Transfer RNA genes of Drosophila melanogaster

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ABSTRACT

Three recombinant plasmids containing randomly sheared genomic D. melanogaster tRNAs have been identified and characterized in detail. One of these, the plasmid 14C4, has a D. melanogaster (Dm) DNA segment of 18 kb, and has three tRNA<sub>Arg</sub> and two tRNA<sub>Asn</sub> genes. The second plasmid, 38B10, has tRNA<sub>His</sub> genes, while the third plasmid, 63H5, contains coding sequences for tRNA<sub>Asp</sub> 2. The Dm DNA segments in each recombinant plasmid are derived from unique cytogenetic loci. 14C4 is from 84 F, 38B10 is from 48 F and 63H5 is from 70 A.

INTRODUCTION

Redundant genes are found in many different organisms from prokaryotes such as E. coli to virtually all higher eukaryotes (1-3). Since these genes typically code for components of the protein synthesis machinery or for proteins involved in the assembly of DNA into chromatin, it is evident that gene reiteration provides an efficient mechanism for supplying large quantities of critical products. In this case, it is clearly important that the functional integrity of these genes be maintained. Indeed, studies on various types of redundant genes have shown that the nucleotide sequence is highly conserved within and even between species (1, 3-7). In this respect, it may be significant that these redundant genes are often arranged in extensive arrays of tandemly repeated units which are composed of the gene sequence plus adjacent spacer, and a number of different mechanisms have been proposed for the maintenance of se-
sequence homogeneity within these gene clusters (1, 3, 6-9). This type of sequence arrangement has been found in D.melanogaster, and recent studies have shown that the 5S RNA genes, the rRNA genes and the histone genes are all arranged in tandemly repeated units which are found clustered at specific cytogenetic loci in the D.melanogaster genome (10-12, 3, 5).

The sequence coding for D.melanogaster tRNAs are also repeated and it has been estimated that there are 600-750 tRNA genes (13, 14). Since there are approximately 60 different tRNA families, it is expected that there are on the average between 10-13 coding sequences for each tRNA species. This average figure is, however, considerably lower than, for example, the 160 repeats of the D.melanogaster 5S RNA gene. In addition to a difference in gene number, the arrangement of the redundant tRNA genes in the D.melanogaster genome appears to be unlike that of the 5S RNA, rRNA or histone genes. While there appear to be clusters of tRNA genes, these clusters are distributed at many cytogenetic loci throughout the D.melanogaster genome and often contain coding sequences for more than one type of tRNA (15-23). These results would suggest that the underlying factors determining the organization of tRNA genes, and the mechanisms ensuring their conservation may differ from those involved with tandemly repeated D.melanogaster genes. As the first step in a detailed analysis of the arrangement, structure and expression of D.melanogaster tRNA genes, we report here the isolation and characterization of three genomic D.melanogaster hybrid plasmids which contain tRNA coding sequences.

MATERIALS AND METHODS

Isolation of tRNA: Transfer RNA was isolated from Drosophila melanogaster (Oregon) flies of mixed age according to the procedure of Kirby (24). The high molecular weight RNAs were either salt precipitated or removed by DEAE-cellulose chromatography (25, 26). The low molecular weight RNAs were further fractionated on a Sephadex G-100 column and the tRNA peak precipitated with ethanol (27).
Preparation of uniformly labeled $^{32}P$-tRNA: 6 $\cdot$ 10$^7$ cells of the Schneider cell line 2 were grown for 24 hours in a phosphate-less medium with 25 mCi $^{32}P$-Na-monophosphate at a density of 1.5 $\cdot$ 10$^7$ cells/ml at room temperature. After pelleting, the cells were washed with cold Tris-buffer (50mM, pH 7.5) and lysed by gentle shaking in Tris-HCl 50mM, pH 7.5; 0.5% SDS; 0.1 vol.% diethylpyrocarbonate. DNase (2 µg/ml) was added and incubated for 10 min. at 0°C. The tRNA was then extracted with phenol and the high molecular weight RNA precipitated with K-acetate. This RNA preparation containing about 25% tRNA was used for hybridization on filters. The specific activity of the RNA was 10$^6$ cpm/µg.

Isolation of plasmid DNA: E.coli strain HB101 containing the ampicillin-resistant plasmids were grown in T-broth containing 200 µg/ml of ampicillin at 37°C. The bacteria were grown in T-broth to an optical density of 1.0 at 650nm. Chloramphenicol was added to a concentration of 100µg/ml and the cultures were shaken at 37°C for another 12 hours. Covalently closed circular plasmid DNA was isolated as supercoils by detergent lysis of the cells, followed by cesium chloride ethidium bromide gradient centrifugation.

Iodination of the tRNA with $^{125}$I: The procedure of Prensky (28) was followed in detail.

Hybridization of filters: The protocol of Clarkson et al. (29) was used. The hybridizations were done in 1.15M Na-acetate, pH 5; 60% formamide at 43°C for at least 8 $\cdot$ t1/2. The value for t1/2 for a pure tRNA is assumed to be 1.1 min. at 3 µg/ml (Clarkson, S., personal communication). The hybridization time for crude tRNA and RNA after K-acetate precipitation was calculated by assuming that crude tRNA contains 60 families and RNA after K-acetate precipitation consists of 25% tRNA. After hybridization the filters were washed for 30 min. in hybridization buffer at 43°C, twice in 2xSSC (1xSSC: 0.15M NaCl, 0.015M Na-citrate) for 20 min. at room temperature, and finally rinsed in 0.1xSSC. For the elution of the $^{32}P$-tRNA the filters were incubated twice for 5 min. in 10mM Tris-HCl, pH 7.6;
90% formamide at 65°C. Both fractions were pooled and Na-acetate, pH 5, added to a concentration of 0.5M. After addition of 400 μg cold carrier tRNA the tRNA was precipitated with 2 1/2 volumes ethanol.

Two-dimensional polyacrylamide gel electrophoresis: The system of Fradin et al. (30) was used with the following modifications. The first dimension was run for 20 hours with 12 V/cm until the cyanol-FF marker had migrated 1 cm above the end of the gel. The second dimension was run at 15 V/cm for 72 hours (31). For analytical purposes the thickness of the gel was 1 mm, for preparative runs 3 mm. The gels were stained with stains-all (32) or ethidium bromide (50 μg/ml) and the radioactive tRNAs located by autoradiography. The elution of the tRNA from the gel was done by pressing the gel pieces through a 1 ml syringe. The gel homogenate was incubated over night at 37°C in a double volume of 0.5M NH₄-acetate; 0.5% SDS; 0.1mM EDTA and 1/5 volume phenol. The phenol was extracted with 1/5 volume chloroform and centrifuged. The water phase was filtered on a Sephadex G25 pasteur pipette column and the RNA ethanol precipitated twice.

Isolation of the aminoacyl-tRNA synthetases, aminoacylation: The aminoacyl-tRNA synthetases were isolated according to Twardzik et al. (33). The aminoacylation conditions were those of White and Tener (34).

DBAE-cellulose chromatography: The procedure described by McCutchan et al. (35) was applied for the fractionation of crude D.melanogaster tRNA. Transfer RNA Pro elutes in the first peak, tRNA AsN in the second.

Reversed-phase chromatography: The RPC-5 system of Kelmers and Heatherly (36) was used. The tRNAs were fractionated on a 0.7x46 cm high performance column at 37°C with a 200 ml NaCl gradient (0.5-0.8M NaCl; 10mM Na-acetate, pH 4.5; 10mM MgCl₂; 1mM 2-mercaptoethanol). One ml fractions were collected at a flow rate of 1 ml/min. The samples were counted in a Packard liquid scintillation counter.

In situ hybridization: In situ hybridization of nick-trans-
lated plasmid DNA was performed essentially as described in (10). For non-stringent hybridization, the temperature was reduced to 58°C and the salt increased to 5xSSC.

RESULTS

Identification of cloned Dm DNA segments containing tRNA genes. A collection of several thousand independent D. melanogaster (Dm) hybrid plasmids constructed by the dA:dT connector method (38, 10) was screened for sequences complementary to in vivo \(^{32}P\) labeled 4 S RNA isolated from tissue culture cells using a modification of the Grunstein and Hogness (39) colony hybridization procedure. Three of the positive clones identified in the screen, called 14C4, 38B10, and 63H5, respectively, were selected for further study. As shown in Figure 1, single and combination restriction enzyme digests of the isolated hybrid plasmid DNAs were used to construct restriction maps for each

![Restriction maps of the recombinant plasmids:14C4, 38B10, and 63H5. Only the Dm DNA segment of each plasmid is shown. The position of tRNA coding sequences are indicated.](image-url)
of the cloned Dm DNA segments. The largest Dm DNA segment is found in the hybrid plasmid 14C4 and it is 18 kb in length, while the Dm segments of 38B10 and 63H5 are 7.5 kb and 2.5 kb respectively.

The positions of the sequences complementary to *D. melanogaster* tRNAs in each of the recombinant plasmids was determined by hybridization of in vivo $^{32}$P labeled 4S RNA to appropriate restriction digests according to the procedure of Southern (40). As indicated in Figure 1, the 4S RNA coding sequences of the hybrid plasmid 14C4 are clustered in a 2 kb Bgl II-Hind III fragment located near the center of the 18 kb Dm DNA insert. The sequences on either side of this Bgl II-Hind III fragment fail to hybridize to 4S RNA, and also show no homology to either total in vivo labeled tissue culture RNA or to in vitro labeled embryonic RNA. The restriction enzyme Eco R1 cleaves the 2 kb Bgl II-Hind III fragment into two fragments of 1.2 kb and 0.8 kb. While both of these are found to be homologous to 4S RNA, the extent of hybridization is not the same. The ratio observed is approximately 4 (1.2 kb fragment) : 1 (0.8 kb fragment) and this would suggest that the tRNA coding sequences are located predominantly in the larger fragment.

Hybridization of 4S RNA to Bgl I-Eco R1 combination digests of 38B10 map the tRNA coding sequences to the central 3.5 kb restriction fragment indicated in Figure 1. It is probable that the sequences complementary to tRNA do not span this entire 38B10 fragment. Thus digestion of the 3.5 kb Bgl I-Eco R1 fragment with Hind III yields one fragment of 2.6 kb and a second fragment of 0.9 kb. Neither of these fragments, however, show detectable labeling with 4S RNA. This finding would suggest that the tRNA coding sequences of 38B10 are cleaved by the restriction enzyme Hind III, and hence this Dm DNA segment may have only a single tRNA gene (see Discussion). 63H5 has the smallest Dm DNA segment of the three hybrid plasmids, and the sequences complementary to 4S RNA in this plasmid map to one side of the insert as shown in Figure 1.
Determination of the tRNA genes encoded by the hybrid plasmids 14C4, 38B10, and 63H5. While the studies described in the previous section map the sequences complementary to tRNA, they provide no information as to the coding properties of the tRNA genes in the three hybrid plasmids. To resolve this question we have used a number of different experimental approaches to determine which tRNA species are encoded in each of the cloned Dm DNA segments. Shown in Figure 2a is the distribution of total D.melanogaster tRNA on a 2-dimensional polyacrylamide gel (see material and methods). Approximately 30 - 40 major and 10 - 20 minor spots can be resolved by this gel system and this corresponds very well with the 56 major and 33 minor isoacceptors identified using RPC-5 chromatography (16). Hence the high resolution of this gel system may be used for a preliminary identification of the tRNA species encoded by each of the cloned Dm DNA segments. For this experiment, denatured DNA from each of the plasmids was immobilized on nitrocellulose filters and incubated under hybridization conditions with total $^{32}$P in vivo labeled RNA (see material and methods). The tRNA was then eluted and co-electrophoresed with crude, cold D.melanogaster tRNA using the 2-dimensional polyacrylamide gel system. The results of these experiments are shown in Figure 2b-d. Only a single $^{32}$P labeled tRNA spot is observed with the plasmid 38B10 (see Figure 2b) and comparison of the position of this tRNA with the distribution of total D.melanogaster tRNA indicates that it corresponds to spot 1 (see Figure 2a). The plasmid 63H5 gives one major spot (see Fig. 2c) which comigrates with spot 4 in the 2-dimensional gel of total tRNA. In addition a second, minor spot is observed in the 63H5 autoradiograph (spot 1). However, the yield of this tRNA species is very low, and hence it may differ from the tRNA in spot 4 only in nucleotide modification.

In contrast to 38B10 and 63H5, two major spots and one minor spot are observed in the autoradiograph of the 14C4 2-dimensional gel (see Fig. 2d). Comparison of the position of the 14C4 spots with total tRNA shows that the 14C4 tRNAs correspond to spots
Figure 2. Determination of the tRNA genes encoded by the plasmids. The [32P] tRNA eluted from the filters was co-electrophoresed on 2-dimensional polyacrylamide gels with crude, cold D. melanogaster tRNA. The autoradiographs were then superimposed on the stains-all stained gels. First dimension: right to left; second dimension: top to bottom. (a) Total tRNA, stains-all staining. Transfer RNAs in the numbered spots are: spot 1: tRNA^{His} and tRNA^{Ala}; spot 2: tRNA^{His}; spot 3: tRNA^{Ala}; spot 4: tRNA^{Asp}; spot 5: tRNA^{Arg}; spot 6: tRNA^{AsN} and tRNA^{Pro}; spot 7: not identified; spot 8: tRNA^{Gly}; spot 9: tRNA^{Ser}. (b, c and d) Autoradiography of 2-dimensional gels containing [32P] labeled tRNAs eluted from nitrocellulose filters having 38B10, 63H5, and 14C4 DNA respectively.

5, 6 and 7 (see Figs. 2a and 2d). The relative yield of the major spots, 5 and 6, is 3:2 suggesting that 14C4 may contain three genes for the first type of tRNA and two genes for the
second. Since the yield of spot 7 was less than 1 (app. 0.1) even when the hybridization was performed at a four times higher $R_t$ value (see materials and methods), it is probable that this spot corresponds to a minor isoacceptor of one of the two 14C4 tRNAs. In this case, it would differ in nucleotide modification rather than in actual sequence.

We have performed a number of experiments to further characterize the tRNAs complementary to each cloned Dm DNA segment. In the first experiment the tRNA species migrating with spots 1, 4, 5, and 6 were eluted from several preparative 2-dimensional gels and tested for aminoacylation with all 20 amino acids. These studies, which are presented in Table 1, permit a tentative assignment of the tRNAs encoded by each hybrid plasmid. Since spot 4 contains only tRNA\textsuperscript{Asp} the plasmid 63H5 must have coding sequences for this tRNA. Similarly, one of the tRNAs encoded by 14C4, spot 5, is tRNA\textsuperscript{Arg}. On the other hand, spot 6 of 14C4 is a mixture of two different tRNA species, tRNA\textsuperscript{Pro} and tRNA\textsuperscript{Asn}, and hence this plasmid may have coding sequences for either one or both of these tRNAs in addition to tRNA\textsuperscript{Arg}. A similar mixture of two tRNA species, tRNA\textsuperscript{Ala} and

<table>
<thead>
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<th>Plasmid</th>
<th>Spot No.</th>
<th>amino acid</th>
<th>pmoles/A\textsubscript{260}</th>
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<tr>
<td>63H5</td>
<td>4</td>
<td>Asp</td>
<td>1080</td>
</tr>
<tr>
<td>14C4</td>
<td>5</td>
<td>Arg</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Pro</td>
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<td></td>
<td></td>
<td>AsN</td>
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<td>38B10</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>His</td>
<td>1250</td>
</tr>
</tbody>
</table>

Determination of the tRNAs species eluted from the polyacrylamide gels by aminoacylation. Transfer RNA from each spot was aminoacylated with all 20 amino acids. Only the positive results are given.
Table 2

<table>
<thead>
<tr>
<th>Plasmid</th>
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<th>cpm bound to filter with DNA</th>
<th>background on filters without DNA</th>
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<tbody>
<tr>
<td>63H5</td>
<td>$^{14}$C-Asp-tRNA</td>
<td>36</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-Ala-tRNA</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>38B10</td>
<td>$^{14}$C-His-tRNA</td>
<td>42</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-Ala-tRNA</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>14C4</td>
<td>$^{14}$C-Arg-tRNA</td>
<td>78</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>$^{14}$C-AsN-tRNA</td>
<td>75</td>
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<td></td>
<td>$^{14}$C-Pro-tRNA</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>63H5</td>
<td>$^{125}$I-tRNA$^{\text{Asp}}$</td>
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<td></td>
<td>$^{125}$I-tRNA$^{\text{His}}$</td>
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<tr>
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<td>$^{125}$I-tRNA$^{\text{His}}$</td>
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<td>2110</td>
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</table>

Confirmation of the tRNA species encoded by the plasmids. Amino acid labeled aminoacyl-tRNAs or purified $[^{125}$I] tRNAs were hybridized to plasmid DNA baked on nitrocellulose filters.

$^{\text{tRNA}}$His, is observed for spot 1 of 38B10. In order to confirm and extend these results, two additional experiments were performed. First, total D. melanogaster tRNA was aminoacylated with amino acids and then hybridized to nitrocellulose filters with each plasmid DNA. Second, highly purified tRNA$^{\text{Asp}}$ and tRNA$^{\text{His}}$ were iodinated with $[^{125}$I] and used as hybridization probes. Although the number of counts observed in the experiments with labeled amino acid-tRNA are low due to deacylation during the hybridization, our findings in both experiments are consistent with the following assignments: 38B10 contains at least one tRNA$^{\text{His}}$ gene; 63H5 codes for tRNA$^{\text{Asp}}$, and 14C4 has genes for tRNA$^{\text{Arg}}$ and tRNA$^{\text{AsN}}$.

Determination of the isoacceptors. In order to determine which isoacceptors are coded by the plasmids, tRNA from spots
1, 4, 5 and 6 (see Figure 2) were aminoacylated with the appropriate [\(^{14}\)C]-amino acid and co-chromatographed with crude tRNA aminoacylated with the same [\(^{3}\)H]-amino acid on RPC-5 columns. As indicated in Figure 3b, the 63H5 spot 1 contains two Asp isoacceptors, tRNA\(_{f_{28}}^{Asp}\) and tRNA\(_{2y}^{Asp}\). This result, in conjunction with the findings reported in the previous sections would be consistent with the suggestion that both of these tRNA\(_{2}^{Asp}\) species may have identical or at least, very similar nucleotide sequences, while the remaining tRNA\(^{Asp}\) isoacceptors must have different nucleotide sequences. Spot 4 from the plasmid 38B10

Figure 3a) RPC-5 chromatography of crude tRNA aminoacylated with [\(^{3}\)H]-arginine co-chromatographed with [\(^{14}\)C]-arginine tRNA from spot 5. b) RPC-5 chromatography of crude tRNA aminoacylated with [\(^{3}\)H]-aspartic acid co-chromatographed with [\(^{14}\)C]-aspartic acid tRNA isolated from spot 4. c) RPC-5 chromatography of crude tRNA aminoacylated with [\(^{3}\)H]-histidine co-chromatographed with [\(^{14}\)C]-histidine labeled tRNA from spot 1.
is found to contain both of the major His isoacceptors, tRNA^{His}_1 and tRNA^{His}_2 (Figure 3c), suggesting that the D. melanogaster His isoacceptors are homogeneic and differ only in modification. As shown in Figure 3a, spot 5 of 14C4 corresponds to the major tRNA^{Arg}_1 isoacceptor Arg₁, and it must differ in nucleotide sequence from the remaining Arg-tRNA species. Similar experiments with the 14C4 tRNA^{AsN} were inconclusive (data not shown).

Cytogenetic origin of 14C4, 38B10 and 63H5. We have determined the cytogenetic origin of the three cloned DNA Dm DNA segments by in situ hybridization to salivary gland polytene chromosomes (10). As shown in Figure 4a and 4b, the plasmids 14C4 and 38B10 hybridize to unique chromosomal sites. 14C4 labels the locus 84 F which is near the base of the right arm of the third chromosome (Figure 4a), while the cytogenetic origin of 38B10 is on the right arm of the second chromosome at 48 F. These findings map genes for tRNA^{Arg}_2 and tRNA^{AsN} to 84 F, and tRNA^{His} genes to 48 F. On the other hand, since short (app. 75 bp) regions of homology would not be detected under the conditions used for in situ hybridization, it is conceivable that Arg and AsN as well as His tRNA coding sequences may be present elsewhere in the D. melanogaster genome. This possibility is supported by results obtained with the hybrid plasmid 63H5. Under stringent hybridization conditions identical to those used in the experiments with 14C4 and 38B10, 63H5 only labels one site, 70A, on the left arm of the third chromosome. However, this is not the case when the hybridization is performed under less stringent conditions. As demonstrated in Fig. 4c, 63H5 shows significant labeling of the cytogenetic locus 42F on the right arm of the second chromosome and weakly labels a number of other sites. These observations would be consistent with the suggestion that there may be multiple tRNA^{Asp} genes distributed at a number of different cytogenetic loci in the D. melanogaster genome.
Figure 4a) In situ localization of 14C4. b) In situ localization of 38B10, and c) hybridization of 63H5 to Dm polytene chromosomes under non stringent conditions.
DISCUSSION

The hybrid plasmid 14C4 has the largest Dm DNA segment of the three recombinant plasmids described here and it is 18 kb in length. Interestingly, the tRNA coding sequences of 14C4 are apparently confined to a short 2 kb region near the center of the insert, and the DNA on either side of this region does not appear to code for either tRNA or for other types of RNA. From the analysis reported here, it is estimated that there are at least 5 tRNA genes in this short 2 kb region - 3 tRNA\textsuperscript{Arg} and 2 tRNA\textsuperscript{AsN} genes. However, we have as yet been unable to discern structural features within this region which are indicative of typical repeating arrays of gene-spacer sequences, and this finding, in conjunction with the results of other workers (22, 23, and R. De Lotto, personal communication), would suggest that, in general, the arrangement of \textit{D.melanogaster} tRNA genes differs substantially from that of 5S RNA, rRNA or histone genes. Moreover, the apparently irregular arrangement of \textit{D.melanogaster} tRNA genes also differs from that observed in \textit{Xenopus} where Clarkson and co-workers have identified a cluster of 4 tRNA genes which are found within a 3.18 kb repeating unit (41). Since the 18 kb Dm insert of 14C4 approaches the size of an average \textit{D.melanogaster} band-interband (app. 26 kb) it is conceivable that the tRNA coding locus at 84F has only 5 tRNA genes. If this supposition is correct, 84F may be an example of a comparatively simple tRNA coding locus. By way of contrast, Yen et al. (22) have isolated a Dm DNA plasmid, pCIT12, having a 9.3 kb insert that is derived from the cytogenetic locus 42A. Recent studies have shown that this plasmid contains at least 6 tRNA genes (Dieter Söll, personal communication). Unlike 14C4, however, the tRNA coding sequences of pCIT12 are distributed throughout the cloned Dm DNA segment, and Yen et al. have suggested that 42A may be a very complex locus which contains many tRNA genes. Similarly, we have recently isolated a series of recombinant DNA clones which are derived from the cytogenetic locus 90BC (R. De Lotto, P. Schedl, S. Artavanis-Tsakonas, in
preparation). Analysis of these clones indicates that the 90BC locus contains multiple copies of genes for at least 4-5 different tRNA species and may span a DNA segment which approaches 20-30 kb in length.

Though the hybrid plasmid 38B10 has a Dm DNA insert of 7.5 kb, we have detected the coding sequences for only one tRNA species, tRNA^{His}. Moreover, our mapping data suggests that this cloned Dm DNA segment may have only a single tRNA^{His} gene which is cut by the restriction endonuclease Hind III. These findings do not, however, necessarily imply that the cytogenetic locus 48F contains only a single tRNA gene. The 38B10 Dm DNA insert is relatively small and could conceivably be derived from the edge of a more extensive array of tRNA genes at 48F. In addition, Clarkson and co-workers (41) have found tRNA genes arranged as inverted repeats, and these genes are often difficult to detect by conventional hybridization procedures. Hence, there may be additional tRNA coding sequences within the 38B10 Dm DNA segment which are arranged in inverted repeats and failed to hybridize to tRNAs under the conditions used in the experiments reported here. Direct sequence analysis of 38B10 should indicate if this is the case. Finally, the plasmid 63H5 was found to code for tRNA^{Asp}_2. Since the 63H5 Dm DNA insert is quite small, the cytogenetic locus, 70A, from which this plasmid is derived may contain coding sequences for tRNAs in addition to tRNA^{Asp}_2. To investigate this possibility further, we are now isolating additional Dm DNA recombinants which are derived from the 70A locus.

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