes. A DNase footprint around this conserved sequence was found in the *VA$_I$* gene *Xba I–Hinc*II restriction fragment (Fig. 1, line 4) in the presence of the yeast fraction (Fig. 2*E*), strengthening the view that the conserved sequence element might be involved with a protein-binding site in DNA. Nucleotides 99–109 and 111–121 of the *VA$_I$* gene show partial matches to the posterior consensus sequence and weaker binding to this region could also be detected.

In fractionating the RNA polymerase III *in vitro* transcription system from HeLa cells, S. A. Fuhrman, in our laboratory, and D. R. Engelke (unpublished data) have already detected a footprint activity on *VA$_I$* DNA with a partly purified fraction that is required for transcription.

Competition Experiments. To determine whether different tRNA genes specifically bind the same protein(s), we turned to competition experiments. Radioactively labeled DNA containing the tRNA$_3^{Leu}$ gene was incubated with a limiting amount of

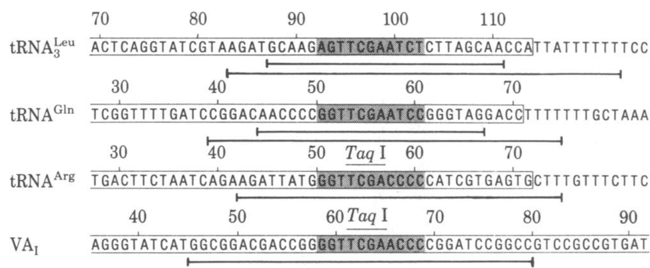

FIG. 3. DNA sequences in the region of protein binding. Transcribed portions are enclosed by horizontal lines and the posterior consensus sequence is shaded. The regions protected from DNase I digestion by yeast protein are indicated by bars. The minimal and maximal extents of the protected regions for the $tRNA_3^{Leu}$ and $tRNA^{Gln}$ genes were derived from comparisons with sequence ladders (Fig. 2 *B* and *C*). The maximal footprint regions of the $tRNA^{Arg}$ and VA_I genes were estimated with the aid of restriction enzyme-cleaved markers (Fig. 2 *D* and *E* and data not shown). DNA sequences: $tRNA^{Gln}$ (20); $tRNA^{Arg}$ (21); VA_I (30); $tRNA_3^{Leu}$ (ref. 29; A. Andreadis, personal communication). Numbers are as in Fig. 2. The *Taq* I sites in the $tRNA^{Arg}$ and VA_I genes, which serve as markers for Fig. 2 *D* and *E*, respectively, are also indicated.

yeast fraction in the presence or absence of a large molar excess of unlabeled supercoiled plasmid DNA: Δ13 ($tRNA^{Tyr}$), YEp13 ($tRNA_3^{Leu}$) or pBR322 (control; no tRNA gene). The tRNA gene-containing plasmid DNA competed for the specifically binding proteins and obliterated the footprint; pBR322 DNA did not (Fig. 4). We conclude that the two tRNA genes compete for the same proteins. Linear Δ13 DNA (cut at the *Eco*RI site, outside the $tRNA^{Tyr}$ gene) also acted as a competitor. When Δ13 DNA was cut with *Taq* I restriction endonuclease, which cuts the $tRNA^{Tyr}$ gene in the middle of the conserved posterior sequence, the competing activity was lost (data not shown). This is consistent with the supposition that the consensus sequence determines the affinity of this DNA for the specifically binding proteins.

Establishing the conditions for competition allowed us to examine the stability of the DNA–protein complex that generated the footprint. The end-labeled DNA probe containing the $tRNA_3^{Leu}$ gene was preincubated with the yeast protein fraction for 5 min at 20°C. Thereafter, an amount of competitor DNA (plasmid Δ13) that could prevent protection of the labeled DNA probe from DNase I digestion if simultaneously present (Fig. 5, lane 7) was added. Samples were taken for DNase I digestion at different times after addition of Δ13 DNA (Fig. 5, lanes 3–6). A footprint was found even after 1 hr. Evidently, the probe-bound protein, which had the opportunity to redistribute to the competing DNA during that hour, failed to do so. This indicates that, once the complex had formed, it was very stable.

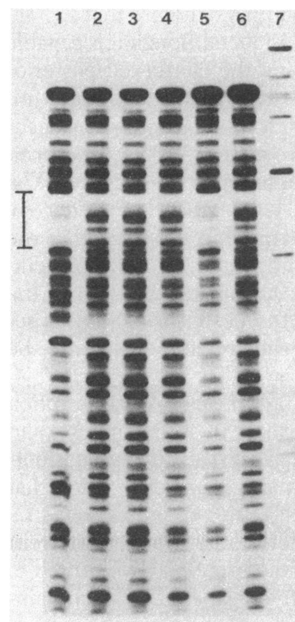

FIG. 4. Footprint competition with supercoiled DNA. Each sample contained ≈1 fmol of 341-base-pair DNA containing the $tRNA_3^{Leu}$ gene, 5′ end-labeled in one strand. Unlabeled competition DNA (162 fmol) was added to the samples for lanes 1–4. (The competitor DNA being entire plasmids, the nucleotide molar excess was in the range of $2–5.6 \times 10^3$.) Yeast protein fraction was added to the samples for lanes 1–5. Lanes: 1, pBR322 DNA competitor (0.46 μg); 2, Δ13 DNA competitor (0.50 μg); 3, pVAwt DNA competitor [containing the VA_I and VA_{II} genes (6); 0.59 μg]; 4, YEp13 DNA competitor (1.15 μg); 5, no competitor; 6, no competitor and no yeast protein; 7, 5′ end-labeled *Hin*f I digest of pBR322 DNA. The bar, which designates the maximal protected region as in Figs. 2 *A* and *B*, covers nucleotides 83–115. (Coordinates are shown in Fig. 2*B*).

DISCUSSION

We have used DNase I digestion patterns to show protection of specific regions of yeast tRNA genes and of the adenovirus VA_I gene by a relatively crude protein fraction of *S. cerevisiae*. Deletion mapping of tRNA genes, combined with *in vitro* and *in vivo* tests of transcribability, suggest that the internal promoters of these genes have two separable regions (8–17). The distal region, encompassing the stretch of DNA that yields the T loop and part of the T stem of the mature RNA, has the consensus sequence G-G-T-T-C-Pu-A-N-Py-C-C. On the basis of certain transcriptional competition experiments (refs. 5 and 8; reviewed in ref. 31), it has been inferred that this distal DNA sequence determines the binding of proteins that are required for correct initiation of RNA synthesis by polymerase III. The footprint signal on which we have focused attention is centered at this region; we surmise that the protein(s) responsible for the footprint pattern may participate in polymerase III transcription. The fraction containing these proteins also gives other footprint signals, which we have noted but have not yet analyzed in detail.

Recent reports have shown that replacing the posterior region of two tRNA genes by a segment of a mouse immunoglobulin G gene (32) or by pBR322 (13) still permitted transcription in a *Xenopus* system. The substituted DNA segments, which lack the original consensus sequences, contain dyad symmetries and are capable of forming stem-and-loop (cruciform) structures similar to those of the tRNA genes. Based on this and other considerations, Hall *et al.* (31) have suggested that the ability of the posterior consensus segments of DNA to assume appropriate stem-and-loop structures and to interact with anterior segments through tertiary base pairing is important for correct transcription by RNA polymerase III. Thus far, our experiments

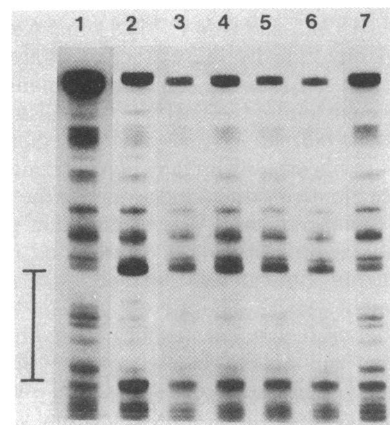

FIG. 5. Stability of a protein–DNA complex. The $tRNA_3^{Leu}$ gene probe was preincubated without (lane 1) or with (lanes 2–7) yeast protein fraction for 5 min at 20°C. Lanes: 3–6, 1 μg of unlabeled competitor Δ13 DNA was added, and incubation was continued at 20°C for 5, 10, 30, or 60 min, respectively; 2, incubation was for 10 min at 20°C without added competitor DNA; 7, unlabeled Δ13 DNA (1 μg) was added to the labeled probe DNA before the yeast protein fraction, and the mixture was then incubated for 10 min at 20°C. The bar designates the maximal protected region.

have been confined to DNA that shares the posterior consensus sequence and so do not directly distinguish between alternative structural models. However, it is worth noting that both strands of tRNA^{Leu} and tRNA^{Gln} DNA are protected from DNase I (Fig. 2). Moreover, we do not see strong competition for the protection-conferring yeast proteins by pBR322 DNA (Fig. 4). Further footprint experiments with appropriate DNA segments would obviously be interesting.

Koski *et al.* (17) have shown that a single-base change in the posterior consensus sequence C → T or G at the fifth nucleotide, leads to a loss of promoter function in a *S. cerevisiae* tRNA^{Tyr} gene. However, Zasloff *et al.* (33) have isolated a human tRNA^{Met} gene with a T instead of the conserved purine at the next (sixth) nucleotide. This tRNA gene is transcribed normally but fails to be properly processed. Thus, the consensus sequence contributes to promoter activity and to other functions as well. Prokaryotic tRNA genes also have the conserved posterior consensus sequence, and the RNA, of course, has the stem-and-loop structure. In fact, the *E. coli* tRNA^{Trp} gene can be transcribed in a eukaryotic transcription system (referred to in ref. 10), although the 5′ flanking region, of course, contains its own prokaryotic promoter (34). It is possible that one of the polymerase III transcription factors has evolved to utilize an even more ancient, conserved sequence or structural feature of tRNA genes. This transcription-oriented discussion notwithstanding, it should be pointed out that we have not yet demonstrated the participation of the DNA-binding protein(s) in polymerase III transcription. A rigorous demonstration of that role would require complete purification. The DNA-binding factor(s) also could be involved in establishing the local structure of chromatin (35). S. Fuhrman and D. Engelke have observed a VA_I gene-binding activity in a partly purified fraction from HeLa cells that has polymerase III transcription factor activity (personal communication).

The competition experiment (Fig. 4) shows that the DNA–protein interaction involves a common (set of) protein(s) binding to several different tRNA genes and to the VA_I gene. We find posterior region footprint patterns spanning ≈25–35 nucleotides in four different genes, all centered on the posterior consensus sequence (Fig. 3). The different genes have divergent nucleotide sequences surrounding this core sequence. Presumably, the region of protected DNA and the required sequences for protein binding are not congruent (29). Sequences at the wings of the binding site might be protected from DNase I attack by steric hindrance; also, the binding proteins might interact predominantly with the sugar-phosphate backbone outside the core of the binding site. In current experiments, the parameters affecting protein–DNA interaction are being explored. The results of the experiment shown in Fig. 5 indicate that one of the protein–DNA complexes is stable for at least 60 min at 20°C. It is interesting that one can use footprint assays in relatively crude fractions to examine these characteristics.

We thank S. M. Brennan, D. A. Brow, J. R. Greene, and G. P. Tocchini-Valentini for helpful and critical comments on the manuscript; S. M. Brennan, S. A. Fuhrman, P. Johnson, R. Ogden, and G. Tschumper for gifts of DNA, plasmids, or polynucleotide kinase; Z. Berry and K. Duquet for preparing plasmid DNA; G. A. Kassavetis for advice; D. R. Engelke and S. A. Fuhrman for numerous discussions; and A. Andreadis for communicating information in advance of publication. R.K. was a postdoctoral fellow of the Swiss National Foundation for Scientific Research. D. J. S. was a predoctoral trainee of a U.S. Public Health Service training grant in Cell and Molecular Biology. Our research was supported by a grant from the National Institute of General Medical Sciences.

1. Bogenhagen, D. F., Sakonju, S. & Brown, D. D. (1980) *Cell* **19**, 27–35.
2. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. (1980) *Cell* **19**, 13–25.
3. Engelke, D. R., Ng, S. Y., Shastry, B. S. & Roeder, R. G. (1980) *Cell* **19**, 717–728.
4. Korn, L. J. (1982) *Nature (London)* **295**, 101–105.
5. Fowlkes, D. M. & Shenk, T. (1980) *Cell* **22**, 405–413.
6. Guilfoyle, R. & Weinmann, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3378–3382.
7. Fuhrman, S. A., Deininger, P. L., LaPorte, P., Friedmann, T. & Geiduschek, E. P. (1981) *Nucleic Acids Res.* **9**, 6439–6456.
8. Kressmann, A., Hofstetter, H., DiCapuo, E., Grosschedl, R. & Birnstiel, M. L. (1979) *Nucleic Acids Res.* **7**, 1749–1763.
9. Telford, J. L., Kressmann, A., Koski, R. A., Grosschedl, R., Muller, F., Clarkson, S. G. & Birnstiel, M. L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2590–2594.
10. Galli, G., Hofstetter, H. & Birnstiel, M. L. (1981) *Nature (London)* **294**, 626–631.
11. Hofstetter, H., Kressmann, A. & Birnstiel, M. L. (1981) *Cell* **24**, 573–585.
12. DeFranco, D., Schmidt, O. & Söll, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3365–3368.
13. Sharp, S., DeFranco, D., Dingermann, T., Farrell, P. & Söll, D. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6657–6661.
14. Garber, R. L. & Gage, L. P. (1979) *Cell* **18**, 817–828.
15. Ciliberto, G., Castagnoli, L., Melton, D. A. & Cortese, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1195–1199.
16. Ciliberto, G., Traboni, C. & Cortese, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1921–1925.
17. Koski, R. A., Clarkson, S. G., Kurjan, J., Hall, B. H. & Smith, M. (1980) *Cell* **22**, 415–425.
18. Sprague, K. U., Larson, D. & Morton, D. (1980) *Cell* **22**, 171–178.
19. Broach, J. R., Strathern, J. N. & Hicks, J. B. (1979) *Gene* **8**, 121–133.
20. Tschumper, G. & Carbon, J. (1982) *J. Mol. Biol.* **156**, 293–307.
21. Schmidt, O., Mao, J., Ogden, R., Beckmann, J., Sakano, H., Abelson, J. & Söll, D. (1980) *Nature (London)* **287**, 750–752.
22. Fuhrman, S. A., VanBeveren, C. & Verma, I. M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5411–5415.
23. Jones, E. (1976) *Genetics* **85**, 23–33.
24. O'Farrell, P. H., Kutter, E. & Nakanishi, M. (1980) *Mol. Gen. Genet.* **179**, 421–535.
25. Sanger, F. & Coulson, A. R. (1978) *FEBS Lett.* **87**, 107–110.
26. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
27. Galas, D. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157–3170.
28. Schmitz, A. & Galas, D. J. (1979) *Nucleic Acids Res.* **6**, 111–137.
29. Venegas, A., Quiroga, M., Zaldivar, J., Rutter, W. J. & Valenzuela, P. (1979) *J. Biol. Chem.* **254**, 12306–12309.
30. Akusjärvi, G., Mathews, M. B., Andersson, P., Vennström, B. & Pettersson, U. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2424–2428.
31. Hall, B. D., Clarkson, S. G. & Tocchini-Valentini, G. P. (1982) *Cell* **29**, 3–5.
32. Carrara, G., Di Segni, G., Otsuka, A. & Tocchini-Valentini, G. P. (1981) *Cell* **27**, 371–379.
33. Zasloff, M., Santos, T. & Hamer, D. H. (1982) *Nature (London)* **295**, 533–535.
34. Siebenlist, U., Simpson, R. B. & Gilbert, W. (1980) *Cell* **20**, 269–281.
35. Wittig, S. & Wittig, B. (1982) *Nature (London)* **297**, 31–38.