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Polyribosomes from Peas

II. POLYRIBOSOME METABOLISM DURING NORMAL AND HORMONE-INDUCED GROWTH[1]

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ABSTRACT

Polyribosomes as large as 10-mers (strands of messenger RNA bearing 10 ribosomes) were isolated from etiolated pea (*Pisum sativum* L. var. Alaska) stem tissue during all stages of development when methods were used which essentially eliminated ribonuclease activity during extraction. Actively growing tissue, harvested from the apical 10 mm, yielded many large polyribosomes and a low (<20%) proportion of monosomes. Similar tissue, allowed to age by applying lanolin to decapitated apices, showed a progressive decrease in number of larger polyribosomes and an increase in the proportion of monosomes. Hormone treatments, which prolonged growth and delayed aging, delayed the loss in large polyribosomes and the increase in proportion of monosomes. Growth-stimulating hormones, added to previously aged tissue, stimulated the production of many large polyribosomes in pre-existing cells.

It is suggested that (*a*) large polyribosomes occur in all regions of the pea stem, (*b*) changes in polyribosome distribution appear to precede changes in growth rate, (*c*) loss of larger polyribosomes is closely related to a decrease in mRNA templates followed more gradually by loss of ribosomes, (*d*) hormone-stimulated continuation of growth is accomplished through maintenance of available mRNA.

Methods are described, involving detailed analysis of polysome distribution, which, although they cannot be used to measure changes in initiation of ribosomes on to mRNA, do permit measurement of the amount of polysomal-associated mRNA present in tissues at different stages of growth. These analyses lead to the further suggestion that hormone stimulation of growth of previously nongrowing tissue is accomplished primarily through an increase in available mRNA prior to synthesis of ribosomes.

In an earlier paper (7), we showed that high yields of large polyribosomes could be obtained from actively growing, etiolated pea stem tissue, homogenized in media buffered with tris-HCl at high concentration (200 mM) and pH (8.5), and that the effectiveness of the buffer was due to its prevention of RNase activity during extraction. We have used these methods to examine the polyribosome distribution in different regions of etiolated pea stem tissue treated in different ways to determine whether the normal distribution of polyribosomes found in the actively growing apical tissue is typical for either growing or nongrowing tissues or both. In addition, we have tried to ascertain whether changes in polyribosome distribution after various hormone treatments are due primarily to changes in the rate of initiation (attachment) of ribosomes on to mRNA or to variations in the amount of available mRNA.

MATERIALS AND METHODS

Seedlings of *Pisum sativum* L. var. Alaska were grown in darkness at 23 to 25 C for 7 to 8 days until the third internode was 2 to 4 cm long (7). In some instances, tissue was excised from different regions of untreated plants. In other instances, plants were decapitated, a mark was made 10 mm below the apex to delineate a segment of tissue, and lanolin with or without additives was applied to the cut end. The segments were excised after various time periods. All operations were conducted under dim green light.

Polyribosomes were isolated as described earlier (7) by homogenizing tissue in a mortar in 5 to 10 volumes of grinding buffer (0.25 M sucrose; 0.2 M tris-HCl, pH 8.5; 60 mM KCl, and 30 mM $MgCl_2$). The resulting brei was centrifuged at 30,000*g* for 20 min. The supernatant was layered on a 4-ml pad of 1.5 M sucrose in gradient buffer (40 mM tris-HCl, pH 8.5; 20 mM KCl; 10 mM $MgCl_2$) and centrifuged at 100,000*g* for 90 min in a Spinco 40 rotor. The polyribosome pellet was resuspended in gradient buffer, and an aliquot was measured at 260 and 280 nm on a Beckman DB spectrophotometer to determine the ribosome concentration. A second aliquot was layered onto a similarly buffered gradient containing sucrose at 150 to 600 mg/ml. The gradient was centrifuged at 34,000 rpm in an SW 36 rotor of a Spinco Model L ultracentrifuge for 75 min. All operations were conducted at 0 to 4 C. The gradients were analyzed on an Isco Model 640 flow analyzer, and the areas under different peaks were measured with a planimeter (7, 14). Frequently, polyribosomes as large as 14-mers could be resolved and occasionally 17-mers were resolved by these methods.

RESULTS AND DISCUSSION

The epicotyl (stem) of the 7- to 8-day-old etiolated pea (with the third internode partially developed) has been the object of a number of studies. Comparisons between the different regions have been made concerning auxin content (25, 27), cellulase and protein distribution (16, 18), anatomy (24), growth rate (1, 21), and cell wall composition (17, 23). Polyribosome yields (and presumably protein synthetic capacity) can, therefore, be related to the above mentioned parameters.

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Accordingly, polyribosomes were isolated from various regions of the stem of 8-day-old etiolated pea plants, and the profiles obtained are depicted in Figure 1. Polyribosomes at least as large as 10 mers were obtained from all tissues examined and by extrapolation to the bottom of the gradient (22), the presence of polyribosomes larger than 20 mers could be deduced (Fig. 1). All regions, except possibly the first node (Fig. 1h), yielded a near normal distribution of polyribosomes with the maximum peak height varying from a 10-mer in the apical 10 mm (Fig. 1c) to a 7-mer in the second internode (Fig. 1g). In those instances where the monosome peak was exceedingly large, *i.e.* plumule (Fig. 1a), hook (Fig. 1b), second node (Fig. 1f), and first node (Fig. 1h), there was a correspondingly large dimer, which may be caused by nonspecific aggregation of monosomes as has been found in *Botryodiplodia theobromae* (4).

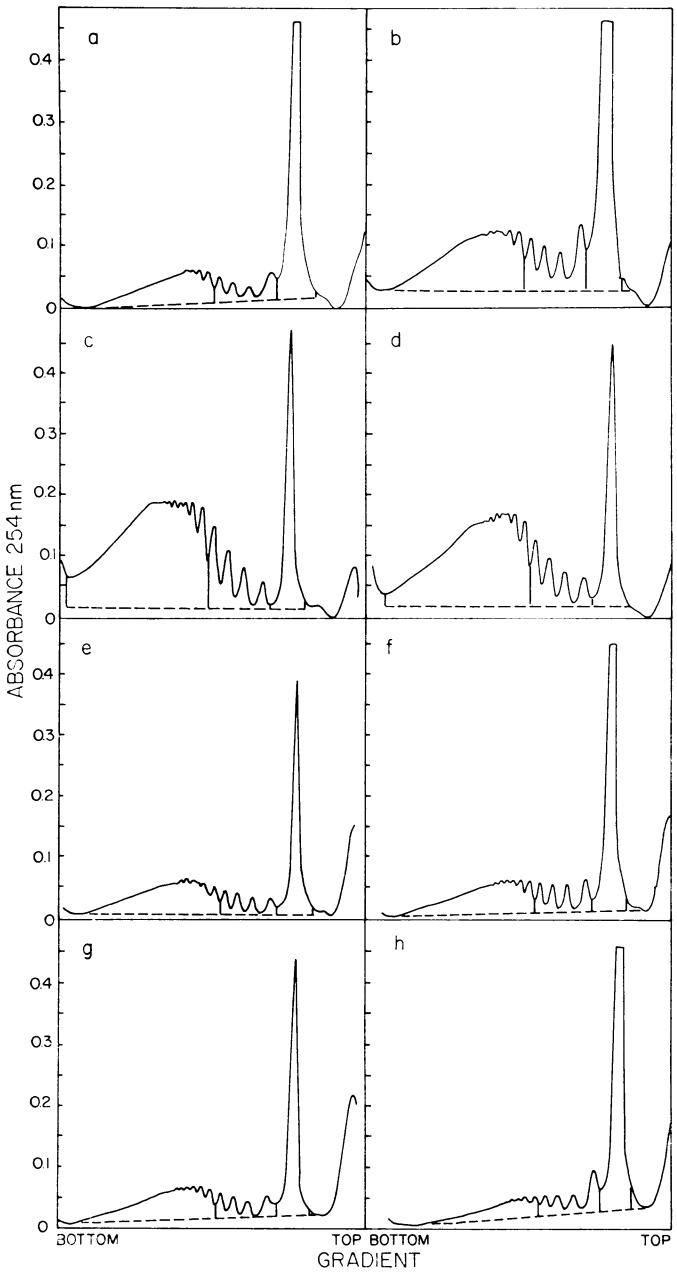

FIG. 1. Distribution of polyribosomes isolated from various regions of etiolated pea stems. Polyribosomes were isolated, as described under "Materials and Methods," from different regions of 8-day-old etiolated pea epicotyls. The amount (fresh wt) of tissue from each region (from apex to base) used for each gradient was: a: plumule, 0.05 g; b: hook, 0.08 g; c: apical 10 mm of third internode, 0.35 g; d: second 10 mm, 0.70 g; e: third 10 mm, 1.10 g; f: second node, 1.32 g; g: second internode, 1.06 g; h: first node, 1.12 g.

Table I. *Polyribosomal Yields from Various Regions of Etiolated Pea Stems*

Data were taken from profiles depicted in Figure 1.

Region (apex to base)	T/10 mg Fresh Wt[1]	L/P[2]	M/T[3]
		%	%
Plumule	200	76.9	54.5
Hook	203	72.6	40.7
Third internode			
First 10 mm	43.5	86.1	17.7
Second 10 mm	23.5	85.8	20.8
Third 10 mm	7.3	86.5	34.8
Second node	7.2	79.5	48.5
Second internode	7.4	74.0	41.9
First node	7.4	56.4	62.0

[1] Total ribosomal (in arbitrary planimeter units) material.

[2] Large polysomes (>5-mers) as a percentage of total polysomes.

[3] Monosomes as a percentage of total material (monosomes + polysomes).

The areas under the different regions of the profiles depicted in Figure 1 (or similar profiles employing less material on the gradient so that the monosome peak did not go off scale) are given in Table I. The plumule, which contains both quiescent, unextended leaf tissue and some meristematic cells (1, 23), and the hook, which is primarily meristematic (1, 23), yielded exceedingly high amounts of total ribosomal material, and the amount decreased in tissues excised farther from the apex. In addition, the plumule and hook had a much higher proportion of monosomes than did the adjacent apical 10 mm, which grows solely or predominantly by cell enlargement (21).

The meristematic tissues also gave a somewhat lower proportion of large polyribosomes (about 75%) than the nonmeristematic regions of the third internode below which contained about 85% large polyribosomes (Table I). Tissues isolated from below the third internode possessed increasing proportions of monosomes and decreasing proportions of large polysomes as they aged, although the total amount of ribosomal material per segment remained essentially constant (Table I). The decrease in large polysomes and increase in monosomes suggest one of two things may be occurring during maturation. There is either a decrease in the number of available mRNA templates (especially the ones coding for larger proteins [26]) or there is a decrease in the rate of initiation of ribosomes onto mRNA (6, 28, 29) or both. It is not possible from these data to favor either explanation. The changes in polyribosome distribution are unlikely to be due to RNase acting *in vitro*, since there would be much higher amounts of smaller polyribosomes, especially 2- to 4-mers (19, 30, 31).

We attempted to determine whether a shortage of mRNA or a decrease in initiation rate was the major factor in the shift in polyribosome distribution accompanying maturation. Accordingly, epicotyls were decapitated and treated in such a way that different growth patterns ensued. Two of these treatments, IAA and IAA plus BA,[2] cause expansion (3, 10, 16); one, GA, causes elongation (8); and one, lanolin alone, pro-

[2] Abbreviations: BA: benzyladenine; FUdR: fluorodeoxyuridine.

motes little growth (8, 10). All hormones were applied at 0.5% (w/w) in lanolin. The polyribosome distributions of tissues treated for 5, 15, and 48 hr are depicted in Figures 2, 3, and 4, respectively, and the data are presented numerically in Table II.

After 5-hr treatment, by which time there was little difference in total size (Table II), there were differences already apparent in polysome distribution. Treatments leading to expansion, *i.e.* IAA (Fig. 2a) and IAA + BA (Fig. 2b) maintained, or slightly increased, the total amount of ribosomal material per segment and caused a substantial decrease in the proportion of monosomes compared with zero time (Fig. 1c, Tables I and II). There was, however, a slight decrease in the total amount of ribosomal material per unit fresh weight, which suggests that ribosome synthesis did not keep pace with cell enlargement. Tissues treated with lanolin (Fig. 2d), which caused little growth, and GA (Fig. 2c), which caused elongation, exhibited a decline in total ribosomal material expressed on both a segment and a fresh weight basis. They also gave a slightly higher proportion of monosomes than the zero time segments (Table II). All treatments yielded virtually identical proportions of large polyribosomes (about 85%) and had the same maximum absorbing peak (a 10-mer).

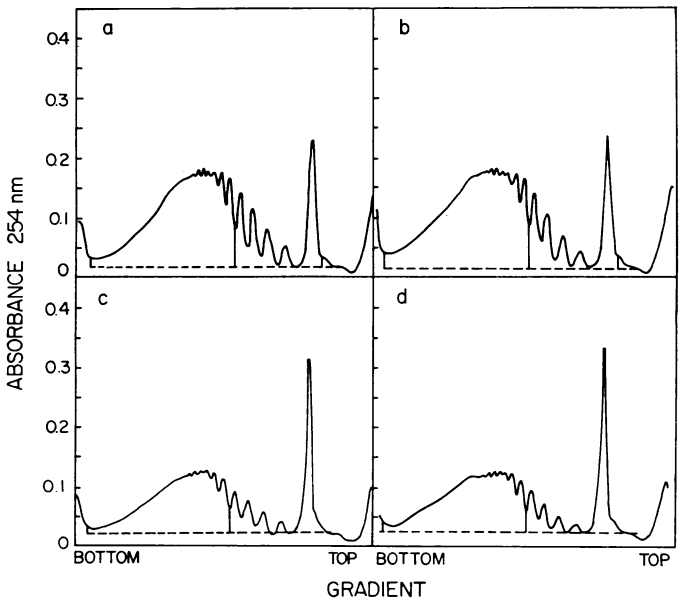

FIG. 2. Distribution of polyribosomes from apical pea stem tissue after 5 hr hormone treatment. Eight-day-old etiolated pea seedlings were decapitated, marked with ink 10 mm from the apex, and lanolin or 0.5% (w/w) hormone in lanolin applied immediately to the cut surface. After 5 hr, 15 segments from each treatment were excised for polyribosome isolation. The amount (fresh wt) of tissue used for each gradient was: a: IAA, 0.40 g; b: IAA + BA, 0.41 g; c: GA, 0.38 g; d: lanolin, 0.36 g.

Table II. *Changes in Polyribosomal Distribution Accompanying Hormone-stimulated Growth*

Data were taken from profiles depicted in Figures 2 to 4. Treatment was applied immediately after decapitation. Final lengths (mm) attained after 2 days were: lanolin, 18.2; GA, 31.2; IAA, 14.5; IAA + BA, 14.2. This gives swelling values (mg/mm) of: lanolin, 2.3; GA, 2.3; IAA, 6.7; IAA + BA, 9.9.

Time and Treatment	Total Material		L/P[1]	M/T[2]	Fresh Wt
	T/segment	*T/10 mg*	%	%	*mg/segment*
Initial	95	45.5	86	17.7	20.8
5 Hr lanolin	73	29.8	85	21.4	24.7
GA	76	30.0	88	19.5	25.8
IAA	101	38.3	86	8.7	26.1
IAA + BA	112	41.9	86	8.8	26.8
15 Hr lanolin	61	20.5	83	40.2	30.0
GA	77	19.5	86	26.4	39.3
IAA	96	20.4	85	17.4	47.3
IAA + BA	112	21.8	89	10.0	51.0
2 Days lanolin	51	12.2	77	70.5	41.8
GA	67	9.4	79	50.6	71.5
IAA	110	11.4	74	46.0	97.0
IAA + BA	142	10.2	75	24.8	140.5

[1] Large polysomes (>5-mers) as a percentage of total polysomes.

[2] Monosomes as a percentage of total material (monosomes + polysomes).

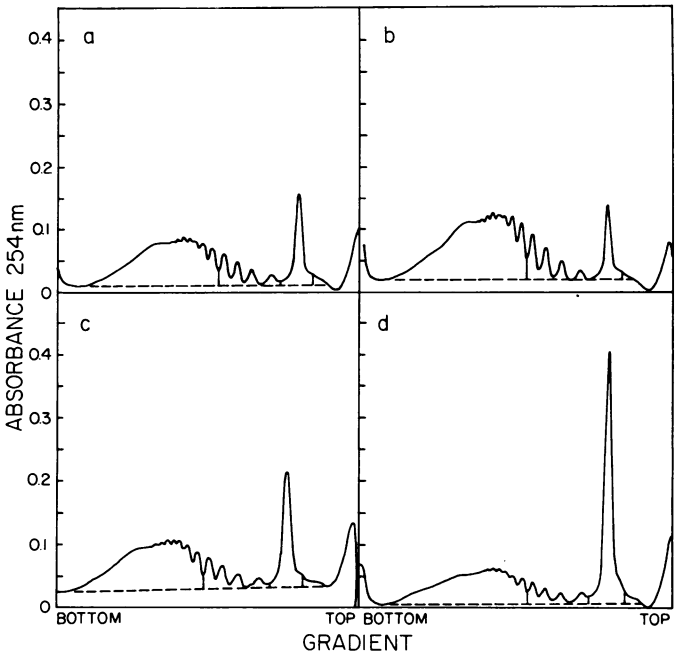

FIG. 3. Distribution of polyribosomes from apical pea stem tissue after 15 hr hormone treatment. Protocol identical to that in Fig. 2, except that tissue was excised after 15 hr. The amount (number and fresh wt) of tissue used for each gradient was; a: IAA, 8 segments, 0.38 g; b: IAA + BA, 8 segments, 0.41 g; c: GA, 9 segments, 0.36 g; d: lanolin, 11 segments, 0.33 g.

After 15-hr treatment (Fig. 3, Table II), the changes in polyribosome distribution became more apparent. All the tissues showed a decrease in total ribosomal material per unit fresh weight and exhibited a similar proportion of large polyribosomes (83–89%). However, the lanolin control yielded a continued decline in ribosomes per segment and a doubling (to 40%) of the proportion of monosomes (Table II). The elongating, GA-treated tissue maintained the same amount of ribosomal material per segment and showed a much smaller increase in proportion of monosomes. The expanding tissues maintained their levels of total ribosomal material compared with the 5-hr treatment and with the zero time tissue, but the IAA-treated tissue showed an increase in proportion of monosomes from 5 hr so that at 15 hr the proportion was similar to zero time. The segments treated with IAA and BA, which expand even more than those treated with IAA alone, maintained their very low level of monosomes (Table II).

After 2 days (Fig. 4, Table II), all tissues again yielded far

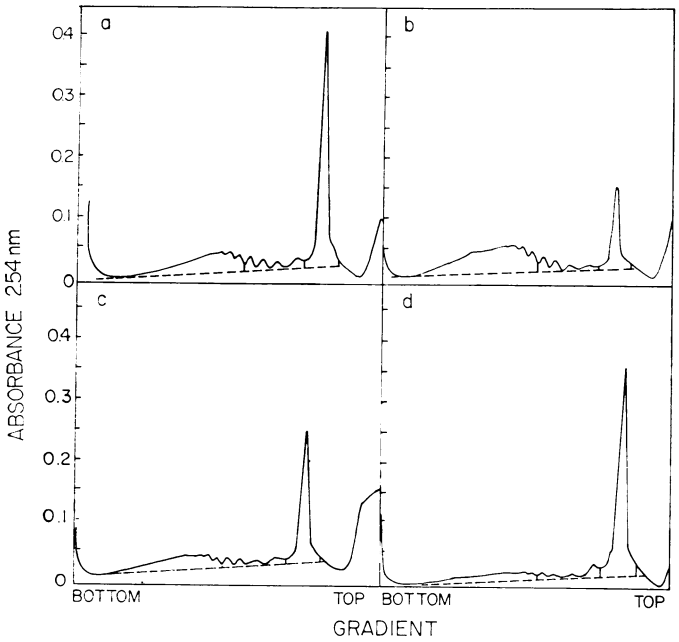

FIG. 4. Distribution of polyribosomes from apical pea stem tissue after 2 days of hormone treatment. Protocol identical to that in Fig. 2, except that tissue was isolated after 2 days. The amount (number and fresh wt) of tissue used for each gradient was: a: IAA, 5 segments, 0.48 g; b: IAA + BA, 3 segments, 0.42 g; c: GA, 6 segments, 0.43 g; d: lanolin, 7 segments, 0.30 g.

fewer total ribosomes per unit fresh weight than at the previous time interval. The levels were only 25% of the level at zero time, and the proportion of large polyribosomes fell somewhat from about 85% to about 75% with all treatments. The lanolin- and GA-treated tissues showed further declines in total ribosomes per segment, whereas the expanding tissues showed a slight increase (Fig. 4, Table II). All tissues yielded greater proportions of monosomes, although the IAA + BA-treated tissue still had 75% polyribosomes. Even though the control tissue had 70% monosomes (Table II), large polyribosomes still persisted and 7-mers could still be resolved (Fig. 4d), and a normal or near normal distribution was obtained in all cases (Fig. 4a–d).

It must be stressed that the techniques employed here probably permitted preferential isolation of free rather than membrane-bound polyribosomes, since many of the latter could have been pelleted along with the mitochondria and others held up by the sucrose pad. This may account for the discrepancy between the results reported here, which show little increase in ribosomes per segment after IAA treatment (Table II) and those reported earlier for similar tissue in which a 3-fold increase in RNA was found (8, 10). It is unlikely that the lower yields found in the present study were a result of incomplete pelleting of monosomes and subunits due to the short initial ultracentrifugation (90 min), because when the duration of this spin was doubled, little difference in yield was obtained. In addition, although unpelleted polysomes could be recovered from the pad and supernatant fluid, preferentially high levels of monosomes were not found unless harsh methods were used in the recovery procedure (data not shown). Further work is being carried out on the metabolism of membrane-bound polysomes as these may well be involved in the hormone-induced synthesis of extracellular, wall-hydrolyzing enzymes, such as cellulase (8, 9, 16).

From the above data, it is apparent that no hormone treatment stimulated marked production of polyribosomes when applied to actively growing tissue, although the treatments which promoted most growth maintained polyribosome levels for a longer period. The other treatments (noticeably lanolin) showed a continued decline in amount of total ribosomal material, a large increase in the proportion of monosomes, and a slight decrease in the proportion of large polysomes. This indicates that there is a maintenance of protein synthetic capacity during periods of active growth and a decline in the capacity coincident with, or prior to, cessation of growth. In addition, the continued presence of near normal distributions of polyribosomes even when the monosomes comprise 70% of the total (Fig. 4d) strongly suggests that the decline in large polyribosomes is caused by a decrease in mRNA templates, rather than a lowering of initiation rates. If it were the latter, one would expect a much more noticeable shift of polysome distributions towards the production of more smaller ones (29). These data do not however, show any remarkable changes in polyribosome distribution in response to hormone treatment, nor do they necessarily show relationships between polyribosomes and cell enlargement, because the two treatments which lead to expansion and preservation of polyribosomes (Table II) also lead to cell division (10).

A system was needed, therefore, in which protein synthesis and growth could be stimulated in previously non-growing tissue, preferably in the absence of cell division, so that the effects on pre-existing cells could be determined. It has been shown previously (8) that tissue allowed to age by decapitation and treatment with lanolin for 2 to 3 days can be stimulated to grow and produce cellulase and other proteins in response to IAA. Accordingly, segments were treated with lanolin and then, after 2 days re-treated either with lanolin or lanolin plus additives. The additives were IAA and IAA + BA with and without fluorodeoxyuridine. FUdR has been shown to be a very effective inhibitor of IAA-induced DNA synthesis and cell division in this system (10).

The results of such tests are shown in Figure 5 and tabulated in Table III. Compared with the lanolin control, all hormone treatments caused a shift in maximum peak height from a 7- to 9-mer (Fig. 5a) to a 10- to 11-mer (Fig. 5b–e). In all cases (Table III), the treatments caused a 2-fold increase in total ribosomal material per segment, an increase (from 30%) to 90% of the proportion of ribosomes bound to polysomes, and an increase (from 73%) to 86 to 89% in the proportion of large polysomes. A 2-fold increase in ribosomes concurrent with a 3-fold increase in the proportion of ribosomes associated in polysome form means there was an actual 6-fold increase in number of ribosomes associated with polysomes after 10 hr of hormone treatment. All of these effects on polyribosomes preceded any noticeable effect on growth which was not discernible until about 15 hr after hormone application. Surprisingly, FUdR appeared to cause a small but consistent increase in the proportion of polyribosomes (Table III). This has been noticed on a number of occasions, but at present there is no way of knowing why it occurs. It is conceivable that in inhibiting DNA synthesis, FUdR prevents the diversion of potential RNA precursors into the DNA pool. It must be emphasized that no detailed investigations on DNA synthesis and its inhibition by FUdR were undertaken in this study, but it seems likely that FUdR would inhibit DNA synthesis in aged tissue since it is so effective in young (immediately treated) tissue (10). In addition. a late consequence of IAA-induced cell division, *i.e.* production of adventitious roots at the stem apex (10, 24) was never seen in FUdR-treated tissues.

From the data as presented in the form shown in Table III and Figure 5, on delayed hormone application, it is impossible

to determine precisely how much of the IAA-induced conversion of monosomes into polysomes was caused by increased mRNA availability and how much was caused by increased initiation of ribosomes. It is possible, however, to demonstrate that some increase in mRNA templates must have occurred. The aged tissue contained only 30% polysomes with a 7- or 9-mer as the highest absorbing peak (Fig. 5a). In order for the number of ribosomes occurring as polysomes to increase 6-fold (as it did after 10 hr hormone treatment), with an increase in initiation rate alone, there would have to have been an average 6-fold increase in the number of ribosomes on each mRNA (13, 29). Hence, polysomes bearing from 40 to 45 ribosomes would have become the most abundant (had the highest absorbing peak). Such large polysomes were not the most abundant; in fact, the maximum size class changed only to 10- to 11-mers (Fig. 5b–e) after hormone treatment.

Although we knew of no way to measure "available" mRNA nor of the factors controlling ribosome initiation in peas, it occurred to us that there may be enough information present in the polyribosome distributions themselves (as hinted by Lecocq *et al.* [14]) to establish which of these events was taking place after hormone application.

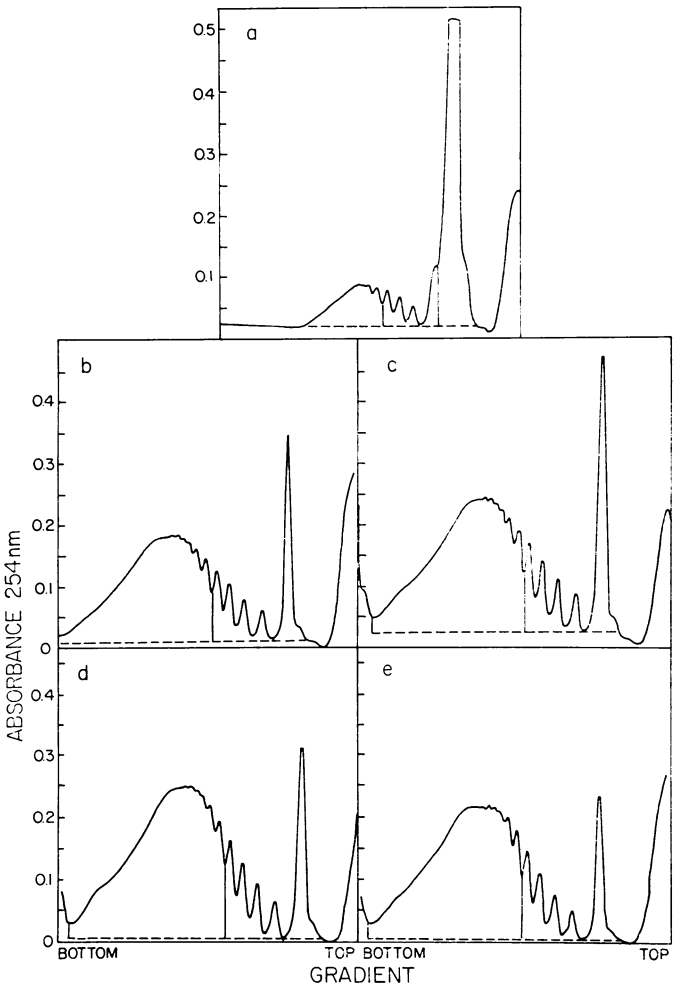

FIG. 5. Distribution of polyribosomes from pea stem tissue aged for 2 days and then treated for an additional 10 hr. Eight-day-old etiolated pea seedlings were decapitated and treated with lanolin. After 2 days, the lanolin was removed, a mark made 10 mm from the apex, and fresh lanolin ± additives applied. After a further 10 hr, the segments were excised for polyribosome isolation. The amount (number and fresh wt) used for each gradient was: a: lanolin, 60 segments, 1.84 g; b: IAA, 50 segments, 1.55 g; c: IAA + BA, 50 segments, 1.51 g; d: IAA + FUdR, 50 segments, 1.50 g; e: IAA + BA + FUdR, 50 segments, 1.61 g. Each hormone was applied at 0.5% (w/w) in lanolin. FUdR was applied at 0.1%.

Table III. *Polyribosome Distribution of Tissue Aged for 2 Days and Subsequently Treated for 10 Hr*

Data were derived from profiles depicted in Figure 5. The terminal 10 mm was harvested from each epicotyl.

Treatment	Total Ribosomal Material	L/P[1]	M/T[2]
	per segment	%	%
Lanolin	65	73	69
IAA	127	89	14
IAA + BA	132	86	11
IAA + FUdR	127	88	9
IAA + BA + FUdR	129	86	7

[1] Large polysomes (>5-mers) as a percentage of total polysomes.

[2] Monosomes as a percentage of total material (monosomes + polysomes).

Table IV. *Analysis of Polyribosome Distribution from Aged and "Rejuvenated" Tissue*

Epicotyls aged for 2 days and then treated with IAA for 3 or 6 hr and the apical 10 mm harvested for analysis. Areas are expressed as planimeter units per 10 segments. Weights of segments were approximately equal.

No. of Ribosomes in Polysome Size Class (N)	Area (A)/Peak (planimeter units)			mRNA/Peak (A/N)		
	Lanolin	3 Hr IAA	6 Hr IAA	Lanolin	3 Hr IAA	6 Hr IAA
1[1]	470	334	173	0	0	0
2	8	7	10	4.00	3.50	5.00
3	11	9	16	3.67	3.00	5.35
4	18	14	28	4.50	3.50	7.00
5	21	18	34	4.20	3.60	6.80
6	23	22	40	3.83	3.67	6.67
7	20	22	42	2.86	3.15	6.00
8	19	21	38	2.38	2.62	4.75
9	16	20	37	1.78	2.12	4.11
10	14	18	33	1.40	1.80	3.33
>10	62	208	344	4.13	13.87	22.94
Total	682	693	795	32.75	40.43	71.93

[1] It is assumed that no monosomes were associated with mRNA.

Accordingly, detailed analyses were made of the profiles obtained from peas treated for 2 days with lanolin and of those subsequently treated for 3 and 6 hr with IAA. These analyses are presented in Table IV. The method used was based on the following suppositions. First, the number of mRNA molecules is equal to the number of polyribosomes (26), and second, the number of polyribosomes (in arbitrary units) can be calculated from the area of each peak divided by the number of ribosomes in polysomes of that size class. For example, if the area under the dimer, trimer, and tetramer peaks was 12 units each, there would be 6, 4, and 3 units of mRNA, respectively. It is theoretically possible to calculate the actual amount of mRNA by these methods from the known molecular weight and absorbance of a known weight of pea polysomes (5). These

latter calculations were considered unnecessary for the conclusions of this study.

There are, however, certain assumptions (approximations) which have to be made when using these analyses. First, the absorbance of mRNA should be negligible compared with the absorbance of the ribosomes. Second, none of the monosomes must be attached to short mRNA species. Third, the area of absorbance of each class size of unresolved (larger) polyribosomes must be approximated. Fourth, RNase activity must be minimal and only intact polysomes (mRNA) can be present. Fifth, some of the dimer must be caused by aggregation of monosomes.

Evidence to support these assumptions (approximations) follows. First, calculations based on data from Noll and coworkers (19, 20), on size distribution of messengers isolated from polysomes of known size, show that more than 98% of the RNA in a polysome is ribosomal and, thus, less than 2% is mRNA. The second assumption is most likely valid, because a 3-fold increase in initiation rate of ribosomes onto short strands of mRNA previously bearing one ribosome would cause a marked increase in the numbers of dimers, trimers and tetramers. This did not occur (Fig. 5). The only other possibility is that many of the monosomes were attached to long strands of mRNA and were converted to large polysomes after hormone treatment. In this case the process could be described more accurately as increase in mRNA availability rather than increase in initiation (because with equal availability, there will be an equal spacing of ribosomes along mRNA's-13). The same argument would hold for mRNA strands with no ribosomes attached. The third approximation has little effect on the final outcome because the unresolved part of the peak contains only large polysomes and so the area has to be divided by a large number. It makes little difference whether a 15-mer or a 20-mer is used as the average size-class for this region from (for example) the 6-hr, IAA-treated tissue, which has the highest amount of unresolved polysome material. In this instance, the total mRNA would drop from about 72 units with a 15-mer as the mean (as shown in Table III) to about 66 units if a 20-mer were used. The fourth assumption, that RNase action is minimal, is supported by the fact that endogenous RNase levels are low in these tissues (3) and that the isolation methods employed here prevent the action of low levels of exogenous RNase (7). The fifth and final assumption has the greatest effect on over-all conclusions because the dimer area will have the smallest divisor (*i.e.* two) and thus the highest number of mRNA molecules per unit area will be found in this region. The validity of this assumption is supported by the fact that in all cases where high monosome levels were found (Fig. 1, a, b, f, and h; Fig. 5a), correspondingly high levels of dimer were also found. Furthermore, the dimer could not be removed by extensive RNase treatment (data not shown) and others have found (4), that a small proportion of isolated monosomes will aggregate spontaneously to form dimers. In all calculations, the size of the dimer was estimated by extrapolation of the normal curve made by the other polysomes; the surplus area of dimer was included in the monosome fraction.

The data in Table IV show that there was little change in total ribosomal material after 3 hr IAA treatment, but there was a great decrease in monosomes (to 70% of the control) and an increase in large polysomes (3-fold that of the control). Interestingly, this corresponds to a loss of 140 units of monosome with a concomitant increase of 140 units of large polysome (>10-mers). After 6 hr, there was a slight (16%) increase in total ribosomes, a further decrease in monosomes (to 30% of the lanolin control) a slight increase in the smaller polysome size classes and a further increase in large (>10-mer) polysomes. Once more, the loss of monosomes (300 units) was about equal to the gain in large polysomes (280 units).

In terms of mRNA per segment, there was little difference between the control and 3-hr IAA-treated tissue over the range 2- to 6-mers, but more mRNA was present in the larger (>7-mer) polysomes from IAA-treated tissue. This strongly suggests that the initial response to IAA (at least as far as polyribosome metabolism is concerned) is an increase in large mRNA species. Between 3 and 6 hr, there is an increase in polysomes of all sizes and some of the decrease in monosomes could have been caused by an increased rate of initiation. There was, however, a much bigger increase in mRNA (120%) than in total ribosomes (16%); thus even at this stage the increases in polysomes might have resulted solely from increases in mRNA. When the data from Tables III and IV are compared, it is apparent that the majority of ribosome synthesis occurs between 6 and 10 hr after hormone treatment. The data from the lanolin treatment were similar in 3 different experiments and results similar to the 3- and 6-hr data shown here were obtained with tissue treated for 3 and 6 hr with IAA + BA, with and without FUdR. The results shown, seem, therefore, to be typical of these treatments.

Although the actual timing of these responses to hormone treatment of aged tissue varied somewhat between experiments, the same sequence of events has always been found, *i.e.* an increase in mRNA (and decrease in monosomes), followed by possible increases in initiation, followed by ribosome production, followed by growth. Aged tissue appears to be far more suitable than actively-growing tissue as a system for studying hormone effects on nucleic acid metabolism. Hormones, applied to growing tissue, merely delay the loss in polyribosomes (Figs. 2–4, Table II), whereas they elicit marked increases in polyribosomes when applied to aged tissue (Fig. 5, Table III). Furthermore, these changes in polyribosome metabolism occur prior to noticeable changes in growth.

Somewhat similar results were found recently with soybean hypocotyls treated with the synthetic auxin, 2,4-D (2). In this system, untreated tissue yielded higher proportions of polyribosomes in meristematic regions than in growing or mature zones (2), whereas in pea, the highest proportion of polysomes occurred in the growing regions (Tables I and II). With basal (aged) hypocotyl tissue there was a 2.5-fold increase in ribosomes in 24 hr (compared with a 2-fold increase in 10 hr in peas, Table III) after auxin treatment. Anderson (2) also found that FUdR did not prevent these responses and, like us, reasoned that auxin must have its effects in pre-existing cells in the absence of DNA synthesis.

There are potentially at least three ways to determine whether there are changes in ribosome initiation rate involved in hormone responses. First, with the aid of an electron microscope, one could examine the spacing of ribosomes along polysomes in intact tissues or in fractionated polyribosomes (14). Second, one could free the mRNA of ribosomes and measure its size, either by sedimentation (20) or electrophoresis (15). Third, one could separate monosomes into free and mRNA-bound ribosomes by density centrifugation (11) or selective proteolytic dissociation (12). We hope to carry out some of these investigations in conjunction with tests involving inhibitors and radioactive precursors in an attempt to find out if the increase in polysome-associated mRNA is due to its synthesis, and whether these increases in available mRNA templates are accompanied by changes in ribosome initiation.

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