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# Messenger RNA Sequence Complexity and Homology in Developmental Stages of *Drosophila*

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Total polysomal RNA and polyadenylated mRNA from third instar larvae, pupae, and adults of *D. melanogaster* were hybridized in vast excess to labeled single-copy DNA in order to measure the sequence complexity of each RNA population. Then, to measure the sequence homology between the populations, each was hybridized to DNA enriched for messenger coding sequences in third instar larvae and to DNA depleted of these sequences. Our results show that a similar number of genes, approximately 16,000, is expressed in larvae, pupae, and adults, and that only one-third of these is expressed as polyadenylated mRNA. Further, the composition of both polyadenylated and nonpolyadenylated mRNA classes is shown to change very little between these three stages of development. Finally, the head of adult *Drosophila* is shown to contain 11,000 RNA species, approximately 70% of the number contained in the entire adult.

## INTRODUCTION

The marked morphological and physiological changes which occur during animal development are considered to be accompanied by or to result from changes in gene expression. Indeed, different sets of genes are expressed during development and in different adult tissues of the sea urchin (Galau *et al.*, 1974; 1976). In *Drosophila*, Levy and McCarthy (1975) used cDNA hybridization kinetics to demonstrate a high degree of sequence homology among the polyadenylated RNAs most abundant in larvae, adults, and Schneider's cells. However, a proportion of the polyadenylated RNA sequences from third instar larvae were shown to be poorly represented or absent in the RNA populations from adults and Schneider's cells. These data are consistent with some measure of differential gene expression, especially among rare-class RNAs, but the technique employed does not allow quantitation of such changes. More recently, Izquierdo and Bishop (1979) employed the same techniques to demonstrate >90% sequence homology between polyadenylated RNA molecules in the cytoplasm of L3 cultured cells and those in larvae, pupae, and adults. Eighty-five to one hundred percent sequence homology was observed when the polyadenylated RNA population from embryo was compared to that from L3 cells and third larval instar. The measurements of sequence homology among polyadenylated RNA populations reported in both of these studies represent minimum estimates, their accuracy limited by the technique employed. Specifically, it was not possible in either study to determine the number of different RNA species represented in a population of molecules shown

by the assay to be nonhomologous. Further, both studies investigated the sequence homology only between populations of polyadenylated RNA molecules. Recently, Zimmerman *et al.* (1980) have demonstrated the presence of a very complex population of RNA molecules associated with polysomes which lacks 3'-polyadenylation. Approximately two-thirds of the single-copy genes expressed in third instar larvae of *D. melanogaster* were shown to be expressed as nonadenylated RNA molecules, none of which were included in the measurements of sequence homology described above.

In the study reported here, we have compared the set of single-copy sequence transcripts, adenylated and nonadenylated, present on polysomes of third instar larvae of *D. melanogaster* with those present on polysomes of pupae and adults. We have approached this problem first by measuring the number of diverse sequences present in each RNA population using RNA-excess saturation hybridization (Galau *et al.*, 1974). Similar experiments were then carried out using a population of DNA molecules greatly enriched for sequences expressed on polysomes of third instar larvae to determine what proportion of this DNA is expressed on polysomes of pupae and adults. DNA greatly depleted of sequences expressed on polysomes of third instar larvae was also obtained to determine what proportion of these sequences is expressed in the later stages. In addition, we examined the RNA sequence diversity on polysomes in the adult head, an enrichment for neural tissue.

We demonstrate here that a similar number of genes is expressed on polysomes of third instar larvae, pupae, and adults, and that approximately one-third of these is

expressed as polyadenylated RNA. Furthermore, we observed a high degree of sequence homology between polyadenylated mRNAs of larvae, pupae, and adults, and between the nonadenylated RNAs of these stages. A small qualitative difference was detected between the polyadenylated mRNA populations, representing approximately 10% of the total number of genes expressed in pupae and adults.

#### MATERIALS AND METHODS

##### *Rearing of Organisms and Collection of Timed Stages*

*Drosophila melanogaster*, strain Oregon R, was maintained in population cages at room temperature, 60% humidity. Eggs were collected for 2 hr on a cornmeal agar food source spread with yeast paste and were incubated at 25°C. Late third instar larvae were collected on Day 5 (120–125 hr). Pupae were collected synchronously 5–7 hr after puparium formation by flotation (Mitchell and Lipps, 1978). Adults were collected within the first day after eclosion, and adult heads were prepared as described (Schmidt-Nielsen *et al.*, 1977).

##### *Preparation of RNA and DNA*

The experimental methods used for the preparation of larval polysomal RNA, poly(A+)RNA, sizing of RNA by electrophoresis in agarose gels in the presence of methyl mercury hydroxide and isolation and labeling of single-copy DNA are as previously described (Zimmerman *et al.*, 1980). For preparation of RNA from isolated polyribosomes, a standard SDS-phenol/chloroform procedure was employed (Galau *et al.*, 1976).

##### *Preparation of mDNA, Null mDNA, (A+)mDNA, Null(A+)mDNA*

<sup>3</sup>H single-copy DNA was fractionated into subsets as illustrated in Fig. 1. As shown, labeled single-copy DNA (~3 μg of which ~10% encodes mRNA) was hybridized to termination ( $R_{ot}$  30,000) with a 50-fold sequence excess of total polysomal RNA (7 mg) from third instar larvae. RNA sequence excess calculations assume that messenger RNA comprises 2% of the mass of total polysomal RNA and that complex mRNA occupies only 10% of the total mRNA mass (Zimmerman *et al.*, 1980). Thus, even rare mRNA species are in 50-fold excess over the coding DNA. The RNA-DNA mixture was then divided into two fractions. Ninety percent of the hybridization mixture was digested with nuclease S<sub>1</sub> ( $1.5 \times 10^5$  units in 5 ml for 30 min at 37°C) and undigested material collected by Sephadex G-100 chromatography. RNA was then removed by hydrolysis with 0.1 N NaOH at 60°C for 1 hr. The remaining single-stranded DNA was further enriched for coding sequences by a second round of

hybridization. The DNA prepared in this manner represents mDNA, a population greatly enriched for messenger RNA coding sequences in third instar larvae. The remaining 10% of the original hybridization mixture was centrifuged twice to equilibrium in a neutral CsCl buoyant density gradient, and after each centrifugation the band of unhybridized DNA was removed. The band was comprised of single-stranded noncoding DNA (94.5%) and DNA-DNA duplex (4.5%). After the second centrifugation the banded DNA was treated with 0.1 N NaOH at 60°C for 1 hr and was neutralized with 2 M Hepes, pH 4. The DNA prepared in this manner represents null mDNA, a population greatly depleted of messenger RNA coding sequences in third instar larvae. (A+)mDNA and null(A+)mDNA were prepared analogously by hybridizing labeled single-copy DNA (~0.8 μg) with a 40-fold sequence excess of polyadenylated mRNA (~10 μg) to termination ( $R_{ot}$  1300). Three rounds of hybridization were required to provide an A+mDNA population sufficiently enriched for coding sequences (see Results).

##### *Hybridization of RNA with [<sup>3</sup>H]DNA and Assay of Hybridization*

<sup>3</sup>H-Labeled single-copy DNA, mDNA, null mDNA, (A+)mDNA and null(A+)mDNA were incubated with a ≥100-fold sequence excess of RNA in 0.75 M NaCl, 0.003 M Pipes, pH 6.8, at 65°C in sealed capillary pipets. Yeast tRNA (10 μg) was added to samples containing poly(A+) polysomal RNA. Hybridization samples were incubated from 1 hr to 4 days, and the  $R_{ot}$  value achieved was calculated based on the concentration of *D. melanogaster* RNA in each sample. The contribution of DNA-DNA self-reassociation to the total amount of hybridization was determined by conducting parallel reactions in which an identical amount of yeast RNA was substituted for *Drosophila* total polysomal or poly(A+)RNA.

Samples were analyzed for hybrid content as described by Zimmerman *et al.* (1980) using TCA precipitation after S<sub>1</sub> nuclease treatment. The percentage DNA-RNA hybridization was calculated by subtracting the percentage S<sub>1</sub> nuclease resistance of the samples containing heterologous RNA (i.e., resistance due to DNA-DNA reassociation and to the inherent resistance of the nonhybridized tracer) from the percent S<sub>1</sub> nuclease resistance of samples containing *D. melanogaster* RNA. The amount of S<sub>1</sub> nuclease-resistant material in control samples containing heterologous RNA was consistently 2–3% of the input DNA.

#### RESULTS

Current estimates of the number of diverse mRNA species present in larvae, pupae, and adults of *D. mela-*

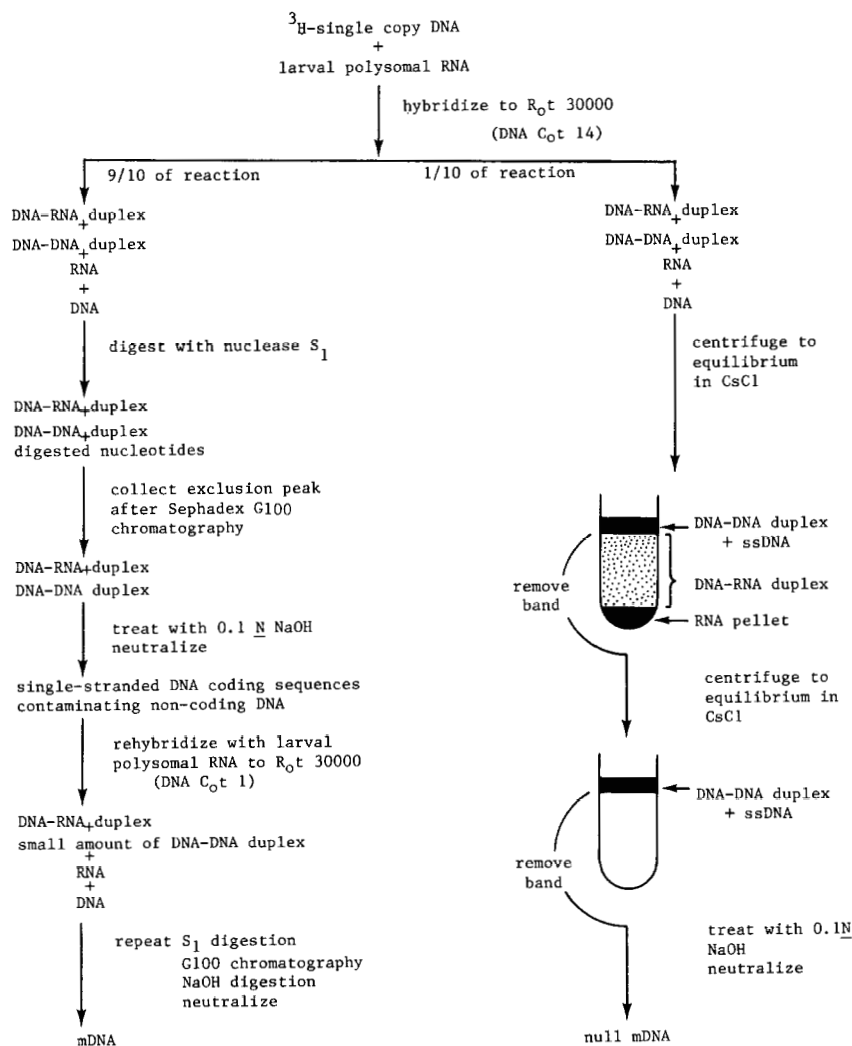


FIG. 1. Preparation of mRNA and null mRNA from *D. melanogaster* single-copy DNA. Populations of DNA enriched for messenger coding sequences in third instar larvae (mDNA) and depleted of those sequences (null mDNA) were prepared as shown and as described in the text. (see Materials and Methods).

*nogaster* are based largely upon analysis of cDNA hybridization kinetics (Levy and McCarthy, 1975; Izquierdo and Bishop, 1979). The results of these studies indicate that in these developmental stages there are between 4900 and 7000 different mRNA molecules. These estimates are derived solely from measurements of poly(A+)RNA, since poly(A-)RNA is refractory to analysis by the cDNA techniques employed. In view of the recent observation (Zimmerman *et al.*, 1980) that much of the complexity of total polysomal RNA in third instar larvae is not adenylated, it was desirable to reexamine the sequence complexity of total polysomal mRNA in both pupae and adults. In these studies, the RNA of interest was hybridized in sequence excess to  $^3\text{H}$  single-copy DNA (see Materials and Methods), and the amount of hybridization observed at saturation was used as a measure of the sequence complexity of the RNA.

The measurements of sequence complexity of total polysomal RNAs are shown in Fig. 2 and are summarized in Table 1. At saturation ( $R_{0t} > 25,000$ ), 10.6% of the single-copy DNA hybridized to polysomal RNA of larvae, a value in excellent agreement with that previously reported by Zimmerman *et al.* (1980). Interestingly, the saturation values for polysomal RNA from pupae and adult (10.3 and 11.0%, respectively) were approximately those observed for larval polysomal RNA. These percentages correspond to RNA sequence complexities of  $1.9 \times 10^7$  to  $2.0 \times 10^7$  nucleotides and represent between 15,000 and 16,000 diverse RNA molecules of average size 1250 nucleotides. A comparison of these measurements with the sequence complexity of poly(A+)mRNA from both pupae and adult (see Table 1; Fig. 3) (Levy and McCarthy, 1975; Izquierdo and Bishop, 1979) clearly indicates that the relative contribution of

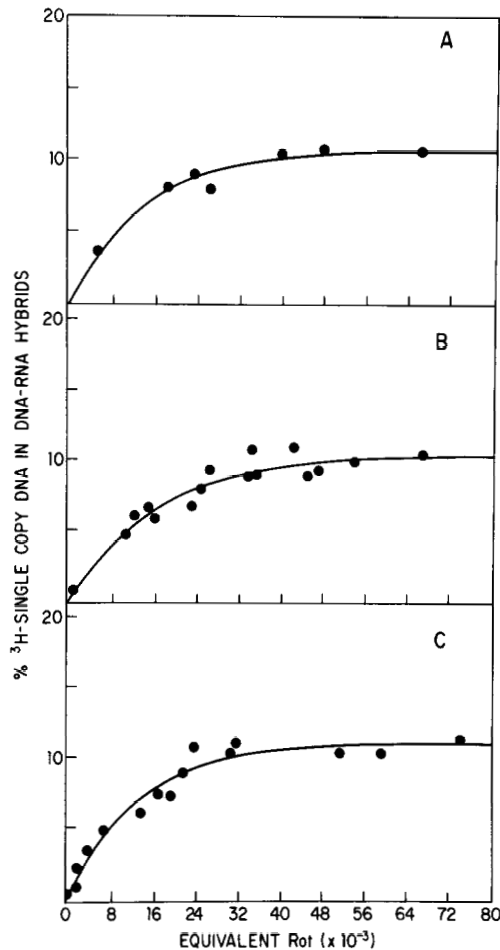


FIG. 2. Saturation hybridization of  $^3\text{H}$  single-copy DNA to *D. melanogaster* polysomal RNA. Trace quantities of  $^3\text{H}$  single-copy DNA (specific activity  $\sim 6 \times 10^6$  cpm/ $\mu\text{g}$ ) were hybridized with a  $\geq 100$ -fold sequence excess of total polysomal RNA from third instar larvae (A), 5 to 7 hr pupae (B) or adults (C). Hybridization was monitored by resistance to  $\text{S}_1$  nuclease digestion. Data points have been corrected for the contribution of tracer self-reassociation and for tracer reactivity ( $\sim 90\%$  when reassociated with an excess of total *D. melanogaster* nuclear DNA).

nonadenylated RNA to the total complexity of polysomal RNA in both pupae and adult is similar to that previously observed for larvae, and is approximately 60%.

#### Sequence Overlap of Total Polysomal RNAs from Different Stages of Development

The measurements of sequence complexity described above indicated that a similar proportion of single copy DNA, 10.3–11.0%, is represented in the polysomal RNA of larvae, pupae, and adults of *D. melanogaster*. We next wished to determine what proportion of these RNA sequences is shared among the stages, and what proportion is unique to a particular stage. To evaluate this, a population of DNA enriched for single-copy sequences ex-

pressed on polysomes of third instar larvae was prepared (mDNA), as was a population of DNA depleted of these sequences (null mDNA). [ $^3\text{H}$ ]mDNA hybridized with polysomal RNA from larvae to a maximum saturation value of 60%, representing a sixfold enrichment for mRNA coding sequences (Fig. 4A). In order to determine the amount of overlap between the mRNA sequences present in larvae and those found on polysomes in pupae and adult, polysomal RNA from pupae and adult was hybridized in sequence excess ( $\sim 150 \mu\text{g}$ ) to trace quantities ( $\sim 5 \times 10^{-4} \mu\text{g}$ ) of the [ $^3\text{H}$ ]mDNA. The amount of [ $^3\text{H}$ ]mDNA which hybridized with the RNAs at saturation may be directly compared with the 60% value observed for the self-reaction (i.e., larval polysomal RNA  $\times$  [ $^3\text{H}$ ]mDNA) to provide a measurement of the amount of sequence overlap. The proportion of the mDNA, 40%, which does not hybridize to larval polysomal RNA is assumed to represent random contamination with the noncoding portion of single-copy DNA. Since at most 11% of this random contamination could represent coding sequences for pupal or adult polysomal RNA, a maximum value of 4.4% ( $0.11 \times 0.4 \times 100\%$ ) of the hybridization observed between the mDNA preparation and polysomal RNA from pupae or adults could be ascribed to this DNA population. The results of this experiment are presented in Fig. 4 and summarized in Table 2.

At saturation, 63 and 59% of the [ $^3\text{H}$ ]mDNA hybridized with polysomal RNA from pupae and adults, respectively (Figs. 4B and C). Therefore, within the limitations of the measurements, it is clear that these RNA populations contain most if not all of the diverse RNA sequences present on polysomes of third instar larvae. Likewise, since the sequence complexity of the pupae and adult polysomal RNAs are approximately equivalent ( $10.3 \pm 0.8$  and  $11.0 \pm 0.8\%$ , respectively), the vast majority of diverse mRNA sequences present in pupae and adults must also be held in common between these two developmental stages.

The results of the above experiments suggest that among the three developmental stages studied, only a small number of mRNA sequences may be unique to any one of the individual stages. To determine more accurately what proportion of the mRNA sequences present in pupae and adult are not present in larvae, trace quantities of  $^3\text{H}$ -null mDNA were hybridized with a sequence excess of polysomal RNA from either larvae, pupae, or adult. The third instar larval polysomal RNA reacted with the null mDNA preparation to a saturation value of only 2.0% as compared to a saturation value of 10.6% for the reaction of this RNA with the starting DNA population. Polysomal RNA preparations from pupae and adults drove 3.0 and 3.2%, respectively, of null mDNA into hybrid form, demonstrating the presence of a small

TABLE 1  
 SEQUENCE COMPLEXITY OF RNA POPULATIONS BY HYBRIDIZATION TO <sup>3</sup>H SINGLE-COPY DNA

| RNA             | Source             | Saturation value <sup>a</sup> (%) | Complexity <sup>b</sup> (nucleotides) | $K_{obs}$ ( $M^{-1} sec^{-1}$ ) | $K_{exp}$ ( $M^{-1} sec^{-1}$ ) <sup>c</sup> | $F^d$                  | No. 1250 NT Sequences |
|-----------------|--------------------|-----------------------------------|---------------------------------------|---------------------------------|--|------------------------|-----------------------|
| Total polysomal | Larva              | 10.6 ± 0.2                        | 1.9 × 10 <sup>7</sup>                 | 7.7 × 10 <sup>-5</sup>          | 2.7 × 10 <sup>-2</sup>                       | 2.8 × 10 <sup>-3</sup> | 15,000                |
| Total polysomal | Pupa               | 10.3 ± 0.8                        | 1.9 × 10 <sup>7</sup>                 | 6.3 × 10 <sup>-5</sup>          | 2.8 × 10 <sup>-2</sup>                       | 2.3 × 10 <sup>-3</sup> | 15,000                |
| Total polysomal | Adult              | 11.0 ± 0.8                        | 2.0 × 10 <sup>7</sup>                 | 7.7 × 10 <sup>-5</sup>          | 2.6 × 10 <sup>-2</sup>                       | 2.9 × 10 <sup>-3</sup> | 16,000                |
| Total polysomal | Adult Head         | 7.7 ± 0.2                         | 1.4 × 10 <sup>7</sup>                 | 7.9 × 10 <sup>-5</sup>          | 3.8 × 10 <sup>-2</sup>                       | 2.1 × 10 <sup>-3</sup> | 11,000                |
| Poly(A+)mRNA    | Larva <sup>e</sup> | 3.7 ± 0.2                         | 6.7 × 10 <sup>6</sup>                 | 4.4 × 10 <sup>-3</sup>          | 7.8 × 10 <sup>-2</sup>                       | 5.6 × 10 <sup>-2</sup> | 5,400                 |
| Poly(A+)mRNA    | Pupa               | 4.5 ± 1.1                         | 8.2 × 10 <sup>6</sup>                 | 7.3 × 10 <sup>-3</sup>          | 6.4 × 10 <sup>-2</sup>                       | 1.1 × 10 <sup>-1</sup> | 6,600                 |
| Poly(A+)mRNA    | Adult              | 4.2 ± 0.2                         | 7.6 × 10 <sup>6</sup>                 | 6.6 × 10 <sup>-3</sup>          | 6.9 × 10 <sup>-2</sup>                       | 9.6 × 10 <sup>-2</sup> | 6,100                 |

<sup>a</sup> Terminal hybridization values described by a least-squares computer solution of the data shown in Figs. 2, 3, and 6A.

<sup>b</sup> Complexity = saturation value × 2 (assuming asymmetric transcription) × (9.1 × 10<sup>7</sup>), where 9.1 × 10<sup>7</sup> nucleotide pairs is the complexity of single-copy DNA from *D. melanogaster* (Manning *et al.*, 1975).

<sup>c</sup> Pseudo-first-order rate constant predicted from an RNA population of known complexity. The predicted rate is calculated from the relationship

$$K_{exp} = \frac{5374 \times 200}{\text{RNA complexity}} \times \left[ \frac{300}{L} \right]^{1/2},$$

where 5374 is the complexity of  $\theta$ X174 RNA and 200  $M^{-1} sec^{-1}$  is the pseudo-first-order rate constant for an RNA excess hybridization between  $\theta$ X174 RNA and 300 nucleotide driver RF DNA (Galau *et al.*, 1977).  $L$  is the mass average length of the *Drosophila* driver RNA (1250 nucleotides). It should be noted that the correction for length of *Drosophila* driver RNA is based on results derived from studies on DNA-DNA reassociation. Similar studies on RNA-DNA reassociation have not been reported.

<sup>d</sup> Fraction of the RNA mass which is driving the reaction as calculated from the ratio of  $K_{obs}$  to  $K_{exp}$  (Galau *et al.*, 1974; Hough *et al.*, 1975).

<sup>e</sup> Data for the RNA sequence complexity of polyadenylated mRNA from larvae are taken from Zimmerman *et al.* (1980).

number of gene sequences (~1600) expressed in these stages which is not expressed in third instar larvae.

#### Sequence Overlap of Poly(A+)mRNA from Different Developmental Stages

The data above indicate that most if not all of the DNA sequences expressed on polysomes of larvae are also expressed on polysomes of pupae and adults, but that some additional sequences are expressed in the later stages.

They do not, however, demonstrate whether the sequences present as polyadenylated RNA on larval polysomes are also present as polyadenylated sequences on the polysomes from pupae and adults. To evaluate this, a DNA population greatly enriched for single-copy sequences expressed as poly(A+)RNA on larval polysomes was prepared [(A+)mDNA], as was a population of DNA depleted of these sequences [null(A+)mDNA]. The logic for utilizing the (A+)mDNA and null(A+)mDNA for determining the amount of overlap in the (A+)mRNAs

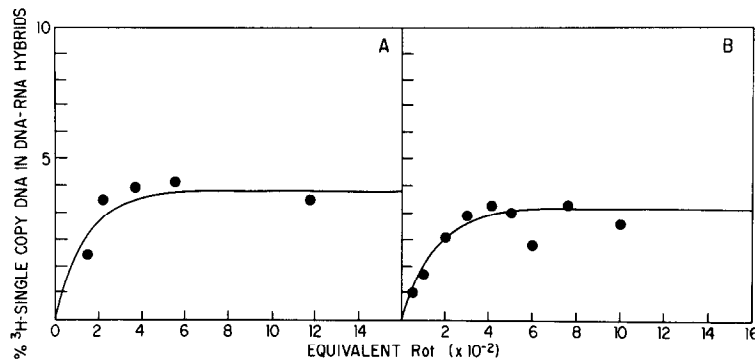


FIG. 3. Saturation hybridization of <sup>3</sup>H single-copy DNA to *D. melanogaster* polyadenylated messenger RNA. Trace quantities of <sup>3</sup>H single-copy DNA (specific activity ~6 × 10<sup>8</sup> cpm/μg) were hybridized with a ≥100-fold sequence excess of polyadenylated messenger RNA from 5 to 7-hr pupae (A) or adults (B). Hybridization was monitored by resistance to S<sub>1</sub> nuclease digestion. Data points have been corrected for the contribution of tracer self-reassociation and for tracer reactivity (~90% when reassociated with an excess of total *D. melanogaster* nuclear DNA).

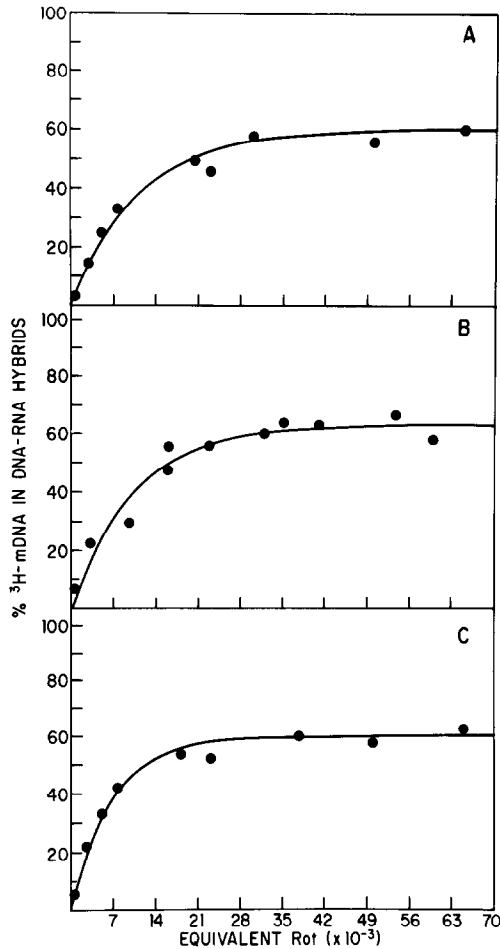


FIG. 4. Saturation hybridization of [<sup>3</sup>H]mDNA to *D. melanogaster* polysomal RNA. Trace quantities of [<sup>3</sup>H]mDNA were hybridized with a  $\geq 100$ -fold sequence excess of total polysomal RNA from third instar larvae (A), 5- to 7-hr pupae (B) or adults (C). Hybridization was monitored by resistance to S<sub>1</sub> nuclease digestion.

from the three stages as well as the number of stage specific (A+)mRNAs is identical to that described above for total polysomal RNA.

<sup>3</sup>H-(A+)mDNA hybridized with poly(A+)mRNA from larvae to a saturation value of 38%, representing approximately a 10-fold enrichment of coding sequences over the 3.3% reported by Zimmerman *et al.* (1980) (Fig. 5A; Table 2). At saturation, 38 and 40% of (A+)mDNA hybridized with poly(A+)mRNA from pupae and adults, respectively (Figs. 5B, C). This result shows that most if not all of the polyadenylated RNA sequences present on polysomes of third instar larvae are also present as polyadenylated RNA on the polysomes of pupae and adults.

<sup>3</sup>H-Null(A+)mDNA hybridized with poly(A+) mRNA from third instar larvae to saturation value of 1.6%. Poly(A+)mRNA from pupae and adults drove 3.3% of <sup>3</sup>H-null(A+)mDNA into hybrid form at saturation, indi-

cating that approximately 2500 diverse RNA sequences not expressed in larvae as polyadenylated polysomal RNA are expressed as such in pupae and adults. This result is consistent with the experiments using <sup>3</sup>H-null mDNA, and suggests that the sequences expressed uniquely in the later stages are expressed as poly(A+)mRNA.

#### *Polysomal RNA Sequence Diversity in a Tissue of the Adult*

The results of the experiments presented above clearly indicate that a similar set of diverse sequences exists on polysomes in three morphologically and physiologically distinct stages of development of *Drosophila*. The question of whether the total mRNA sequence complexity of the whole organism represents the sum of the different mRNA sequence complexities of various organs and tissue types, or whether all organs and tissues contain mRNA with a sequence complexity equal to that of total is not addressed by these studies. Some insight into this question can, however, be obtained from the studies of Galau *et al.* (1976). Here, it has been clearly demonstrated that several adult tissues in the sea urchin share a substantial subset of their mRNA population. However, it is apparent that individual tissues of the adult exhibit a population of mRNA unique to that tissue. In view of these results, we chose to measure and subsequently contrast the RNA sequence complexity of an adult structure significantly enriched for a specific tissue type to that observed for the total organism.

We chose to investigate the sequence complexity of the mRNA population present in the head of adult *Drosophila* for the following reasons. Although the head

TABLE 2  
SEQUENCE HOMOLOGY BETWEEN LARVAL, PUPAL, AND ADULT mRNA POPULATIONS

| RNA             | Source | DNA                                  | Saturation value <sup>a</sup> (%) |
|-----------------|--------|--------------------------------------|-----------------------------------|
| Total polysomal | Larva  | [ <sup>3</sup> H]mDNA <sup>b</sup>   | 60 ± 0.7                          |
| Total polysomal | Pupa   | [ <sup>3</sup> H]mDNA                | 63 ± 2                            |
| Total polysomal | Adult  | [ <sup>3</sup> H]mDNA                | 59 ± 0.6                          |
| Poly(A+)mRNA    | Larva  | <sup>3</sup> H-(A+)mDNA <sup>c</sup> | 38 ± 0.2                          |
| Poly(A+)mRNA    | Pupa   | <sup>3</sup> H-(A+)mDNA              | 38 ± 0.3                          |
| Poly(A+)mRNA    | Adult  | <sup>3</sup> H-(A+)mDNA              | 40 ± 0.1                          |

<sup>a</sup> Terminal hybridization values described by a least squares computer solution of the data shown in Figs. 4 and 5.

<sup>b</sup> [<sup>3</sup>H]mDNA represents a radioactively labeled population of single-copy DNA greatly enriched for messenger coding sequences in third instar larvae.

<sup>c</sup> <sup>3</sup>H-(A+)mDNA represents a radioactively labeled population of single-copy DNA greatly enriched for polyadenylated messenger coding sequences in third instar larvae.

does not represent an anatomical structure containing an individual organ or tissue type, it does represent a significant enrichment for neural tissue. This is evident from the fact that, although the head comprises only 10% of the body mass, it contains ~50% of the total neural tissue of the adult (Demerec, 1950). In keeping with this anatomical distribution, 56% of the acetylcholine receptor sites in adult flies have been found to be present in head tissue (Schmidt-Nielsen *et al.*, 1977). Furthermore, certain tissue types and organs are clearly absent in the head, such as the reproductive organs and the vast majority of the digestive and excretory systems. Thus, the head represents a structure which is devoid of many tissue types while containing a significant enrichment for neural tissue.

The sequence complexity of head polysomal RNA was measured by hybridization of an excess of polysomal

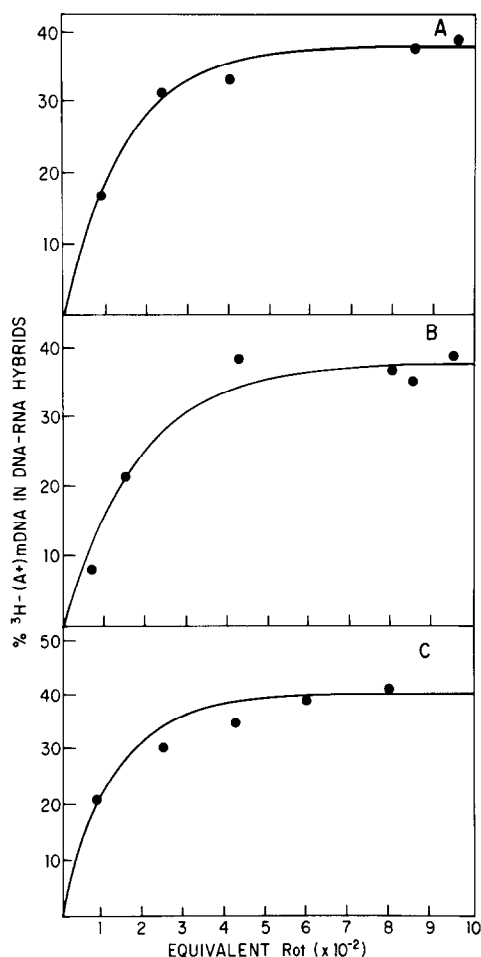


FIG. 5. Saturation hybridization of  $^3\text{H}$ -(A+)mDNA to *D. melanogaster* polyadenylated messenger RNA. Trace quantities of  $^3\text{H}$ -(A+)mDNA were hybridized with a  $\geq 100$ -fold sequence excess of polyadenylated messenger RNA from third instar larvae (A), 5- to 7-hr pupae (B), or adults (C). Hybridization was monitored by resistance to  $\text{S}_1$  nuclease digestion.

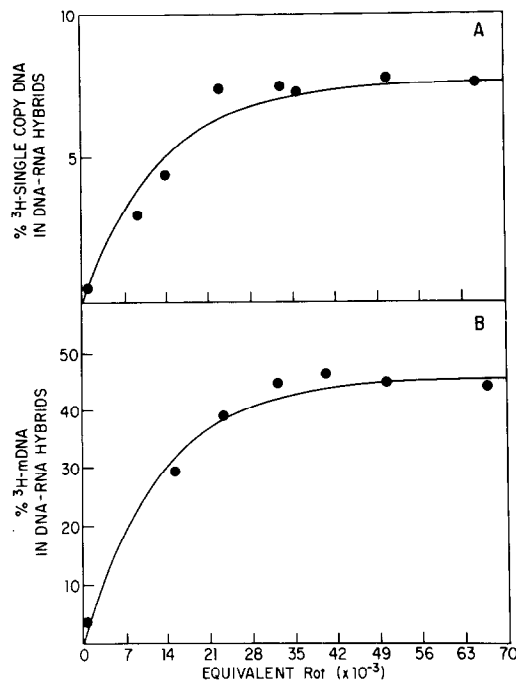


FIG. 6. Saturation hybridization of  $^3\text{H}$ DNA to *D. melanogaster* polysomal RNA from adult heads. Trace quantities of  $^3\text{H}$  single-copy DNA (A) or  $^3\text{H}$ mDNA (B) were hybridized with a  $\geq 100$ -fold sequence excess of total polysomal RNA from adult heads. Hybridization was monitored by resistance to  $\text{S}_1$  nuclease digestion. Data points in (A) have been corrected for the contribution of tracer self-reassociation and for tracer reactivity ( $\sim 90\%$  when reassociated with an excess of total *D. melanogaster* nuclear DNA).

RNA to  $^3\text{H}$  single-copy DNA (Fig. 6A). At saturation, 7.7% of the single-copy DNA hybridized to the polysomal RNA, representing an RNA sequence complexity of  $1.4 \times 10^7$  nucleotides, or about 11,000 diverse RNA species. This represents  $\sim 69\%$  (11,000/16,000) of the RNA sequence complexity observed for total adult polysomal RNA. Hybridization of  $^3\text{H}$ mDNA with excess head polysomal RNA (Fig. 6B) showed that at saturation only 46% of the mDNA hybridized with the RNA from adult heads. These two observations confirm that the particular set of tissues found in the head contain only a subset of the RNA sequence complexity of the entire adult animal and agree in establishing that approximately 70–75% of the RNA diversity of the entire adult is expressed in the head.

#### DISCUSSION

In this report, measurements of sequence complexity using RNA excess saturation hybridization demonstrate that 15,000–16,000 diverse RNA sequences of average size are present on the polyribosomes of third instar larvae, 5- to 7-hr pupae and adults of *D. melanogaster*, a number consistent with that previously measured for third instar larvae (Zimmerman *et al.*, 1980). Interest-



ingly, a similar number of genes is expressed in a large variety of eukaryotic organisms whose genomic sequence complexities range in size from 0.3 to 20 times that of *Drosophila* (Galau *et al.*, 1976; Hastie and Bishop, 1976; Van Ness and Hahn, 1980; Lanar, Levy, and Manning, 1981).

As reported previously for larvae (Zimmerman *et al.*, 1980), the poly(A+)mRNA of pupae and adults constitutes an unusually small proportion of the mass of polysomal RNA, 0.2–0.5%, and contributes only approximately one-third of its sequence diversity. Specifically, our measurements show that pupal poly(A+)mRNA contains approximately 6500 different sequences, while 15,000 diverse sequences are present in total polysomal RNA. Similarly, adult poly(A+)mRNA contains 6100 different sequences, with 16,000 present in total polysomal RNA. Therefore, in larvae, pupae, and adults, nonadenylated RNA molecules associated with polysomal RNA constitute approximately 60% of the RNA sequence diversity.

The heads of adult flies were prepared to represent an enrichment for neural tissue (Demerec, 1950). Polysomal RNA from heads has a complexity which corresponds to 11,000 different RNA sequences. The sequence diversity represents a subset, ~70%, of that observed in the entire adult. This result suggests that different adult tissues may contain somewhat different subsets of sequences on polysomes, all of which total the 16,000 sequences observed on polysomes of the entire adult. Indeed, qualitative differences in the RNA sequences on polysomes from several adult tissues of the sea urchin have been well characterized (Galau *et al.*, 1976). The distribution of the polysomal sequence complexity from adult heads among adenylated and nonadenylated classes was not measured because of scarcity of material.

Thus far, the results indicate that a similar number of diverse sequences exists on polyribosomes in three stages of development of *Drosophila*, and suggest that there may be little qualitative difference between the RNA populations in these organisms. Our sequence overlap studies using mDNA demonstrate that, indeed, virtually all of the DNA coding sequences expressed on polysomes in larvae are also expressed on polysomes in pupae and adults. Within the limits of detection of our technique, therefore, coding sequences represented on polysomes during larval development continue to be present throughout the life of the animal. It is possible that during development, such sequences change their distribution among the adenylated and nonadenylated classes of RNA; however, our overlap studies using (A+)mDNA show this not to be the case. In fact, all of the polyadenylated sequences on polysomes of larvae also appear as adenylated molecules on polysomes of pupae and adults. Therefore, polyadenylation, or the

lack of it, is a nonrandom, constant characteristic of a particular RNA species expressed on polyribosomes during these stages of development in *Drosophila*.

Hybridizations using null mDNA indicate that a small number of new sequences, ~2000, are expressed on polysomes of pupae and adults which were not expressed in larvae. Furthermore, these new sequences appear in the polyadenylated class as evidenced by an increased hybridization of larval null(A+)mDNA with poly(A+)mRNA from pupae and adults. We report that the number of genes expressed in poly(A+)mRNA of pupae and adults is 6500 and 6100, respectively, an increase of 700–1100 over the value for larvae reported by Zimmerman *et al.* (1980) from experiments performed exactly as those reported here. Kinetic measurements which employed [<sup>3</sup>H]cDNA to poly(A+) cytoplasmic RNA have reported the number of genes expressed in larvae to be  $\geq 4900$  (Izquierdo and Bishop, 1979) and 6900 (Levy and McCarthy, 1975) and the number expressed in pupae and adults to be 6900 and  $\geq 4900$ , respectively (Izquierdo and Bishop, 1979). In addition, the fact that we observe little if any increase in sequence diversity of total polysomal RNA from the later stages is evidence that the number of new sequences detected by our overlap studies is quite small.

Thus, very little qualitative change occurs in the adenylated and nonadenylated classes of polysome associated RNAs during *Drosophila* development. Such extensive RNA sequence homology has been previously reported to exist between tissues which differ dramatically in morphology and physiology. For example, cultures of undifferentiated chick myoblasts appear to share the same set of approximately 17,000 different messenger RNA sequences with differentiated myofibrils *in vitro* (Paterson and Bishop, 1977). Two highly differentiated chicken tissues, liver and oviduct, have been shown by hybridization kinetics to contain 12,000–15,000 diverse mRNA species, of which at least 85% are held in common (Axel *et al.*, 1976). In addition, extensive homology between the mRNAs of normal and chemically or virally transformed cells has been reported, although transformation results in a large number of phenotypic changes (Getz *et al.*, 1977; Rolton *et al.*, 1977; Williams *et al.*, 1977).

In contrast to the studies discussed thus far, a variety of sea urchin embryo and adult tissues share only a small proportion of their total mRNA sequence diversity. For example, of the 14,000 mRNA species found in gastrula, 11,000 are also expressed in pluteus, and only 1000–1500 in three adult tissues (Galau *et al.*, 1976). Thus, it is clear that qualitatively distinct sets of single-copy sequence transcripts appear on polyribosomes during sea urchin development.

Although large qualitative changes in mRNA popula-

tions do not accompany phenotypic change in *Drosophila* and in many other systems studied, the occurrence of quantitative changes in RNA populations has been well characterized, i.e., changes in the relative abundance of particular RNA species (Axel *et al.*, 1976; Getz *et al.*, 1976; Paterson and Bishop, 1977; Wilkes *et al.*, 1979). Our study does not address this question, although recent reports have demonstrated that changes in the relative abundance of individual polyadenylated RNAs accompany development in *D. melanogaster* (Biessman, 1981) and in *Xenopus laevis* (Dworkin and Dawid, 1980). Such quantitative changes may influence the phenotype of the developing system more profoundly than the presence on polyribosomes of an apparently constant sequence diversity.

In summary, our studies confirm and extend previous reports that the majority of polyadenylated mRNA sequence diversity in *Drosophila* is retained throughout development (Levy and McCarthy, 1975; Izquierdo and Bishop, 1979; Arthur *et al.*, 1979). Further, we report that the nonadenylated sequences present on polyribosomes represent a discrete class of molecules present continually during three stages of development in *Drosophila*. Thus, it is clear from our studies and others that development or differentiation is not necessarily accompanied by sweeping qualitative changes in gene expression.

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