

ANALYSIS OF tRNA POPULATION FROM *DROSOPHILA MELANOGASTER* BY MEANS OF POLYACRYLAMIDE GEL MAPPING

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1. Introduction

Two-dimensional polyacrylamide gel electrophoresis of tRNA [1] is becoming a remarkable tool to investigate various problems dealing with the regulation of protein synthesis: accurate quantitation of mature tRNA species [2–4]; purification of individual tRNA species [3,5,6]; identification and quantitation of tRNA precursors [4,7] and maturation products [8]; turnover rates [4]; distribution and arrangement of tRNA genes [4,9,11]; test of purity of [^{125}I]iodo-tRNA [12,13]. In each case, however, the tRNA spots on the gel map must be identified. This relatively tedious work limits the use of polyacrylamide gel electrophoresis (PAGE) of tRNA. So far, the only available maps are those of *Escherichia coli* [2], yeast [1,11] and *Bombyx mori* silkworm [3,4].

In order to quantitate *Drosophila* tRNA genes and to compare their distribution with those of other species, we first analysed the spots obtained from *D. melanogaster* tRNA (third instar larvae and adult) fractionated by electrophoresis on two-dimensional gels. In the present study, we have been able to define the nature of 32 out of the 40 visible spots. For this, we have used preacylated species and enriched tRNA fractions from benzoylated DEAE-cellulose chromatography. ^{32}P -labelled tRNA from cell cultures has been used for quantitation of each spot.

2. Materials and methods

Freeze-dried tRNA samples from third larvae and

wild-type adult flies from the Samarkand strain of *Drosophila melanogaster* were provided by Dr B. N. White as well as enriched fractions (A–D) from a benzoylated DEAE-cellulose column [14] containing tRNA^{Ala}₁, tRNA^{Asp}₁, tRNA^{Gln}, tRNA^{Gly}₁, tRNA^{Met}, tRNA^{Ser}₂, tRNA^{Trp} and tRNA^{Val}.

^{32}P -labelled tRNA was prepared from Oregon R strain cells in culture maintained in D 22 medium according to [15]. The cells were exposed to [^{32}P]orthophosphate (1 mCi/ml) for 3 h at 23°C.

The electrophoretic separation of tRNA on polyacrylamide gels was performed as in [3,4] after acylation and stabilization of the phosphodiester bond by nitrosation [16]. After electrophoresis, [^3H]- and [^{14}C]aminoacyl-tRNA spots were cut out of the gel and digested either with 0.5 ml H_2O_2 at 50°C for 4 h or 0.5 ml NH_4OH 10% at 25°C overnight before addition of 5 ml of an appropriate scintillation cocktail (usually Aquasol or PCS). Kodak X-omat XR1 films were used for gel autoradiography.

3. Results

3.1. Identification of tRNA species

Total tRNA from *Drosophila* yields 13 bands when subjected to polyacrylamide gel electrophoresis under the conditions used for the first dimension. No significant difference is observed between the densitometric profiles of tRNA from third instar larvae and from adult flies (fig.1, bands 1,2). These tRNA are resolved into 16 bands when subjected to electro-

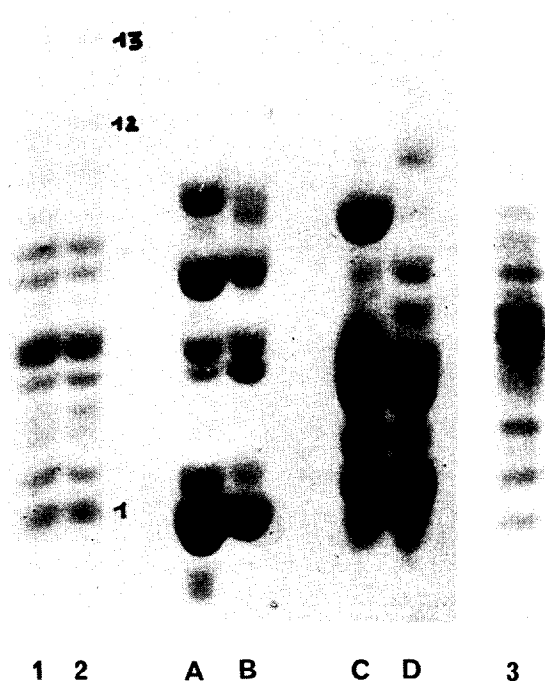


Fig. 1. Polyacrylamide gel electrophoresis of total *D. melanogaster* tRNA and enriched fractions. Total tRNA from third instar larvae (band 1) and adult fly (band 2) and chromatographic fractions (A, B, C and D) from a BD-cellulose column of adult fly tRNA were applied on a 9.6% polyacrylamide gel and run in a Tris-borate buffer (pH 8.3), 7 M urea during 48 h at 470 V at 4°C using a slab gel of 1.5 × 200 × 400 mm. Gels were stained in a 0.2% methylene blue solution in acetate buffer (pH 4.7) for 1 h and destained in running water for several hours. Band 3 represents an autoradiography of tRNA from cell cultures (320 000 cpm of ^{32}P -labelled tRNA applied).

phoresis in the conditions used for the second dimension (expt. not shown). Analysis of enriched tRNA samples from BD cellulose fig. 1, bands A–D allow one to assign the faster running band 1 of fig. 1, 1 to $\text{tRNA}_{1}^{\text{Ala}}$ and $\text{tRNA}_{1}^{\text{Gly}}$. It represents $9.7 \pm 0.3\%$ of total tRNA. The slower running bands 12, 13 (which can be assigned to $\text{tRNA}_{2}^{\text{Ser}}$ and $\text{tRNA}_{1}^{\text{Leu}}$ by gel mapping) (fig. 4) represent $4.6 \pm 0.2\%$ and $4.0 \pm 0.1\%$, respectively. Autoradiograms of ^{32}P -labelled tRNA from cultured cells also reveal 14 bands (fig. 1–3).

The electrophoretic behavior of some preacylated tRNA species after a single separation is shown in fig. 2. It offers a way of analysing the number, importance and position of different isoacceptors

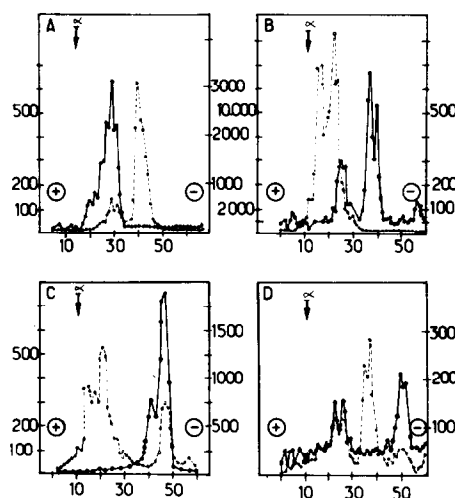


Fig. 2. Polyacrylamide gel electrophoretic patterns of tRNA species from *D. melanogaster*. [^{14}C]- and/or [^3H]nitroso acylated tRNA species were subjected to 9.6% polyacrylamide gel electrophoresis as described in fig. 1. After staining with 0.2% methylene blue, the gel strips were cut in 1.1 mm wide pieces, digested in 0.5 ml NH_4OH 10% at 25°C overnight and counted in 5 ml Aquasol scintillation cocktail. Migration is from right to left. α is a well defined stained band used as internal marker. A, (—○—) Isoleucine, (—●—●—) valine; B, (—○—) arginine, (—●—●—) leucine; C, (—○—) asparagine, (—●—●—) lysine; D, (—○—) proline, (—●—●—) glutamine.

corresponding to one amino acid. Several analyses can be done on the same gel. By combining two separate electrophoresis, one 10% acrylamide, 7 M urea and one 20% acrylamide, 4 M urea, it is possible to construct a map of many isoacceptors which can be compared to that obtained by two successive electrophoreses (10% and 20% acrylamide). This helps for the identification of some spots.

Another partial analysis of tRNA spots is obtained by two-dimensional gel electrophoresis of tRNA fractions enriched by BD-cellulose chromatography, as shown in fig. 3. The major spots have been assigned to the preponderant tRNA species of the different fractions, at least for $\text{tRNA}_{1}^{\text{Ala}}$ (spots 1, 3), $\text{tRNA}_{1,2}^{\text{Asp}}$ (spots 34, 35), $\text{tRNA}_{1}^{\text{Gly}}$ (spot 13), $\text{tRNA}_{2}^{\text{Ser}}$ (spot 24) and $\text{tRNA}_{1}^{\text{Val}}$ (spots 29, 30). Their identification has been further confirmed by acylation. In addition, coelectrophoresis with ^{32}P -labelled tRNA from *B. mori* posterior silk gland (not shown here) indicates similar migration in both dimensions for *Drosophila*

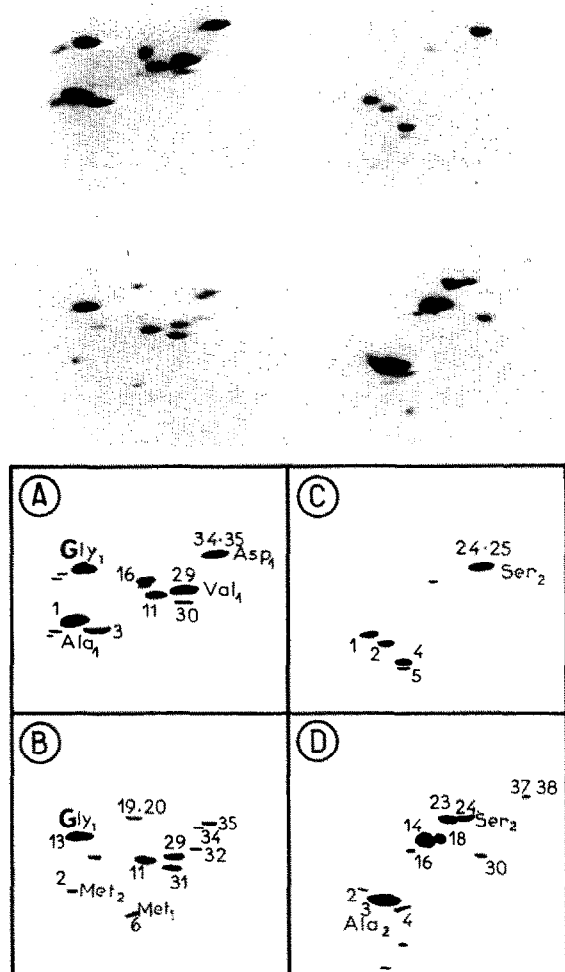


Fig.3. Polyacrylamide gel electrophoretic maps of enriched fractions of *D. melanogaster* tRNA. 0.3–0.5 A_{260} unit of tRNA from BD-cellulose columns (first eluted A–D fractions) [14] was subjected to two-dimensional polyacrylamide gel electrophoresis and stained with 0.2% methylene blue. Identification of tRNA spots was done by acylation and nitrosation prior to electrophoresis. The spot numbers are the same as in fig.4 and table 1 and in the elution profile of BD-cellulose chromatography [14,22].

tRNA^{Ala}_{1,2} and *Bombyx* tRNA^{Ala}_{2b} [3,5,17] as well as for homologous major species like tRNA^{Gly}₁, tRNA^{Leu}₁ and tRNA^{Lys}. Close homologies between the sequences of Diptera and Lepidoptera can be

expected for these tRNA species, especially for their anticodon, suggested in table 1.

Electrophoretic maps of total *Drosophila* tRNA are shown in fig.4. 40 spots are visible, 32 of them have been identified using preacylated species (see table 1). Long tRNA molecules (tRNA^{Leu} and tRNA^{Ser} with

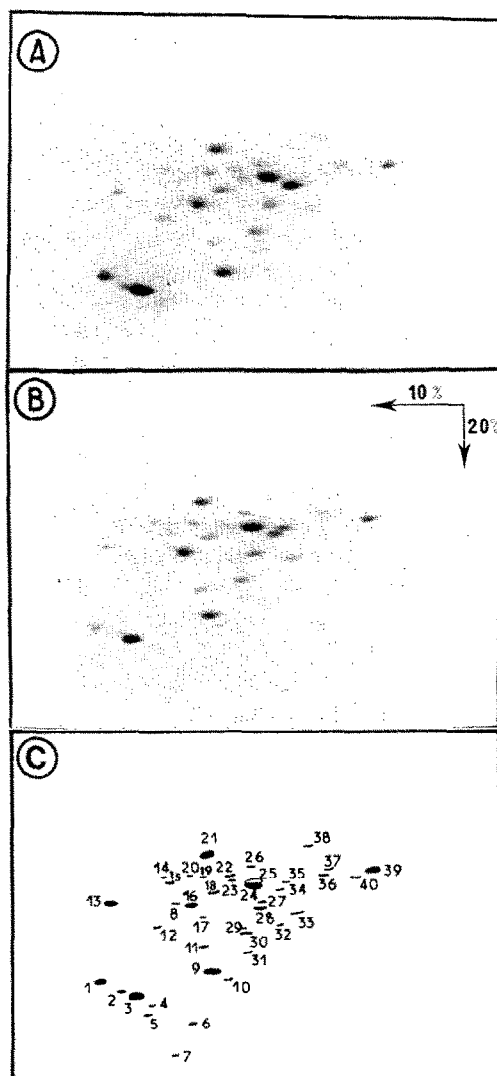


Fig.4. Polyacrylamide gel electrophoretic map of *D. melanogaster* tRNA. One A_{260} unit of total RNA from third instar larvae (A) or wild-type adult fly (B) was analysed on two-dimensional polyacrylamide gel electrophoresis as in fig.3 [1,3]. The diagram (C) represents detectable tRNA species identified in table 1.

Table 1
Identification and quantitation of *Drosophila* tRNA spots from polyacrylamide gel maps

Spot number	tRNA species	Anticodon (possible)	% from total	Ref.
1	Alanine 1		2.2 ± 0.3	
2	Methionine	(CAU)		
3	<u>Alanine 2</u>	(IGC)	4.1 ± 0.7	
6	Initiator	CAU	1.1 ± 0.3	26
9,10	<u>Arginine 1</u>		4.9 ± 0.5	
12	Tyrosine		2.2 ± 0.2	
13	<u>Glycine 1</u>	(GCC)	5.3 ± 0.4	
14	<u>Glycine 2</u>			
	Arginine 2			
15	Tryptophane			
16,17	Valine 2	(U ^X AC)		23
19	Arginine 3			
21	Cysteine			
	Isoleucine			
	<u>Lysine 2</u>	CUU	3.1 ± 0.3	24
22	Lysine 1	(U ^X UU)		
24	Serine 2	GCU	4.8 ± 0.8	14
25	Leucine 2	(GAG)		
27 or 28	Valine 3	(CAC)	5.5 ± 0.6	23
29,30	<u>Valine 1</u>	(IAC)		23
33	Leucine 4			
34	Aspartate	(GUU)	4.1 ± 0.5	25
35	Aspartate	(QUU)		25
36	Leucine 3			
37	Serine			
38	<u>Serine 4</u> (and/or 7)	IGA	2.8 ± 0.4	14
39	<u>Leucine 1</u>	(CAG)	3.0 ± 0.4	
40	Serine			

The number of each spot refers to the diagram of fig.4c. Major iso-tRNA species are underlined. Tentative assignment of anticodons is based on structural data [14,23–26] and electrophoretic homologies with *B. mori* tRNA species (Ala and Gly). Quantitations (%) derive from ³²P-labelled tRNA mapping (see fig.5) from cell cultures (mean of 3 independent determinations)

about 85 residues run slowly in the two dimensions of the gel. Only one difference is visible between tRNA from larvae and adult fly. Spots 34 and 35 have been identified as tRNA^{Asp} (fig.3,4). One spot (34) is visible in larval tRNA and two (34+35) in adult tRNA. A similar situation has been observed on RPC-5 [18]. The larval tRNA^{Asp} has a G base in the wobble position whereas the adult tRNA^{Asp} (spot 35) has a modified 7-deazaguanosine or Q base in the wobble position. Other species, tRNA^{His} and tRNA^{Tyr}, which are known to possess a Q base in the wobble position of several eukaryotic species, correspond to single spots in our map.

3.2. Quantitation of tRNA species

Polyacrylamide gel electrophoresis maps of tRNA species offer the most suitable analytical tool for accurate quantitation of each mature tRNA species, regardless of acylation rate. For this purpose, we have used ³²P-labelled tRNA from *Drosophila* cell cultures labeled for 3 h [15] (fig.5). Preliminary results are given in table 1. The intracellular level of tRNA^{Met} is lower than that observed [18] based on amino acid acceptance, but in agreement with the methionine content in proteins. Furthermore, the levels for tRNA^{Ala}, tRNA^{Leu}, tRNA^{Ser} and tRNA^{Val} species are consistent with an adaptive ratio to the

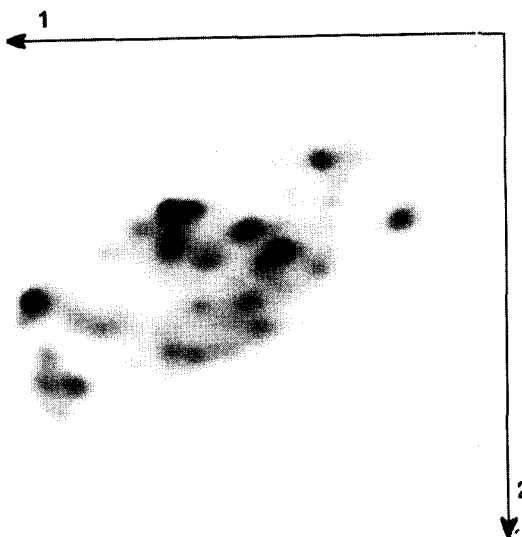


Fig.5. Autoradiography of ^{32}P -labelled tRNA from *D. melanogaster* run on two-dimensional polyacrylamide gel electrophoresis. ^{32}P -labelled tRNA from Oregon R strain cells labeled during 3 h was subjected to a two-dimensional polyacrylamide gel electrophoresis as in fig.4. After staining in methylene blue and autoradiography, tRNA spots were cut out. Radioactivity of gel pieces was determined by Cerenkov counting (172 000 cpm recovered). Identification of major spots was done according to the diagram of fig.4C.

average amino acid content of proteins being synthesized [19]. In addition, isoaccepting-tRNA levels seem to be balanced with the frequency of their cognate codons in eukaryotic mRNAs, usually mammalian messengers [20,21]. However, conclusive remarks will need more details on the anticodon structure of *Drosophila* iso-tRNA species and simultaneously on the codon usage of insect mRNAs.

4. Discussion

4.1. Resolution of the gel electrophoresis: number of tRNA species

The number of isoaccepting tRNA species (iso-tRNA) fractionated on the basis of chain length and net charge in denaturing conditions (7 M urea in 10% acrylamide gels) agrees with that revealed by BD-cellulose and reversed-phase chromatography [18,22]. For tRNA species able to decode 2 codons ending with a purine base (MNA and MNG, where M and N

stand for any base), we found two iso-tRNA species with unbalanced ratios (Gln and Lys of fig.2). For tRNA which decode NAY codons (Y represents pyrimidines for Tyr, His, Asn and Asp), the electrophoretic resolution seems to be lower than that of the RPC [18]. We fractionated two homodecoding tRNA^{Asn} species (fig.2), probably on the basis of their wobble base (G and Q) (see further discussion of this point with tRNA^{Asp} spots). The three isoleucine codons are decoded by the usual major and minor tRNA^{Ile} species (fig.2). The four proline and valine codons are recognized by two iso-tRNA^{Pro} species and possibly 3 closely related iso-tRNA^{Val} species, which are better fractionated on hydrophobic support than on electrophoretic gel [23] (fig.2). For the 6 arginine and leucine codons, we found the expected number of iso-tRNA species: 3 for tRNA^{Arg} and 5 for tRNA^{Leu} with one preponderant species (fig.2). For further comments on isoaccepting and homodecoding tRNA species, see [20].

Electrophoretic maps (two-dimensional gel electrophoresis) of total *Drosophila* tRNA revealed 40 spots (fig.4c). This number is lower than that observed using fractionation on RPC-5 columns [18], but close to those found for other eukaryotes [1,3,4,11]. From simple considerations based on codon-anticodon interactions, the minimum number of eukaryotic tRNA species required for decoding the 61 codons can be estimated at 39 including initiator tRNA. Identified spots of tRNA on two-dimensional gel reveal that some spots have equivocal responses (spots 14 and surprisingly spot 21, which is able to recognize isoleucine and lysine). Despite close structural relationships between some tRNA species, we believe that our gel maps resolve most of the 39 standard tRNA species. Running on longer plates for the second dimension would improve fractionation, especially the crowded area between spots 15 and 25.

The relatively low number of tRNA spots resolved on two-dimensional gels compared to RPC-5 chromatography suggest that post-transcriptional methylations, which are known to influence hydrophobic binding with the chromatographic support, do not sufficiently change the pK of the base and consequently the mobility of the tRNA run on the gel. In most cases, these additional species correspond to homodecoding tRNA species carrying the same anticodon (see [20]).

Table 2
Detectable tRNA spots on two-dimensional polyacrylamide gel electrophoresis

Organism	Spot no.	Ref.
<i>Escherichia coli</i>	36	[2] A. Chevallier (unpublished)
Yeast mitochondria	27	(11)
Yeast	40	(1)
<i>Dictyostelium discoideum</i>	40	C. M. Palatnik, A. Chevallier (unpublished)
<i>Artemia salina</i>	42	J. P. Garel (unpublished)
<i>Drosophila melanogaster</i>	40	This paper
<i>Bombyx mori</i>	53	[3,4]
<i>Xenopus laevis</i>	50–52	A. Mazabraud, J. P. Garel (unpublished)
Chick embryo	45	H. Drabkin, J. Rosenbloom, J. P. Garel (unpublished)
Chicken culture cells	65	Th. Heymann (unpublished)
Rabbit liver, reticulocyte	56	F. Varricchio (unpublished)
Calf liver, lens, brain	45–50	J. P. Garel, R. Camato (unpublished)
Ewe liver	55	R. M. Landin and G. Petrisan (unpublished)
KB cells	47	[27]

4.2. Evolution of the tRNA population among organisms

Two-dimensional electrophoresis of tRNA populations from different organisms shows a slight increase in the number of tRNA species during evolution, but few variations from one tissue to another (see table 2). In addition to changes in the decoding pattern of tRNA between pro- and eukaryotes (from 39 tRNA species in *E. coli* to ~43 in plants and animals), the homodecoding species are more numerous among eukaryotes. These additional species are often detectable by gel electrophoresis. They correspond to a limited increase in number of the gene copies for each iso-tRNA species (one copy for several viral and bacterial tRNA species, 8 ± 2 for yeast and an average of 30 ± 10 for mammals) as well as to post-transcriptional modifications.

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