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Nucleic Acid Synthesis during the Hormone-Stimulated Growth of Excised Oat Coleoptiles*

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ABSTRACT: The effect of the plant hormone, indoleacetic acid, on nucleic acid synthesis during the hormone-stimulated elongation of oat coleoptile sections was studied by incubating the sections with and without the hormone in the presence of $H_3{}^{32}PO_4$ for 6 hr, following which time the total nucleic acid complement of the sections was extracted and fractionated on MAK columns, and the extent of isotope incorporation into the various nucleic acid species was determined. The contribution to the radioactivity profiles made by contaminating bacteria in the incubation media was visualized and then made negligible by the inclusion of gramicidin in the medium. The hormone was found to stimulate twofold the incorporation of isotope into soluble ribonucleic acid and ribosomal ribonucleic acid, although there was no net increase in either species of nucleic acid during incubation. The hormone stimulated fourfold the isotope incorporation into a ribonucleic acid fraction that is tenaciously bound to methylated albumin kieselguhr columns and not elutable with a salt gradient at neutral pH. In addition, the base composition of this tenaciously bound ribonucleic acid from hormone-treated tissue was found to resemble that of oat deoxyribonucleic acid when the composition was based on the radioactivity of the ribonucleotides.

Many reports have linked mammalian hormone action with DNA-directed RNA synthesis. More recently the action of several plant hormones has been related to RNA synthesis. The effects of IAA,[1] 2,4-D, and gibberellic acid on excised plant tissues have been shown to be actinomycin D sensitive (Nooden and Thimann, 1963; Venis, 1964; Hamilton *et al.*, 1965; Roychoudhury and Sen, 1964; Key and Shannon, 1964; Chandra and Varner, 1965). In several of these studies an enhanced incorporation of radioactive precursors into RNA has been shown to accompany the hormone-stimulated growth of these tissues (Key and Shannon, 1964; Roychoudhury and Sen, 1964; Hamilton *et al.*, 1965).

In this report we present data that show an enhanced incorporation of radioactive precursors into RNA in excised oat coleoptiles incubated with the hormone IAA at a concentration that stimulates markedly the elongation of this excised tissue. This corroborates the findings of Hamilton *et al.* (1965), who have also reported such an enhancement in this tissue. In addition, we have attempted to effect a quantitative extraction of the tissue nucleic acid, to characterize by MAK chromatography the species of RNA whose synthesis is stimulated by the hormone, and to characterize the artifactual contribution by contaminating bacterial nucleic acid synthesis to the radioactivity profiles of the tissue nucleic acids.

Materials and Methods

Preparation and Incubation of Tissue. Seeds of *Avena sativa* L. (Svalöf's Original Victory I: Allmänna Svenska

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[1] Abbreviations used: IAA, indoleacetic acid; TB-RNA, tenaciously bound RNA; 2,4-D, 2,4-dichlorophenoxyacetic acid; SDS, sodium dodecyl sulfate; MAK, methylated albumin kieselguhr; SSC, standard saline citrate (0.015 M NaCl and 0.0015 M trisodium citrate, pH 7); AMP, CMP, GMP, TMP, and UMP, adenosine, cytosine, guanosine, thymidine, and uridine monophosphates.

Utsädes AB, Svalöv, Sweden) were soaked in running water for 2 hr and germinated in darkness for 72 hr. One-hundred 10-mm coleoptile sections were cut under dim red light starting 5 mm below the tip. After removal of the primary leaf the sections were placed into 30 ml of incubation medium and rinsed thoroughly. After draining the sections, they were returned to 10 ml of incubation medium (2% sucrose, 0.0025 M potassium succinate (pH 4.5), 10^{-4} M penicillin G (K), 10^{-4} M streptomycin sulfate, and $H_3{}^{32}PO_4$ to give 100 μc/ml). Gramicidin D was added to give a concentration of 3×10^{-6} M as indicated in the text. In incubations testing the effect of the hormone on RNA synthesis, IAA was added to give a final concentration of 1×10^{-5} M (1.75 μg/ml). The sections were incubated in darkness at 26° with shaking for 6 hr, after which time they were rinsed with cold 0.1 M sodium acetate (pH 6), 0.001 M EDTA, and 1 mg/ml of bentonite (prepared according to Fraenkel-Conrat *et al.*, 1961). During this incubation, the control sections elongate 20% of their initial length while the hormone-treated sections elongate 60%.

Extraction of Tissue for Total Nucleic Acid. The sections were homogenized in 10 ml of 0.1 M sodium acetate (pH 6), 0.001 M EDTA, and 1 mg/ml of bentonite with a loose-fitting Duall homogenizer driven at 100 rpm in an ice bath. An equal volume of redistilled phenol, saturated with the above buffer, was added to the homogenate and the mixture was shaken for 30 min at 1°. The phases were separated by centrifugation and the aqueous layer was removed and frozen. The phenol and interface layers were next extracted with 10 ml of 0.1 M Tris-succinate (pH 8.5), 2% SDS, 0.1 M NaCl, 0.001 M EDTA, and 1 mg/ml of bentonite at room temperature for 30 min. After centrifugation, the aqueous phase was removed and frozen. The phenol and interface layers were finally extracted with 10 ml of 0.1 M Tris-succinate (pH 8.5), 2% SDS, 0.7 M NaCl, 0.001 M EDTA, and 1 mg/ml of bentonite at 50° for 15 min. After centrifugation of the last extraction mixture, the three aqueous layers were combined, made 2% in potassium acetate (pH 5), and then made 70% in cold ethanol. The nucleic acids were allowed to precipitate overnight at −20°. The resulting precipitate was collected by centrifugation, resuspended in 10 ml of 0.01 M Tris-succinate (pH 7.8), 0.01 M NaCl, and 0.001 M EDTA, and the ethanol precipitation step was repeated. The nucleic acids thus obtained were solubilized in the Tris-succinate–NaCl–EDTA buffer and centrifuged to remove traces of bentonite and other insolubles. The purity of these preparations was determined by their ultraviolet absorption spectra, using a Bausch & Lomb 505 spectrophotometer.

Extraction of Incubation Medium for Total Nucleic Acid. In order to determine the extent of bacterial nucleic acid synthesis occurring in the incubation medium, this solution was also extracted for total nucleic acids. After the 6-hr incubation, the medium was drained from the sections which were then rinsed with 10 ml of the pH 6 homogenizing solution. The original medium and the rinse were then pooled, and 5 A_{260} units of purified oat DNA and 10 A_{260} units of purified oat rRNA were added as carrier nucleic acid. The resulting solutions were then extracted by the same three-step phenol method used for the tissue homogenates.

Procedure for Labeling the Nucleic Acids of Intact Plantlets. After soaking, the seeds were germinated between layers of Miracloth sandwiched between layers of moist sand. After 72-hr germination at 26°, 100 plantlets were placed upright in a 75 × 150 mm crystallization dish. $H_3{}^{32}PO_4$ was added in glass-distilled water at 100 μc/ml in an amount that just covered the plantlet roots, and the plantlets were incubated in the dark for 10 hr. Coleoptile sections were obtained which correspond to those used in the floating incubations, and extracted by the three-step phenol procedure.

Preparation of rRNA for Sucrose Density Gradient Centrifugation. The coleoptile and primary leaf were excised from 72-hr germinated plantlets and immediately extracted by the three-step phenol method. The purified nucleic acids which resulted were solubilized in 0.01 M Tris-succinate (pH 7.8) and 0.001 M EDTA. The resulting solution was made 1 M in NaCl and kept at 1° for 24 hr. The nucleic acids (primarily rRNA) which precipitated were collected by centrifugation and solubilized in 0.01 M Tris-succinate (pH 7.8) and 0.001 M EDTA to give a concentration of 4 mg of RNA/ml.

Preparation of DNA. The 1 M NaCl supernatant from the above procedure was carefully overlayed with two volumes of cold ethanol and held at −20° in the freezer for 30 min after which time the fibrous DNA was wound out at the interface on a glass rod. The DNA was drained of ethanol and dissolved in dilute SSC. RNase was added to give a concentration of 50 μg/ml and the mixture was incubated for 30 min at 37°. Following this digestion, the mixture was deproteinated by the phenol procedure, and the DNA was again wound out from an aqueous ethanol interface, and resolubilized in dilute SSC. This last step was repeated until all traces of phenol were removed.

Sucrose Density Gradient Fractionation. RNA was fractionated on a 25-ml linear sucrose gradient (6–22%) containing 0.01 M Tris-succinate (pH 7.8), 0.001 M EDTA, and 0.05% SDS. The gradients were centrifuged at 25,000 rpm for 20 hr. The absorbancy profiles of the resulting RNA gradients were obtained by using an ISCO gradient fractionator with automatic recording of absorbancy at 254 mμ.

MAK Column Chromatography of Extracted Nucleic Acids. Methylated albumin was prepared and adsorbed onto Celite 545 by the method of Mandel and Hershey (1960). The column consisted of one layer of MAK (column volume, 20 ml) overlayed with a layer of Celite 545. The purified nucleic acids from 100 sections were adsorbed onto the column in 0.3 M KCl and 0.05 M potassium phosphate (pH 6.7) and eluted with a linear gradient made from 70 ml of 0.5 M KCl and 70 ml of 1.7 M KCl buffered with 0.05 M potassium phosphate (pH 6.7). The gradient was pumped at 1 ml/min through an ISCO column monitor with recorder. After the salt gradient was terminated, the column was washed with

1.5 M NH_4OH to elute those nucleic acids not elutable with salt. Fractions (1 ml) of the gradient and NH_4OH eluate were collected and the radioactivity of 0.1-ml aliquots of each was determined by the paper disk method of Bollum (1966).

The MAK column was washed with the NH_4OH after completion of the salt gradient in order to elute a fraction of nucleic acid that is retained by the column during the salt elution. This fraction is referred to as tenaciously bound nucleic acid after Ellem and Sheridan (1964) who reported that this fraction could be eluted with NH_4OH. We have observed this fraction in all MAK elutions of total nucleic acids from higher plant material when the column is washed with NH_4OH. The spectrum of this fraction invariably reveals some contamination with the methylated albumin from the column, and consequently only the radioactivity eluting with this fraction is plotted in the figures.

This failure to achieve a complete elution of nucleic acid from the MAK column with a salt gradient is apparently due to nucleic acid aggregation at low charge densities, and the yield with salt elution can be improved by using KCl rather than NaCl as the eluent (V. J. Medina and L. S. Dure, in preparation).

Nucleotide Composition of Nucleic Acid Fractions. The nucleic acid which was eluted by NH_4OH from the MAK column was precipitated by ethanol after the addition of 8 A_{260} units of purified rRNA. The precipitated nucleic acids were solubilized in 1 ml of 0.3 M KOH and incubated at 37° for 18 hr. The hydrolysates were next chilled in an ice bath and neutralized with perchloric acid. After removing the insoluble $KClO_4$ by centrifugation, the supernatant was made 0.5 N HCl and adsorbed onto Barneby–Cheney charcoal which was first deactivated with toluene. After washing the column to neutrality with glass-distilled water the nucleotides were eluted with a solution of ethanol–water–ammonium hydroxide (2:2:1, v/v). The eluate was reduced to dryness by flash evaporation and the nucleotides were solubilized in 95% ethanol. The nucleotides were then separated by the paper chromatography method of Lane (1963). The resulting chromatogram was analyzed for radioactivity with a Vanguard 880 strip counter and the individual nucleotide spots were cut from the chromatogram and their relative amounts were determined on the basis of their radioactivity in a Tri-Carb liquid scintillation spectrophotometer.

The nucleotide composition of rRNA was determined on pooled fractions from sucrose density gradients by the method of Katz and Comb (1963).

The following procedure was followed to determine the nucleotide composition of oat DNA. Purified DNA was solubilized in dilute SSC to give a concentration of 1 mg/ml. This preparation (15 ml) was made 0.1 M KOH and incubated at 37° for 1 hr. After cooling and neutralizing with HCl, the preparation was subjected to chromatography on Sephadex G-25 to remove ribonucleotides. The DNA fraction from the Sephadex chromatography was precipitated with cold ethanol, collected by centrifugation, solubilized in 6 ml of 0.015 M NaCl, and brought to 95° to remove traces of ethanol. After cooling, the solution was made 0.01 M Tris-succinate (pH 7.5) and 0.005 M $MgCl_2$. A crystal of DNase I was added and the solution was incubated at 37° for 1 hr. The solution was next made 0.05 M Tris-succinate (pH 8.5) and 0.1 mg of phosphodiesterase was added. This was incubated at 37° for 1.5 hr. Next, the pH was carefully adjusted to 4.2 with HCl and the solution was placed in a boiling water bath for 7 min to inactivate phosphomonoesterases. After chilling, the preparation was centrifuged at 15,000*g* for 25 min and the supernatant containing the deoxyribonucleotides was analyzed by the methods of Cohn (1955) and Katz and Comb (1963).

Source of Chemicals. Carrier-free $H_3{}^{32}PO_4$ was obtained from New England Nuclear Corp. Sucrose and SDS were purchased from the Mann Research Laboratories, Inc. IAA, penicillin G (K), and streptomycin sulfate were purchased from Calbiochem. Gramicidin D was purchased from Sigma Chemical Co. DNase I (electrophoretically purified), RNase, and snake venom phosphodiesterase were obtained from Worthington Biochemical Corp.

Results

Extraction of the Total Nucleic Acid Fraction from the Tissue. Utilizing the differential extraction procedure outlined in the Methods section, quantitative extraction of all the nucleic acid in an undegraded condition was achieved. Subsequent extracts of the residues with boiling 1 M NaCl did not liberate further material with a ultraviolet absorption spectrum resembling nucleic acids. The nucleic acid preparations routinely had the spectral properties typical of uncontaminated nucleic acids. The 260:280 and 260:231 absorbancy ratios were at least 2, and the 260:220 ratio was greater than 1.

MAK column chromatography was used to determine the amounts of sRNA, DNA, and rRNA in the 10-mm coleoptile section at this stage of seedling growth and after 6-hr incubation. All of the ultraviolet-absorbing material of these preparations adsorbed onto the MAK columns in 0.3 M KCl. Since the MAK column retains approximately 5–10% of the rRNA and a small amount of DNA, the yields of these species of nucleic acid are not precise values. These yields are given in Table I and are A_{260} units/100 coleoptile

TABLE I: Yields of Different Species of Nucleic Acid/100 Sections.[a]

Fraction	sRNA	rRNA	DNA
Before incubation	2.50	11.94	2.49
After incubation			
Control sections	2.55	12.00	2.45
Hormone-treated sections	2.52	11.80	2.68

[a] Absorbancy units at 260 mμ.

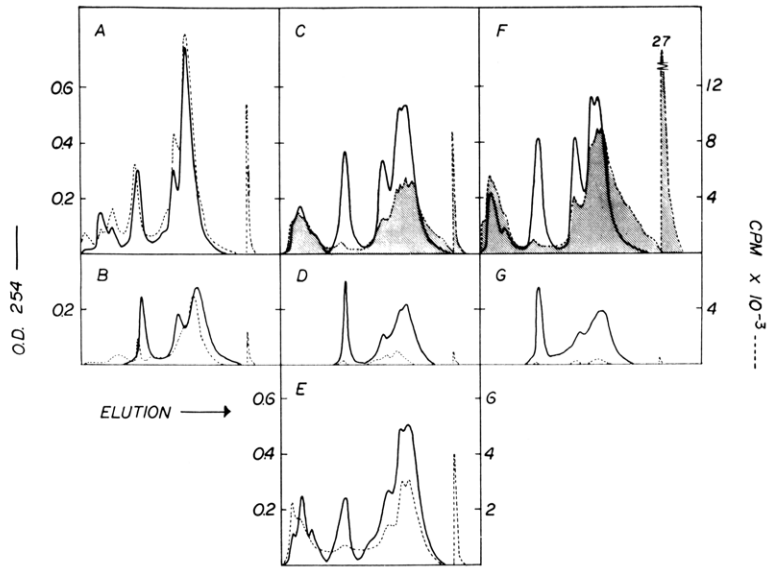

FIGURE 1: MAK profiles of absorbancy and radioactivity of the total nucleic acids from 100 coleoptlie sections. (A) From sections incubated in penicillin and streptomycin. (B) From incubation medium of A (carrier DNA and rRNA added). (C) From sections incubated in penicillin, streptomycin, and gramicidin. (D) From incubation medium of C (carrier DNA and rRNA added). (E) From coleoptile sections labeled while on intact plantlet. (F) From sections incubated in penicillin, streptomycin, gramicidin, and IAA. (G) From incubation medium of F (carrier DNA and rRNA added). Radioactivity profiles shaded in 1C and 1F for emphasis.

sections. No significant difference in the yield of nucleic acid was observed between the control tissue and that incubated with the hormone, nor was there a net increase in any species of nucleic acid observed during the 6-hr incubation.

Isotope Incorporation into Nucleic Acids by Sections Incubated with Streptomycin and Pencillin. When excised plant tissue is incubated for extended periods in sugar media, bacterial contamination is an expected consequence. Although the elongation growth of the oat coleoptiles is not affected by a small bacterial contamination of the medium, the measurement of nucleic acid synthesis by radioactive precursor incorporation may be enormously affected by the contribution of radioactive nucleic acid synthesized by the bacteria. Antibiotics in general fail to prevent all traces of bacterial growth in such incubations.

Figure 1A is the MAK profile of absorbancy and radioactivity of the total nucleic acid complement from 100 coleoptile sections after incubation for 6 hr in the incubation medium containing streptomycin and penicillin. In order to estimate the amount of nucleic acid radioactivity contributed by bacterial synthesis in the medium, the incubation medium itself, after removal of the tissue, was extracted for nucleic acids using the phenol method. Five A_{260} units of oat DNA and ten A_{260} units of oat rRNA were added to the medium as carrier nucleic acid. Figure 1B shows the MAK profile of radioactivity extracted from the medium and of the absorbancy of the carrier nucleic acid. The radioactivity profile of Figure 1B may reflect nucleic acids synthesized by bacterial contaminants, or conversely, it may represent leakage of radioactive nucleic acids from the cut surfaces of the tissue. In either case the two figures reveal that approximately 23% of the total radioactivity incorporated into the nucleic acids extracted from both the tissue and the medium after 6-hr incubation is found in the medium, whereas no discernible amount of nucleic acid absorbance can be isolated from the medium without carrier.

In addition the MAK profiles of radioactivity in Figure 1A,B show an incorporation of the isotope into a species of DNA that elutes from the column just prior to the bulk of the oat DNA absorbancy. This radioactivity is presumed to be DNA since it is not rendered acid soluble by alkaline hydrolysis.

Isotope Incorporation in the Presence of Gramicidin. Figure 1C,D shows the absorbancy and radioactivity profiles resulting from an experiment identical with that shown by Figure 1A,B except that gramicidin, a broad spectrum antibiotic, has been added to the incubation medium in addition to streptomycin and penicillin. Figure 1C reveals that the incorporation of $^{32}PO_4^{3-}$ into all species of tissue nucleic acid has been reduced by the gramicidin and Figure 1D shows that with gramicidin in the medium very little radioactive nucleic acids are extracted from the medium. This suggests that perhaps some of the radioactivity associated with the nucleic acid preparations from the tissue incubated with only penicillin and streptomycin is bacterial nucleic acid, the synthesis of which can be retarded by the gramicidin inhibition of bacterial growth.

The most extensive effect of gramicidin is to de-

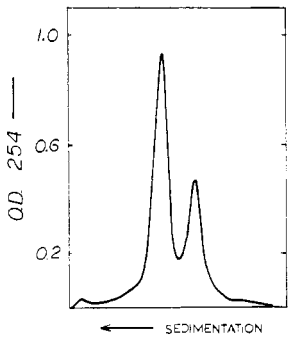

FIGURE 2: Sucrose density gradient absorbancy profile of carrier rRNA.

crease the radioactivity eluting just prior to the tissue DNA. Several investigators have reported a peak of radioactivity eluting prior to the DNA absorbancy peak in the MAK column chromatography of plant nucleic acids after incubation of the tissue with radioactive precursors (Ingle *et al.*, 1965; Cherry, 1964; Sampson *et al.*, 1965). In all of these cases the isotope incubation involved the incubation of excised tissue with isotope in aqueous solutions. In one case (Ingle *et al.*, 1965) streptomycin was present in the incubation mixture, while in the others no antibiotic was reported to have been used. This radioactivity eluting prior to the principal DNA absorbancy was reported to be in DNA (Ingle *et al.*, 1965; Sampson *et al.*, 1965) and in a DNA–RNA hybrid (Cherry, 1964).

The synthesis of nuclear DNA in this incubated coleoptile section would be surprising in that no cell division is thought to take place in this tissue at this stage of growth (Avery *et al.*, 1945). However, recently data have been presented (Nitsan and Lang, 1966) showing that a considerable amount of radioactivity eluted with the DNA isolated from a tissue that was incubated with [^{3}H]thymidine at a stage in which no cell division was thought to take place.

The synthesis of a species of DNA eluting from MAK columns prior to the nuclear DNA as is seen in Figure 1A could reflect the synthesis of the DNA of cytoplasmic organelles or that of bacteria. In view of the effect of gramicidin, it would seem that in this case, the majority of the radioactivity eluting with the leading edge of the DNA absorbancy is contained in bacterial DNA.

To further test the validity of the radioactivity profile observed from the tissue incubated with gramicidin, and to determine if any DNA synthesis can be observed in this tissue section when not dissected from the growing plantlet, 100 intact oat seedlings were allowed to absorb $^{32}PO_4^{3-}$ through their root systems for 10 hr. After this time the same coleoptile section as used in the excised tissue incubations was removed, and the nucleic acids were extracted and subjected to MAK chromatography (Figure 1E). Since in this experiment the isotope must be absorbed by the roots and translocated to the aerial coleoptile, the amount of isotope actually incorporated into the coleoptile nucleic acids is not comparable to the amount incorporated by the excised coleoptile sections incubated in liquid media. However, the relative extent of isotope incorporation into the various species of nucleic acids by the intact coleoptile section and the excised section should indicate the extent to which bacterial nucleic acid synthesis influences the radioactivity profile of the nucleic acid preparations. Figure 1E shows that there is very little radioactivity eluting with the DNA from the intact seedling nucleic acid preparations. Consequently, we feel that the MAK profile of isotope incorporation into the nucleic acids of the excised sections incubated with penicillin, streptomycin, and gramicidin is a valid measurement uninfluenced by bacterial nucleic acid synthesis.

Isotope Incorporation by Sections Incubated with Penicillin, Streptomycin, Gramicidin, and IAA. Figure 1F shows the absorbancy and radioactivity profiles of the total nucleic acid fraction from 100 sections whose incubation medium contained all three antibiotics and also IAA at the concentration most stimulatory to cell elongation in this tissue. Figure 1G, which gives the profiles of the incubation medium preparation, shows again that gramicidin has reduced bacterial nucleic acid synthesis markedly when compared to the radioactivity profile of the medium not containing gramicidin (Figure 1B). Figure 1F further shows that the inclusion of this concentration of IAA in the incubation medium has increased the incorporation of isotope into sRNA and rRNA about twofold over that obtained in the absence of IAA (Figure 1C). More interestingly the hormone has increased the incorporation of isotope into the TB-RNA fourfold. The base composition of the radioactive TB-RNA from the control preparation (Figure 1C) and from the IAA-treated tissue (Figure 1F) was determined by comparing the relative amount of radioactivity of the ribonucleotides. These data are presented in Table II. In addition, the base composition of the two rRNA species and of oat DNA was determined by absorbancy methods and is also presented in Table II.

The radioactive TB-RNA of the control tissue has a very high molar percentage of AMP (38.9%) and a very low percentage of GMP (17.6%), which is quite distinct from either of the species of rRNA. Its AMP:UMP ratio shows it to be quite unlike DNA in base composition. On the other hand, the TB-RNA from the hormone-treated tissue has a base composition that is remarkably similar to that of DNA. Its AMP:UMP ratio approaches 1 and its AMP + UMP:GMP + CMP ratio is very close to the equivalent ratio for DNA.

MAK Chromatography Profiles. In all the MAK chromatography elution profiles of these nucleic acid preparations, the RNA in the region of the rRNA elution is very poorly resolved. In some cases an additional peak is observed. This poor resolution and the additional peak are thought to be the result of

TABLE II: Nucleotide Composition (molar percentage) of RNA and DNA.

Fraction	AMP	GMP	CMP	UMP-(TMP)	CMP: GMP	AMP: UMP	AMP + UMP(TMP): GMP + CMP
Light rRNA	22.9	29.7	23.2	24.2	0.78	0.95	0.89
Heavy rRNA	22.0	31.9	24.2	21.9	0.76	1.01	0.78
Control TB-RNA	38.9	17.6	23.4	20.1	01.33	1.93	1.44
Hormone TB-RNA	26.3	20.6	24.6	28.5	01.19	0.93	1.21
DNA	27.5	21.6	22.2	28.7	01.03	0.96	1.28

nucleic acid intermolecular binding during elution, and can be resolved into the two species of rRNA with the theoretical yield of each on the MAK column upon further treatment of the nucleic acid preparation and the MAK column (V. J. Medina, and L. S. Dure, in preparation). The RNA of this elution region can also be shown to consist principally of the two species of rRNA by sucrose density gradient centrifugation in the presence of 0.05% SDS. Figure 2 is the sucrose density gradient absorbancy profile of 10 A_{260} units of purified oat rRNA from the same preparation that was added to the incubation medium as carrier. This preparation is poorly resolved on the MAK column as shown by Figure 1B,D,G. However, on the sucrose density gradient it is shown to contain almost exclusively the two species of rRNA with twice the area under the absorbancy curve of the heavy rRNA as is under that of the light rRNA. Hence the additional peaks and shoulders seen on the MAK chromatography profiles are considered artifacts and not new or unusual RNA species.

Discussion

The plant hormone, IAA, at 10^{-5} M, a concentration that stimulates maximally the elongation of the excised segment of the oat coleoptile, is shown by these data to bring about a parallel increase in the extent of isotope incorporation into sRNA and rRNA. In addition, an even greater enhancement of isotope incorporation into RNA that is tenaciously bound to the MAK column is observed. The bulk of the RNA tenaciously bound to the MAK column is found to be rRNA in most cases when the base composition of this fraction is based on absorbancy. However, when the base composition of this fraction is determined by isotope incorporation, a very high specific activity species of RNA with a base composition unlike rRNA is shown to contribute the bulk of the radioactivity in this TB-RNA. Furthermore, the data indicate that the base composition of this nonribosomal TB-RNA fraction is distinctly modified when this tissue is incubated with IAA.

Further characterization of this RNA fraction by rechromatography on MAK has not been fruitful, since it appears to have suffered partial hydrolysis during the NH_3OH elution, and consequently gives a broad elution profile on rechromatography.

It could be said that this tenaciously bound radioactive RNA fraction has not only been increased by incubation with IAA but has become DNA-like RNA, an RNA fraction reported from many tissues by numerous investigators. Ellem (1966) has recently reported that a rapidly labeled, DNA-like RNA fraction isolated from HeLa cells is tenaciously bound to MAK columns.

It is tempting to speculate that the radioactive RNA eluting with NH_4OH is principally mRNA; that the hormone has increased the synthesis of mRNA as shown by its increased radioactivity; and that this stimulated synthesis represents a general stimulation of mRNA synthesis since the base composition of this fraction as determined by radioactivity has become very similar to that of oat DNA. However, without an adequate characterization of the RNA that elutes with NH_4OH and without an unequivocal assay for mRNA, such speculations should be made with caution. At present, there is no compelling reason for assuming that the mRNA synthesized at any one period in a tissue's existence should reflect a DNA-like base composition, unless one assumes that a generalized stimulation of transcription that involves a small portion of the DNA may be representative of the total DNA base composition. On the other hand, there exists evidence that only one strand of the DNA is transcribed which weakens this assumption (Hayashi *et al.*, 1963).

Several reports (Hamilton, 1964; Notides and Gorski, 1966; Wool and Cavicchi, 1966) present data suggestive that the initial action of several mammalian hormones is to promote a specific and hitherto blocked translation from which a generalized stimulation of transcription may follow. The fact that this hormone in this system, similar to other plant and mammalian hormones, is shown to bring about a general stimulation of the synthesis of all species of RNA, rather than to stimulate the synthesis of a specific mRNA fraction, also tends to subvert the idea that this hormone's direct and primary action is upon transcription.

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Cell-Free Synthesis of Thyroglobulin*

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ABSTRACT: Microsomal and polyribosomal fractions, prepared from calf thyroid glands, incorporate radioactive amino acid into both particle-bound and soluble proteins. In both cases, the incorporation of leucine into internal peptide linkage resembles that of protein synthesis in other mammalian systems. Sucrose density gradient centrifugation analysis shows the radioactive protein extractable from microsomes or ribosomes to sediment at 3–12 S, while a considerable portion of the labeled soluble protein released into the medium during incubation of microsomes but not of ribosomes resembles thyroglobulin in sedimentation and immunological properties. By the double-antibody technique, some of the labeled soluble protein sedimenting at 3–8 S was shown to be immunologically related to thyroglobulin and presumably subunits of thyroglobulin. These studies suggest a role for membrane-containing structures in the biosynthesis of thyroglobulin.

Thyroglobulin, a complex carbohydrate and iodine-containing protein with a sedimentation constant of 19 S and a molecular weight of 660,000, is the major protein synthesized in the thyroid gland. Previous reports from this laboratory and others (Seed and Goldberg, 1963, 1965a; Lissitzky *et al.*, 1965; Nunez *et al.*, 1965b) have shown that thyroid slices incorporate [^{14}C]amino acids into protein of one-quarter (3–8 S) and one-half (12 S) the size of thyroglobulin and that this isotope is "chased" by continued incubation with unlabeled amino acids from the smaller proteins into thyroglobulin-like material. The latter sediments at 17–18 S, is specifically precipitated with antithyroglobulin antibodies, and is converted to a material sedimenting at exactly 19 S by treatment with a chemical iodinating system (Goldberg and Seed, 1965; Nunez *et al.*, 1965b). These and other observations (Seed and Goldberg, 1965a; Sellin and Goldberg, 1965) have been interpreted to indicate

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