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Synthesis of Two Classes of Small RNA Species in Vivo and in Vitro†

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ABSTRACT: A set of low molecular weight RNA species has been described in mammalian cells. These RNAs are localized primarily in the nucleus and are not involved in protein synthesis (Zieve and Penman, 1976). In the HeLa cell these small RNA species are divisible into two distinct families that have different methods of formation: the class I small RNAs which may be formed by a polymerase type I activity and the class III small RNAs which are clearly synthesized by RNA polymerase III. The class III small RNAs include tRNA, 5S RNA, and species K and L. They are synthesized in HeLa cell nuclei incubated in vitro where their synthesis is unaffected by low levels of α-amanitin but inhibited by high levels of α-amanitin which indicates that they are products of RNA polymerase III. In vivo, the synthesis of the class III small RNAs is insensitive to several inhibitors and to alterations in cellular metabolism that severely inhibit the synthesis of other types of cellular RNA including the class I small RNAs. The class I small RNAs include the nuclear species A, C, D, F, and H. They are not made in detectable amounts in vitro in isolated nuclei which prevents a direct determination of the RNA polymerase responsible for their synthesis. In contrast to the class III small RNA species, their synthesis in vivo is sensitive to inhibitors and to alterations in cellular metabolism which also inhibit the transcription of the 45S rRNA precursor, suggesting that they are transcribed by RNA polymerase I. The small RNA species of *Drosophila melanogaster* cells also form two distinct families, one whose synthesis is sensitive to α-amanitin and high temperature and one which is resistant.

The low molecular weight RNA species, in eukaryotic cells, have been described by several authors (Weinberg and Penman, 1968; Hodnett and Busch, 1968; Goldstein and Ko, 1974; Zieve and Penman, 1976). In mammals, these RNA molecules form a distinct class of at least eight well-defined species that range in size from 4 to 7 S. They have been shown to be relatively abundant and stable (Weinberg and Penman, 1969; Frederiksen et al., 1974). These RNAs can be found associated with different cellular fractions including cytoplasmic membranes (species L) (Zieve and Penman, 1976), the nucleolus (species A) (Weinberg and Penman, 1968; Prestayko et al., 1971) and with nuclear ribonucleoprotein particles containing hnRNA (Deimel et al., 1977; Zieve and Penman, in preparation). They appear to be tightly bound to specific subcellular structures suggesting that they may play a structural role in some cell organelles. The 5S RNA and tRNA are known to be transcribed by RNA polymerase III (McReynolds and Penman, 1974; Weinmann and Roeder, 1974; Marzluff et al., 1974); however, little is known so far about the biosynthesis

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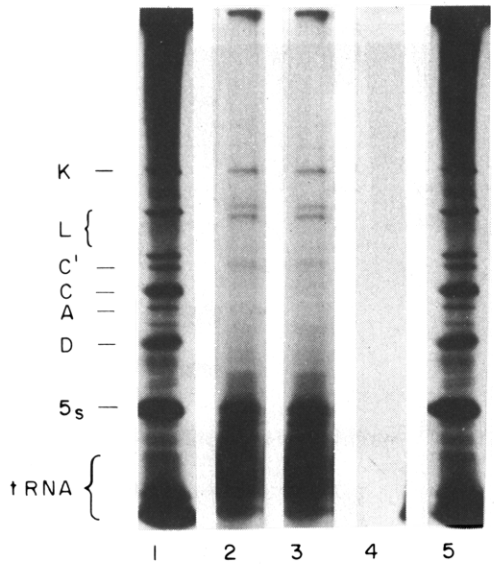

FIGURE 1: The small molecular weight RNAs synthesized in vitro by HeLa cell isolated nuclei. The 2×10^6 HeLa cell isolated nuclei were incubated 30 min as previously described (McReynolds and Penman, 1974). The nuclei were sedimented by centrifugation and the RNA in the supernatant was analyzed on 6–15% gradient gels. (1 and 5) Total HeLa cell small molecular weight RNAs from 1×10^6 cells labeled for 2 h. (2) In vitro products synthesized in the absence of α-amanitin. (3) In vitro products synthesized in the presence of 0.5 μg/mL α-amanitin. (4) In vitro products synthesized in the presence of 150 μg/mL α-amanitin.

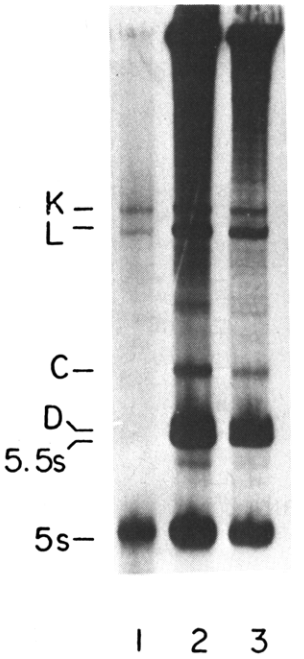

FIGURE 2: The 7 M urea gel of products of HeLa cell isolated nuclei. (1) RNA synthesized in vitro in HeLa cell isolated nuclei in the absence of α-amanitin. (2) Cytoplasmic fraction from 4×10^5 HeLa cells labeled for 16 h with 5 μCi/mL of [^{3}H]uridine. (3) Cytoplasmic fraction from 2×10^5 HeLa cells labeled for 48 h with 5 μCi/mL of [^{3}H]uridine.

of the remaining small RNA species and the RNA polymerases involved. The present report analyzes the formation of these RNA species both in isolated HeLa cell nuclei in vitro and under a variety of different conditions in vivo. It is shown that there are two distinct families of small RNAs both in HeLa cells and *Drosophila* cells which differ in their mechanisms of formation.

Materials and Methods

Cell Culture. HeLa S3 cells were grown in suspension at a density of 4×10^5 cells/mL (Eagle, 1959). They were routinely tested by H.E.M. Research (Rockville, Md.) and found to be PPLO negative.

Schneider line 2 *Drosophila* cells, adapted for suspension growth, were cultured as described by Lengyel et al. (1974).

Cell Labeling and Fractionation. For in vivo metabolic studies HeLa cells were concentrated fivefold to 200×10^4 cells/mL. Cells were incubated with 50 μg/mL α-amanitin (Sigma) for 4 h, 0.1 μg/mL actinomycin D (Sigma) for 30 min, 1 μg/mL of camptothecin (N.C.I.) for 30 min, 5 μg/mL of toyocamycin (NCI) for 30 min, and exposed to the altered cell culture conditions of 43 °C for 15 min and a doubled tonicity of the media of 294 mM NaCl for 15 min, prior to labeling with [5-^{3}H]uridine (New England Nuclear, Boston) as described in the text. Cytoplasmic and nuclear fractions were prepared as previously described (Zieve and Penman, 1976).

Drosophila cells were concentrated fivefold to 1×10^7 cells/mL for in vivo metabolic studies. Cells were incubated with 50 μg/mL α-amanitin (Sigma) for 4 h or warmed to 37 °C for 10 min prior to the addition of [5-^{3}H]uridine (New England Nuclear, Boston) as described in the text. *Drosophila* cells were fractionated into cytoplasm and nuclei as described by Lengyel et al. (1974).

Labeling of RNA in HeLa Cell Nuclei in Vitro. HeLa cell nuclei were prepared and incubated for in vitro RNA synthesis as described by McReynolds and Penman (1974). After the incubation the nuclei were pelleted by centrifugation for 3 min at 2K in the Sorvall RC-3. The labeled RNA released from the nuclei into the supernatant was extracted with phenol and chloroform and then precipitated with 2 vol of ethanol.

Electrophoresis. Electrophoresis in 6–15% acrylamide gradient gels and 8% urea gels and fluorography were performed as previously described (Zieve and Penman, 1976).

Results

Synthesis of the Small RNA Species in Isolated Nuclei from HeLa Cells. Isolated HeLa cell nuclei synthesize RNA in limited amounts when incubated with all four triphosphates under the proper ionic conditions (Zylber and Penman, 1971; Marzluff et al., 1973). In particular, the polymerase III activity in these nuclei reinitiates and synthesizes new tRNA and 5S RNA, de novo (McReynolds and Penman, 1974; Udvardy and Seifart, 1976). The mycotoxin α-amanitin, a potent and selective inhibitor of RNA polymerases II and III, is used to determine which RNA polymerase is responsible for a specific RNA transcript.

The gel electrophoretic pattern in Figure 1 shows the distribution of small RNA molecules synthesized in isolated HeLa cell nuclei and released into the supernatant. In control nuclei incubated in the absence of α-amanitin the prominent RNA species seen are 5S RNA, tRNA, and species K and L, the class III small RNAs (Figure 1, lane 2). The identification of K and L with the in vivo products is based on comigration in both aqueous and urea gel systems (Figures 1 and 2). Under nondenaturing conditions, species L migrates in gels as a main band and one of several faster moving conformers (Zieve and Penman, 1976; Frederiksen et al., 1974, Figure 1). However, under denaturing conditions, species L migrates as a single band during gel electrophoresis (Figure 2).

The synthesis of tRNA precursor and 5S RNA as well as species K and L is resistant to low levels of α-amanitin (1 μg/mL) (Figure 1, lane 3) which inhibit polymerase II selectively (Kedinger et al. 1970). As expected for polymerase III

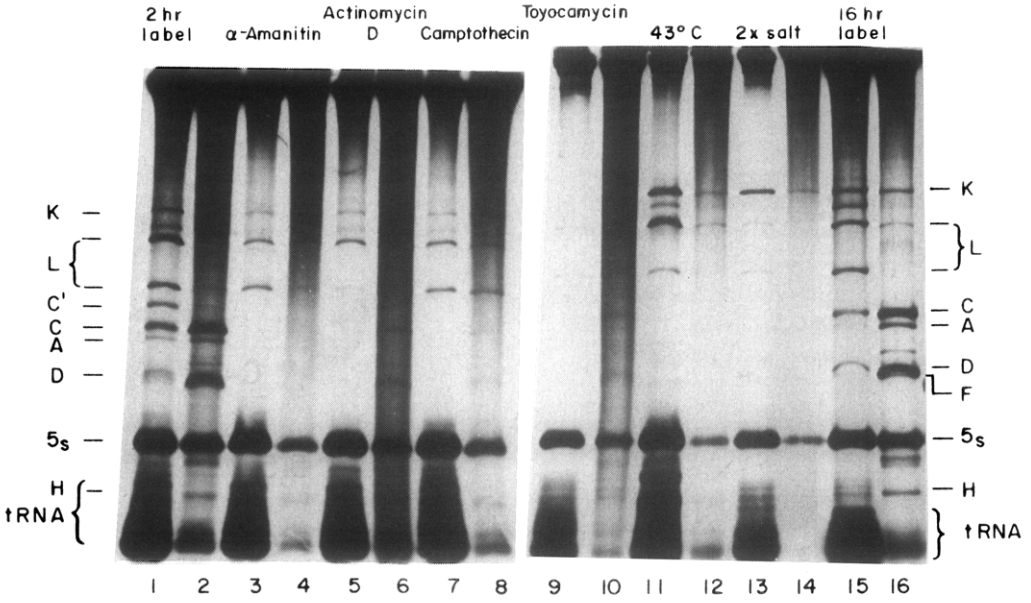

FIGURE 3: Resistance of class III RNAs to in vivo drug treatments and metabolic alterations. Each pair of lanes represents the small cytoplasmic and nuclear RNAs from 4×10^7 HeLa cells pretreated in the described manner and then labeled under those same conditions for 2 h with 40 μCi/mL of [^{3}H]uridine with the exception of lanes 15 and 16 which are from cells labeled for 16 h. Cytoplasmic and nuclear fractions were prepared and electrophoresed on 6–15% gradient slab gels as described in Materials and Methods. (1) Cytoplasmic and (2) nuclear fractions from control cells labeled for 2 h. (3) Cytoplasmic and (4) nuclear fractions from cells pretreated with 50 μg/mL α-amanitin for 4 h and then labeled for 2 h. (5) Cytoplasmic and (6) nuclear fractions from cells pretreated with 0.1 μg/mL actinomycin D for 30 min and then labeled for 2 h. (7) Cytoplasmic and (8) nuclear fractions from cells pretreated with 1 μg/mL camptothecin for 30 min and then labeled for 2 h. (9) Cytoplasmic and (10) nuclear fractions from cells pretreated with 5 μg/mL toyocamycin for 30 min and then labeled for 2 h. (11) Cytoplasmic and (12) nuclear fractions from cells elevated to 43 °C for 15 min and then labeled for 2 h. (13) Cytoplasmic and (14) nuclear fractions from cells with the tonicity of their media doubled to 294 mM NaCl for 15 min and then labeled for 2 h. (15) Cytoplasmic and (16) nuclear fractions from control cells labeled for 16 h.

products, their synthesis is completely inhibited by high levels of α-amanitin (150 μg/mL) (Figure 1, lane 4) (Weinman and Roeder, 1974). The insensitivity to low levels of α-amanitin but inhibition at high concentrations of α-amanitin indicates that the class III small RNAs which are formed in vitro are products of polymerase III.

The remaining species of small RNA (previously designated A, C, D, F, and H) are not synthesized in detectable amounts in isolated nuclei. The fact that they all are similar in this respect is an indication that they may all be formed by a similar mechanism. These species share a number of distinct properties and, in particular, their syntheses in vivo show similar sensitivities to inhibition by alterations in cell culture conditions and exposure of the cells to inhibitors of RNA synthesis. We identify them as forming a distinct class which is tentatively designated class I since a polymerase I type of activity may be involved in their formation.

G′, a methylated RNA with an electrophoretic mobility corresponding to 5 S (Weinberg and Penman, 1969), has not been analyzed in these experiments because of its close proximity to ribosomal 5S in our gel system.

Differential Inhibition of the Class I and III Small RNAs in Vivo. Several inhibitors of RNA synthesis preferentially affect the formation of class I RNA and have little or no effect on the synthesis of class III species. These include actinomycin D, α-amanitin, camptothecin, and toyocamycin. In addition, the exposure of cells to elevated temperature or to an increase in the tonicity of the culture medium severely inhibits class I synthesis relative to class III. The inhibition of class I RNA formation closely parallels the response of rRNA precursor synthesis. Figure 3 shows the patterns of small RNA synthesis in untreated cells labeled for 2 h and 16 h with [^{3}H]uridine and in cells pretreated in the six above-mentioned ways and then labeled for 2 h. These data, along with the responses of the other major classes of RNA (ribosomal, messenger and hnRNA) in the cell to these metabolic alterations, are tabulated in Table I.

Actinomycin D has been shown to have a differential effect on the synthesis of different RNA species in mammalian cells (Reich and Goldberg, 1964; Perry and Kelley, 1970), most likely by interacting with the DNA and interfering with the RNA polymerases (Muller and Cruthers, 1968). Ribosomal RNA is the most sensitive to inhibition by actinomycin and its synthesis is totally inhibited in HeLa cells by 0.04 μg/mL actinomycin D (Penman et al., 1968). At 0.1 μg/mL of actinomycin D, the class I small RNA species A, C, D, F, and H and the cytoplasmic precursors of C and D, C′ and D′ respectively (Zieve and Penman, 1976; Elicieri and Sayaveda, 1976) are almost totally inhibited (Figure 3 and Table I). Figure 3, lanes 5 and 6, shows that K, L and 5S RNA and tRNA are still synthesized at this concentration of the drug. Apparently the synthesis of the class III small RNAs, which are transcribed by polymerase III, is much less affected by actinomycin D.

The effect of α-amanitin in vivo is paradoxical. Although it has no effect on polymerase I in vitro (Seifart and Sekeris, 1969; Kedinger et al., 1970), in the intact cell α-amanitin has been shown to inhibit the synthesis of nucleolar RNA by polymerase I (Schmid and Sekeris, 1973; Jacob et al., 1970) in addition to inhibiting polymerase II. Tissue culture cells are not readily permeable to α-amanitin (Kuwano and Ikehara, 1973). However, if cells are incubated in the presence of relatively high concentrations of the drug for an extended time, sufficient amounts enter the cell to affect RNA synthesis. After a 5-h incubation in media containing 50 μg/mL α-amanitin, the synthesis of hnRNA by polymerase II is inhibited more than 95% (Table I). Under these conditions ribosomal RNA synthesis is also inhibited and there is an 85% reduction in the synthesis of the 45S ribosomal precursors (Table I). Figure 3 (lanes 3 and 4) shows that class I small RNA species A, C, D, F, and H are totally inhibited while the synthesis by polymerase

TABLE I: Inhibition of Synthesis of Different Classes of Cellular RNA in HeLa Cells by in Vivo Drug Treatments and Metabolic Alterations.[a]

	Labeling remaining after treatment (%)						
	50 μg/mL α-amanitin	0.1 μg/mL actinomycin D	1 μg/mL camptothecin	5 μg/mL toyocamycin	43 °C	2 × salt	Control
Class III RNAs							
K	80	45	75	55	125	180	100
L	90	40	80	50	105	65	100
5S, tRNA	80	80	90	80	115	75	100
Class I RNAs: A, C, D, H	<5	<10	<15	<15	<5	<5	100
45S rRNA	<15	<2	<2	<2	<10	<4	100
hnRNA	<5	90	55	10	85	20	100
A + mRNA	<5	15	35	15	20	20	100

[a] Cells were pretreated with the indicated drug treatments or metabolic alterations as described in the legend to Figure 3 and the syntheses of the small RNAs, 45S rRNA, hnRNA, and adenylated mRNA were determined. The amount of these different species of RNA labeled after the indicated pretreatment was expressed as a percentage of the labeling in the untreated controls. The labeling of the small RNAs was determined by labeling 4×10^6 HeLa cells for 2 h with [^{3}H]uridine and analyzing the cytoplasmic and nuclear fractions on 6–15% polyacrylamide slab gels as described in the legend to Figure 3. The amount of label incorporated into the small RNAs was determined by densitometry and was expressed as a percentage of the incorporation into untreated controls. 45S ribosomal RNA precursor synthesis was determined by pretreating cells in the indicated manner and then labeling 4×10^6 cells for 20 min with 10 μCi/mL of [^{3}H]uridine. Nucleoli were prepared according to the procedure of Weinberg and Penman (1968). Nucleolar RNA was analyzed on 15–30% sucrose gradients as described by Penman (1966) and the synthesis of labeled 45S RNA was expressed as a percentage of the label incorporated into 45S RNA in untreated controls. hnRNA synthesis was determined as the synthesis of nonribosomal nuclear RNA. Cells were pretreated in the indicated manner but in addition 0.04 μg/mL of actinomycin D was added 20 min prior to the addition of label to inhibit ribosomal RNA transcription (Penman et al., 1968). Cells were labeled with 10 μCi/mL of [^{3}H]uridine for 20 min and nuclei were prepared. The amount of Cl_3CCOOH-precipitable radioactivity in the nuclei was considered hnRNA. The amounts labeled were expressed as a percentage of the incorporation in untreated controls. A + mRNA synthesis was determined by pretreating 4×10^6 cells in the indicated fashion and then labeling for 2 h with 20 μCi/mL [^{3}H]uridine. A + mRNA was prepared according to the method of Singer and Penman (1973). The amount of A + mRNA labeled was expressed as a percentage of the incorporation in untreated controls.

III of the class III small RNAs, K, L, and 5S rRNA and tRNA, are relatively unaffected. Apparently the amount of α-amanitin which enters the cell is insufficient to inhibit polymerase III.

The metabolic inhibitors camptothecin and toyocamycin also have a greater effect on the appearance of the class I small RNA species A, C, D, F, and H and their cytoplasmic precursors C′ and D′ than on the synthesis of the class III small RNAs. The plant alkaloid camptothecin rapidly inhibits DNA synthesis and interrupts high molecular weight RNA transcription resulting in the formation of aberrantly shortened molecules which are not properly processed (Abelson and Penman, 1973; Wu et al., 1971) while leaving mitochondrial macromolecular processes unaffected (Abelson and Penman, 1972). The adenosine analogue toyocamycin is incorporated into newly synthesized RNA and at the proper concentration selectively inhibits rRNA synthesis by interfering with the processing of the 45S rRNA precursor (Tavitan et al., 1968; Sverak et al., 1970). Figure 3, lanes 7–10, shows that at moderate concentrations of these drugs the class I small RNA species are strongly inhibited while the class III RNA species K, L, 5S RNA and tRNA are still synthesized. hnRNA synthesis and message production continues, although at reduced rates (Table I), but ribosomal RNA synthesis is totally inhibited by these agents. As in the previous experiments, the inhibition of the class I small RNAs is strongly correlated with the inhibition of ribosomal precursor synthesis which is known to be transcribed by polymerase I (Zylber and Penman, 1971; Roeder and Rutter, 1970). This correlation was also found in two alterations of the cell culture conditions that drastically affect cellular RNA metabolism.

When cells are incubated at an elevated temperature of 43 °C, the class I small RNAs are completely inhibited while the class III RNAs are still synthesized (Figure 4, lanes 11 and 12).

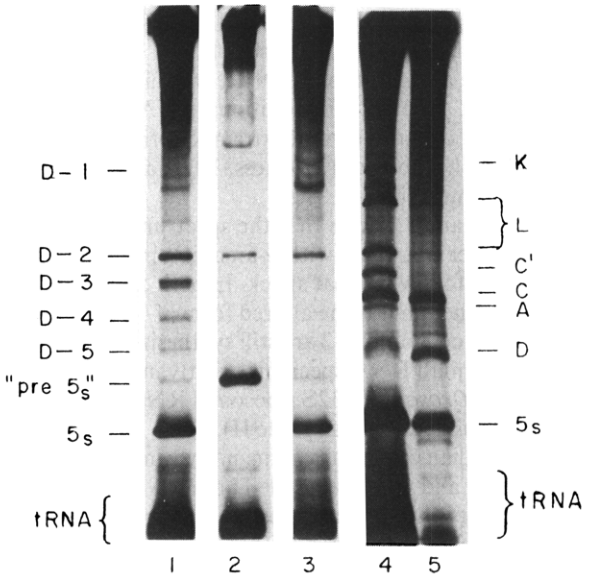

FIGURE 4: Small RNAs of *Drosophila melanogaster*. Lanes 1, 2, and 3 represent the cytoplasmic fraction of 3×10^7 *Drosophila* cells pretreated as indicated and then labeled under the same conditions for 2 h with 40 μCi/mL of [^{3}H]uridine. Over 80% of the small RNAs of *Drosophila* cells appear in the cytoplasmic fraction of an aqueous fractionation. Lanes 4 and 5 represent small RNAs from 4×10^6 HeLa cells. (1) Cytoplasmic fraction from control cells labeled for 2 h. (2) Cytoplasmic fraction from cells elevated to 37 °C for 10 min and then labeled for 2 h. (3) Cytoplasmic fraction from cells pretreated with 50 μg/mL of α-amanitin for 4 h and then labeled for 2 h. (4) Cytoplasmic and (5) nuclear fraction from HeLa cells labeled for 2 h.

At this temperature hnRNA and mRNA are still produced, although at reduced rates (Table I); however, rRNA synthesis is totally inhibited. As before, the synthesis of class III small

RNAs by polymerase III is very little affected by the alteration in cell metabolism, while the production of class I small RNA and rRNA precursor is coordinately suppressed.

Another metabolic alteration was effected by an increase in tonicity of the media. Pederson and Robbins (1970) showed that exposure of cells to increased ionic strength caused alterations in cell metabolism similar to the changes that take place during mitosis. Chromatin condenses, the nuclear envelope is dispersed, and there are significant shifts in the pattern of RNA synthesis. When the tonicity of the media is twice normal, rRNA synthesis is totally inhibited, hnRNA synthesis is reduced, and 5S RNA and tRNA are synthesized at close to the rates in control cells. Figure 3 (lane 13 and 14) and Table I show that under these conditions the class I of small RNAs are totally inhibited, while the class III species K and L together with 5S RNA and tRNA are still synthesized. The response of species K is unusual in that it is synthesized at twice the rates found in controls under these conditions. Here again, the inhibition of synthesis of the class I small RNAs correlates with the inhibition of rRNA transcription.

The Small RNAs of Drosophila melanogaster. Drosophila melanogaster has a spectrum of small RNA species that are dissimilar (in electrophoretic mobilities) to those of mammalian cells. Figure 4 shows an electropherogram of the small RNA species of *Drosophila* cells run with HeLa cell markers and their response to elevated temperature and pretreatment with α-amanitin in vivo. The nomenclature used is that of Spradling et al. (1977) with some additions.

The response of *Drosophila melanogaster* RNA metabolism following a sudden increase in temperature (heat shock) has been widely studied (Spradling et al., 1977; Rubin and Hogness, 1975; McKenzie et al., 1975). There is a drastic reduction in the production of cytoplasmic RNA with the continued production of only a limited spectrum of RNA molecules. These include a small set of mRNAs, and mitochondrial RNA (Spradling et al., 1977). In addition, tRNA, a novel form of 5S RNA that contains several extra nucleotides and one small RNA species (Rubin and Hogness, 1975; and Figure 4, lane 2) are still synthesized.

Figure 4, lane 3, shows that the spectrum of small RNAs produced after treatment with α-amanitin in vivo is similar to the response following heat shock. In both cases, tRNA, and 5S RNA, although it is the altered form of 5S RNA found in heat shocked cells, and D-2 are still synthesized. The synthesis of the other small RNA species is greatly inhibited. The synthesis of the *Drosophila* 32S ribosomal RNA precursor is inhibited by both treatments (Table II). These data suggest that, as in mammalian cells, α-amanitin, in vivo, interferes with both polymerase I and polymerase II. Those RNAs synthesized in the presence of this drug are most likely polymerase III products. This included 5S RNA, tRNA, and, in the case of *Drosophila*, one higher molecular weight small RNA designated D-2. Also, it is apparent that the small RNAs produced after heat shock are the same products obtained with α-amanitin and that the syntheses of the other small RNAs are inhibited. This response is similar to the HeLa cells where the synthesis of polymerase III products is the most resistant to elevated temperature and α-amanitin.

TABLE II: Inhibition of *Drosophila* Ribosomal RNA Transcription.[a]

	32S ribosomal RNA (%)
Control	100
37 °C	10
α-Amanitin	35

[a] The 2×10^7 *Drosophila* cells were concentrated fivefold to 1×10^7 cells/mL. Cells were pretreated with 50 μg/mL α-amanitin for 4 h and by elevations of the culture media to 37 °C for 10 min. The cells were then labeled for 15 min with 10 μCi/mL of [^{3}H]uridine. Cells were then fractionated and the nuclear RNA was analyzed on sodium dodecyl sulfate–sucrose gradients as described by Lengyel and Penman (1975) and in Materials and Methods. The amount of label incorporated into the 32S rRNA precursor was quantitated and expressed as a percentage of the label incorporated into 32S rRNA in the untreated control.

Discussion

The in vivo and in vitro results indicate that the HeLa cell small RNA species K and L are products of polymerase III along with 5S RNA and tRNA. This was shown in vitro in isolated nuclei where the synthesis of these "class III" small RNAs is resistant to low levels of α-amanitin but sensitive to high levels. In addition, these four species of RNA showed similar resistance to a variety of metabolic alterations in vivo that drastically affected the synthesis of other classes of RNA in the cell, especially the rRNA precursor and class I small RNA. Polymerase III apparently continues to transcribe class III RNAs under conditions where polymerase I and polymerase II activity is reduced by varying degrees. In addition to the metabolic alterations discussed in this paper, polymerase III has been shown to be active during mitosis when the other polymerases are both inactive (Zylber and Penman, 1971). Apparently, polymerase III continues to function under a variety of conditions where the other RNA polymerases in the cell are largely inhibited.

All of the five class I small RNAs, species A, C, D, F, and H, appear to be formed by mechanisms having identical sensitivities to inhibitors of RNA synthesis and to alterations in cell metabolism produced by elevated temperature and high external tonicity. In addition, the in vivo studies have shown a strong correlation between the inhibition of the synthesis of the class I small RNAs and the inhibition of transcription of nucleolar RNA. This inhibition occurs under conditions where hnRNA is often still transcribed by polymerase II and mRNA appears in the cytoplasm and where polymerase III products are still made at nearly normal rates. These data suggest the possibility that the class I small RNAs are synthesized by polymerase I and that the inhibitions studied may interfere with the transcription of the class I small RNAs by polymerase I, although proof will be possible only when the precursors to these molecules are identified. It is also possible that these various treatments prevent the formation of these small RNAs by interfering with their processing from the putative precursor which could be synthesized by any of the polymerases in the cell. However, it is difficult to understand the sensitivity to transcription inhibitors if processing is the principal target.

The data obtained with *Drosophila* cells indicates that tRNA, 5S RNA, and D-2 are similar to the HeLa cell class III RNAs in that their synthesis is resistant to high temperatures and α-amanitin. Based on the results with HeLa cells, it would be expected these RNAs would be products of polymerase III, although studies using isolated nuclei have not been carried out with the *Drosophila* cells. Species D-2 is similar to HeLa cells species K and L in that it is not methylated (R. Levis, personal communication). The other *Drosophila* small RNAs whose synthesis is sensitive to high temperature and α-amanitin are all methylated. The spectrum of small RNAs in *Drosophila* cells is obviously quite different from that seen in mammalian cells and little is known about their function.

The data presented here show that the small RNA species of the HeLa and *Drosophila* cells can be grouped into two

distinct classes. In HeLa cells, the group III small RNAs which include species, K, L, 5S RNA, and tRNA are products of polymerase III and their transcription is relatively resistant to alterations in normal cell metabolism. The group I small RNAs which include species A, C, D, F, and H are regulated in a different manner and their synthesis is inhibited by six treatments that also inhibit rRNA precursor synthesis by polymerase I but leave the synthesis of the class III small RNAs unaffected. *Drosophila* cells have a different spectrum of small RNAs but they also can be grouped into two families that have different sensitivities to α-amanitin and high temperature.

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