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The initiator tRNA genes of Drosophila melanogaster: evidence for a tRNA pseudogene

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ABSTRACT

We have isolated four segments of Drosophila melanogaster DNA that hybridize to homologous initiator tRNA^{Met}. Three of the cloned fragments contain initiator tRNA genes, each of which can be transcribed in vitro. The fourth clone, pPW568, contains an initiator tRNA pseudogene which is not transcribed in vitro by RNA polymerase III. The pseudogene is contained in a 1.15 kb DNA fragment. This fragment has the characteristics of dispersed repetitive DNA and hybridizes in situ to at least 30 sites in the Drosophila genome. The arrangement or the initiator tRNA genes we have isolated, is different to that of other Drosophila tRNA gene families. The initiator tRNA genes are not clustered nor intermingled with other tRNA genes. They occur as single copies within an approximately 415-bp repeat segment which is separated from other initiator tRNA genes by a mean distance of 17 kb. In situ hybridization to polytene chromosomes localizes these genes to the 61D region of the Drosophila genome. Hybridization analysis of genomic DNA indicates the presence of 8-9 non-allelic initiator tRNA genes in Drosophila melanogaster.

INTRODUCTION

In the haploid genome of Drosophila melanogaster there are 600 to 750 tRNA genes (1-3) which give rise to approximately 90 different (56 major and 33 minor) tRNA species (4). Some of these tRNA species may have the same nucleotide sequence but differ in their extent of base modification. Weber and Berger (3) estimate that there are 59 families of kinetically distinct tRNAs. Therefore, there is an average of about 10 genes for each tRNA. Members of such reiterated tRNA gene families have been localized to several regions throughout the genome by in situ hybridization to polytene chromosomes (5-8). So far, the analysis of cloned Drosophila DNA fragments that contain tRNA genes has not been exhaustive. However, the results have demonstrated that some Drosophila tRNA genes are locally clustered (9-13). The tRNA genes contained within such clusters may code for the same, or different RNA species which are intermingled, irregularly spaced and may have

the same or opposite polarity (14-17). In general, the 5'- and 3'-flanking sequences of tRNA genes, which have the same mature-tRNA coding sequence, are not highly conserved. The greatest homology among flanking sequences thus far observed, has been for the sequences flanking the $tRNA^{Gly}$ genes of chromosomal locus 56F. Single *Drosophila* $tRNA^{Gly}$ genes are encoded in two 1.1 kb EcoR1 DNA fragments. These fragments are separated by a distance of approximately 1 kb and the two genes are contained within a repeated sequence of 280 bp (16).

To further our understanding of the arrangement of *Drosophila* tRNA genes, other tRNA gene families need to be characterized. Since the initiator tRNA genes represent the only tRNA gene family that has been studied in detail in several higher eukaryotes we have attempted to characterize this tRNA gene family in *Drosophila*. In *Xenopus* tandem initiator tRNA genes and 6 other tRNA genes are contained within a 3.18 kb DNA segment. This segment is repeated approximately 300 times in the haploid genome (19, 20). In the human genome the 12 initiator tRNA genes comprise a dispersed multigene family (21). In this paper we describe the structure and arrangement of the initiator tRNA genes of *Drosophila*.

MATERIALS AND METHODS

DNA. Clones pPW539, pPW568 and pPW591 were selected from a *Drosophila melanogaster* (Oregon R) DNA clone bank which was prepared (22) by ligating size fractionated randomly sheared embryo DNA into the single Eco R1 site of pMB9 by the A:T tailing method. Recombinant plasmid pTR18EH was selected from a *D. melanogaster* (Oregon R) clone bank which had been prepared by ligating HindIII digested embryo DNA into the HindIII site of pBR322 (12). Moderately repetitive *Drosophila* DNA was isolated as described (13). Plasmid DNA was propagated in *Escherichia coli* K12 strain HB101 by chloramphenicol amplification and purified by CsCl-ethidium bromide density gradient ultracentrifugation according to published procedures (23).

Restriction Enzyme Mapping. Restriction enzymes were obtained commercially and used according to the suppliers' specifications. Digested DNA was analyzed by agarose gel electrophoresis essentially as described by Sharp *et al*. (24) or on vertical acrylamide gels (25) using the mapping method of Smith and Birnstiel (26). DNA was transferred from agarose gels to nitrocellulose membranes (27). For hybridization to genomic DNA blots ^{32}P-labeled DNA was prepared from pTR18EH. Very high specific activity (109 cpm/ug DNA) DNA probe was prepared using the T4 DNA polymerase replacement

synthesis procedure of P. O'Farrell (personal communication). The probe used was the 79 bp TaqI fragment (coordinates 31-110 in Figure 3).

DNA Sequence Analysis. 5'- and 3'- end group labeling and DNA sequence analysis were performed according to the procedures of Maxam and Gilbert (28).

In vitro Transcription. Transcription of tRNA genes in a Drosophila Kc cell extract and subsequent RNA analyses were performed as described (29).

End Labeling of tRNA. D. melanogaster unfractionated embryo tRNA or purified initiator tRNA (30) was labeled at the 3'-terminus using T4 RNA ligase by the method of Bruce and Uhlenbeck (31). 5'-terminal labeling of tRNA was performed as follows: Approximately 20 - 60 pmole of tRNA in 5 ul 50 mM Tris-HCl, pH 8.3 was de-phosphorylated using 0.005 units of calf alkaline phosphatase (nuclease free; ref. 32). After 30 minutes incubation at 37°C the reaction was terminated by the addition of 0.5 ul of 0.1 M potassium phosphate, pH 9.5. To this mixture was added a solution of 0.1 M $MgCl_2$ - 20 mM spermine - 1 M KCl (1 ul), 50 - 250 uCi (γ-^{32}P)ATP (specific activity 3,000-8,000 Ci/mmole; ref. 33) and 5 Richardson units of T4 polynucleotide kinase to bring the final volume to 0.01 ml. The reaction was incubated at 37°C for 30 minutes. 5'-and 3'-terminus labeling reactions were loaded directly, and labeled tRNA purified on, 12% polyacrylamide thin-gels (34).

In situ Chromosomal Hybridization. The salivary glands from larvae bearing the mutation giant (gt, 1-0.9) were dissected and the chromosomes prepared for hybridization according to the method of Spradling et al., (35). Tritium labeled plasmid DNA (specific activity 1-5 x 10^6 cpm/ug) was prepared by the nick translation method of Maniatis et al. (36) and used for in situ hybridization as follows: The ^{3}H-DNA was denatured by boiling for 5 minutes shortly before hybridization. Approximately 0.5-2 x 10^5 cpm were used per slide in a hybridization mix which contained the following: 0.1 M Pipes, pH 6.8 - 0.45 M NaCl - 0.045 M sodium citrate (3 x SSC) -Denhardt's solution (37); 50% (v/v) deionized formamide and 2 ug (per slide) salmon sperm carrier DNA. Hybridization was performed at 38°C for 15 h. Each slide was then washed in 25 ul of each of the following solutions for the times indicated: three times 15 min at 36°C in 3 x SSC -50% (v/v) formamide; two times 15 min at 25°C in 2 x SSC; 10 min in 70%(v/v) ethanol; 10 min in 100% ethanol; air dried.

RESULTS

Isolation of Initiator tRNA Genes. Two Drosophila melanogaster clone

banks were hybridized with unfractionated ^{32}P-tRNA and all positive colonies were picked and re-screened using 5'- or 3'-labeled initiator tRNA. Only four clones from the two respective clone banks hybridized to the initiator tRNA. Their designations and sizes of Drosophila DNA insert are: pPW539 (17 kb), pPW568 (18 kb), pPW591 (8 kb) and pTR18EH (12 kb). In preliminary screenings the isolated plasmid DNA from these clones did not hybridize to any other Drosophila tRNA. pPW568 DNA, however, hybridized to purified moderately repetitive DNA ($0.05 \leq Cot \leq 3.0$, 13).

Restriction Enzyme and tRNA Gene Mapping. The DNA of the four plasmids was digested using a variety of restriction endonucleases in order to construct physical restriction maps (Fig.1). As determined by restriction enzyme analysis and hybridization to initiator tRNA (and subsequent DNA sequencing) each of the recombinant plasmids represents a unique fragment of Drosophila genomic DNA. The DNA inserts of pPW591 and pTR18EH, however, overlap within the tRNA gene region. Southern blot analysis of AvaII, HaeIII, HhaI or TaqI digests of the four plasmids using 5'- and 3'-labeled unfractionated tRNA or tRNA^{Met}, revealed that the initiator tRNA was the only tRNA that hybridized to these plasmids (results not shown). This evidence suggests that the

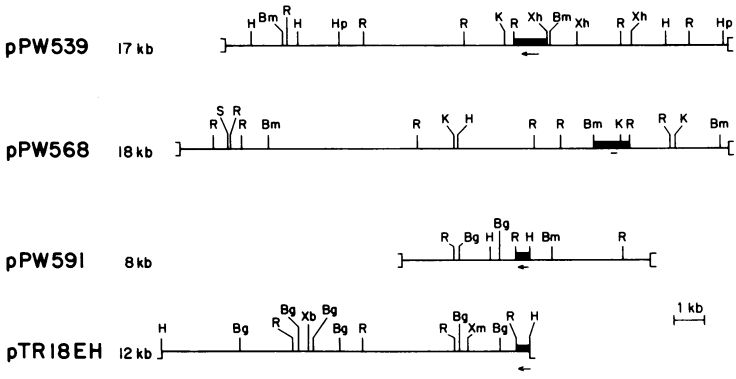

Figure 1. Restriction Maps of Cloned Drosophila DNA. Sites of cleavage by restriction endonucleases are indicated: BglII, Bg; BamHl, Bm; EcoRl, R; HindIII, H; HpaI, Hp; KpnI, K; SmaI, S; XhoI, Xh. DNA regions to which initiator tRNA hybridized are shown by the heavy lines. The arbitrary orientation of these maps are such that the initiator genes are transcribed from right to left, indicated by the arrows. In all cases the plasmids are circular and the vector plasmid DNA is not shown; for plasmids pPW539, pPW568 and pPW591 the vector is pMB9 and each is orientated so that the right hand side of the fragment is close to the single HindIII site in pMB9. The orientation of the Drosophila insert of pTR18EH in pBR322 is such that the right hand side is closest to the single EcoRl site of pBR322.

initiator tRNA gene is the only tRNA gene present in these plasmid DNAs.

Nucleotide Sequence Analysis. Sequencing of each of the four hybridizing plasmid DNAs using the strategies indicated (Fig. 2) revealed that pPW539, pPW591 and pTR18EH each contain a single initiator tRNA gene. Furthermore, each gene was contained within an approximately 415 bp repeated sequence (Fig. 3). The only nucleotide difference found between the coding sequence of any of the gene copies and the tRNA sequence (30), was within pPW591 (MET 3) which in position 30 had a T instead of a G (Fig. 3). This G→T transversion introduced a second AvaII restriction site into the gene coding sequence (Figs. 2 and 3). A computer search (38) for sequences (at least 10 bp in length) homologous to initiator tRNA located outside the coding region of the gene, revealed the sequence shown in Figure 3. We have indicated the tRNA co-ordinates to which this homology corresponds, but at present we cannot comment on its significance.

Nucleotide Sequence Analysis of pPW568. As already mentioned, the Drosophila DNA insert in pPW568 hybridized to moderately repetitive DNA

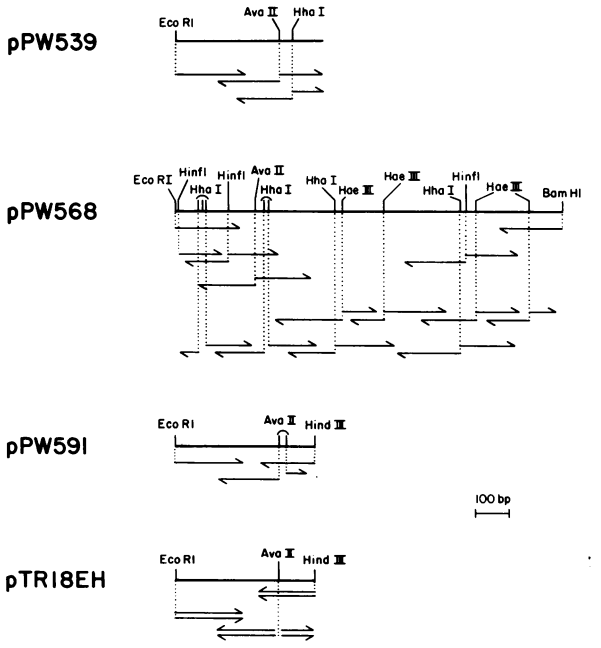

Figure 2. DNA Sequencing Schemes. The direction and extent of sequencing from ^{32}P-labeled 5'-termini is shown by arrows. For pTR18EH sequencing was also performed from ^{32}P-labeled 3'-termini.

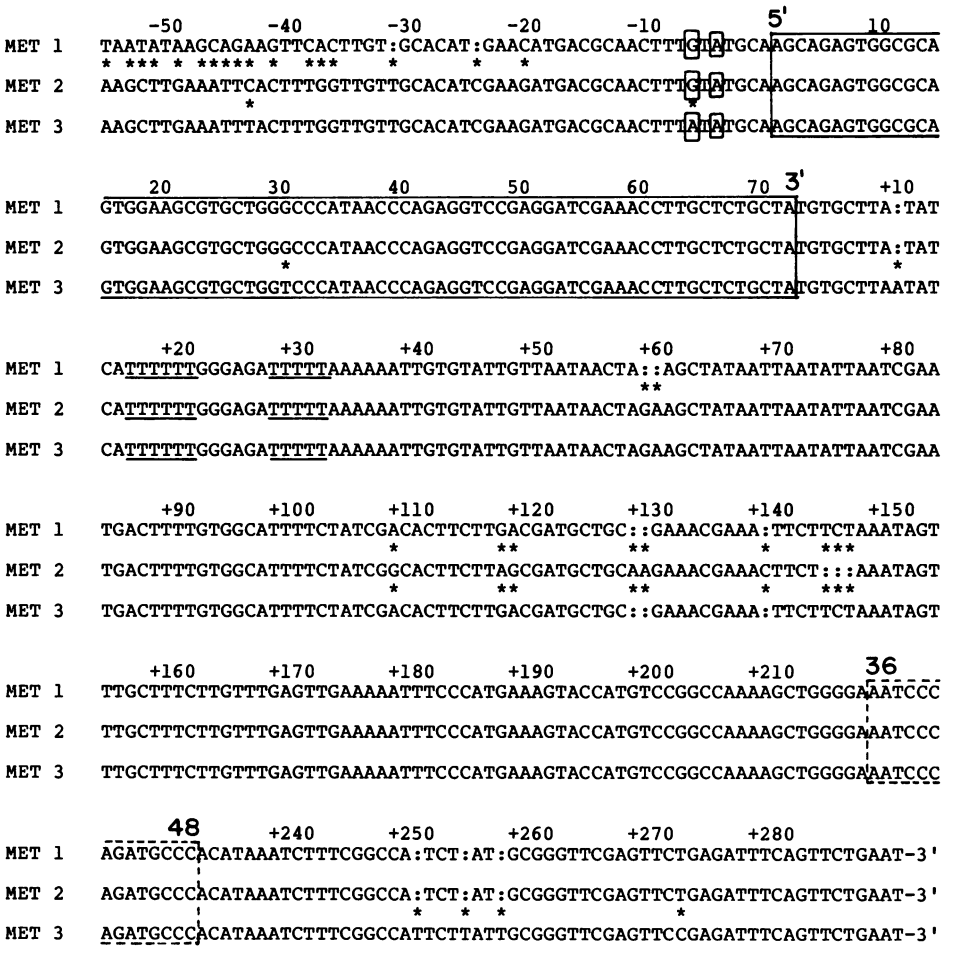

Figure 3. DNA Sequences of tRNA Gene Regions of pPW539 (MET 1), pPW591 (MET 3) and pTR18EH (MET 2). (The noncoding strand is shown.) An asterisk indicates a difference compared to the MET 2 sequence and colons have been inserted to provide maximum homology alignment. The outlined region indicates the mature-tRNA coding region. The boxed nucleotides show the initiating nucleotides for transcription and the underlined sequence represents termination of transcription. The dashed underlined sequence is homologous to the nucleotide co-ordinates of initiator tRNA as indicated by bold-printed numbers.

(13). Since a complete characterization of pPW568 was not within the scope of the present study we concentrated on defining the DNA element of pPW568 that hybridized to initiator tRNA. The approximately 1150 bp EcoRl/BamHl fragment that hybridized to $tRNA^{Met}$ (Fig. 1), was sub-cloned by standard procedures into pBR322. This newly generated recombinant plasmid was designated pYD6. While pYD6 DNA hybridized strongly to initiator tRNA, DNA sequencing revealed that a complete initiator tRNA gene sequence was not contained within the DNA (Fig. 4). A computer search (38) for sequences (at least 10 bp in length) homologous to initiator tRNA revealed four regions that displayed significant sequence homology. These regions are indicated in Figure 4. The largest region of homology (34 bp) probably contributes the most to the strong hybridization of initiator tRNA to pYD6. However, the smaller homologous sequences may also contribute. The larger homologous sequence which we designate as pseudogene, corresponds to the region in initiator tRNA between co-ordinates 7 and 39, which represents approximately 50% of an intact initiator tRNA sequence. This is the first indication that pseudogenes may be found among tRNA gene families.

A search for repeated sequences (at least 9 bp in length) within pYD6 revealed a set of 29 oligonucleotides scattered throughout the 1154 bp sequence. We could not determine the relation of any of these repeated segments to the occurrence of the initiator tRNA pseudogenes.

In vitro Transcription of Initiator tRNA Genes. Covalently closed circular pPW539, pYD6, pPW591 and pTR18EH DNAs were transcribed in *Drosophila* Kc cell extracts (29). Two primary transcription products resulted from each gene (designated p_1tRNA and p_2tRNA in Figure 5). The relative ratio of the occurrence of these two RNAs was usually 4:1. Using (α-^{32}P)UTP in transcription reactions of pPW539 and pTR18EH and subsequent 5'-terminal analysis or the isolated primary transcripts, we found that the 5'-terminus of p_1tRNA was pppG and of p_2tRNA was pppA. Thus the initiator tRNA genes of pPW539 and pTR18EH predominately initiated with G at nucleotide -7, whereas the minor primary transcript initiated with A at nucleotide -5. The coding and flanking sequences of the tRNA gene in pPW591 are essentially the same as those for pPW539 and pTR18EH except that it contains an A at position -7. End group analysis of primary transcripts of pPW591 revealed only pppA. From these results and from the identical transcription pattern for each of the initiator tRNA genes, we conclude that the genes on pPW591 also initiate at nucleotides -7 and -5. The erficiency of tRNA gene transcription in each of the clones was equal and transcription termination appeared to occur

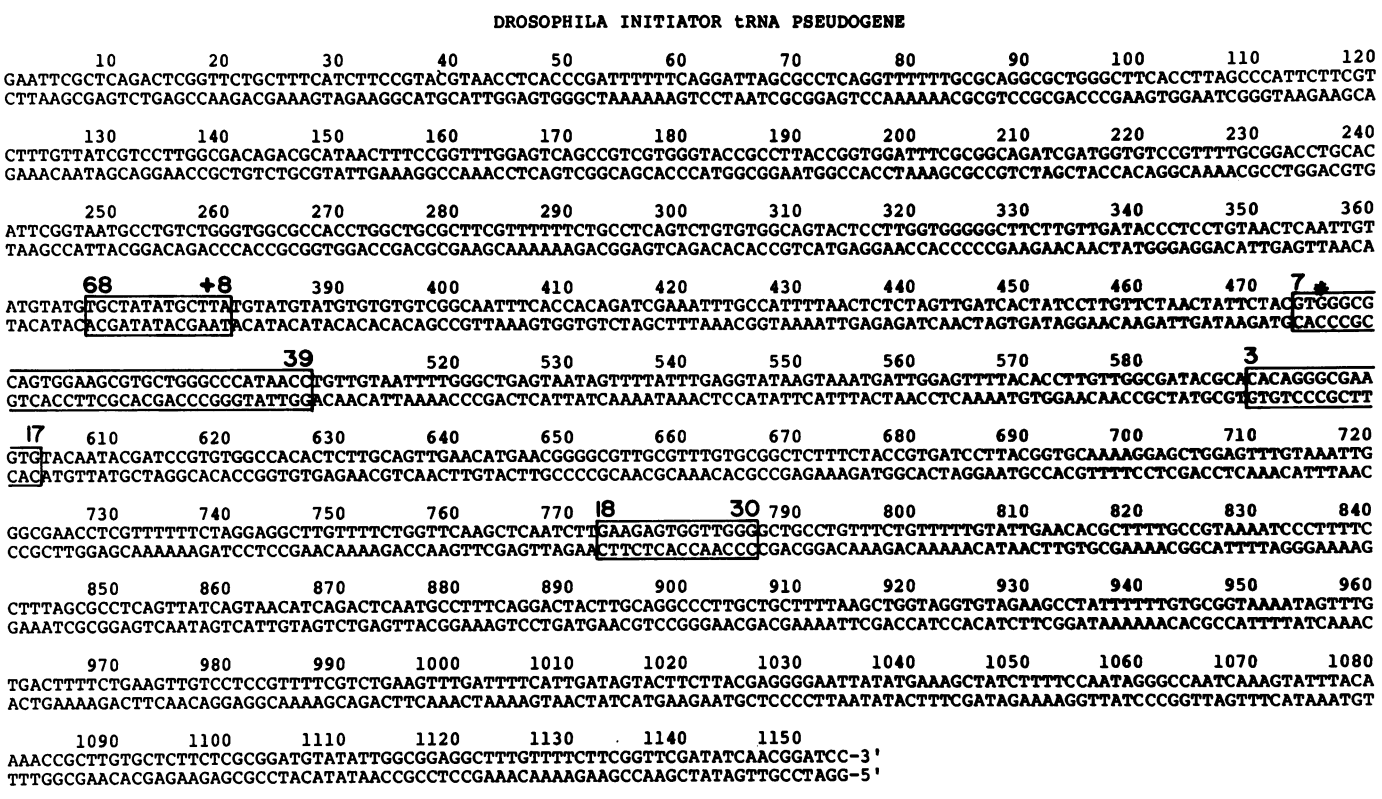

Figure 4. The DNA sequence of pYD6. The sequences homologous to initiator tRNA are boxed. The nucleotide at co-ordinate 476, marked with an asterisk, is not found in initiator tRNA. Bold-printed numbers are co-ordinates from Fig. 3 and represent homologous sequences to initiator tRNA gene.

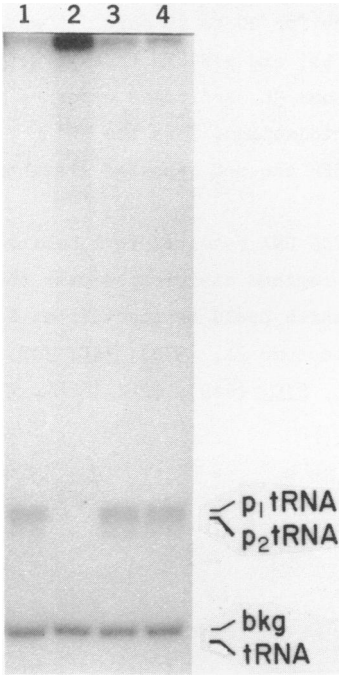

Figure 5. Autoradiography of a gel electrophoretic separation of the ^{32}P-labeled transcription products of (1) pPW539 (2) pYD6 (3) pPW591 (4) pTR18EH in a Drosophila Kc cell extract. Precursor tRNA, ptRNA; tRNA, mature-size tRNA; bkg is not a transcription product (29). Precursor tRNA processing activity in this extract was low.

within the first oligothymidylate stretch following the mature-tRNA coding region (Fig. 3). The transcription products of the initiator tRNA genes follow the trend observed for transcription of other Drosophila tRNA genes. Initiation occurs within the first eight nucleotides of the 5'-flanking sequence and termination within an oligothymidylate stretch in the 3'-flanking region closely located to the mature tRNA sequence.

Transcription of pYD6 in vitro did not give detectable RNA product. It appears that the transcription control regions are not intact in this truncated pseudogene. This is in agreement with the recent determination of the regions within tRNA genes involved in transcription control (19, 39). This may explain the inability of pYD6 to support RNA synthesis. However, we cannot exclude the possibility that pYD6 may form an unstable transcript.

Chromosomal Localization. Tritium-labeled plasmid DNAs were hybridized

to polytene chromosome squashes of third instar larval salivary glands. After autoradiographic exposure for 20 to 60 days, it was observed that pPW591 (Fig. 6a), pPW539 (Fig. 6b) and pTR18EH (not presented), hybridized only to region 61D on chromosome 3L. Prolonged exposure did not reveal any additional sites of hybridization. Thus the DNA regions corresponding to pPW539, pPW591 and pTR18EH are not repeated elsewhere in the Drosophila chromosomes.

Hybridization of pYD6 DNA resulted in labeling at the centromere region and about 30 additional regions distributed over the whole genome. This is a summary of those sites which could be identified: X chromosome, 19E and 3 non-identified regions; chromosome 2L, (37D), 34C, 30B, 25F, 25A; 2R, 42A, 42B, 42DE, 46AB, 46C, 47B; 3L, 61C, (61D), 62F, (66B), 77C, 79A.; 3R, 82CD,

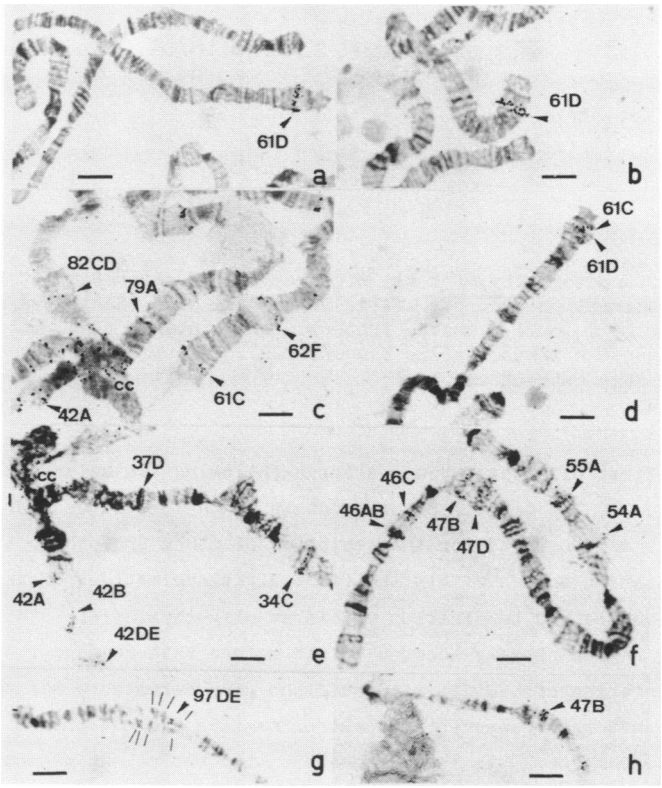

Figure 6. In situ hybridization of nick-translated plasmid DNA to larval polytene chromosome squashes (a) pPW591 (b) pPW539 (c-h) pYD6. Centromere region, CC. The line is equivalent to 10 um.

85F, 86DE, (97DE). Those sites found hybridizing frequently are underlined, those rarely found (a few slides) are bracketed. In contrast to pPW539 and pPW591 (pTR18EH), pYD6 showed strong labeling at 61C (Fig. 6c) and only in rare cases weak labeling at 61D (Fig. 6d). Within some regions sequences complementary to pYD6 were not equally represented on sister chromatids. For example, labeling at 46C, 97DE and 47B occurred only on one sister chromatid (Fig. 6, f-h). In addition, an extended asynapsis was observed within chromosomal region 97 for the fly stock used in the *in situ* hybridization experiments (Fig. 6g). The pattern of *in situ* hybridization for pYD6 is indicative of a mobile DNA element. The chromosomal origin(s) of the DNA cloned in pPW568 was not determined.

Chromosomal Number of Initiator tRNA Genes. Each initiator tRNA gene contains an AvaII restriction site at position 44 (Fig. 3). When ^{32}P-labeled initiator tRNA was used to probe AvaII digests of each of the four plasmid DNAs, a single band from each plasmid exhibited hybridization. In each instance only the fragment containing the 5' half of the tRNA gene hybridized. AvaII digestion of genomic DNA and subsequent hybridization to the $tRNA^{Met}$ complementary DNA probe revealed seven hybridizable fragments (Fig. 7, lane 1). These fragments contain the 5' half of the tRNA gene (Fig. 7). A combined AvaII/ HindIII digest demonstrated a change in mobility of band 2 which corresponds to pPW591 or pTR18EH DNA. The smaller fragment that resulted was not detected but is known from mapping and sequencing to be 84 bp for pPW591 and 99 bp for pTR18EH (Figs. 1 and 3). This digest also revealed band 8 (lane 2), which in lane 1 probably co-migrated with band 1. Band 3 (lane 1) which has the same relative intensity as band 1 (lane 1) may also have co-migrating fragments. Thus, there are at least eight, perhaps nine, initiator tRNA genes in *D. melanogaster*.

Digestion of genomic DNA using EcoR1/BamH1 revealed four fragments hybridizing to the $tRNA^{Met}$ complementary DNA probe (Fig. 7, lane 5). Since there are eight copies of the tRNA gene, five of them, (including the genes of pPW539 and pPW591) appear to be located within EcoR1/BamH1 fragments of equal size of approximately 1150 bp (Fig. 1). When a DNA probe prepared from the HindIII/EcoR1 415 bp repeat (Fig. 3) was hybridized to a genomic HindIII/EcoR1 digest, three bands gave a strong and two bands a weak signal (results not shown). This observation and the EcoR1/BamH1 hybridization data (Fig. 7) suggest that the sequences surrounding at least five of the initiator tRNA gene copies are highly conserved within a fragment size of at least 1150 bp. A fragment equivalent in size to the expected tRNA hybridizing

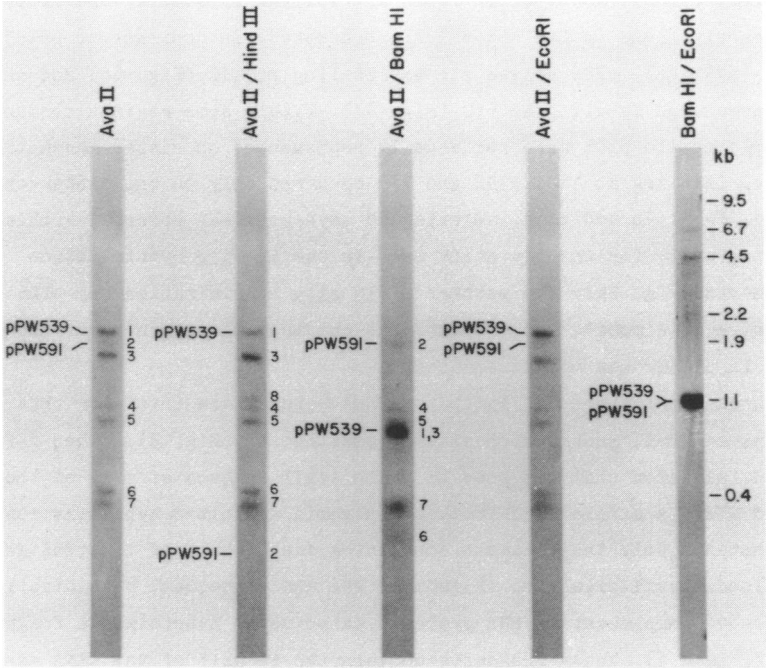

Figure 7. Genomic DNA hybridization. Genomic DNA was digested as indicated and probed using a $tRNA^{Met}$ complementary DNA fragment. The mobilities of the fragments generated from the cloned initiator tRNA genes is indicated. Each uniquely hybridizing fragment size is numbered 1-8. AvaII/EcoR1 digestion results in detection of the same fragments as for AvaII digestion.

AvaII fragment of pPW568, was not observed under these hybridization conditions. More probably the fly stock used as the source of DNA for the genomic blotting experiments, did not have initiator tRNA gene sequences associated with the dispersed repetitive DNA of pPW568.

DISCUSSION

We have isolated four segments of *Drosophila* DNA that hybridize to homologous initiator tRNA. DNA sequence analysis of the hybridizing regions revealed that three of the isolated recombinant DNAs (pPW539, pPW591 and pTR18EH) contained the sequence complementary to *Drosophila* initiator tRNA. From restriction mapping of isolated DNA it appears that the DNA inserts of pPW591 and pTR18EH overlap. Thus each of these inserts may be representative of the same gene copy (allelic). Knowledge of the restriction maps of each of the recombinant DNAs led us to conclude from genomic blotting

experiments that D. melanogaster contains 8-9 non-allelic initiator tRNA genes. The DNA inserts of pPW539 and pPW591(pTR18EH) represent two of these.

The Drosophila DNA inserts of pPW539 and pPW591(pTR18EH) localize to region 61D of D. melanogaster chromosome 3L. Localization of initiator tRNA genes by in situ hybridization using tRNA as a probe unambiguously identified the regions 61D and 70DE (40). Two other sites hybridized but these were concluded to result from a contaminating tRNA (40). The kinetic analysis or in situ hybridization of Drosophila initiator tRNA to polytene chromosomes indicated the presence of 0.5 and 0.7 tRNA genes at 61D and 70DE, respectively (40). Our study shows that there are at least two genes located at region 61D. If the ratio of the gene numbers obtained from the in situ studies is correct we would predict the presence of 3 initiator tRNA genes at 61D and 5 at 70DE. While the reason for these low gene number estimates is not clear (40), several factors could account for this low efficiency. Tightly clustered tRNA genes appear to enhance in situ hybridization within a single region (9). Therefore, the lack of clustering of the initiator tRNA gene at region 61D could account for the low estimate at this site. Also, a source for ambiguity in in situ hybridization of tRNAs stems from the presence of inverted repeat structures making tRNA gene detection by hybridization very difficult (14).

We were surprised to find initiator tRNA gene fragments (pseudogenes) in pPW568. To date, incomplete tRNA gene seqeuences have not been observed in Drosophila DNA. The subcloned 1150bp fragment (pYD6) not only hybridizes strongly to initiator tRNA, it also displays properties of moderately repetitive DNA (13,41). In situ, this DNA hybridized to approximately 30 sites dispersed throughout the chromosomes. In chromosome regions where the maternal and paternal homologs failed to synapse, the in situ hybridization of pYD6 was sometimes restricted to one of the homologs, indicating heterozygosity at these sites. Heterozygosity was also observed at some sites where asynapsis was not immediately evident. These observations indicate that the Drosophila DNA component of pYD6 is mobile (41). The initiator tRNA pseudogene(s) may have been created by repeated insertion and excision of a transposable element into an intact tRNA gene. From its hybridization properties the DNA surrounding the pseudogene can be classified as dispersed repetitive. Further studies would be needed to show whether it corresponds to a transposable element. In this context it is interesting to note that the transposable element originating from the right part of white locus and roughest locus, designated TE1, has been found inserted at 61D (TE51) (41).

The initiator tRNA genes contained on pPW591 and pTR18EH may be allelic. Thus the several differences observed in their sequence may reflect polymorphism between two fly stocks or may be due to the presence of nonidentical homologous alleles of this particular initiator tRNA gene. Alternatively, the genes contained on pPW591 and pTR18EH may represent repeated sequences in the DNA of the same chromosome. The differences in the DNA sequences are the G→A change at co-ordinate -7 (Fig. 3) which results in a different nucleotide initiating transcription. The other more notable difference is the G→T transversion within the mature-tRNA coding sequence, at co-ordinate 30 (Fig. 3). Obviously, the two genes code for methionine isoacceptors.

This raises the general question of which genes of a tRNA multigene family are actually expressed in the cell. In the developing organism it has not been established whether specific tRNA gene expression is induced or whether tRNA genes are expressed constitutively. This question at present, remains open. Each of the tRNA genes MET 1 and MET 3 transcribe equally well *in vitro*. However, tRNA sequence analysis gave no indication of heterogeneity in the isolated *Drosophila* initiator tRNA (30).

There are other examples of the sequence of possible tRNA coding regions being different to the sequence of the isolated tRNA. These include the *Drosophila* genes for $tRNA_5^{Lys}$ (D. Cribbs, D. DeFranco, S. Hayashi, D. Söll and G.M. Tener, unpublished), $tRNA_4^{Val}$ (18), $tRNA^{His}$ (L. Cooley and D. Söll, unpublished) and $tRNA^{Glu}$ (12), human $tRNA^{Met}$ (21) and an *S. pombe* $tRNA^{Glu}$ gene (J. Mao, V. Gamulin and D. Söll, unpublished). Transfer RNAs resulting from transcription of such genes could be minor chromatographic species and thus not readily purified or, conversely, may be rapidly degraded in the cell. If tRNA gene expression is constitutive then the levels of fully modified tRNA may be controlled in the cell by specific ribonuclease activity and tRNA-modifying enzymes.

The arrangement of the *Drosophila* initiator tRNA genes is unlike that observed for other *Drosophila* tRNA gene families. The arrangement more closely resembles that found for the human initiator tRNA genes (21). In *Drosophila* the initiator tRNA genes we have isolated are not clustered nor intermingled with other tRNA genes. They occur as single copies within an approximately 415-bp repeat segment which is separated from other initiator tRNA genes by a mean distance of 17 kb. The finding that large regions surrounding the initiator tRNA genes have extensive sequence conservation contrasted with the existence of an initiator tRNA pseudogene(s). This raises questions on maintaining identical tRNA genes within the genome. A

major step in answering this will be the determination of whether tRNA genes are expressed constitutively and also whether the amount of a chargeable tRNA species corresponds to the number of its genes.

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