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Biochemistry

Transcription and processing of a yeast tRNA gene containing a modified intervening sequence

(tRNA$_3^{Leu}$/*lac* operator/splicing)

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ABSTRACT **The tRNA$_3^{Leu}$ gene from yeast contains an intervening sequence of 32 nucleotides not present in mature tRNA. This sequence is transcribed and subsequently removed during the maturation of the RNA. To probe the involvement of this region of the gene in transcription and processing of the pre-tRNA$_3^{Leu}$, the yeast DNA was cloned in plasmid pBR322 and a 21-base-pair DNA fragment corresponding to the *lac* operator was inserted into the intervening sequence. Insertion was done at a cleavage site for the restriction endonuclease *Hpa* I that occurs 19/20 base pairs from the 5′ end of the intervening sequence. The parent and modified plasmids were then transcribed in a *Xenopus* germinal vesicle extract. RNA-fingerprint analysis of the transcription products revealed that both the tRNA$_3^{Leu}$ gene and its modified counterpart were accurately transcribed. Transcription products corresponding to mature tRNA$_3^{Leu}$ and pre-RNA$_3^{Leu}$ with the normal and *lac*-containing intervening sequence were identified. Precursors extended at their 5′ and 3′ ends were also present. Both parent and modified genes were transcribed efficiently, and the various products accumulated in similar amounts, indicating that no deleterious effects on transcriptional competence, stability of the transcripts, or processing result from insertion of the 21-base-pair *lac* operator DNA. Incubation of pre-tRNA molecules that contained intervening sequences but were 5′ and 3′ mature with a yeast ribosomal wash fraction resulted in excision of the intervening sequence and, in the presence of ATP, ligation of the resulting half-tRNA molecules. The presence of RNA complementary to *lac* operator DNA neither inhibited the excision and splicing activities nor altered the site of the junction.**

Some but not all yeast tRNA genes contain an intervening sequence (IVS). Evidence obtained from the characterization of a set of tRNA precursors accumulating in the yeast mutant ts 136 (1) indicates that there are approximately 10 sets of tRNA genes that contain an IVS. In the cases in which the sequences are known, the IVS occurs at the same place, interrupting the mature sequence at a position one base from the 3′ side of the anticodon. In general, the IVS in each member of a family of tRNA genes is similar but is different in size and sequence from the IVS in other families. Efforts to understand the RNA processing steps involved in the synthesis of these tRNAs have yielded some results (2–4), but as yet we do not understand why some genes have IVSs and others do not.

A possible function for the IVSs in the genes transcribed by RNA polymerase III has been suggested by the findings of Brown and his colleagues (5, 6). In their studies of the transcription of the *Xenopus borealis* 5S RNA gene it was discovered that the control region for specific transcription of the 5S gene is localized in the center of the gene between residues 50 and 80. If the control region in tRNA genes is also located in the middle of the gene, the IVS would be part of that region. We acknowledge that the existence of tRNA genes without IVSs and also the heterogeneity in length of the IVSs pose problems for this theory. Nonetheless, a direct test of the involvement of the IVS in transcription, processing, or both can be made by altering the IVS, either by an insertion or a deletion.

The gene we have chosen for our studies is a tRNA$_3^{Leu}$ gene isolated from yeast (7). This gene contains an IVS of 32 base pairs (bp) that includes a *Hpa* I restriction endonuclease site near the middle of the IVS at position 57/58 (8). We have altered the IVS in this gene by insertion of the 21-bp synthetic *lac* operator DNA (9) into the *Hpa* I site. The transcription of the normal and modified genes has been examined *in vitro* by using a germinal vesicle extract prepared from *Xenopus laevis* oocytes (10–12). Transcription of the normal and modified genes was found to be essentially equal. The products produced *in vitro* were, in both cases, mature tRNA$_3^{Leu}$ and precursors containing the IVS (with either mature or extended 5′ and 3′ ends). The precursors produced by the modified gene were longer because they contained the 21 bases complementary to the inserted *lac* operator.

Thus, the insertion of 21 bp into the central 50–80-bp region of the tRNA$_3^{Leu}$ gene did not affect either the transcription of the gene or the processing of the precursor. Further, we have shown that the precursor containing the *lac* sequences can also be processed *in vitro* by the yeast splicing activity.

MATERIALS AND METHODS

Plasmid Construction. The yeast tRNA$_3^{Leu}$ gene from a *Hin*dIII digest of yeast DNA was inserted into the vector pBR313 to produce a chimeric plasmid pJB2k (7). A 2.5-kilobase (kb) *Eco*RI fragment from pJB2k bearing the tRNA$_3^{Leu}$ gene was ligated into the *Eco*RI cleavage site of the plasmid pBR322 and the DNA was used to transform *Escherichia coli* strain C600SF8 (13). Transformants were selected by their resistance to tetracycline and ampicillin, then screened by colony hybridization to a [^{32}P]tRNA$_3^{Leu}$ (14). This plasmid, pJD137, was then modified by digestion with *Hpa* I and blunt-end ligation to a 21-bp DNA molecule, produced by chemical synthesis, with a sequence that corresponds to that of the *lac* operator DNA (9),

A-A-T-T-G-T-G-A-G-C-G-G-A-T-A-A-C-A-A-T-T
T-T-A-A-C-A-C-T-C-G-C-C-T-A-T-T-G-T-C-A-A

These recombinant DNA molecules were also cloned in *E. coli* C600SF8. Colonies were selected for both antibiotic resistance and the *lac*-constitutive phenotype, the latter being caused by the extra copies of operator DNA in the cells, which bind repressor. DNA was isolated from transformant colonies that appeared blue when grown in the presence of the indicator 5-bromo-4-chloro-3-indoyl β-D-galactoside (15) and digested

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Abbreviations: IVS, intervening sequence; bp, base pair(s); kb, kilobase(s).

with the restriction endonucleases *Hpa* I, *Eco*RI, and *Hae* III. The presence of both the $tRNA_3^{Leu}$ gene and the *lac* DNA in the restriction fragments was confirmed by filter hybridization (16) to $tRNA_3^{Leu}$ and *lac* operator DNA. The two probes were labeled to specific activities of $1–5 \times 10^6$ cpm/μg by incubation with [γ-^{32}P]ATP and polynucleotide kinase. The recombinant DNA experiments were performed under P2–EK1 conditions as specified by the National Institutes of Health guidelines.

Transcription *In Vitro*. Germinal vesicle extracts were prepared from *X. laevis* oocytes as described (10, 12). Then 270 ng of DNA from the plasmids pBR313, pBR322, pJB2k, pJD137, and pJD110 were each incubated with 10 μl of germinal vesicle extract and 20 or 30 μCi (1 Ci = 3.7×10^{10} becquerels) of [α-^{32}P]UTP (56 Ci/mmol) or [α-^{32}P]ATP (62 Ci/mmol), respectively, in a total volume of 15 μl.

Transcription was allowed to proceed for 180 min at 23–24°C. After this incubation, each reaction was terminated by addition of 5 μl of 10% sodium dodecyl sulfate, 10 μl of proteinase K at 10 mg/ml, 5 μl of poly(U) at 10 mg/ml, and 65 μl of 0.15 M NaCl/0.01 M Tris·HCl (pH 7.8)/5 mM EDTA. Incubation was then continued for 30 min at 37°C. After a phenol extraction and ethanol precipitation, the reaction mixtures were subjected to electrophoresis in 10% polyacrylamide gels containing 4 M urea (12).

RNA Fingerprinting. RNA products of the germinal vesicle transcriptions were recovered from polyacrylamide gels by extraction at 37°C with 0.3 M NaCl/10 mM Tris·HCl (pH 7.4)/1 mM EDTA/1% phenol and ethanol precipitation. The ^{32}P-labeled RNA was then analyzed by using the fingerprinting techniques of Brownlee (17).

Splicing of *In Vitro* Transcription Products by Yeast Ribosomal Wash Enzymes. Samples containing 10^4 cpm of pre-$tRNA_3^{Leu}$ molecules identified as having mature 5′ and 3′ ends but still containing IVSs were incubated with a yeast extract as described (1), except that the incubations were for 30 min, additional poly(U) was not added, in some cases ATP was not included in the reaction mixture and the final incubation volume was 10 μl. Termination of the reactions and polyacrylamide gel electrophoresis of the products were essentially as described for transcription *in vitro*.

$tRNA_3^{Leu}$ was the gift of S. H. Chang. All restriction endonucleases were purchased from New England BioLabs except for *Hae* III, which was generously donated by Roberto Kolter. Polynucleotide kinase was purchased from Miles Laboratories. [γ-^{32}P]ATP (4000–7000 Ci/mmol) was the gift of M. Simon. DEAE-cellulose paper for RNA fingerprinting was from either Whatman or Nucleopore Corp.

RESULTS

Construction of an Altered $tRNA_3^{Leu}$ Gene. We had originally isolated a number of $tRNA_3^{Leu}$ genes by cloning *Hin*dIII fragments of yeast DNA in the plasmid pBR313 (7). One of these plasmids, pJB2k, was further characterized by restriction mapping and nucleotide sequence determination of the $tRNA_3^{Leu}$ gene (8). Fig. 1 schematically depicts the subcloning of a 2.5-kb *Eco*RI fragment from pJB2k into pBR322. This was done to remove a second *Hpa* I site that existed in the parent clone. The single *Hpa* I site in the pBR322 recombinant, pJD137 (Fig. 1), is located near the middle of the IVS of the $tRNA_3^{Leu}$ gene.

To insert the 21-bp *lac* operator DNA into this site, pJD137 DNA was cleaved with *Hpa* I and ligated to synthetic *lac* operator DNA (9). The recombinant plasmid was introduced into *E. coli* by transformation and tetracycline–ampicillin-resistant, *lac*-constitutive colonies were isolated. One of the resulting plasmids, pJD110 (Fig. 1), was chosen for further study. The presence and location of the operator sequence in this plasmid

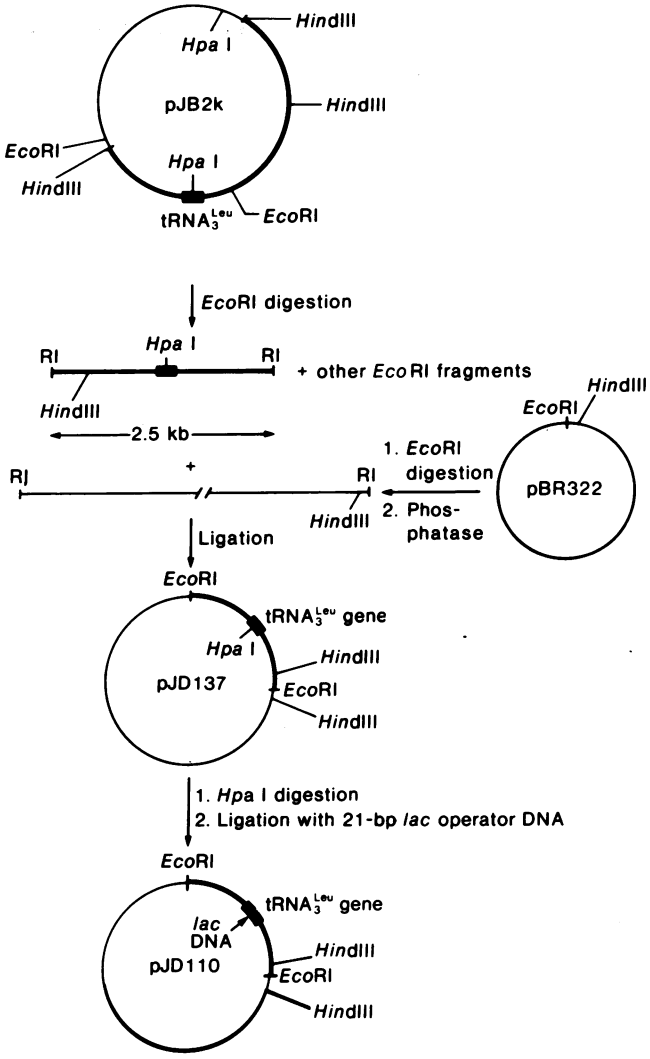

FIG. 1. Cloning of the yeast $tRNA_3^{Leu}$ gene. *Hin*dIII fragments of yeast genomic DNA were inserted in the vector pBR313 and screened by hybridization to purified yeast tRNAs (7). A clone hybridizing to $tRNA_3^{Leu}$, pJB2k, was identified. An *Eco*RI fragment that contains the $tRNA_3^{Leu}$ gene from pJB2k was subcloned into pBR322. The orientation of the yeast fragment in pJD137 was deduced from the observation that, after *Hin*dIII digestion, the $tRNA_3^{Leu}$ gene was found in a fragment that comigrates with linear plasmid DNA. This subclone, pJD137, was made linear by digestion with *Hpa* I and then ligated to a 21-bp DNA molecule with a sequence corresponding to *lac* operator to produce the plasmid pJD110.

were determined as follows: *Hpa* I did not cleave pJD110 DNA as it does the parent pJD137. pJD110 DNA was cleaved with *Hae* III restriction endonuclease and the fragments were separated by agarose gel electrophoresis and transferred to nitrocellulose paper by using the method of Southern (16). Hybridization of these fragments with [^{32}P]$tRNA_3^{Leu}$ revealed that the *Hae* III fragment containing the gene in pJD110 is about 20 bp larger than is the corresponding parent fragment from pJD137 (Fig. 2*A*). *Hpa* I digestion of the *Hae* III fragments containing the $tRNA_3^{Leu}$ gene indicated the pJD137 DNA is cleaved whereas pJD110 is not (Fig. 2*A*, lanes c and d). The [^{32}P]$tRNA_3^{Leu}$ was stripped from the filter by incubation at 65°C in 50% (vol/vol) formamide/0.9 M NaCl/0.09 M sodium citrate and the DNA remaining on the filter was rehybridized to *lac* [^{32}P]DNA. Fig. 2*B* shows that the *lac* probe hybridized only in the pJD110 lane of the gel and to a fragment with the same mobility as the fragment that had hybridized to [^{32}P]$tRNA_3^{Leu}$. From these data, we conclude that the *lac* DNA was inserted at this *Hpa* I cleavage site in the IVS of the $tRNA_3^{Leu}$ gene.

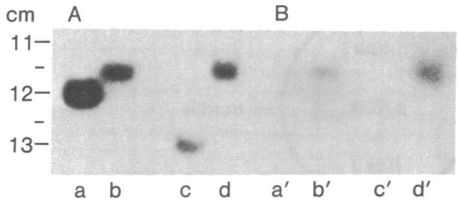

FIG. 2. Autoradiography of Southern filter hybridization of DNA from the plasmids pJD137 and pJD110 digested with restriction endonucleases. Restriction endonuclease fragments were electrophoresed in a 1.5% agarose gel; migration is from top to bottom. Bands in lanes a and b were determined by comparison with a *Hae* III digest of pBR322 to contain DNA fragments of 150 and 170 bp, respectively. (*A*) Hybridization to ^{32}P-labeled $tRNA_3^{Leu}$; (*B*) hybridization to ^{32}P-labeled *lac* operator DNA. Lanes a and a', pJD137 DNA digested with *Hae* III; lanes b and b', pJD110 DNA digested with *Hae* III; lanes c and c', pJD137 DNA digested with *Hae* III and *Hpa* I; lanes d and d', pJD110 DNA digested with *Hae* III and *Hpa* I. Distances indicated are from the origin.

Transcription *In Vitro*. To test the effect on transcription of the 21-bp *lac* operator inserted into the IVS of the $tRNA_3^{Leu}$gene, we have used the *Xenopus* oocyte germinal vesicle system. This *in vitro* system has previously been shown to be active in the transcription and processing of a number of RNA polymerase III transcripts, including yeast tRNA genes (10–12). DNA from the plasmid pJD137 (containing the $tRNA_3^{Leu}$ gene) and pJD110 (containing the modified gene) were incubated in a *X. laevis* germinal vesicle extract. The RNA transcripts were labeled with either [α-^{32}P]ATP or [α-^{32}P]UTP. RNA polymerase II transcription was inhibited by addition of 2 μg of α-amanitin per ml. The products of the reaction were separated by electrophoresis in a 10% polyacrylamide gel containing 4 M urea.

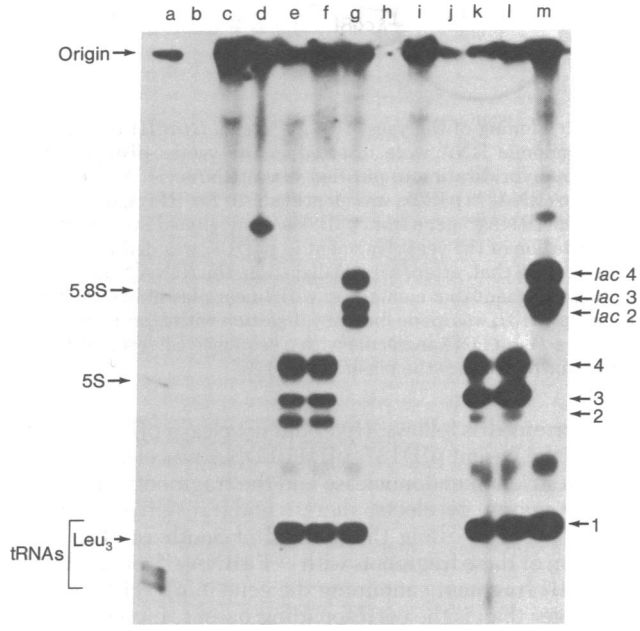

FIG. 3. Autoradiography of the [^{32}P]RNA products of transcription of cloned yeast $tRNA_3^{Leu}$ genes in *Xenopus* germinal vesicle extracts. Equivalent amounts of DNA from the vectors and recombinant plasmids were incubated with an extract of *X. laevis* stage V and VI oocytes. The reaction mixtures contained either [α-^{32}P]ATP (lanes b–g) or [α-^{32}P]UTP (lanes h–m). The reaction mixtures were then subjected to a protease digestion followed by phenol extraction and ethanol precipitation. The RNA was then electrophoresed on a 10% polyacrylamide gel containing 4 M urea. ^{32}P-Labeled yeast 4S, 5S, and 5.8S RNAs were included as markers (lane a). Lanes b and h, no DNA; lanes c and i, pBR322; lanes d and j, pBR313; lanes e and k, pJB2k; lanes f and l, pJD137; lanes g and m, pJD110.

Fig. 3 shows that little transcription is observed from the DNAs of the parent plasmid vectors, pBR313 and pBR322, but that four distinct products are observed when the system is programmed by pJB2k, pJD137, or pJD110 DNA in the presence of [α-^{32}P]ATP or [α-^{32}P]UTP.

We have characterized these products by RNA fingerprint analysis. In each case there is a good yield of band 1, the fastest-moving product. Analysis of this band from each of the reaction mixtures (data not shown) proved that this RNA is mature $tRNA_3^{Leu}$. Thus, both the normal and the modified tRNA genes are transcribed and the IVS is removed to yield the mature tRNA molecule.

The pJB2k and pJD137 reaction mixtures show identical patterns, each with products larger than the mature tRNA. Analysis of band 3 RNA from these reactions showed that it contains the mature 3' and 5' ends but retains the 32-base IVS. This $tRNA_3^{Leu}$ precursor is therefore analogous to the pre-$tRNA_3^{Leu}$ precursor that accumulates at the nonpermissive temperature in the yeast mutant ts136 (1). The largest product, band 4, is immature at both ends. Preliminary analysis of this RNA suggests that it contains one or two extra nucleotides at the 5' end and at least five extra nucleotides at the 3' end, extending up to a stretch of nine T residues found in the DNA sequence (8). We have not characterized band 2 RNA, but its size is consistent with its being the band 3 precursor without the C-C-A end which, for $tRNA_3^{Leu}$, must be added in a posttranscriptional reaction.

The analogous three products are seen when the modified $tRNA_3^{Leu}$ gene is transcribed (lanes g and m), but these products all migrate slower than their counterparts in lanes f and l. Pancreatic RNase digestion products of the [α-^{32}P]UTP-labeled *lac* 3 RNA (lane l) were separated in two dimensions (Fig. 4*A*). The identities of individual spots, as indicated in Fig. 4*B*, were deduced by their relative positions on the map and confirmed by molar yield measurements (data not shown) as well as analysis of ^{32}P-labeled products from RNase T1 digestions of individual oligonucleotides (data not shown). The open spots in Fig. 4*B* are derived from the portion of the molecule that corresponds to mature $tRNA_3^{Leu}$, or they are common to mature tRNA and the normal IVS. Spot P17 is the matured 5' end of the molecule.

An oligonucleotide representing the matured 3' end was identified in an RNase T1 digest of the corresponding RNA

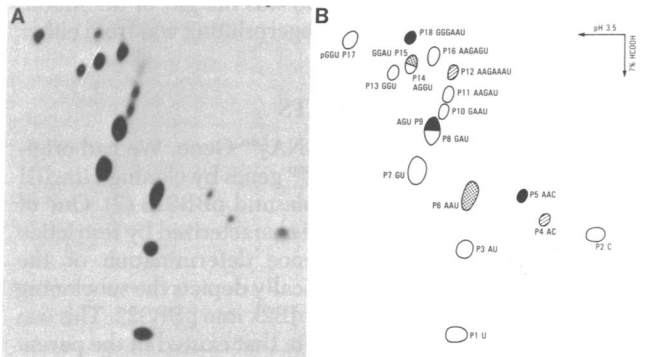

FIG. 4. Fingerprint analysis of *lac* 3 RNA. (*A*) Autoradiogram of the digestion products. [α-^{32}P]UTP-labeled RNA, isolated from the *lac* 3 band of lane m (Fig. 3) was digested with pancreatic RNase and the oligonucleotides were separated by two-dimensional paper electrophoresis (17). (*B*) Positions of the oligonucleotides. Open symbols denote oligonucleotides that derive from pre-$tRNA_3^{Leu}$ and the *lac* fragment (P1 and P7). Closed symbols denote those oligonucleotides uniquely derived from the normal IVS. Hatched symbols indicate the two oligonucleotides that span the junctions between the tRNA and the IVS. Stippled symbols denote oligonucleotides that are unique to transcription of the 21-bp *lac* insertion.

labeled with [α-^{32}P]ATP (data not shown). All other nucleotides present in mature tRNA$_3^{Leu}$ can be accounted for in the pancreatic RNase digest. The hatched spots, P4 and P12, are oligonucleotides that span the junction of the IVS and tRNA portions of the transcript. The black spots, P5, P9, and P18, are derived uniquely from the normal IVS. The stippled spots, P6 and P15, are generated uniquely by transcription of the *lac* DNA insertion in the gene. The presence of P15, G-G-A-U, and the complete absence of G-C in this RNA indicates that the orientation of the *lac* sequence in the transcript is as shown in Fig. 5. The molar yields of spots P3 and P7 and P14-15 confirm this orientation. The extra nucleotides in the *lac* 4 RNA (Fig. 3) appear to be exactly analogous to those in band 4 RNA (Fig. 3) from both pJB2k- and pJD137-directed transcription.

In summary, the results of this experiment indicate that, regardless of the presence of the *lac* insertion, the tRNA$_3^{Leu}$ gene is transcribed to give a set of three precursor RNAs and a mature tRNA molecule. The yields of these products are similar, but when an insertion is present in the tRNA the three precursors are larger because they contain 21 bases complementary to one strand of the *lac* operator insertion. The insertion does not seem to affect the processing of the precursor.

Processing of Pre-tRNA$_3^{Leu}$ by the Yeast Splicing System. The experiments described above indicate that the insertion of 21 bases into the IVS of pre-tRNA$_3^{Leu}$ does not affect the *Xenopus* splicing reaction. We have investigated this point further with the yeast splicing system, about which more is known. The splicing has been shown to be a two-step reaction involving an endonucleolytic excision of the IVS followed by an ATP-dependent ligation of the two half-tRNA molecules (3, 4). Incubation of the *X. laevis* band 3 transcripts with the yeast extract in the absence of ATP results in appearance of RNA molecules with the size expected of 5′ and 3′ halves of tRNA$_3^{Leu}$ and an IVS that is smaller than the half tRNA molecules in the case of pJD137 transcripts but larger in the case of pJD110 (Fig. 6, lanes c and d). The electrophoretic mobility of the IVS bands indicates their length as 32 and 47 bases, respectively, for pJD137 and pJD110; however, the relationship between size and mobility may be influenced by secondary structure in this gel system. Inclusion of 1.0 mM ATP in the incubation of band 3 RNA with yeast ribosomal wash enzymes results in the appearance of tRNA$_3^{Leu}$-sized molecules and the concomitant loss

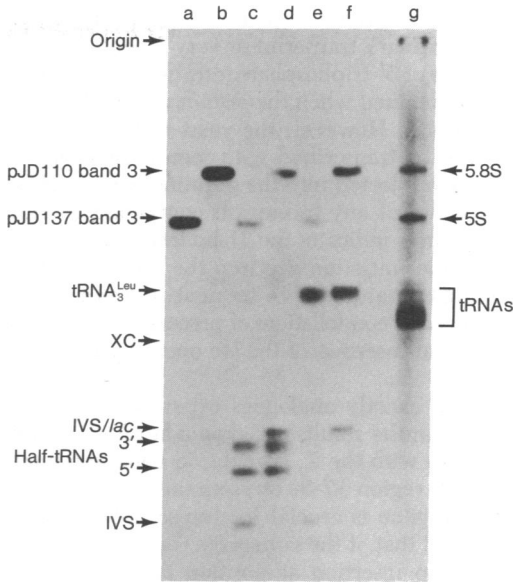

FIG. 6. Splicing of pre-tRNA$_3^{Leu}$ from *Xenopus* germinal vesicle transcription by yeast ribosomal wash proteins. Pre-tRNA$_3^{Leu}$ isolated from band 3 of lane l and *lac* 3 of lane m in Fig. 4 were incubated with a yeast ribosomal wash fraction known to be capable of excising the IVS from yeast pre-tRNA molecules synthesized *in vivo* (1). The reaction mixtures were electrophoresed on 10% polyacrylamide gels containing 4 M urea and autoradiographed. Band 3 RNA from pJD137 and *lac* 3 RNA from pJD110-directed transcription are in lanes a, c, and e and b, d, and f, respectively. Reaction mixtures in lanes a and b were incubated without ribosomal wash protein, lanes c and d with ribosomal wash, and lanes e and f have ribosomal wash plus 1.0 mM ATP. Lane g, marker RNAs.

of half-tRNA-sized molecules (Fig. 6, lanes e and f). The IVS bands persist in the presence of ATP.

DISCUSSION

The insertion of the 21-bp *lac* operator at position 57/58 of the tRNA$_3^{Leu}$ gene has no apparent affect on the transcription of the gene in the *X. laevis* oocyte germinal vesicle system under the conditions used. That transcription is completely unaffected is not proven, however, because we have not established with

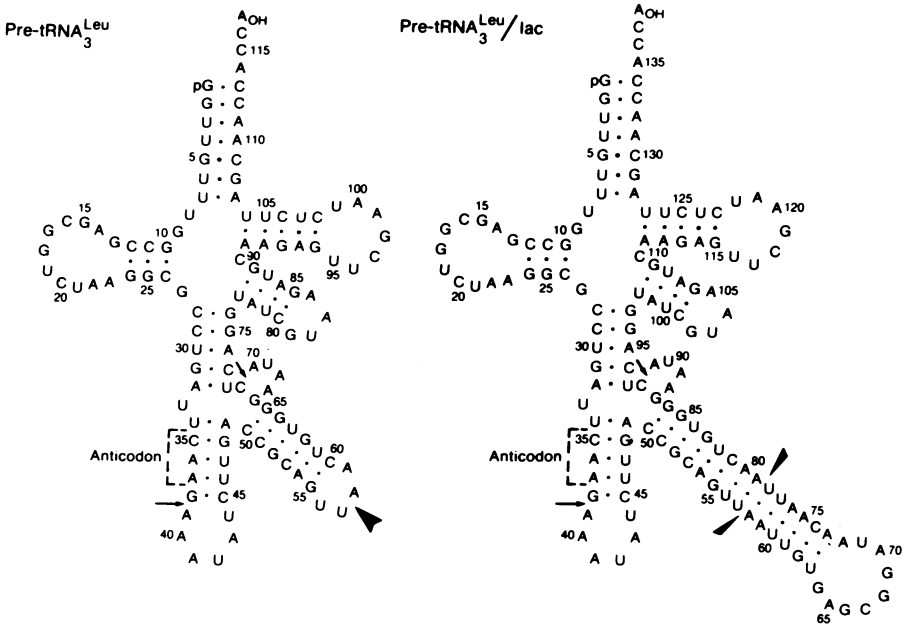

FIG. 5. Effect of *lac* DNA insertion on the structure of tRNA$_3^{Leu}$ precursor molecules that contain the IVS. Pre-tRNA$_3^{Leu}$: the structure of the precursor with mature 5′ and 3′ ends but still containing an IVS colinear with the gene as identified from *in vitro* transcription of the plasmids pJB2k and pJD137 (Fig. 3) as well as *in vivo* transcription in the yeast *rna1* mutant ts136 (8). The small arrows denote the sites for cleavage and ligation of the precursor to form mature tRNA$_3^{Leu}$. The large arrowhead shows the point at which *Hpa* I cuts the gene. Pre-tRNA$_3^{Leu}$/*lac*: the structure of the analogous pre-tRNA$_3^{Leu}$ molecule produced by *in vitro* transcription of the plasmid pJD110 that includes the *lac* DNA insertion. The position and nucleotide sequence of the modified IVS have been determined from the position of the *Hpa* I cleavage site (split arrowheads), the sequence of the DNA insert (9), and the results of RNA-fingerprinting analyses (Fig. 4).

certainty the transcription start site. We believe that band 4 (Fig. 4) is the primary transcript or very close to it. Evidence for the presence of 5′-triphosphate termini in analogous products has been obtained when the *Bombyx mori* $tRNA_1^{Ala}$ gene is transcribed (18). However, the yeast $tRNA^{Tyr}$ genes have been shown to be transcribed with somewhat longer leader sequences when injected into the germinal vesicle of oocytes (19). The absence of any transcripts containing 5′-truncated $tRNA_3^{Leu}$ sequences indicates the 21-bp insertion does not shift the transcription initiation site into the portion of the gene corresponding to mature tRNA sequences. The similar levels of transcription and constellations of precursor bands encourage us to believe that insertion of the *lac* operator does not affect transcription.

Although an exactly analogous experiment has not been performed, from the results of Sakonju *et al.* (5) and Bogenhagen *et al.* (6) with the *X. borealis* 5S gene, the nucleotide sequence in the region 50–80 bp from the 5′ end of the coding portion of the gene is crucial for transcription. One would therefore expect that, if the same were true for the yeast tRNA gene, the 21-bp insertion at position 57/58 would abolish transcription because 14 changes in sequence are affected by the insertion over the domain extending between positions 50 and 80. There is, however, only one change between positions 55 and 62, a region containing obvious homology among a series of four genes transcribed by RNA polymerase III (6). But neither the $tRNA_3^{Leu}$ gene nor the *lac*-altered gene has that conserved sequence A-G-C-A-G-G-G-T (8).

Thus, we are led to the tentative conclusion that there must be a different mechanism for the recognition of tRNA and 5S genes by RNA polymerase III. In a set of experiments with some similarities to those reported here, Kressmann *et al.* (20) have concluded that the $tRNA^{Met}$ gene of *X. laevis* must be transcribed by a different mechanism from the 5S gene. The reason for this difference is probably that different transcription factors are required for different types of RNA polymerase III transcription units. Ng *et al.* (21) have purified a transcription factor that is required for correct transcription of 5S genes by RNA polymerase III. More recently, Engelke *et al.* (22) have shown that it is this factor, independent of RNA polymerase III, that binds to the intragenic regions of the 5S gene. This factor is not active in promoting the transcription of the *Xenopus* $tRNA_1^{Met}$ gene.

Factors are now being characterized that do promote tRNA transcription (23), and it will be of great interest to learn their recognition sites and mode of action. Whatever the interaction site may be, the information currently available would suggest that it is contained largely within the structural gene. The comparisons of sequences at the 5′ termini of a number of yeast tRNA genes have not revealed any impressive homology (8, 24, 25). In addition, it has been shown that all but six nucleotides of the 5′ leader sequence of the *B. mori* $tRNA_2^{Ala}$ gene can be removed without affecting the *in vitro* transcription of the gene (26).

It is also of interest that insertion of the 21-bp *lac* sequence into the $tRNA_3^{Leu}$ gene does not affect the processing of the precursor in either the *Xenopus* or yeast systems. The correct 5′ and 3′ ends are produced in precursors containing the modified IVS. It would thus seem that the structure of the IVS is not a crucial factor in the recognition of pre-tRNA molecules by either the nuclease(s) responsible for end trimming or the nucleotidyl transferase enzyme. Furthermore, the endonuclease responsible for excising the IVS is able to recognize the modified substrate and cleave it at precisely the same sites as the unmodified pre-$tRNA_3^{Leu}$. Thus, the positions of cutting for excision of the IVS are not determined by the size of the base-paired region in the central part of the IVS.

It may be that recognition of the precursor is not affected because the *lac* operator sequence is particularly well engineered to fit into this molecule. Indeed, this is part of the reason we chose to use it in this experiment. It can be seen in Fig. 5 that the base-paired stem present in the IVS of $tRNA_3^{Leu}$ is extended in the altered gene. There is a stem of 14 bp with a loop of 9 bases in the altered IVS replacing a stem of 6 bp and a loop of 4 bases. Alternatively, the processing system may recognize only the tRNA portion of the substrate, so that the IVS region of the precursor could be altered in almost any way without adverse effects on the maturation of the tRNA. Construction of different $tRNA_3^{Leu}$ altered genes should allow us to distinguish between these possibilities.

Initial experiments on the transcription of the $tRNA_3^{Leu}$ gene in the germinal vesicle system were performed by A. Sarabhai and H. Lamfrom. We thank Craig Peebles for help with the yeast splicing enzyme experiment. This research was supported by National Institutes of Health Grants CA10984 (to J.A.) and GM24393 (to K.I.).

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