Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 8763–8767, December 1987
Biochemistry

Comparative mutational analysis of wild-type and stretched $tRNA_3^{Leu}$ gene promoters

(site-directed mutagenesis/DNA-binding proteins)

PATRIZIA FABRIZIO*, ANNA COPPO*, PAOLO FRUSCOLONI†‡, PIERO BENEDETTI†, GIANFRANCO DI SEGNI†§, AND GLAUCO P. TOCCHINI-VALENTINI†

*Dipartimento di Biologia Cellulare e dello Sviluppo, Sez. Scienze Microbiologiche, Università degli Studi La Sapienza, 00185 Rome, Italy; †Istituto di Biologia Cellulare, Consiglio Nazionale delle Ricerche, 00196 Rome, Italy; and ‡Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637

Communicated by E. Peter Geiduschek, July 28, 1987

ABSTRACT **We demonstrate that, when the yeast $tRNA_3^{Leu}$ gene is stretched so that the distance between the two portions of the intragenic promoter is increased to 365 base pairs, the A and B blocks remain functional. Mutations in the A block, which show a weak phenotype when inserted in the wild type, exert a dramatic effect when inserted into the stretched gene. Experiments with extensively purified transcription factor τ indicate that the τ_B–B block interaction is not influenced by A–B distance; only the ability of τ_A to interact with A block sequences is affected, possibly because of the additional free-energy cost of forming a large loop of the intervening DNA.**

There are many examples in prokaryotes and eukaryotes of split regulatory sites; it is important to establish, in each case, the type of mechanism utilized in the regulation of transcription. For example, the intragenic eukaryotic tRNA promoter contains two sequences, termed the A and B blocks, that are set far apart. In natural yeast tRNA genes the distance between the two blocks can vary from 31 to 93 base pairs (bp), the variability being due to the length of the extra arm and the presence, within certain tRNA genes, of an intervening sequence. It has been established that the transcription factor τ, through its τ_A and τ_B domains, interacts with the intragenic promoter (1–4). The B block exerts a dominant effect and the A block assists in the stabilization of the binding of τ (4, 5). How does the arrangement of the two blocks affect the interaction of this factor? Baker *et al.* (6) established the lower limit of the A-to-B distance in the yeast $tRNA_3^{Leu}$ gene that allows optimal factor interaction. We have not found the corresponding evidence for an upper limit. In this paper we report that extending the A-to-B distance from 74 to 365 bp within the same gene still allows some transcription to occur in the homologous cell-free system. We used template activity, transcription competition measurements and "footprinting" to characterize A and B block mutations in the wild type and in the stretched genes. Our comparative mutational analysis demonstrates that the A and B blocks are functional even at a large distance apart. We suggest that the τ protein, when bound to the two sites, induces looping of the intervening DNA.

MATERIALS AND METHODS

Plasmids. Plasmid pT0 was constructed by subcloning the 2.5-kilobase (kb) *Eco*RI DNA fragment containing the yeast $tRNA_3^{Leu}$ gene from pJB2K (7) into pBR322 at the *Eco*RI site, as described by Johnson *et al.* (8). Plasmids pT1 and pT2 contain a modified yeast $tRNA_3^{Leu}$ gene, obtained by insertion of a 293-bp IgG-encoding fragment from the MOPC 141 mouse myeloma (9) at the *Hpa* I site in the middle of the tRNA gene, in the orientation shown in Fig. 1 (pT2) or its inverse (pT1). During the insertion procedure, two adenylates of the *Hpa* I site of pT0 were lost. The restriction maps of these plasmids are shown in the paper of Carrara *et al.* (10). The yeast *SUP4-o* (ochre suppressor) $tRNA^{Tyr}$ gene is contained in a 4.5-kb *Eco*RI–*Hin*dIII DNA fragment inserted into pBR322 (11) and was kindly provided by B. D. Hall. The construction of mutants M1, M2, and M3 was previously described by Mattoccia *et al.* (12) and Baldi *et al.* (13). The oligodeoxyribonucleotide used to construct G_{56} was 5′ AGAGATTCCAACTCTTG 3′. Mutation M1 differs from the wild-type gene at positions 10, 11, and 12, where three A residues substitute for the sequence GCC in the noncoding strand. M3 differs from the wild-type sequence at positions 24, 25, and 26, where three T residues substitute for GGC. M2, a double mutant, is characterized by both substitutions described above, at positions 10, 11, and 12 and 24, 25, and 26. G_{56} differs from wild type at position 97, where one G substitutes for C in the noncoding strand; this nucleotide corresponds to position 56 in the standard tRNA numbering system (14).

Preparation of Extracts. Yeast extract was prepared essentially according to Koski *et al.* (15), with the following modifications. We used the *Saccharomyces cerevisiae* strain SX4-6A (*his-532, trp1-289, ura3-1, ura3-2, ade2*; kindly supplied by P. L. Donini). The cells were disrupted by agitation in a Bead-Beater (Biospec Product, Bartlesville, OK) for a total of 5 min with intermittent cooling. In some preparations, the extract, in 650 mM ammonium sulfate, was loaded onto a DEAE-Sephadex column (A25, Pharmacia) that had been previously equilibrated with buffer A [50 mM Tris·HCl, pH 7.9/100 mM ammonium sulfate/0.1 mM dithiothreitol/0.01 mM phenylmethylsulfonyl fluoride/25% (vol/vol) glycerol]. The extract was eluted with buffer A in the flow-through fractions. We have found that the DEAE-Sephadex column, loaded at high-salt conditions, removed an inhibitory activity more efficiently than the Sephadex G-25 column (15, 16). Factor τ was prepared and assayed as described by Ruet *et al.* (17). Some modifications were introduced; 90 ml of extract, obtained from 40 g of yeast cells, was applied to a heparin-Sepharose column (CL-6B, Pharmacia; 1.5 × 22 cm). The fractions active in reconstitution of transcription were pooled, precipitated by ammonium sulfate, dialyzed, and subjected to sedimentation on 4.5 ml of a 10–30% (vol/vol) glycerol gradient in Tris·HCl, pH 8/1 mM EDTA/10 mM 2-mercaptoethanol/300 mM ammonium sulfate. The gradient was centrifuged in a Beckman SW 50.1 rotor for 18 hr at 45,000 rpm, at 2°C.

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: Department of Genetics, SK 50, University of Washington, Seattle, WA 98195.

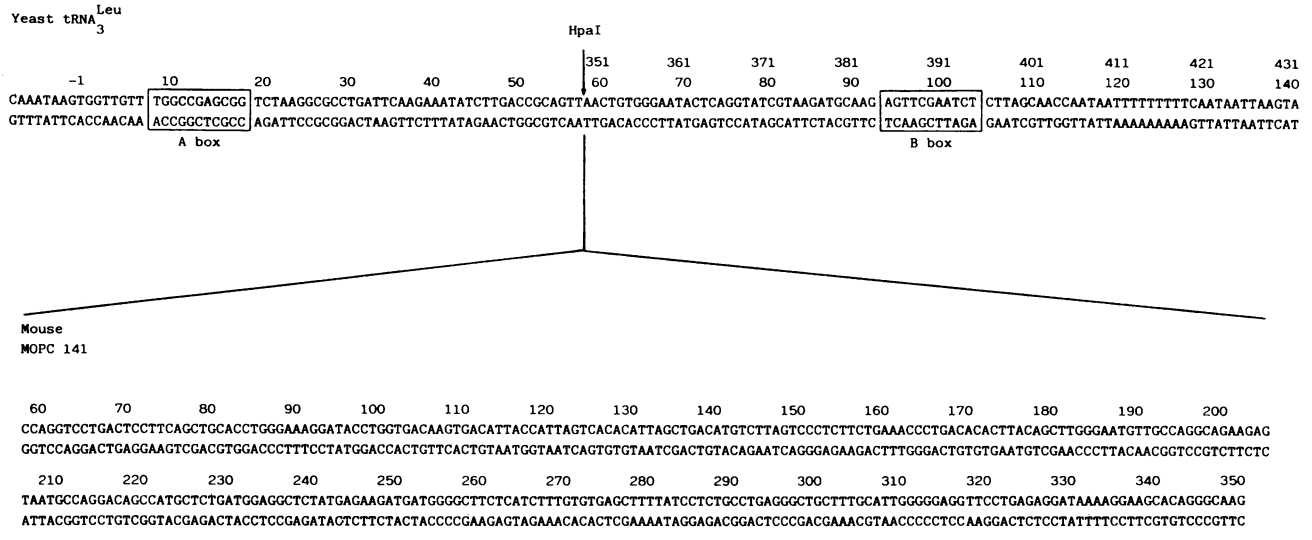

FIG. 1. General organization and sequence of the tRNA$_3^{Leu}$ gene and of an IgG gene fragment from the MOPC 141 mouse myeloma. The internal tRNA$_3^{Leu}$ gene promoter sequences (A and B blocks) are boxed. The tRNA$_3^{Leu}$ coding region starts at nucleotide 1 (G) and stops at nucleotide 114. The numbers from 351 to 431 on the top line indicate positions of tRNA gene nucleotides in pT2 plasmid. The RNA polymerase III termination site corresponds to the T cluster at position 119–127 in pT0 and at position 410–418 in pT2. The fortuitous RNA polymerase III termination site in pT2 corresponds to the T cluster at position 278–281.

Transcription Assay. The standard transcription reaction mixture contained, in a total volume of 20 μl, 3 μl of yeast extract and 250 ng of tRNA gene-containing plasmid DNA. Final concentrations of buffer components were 20 mM Hepes–KOH, pH 7.9, 5 mM $MgCl_2$; 130 mM KCl; 0.1 mM EDTA; 25% (vol/vol) glycerol; 0.1 mM dithiothreitol; 0.005 mM phenylmethylsulfonyl fluoride; 500 μM each of ATP, CTP, and UTP; and 50 μM [α-^{32}P]GTP (15–20 μCi/mmol, Amersham; 1 Ci = 37 GBq). Reaction mixtures were incubated at 25°C for 30–40 min and reactions were terminated as described by Birkenmeier *et al.* (17). The RNA products were dissolved in TBE buffer (80 mM Tris borate, pH 8.3/1 mM EDTA) containing 10% sucrose, 7 mM urea, and 0.05% each of bromophenol blue and xylene cyanol FF and were subjected to electrophoresis in thin (0.4 mm) 8% or 10% polyacrylamide gels (29:1, acrylamide/bisacrylamide) in TBE buffer containing 4 M urea. Gels were exposed wet to Kodak X-Omat S films at −50°C, with intensifying screens. RNA bands were excised from the gel and Cerenkov radiation was quantified to determine the relative amounts of transcripts.

Competition Assay. Samples (250 ng) of plasmids containing the gene (wild type or mutant) to be tested were incubated for 3 min at 25°C with 3–6 μl of yeast extract in 17.5 μl (total volume) of buffer in the standard transcription conditions. Then 250 ng of plasmid containing the *SUP4-o* tRNA^{Tyr} gene was added in 2.5 μl of the same buffer, incubation was continued for 30 min, and the reaction was terminated as the standard reaction was. Relative competitive strengths are given as percentage of blocked *SUP4-o* tRNA^{Tyr} gene transcription [with wild-type tRNA$_3^{Leu}$ gene (pT0) competitive strength normalized to 100%].

Footprint Assay. Plasmid pT0 or plasmid pT2 was cut with restriction enzyme *Xba* I (New England Biolabs). The DNA was treated with alkaline phosphatase in 10 mM Tris·HCl, pH 8, for 45 min at 45°C. After extraction with phenol and precipitation with ethanol, the DNA was labeled at the 5′ end with phage T4 polynucleotide kinase (Promega Biotec, Madison, WI) and [γ-^{32}P]ATP (Amersham). The double-end-labeled DNA was cut with *Eco*RI to produce one-end-labeled fragments. Labeled restriction fragments were separated on 4% polyacrylamide gels; bands were cut out of the gel and the DNA was eluted by diffusion. For the footprint assay 6 μl of yeast extract, 15–25 fmol of labeled DNA fragment (15,000–20,000 cpm), and 75 ng of unlabeled pBR322 carrier DNA were incubated in 25 μl of footprinting buffer [20 mM Tris·HCl, pH 7.5/100 mM NaCl/7 mM $MgCl_2$/1.25 mM dithiothreitol/7% (vol/vol) glycerol/5% (vol/vol) dimethyl sulfoxide] for 20 min at 20°C. Then 300–400 ng of DNase I (Miles) freshly prepared from a 1 mg/ml stock solution in footprinting buffer containing 0.5 mM $CaCl_2$ was added in 3 μl and the digestion was allowed to proceed at 20°C for 30 sec. The reaction was stopped by adding 75 μl of 100 mM Tris·HCl, pH 8/20 mM EDTA/0.6% $NaDodSO_4$ containing sheared calf thymus DNA (Sigma) at 8 μg/ml, and the sample was boiled for 3 min. Samples were digested with 10 μl of proteinase K (Boehringer Mannheim) at 1 mg/ml for 30 min at room temperature, extracted with phenol, and precipitated with ethanol. Samples were electrophoresed on 6% polyacrylamide/7 M urea sequencing gels according to standard procedures and were exposed wet to Kodak X-Omat S films at −50°C with intensifying screens.

Exonuclease III Assay. One-end-labeled *Xba* I/*Eco*RI DNA fragment, 10,000–30,000 cpm, representing about 4 ng of DNA, was incubated for 15–20 min at 25°C in 50 μl of footprinting buffer, with 0.5 μg of pBR322 DNA cut with *Eco*RI, 10 μg of total yeast tRNA (Sigma), 1 μg of poly(dG) (P-L Biochemicals), 2 mM sodium phosphate buffer at pH 7, and 10 μl of fractions from the preparative glycerol gradient containing factor τ. After the binding step, 100 units of exonuclease III (New England Biolabs) was added, and the mixture was allowed to digest for 45 min at 25°C. The reaction was terminated as described under *Transcription Assay*. The DNA was purified by extraction with phenol and precipitation with ethanol and was electrophoresed as described in *Footprint Assay*.

RESULTS

Effect of the Mutations on Transcription. The wild-type yeast tRNA$_3^{Leu}$ gene (pT0) has 74 bp between the last base pair of its A block sequence (gene position 18) and the first base pair of its B block sequence (gene position 93). [These positions correspond to 18 and 52 in the standard tRNA numbering system (14).] We constructed two stretched tRNA genes (pT1 and pT2) by inserting a 293-bp DNA fragment, in either orientation, into the *Hpa* I site in the middle of the wild-type gene (Fig. 1) (10).

To study the functional relationship between the A and B blocks in pT0 and pT2 we have produced the same mutations in both genes. Mutations in the wild type were introduced through the use of short synthetic oligonucleotides as site-specific mutagens, following a strategy derived from the one described by Wallace *et al.* (19). To construct mutations in the stretched gene, heteroduplex molecules were obtained by hybridizing linear single-stranded DNA, produced by denaturing the mutant DNAs (see Table 1) linearized at the *Hpa* I site, to single-stranded circles, derived from pT2 DNA.

The sequence changes in the various mutants used in this work are shown in Table 1. We used a *S. cerevisiae* extract as the source of RNA polymerase III and transcription factors (15, 20). In assaying template activity of the mutants, we used a template DNA concentration (12.5 μg/ml) that is limiting. With wild-type DNA (pT0) as the template at a concentration of 12.5 μg/ml, the amount of transcript produced was about half that obtained when saturating amounts (50 μg/ml) of pT0 DNA were used (not shown).

The results of such template activity measurements for the various mutant genes are shown in Fig. 2 and Table 1.

Mutations in the A block (M1; Fig. 2, lane 3) and in the B block (G_{56}; Fig. 2, lane 6) cause decreased accumulation of transcripts when inserted in the wild type. The mutation M3, localized immediately downstream of the A portion of the promoter, has no effect (Fig. 2, lane 5). The effect of G_{56} is more dramatic than that of M1 (Table 1). These results confirm the findings reported by Newman *et al.* (16) and indicate that the B block has a dominant role in tRNA transcription. This conclusion is in keeping with the idea that the anchorage of τ to the B block constitutes the first step in the initiation process.

The two stretched genes are both transcriptionally active, although they are weaker templates than the wild type. The two major transcripts observed with pT1 and pT2 (Fig. 2, lanes 7 and 8) correspond to the primary transcript. The faster migrating bands correspond to products terminating at the fortuitous termination site present in the inserted DNA. The sequences present in the RNAs were verified by Southern blotting and by S1 nuclease mapping (data not shown).

It is important to establish that the promoter of the stretched gene still consists of A and B blocks. We introduced into pT2 the same mutations that had been inserted in the wild type. Table 1 shows that, as in the wild type, the mutations M1 (Fig. 2, lane 9) and G_{56} (Fig. 2, lane 12) cause decreased accumulation of transcripts. We conclude that these A and B blocks are functional even at a large distance. Surprisingly, the mutation M1 in the stretched gene has a more dramatic effect than does G_{56}. Does this result mean that τ is not anchored to the B block in the stretched gene? To address this issue we performed a competition assay in which the competitive template, during the preincubation with the extract, is given the opportunity to sequester τ that is present in limiting amounts. A plasmid containing the yeast $tRNA^{Tyr}$ gene was used as the second template. Table 1 indicates that

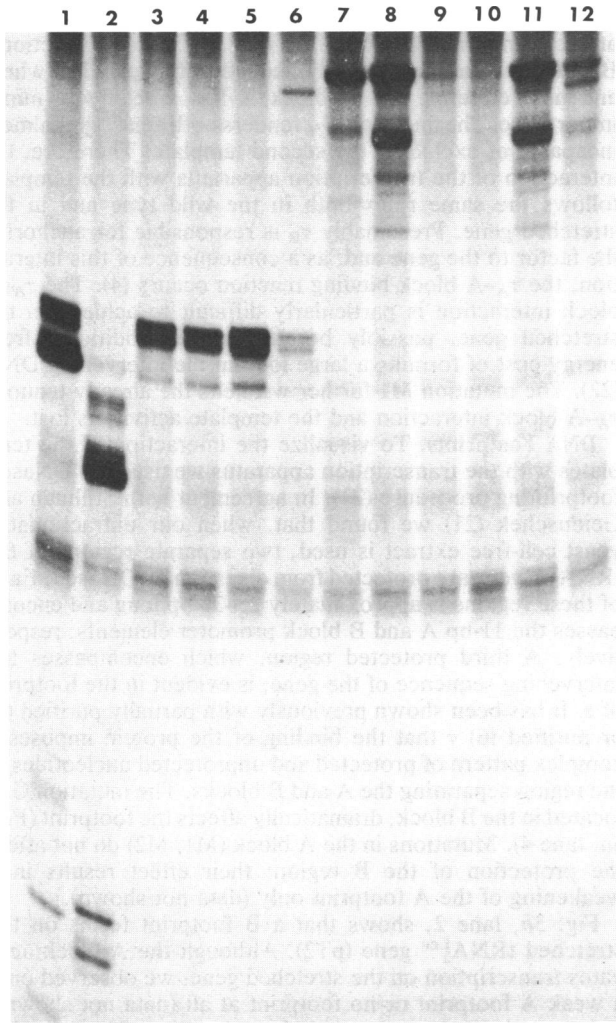

FIG. 2. *In vitro* transcription reaction of wild-type and mutant yeast tRNA genes. Lane 1, wild-type $tRNA_3^{Leu}$ gene (pT0); lane 2, wild-type *SUP4-o* $tRNA^{Tyr}$ gene; lanes 3, 4, 5, and 6, mutations M1, M2, M3, and G_{56}, respectively, of the pT0 gene (see Table 1); lane 7, stretched gene pT1; lane 8, stretched gene pT2; lanes 9, 10, 11, and 12, mutations M1, M2, M3, and G_{56}, respectively, of the stretched gene pT2.

Table 1. Organization, transcription, and competition of altered $tRNA_3^{Leu}$ genes

A block	B block	Template	A–B distance = 74 bp: Transcription efficiency, %	A–B distance = 74 bp: Competitive strength, %	Stretched template	A–B distance = 365 bp: Transcription efficiency, %	A–B distance = 365 bp: Competitive strength, %
10 11 12	24 25 26 97 (56)						
G–C–C	---G–G–C--- C	pT0	100	100	pT2	100*	75
A–A–A	----------	M1	40	68	M1	0	65
A–A–A	---T–T–T---	M2	36	51	M2	0	65
	---T–T–T---	M3	90	100	M3	95	70
	---------- G	G_{56}	13	10	G_{56}	17	10

Transcription efficiencies are given as percentage of pT0 or pT2 transcription. Bands corresponding to transcription products were quantitated by Cerenkov counting.

*Transcription efficiency of pT2 was 80% that of pT0.

both stretched and unstretched genes work reasonably well at excluding transcription of the $tRNA^{Tyr}$ gene. A functional B block is what is principally needed to tie up τ (21); where and how effective the A block is has a relatively minor importance. The mutation G_{56} renders both gene types almost incapable of excluding the second template. Therefore, the interaction of the transcription apparatus with the template follows the same rules both in the wild type and in the stretched gene. Presumably τ_B is responsible for anchoring the factor to the gene and, as a consequence of this interaction, the τ_A–A block binding reaction occurs (4). The τ_A–A block interaction is particularly difficult to achieve in the stretched gene, possibly because of the additional free-energy cost of forming a large loop in the intervening DNA (22). The mutation M1 further weakens the already tenuous τ_A–A block interaction and the template activity is lost.

DNA Footprints. To visualize the interaction of the templates with the transcription apparatus we used the DNase I footprinting procedure (23). In agreement with Stillman and Geiduschek (21) we found that, when our unfractionated yeast cell-free extract is used, two separate regions of the $tRNA_3^{Leu}$ gene are protected from digestion by DNase I. Each of these regions is approximately 25–30 bp long and encompasses the 11-bp A and B block promoter elements, respectively. A third protected region, which encompasses the intervening sequence of the gene, is evident in the footprint of τ. It has been shown previously with partially purified (1) or purified (6) τ that the binding of the protein imposes a complex pattern of protected and unprotected nucleotides in the region separating the A and B blocks. The mutation G_{56}, located in the B block, dramatically affects the footprint (Fig. 3*a*, lane 4). Mutations in the A block (M1, M2) do not affect the protection of the B region; their effect results in a weakening of the A footprint only (data not shown).

Fig. 3*b*, lane 2, shows that a B footprint forms on the stretched $tRNA_3^{Leu}$ gene (pT2). Although the A block activates transcription on the stretched gene, we observed only a weak A footprint or no footprint at all (data not shown). Baker *et al.* (6) show that τ binds optimally to DNAs having A–B distances of 30–60 bp, the distance found in the majority of yeast tRNA genes; when the A–B distance is larger than 53 bp it becomes difficult to see protection in the A region. The mutation G_{56} dramatically affects the footprint as with the wild-type gene.

The studies described above were carried out with a complete polymerase III transcription system; in the subsequent part of this paper, we used a purified preparation of τ.

Using purified τ, Baker *et al.* (6) show that when τ complexes, formed with the wild-type $tRNA_3^{Leu}$ gene, are digested from the 5′ end with phage λ exonuclease, two discrete protected fragments are produced. The differential stability of τ binding at the two intragenic regions is responsible for the alternative pause sites (24). We observed a stop to digestion 8 bp upstream of the A block; the other stop is at a position 13 bp preceding the B block (Fig. 4, lane 2). The upstream border is not observed when τ complexes, formed with the stretched gene, are digested with the exonuclease (Fig. 4, lane 3). Since the stop upstream of the B block is observed even when the A–B distance is increased, we conclude that varying the A–B distance primarily affects the ability of τ to interact with the A block.

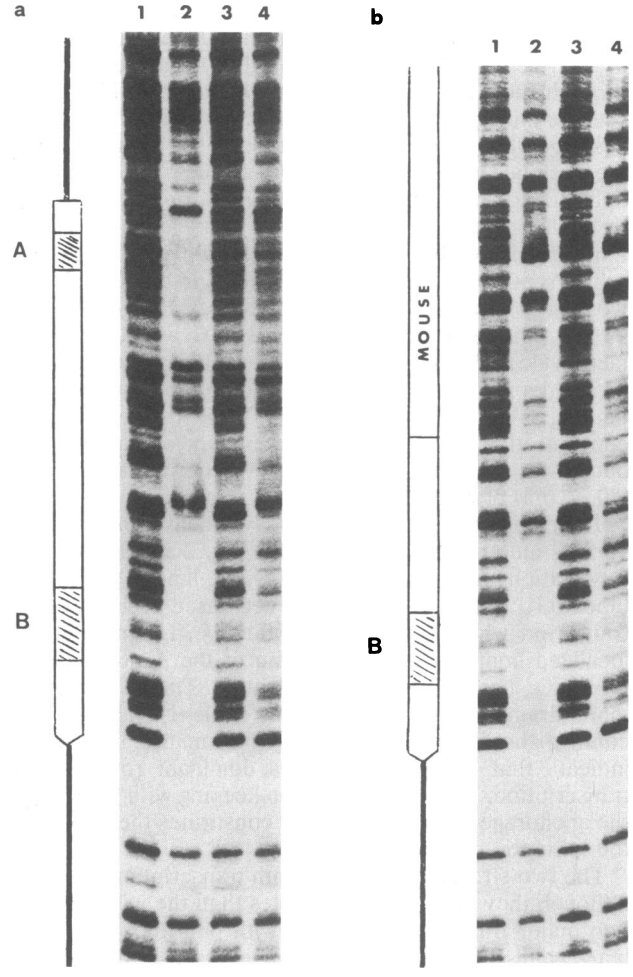

FIG. 3. Footprinting analysis. DNase I protection of wild-type and mutant $tRNA_3^{Leu}$ genes. *Xba* I–*Eco* RI fragments, of lengths 660 bp for pT0 and 951 bp for pT2, labeled at the 5′ end of the coding strand, were digested with DNase I after incubation with the yeast extract. (*a*) pT0; (*b*) pT2 (stretched gene). The genes are shown schematically on the left of each panel. Lanes 1 and 3 show the DNase I digestion pattern in the absence of the extract, and lanes 2 and 4 show the pattern in the presence of the yeast extract for the wild-type sequences (lanes 1 and 2) and for G_{56} mutants (lanes 3 and 4), respectively.

DISCUSSION

There is evidence, in both prokaryotes and eukaryotes, for the control of transcription by regulatory proteins that bind to separate DNA sites.

In a recent review Ptashne suggested a unified view of the regulation of disparate types of genes (25). In its simplest form this view can be described in two statements. First, regulatory proteins recognize specific structures in double-stranded DNA; second, DNA-bound regulatory proteins influence transcription by touching other DNA-bound proteins. This description, according to Ptashne, holds whether the interaction proteins are adjacent or whether they are separated on the DNA; in the latter case the DNA in between the separate sites loops out to allow protein–protein interaction.

tRNA genes are relatively simple, and therefore we anticipate that a description of the interaction of their intragenic promoter with the transcription apparatus will provide a unique window on to the problem of gene regulation. Experiments with partially purified and purified τ factor (3, 21) indicate that a single protein, although probably multipartite, interacts with both portions of the promoter. Our studies demonstrate that the τ_B–B block interaction is not greatly influenced by A–B distance; only the ability of τ_A to interact with A block sequences is affected. Baker *et al.* (6), working with the yeast $tRNA_3^{Leu}$ gene, established that there is a lower limit of 21 bp to the A–B distance. Our results indicate that even when the A–B distance is increased to 365 bp the two blocks remain functional.

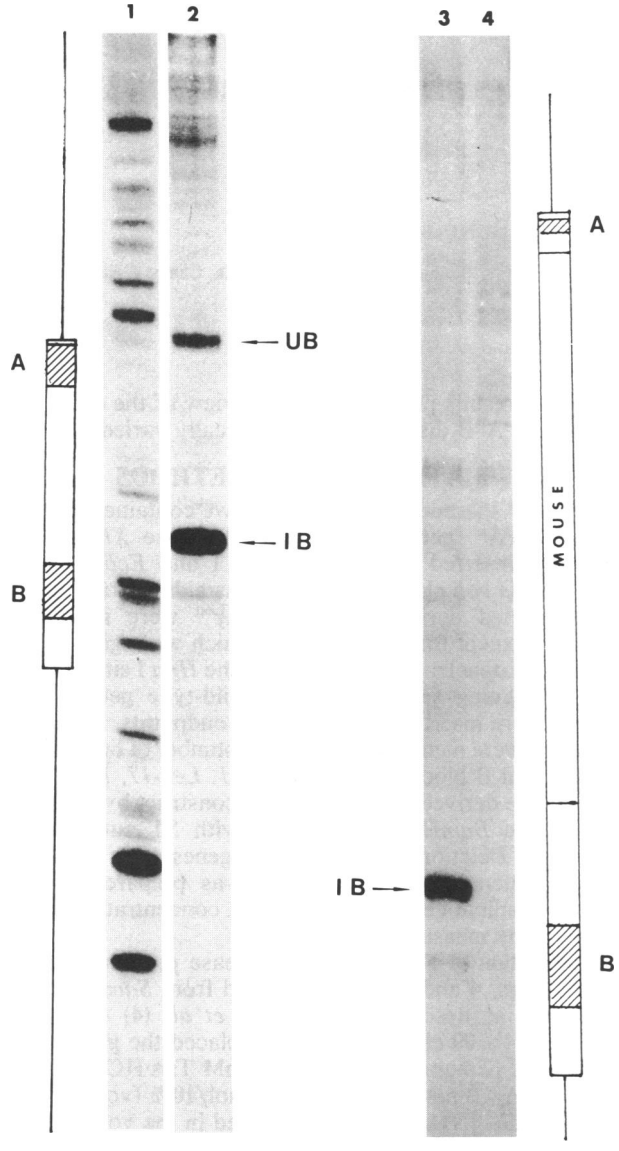

FIG. 4. Exonuclease III digestion. The *Xba* I–*Eco*RI fragments of pT0 (lanes 1 and 2) and pT2 (lanes 3 and 4), labeled at the 5′ end of the coding strand, were complexed with purified transcription factor and subsequently treated with exonuclease III. Lanes 1 and 4, controls without factor; lanes 2 and 3, nuclease-resistant DNA bands in the presence of factor. UB, upper border; IB, internal border.

In one model, the B block acts as the primary binding site for τ, a protein characterized by two binding domains: one for the A block and the other for the B block. The DNA present between the two blocks is so positioned that it allows τ to contact the A region. When the A–B distance is enormously increased a loop must be formed. Mutations in the A block exert a dramatic effect when inserted into the stretched gene, possibly because of the additional free-energy cost of forming a large loop in the intervening DNA.

In addition to looping, bending, or unwinding of the DNA may contribute to bring the A and B sites close together.

Although the anchorage of τ_B to the B block constitutes the first step in the initiation of transcription, the A block sequence is probably responsible for the selection of the initiation site (26). The residual activity observed with $tRNA_3^{Leu}$ genes lacking the B block finds an explanation in this hypothesis (10). Accordingly it was observed that, to overcome the predictable low affinity for τ of the genes that had undergone deletions, higher than normal concentrations of template were required (27).

We are grateful to B. D. Hall, R. Haselkorn, L. Rothman-Denes, and S. Ziemin for a critical reading of the manuscript. We thank G. Guidi and G. Di Franco for excellent technical assistance and A. Sebastiano for typing the manuscript. This work was supported in part by the Progetto Finalizzato Oncologia, by the Progetto Finalizzato Ingegneria Genetica e Basi Moleculari delle Malattie Ereditarie, and by a grant from Enichem. G.D.S. was awarded a fellowship from the A.I.R.C. (Italian Association for Cancer Research). A.C. was supported by the Fondazione Istituto Pasteur, Fondazione Cenci Bolognetti.

1. Klementz, R., Stillman, D. J. & Geiduschek, E. P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6191–6195.
2. Baker, R. E. & Hall, B. D. (1984) *EMBO J.* **3**, 2793–2800.
3. Marzouki, N., Camier, S., Ruet, A., Moenne, A. & Sentenac, A. (1986) *Nature (London)* **323**, 176–178.
4. Stillman, D. J., Caspers, P. & Geiduschek, E. P. (1985) *Cell* **40**, 311–317.
5. Baker, R. E., Gabrielsen, O. & Hall, B. D. (1986) *J. Biol. Chem.* **261**, 5275–5282.
6. Baker, R. E., Camier, S., Sentenac, A. & Hall, B. D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8768–8772.
7. Beckman, J. S., Johnson, P. F., Abelson, J. & Fuhrman, S. A. (1977) in *Molecular Approaches to Eucaryotic Systems*, eds. Wilcox, G., Abelson, J. & Fox, C. F. (Academic, New York), pp. 213–226.
8. Johnson J. D., Ogden, R., Johnson, P., Abelson, J., Dembeck, P. & Itakura, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2564–2568.
9. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. (1986) *Nature (London)* **286**, 676–683.
10. Carrara, G., Di Segni, G., Otsuka, A. & Tocchini-Valentini, G. P. (1981) *Cell* **27**, 371–379.
11. Kurjan, J., Hall, B. D., Gillam, S. & Smith, M. (1980) *Cell* **20**, 701–709.
12. Mattoccia, E., Baldi, M. I., Pande, G., Ogden, R. & Tocchini-Valentini, G. P. (1983) *Cell* **32**, 67–76.
13. Baldi, M. I., Mattoccia, E. & Tocchini-Valentini, G. P. (1983) *Cell* **35**, 109–115.
14. Gauss, D. H. & Sprinzl, M. (1984) *Nucleic Acids Res.* **12**, 59–131.
15. Koski, R. A., Allison, D. S., Worthington, M. & Hall, B. D. (1982) *Nucleic Acids Res.* **10**, 8127–8143.
16. Newman, A. J., Ogden, R. C. & Abelson, J. (1983) *Cell* **35**, 117–125.
17. Ruet, A., Camier, S., Smagowicz, W., Sentenac, A. & Fromageot, P. (1984) *EMBO J.* **3**, 343–350.
18. Birkenmeier, E. H., Brown, D. D. & Jordan, E. (1978) *Cell* **15**, 1077–1086.
19. Wallace, R. B., Schold, M., Johnson, M. J., Dembeck, P. & Itakura, R. (1981) *Nucleic Acids Res.* **9**, 3647–3656.
20. Klekamp, M. S. & Weil, P. A. (1982) *J. Biol. Chem.* **257**, 8432–8441.
21. Stillman, D. J. & Geiduschek, E. P. (1984) *EMBO J.* **3**, 847–853.
22. Jacobson, H. (1969) *Macromolecules* **2**, 650–662.
23. Galas, D. J. & Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157–3170.
24. Camier, S., Gabrielsen, O., Baker, R. & Sentenac, A. (1985) *EMBO J.* **4**, 491–500.
25. Ptashne, M. (1986) *Nature (London)* **322**, 697–701.
26. Ciliberto, G., Raugei, G., Costanzo, F., Dente, L. & Cortese, R. (1983) *Cell* **32**, 725–733.
27. Wilson, E. T., Larson, D., Young, L. S. & Sprague, K. U. (1985) *J. Mol. Biol.* **183**, 153–163.