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EFFECTS OF HORMONES ON CHANGES IN POLYRIBOSOMAL DISTRIBUTION

by

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SUMMARY

The influence of some hormones on polyribosome level in pea apicotyl was studied. Profiles polyribosomes were obtained from etiolated stem segments of *Pisum sativum* L. var. Alaska.

In terms of polyribosome metabolism, expanding tissues yielded large increases in polyribosomes on both a segment and fresh weight basis. Elongating tissues yielded more polyribosomes per segment, but only maintained them on a fresh weight basis,.Non growing tissue yielded fewer polysomes per segment or per unit fresh weight. These results were found whether the hormones were applied immediately after decepitation or after 1 day delay.

In this paper, the main purpose is to study the effect of hormones on the level of polyribosomes in pea epicotyl and to relate this information to what is known about hormones effect on protein.

Introduction

Ribosomes were first isolated from plants by TS'O et al. (1956). Since proteins are synhesized on polyribosomes, the level of protein synthesis in some way be related to the level of polyribosomes in the cell (SCHWEET et al., 1965; WETTSTEIN et al., 1963).

TREWAVAS (1968) reported that IAA treatment of excised etiolated pea stem tissue yielded an increased polyribosome level over non treated tissue. In an earlier work, DAVIES et al. (1972) showed that high yields of large polyribosomes could be obtained from actively growing, etiolated pea stem tissue. GONZALES (1980) found a 50 %stimulation of polysome number by Giberellic Acid (GA_3) and has claimed that GA_3 applied to 48 hours germinated seedlings caused a 50 % stimulati-

on in amount of membrane-bound polysomes, but no stimulation in cytosolic (free) polysomes. However she did find a 55 % stimulation of the amount of cytosolic monosomes by GA_3. In an another study, MARTIN and NORTHCOTE (1983) have shown that GA stimulated not only polysome formation but also the amount of mRNA about 2 fold. On the other hand, SCHUSTER and DAVIES (1983) have been able to show that in the aging of actively growing etiolated pea tissues, ribosomal RNA and messenger RNA contents declined, polyribosomes disaggregated, and the protein synthesizing capacity of polysomes decreased.

In this study, we attempt to determine the distribution of polyribosomes after different hormone treatments of immediately treated or previously aged tissue and also to examine whether changes in polyribosome distribution could be related to changes in growth rate and or to changes in mRNA templates.

Material And Methods

Pea seeds (*Pisum sativum* L. var. Alaska) were soaked for 30 min in 10 % (v/v) Clorox and then allowed to imbibe in tab water overnight. The seeds were sown in moist vermiculite and placed in a dark room for germination and seedling growth. The seedlings with third internodes longer that 10 mm were either treated with about 2 to 2.5 mg lanolin paste in which Indoleacetic acid (IAA) or other hormones were suspended at varying concentrations or treated for the aging process. Aging was initiated by excising the hooks and plumules and applying lanolin to the cut apex. The seedlings were aged for up to 1 day. At various times during the treating and aging period, the plants were harvested and the apical 10 mm marked segments used for experimental analysis. All manipulations were carried out under dim green light (DAVIES and ÖZBAY, 1975; ÖZBAY, 1978).

Polyribosome isolation was perfomed according to LARKINS and DAVIES (1975) with certain modifications. In our study 15 apical 10 mm marked segments were ground in a mortar with 5 volumes of grinding buffer A (0.25 M sucrose; 0.2 M Tris-HCl, pH 8.5; 60 mM KCl; 30 mM $MgCl_2$ – DAVIES et al., 1972). The resulting Brei was strained through nylon cloth and the filtrate was centrifuged at 17.300 x g for 15 min The post mitochondrial supernatant was layered on a 4 ml pad of 1.5 M sucrose in buffer B (40 mM Tris-HCl, pH 8.5; 10 mM $MgCl_2$; 20 mM KCl) and centrifuged for 120 min at 95.000 x g in the rotor of

a SPINCO Model L Ultracentrifuge. The pellet was rinsed gently and resuspended in 0.5 ml buffer B by means of a Votex-Genie mixer. Aliquots (usually 0.5)ml) of resuspended polyribosomes were layered onto linear (150–600 mg/ml) sucrose gradients in buffer C (20 mM Tris-HCl, pH 8.5; 10 mM $MgCl_2$; 20 mM KCl) and centrifuged at 122.000 x g for 75 min in a SW-36 rotor. The gradients were prepared by layering 2 ml of sucrose at 600 mg/ml cellulose nitrate tubes followed by 4 ml at 450 mg / ml, 4 ml at 300 mg/ml, and 2 ml at 150 mg/ml and equilibrated for 48–72 hours at 2°C (BRAKKE, 1967). all manipulations were performed at 4°C.

These gradients scanned at 254 nm using an ISCO UA-640 Manitor. The polysome profiles were used to calculate the relative amounts of subunits, monosomes, small polysomes and large polysomes by measuring areas under the peaks with a planimeter (DAVIES and LARKINS, 1973). Equilibrated blank gradients were always monitored because it was found that the base line varied from to time (DAVIES et al., 1972). The base line is reported for each figure and the area below excluded from calculations.

Results And Discusion

In an earlier work, DAVIES and LARKINS (1973), have been shown that the actively growing tissue, harvested from the apical 10 mm, yielded many large polyribosomes and a low (20 %) proportion of monosomes. In this study, the same methods were used to examine the distribution of polyribosomes after different hormone treatments.

Since it has been previously shown that lanolin alone causes little growth, high levels of IAA promote expansion, low levels of IAA and high levels of GA cause elongation (DAVIES and ÖZBAY, 1975; ÖZBAY, 1978) when decapitated epicotyls were treated with these treatments for different growth periods. We attempted to determine whether changes in polyribosome distribution could be related to changes in growth rate and or to changes in mRNA templates.

Segments were harvested at 24 and 48 hours after treatment to examine the distribution of polyribosomes. The resulting profiles are depicted in Figures 1 and 2 and analysis of the data shown in Table 1. After 24 hrs the treatment which caused most expansion (0.5 % w/w IAA) increased the total amount of ribosomal material per segment (T/ seg) about 125 % and caused a substantioal decrease in the propor-

TABLE I

Effect of Hormones on Changes in Polyribosomal Distribution

Time and Treatment	Total (T/seg)	Material[1] (T/10 mg)	L/P[2] (%)	M/T[3] (%)	Fresh Weight (mg/seg)
24 Hours					
Lanolin (Control)	80.0	18.4	80.2	42.2	43.5
High IAA[4]	181.0	27.6	81.2	11.6	65.5
High GA	119.3	18.6	86.0	16.0	64.1
Low IAA[5]	74.3	16.6	84.6	32.1	44.8
48 Hours					
Lanolin (Control)	41.5	10.6	67.8	53.4	39.2
High IAA	247.5	37.2	81.7	20.6	66.6
High GA	53.5	10.6	71.1	45.0	50.7
Low IAA	46.0	12.0	70.4	51.0	38.2

1 Total ribosomal material (in arbitrary planimeter units).

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

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

4 High concentration = 0.5 % w/w ($\cong$ 10 µg/ plant)

5 Low concentration = 0.0005 % w/w ($\simeq$ 0.01 µg / plant)

Data were taken from profiles decpicted in Figures 1 to 2. Treatment was applied immediately after decapitation. Final lengths (mm) and swelling (mg/mm) values after 2 days were: lanolin, 14.4, 2.7; high IAA, 13.9, 4.7; high GA, 21.1, 2.8; low IAA, 16.6, 2.5 Valus are average of at least 2 experiments.

tion of monosomes (M/T) compared with the lanolin control (Table 1, Fig 1b and 1a). The treatment which promoted the most elongation (0-.5 % w/w GA) gave only a 50 % increase in (T/seg) and had less effect in decreasing (M/T) than did high IAA (Table 1, Fig c) The low level of IAA (0.0005 % w/w) which caused only slight elongation was little different from the lanolin control (Fig 5 d, Table 1). Although there was a 50 % increase in the total amount of ribosomal material per unit fresh weight with high IAA (Table 1), high GA have no significant increase compared with the lanolin control. All treatments yielded almost identical proportions of large polyribosomes.

After 48 hours (Table 1, Fig b) the changes in polyribosome distribution became more apparent. In the expanding tissue, the total amount of ribosomal material per segment increased by about 500 %, the amount of material per unit fresh weight increased about 250 % and there was about a 60 % decrease in the proportion of monosomes compared with lanolin control (Table 1). The elongating, GA treated tissue gave about 30 % increase in the total amount of ribosomal material per segment (Table 1), no increase in materyial per unit fresh weight and a

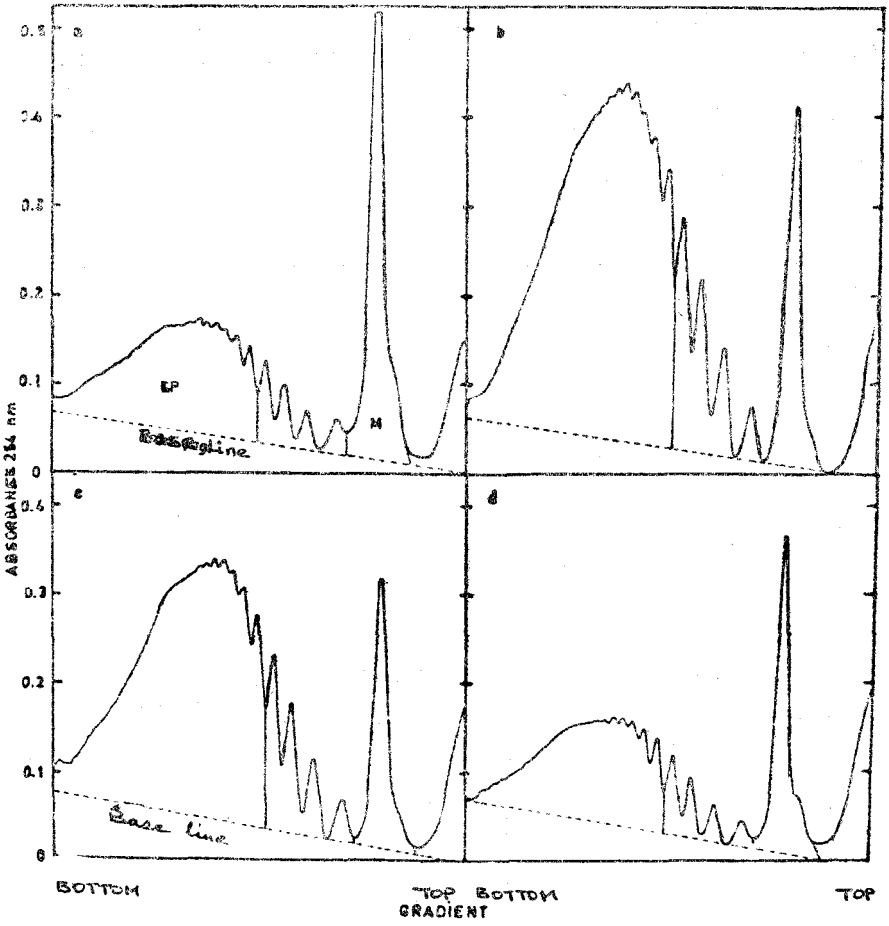

FIGURE 1. Distribution of Polyribosomes From Apical Stem Tissue 24 hrs After Hormone Treatment.

Lanolin or 0.5 % w/w(high level), 0.005 /w/w (low level) IAA or GA in lanolin paste applied immediately to the cut surface. Fifteen segments from each treatment were excised for polyribosome isolation after 24 or 48 hrs.

The amount (fresh weight) of tissue used for each gradient was: a) lanolin, 0.811 g; b) high IAA, 1.216 g ; c) high GA, 1.260 g; d) low IAA, 0.838 g.

15 % decrease in the proportion of monosomes. The low IAA level again showed little changes in polyribosome distribution compared with the lanolin control. but in this experiment it also caused little growth (Table 1). At 48 hrs, the differences in proportions of large polyribosomes and

in fresh weight (mg/seg) for IAA and GA treatments were much more apparent than those at 24 hrs (Table 1, Fig 1b). It seems that probable the polyribosomes isolated by these technique were free rather than membrane-bound, because some of the latter would have been pelleted with the mitchondria and others held up by the sucrose pad (LARKINS and DAVIES, 1974).

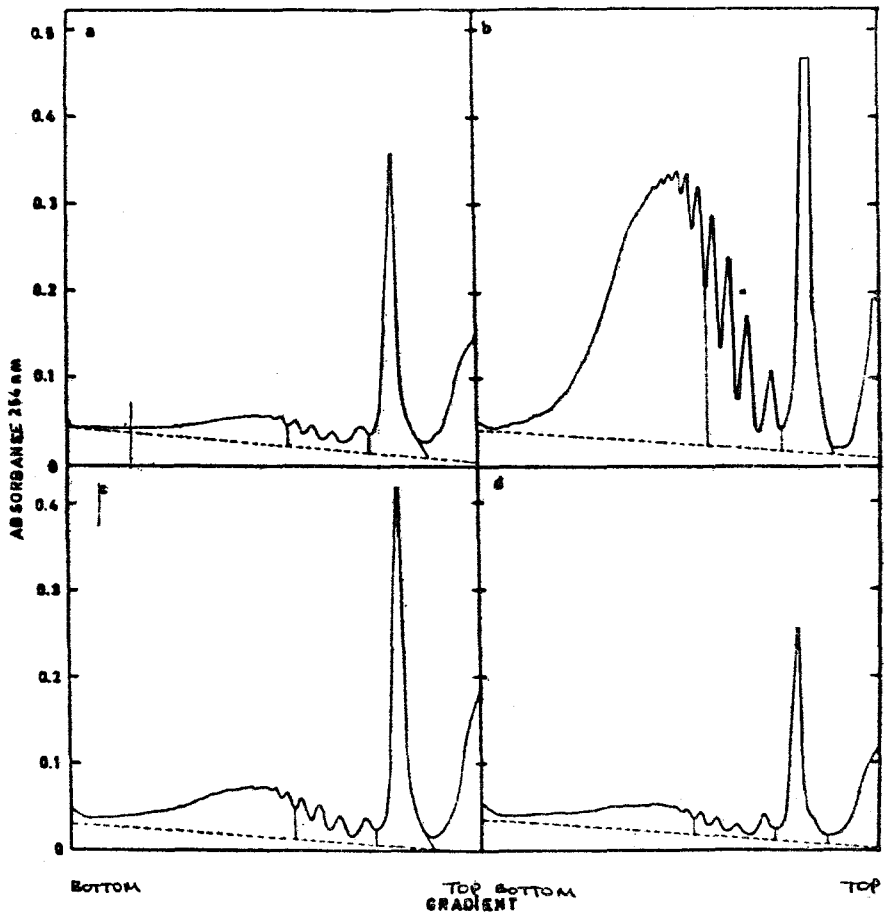

FIGURE 2. Distribution of Polyribosomes From Apical Stem Tissue 48 hrs After Hormone Treatment.

Methods were the same as in Fig. 1, but segments from each treatment were excised for polyribosome isolation after 48 hrs. The amount of tissue used for each gradient was: a) lanolin, 0.573 g; b) high IAA, 0.971 g; c) high GA, 0.874 g; d) low IAA, 0.611 g.

From the data shown here, it is clear that IAA treatment stimulated marked production of polyribosomes, after its application to actively growing tissue, whereas GA treatment showed little stimulation and generally just maintained the endogenous polyribosome levels.

The data shown in Table 1 indicate that high IAA was most effective in decreasing the proportion of monosomes even though at 48 hrs it also caused a 5–fold increase in total ribosome material per segment compared with the lanolin control. This 5–fold increase in ribosomes accompanied by a decrease in the proportion of monosomes means that IAA must have caused a greater than 2–fold increase in polysome-associated messenger- RNA. This IAA induced increase in polyribosomes would presumably suggest more synthesis of protein which would be required for greater growth. An earlier work showed that hormone-stimutaled growth is accomplished through maintenance of available mRNA, and they deduced that the loss of larger polyribosomes was closely related to a decrease in mRNA templates (DAVIES and LARKINS, 1973). In contract to these earlier studies the data presented here suggest that IAA causes a marked increase in the amount of mRNA, whereas GA merely maintains its level. This is in accord with the suggestion that IAA is the dominant hormone and that GA acts merely to maintain the endogenous growth rate, and polysome content and presumably the IAA content.

Because earlier growth studies (DAVIES and ÖZBAY, 1975; ÖZBAY, 1978) had shown that GA was far less effective after a 24 hrs delay in its application, hormone effects on polyribosome metabolism of 24 hrs aged tissue were examined. The polysome profiles are depicted in Fig 3 and 4 and the data analysis is presented in Table 2.

The results are essentially similar to those obtained with immediate application (Fig 1,2; Table 1) except that the effect of high IAA is even more valuable.

The lanolin, GA and low IAA-treated tissues showed declines in total ribosomes on both a segment and a fresh weight basis compared with the 1 day lanolin value (Table 1). However the expanding tissues yielded greater amounts of polyribosomes per segment and per unit fresh weight and a much lower proportion of monosomes. All tissues yielded almost the same proportions of large polyribosomes.

The data presented in Table 2 and Figures 3 and 4 on delayed hormone application show that high IAA caused a considerable conver-

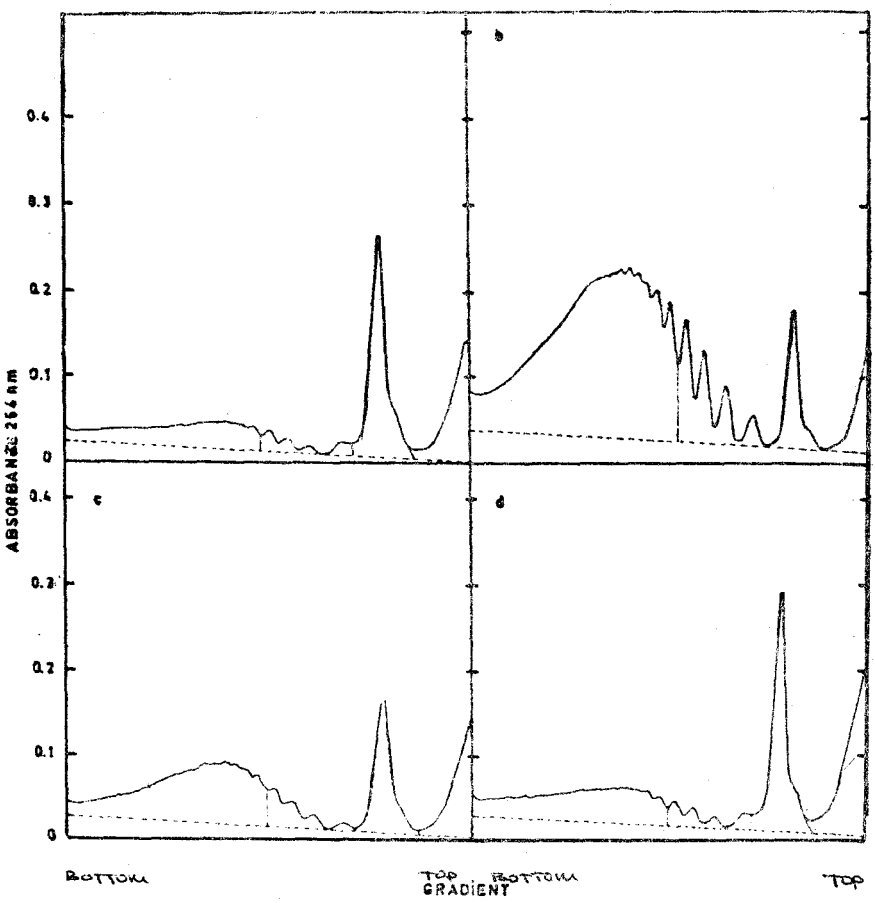

FIGURE 3. Distribution of Polyribosomes 24 hrs After Hormones Treatment of 1 Day Aged Peas Stem Tissues.

Methods were the same as in Fig. 1, except that decapitated epicotyls were treated with plain lanolin for 24 hrs and after 1 day delay, lanolin was removed, a mark made 10 mm from the cut apex and seedlings were treated with lanolin + 0.5 % ,00005 % w/w IAA or 0.5 % w/w GA. Fifteen segments were excised for the isolation of polyribosomes after 1 more days growth. The amount of tissue used for each gradient for 24 hrs was: a) lanolin, 0. 407 g; b) high IAA, 1.057 g; c) high GA, 0.708 g; d) low IAA, 0. 497 g.

sion of monosome into polysomes and, hence, in availability of mRNA (DAVIES and LARKINS, 1973). This can be related to an increase in the synthesis of protein and presumably an increase in growth rate. However, it is difficult to determine exactly how much of the IAA induced conversion of monosomes into polysomes was caused by increas-

TABLE II

Effect of Hormones on Changes in Polyribosomal Distribution of 1 Day Aged Tissue of Pea Seedlings.

Time and Treatment	Total (T/seg)	Material (T/10mg)	L/P (/)	M/T (%)	Frech Weight (mg/seg)
24 Hours					
Lanolin (Control)	38	14.1	78.1	43.8	27.1
High IAA	130	18.6	86.1	6.5	70.5
High GA	52	11.1	83.6	21.8	47.2
Low IAA	49	14.8	82.2	39.2	33.1
48 Hours					
Lanolin (Control)	33	12.2	80.0	50.0	26.7
High IAA	227	29.1	81.1	22.6	77.5
High GA	53	9.6	80.8	40.5	55.0
Low IAA	46	11.08	75.7	44.8	37.5

Data were taken from profiles depicted in Figures 3 to 4. Treatments were applied 24 hrs later on previously decapitated seedlings Final lengths (mm) and swelling (mg/mm) values after 3 days were: lanolin, 11.9,2.2; high IAA, 14,3, 4.4; high GA, 18.7, 2.9; low IAA, 14.5, 2.6.

ed mRNA availability and how much was caused by increased initiation of ribosomes. The IAA-induced increase in polyribosome in the apical region of pea found in this study resembled the results in some previosly reported studies (DAVIES et al., 1972; DAVIES and LARKINS, 1973; DAVIES, 1976; LARKINS and DAVIES, 1975. ANDERSON (1972) reported in his thesis that GA did not appreciably affect the level of polyribosomes in excised soybean hypocotyl. Our findings confirm the same effect of GA in pea epicotlys. But GONZALES (1980) has claimed that GA_3 applied to 48 hrs-germinated seedlings caused a a 50 % stimulation in amount of membrane-bound polysomes, but no stimulation in cytosolic polysomes. However, she did find a 55 % stimulation of the amount of cytosolic monosomes by GA_3. The method employed by GONALES might have involved contamination of the membrane fraction with cytosolic polysomes. Other workers have reported cytosolic polysome contamination as a considerable problem (CARDIELLI et al., 1981). MARTIN and NORTHCOTE (1983) have found that GA_3 appears to stimulate the production of mRNA and rRNA in early germination and accelerate their disappearance later. The stimulation of mRNA and rRNA appearance is probably brought about by increased transcription.

During aging, a disaggregation of free polyribosomes happens (DAVIES and LARKINS, 1973). Ageing of apical pea stem tissue results in decreased protein synthesizing activity. SCHUSTER and DA-

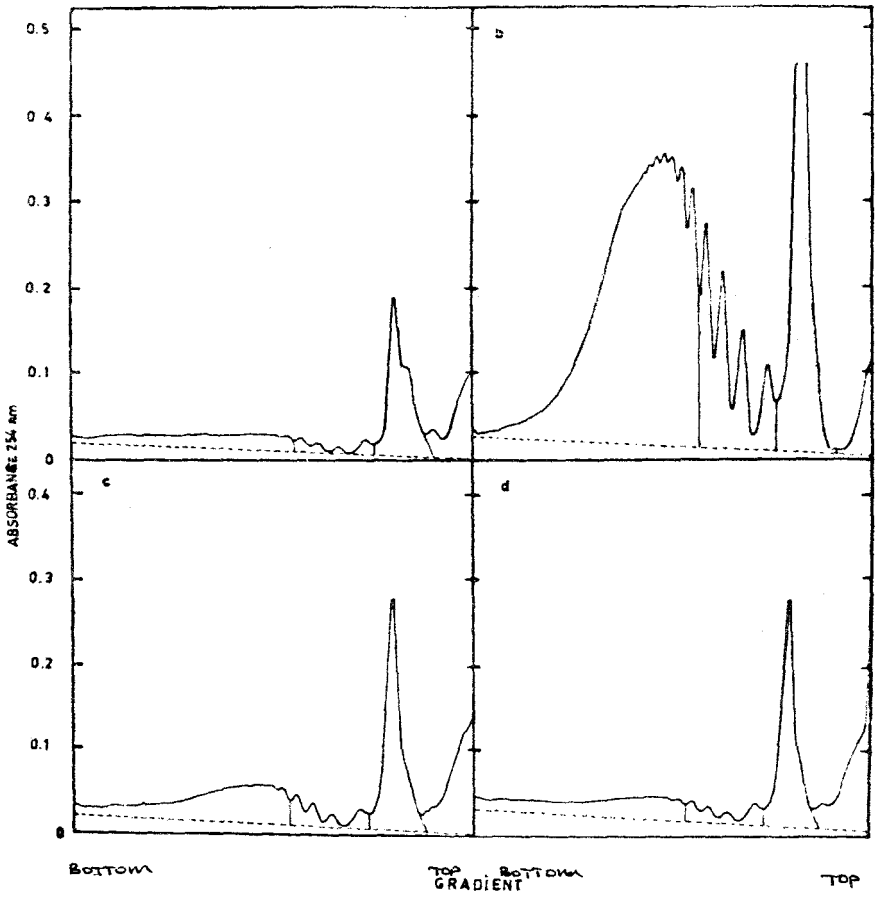

FIGURE 4. Distribution of Polyribosomes 48 hrs After Hormone Treatment of 1 Day Aged Peas Stem Tissues.

Methods were the same as in Fig.3, except that 15 segments were excided for the isolation of polyribosomes after 2 more days growth. The amount of tissue used for each gradient for 48 was: a) lanolin 0.4 g; b) high IAA, 1.163 g; c) high GA, 0.825 g;) low 0.563 g.

VIES (1983), have been able to show that in the aging of actively growing etiolated pea tissues, ribosomal RNA and mesenger RNA contents declined polyribosomes disaggregated, and the protein synthesizing capacity of polysomes decreased. Some of our findings also confirm the same results for aging process in pea epicotyls.

The overall results suggest that effect of IAA may be initiated in the nucleus by the synthesis of new kinds of RNA which have some

of the properties of mRNA. The increases in the relative rate of synthesis of ribosomal RNA and the increases in polysome production, may be a primary requirement for the stimulation of protein synthesis by auxins which is observed in whole plants.

Conclusions

In this study, the most striking results are as follows:

Expanding tissues yielded large increases in polyribosomes on both a segment and fresh weight basis.

Elongating tissues yielded more polyribosomes per segment, but only maintained them on a fresh weight basis.

Non - growing tissues yielded fewer polysomes per segment or per unit fresh weight.

All treatments yielded almost identical proportions of large polyribosomes.

IAA treatment stimulated marked production of polyribosomes, whereas GA treatment showed little stimulation and generally just maintained the endogenous polyribosome levels.

This IAA-induced increase in polyribosomes would presumably suggest more synthesis of protein which would be required for greater growth

All of these findings are in accord with the suggestion that IAA is the dominant hormone which causes a marked increase in the amount of mRNA, wheras GA merely maintains its level and acts to maintain the endogenous growth rate, and polysome content and presumably the IAA content.

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ÖZET

Bezelye epikotili ribozom seviyesine bazı hormonların etkileri çalışılmıştır. İlgili poliribozom profilleri, etiyole olmuş (karanlıkta büyütülmüş) *Pisum sativum* L. var Alaska bitkisine ait gövde parçalarından elde edilmiştir.

Poliribozom metabolizmasıyla ilgili olrak, şişme gösteren dokular, gerek parça ve gerekse taze ağırlık başına düşen poliribozom miktarından fazlaca artış göstermiştir. Uzama gösteren dokular, parça başına düşen poliribozom miktarında artış gösterdiği halde, taze ağırlık esasına göre miktarlarını düzenlediği kaydedilmiştir. Büyüme göstermeyen (kontrol) doku ise birim taze ağırlığa veya parça başına bir kaç adet poliribozom vermiştir. Bezelye fidelerinin uçları koparıldıktan sonra hormonarın derhal veya 1 günlük gecikmeden sonra bitkiye tatbikinde sonuçlar değişmemiştir.

Bu çalışmada, bezelye epikotilindeki poliribozomların miktarına hormonların nasıl etki ettikleri ve elde edilen bulguların, hormonların protein sentezi üzerindeki etkilerine ait mevcut bilgilerle ilişkisini belirlemek, amaç edinilmiştir.