Perreault, W. J., B. P. Kaufmann and H. Gay. University of Michigan, Ann Arbor, Michigan. A DNA-associated RNA in Drosophila melanogaster adults.

A DNA-associated RNA isolated from pulse-labeled Drosophila melanogaster larvae was shown by Mead (1964) to possess many of the properties of messenger RNA. We have extracted a similar RNA from adult flies that had been labeled for only a short period of time with P^{32}.

Nucleic acids were isolated by Mead's procedure from five-gram lots of unlabeled adult Swedish b flies, and subjected to hydrolysis and separation of mononucleotides by the Katz and Comb (1963) technique. These procedures afforded the data contained in Table 1.

Table 1. Nucleotide analysis of RNA isolated from unlabeled XX, XY and unseparated (mixed) Drosophila melanogaster adults.

Source	N	AMP	UMP	GMP	CMP	A+U/G+C
DNA-associated RNA						
Mixed	4	28.2±0.3	28.5±0.6	24.2±0.8	19.1±0.7	1.31
XY Male	2	28.2±0.1	28.6±0.2	24.5±0.5	18.6±0.2	1.32
XX Female	4	28.2±0.2	28.4±0.4	24.7±0.2	18.7±0.2	1.30
Microsomal RNA						
Mixed	3	28.7±0.5	30.5±0.6	24.7±0.2	16.1±0.4	1.45
XY Male	2	28.6±0.6	30.6±1.1	23.9±0.3	16.9±0.7	1.45
XX Female	4	28.5±0.4	30.1±0.2	24.8±0.3	16.6±0.4	1.41

These experiments were designed to see if the Y chromosomes has any qualitative effect on the composition of the DNA-associated RNA. Microsomal RNA's were included as a control since they would not be expected to vary within a given species. The A+U/G+C ratios of DNA-associated RNA are nearly identical in the karyotypes analyzed. The nucleotide ratios obtained from microsomal RNA differ slightly but significantly from those of the DNA-associated RNA at the 5% level of confidence. Because of the slight difference in nucleotide composition between microsomal and DNA-associated RNA we attempted to determine whether the DNA-associated RNA might be an artifact resulting from binding of microsomal RNA to DNA during the isolation procedures. To investigate this possibility we isolated DNA-associated RNA from 5 grams of flies and included in the isolation medium 375 µg of P^{32}-microsomal RNA. Upon recovery of the DNA-associated RNA, only 2.4 µg out of a total yield of 270 µg carried the label. This results suggests that a small part of the DNA-associated RNA may be derived as an artifact of isolation, but that the major portion is not.

To incorporate label into the DNA-associated RNA of adults, Sw-b flies were kept in empty half-pint bottles for twelve hours at 25°C., after which a filter paper containing 0.5 mc P^{32}-orthophosphate in 1.0 ml of grape juice was introduced, and the flies were allowed to feed for one hour. The papers were removed, and the flies were frozen for isolation of nucleic acids. As many as one thousand flies may be treated in one bottle, so that 5 mc of P^{32} will suffice for ten thousand flies, or about ten grams wet weight.

An experiment conducted in this fashion demonstrated that the DNA-associated RNA incorporates P^{32} at several times the rate of either microsomal RNA or DNA (Table 2).

Table 2. Incorporation of P^{32} in a one-hour-feeding experiment.

	O.D. units* (260 mµ)	CPM	CPM/O.D. unit (Specific Activity)
DNA-associated RNA	6.70	16,540	2,469
Microsomal RNA	6.40	3,490	545
DNA	9.80	1,700	173

* One O.D. unit equals 45 µg nucleic acid/ml.

That the label actually resides in the RNA mononucleotides was demonstrated by an experiment in which the mononucleotides were separated, and optical density and radioactivity were plotted on the same set of axes (Figure 1). In this experiment 86% of the radioactivity was recovered within the optical density peaks of the mononucleotides. The nucleotide composition of this DNA-associated RNA from labeled flies is not significantly different from that given in the upper part of Table 1.

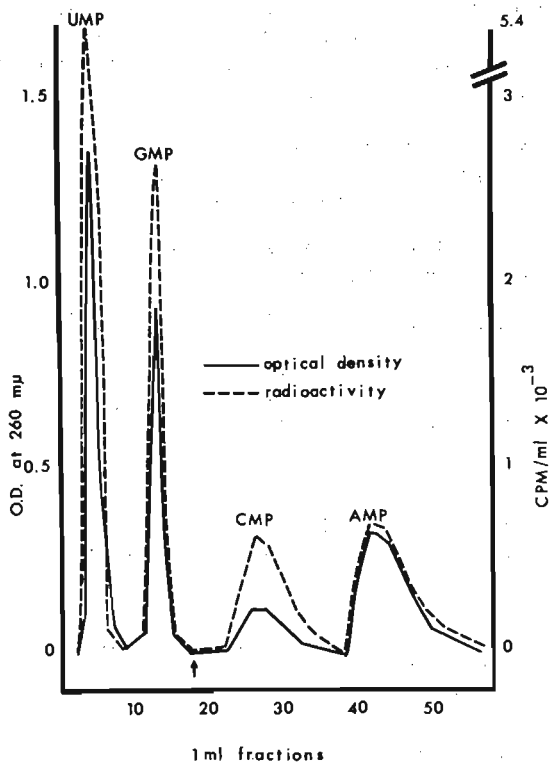

Figure 1. Separation of P^{32} labeled mononucleotides. UMP and GMP were eluted from Dowex 50 (H^+). At fraction 18 (see arrow) effluent was transferred to a Dowex 1 column and CMP and AMP collected.

The administration of actinomycin D (100 ug per filter paper) either fifteen minutes before addition of P^{32}, or concurrent with it, resulted in ca. 13-15% inhibition of P^{32} incorporation (Table 3). It is not clear whether this inhibition is due to interference with transcription, or simply a poisoning effect on the overall metabolism of the treated flies. In Table 3, experiment 1 involved the simultaneous administration of actinomycin D and P^{32} for one hour without any prior treatment, whereas experiments 2 and 3 featured a fifteen minute preliminary exposure of the dehydrated flies, namely, to AMD in the treated series and to water in the controls. The reduction in total P^{32} incorporation in those control flies subjected to the fifteen-minute pretreatment (experiments 2 and 3) indicates that dehydrated flies do considerable drinking during the first quarter hour that fluid is available. This fact should allow studies on adults in which the period utilized for incorporation of isotope is much less than one hour.

Table 3. The effect of actinomycin D on P^{32} incorporation.

	Treated with actinomycin D			Controls without actinomycin D			
Experiment	O.D.	CPM	Specific Activity	O.D.	CPM	Specific Activity	% Inhibition
1	12.3	46,310	3,764	11.4	61,220	5,347	14.5
2	9.0	15,350	1,696	6.2	15,460	2,493	14.7
3	12.5	20,840	1,669	10.2	23,857	2,150	12.9

We have concluded that adult D. melanogaster flies contain a species of RNA which is bound to DNA in an unknown fashion. This RNA is distinguished by a characteristic nucleotide composition and the rapid uptake of P^{32}. It seems probable that at least some of this RNA is mRNA, and that by further refinements of technique an adult messenger RNA fraction may be isolated.

References: Mead, C.G., J. Biol. Chem. 239:550 (1964)
Katz, S. and D. G. Comb, J. Biol. Chem. 238:3065 (1963)

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