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Each element of the *Drosophila* $tRNA^{Arg}$ gene split promoter directs transcription in *Xenopus* oocytes

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The intragenic control regions of a eukaryotic tRNA gene have been examined by transcribing mutant forms of a Drosophila $tRNA^{Arg}$ gene either by injection into the nucleus of Xenopus oocytes or in extracts prepared from isolated oocyte nuclei. These experiments demonstrate that the selection of the transcription initiation site is a complex mechanism that involves the T-control region, the D-control region, and sequences 5' adjacent to the D-control region. In this study either "half" of the Drosophila $tRNA^{Arg}$ gene promoted transcription in Xenopus oocytes. This finding supports a recent model for eukaryotic tRNA gene transcription (Dingermann et al., 1983, J. Biol. Chem. 258, 10395-10402) that proposes transcription initiation is dependent on the ability of specific DNA sequences to sequester two RNA polymerase III transcription factors.

INTRODUCTION

The transcription of nuclear eukaryotic tRNA genes is controlled by two noncontiguous intragenic sequence blocks. These control regions were defined qualitatively by the ability of deletion and insertion mutant tRNA genes to direct transcription in vitro or in vivo. By this methodology the intragenic control regions for the Xenopus initiator $tRNA^{Met}$ (1) and $tRNA^{Leu}$ (2) genes, the Drosophila $tRNA^{Arg}_2$ gene (3,4), the Caenorhabditis elegans $tRNA^{Pro}$ gene (5,6) and the yeast SUP4-0 $tRNA^{Tyr}$ gene (7) have been defined. For the Drosophila $tRNA^{Arg}$ gene one promoter element was located within coordinates 8-25 (D-control region) and the other within coordinates 50-58 (T-control region) of the mature tRNA coding region (3). There are qualitative differences in the defined boundaries of the intragenic control regions for the different tRNA genes studied. A major difference between the transcription properties of deletion mutant $tRNA^{Arg}$ genes and those of Xenopus $tRNA^{Met}$, Xenopus $tRNA^{Leu}$, and C. elegans $tRNA^{Pro}$ genes is that the cloned 5' halves of these genes do not support RNA synthesis. The cloned 5' half of the Drosophila $tRNA^{Arg}$ gene supports RNA synthesis in Drosophila Kc cell and Xenopus whole oocyte transcription systems (3). Similarly, deletion of the 3' half of the yeast $tRNA^{Leu}_3$ gene did not abolish promoter function in vitro (8). The cloned 5' half of the $tRNA^{Arg}$ gene, which contains the D-control region, only supports RNA

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synthesis when the D-control region is located within the context of wild-type adjacent DNA sequences. Deletion of 5' flanking and tRNA 5' stem-coding sequences results in abolition of transcription directed solely by the D-control region in Drosophila Kc cell and Xenopus whole oocyte extracts (9).

Studies which involved measuring the ability of mutant $tRNA^{Arg}$ genes to form stable transcription complexes led to a model for transcription factor involvement in tRNA gene transcription (4,10) that is consistent with the observed ability of 5' half $tRNA^{Arg}$ genes to support RNA synthesis. This model proposes recognition of sequences within the D- and T-control regions respectively by two transcription factors, δ and τ (4). A consideration in this model for tRNA gene transcription that is still outstanding however is the inability of 5' deletion mutant $tRNA^{Arg}$ genes to support RNA synthesis in these cell-free transcription extracts.

In the transcription studies involving mutant forms of the Xenopus $tRNA^{Met}$ and $tRNA^{Leu}$ genes and the C. elegans $tRNA^{Pro}$ gene, the nucleus of live Xenopus oocytes was used as the transcription system (1,2,5,6). To compare the transcription properties of mutant forms of the Drosophila $tRNA^{Arg}$ gene to those of the $tRNA^{Met}$, $tRNA^{Leu}$, and $tRNA^{Pro}$ genes, and in particular to correlate the transcriptional activity of cloned 5' and 3' halves of tRNA genes we have tested the transcription of the $tRNA^{Arg}$ gene mutants in systems which use the Xenopus oocyte nucleus (germinal vesicle, GV). At present this system offers the only representation of an "intact" RNA polymerase III transcription apparatus for general transcription of RNA polymerase III Class 1 genes (11).

MATERIALS AND METHODS

Recombinant DNA

The plasmid pArg (pYH48) consists of a 508 bp HindIII Drosophila DNA fragment carrying the gene for the major $tRNA^{Arg}$ species inserted into the HindIII site of pBR322 (12). The construction of 5' and 3' deletions within this gene using BAL-31 has been described (3). The 5' deletion clones are named as pArg5. and 3' deletion clones as pArg3.; the second number in the designation (e.g., pArg5.7) indicates the extent of deletion, the number being the first (5' deletion) or the last (3' deletion) nucleotide that remains from the wild-type Drosophila $tRNA^{Arg}$ sequence. Two $tRNA^{Arg}$ minigenes pArg7/26 and pArg7/58 were constructed by joining the deletion clones pArg5.7 and pArg3.26 as well as pArg5.7 and pArg3.58 at their common HhaI site (9). pArg7/26 therefore contains only nucleotides 7 to 26 of the mature tRNA coding sequence while the plasmid pArg7/58 contains nucleotides 7 to 58 of the mature tRNA coding sequence (9). Three plasmids with mutations between the two intragenic control regions were

constructed (4). A linker sequence of 21 nucleotides (GGAATTCCTCGAGGGATCCGG) joins Drosophila $tRNA^{Arg}$ gene coordinastes 26 and 21 (pArg 26x21), 26 and 36 (pArg 26x31), and 26 and 55 (pArg 26x55) (4).

Transcription Analysis

Plasmid DNAs were injected at a concentration of 200-300 μg/ml (35 nl/oocyte) into the nucleus of intact X. laevis oocyte GV together with 10 mCi/mmol of [α-^{32}P]GTP (350 Ci/mmole) as described (13). Oocytes were incubated, homogenized and digested in proteinase K, and the synthesized RNAs were isolated and electrophoresed in 8% polyacrylamide gels containing 7 M urea as described previously (14).

The oocyte GV extract was prepared by the procedure of Birkenmeier et al. (15) and transcription reactions were performed as described (16). Synthesized RNA was isolated and electrophoresed using 8% polyacrylamide gels containing 8.3 M urea (17).

Nuclease S1 Protection Mapping

RNAs formed in transcription reactions in the presence of [α-^{32}P]GTP were recovered from gels and hybridized to 5'-end labeled complementary DNA as described (4). Hybridization and subsequent digestion by nuclease S1 was performed by the procedure of Weaver and Weissmann (18) using 300 units of nuclease S1 per ml. Complementary DNA was prepared by HinfI digestion of each different 5'-deletion mutant DNA which was subsequently ^{32}P-labeled at the 5'-end. Single-stranded DNA was generated by treating the labeled DNA with T4 DNA polymerase in the absence of nucleoside triphosphates (19). This DNA was used directly in hybridization reactions.

RESULTS

Transcription of 5'-Deletion Templates In Vivo

A cloned wild-type $tRNA^{Arg}$ gene of Drosophila and various 5'-deletion mutants of this gene (3) were injected into the nucleus of Xenopus oocytes to determine which of these templates could direct RNA synthesis in vivo. All tested templates supported RNA synthesis efficiently and transcripts were identified from their electrophoretic mobilities on polyacrylamide gels (Fig. 1A). The wild type gene, pArg, and the 5'-deletion templates pArg5.-10 and pArg5.-8 directed the transcription of a precursor RNA which was processed to form a mature sized RNA. pArg5.7 was transcribed resulting in the formation of a primary transcript which had a similar size as the product of pArg. Each of the 5'-deletion templates pArg5.9, pArg5.12, and pArg5.21, which have part of the D-stem and D-loop structure of the mature tRNA deleted (D-control region), supported efficient synthesis of a heterogeneous array of RNA (Fig. 1A). Most of the transcription products formed from pArg5.9, pArg5.12 and pArg5.21, however, were longer than the precursor product of pArg. Previously, in cell-free transcription reactions these deletion mutant

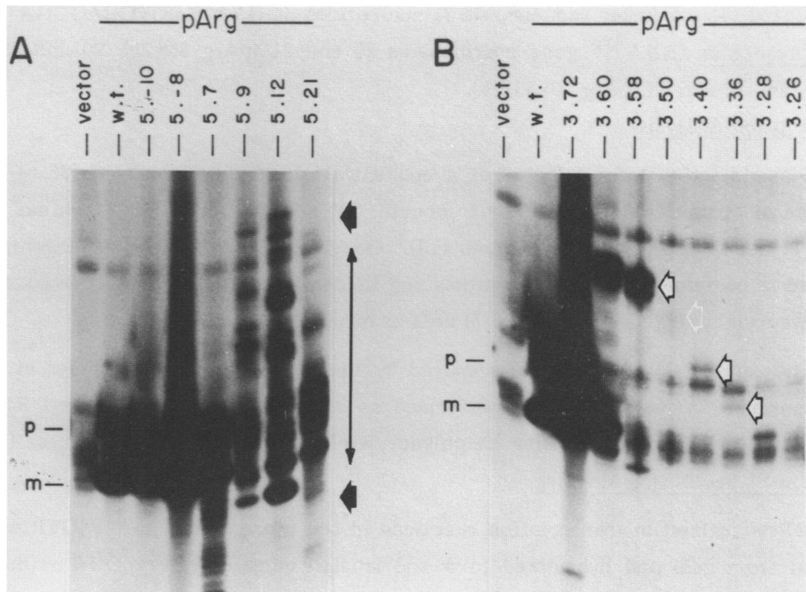

Figure 1 - Transcription of Drosophila Deletion tDNAs in Live Oocytes. Autoradiographs of polyacrylamide gel electrophoretic separation of ^{32}P-labeled RNAs which have been synthesized in oocyte GV's after injection of plasmid DNA. Ten oocytes were injected for each DNA. Transcripts were collected and pooled and the equivalent of two oocytes was loaded onto the gel. Exposure of the gel to Kodak XRP x-ray film was for three days at -70°C with an intensifying screen. The gels were overexposed in this manner in order to reveal, and compare, the low level of transcription directed by the 3' deletion mutants devoid of the T-control region (indicated by open arrows in B). (A) 5' deletion tDNAs; The region outlined by the arrows indicates the size range of specific transcription products. (B) 3' deletion tDNAs; the open arrows indicate specific transcripts generated from 3' deletion mutant tDNAs. pArg 3.58 has a relatively efficient level of transcription whereas, the transcripts pArg 3.50, pArg 3.40 and pArg 3.36 are barely distinguishable compared to the control vector transcription. p and m respectively refer to the electrophoretic mobility of the precursor and mature tRNAs resulting from transcription of pArg (wild-type, w.t.).

tDNAs did not support RNA synthesis.

Transcription of 3'-Deletion Templates In Vivo

Each 3'-deletion tRNA^{Arg} gene was also separately injected into the nucleus of Xenopus oocytes (Fig. 1B). Genes containing both of the intragenic transcription control regions efficiently directed transcription (pArg3.72, pArg3.60, pArg3.58). Other 3'-deletion DNAs tested, pArg3.50, pArg3.40, pArg3.36, pArg3.28, pArg3.26 (5' half tDNAs), supported a greatly reduced level of RNA synthesis in the Xenopus oocyte GV compared to the level supported by pArg (Fig. 1B). Relative to the level of RNA synthesis sup-

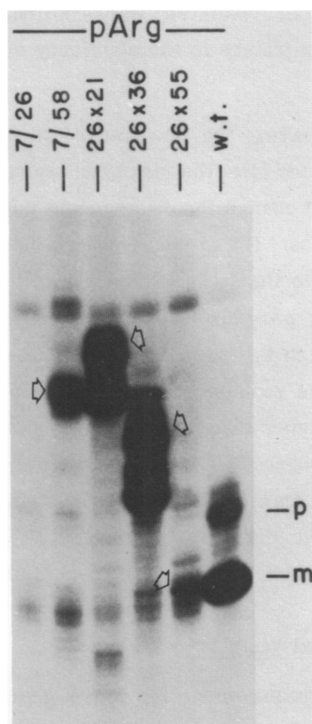

Figure 2 - Transcription of $tRNA^{Arg}$ Mini- and Maxigenes in Live Oocytes. These experiments were performed as described in Figure 1. The open black arrows indicate the major transcripts produced from mutant tDNAs.

ported by pArg the levels of transcription supported by the 3'-deletion tDNAs was lower in vivo than it was using cell-free extracts; for some clones (pArg3.50, pArg3.28) in vivo RNA synthesis was barely evident.

Transcription of Minigenes and Maxigenes In Vivo

The 5'- and 3'-deletion pArg templates have intact either the wild-type 5' part of the gene and its 5'-flanking region or the wild-type 3' part of the gene and the 3'-flanking region. We constructed two minigenes, one of which contains only the D-stem and D-loop sequences (D-control region, pArg7/26), and the other has the D-stem and D-loop plus the anticodon-stem and -loop and the 5' part of the T-stem and -loop sequences (D-control plus T-control regions, pArg7/58) in the wild-type configuration. In pArg7/58 the acceptor stem, the 3'-part of the T-loop and T-stem and the entire 3'- and 5'-flanking regions have been replaced by pBR322 sequences (7). In the Xenopus oocyte GV pArg7/58 supported relatively efficient RNA synthesis whereas for pArg7/26 transcription was not detectable (Fig. 2).

Although pArg7/58 supported RNA synthesis the level was reduced compared to the transcription efficiency of pArg. This indicates that even though the two internal con-

trol regions are sufficient to direct RNA-polymerase III mediated transcription, sequences adjacent to these control regions significantly contribute to the efficiency of in vivo transcription.

To determine the functional role of sequences separating the two transcription control regions of a tRNA gene "maxigenes" were constructed (4). The maxigene series of mutant tDNAs maintain the two control regions intact but change the distance and the sequence of the region that separates the two control regions. The in vivo transcription properties of the maxigene series was qualitatively similar to that observed earlier in in vitro transcription experiments. Of the three maxigenes, pArg26x36, was more efficiently transcribed in vitro and in vivo (Fig. 2). Even though this gene has the nucleotides between the coordinates 26-36 of the wild-type gene replaced by the longer 21 nucleotide sequence, it directed a level of transcription higher than pArg. Due to the inserted spacer sequences the product is 12 nucleotides longer than the wild-type precursor product. The mutant gene pArg26x21 also was transcribed better in vivo, however, the level of RNA synthesis was less than that directed by pArg26x36 . pArg26x55 was transcribed in vivo with low efficiency, which may be due to partial deletion of the T-control region.

Transcription Using Extracts Prepared from Isolated Germinal Vesicles

The D-control region was shown to be an essential requirement for tRNA gene transcription in cell-free extracts. However, in the present study mutant genes devoid of the D-control region, supported transcription. The same mutant tDNAs were transcriptionally inactive in extracts prepared from whole oocytes (3). This difference in the two Xenopus oocyte systems was further explored by also testing the transcription of the pArg deletion mutants in extracts prepared from isolated GV's. These experiments would then provide transcription information for each of the current Xenopus oocyte transcription systems: (i) injected GV; (ii) GV extract; and (iii) whole oocyte extract, using the same series of mutant tDNAs.

The transcription levels of the tDNAs having deletions extending into or removing the D-control region (5' deletions) were comparatively lower in GV extracts than in GV (Fig. 3A). Thus the use of GV extract led to a reduction in a transcription activity. Since these 5' deletion tDNAs do not support any RNA synthesis in the whole-oocyte transcription system (3,9), the whole oocyte extract has a less activity than the GV extract.

The relative transcription levels of the 3'-deletion DNAs devoid of the T-control region were higher in the GV extract than in the intact GV (Fig. 3B). In this respect the GV extract mirrors the transcription activity observed using whole-oocyte extract (3). It appears that in vivo there is a greater dependence on the T-control region for efficient

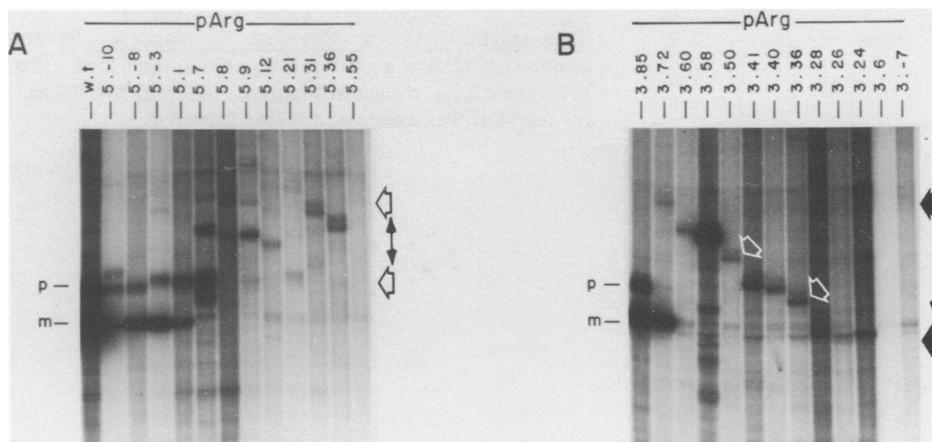

Figure 3 - Transcription of Drosophila Deletion tDNAs in Germinal Vesicle Extracts. Transcription analyses using GV extract performed as described in Materials and Methods. (A) 5' deletion tDNAs; the open black arrows indicates the size range of 5' deletion mutant transcripts. This transcription profile is qualitatively similar to that observed in Figure 1A. (B) 3' deletion tDNAs; the open black arrows indicate the size range of 3' deletion mutant transcript RNAs. Transcript RNAs become shorter between tDNAs pArg3.72 and pArg 3.36 due to deletion of Drosophila DNA while maintaining the same transcription termination sequence. Compare the relatively higher intensities of the transcripts indicated by open white arrows to their intensities in Figure 1B.

transcriptional activity. For the Drosophila $tRNA^{Arg}$ gene this high dependence was reduced by changing from the use of GV to GV extract (or also for the 3'-deletion tDNAs, whole oocyte extract).

Analysis of Transcripts Formed from 5'-Deletion Mutant tDNAs.

Longer-sized transcription products were formed from the 5' deletion tDNAs having seven or more base pairs of Drosophila DNA deleted 5' adjacent to the D-control region (e.g, pArg5.7 and pArg5.12). These transcripts may be formed by either transcription initiation occurring further upstream or by incorrect termination which would occur further downstream from the wild-type $tRNA^{Arg}$ gene termination sequence. To test these possibilities selected 5' deletion mutant DNAs were transcribed in the GV extract in the presence of [α-^{32}P]UTP (Fig. 4). The RNA products indicated were recovered and subjected to fingerprint analysis after digestion with ribonuclease T1 (20,21). Each of these RNAs displayed the same uridylate-rich oligonucleotides representing transcript 3'-end oligonucleotides, $AA(U)_{3-5}\ U_{OH}$ (12), which derive from the wild-type $tRNA^{Arg}$ gene transcription termination sequence (results not shown). The approximate 5' termini of these transcripts were determined by nuclease S1 protection mapping. Each of the

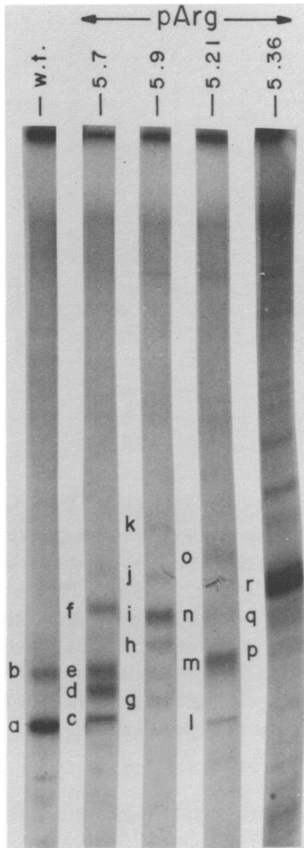

Figure 4 - Preparative Electrophoresis of Transcript RNAs for Nuclease S1 Mapping. Several 5' deletion tDNAs as indicated, were transcribed using GV extracts as shown in Figure 3. Each of the RNAs for analysis was assigned a letter from a-r.

transcripts indicated in Figure 4 was hybridized to its complementary plasmid DNA as described in Materials and Methods and after nuclease S1 digestion of the hybrids, the protected DNA fragments were resolved by polyacrylamide gel electrophoresis. The conditions of nuclease S1 digestion were not optimized for these hybrids and so in each instance a set-array of protected fragments resulted. A summary of the nuclease S1 results is presented in Table 1. In several instances the observed protected fragment did not align in size with the fragment size that was expected. We attribute this to non-optimizing the conditions of hybridization and subsequent nuclease S1 digestion.

While precise transcription starts cannot be assigned from this analysis the observed protection of the ^{32}P-DNA probe indicates that the transcripts resulting from D-control region deletion tDNAs are longer and heterogeneous in the 5' end of the RNA. We conclude that the differences in the transcript sizes is due to differences in the transcription initiation start sites rather than a difference in the termination site.

TABLE 1
Nuclease S1 Mapping of 5'
Deletion tDNA Transcripts

Transcript	Size of Protected Fragment: Expected	Size of Protected Fragment: Observed
a	48	48,71
b	55	51,71
c	49	47
d	53	46-50
e	55	47-54
f	68	65-69
g	—	—
h	60	61
i	64	64-70
j	82	—
k	—	—
l	47	—
m	56	51-57
n	64	—
o	90	88-92
p	56	—
q	64	60-74
r	74	68-76

a-r were hybridized to 5' 32-P labeled complementary DNA. The hybrids were digested with nuclease S1 and the protected DNA fragments were sized on a denaturing polyacrylamide gel. The expected size of the fragments was assessed from the electrophoretic mobility of the RNA transcripts of Figure 4.

The sizes of the nuclease S1 protected fragments were approximated and superimposed onto the DNA sequences of the tDNAs from which they were formed (Fig. 5). This gives an indication of the possible initiation sites for each of the transcripts.

In these experiments the tDNA coding strand has been 5'-labeled at a HinfI restriction endonuclease digestion site occurring at position 48 within the mature $tRNA^{Arg}$ coding sequence. Initiation sites were not detected within 45 nucleotides of the labeling site. In the wild-type tRNA gene the 45 position would correspond to nucleotide 4 in the 5' stem of the amino acid acceptor arm; or for a more convenient reference, it corresponds to 52 nucleotides upstream of nucleotide C56 in the T-control region. (This reference was chosen because nucleotide C56 was shown to be an essential nucleotide for promotion of tRNA gene transcription; ref. 22). Alternatively, once the D-control region had been partially or completely removed there does not appear to be a limitation on the transcription complex to select an initiation site further away since initiation sites were even selected up to several hundred nucleotides upstream of C56 (Fig. 1A, 3A). Once a particular sequence had been selected for use as a transcription initiation site, its use as

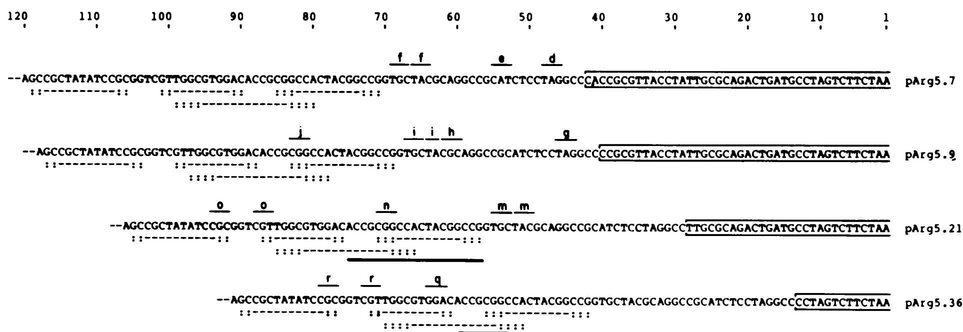

Figure 5 - The Initiation Sites of 5' Deletion tDNA Transcripts. The DNA sequences of the coding strand of the 5' deletion mutants used in the nuclease S1 mapping experiments are shown. The direction of transcription is from left to right. The boxed sequences represent Drosophila tDNA sequences that are remaining in the deletion clones. Reading from right to left nucleotide 1 represents the ^{32}P-labeled HinfI site (tRNA^{Arg} coordinate 48) and the number above refers to the size of the transcript as determined by the nuclease S1 mapping procedure. Each lower case letter refers to the RNA transcript of Figure 4 and these are positioned according to their approximate 5' terminus as deduced. Potential stem and loop structures (stems containing 2-4 paired nucleotides) are indicated by underlining (::—::). Sequences having homology to the D-control region consensus sequence are underlined using a solid bar (——).

a start site continued in the successive tDNA deletion clones until the particular sequence was within 52-58 nucleotides upstream of C56 (Fig. 5).

Since the tRNA^{Arg} gene deletion mutants were constructed by substituting Drosophila DNA sequences with pBR322 sequences perhaps transcription of the 5' deletion tDNAs in the GV was directed by a substitute D-control region formed during construction of the deletion mutants. The possible occurrence of a substitute T-control region in the Drosophila tRNA^{Arg} gene 3' deletion mutants, was recently discussed (23); subsequent transcription-competition experiments with these mutant tDNAs demonstrated that a substitute T-control region had not been introduced (10). In the present study to test for the possibility of a substitute D-control region in 5' deletion tDNAs we have searched the upstream sequences of the 5' deletion tDNAs for the presence of tRNA D-stem and D-loop-like structures and for sequences corresponding to a D-control region consensus sequence ($\genfrac{}{}{0pt}{}{G}{A}$ T $\genfrac{}{}{0pt}{}{G}{A}$ G $\genfrac{}{}{0pt}{}{C}{T}$ N N A G T . . G G T . . A $\genfrac{}{}{0pt}{}{G}{A}$; 2,3,11). These structures have been superimposed onto the sequences of the 5' deletion tDNAs in Figure 5. From the locations of most of the identified structures relative to the corresponding transcription initiation sites, it appears they do not contribute in directing the transcription of the 5' deletion tDNAs in the GV transcription system.

DISCUSSION

We have tested the ability of a series of 5' and 3' deletion mutants of a Drosophila $tRNA^{Arg}$ gene to support RNA synthesis in the nucleus of live Xenopus oocytes and in GV extracts. The result of this study was that deletion tDNAs, devoid of an intact D-control region, directed efficient RNA synthesis. This is in contrast to the results obtained using cell-free extracts. The present results show that the T-control region of the $tRNA^{Arg}$ gene is sufficient to ensure an efficient level of in vivo transcription. Previously, using cell-free transcription extracts, the T-control region was demonstrated as being the control region essential for the formation of a stable transcription complex (10). While stable complex formation in vitro does not necessarily ensure active transcription, it seems that tDNAs that have this ability, also serve as active transcription templates in vivo. Indeed, 3' deletion mutant tDNAs devoid of the T-control region, demonstrated not to form stable transcription complexes in vitro, are very poorly transcribed in vivo. We predict from these results that tDNAs that are able to bind transcription factor at the T-control region (10) and subsequently form a stable complex, will be actively transcribed in vivo.

The altered transcription initiation sites observed for the transcripts of the 5' deletion tDNAs re-emphasizes the importance of 5' flanking sequences in the initiation of tRNA gene transcription. Transcription initiation by RNA polymerase III uses a purine nucleotide and (comparing the transcription of tRNA genes so far studied), the initiation sites occur within 10-22 nucleotides from the 5' border of the D-control region (nucleotide position 8 in the mature tRNA coding sequence). Comparing the transcripts formed from the deletion clones pArg5.1 and pArg5.7 in the GV extract, it appears that deletion of the sequence 5' adjacent to the D-control region relaxes the stringency in selecting the initiation nucleotide. This sequence codes for the 5' stem of the amino acid acceptor arm. All stringency, however, was lost only after deletion of the nucleotides at position 7 and 8 in the mature tRNA coding sequence (compare pArg5.7, pArg5.8, and pArg5.9 in Figs. 1A and 3A). In the injected GV, however, stringency was maintained up to removal of the 5' stem and was lost only after deletion of nucleotides 7 and 8.

Several Drosophila tRNA genes that code for the same tRNA isoacceptor and therefore have the same promoter sequences as well as 5' stem sequences, direct selection of different initiation sites (24-26). These tRNA genes have different 5' flanking sequences and this observation, combined with experiments involving exchanging 5' flanking regions between different genes, demonstrated that the 5' flanking sequence participated in directing selection of the initiation nucleotide (24-26). The present results demonstrate that the 5' stem encoding region together with nucleotide 8 within the wild-type tRNA coding region, imparts specificity on the RNA polymerase III transcription apparatus for the selection of the initiation site.

Transcriptional analysis of 5' deletion mutants of the X. borealis somatic 5S RNA gene first demonstrated that selection of the transcription initiation site was "measured" from the Internal Control Region (ICR) (27). In a separate study Sakonju et al. (28) suggested that the Xenopus 5S RNA gene contained two interrelated regions within the ICR. Recently, the ICR of the 5S RNA gene was indeed shown to be comprised of a split promoter in which the 5' promoter element is responsible for "measuring" upstream to select the transcription initiation site (11). Moreover, the 5' promoter element of the 5S RNA gene ICR was functionally interchangeable with the 5' promoter element (A box or D-control region) of the C. elegans $tRNA^{Pro}$ gene (11). The mechanism of transcription initiation is more complex than appears however, since mutant genes comprising the 5' element of the $tRNA^{Pro}$ gene and the 3' element of the 5S RNA gene directed 5S RNA-type transcription initiation (11). The present study demonstrates that the $tRNA^{Arg}$ gene D-control region mediates the "measurement" function of RNA polymerase III for tRNA gene transcription. We suggest that the "measurement" mechanism is a function mediated by the factor that interacts with the D-control region (δ-factor).

The ability of a transcription factor to bind to 5' deletion tDNAs, which are unable to support transcription, has been observed for Class 1 genes (28-33) and for the Xenopus 5S RNA gene (34). In the latter case, the transcription factor involved is TFIIIA (35) and binding of this factor is dependent on the 3' half of the 5S RNA gene ICR (28). The tRNA gene τ-factor appears to have functional equivalence to TFIIIA in that footprinting analyses of several Class 1 genes demonstrated nuclease protection of each respective T-control region (36). Of the mutant genes comprising only their 3' promoter element, which have been examined in GV transcription systems, the 5' deletion mutants of the Drosophila $tRNA^{Arg}$ gene provide the only example for supporting RNA synthesis. Since each of the cloned halves of this gene is able to support limited forms of transcription, in various transcription systems each half of the $tRNA^{Arg}$ gene represents a "strong" promoter element. Clearly within the wild type tRNA gene the "strength" of a promoter element must be defined by sequences outside the internal control regions (5,9,37,38), as well as by nucleotides within the semi-invariant sequence of the D-control region (38,39).

The transcription systems of Xenopus oocytes in order of increasing manipulation required for their preparation are: (i) injection of the GV within a live oocyte; (ii) extract prepared from isolated GVs; and (iii) extract prepared from whole oocytes. Comparing each of the Xenopus transcription systems in regard to their abilities to transcribe the same set of Drosophila $tRNA^{Arg}$ gene mutants, reveals a changing transcriptional trend. The differences in transcription of mutant $tRNA^{Arg}$ genes, compared to the wild-type gene, as this progression is made is: 5' deletion mutants support a lower level of RNA synthesis whereas 3' deletion mutants support an increasing level of RNA synthesis. These changes in transcriptional properties might be attributable to the

increased manipulation of the tissue leading to the loss of a transcriptional function. This loss may involve destruction of nuclear integrity important for transcription, loss of a general DNA binding protein(s) for example, or loss of a specific transcription factor(s) activity. The latter could occur by a decrease in the concentration or amount, of the factor in the systems or by a reduction in the single activity of a multifunctional factor.

The precise function(s) that is affected in the transcription systems at the moment is not known. Nevertheless, the different transcription activities of the same tRNA gene mutants in the different Xenopus systems is suggestive of potential transcriptional deficiencies in cell-free extracts. It is striking that the transcriptional behaviour of the pArg deletion mutants are qualitatively similar in Xenopus whole oocyte extract as in the Drosophila Kc cell transcription extract (3,9). Whether this is coincidental or indicative of a transcriptional deficiency in the Kc cell-free extract compared to in vivo in Drosophila needs to be examined.

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