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Metabolism of Poly(A) in Plant Cells

DISCRETE CLASSES ASSOCIATED WITH FREE AND MEMBRANE-BOUND POLYSOMES[1]

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ABSTRACT

In the subapical region of dark-grown pea epicotyls about 40% of the total polysomes are associated with membranes. The presence of poly(A) in polysomal mRNA was detected by hybridization of unlabeled RNA with ^{3}H-poly(U). Both free mRNA and messenger ribonucleoprotein particles in polysomes hybridize with ^{3}H-poly(U) quantitatively. The binding of ^{3}H-poly(U) to polysomes is increased by treatment with the detergent sodium dodecyl sulfate. Since detergent influenced the ^{3}H-poly(U) binding more in membrane-bound polysomes than in free, there may be more protein(s) associated with the poly(A) portion of the mRNA in membrane-bound polysomes. Analysis of the poly(A) segments isolated from the mRNA of these two classes of polysomes indicates that there are discrete classes of poly(A) and they appear to be differentially associated with free and membrane-bound polysomes. Mean size distribution of poly(A) in free polysomes is larger than in membrane-bound polysomes.

Following treatment (2 days) with the plant growth hormone indoleacetic acid, there is a gradual decrease in the mean length of total poly(A), which appears to correspond to a decrease in the size of the polysomes and their associated mRNA.

Poly(A) sequences 50 to 200 nucleotides long have been found attached to the 3'-end of most eucaryotic mRNA from animal cells (5, 8, 13, 16) as well as in plant cells (11, 17, 22). However, its exact role in RNA metabolism is not yet understood. Although there is a correlation between the length of poly(A) in mRNA and the age of the message (9, 23), neither the stability nor the translational capacity of mRNA appears to depend on its presence (2). Under steady state conditions, the poly(A) population is heterogeneous (8). The poly(A) profiles generally reveal one major peak (corresponding to approximately 150 nucleotides), but in some cases discrete populations of lower mean sizes have been observed (7). Whether these classes represent functional entities or degradation products remains to be determined.

Poly(A) has been reported (1, 15, 18) to be associated with mRNA from both free and membrane-bound polyribosomes. The poly(A) of membrane-bound polysomes is tightly attached to the membrane and is almost the same average length as poly(A) of the total cytoplasmic mRNA. Since the size distribution of poly(A) is apparently similar in these two polysome populations, it was concluded that poly(A) is not involved in directing mRNA into one particular group of polysomes.

The present study indicates the existence of several discrete size classes of poly(A) which are differentially associated with mRNA of both free and membrane-bound polysomes in pea epicotyls. The poly(A) segment of the mRNA in membrane-bound polysomes appears to be more masked by proteins than that of free polysomes. This re-opens the possibility (1) that the size of the poly(A) strand may influence the discriminatory binding of proteins and direct mRNA to one or the other class of polysomes.

Treatment of pea epicotyls with the plant growth hormone, IAA, results in the induction of specific species of mRNA (24) and a concomitant increase in the net amount of polysomes/cell. After 3 to 4 days, the supply of IAA is probably exhausted and the percentage of ribosomes in polysomes decreases. There is a selective loss of large size polysomes which appears to be closely related to a decrease in mRNA template. In the present study, this system is used for assaying the metabolism of poly(A)-RNA during hormone-induced growth and eventual senescence. We found that the average length of poly(A) gradually decreases after 48 hr of IAA treatment and appears to be correlated with the decrease in the size of polysomes and their associated mRNA.

MATERIALS AND METHODS

Growth and Treatment of Plants. Seeds of *Pisum sativum* L. var. Alaska were grown in darkness as described previously (4). Seven-day-old seedlings in which the third internode was 3 to 5 cm long were decapitated and 0.5% (w/w) IAA in lanolin paste was applied to the apices. Growth continued in darkness by lateral cell expansion (swelling) with little cell elongation. Subapical segments (1 cm) were excised at different time intervals after hormone treatment and used for isolation of poly(A)-RNA.

Isolation of Polysomes. Tissue segments from control and IAA-treated epicotyls were frozen in liquid N_2 immediately after excision, ground with a mortar, and homogenized in 6 ml of "polysomal extraction medium" (150 mM tris-acetate, pH 8.5, 200 mM sucrose, 50 mM KCl, 20 mM magnesium acetate, and 0.4% Nonidet P-40 [Shell Chemical Co.]). The slurry was centrifuged for 10 min at 23,000*g* and the postmitochondrial supernatant was layered on a 2-ml sucrose cushion (containing 1.5 M sucrose, 50 mM tris-acetate, pH 8.5, 20 mM KCl, and 10 mM magnesium acetate) and centrifuged for 90 min at 105,000*g*. Using these techniques, undegraded polysomes and mRNA are obtained (24, 26).

Isolation of Free and Membrane-bound Polysomes. These two classes of polysomes were isolated by a modification of the above procedure. The frozen tissue was first homogenized in 6 ml of a solution containing 50 mM tris-acetate, pH 8.5, 50 mM KCl, 20 mM magnesium acetate, and 200 mM sucrose. The homogenate was centrifuged (27,000*g*, 10 min) to yield a pellet containing membrane-bound material and a supernatant containing free polysomes. The pellet was re-extracted in 6 ml of the polysomal extraction medium. Both supernatants were layered on 1.5-ml sucrose cushions and the polysomes were obtained as described

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above. Total polysomal RNA was extracted from these preparations as described previously (24, 26). The concentration of RNA was determined by measuring the absorbance at 254 nm (20 *A* units = 1 mg RNA/ml).

Isolation of Poly(A). Total polysomal RNA was incubated for 30 min at 30 C in 0.1 ml ribonuclease (5 μg/ml pancreatic and 10 units T_1 RNase, 100 mM NaCl, 10 mM EDTA, and 10 mM tris-acetate, pH 7.4). After incubation, RNA was re-extracted with phenol-chloroform. It was precipitated by the addition of 2.5 volumes of ethanol and 2% potassium acetate, pH 5.5 (50 μg wheat germ tRNA added as carrier), and left overnight at −20 C. The poly(A) obtained by centrifugation at 17,000*g* for 10 min was dried and dissolved in 50 μl of a buffer containing 40 mM tris-acetate, pH 7.8, 20 mM sodium acetate, and 2 mM EDTA. The samples were layered on 10% polyacrylamide gels (0.6 × 8 cm) and electrophoresed in 0.04 M tris-acetate, pH 7.8, 0.02 M sodium acetate, and 2 mM EDTA at 3 mamp/gel. The gels were frozen in dry ice and fractionated into 2-mm slices with a Brinkman gel slicer. The slices were eluted overnight in 0.5 ml of buffer (10 mM tris-HCl, pH 7.8, 200 mM NaCl, and 5 mM magnesium chloride) and assayed for the presence of poly(A). Mean length of poly(A) was calculated on the basis of *Escherichia coli* 4S and 5S RNA markers using an equation similar to that described by Palmiter (19).

^{3}H-Poly(U) Hybridization Assay. Aliquots of each fraction were added to 50 μl of a reaction mixture containing 100 mM tris-acetate, pH 7.6, 2 M NaCl, 50 mM $MgCl_2$, and 0.01 μCi/ml ^{3}H-poly(U) (47 μCi/mol phosphate) and brought to a final volume of 0.5 ml. After incubation at 25 C for 15 min, 20 μl pancreatic RNAse (20 μg/ml) were added to each of the tubes and the incubation was continued for 30 min. Reactions were stopped by the addition of 1 ml 15% trichloroacetic acid and 3 ml of 5% trichloroacetic acid (100 μg wheat germ tRNA added as carrier). The cold trichloroacetic acid-precipitable material was collected on Whatman GF/C filters and washed with 5% trichloroacetic acid. Radioactivity was measured in 10 ml scintillation fluid (5 g PPO and 30 mg POPOP/l toluene) in a Beckman CPM-100 scintillation counter. When polysomes were treated with SDS before hybridization to poly(U), the ribonuclease concentration was increased 2-fold since SDS inhibits some ribonuclease activity.

RESULTS

Free and Membrane-bound Polysomes in Pea Epicotyls. Two classes of polysomes were isolated from the apical regions of dark-grown pea epicotyls as described under "Materials and Methods," and analyzed on sucrose gradients. Approximately 40% of total ribosomes are associated with membranes (24). The bound population (Fig. 1B) appears to be composed of polysomes with a smaller mean size distribution as compared to free (Fig. 1A). The question is whether this reflects the native configuration of polysomes or arises due to degradation during isolation. The particular conditions used for isolation of these polysomes have been shown (6) to be highly resistant to ribonuclease action, *i.e.* even if purified ribonuclease is added during homogenization, it does not cause significant damage to the polysomes. Using this technique, we have isolated polysomes from several plant tissues (24–26) and have obtained intact translatable mRNA for specific proteins (24, 26). Since the conditions used for isolation of membrane-bound ribosomes were even more inimical to ribonuclease action, *i.e.* the tris concentration was raised from 0.05 to 0.15 M, the observed profiles (Fig. 1B) may not be attributed to degradation by endogenous nucleases (see also "Discussion"). Therefore, it appears that free and bound polysomes are distinct populations which represent the *in vivo* situation in actively growing pea epicotyl tissue. We have demonstrated (24) that the latter population specifically contains a hormone-induced message in this tissue.

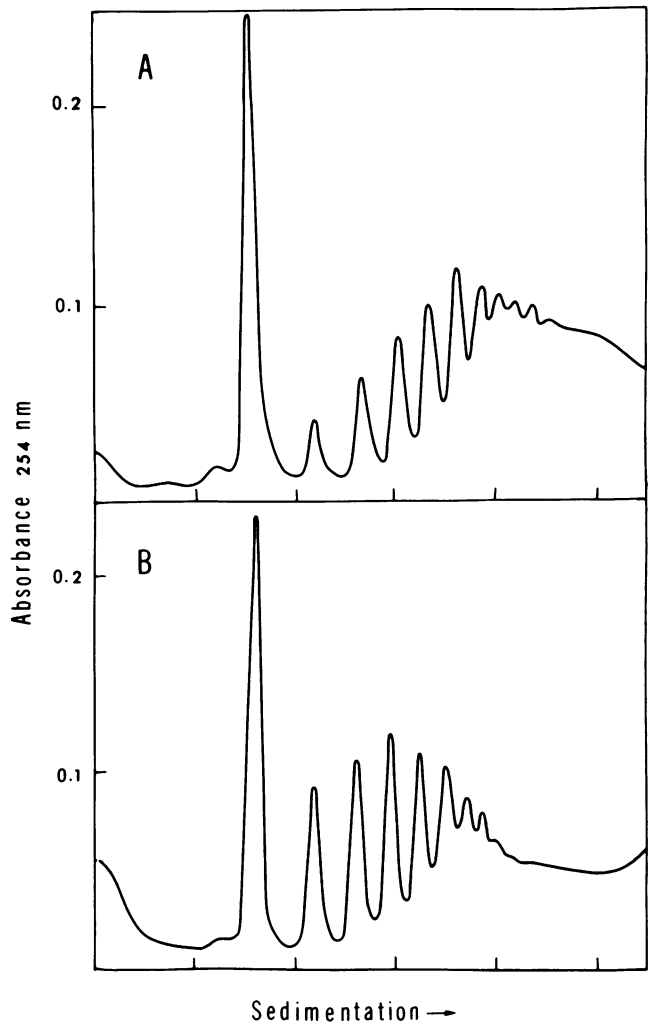

FIG. 1. Sucrose gradient analysis of free (A) and membrane-bound (B) polysomes of dark-grown pea epicotyls. The polysomes are prepared as described under "Materials and Methods" and 50 to 150 μg of ribosomes were layered on 10 to 30% linear sucrose gradients and centrifuged for 45 min in a Beckman SW 50.1 rotor. The optical profile at 254 nm was monitored through an ISCO UV analyzer.

Poly(A) in mRNA of Free and Membrane-bound Polysomes. In pea epicotyls it is difficult to label RNA *in vivo* to a high specific radioactivity. In order to detect the presence of poly(A) in mRNA, ^{3}H-poly(U) was quantitatively hybridized *in vitro* to unlabeled mRNA (24, 26). Using this technique, we attempted to locate the poly(A) containing mRNA in free and membrane-bound polysomes. Figure 2A shows that mRNA in both membrane-bound and free polysomes hybridizes with ^{3}H-poly(U) quantitatively over a wide range of concentrations, indicating the presence of poly(A) in mRNA of both classes of polysomes. Since the percentage of total ribosomes in polysomes is similar in both free and membrane-bound polysomes (Fig. 1), assuming that they bear the same size of mRNA, they would be expected to bind equal amounts of poly(U)/unit of polysomes. However, Figure 2A shows that the membrane-bound polysomes hybridize less poly(U)/unit of polysomes than free polysomes. This could be due either to a smaller mean size distribution of poly(A) in mRNA of membrane-bound than in free polysomes, or to its inaccessibility to hybridization, *i.e.* masking by protein(s).

We tested the effect of detergent SDS on the hybridization of

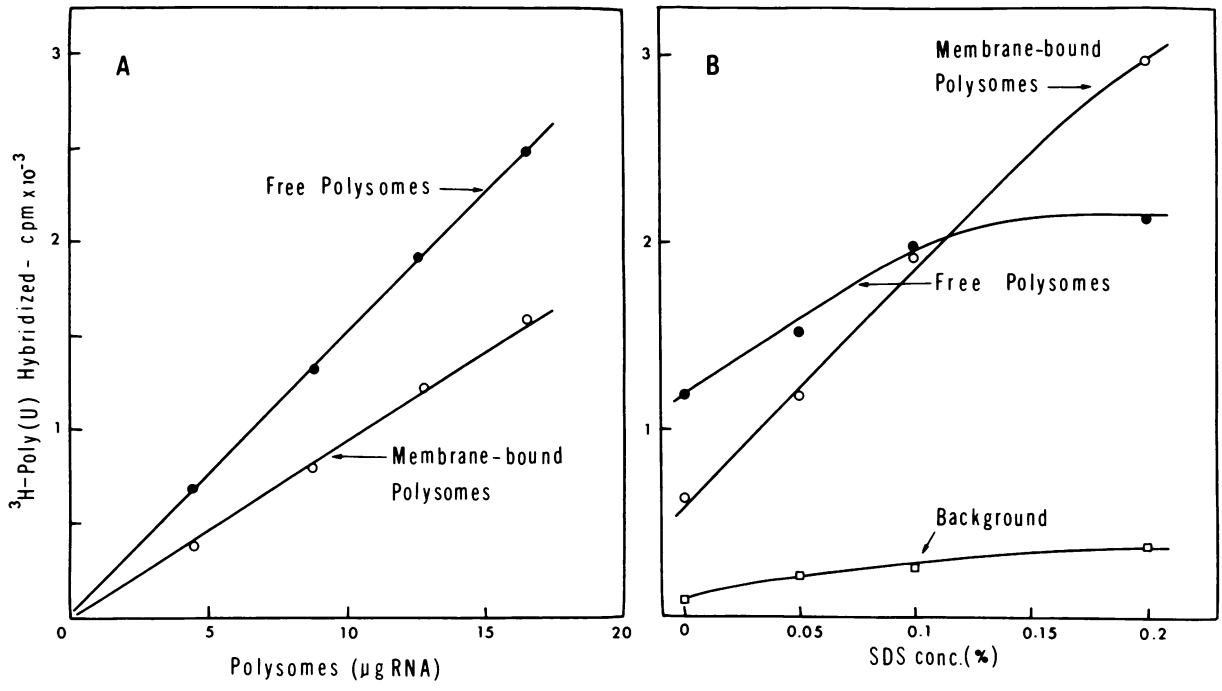

FIG. 2. A: ^{3}H-poly(U) hybridization with mRNA in free and membrane-bound polysomes. Polysomes were prepared as in Fig. 1 and an aliquot was hybridized with ^{3}H-poly(U). Cold trichloroacetic acid-precipitable counts were measured on GF/C filters. B: effect of sodium dodecyl sulfate on the hybridization of ^{3}H-poly(U) with free and membrane-bound polysomes. Equal aliquots of polysomes (10 μg RNA) were hybridized with poly(U) in the presence of increasing concentrations of SDS. Note the effect of detergent on the background since it interferes with ribonuclease digestion. Values for background were subtracted from those for polysomes.

poly(U) to the total free and membrane-bound polysomes. Free polysomes (Fig. 2B) show only a small increase in poly(U) binding with increasing concentrations of detergent, while membrane-bound polysomes show a linear increase up to 0.2% of SDS. Higher concentrations of this detergent become inhibitory for this assay. This suggests that at least some of the increased accessibility to poly(U) hybridization of membrane-bound polysomes is due to proteins which can be dissociated from the poly(A) part of mRNA.

Size of Poly(A) in mRNA from Free and Membrane-bound Polysomes. The size of poly (A) segments in the two classes of polysomes was measured using acrylamide gels after digestion of mRNA by nucleases. The presence of poly(A) was detected by ^{3}H-poly(U) hybridization. Figure 3 shows that poly(A) exists in discrete size classes which have different distributions in free and membrane-bound fractions (*cf.* 1 and 18). In contrast, total poly(A) from most eucaryotic mRNA appears to be heterogeneous. Similarly, in this case, if poly(A) is derived from a total population of polysomes (free plus membrane-bound), the distinctions between size classes is less evident (data not shown).

The first high mol wt peak, corresponding to a poly(A) of approximately 200 nucleotides, is a major component in mRNA of free polysomes while a minor component in membrane-bound polysomes. There are at least three discrete size class components of poly(A) with a mobility lower than that of 5S RNA. The ratio of poly(A) size classes which have a mean size of less than 5S to those which are more than 5S is 1.7 in free and 4.5 in membrane-bound mRNA. The mean number of nucleotides is approximately 145 in free and 100 in membrane-bound polysomes.

In order to rule out the possibility that degradation of poly(A) or contamination from organelles accounts for its smaller mean size in membrane-bound polysome fractions, the following control experiment was carried out. L-cells were labeled with ^{14}C-adenosine and mixed with unlabeled pea tissue before homogenization. Both were homogenized and processed together. The

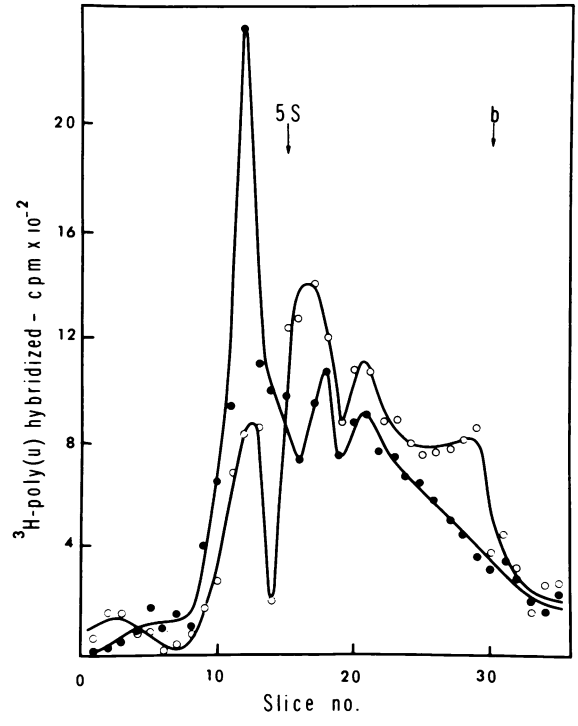

FIG. 3. Size distribution of poly(A) from mRNA of free and membrane-bound polysomes. The mRNA was isolated from polysomes and treated with T_1 and pancreatic ribonuclease to obtain poly(A) segments which were then analyzed in 10% acrylamide gels. Gels were cut into 2-mm slices which were eluted with binding buffer and hybridized with ^{3}H-poly(U). ^{14}C-Labeled 4S and 5S RNA were used as markers. b: bromophenol blue; ●: poly(A) in mRNA of free polysomes; ○: poly(A) in mRNA of membrane-bound polysomes.

RNA was isolated and poly(A) segments were analyzed on gels. The results of this experiment (Fig. 4) indicate that no breakdown of L-cell poly(A) occurs during coextraction with pea tissue and the techniques used do not show any mitochondrial contamination. It should be noted that if a labeled homo-oligonucleotide such as ^{3}H-poly(A) is added to pea extract, complete breakdown occurs in 2 to 5 min. This suggests that free naked RNA is highly accessible to ribonucleases which are present in plant tissues. However, the messenger ribonucleoprotein particles which are the natural form of message are less susceptible under these conditions (Fig. 4). Thus the presence of the discrete size classes of poly(A) in both free and membrane-bound polysomes does not appear to be due to degradation during isolation. Furthermore, small poly(A) segments are due neither to mitochondrial contamination (as shown by above experiment) nor any contamination from chloroplasts since this tissue does not have an appreciable amount of chloroplasts at this stage of development.

The existence of discrete size classes of poly(A) in pea epicotyls was confirmed by another method. The mRNA was labeled at the 3'-OH terminal with ^{3}H-borohydride and was subsequently processed with ribonucleases to obtain individually labeled poly(A) segments. The size distribution of such labeled poly(A) derived from free and membrane-bound polysomes was qualitatively similar to that in Figure 3 (data not shown). However, the relative sizes of the peaks obtained were not the same in the two experiments since, in the hybridization assay of poly(A), the poly(U) binding in each peak is proportional to the number of adenylic acid residues and not to the number of poly(A) tracts.

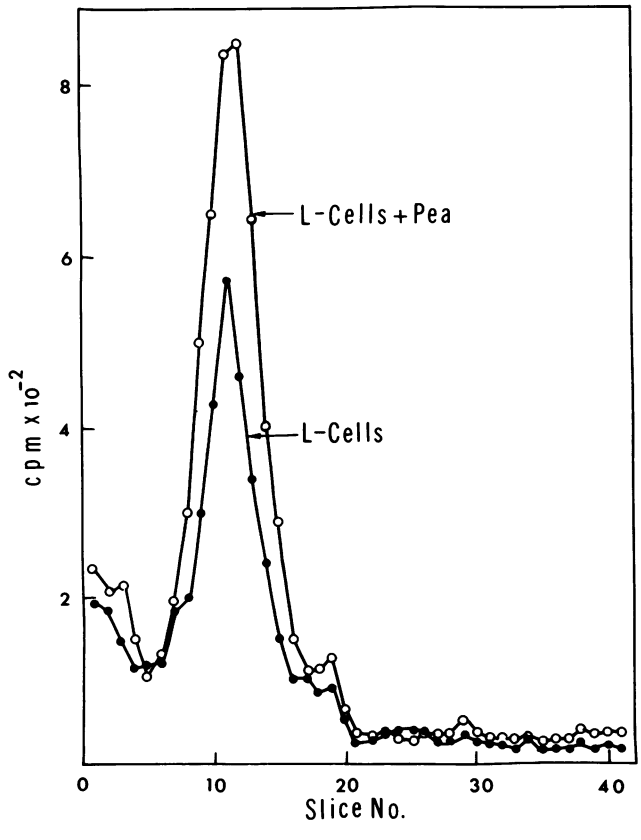

FIG. 4. Poly(A) size distribution in L-cells and effect of co-extraction and processing with pea tissue. L-cells were incubated with ^{14}C-adenosine (0.1 μCi/ml) for 1 hr. RNA was isolated directly from half of the cells, and the other half were mixed with pea tissue and co-extracted. Samples were processed as in Fig. 5. Note that the presence of pea RNA results in a higher yield of L-cell poly(A) and no degradation occurs.

Polysome Population following Treatment with Growth Hormone. Treatment of pea epicotyl with the auxin type of plant growth hormone, IAA, results in an increase in both RNA and protein. In control tissues 80 to 90% of the total ribosomes are present in polysomes (Fig. 1 and ref. 24). This proportion stays relatively high up to 48 hr after hormone treatment, although a net increase in polysomes occurs as a result of both ribosome and mRNA synthesis. While ribosome synthesis continues for several days, the amount of polysomes/segment starts to decline after 48 hr and within 5 days there are only about 30% of the ribosomes in polysomes as compared to zero time (Table I). This is also accompanied by a decrease in the mean size of polysomes (Fig. 5) and a gradual reduction in the larger size polysomes without a proportional increase in small polysomes. Total polysomes were isolated and no attempt was made to resolve free and membrane-bound fractions (Table I and Fig. 5).

The decrease in polysome size distribution is not due to mRNA degradation during isolation (see above). Treatment of intact pea epicotyls with cycloheximide before harvesting, at a dose which inhibits about 50% protein synthesis, does not change the size distribution of polysomes, indicating that peptide chain initiation is not limiting under these conditions, [also (24)]. This could be due to either a selective loss of larger mRNA in hormone-treated tissue and/or a synthesis of mRNA with a relatively small mean size.

Metabolism of Poly(A)-RNA following Treatment with Hormone. Total polysomal RNA (including free and membrane-bound) was extracted from pea epicotyls that had been treated with IAA up to 5 days. Poly(A) was isolated by RNAse treatment, separated according to size by gel electrophoresis, and assayed by ^{3}H-poly(U) hybridization. There is no significant change in the length of the poly(A), or in amount/unit RNA up to 2 days after hormone treatment (Table I). After 2 days there is a gradual decrease in the mean length and amount of poly(A)/unit RNA. Within 5 days, the mean number of nucleotides in poly(A) decreases from approximately 145 to 100 (as measured by gel electrophoresis, data not shown) concurrently with the decrease in size of polysomes (Fig. 5).

Table I. *^{3}H-Poly(U) Binding to Total Polyribosomal RNA following Treatment with Hormone*

Time after IAA treatment	Ribosomes in polysomes	Mean length of poly(A)	^{3}H-poly(U) bound/100 µg RNA[a]	Expected binding	Excessive binding	Increase in mRNA molecules[b]
(days)	(%)	(nucleotides)	(cpm)	(cpm)	(cpm)	(fold)
0	86.3	141	6629	6629	-	0
1	79.5	139	6876	6106	770	1.1
3	47.0	129	4935	3646	1289	1.5
5	26.7	108	4144	2050	2094	2.6

[a] Total polysomal RNA (including free and membrane-bound) was extracted from ribosomal pellets following hormone treatment and ^{3}H-poly(U) was hybridized as under "Materials and Methods."

[b] If the mean size of polysomes and length of poly(A) in mRNA does not change after hormone treatment, the ^{3}H-poly(U) binding would be proportional to the percentage of ribosomes in polysomes. However, this relationship was not observed, *i.e.* there is excessive binding as percentage and size of polysomes decline. Since the size of poly(A) decreases after hormone treatment, the expected binding should be further lowered proportionately with the decline in mean length of poly(A). The relative number of mRNA molecules can be calculated as follows: Relative no. of mRNA molecules, *e.g.* at 5 days after hormone treatment.

$$\frac{\text{Observed binding (4144)}}{\text{Expected binding (2050)}} \times \frac{\text{length of poly(A) at 5 days (108)}}{\text{length of poly(A) at 0 time (141)}} = 2.6$$

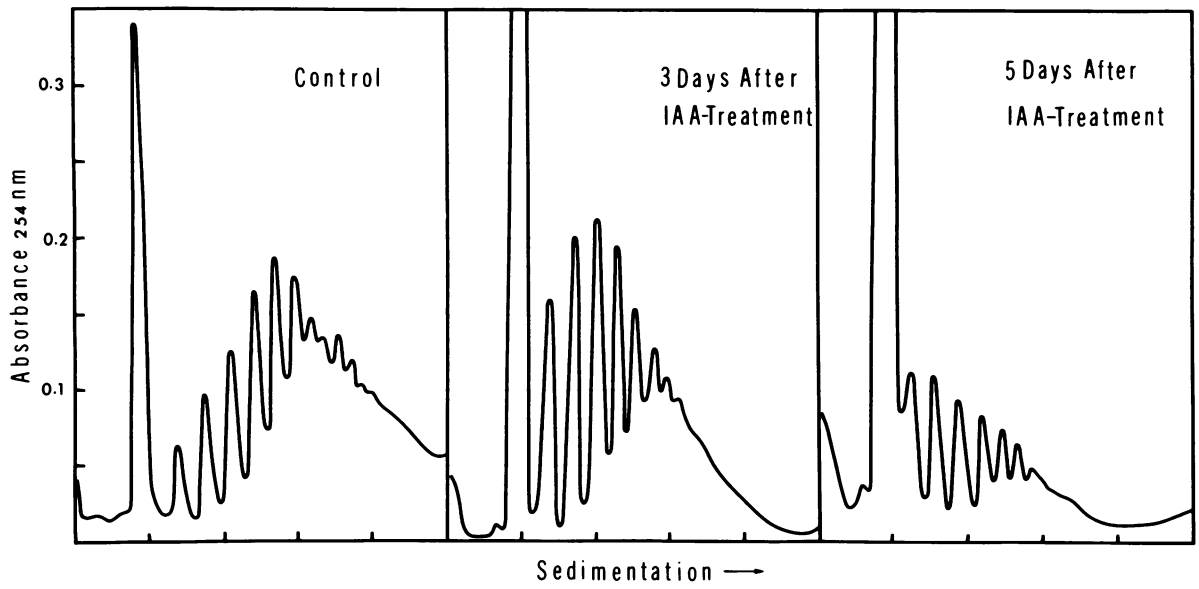

FIG. 5. Size distribution of polysomes following treatment with IAA. Total polysomes were isolated and an aliquot (100 μg RNA) was analyzed on sucrose gradients. The area of profiles representing polysomes is calculated as a percentage of total ribosomes (see Table I).

The binding of ^{3}H-poly(U) to total polysomal RNA after hormone treatment does not decrease proportionately to the percentage of ribosomes in polysomes (Table I). Since the mean size of poly(A) is smaller in hormone-treated tissue, the higher poly(U) binding could not be due to an increase in the number of poly(A) containing messages/unit polysomes. Since small polysomes would have a greater number of messages/ribosome, they would be expected to show more binding of ^{3}H-poly(U)/unit of polysomal RNA. When ^{3}H-poly(U) was hybridized across the polysome gradient, indeed a higher specific radioactivity was observed in small polysomes and very little poly(A) was found in monosomes. From the data (Table I), it can be calculated that the number of poly(A) containing mRNA molecules in polysomes increases approximately 2- to 2.5-fold in 5 days as a result of hormone treatment. Due to the difficulty of pulse and chase labeling in these experiments, it was not possible to distinguish between the loss of larger mRNA template and the selective formation of smaller mRNA. Furthermore, no attempt was made to assess the amount of nonpolyadenylated mRNA with and without hormone treatment. In any event, a population of small messages bearing a short poly(A) strand which is associated with small polysomes appears with time.

DISCUSSION

Most studies on poly(A) in plant cells have been confined to total cellular or polysomal RNA. Since a large percentage (up to 40%) of the polysomes in pea epicotyl are associated with membrane (24), we attempted to analyze the poly(A) content of mRNA from free and membrane-bound polysomes and to determine the relationship between the size of mRNA and the length of poly(A) in the two classes of polysomes. Under steady state conditions, the sizes of the poly(A) segments in mRNA of free and membrane-bound polysomes exist as discrete populations. The mean size distribution of the polysomes in the membrane-bound fraction is smaller than that in free, which is not due to lack of initiation.

The binding of ^{3}H-poly(U) can be achieved not only to free mRNA or poly(A) segments, but also to polysomes directly, which suggests that a portion of the poly(A) segment is exposed in messenger ribonucleoprotein particles of polysomes. This binding can be increased by treatment with detergents such as SDS which effectively remove proteins. The data (Fig. 2) indicates that more protein is associated with the poly(A) part of mRNA in membrane-bound than in free polysomes. Functional mRNA in polysomes exists as a messenger ribonucleoprotein complex (21) and specific proteins have been found associated with the poly(A) strand of mRNA (3, 14). Recently, it has been shown (15, 18) that poly(A) itself may be buried in membranes. In pea epicotyl membrane-bound polysomes (Fig. 1B), the membranes were removed with detergent (Nonidet P40) before hybridization with poly(U) (*cf.* 15). Thus, the relatively low hybridization observed/unit of membrane-bound polysome (Fig. 2) is in part due to masking by proteins which can be dissociated by SDS. In addition the mean size of poly(A) in mRNA of membrane-bound polysomes is smaller compared to that of free polysomes (Fig. 3).

The finding that poly(A) in mRNA of both free and membrane-bound polysomes exists in pea epicotyl as discrete size classes (Fig. 3) could be due to the fact that this tissue contains a variety of cell types, and under steady state conditions messenger molecules of different ages would exist. Due to the difficulty in labeling, it cannot be determined if the smaller poly(A) is derived from larger poly(A), or if they represent endogenous separate size classes. Discrete size classes of poly(A) have been observed as a function of age of globin mRNA (7).

Actively growing pea epicotyl tissue contains 80 to 90% of its ribosomes in polysomes (24) and, when such tissue is treated with growth hormones, the percentage of active polysomes remains unchanged for up to 2 days [*cf.* oviduct-stimulation, (20)]. During this period, a gradual net increase in polysomes occurs as a result of both new mRNA and ribosome synthesis. An increase in RNA polymerase I activity has been observed after auxin treatment (10) and gibberellic acid-treated nuclei have been shown to synthesize more RNA than controls (12). Two days after IAA treatment the polysome population starts to decline. This is accompanied by a decline in the mean size distribution of polysomes (Fig. 5). Since ribosome synthesis continues for 3 to 4 days after hormone treatment, a continuous decrease in percentage of polysomes results in a corresponding increase in free ribosomes. This situation may have resulted from lack of coordination in the synthesis of ribosomes and mRNA during hormone-induced growth. About a week after such treatment, gradual senescence finally results in death of most of the tissue.

That the size of mRNA is smaller in small polysomes is indirectly derived from the observation that the rate of initiation is not limiting after hormone treatment. The mean size of the poly(A) segments in these polysomes is smaller than control. The data in Table I reinforces this conclusion because if the change in the size of polysomes was due to degradation, the poly(A) in the mRNA population would stay constant, or change in proportion to the percentage of polysomes. The fact that ^{3}H-poly(U) binding is not proportional to percentage polysomes indicates that a larger number of intact mRNA molecules are present in small polysomes. This increase in the number of mRNA molecules can be calculated from the data in Table I and is found to be 2- to 2.5-fold after 4 to 5 days of hormone treatment. Although the significance of these findings is not clear, it may be that hormone induces the selective synthesis of relatively small proteins in this tissue, such as various hydrolases which are likely to be translated by membrane-bound polysomes, as shown for wall hydrolyzing enzyme cellulase (24). An increase in the length of RNA synthesized by chromatin-bound polymerase from auxin-treated soybean has been observed (10). Since this activity represented ribosomal RNA polymerase under the assay conditions which are not physiological, *a priori* there is no reason to believe that mRNA polymerase would behave similarly in auxin-treated tissue. Apart from initial transcript, other factors such as processing, maturation, transport, and stability may regulate the size of mRNA and poly(A). These processes may be modulated with hormone treatment.

Note Added in Proof. Using poly(A) standards of known mol wt from Miles Lab., the electrophoretic migration of poly(A) does not correspond to the mol wt calculated using 4S and 5S RNA as markers. Thus, the values calculated in this paper should be considered relative and not absolute.

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