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Nonadenylylated mRNA is present as polyadenylylated RNA in nuclei of *Drosophila*

(RNA·DNA hybridization/DNA subsets)

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ABSTRACT **The sequence complexity of nuclear total RNA and nuclear poly(A)$^+$RNA from *Drosophila* third-instar larvae was determined by hybridization of these RNAs to labeled single-copy DNA. At saturation, the nuclear poly(A)$^+$- and total RNA hybridized to 11% and 22.5% of the single-copy DNA, respectively. The increase in complexity of nuclear total RNA over that observed for nuclear poly(A)$^+$RNA indicates the presence of a discrete class of nonadenylylated nuclear RNA molecules. The relationship between DNA sequences coding for nuclear RNA and mRNA was then determined by hybridization of nuclear total and poly(A)$^+$RNA to DNA enriched for mRNA coding sequences. The results of these studies show that those single-copy DNA sequences that are represented in either the poly(A)$^+$- or poly(A)$^-$mRNA population are transcribed into RNA molecules that appear in the nuclear poly(A)$^+$RNA population.**

It has been proposed that high molecular weight nuclear RNA serves several functions within the eukaryotic cell, the most widely accepted of which is as a precursor to cytoplasmic mRNA (1). Although little direct evidence for this role existed for several years, recent advances in molecular techniques have resulted in the identification and characterization of nuclear precursors for several specific mRNAs (2–5). In these studies, it has been clear that the mRNA sequences represent only a portion of the primary transcripts, and hence specific processing appears to be an essential feature and potential regulatory event in mRNA biogenesis. In lower eukaryotes (i.e., fungi) there is little or no difference in either the complexity or the size of the RNAs found in the nucleus and the cytoplasm (6, 7); in these systems the relationship between nuclear transcripts and translated mRNAs appears to be direct. In higher eukaryotes, where nuclear RNA is several times more complex than mRNA, the relationship between the nuclear and mRNA species is not as clearly understood. In mouse and rat brain (8, 9), RNA·DNA hybridization experiments clearly show that the polyadenylylated nuclear RNA population contains all of the polyadenylylated cytoplasmic RNA sequences. However, it is also clear that both the nuclear RNA and mRNA are composed of poly(A)$^+$- and poly(A)$^-$RNA populations (8–10). At present, the relationship between the nonadenylylated nuclear RNA and mRNA in these systems is unknown.

In *Drosophila*, however, a recent observation by Lengyel *et al.* (11) clearly indicates that the transcribed region of one of the major *Drosophila* heat shock puff sites, 93D, is represented almost exclusively by poly(A)$^-$RNA in the cytoplasm, whereas it is represented by both poly(A)$^+$- and poly(A)$^-$RNA in the nucleus.

In view of this fact, and because we have determined that the mRNA of *Drosophila* third-instar larvae is composed of both adenylylated and nonadenylylated species (12), we have measured the sequence complexity of poly(A)$^+$- and total nuclear RNA present in these larvae. We report here that, as was observed in larval mRNA, the nuclear RNA is composed of both an adenylylated and a nonadenylylated class. In the nucleus, these two populations each contribute approximately half of the total nuclear RNA complexity. In addition, we have investigated relationships between the nuclear and polysomal RNAs in the adenylylated and nonadenylylated classes. Surprisingly, these studies revealed that the nuclear poly(A)$^+$RNA contains many, and possibly all, of the sequences present in the total polysomal mRNA population.

MATERIALS AND METHODS

Isolation and Purification of Nuclear RNA and Polysomal RNA from *D. melanogaster* Larvae. *Drosophila* third-instar larvae (120–125 hr) were homogenized in extraction buffer (13) containing 4 mM spermidine, 1 mM spermine, 10 mM EDTA, 100 mM KCl, 10 mM Tris (pH 7.9), 14 mM mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, and 0.5 M sucrose. After filtration through sterile cheesecloth, a crude nuclear pellet was obtained by centrifugation at 8,000 × *g* for 5 min. The pellet was gently dispersed in extraction buffer containing 0.5% Nonidet P-40, and nuclei were pelleted by centrifugation at 3,000 × *g* for 5 min.

Nuclei were lysed at 4°C in RNA extraction buffer (0.1 M NaCl/0.1 M NaOAc/5 mM $MgCl_2$, pH 5.1) to which 2% (wt/vol) $NaDodSO_4$ and an equal volume of buffer-saturated phenol had been added. Chloroform/isoamyl alcohol, 24:1 (vol/vol), was then added to a volume equivalent to that of the phenol. The mixture was gently agitated for ≈5 min, and the aqueous and organic phases were separated by centrifugation. The aqueous phase plus interface was reextracted four times, twice with phenol/chloroform/isoamyl alcohol, and twice with chloroform/isoamyl alcohol. The RNA was then precipitated at −20°C by addition of 2 vol of 95% ethanol. The RNA was further purified by pelleting through 6 M CsCl (14).

Poly(A)$^+$RNA was isolated from total nuclear RNA by chromatography on either poly(U)-Sepharose or oligo(dT)-cellulose. Both procedures revealed that approximately 1% of total nuclear RNA is polyadenylylated; oligo(dT)-cellulose chromatography was routinely used for the preparative isolation of nuclear poly(A)$^+$RNA. The preparation of total polysomal RNA and of poly(A)$^+$mRNA has been described (12).

Size Measurements of Nuclear RNA. The mass average size of nuclear poly(A)$^+$RNA was measured by gel electrophoresis

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Abbreviations: mDNA, DNA complementary to total polysomal mRNA; (A)$^-$DNA, DNA complementary to poly(A)$^-$RNA.

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in the presence of the denaturant methyl mercury hydroxide (15).

In addition, the size of newly synthesized nuclear RNA was measured after selective labeling of this class of RNA. Briefly, Schneider's cells, grown in Schneider's *Drosophila* medium enriched with 15% fetal calf serum (GIBCO) were incubated with [^{3}H]uridine at 11.0 μCi/ml (1 Ci = 3.7 × 10^{10} becquerels) for 15 min in the presence of 5-fluorouracil (5 μg/ml; 1-hr preincubation) as described (12). Cells were pelleted at 4,000 × *g* and nuclear RNA was prepared as described above. Transfer RNAs, which are labeled in the presence of 5-fluorouracil, were removed by a high-salt precipitation of the nuclear RNA (16). Pulse-labeled nuclear RNA was sedimented through a linear 5–20% (wt/vol) sucrose velocity gradient at 25,000 rpm for 15.5 hr in a Beckman SW 41 rotor. The gradient was fractionated and the amount of radioactivity in each fraction was determined.

Preparation of Single-Copy DNA and DNA Complementary to Total Polysomal mRNA (mDNA). Single-copy nuclear DNA was prepared by several cycles of reassociation and hydroxyapatite fractionation and was labeled *in vitro* with [^{3}H]TTP as described (12). Labeled single-copy DNA prepared in this manner had an average single-strand length of 210 nucleotides and a specific activity of 6–7 × 10^6 cpm/μg. Approximately 90% of this DNA reassociated when mixed in trace quantities with excess amounts of total *D. melanogaster* nuclear DNA.

mDNA from third-instar larvae was prepared as described (17).

From the mDNA, a population of DNA complementary to nonadenylylated mRNA was prepared [i.e., (A)$^-$mDNA]. This population was prepared by removal from mDNA of those sequences complementary to poly(A)$^+$mRNA. To achieve this, poly(A)$^+$mRNA (10 μg) was hybridized in >100-fold sequence excess with [^{3}H]mDNA (5 × 10^{-3} μg). DNA in hybrid form was separated from unhybridized DNA by centrifugation to equilibrium twice in a neutral CsCl gradient as described (17). A band of single-stranded, noncomplementary DNA was isolated from the gradient, and contaminating RNA was removed by treatment with 0.1 M NaOH at 60°C for 30 min followed by neutralization with 2 M Hepes (pH 4). The (A)$^-$mDNA prepared in this manner was shown to be substantially depleted of DNA sequences encoding poly(A)$^+$mRNA (see *Results*).

Hybridization of RNA with [^{3}H]DNA and Assay of Hybridization. ^{3}H-Labeled single-copy DNA, mDNA, and (A)$^-$mDNA were incubated with a 50- to 100-fold sequence excess of RNA as described (12). The contribution of DNA·DNA self-reassociation to the total amount of hybridization was determined by assaying parallel reactions in which an identical amount of yeast soluble RNA was substituted for *Drosophila* RNA.

Samples were analyzed for hybrid content as described by Zimmerman *et al.* (12) by using trichloroacetic acid precipitation after S1 nuclease treatment. The percentage DNA·RNA hybridization was calculated by subtracting the percentage S1 nuclease resistance of the samples containing heterologous RNA (i.e., resistance due to DNA·DNA reassociation and to the inherent S1 nuclease resistance of the nonhybridized tracer) from the percentage S1 nuclease resistance of samples containing *D. melanogaster* RNA. The amount of S1 nuclease-resistant material in control samples containing heterologous RNA was consistently 2–3% of the input DNA.

RESULTS

Size of Nuclear RNA. The mean size of newly synthesized nuclear RNA was determined by sucrose gradient centrifugation (Fig. 1*A*). In agreement with previous studies (18), pulse-labeled nuclear RNA showed a heterogeneous distribution of size with a mean sedimentation coefficient of approximately 26 S. However, the mass average size of steady-state nuclear RNA, as determined by electrophoretic mobility in denaturing gels (Fig. 1*B*), was significantly smaller with a mean length of approximately 1,600 nucleotides. A similar value has been observed for steady-state nuclear RNA (19). These data indicate that, although newly synthesized nuclear RNA is perhaps 3 times as long as polysomal poly(A)$^+$RNA, the average length of poly(A)-containing molecules that accumulate in the nucleus is approximately that observed for polysomal poly(A)$^+$RNA.

Sequence Complexity of Nuclear RNA. It has been shown that there are two classes of mRNAs in *Drosophila* third-instar

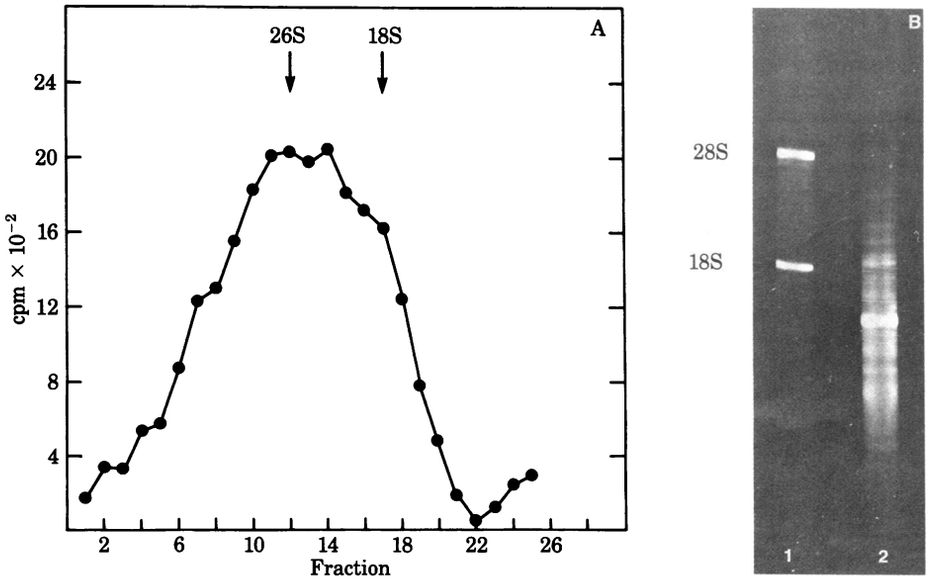

FIG. 1. Size of nuclear RNA in *Drosophila* larvae. (*A*) The size of pulse-labeled nuclear RNA was measured by sedimentation through a linear sucrose gradient [5–20% (wt/vol)]. Radioactivity in each fraction was assayed by liquid scintillation counting. (*B*) The mass average size of nuclear poly(A)$^+$RNA was determined by gel electrophoresis in the presence of the denaturant methylmercuryhydroxide. rRNA from HeLa cells was run in parallel as a size standard. Gels were stained with ethidium bromide (2 μg/ml) and photographed by using ultraviolet light.

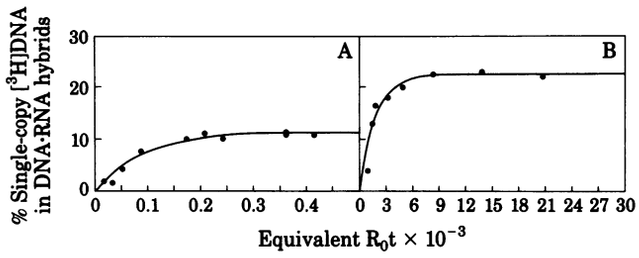

FIG. 2. Saturation hybridization of single-copy [^{3}H]DNA to RNA from third-instar larvae of *D. melanogaster*. Trace quantities of single-copy [^{3}H]DNA (specific activity, $\approx 6 \times 10^6$ cpm/μg) were hybridized with a 50- to 100-fold sequence excess of nuclear poly(A)$^+$RNA (*A*) or nuclear total RNA (*B*). Hybridization was monitored by resistance to S1 nuclease digestion. Data points have been corrected for the contribution of tracer self-reassociation and for tracer reactability ($\approx$90% when reassociated with an excess of total *D. melanogaster* nuclear DNA).

larvae—those that bind to oligo(dT)-cellulose [poly(A)$^+$RNA], and those that do not [poly(A)$^-$mRNA] (12). In order to ascertain whether the nuclear RNA from third-instar larvae is also composed of an adenylylated and a nonadenylylated class of molecules, the sequence complexity of nuclear poly(A)$^+$RNA and total RNA was measured by RNA-excess saturation hybridization with single-copy [^{3}H]DNA. The presence of a nonadenylylated class of nuclear RNA would be indicated if the sequence complexity of total nuclear RNA significantly exceeded that observed for nuclear poly(A)$^+$RNA. Samples were removed at appropriate R_0t values and assayed for hybrid content. The amount of single-copy [^{3}H]DNA that hybridizes with the RNA at saturation provides a direct estimate of the nuclear poly(A)$^+$RNA complexity. The results of this experiment are presented in Fig. 2*A* and are summarized in Table 1. At saturation ($R_0t > 300$), 11.1% of the single-copy [^{3}H]DNA hybridized with the nuclear poly(A)$^+$RNA. After correction for asymmetric transcription, 22.2% of the single-copy DNA in the *Drosophila* genome is represented in larval nuclear poly(A)$^+$RNA. This corresponds to $\approx 2.0 \times 10^7$ nucleotides of coding DNA.

The sequence complexity of nuclear total RNA was measured by using exactly the same method. The results are presented in Fig. 2*B* and are summarized in Table 1. At saturation ($R_0t > 9{,}000$), 22.5% of the single-copy [^{3}H]DNA hybridized with nuclear total RNA. This value is in good agreement with that reported (19) and, after correction for asymmetric transcription,

Table 1. Sequence complexity of RNA populations by hybridization to single-copy [^{3}H]DNA

RNA	Saturation value,* %	Complexity† × 10^{-7}	F‡ × 10^2
Nuclear total	22.5 ± 0.3	4.1	4.9
Nuclear poly(A)$^+$	11.1 ± 0.1	2.0	50
Polysomal total§	10.6 ± 0.2	1.9	0.28

* Terminal hybridization values described by a least-squares computer solution of the data shown in Fig. 2, expressed as mean ± SD.

† Complexity = saturation value × 2 (assuming asymmetric transcription) × (9.1×10^7); 9.1×10^7 nucleotide pairs is the complexity of single-copy DNA from *D. melanogaster* (20).

‡ Fraction of the RNA that is driving the reaction as calculated from the ratio of K_{obs} to K_{exp} (21, 22). K_{exp} was calculated according to Galau *et al.* (23) with 1,600 nucleotides as the mass average length of *Drosophila* nuclear RNAs. 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.

§ Data for the RNA sequence complexity of polysomal total RNA from larvae (12), included for comparison.

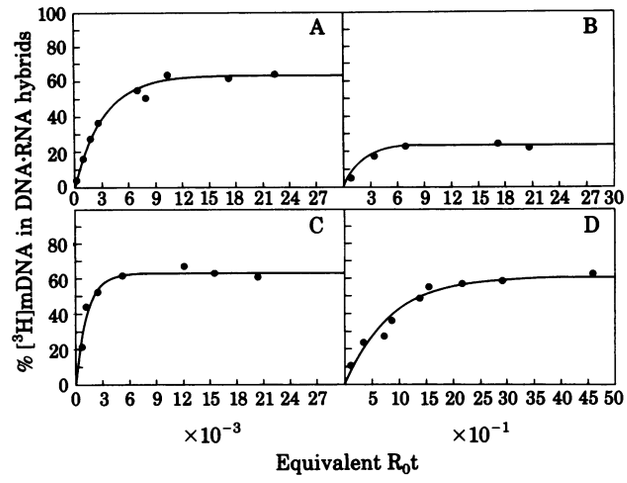

FIG. 3. Saturation hybridization of [^{3}H]mDNA to RNA from third-instar larvae of *D. melanogaster*. Trace quantities of [^{3}H]mDNA were hybridized with a $\geq$100-fold sequence excess of polysomal total RNA (*A*), polyadenylylated mRNA (*B*), nuclear total RNA (*C*), and nuclear polyadenylylated RNA (*D*). Hybridization was monitored by resistance to S1 nuclease digestion.

corresponds to 45.0% of the single-copy DNA complexity, or $\approx 4.1 \times 10^7$ nucleotides.

The data in the above experiments show that the sequence complexity of nuclear total RNA is approximately twice that of nuclear poly(A)$^+$RNA. This indicates that a discrete class of highly complex nonadenylylated RNA molecules exists in the nucleus of *Drosophila* third-instar larvae.

mRNA Sequences Present in Nuclear RNA. In previous studies (12, 17), we have shown that approximately two-thirds of the diverse mRNAs on polysomes are found only in the nonadenylylated RNA population. The above studies indicate a similar sequence distribution for nuclear total RNA. The presence of a discrete subpopulation of nonadenylylated RNA molecules in both the mRNA and the nuclear RNA suggests the possibility that these nonadenylylated RNAs may be homologous. In order to investigate this possibility, we first prepared a population of DNA molecules highly enriched for mRNA coding sequences (i.e., mDNA). To measure the amount of enrichment achieved, and to ensure that the DNA fragments represented both nonadenylylated and adenylylated mRNA coding sequences, polysomal total RNA and poly(A)$^+$mRNA were hybridized in excess to trace quantities of [^{3}H]mDNA. The results of this experiment are presented in Fig. 3 and are summarized in Table 2.

Table 2. Sequence homology among mRNA, nuclear RNA, and nuclear polyadenylylated RNA populations

RNA	DNA*	Saturation value,† %
Polysomal total	[^{3}H]mDNA	63.5 ± 0.7
Poly(A)$^+$mRNA	[^{3}H]mDNA	23.0 ± 0.1
Nuclear total	[^{3}H]mDNA	63.0 ± 1.1
Nuclear poly(A)$^+$	[^{3}H]mDNA	61.0 ± 1.1
Poly(A)$^+$mRNA	[^{3}H](A)$^-$mDNA	6.0 ± 0.1
Nuclear poly(A)$^+$	[^{3}H](A)$^-$mDNA	54.5 ± 0.1
Nuclear total	[^{3}H](A)$^-$mDNA	53.0 ± 0.7

* [^{3}H]mDNA represents a radioactively labeled population of single-copy DNA enriched for mRNA-coding sequences in third-instar larvae. [^{3}H](A)$^-$mDNA represents a radioactively labeled population of single-copy DNA greatly enriched for nonadenylylated mRNA coding sequences in third-instar larvae.

† Terminal hybridization values described by a least-squares computer solution of the data shown in Figs. 2 and 3. Results are shown as mean ± SD.

Hybridization of the mDNA with polysomal total RNA revealed a saturation level of 63.5% at $R_0t > 9{,}000$ (Fig. 3A). This represents an approximately 6-fold enrichment of mRNA coding sequences (i.e., 63.5/10.6 = 6). However, when the mDNA was hybridized with poly(A)$^+$mRNA, only 23% of the mDNA hybridized at saturation [$R_0t > 80$ (Fig. 3B)]. This represents ≈36% of the hybridization with polysomal RNA (23/63.5) and is in good agreement with previous studies (12, 17). The additional complexity observed in the polysomal total RNA hybridization must be due to the reaction of nonadenylylated mRNA with sequences present in the mDNA. Therefore, the mDNA contains sequences complementary to both adenylylated and nonadenylylated mRNAs in the expected proportions and is thus representative of the mRNA coding sequences that are expressed in third-instar larvae.

Hybridization of the [^{3}H]mDNA with a sequence excess of total nuclear RNA revealed a saturation value of 63% (Fig. 3C). Comparison of this saturation value with that observed for the self-reaction (i.e., polysomal RNA × [^{3}H]mDNA; 63.5%) indicates that the nuclear total RNA population contains most, if not all, the sequence complexity present in polysomal RNA.

To determine what fraction of the polysomal total mRNA sequences are represented in the nuclear poly(A)$^+$RNA, [^{3}H]mDNA was allowed to react to saturation with an excess of nuclear poly(A)$^+$RNA (Fig. 3D). At saturation, the nuclear poly(A)$^+$RNA hybridized to 61% of the [^{3}H]mDNA. Within the limitations of these experiments, this saturation value is essentially identical to the value, 63.5%, observed for the self-reaction. This leads to the surprising conclusion that the vast majority of all polysomal mRNA transcripts, both adenylylated and nonadenylylated, are present as polyadenylylated species in the nucleus. It further indicates that the discrete subpopulation of nonadenylylated RNA species in the nucleus is not homologous with the subpopulation of nonadenylylated mRNAs present on polysomes. We base this conclusion on the following. The total sequence complexity of polysomal mRNA is present in nuclear poly(A)$^+$RNA. Because the subpopulation of nonadenylylated nuclear RNA is by definition nonhomologous to the nuclear poly(A)$^+$RNA, it must also lack homology to the nonadenylylated class of mRNA.

In view of this surprising observation, we chose to measure directly the extent of hybridization between nuclear poly(A)$^+$RNA and DNA fragments enriched for poly(A)$^-$mRNA coding sequences. A fraction of [^{3}H]mDNA, enriched for poly(A)$^-$mRNA coding sequences [i.e., (A)$^-$mDNA], was prepared and hybridized to an excess of poly(A)$^+$mRNA, nuclear total RNA, or nuclear poly(A)$^+$RNA. The results of these hybridizations are presented in Fig. 4 and are summarized in Table 2. At saturation ($R_0t > 100$), 6% of the [^{3}H](A)$^-$mDNA hybridized to the polysomal poly(A)$^+$mRNA compared to 23% in the original mDNA, thus showing an ≈75% decrease in the poly(A)$^+$mRNA coding sequences (Fig. 4A). Hybridization of nuclear poly(A)$^+$RNA and total RNA to the [^{3}H](A)$^-$mDNA resulted in essentially identical saturation values of 54.5% and 53%, respectively. These results confirm that DNA sequences that are represented in polysomal RNA as poly(A)$^-$mRNA are represented in the nucleus as poly(A)$^+$RNA.

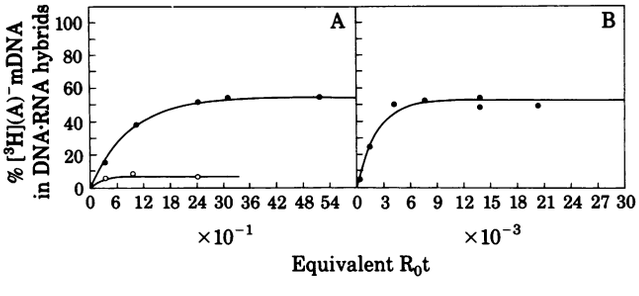

FIG. 4. Saturation hybridization of [^{3}H](A)$^-$mDNA to RNA from third-instar larvae of *D. melanogaster*. Trace quantities of [^{3}H](A)$^-$mDNA were hybridized with a ≥100-fold sequence excess of poly(A)$^+$mRNA (open circles, *A*), nuclear poly(A)$^+$RNA (closed circles, *A*), and nuclear total RNA (*B*). Hybridization was monitored by resistance to S1 nuclease digestion.

DISCUSSION

Our results point to three principal conclusions. First, the increase in complexity observed in comparing nuclear total RNA to nuclear poly(A)$^+$RNA is the result of the presence of a complex class of nonadenylylated nuclear RNA molecules. Accordingly, the nuclear poly(A)$^+$RNA must represent a discrete subpopulation of the nuclear total RNA. Second, those single-copy DNA sequences that are represented in both the poly(A)$^+$- and poly(A)$^-$mRNA populations are transcribed into RNA molecules that appear in the nuclear poly(A)$^+$RNA population. Lastly, because the entire complexity of the polysomal mRNA population is represented in the nuclear poly(A)$^+$RNA, the additional complexity present in the nonadenylylated nuclear RNA population must represent transcription of DNA sequences other than those represented on polysomes.

The observation that the total sequence complexity of polysomal mRNA is present in nuclear poly(A)$^+$RNA is surprising in that it indicates that poly(A)$^-$mRNAs have adenylylated counterparts in the nucleus. Such an observation clearly has an implication on the origin of poly(A)$^-$mRNA. One of the simplest explanations for this observation is that specific polysomal mRNAs, which are destined to lack poly(A) tracts, are synthesized as poly(A)$^+$ molecules and subsequently are processed, either in the nucleus or in the cytoplasm, to poly(A)$^-$mRNA. Because both poly(A)-specific exoribonuclease and endoribonuclease activities have been identified in the nuclei of several diverse organisms and tissues (24–28), the possibility of nuclear processing of poly(A) chains is certainly viable. Alternatively, the possibility of cytoplasmic processing is supported by the results of studies on the biogenesis of myosin heavy chain mRNA during myogenic differentiation (29, 30). Essentially all of the myosin heavy chain mRNA that accumulates in L6E9 myotubes during myogenic differentiation has either short (<20) poly(A) tails or lacks poly(A) tails. In contrast, newly synthesized myosin heavy chain mRNA appears in the cytoplasm of differentiated L6E9 myotubes as containing long poly(A) tails. These results imply that the newly synthesized myosin heavy chain poly(A)$^+$RNA undergoes a processing event(s) in the cytoplasm which removes or shortens the poly(A) chain. The possibility of cytoplasmic processing of poly(A) is further supported by the observations of Bergmann and Brawerman (31). When ascites cell polysomes were exposed to either micrococcal nuclease or ascites cell extracts, individual mRNA species showed different susceptibilities to the loss of 3′-terminal poly(A) sequences. Thus, under both *in vivo* and *in vitro* assay conditions, cleavage of poly(A) on mRNA in polysomes appears to be quite selective. At present, we do not know whether individual poly(A)$^-$mRNAs in *Drosophila* first appear in the cytoplasm as poly(A)$^+$ molecules and are subsequently processed and accumulated as poly(A)$^-$mRNA or whether the processing activity for removal or shortening of the poly(A) tail resides in the nucleus. An investigation of these two possibilities is best undertaken by studying the biogenesis of individual poly(A)$^-$mRNAs.

The observation that nuclear poly(A)$^+$RNA contains the total sequence complexity of polysomal mRNA has certain implications for the precursor–product relationship of nuclear RNA and

polysomal mRNA. Because the sequence complexity of nuclear poly(A)$^+$RNA and polysomal total mRNA are similar, 11.1 ± 0.1% and 10.6 ± 0.2%, respectively, the average size of nuclear poly(A)$^+$RNA would be expected to be about that of polysomal mRNA. Although pulse-labeling studies revealed that nuclear poly(A)$^+$RNA is 2–3 times larger than poly(A)$^+$mRNA (ref. 18; Fig. 1), the mean size of poly(A)$^+$RNA that accumulates in the nucleus (i.e., the steady-state size) is ≈1,600 nucleotides (ref. 19; Fig. 1*B*) which is not substantially greater than that of poly(A)$^+$mRNA. Recent results on processing times for individual HeLa cell hnRNAs (32) suggest that these size differences most likely reflect rapid *in vivo* processing events. In any event, the average size of steady-state nuclear poly(A)$^+$RNA is about that of polysomal total mRNA.

In regard to the class of nonadenylylated nuclear RNA which lacks a poly(A)$^+$ counterpart, we have no direct experimental evidence as to the function of this class of RNA. It is possible that it may represent mRNAs that are expressed in developmental stages other than third larval instar. It is evident that, in other organisms, RNA transcripts can be found in the nucleus for genes that are not expressed in that particular developmental stage or tissue but are expressed in other stages or tissue types (33, 34). Our previous results (17), which indicate little qualitative change in the polysomal mRNA population in adults, larvae, and pupae, do not support this interpretation. However, it should be noted that these stages represent a limited sampling of the development of *Drosophila*. It is clear that in third-instar larvae this nonadenylylated class of nuclear RNA does not contain sequences found on polysomes.

In summary, the data presented here indicate that two classes of transcripts are found in the nuclei of *Drosophila* larvae. The polyadenylylated nuclear RNA comprises approximately half of the total nuclear sequence complexity and may function as the precursor of polysomal mRNAs of both the adenylylated and nonadenylylated classes. In contrast, the remaining complexity of the nuclear RNA is present as nonadenylylated RNA molecules which appear to have no polysomal RNA counterpart. As yet, no function can be assigned to this class of molecules.

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