tionen des lignins bei der Sulfatkochung. II. Modellversuche zur Spaltung von Arylalkyllatherbindingungen durch Alkali. Acta Chem. Scand. 16: 1713–29.
8. HANSON, K. R. AND M. ZUCKER. 1963. The biosynthesis of chlorogenic acid and related conjugates of the hydroxycinnamic acids. J. Biol. Chem. 238: 1105–15.
9. HIGUCHI, T. 1957. Biochemical studies of lignin formation. II. Physiol. Plantarum 10: 621–32.
10. HIGUCHI, T. 1958. Studies on the chemical properties of bamboo stalk. J. Biochem. (Tokyo) 45: 675–85.
11. HIGUCHI, T. AND S. A. BROWN. 1963. Studies on lignin biosynthesis using isotopic carbon. XI. Reactions relating to lignification in young wheat plants. Can. J. Biochem. Physiol. 41: 65–76.
12. HIGUCHI, T. AND S. A. BROWN. 1963. Studies of lignin biosynthesis using isotopic carbon. XIII. The phenylpropanoid system in lignification. Can. J. Biochem. Physiol. 41: 621–8.
13. LEMON, H. W. 1947. The effect of alkali on the ultraviolet absorption spectra of hydroxyaldehydes, hydroxyketones, and other phenolic compounds. J. Am. Chem. Soc. 69: 2998–3000.
14. LEVY, C. C. AND M. ZUCKER. 1960. Cinnamic and *p*-coumaryl esters as intermediates in the biosynthesis of chlorogenic acid. J. Biol. Chem. 235: 2418–25.
15. RICHTZENHAIN, H. 1949. Enzymatische Versuche zur Entstehung des Lignins. IV. Mittel Dehydrierung in der Guajacolreihe. Chem. Ber. 82: 447–53.
16. SIEGEL, S. M. 1954. Studies on the biosynthesis of lignins. Physiol. Plantarum 7: 41–50.
17. SIEGEL, S. M. 1957. Non-enzymic macromolecules as matrices in biological synthesis: The role of polysaccharides in peroxidase catalyzed lignin polymer formation from eugenol. J. Am. Chem. Soc. 79: 1628–32.
18. SMITH, D. D. C. 1955. Ester groups in lignin. Nature 176: 267–68.
19. STAFFORD, H. A. 1960. Differences between lignin-like polymers formed by peroxidation of eugenol and ferulic acid in leaf sections of Phleum. Plant Physiol. 35: 108–14.
20. STAFFORD, H. A. 1960. Comparison of lignin-like polymers produced peroxidatively by cinnamic acid derivatives in leaf sections of Phleum. Plant Physiol. 35: 612–18.
21. STAFFORD, H. A. 1962. Histochemical and biochemical differences between lignin-like materials in *Phleum pratense* L. Plant Physiol. 37: 643–49.
22. STAFFORD, H. A. AND I. HELLER. 1963. Lignin production in tissues of Elodea and Phleum. Plant Physiol. 37: lvi.

Enhancement by Auxin of Ribonucleic Acid Synthesis in Excised Soybean Hypocotyl Tissue [1, 2, 3]

Joe L. Key and Jack C. Shannon

Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana

Many changes in RNA metabolism occur during normal (3, 4, 9, 16, 27, 28) and auxin-modified growth (1, 2, 7, 8, 10, 11, 12, 13, 14, 25) of plant cells. Skoog (18) suggested in 1954 that the action of auxin in regulating growth is concerned with nucleic acid metabolism. Yet, there is no evidence that the influence of auxin on RNA metabolism is more primary than other metabolic responses of auxin.

The experiments reported in this and the following paper (11) were done to gain additional information on the regulation by auxin of growth and development of plant cells as related to RNA metabolism. In general, growth promotive concentrations of auxin stimulated C^{14}-nucleotide incorporation into RNA of elongating cells whereas inhibitory concentrations decreased incorporation. In fully elongated cells, auxin induced a net synthesis of RNA, the increase occurring primarily in the ribosomal fraction. The data reported provide evidence for the DNA dependence of ribosomal RNA synthesis.

Materials and Methods

Soybean seeds (*Glycine max*, var. Hawkeye) were planted between layers of Krum moistened with distilled water in 22 × 33 cm Pyrex baking dishes covered with Saran wrap (perforated for aeration). After 48 hours at 27 to 29° in the dark, the Saran cover was removed, and 150 ml of solution containing 1×10^{-3} M $CaCl_2$, 3×10^{-4} M $MgCl_2$, and 3×10^{-3} M KCl were added to each tray. Experimental tissue was harvested after an additional 24 hours of growth.

Tissue sections were incubated in Erlenmeyer flasks in a 30° water-bath shaker. After incubation, the sections were blotted to remove excess moisture, weighed, and homogenized in ice-cold deionized water containing one drop of antifoam, unless otherwise stated. The homogenates were filtered through glass wool, and aliquots removed for RNA determinations. In experiments where C^{14}-nucleotide was added to the incubation medium, aliquots of the homogenates were removed, dried, and counted for total nucleotide uptake. RNA analyses were made by a modified Smillie and Krotkov procedure (19). The aliquots were made to 0.2 N with respect to $HClO_4$, thoroughly mixed, and centrifuged at 1100 × *g* for 10 minutes. The pellets were then suspended and twice washed in 0.2 N $HClO_4$ and once in methanol containing 0.02 M formic acid. All steps including centrifugation were carried out at 2 to 4°. The washed pellets were twice extracted at 37° for 30 minutes in a 2: 2: 1 mixture of ethanol-ether-chloroform to remove lipids. RNA was hydrolyzed in 0.3 N KOH for 18 hours at 37°.

[1] Aug. 5, 1963.
[2] This work was supported by Public Health Service Research Grant GM 10157–03 from the National Institutes of Health.
[3] Purdue Agriculture Experiment Station Journal Paper 2186.

After chilling, $HClO_4$ was added to a final concentration of about 0.3 N followed by centrifugation to remove the $KClO_4$ precipitate, protein, and DNA. Absorbance of samples of RNA was measured at 260 and 290 mμ with a Zeiss PMQII Spectrophotometer. The absorption difference was referred to a standard conversion factor obtained in the following manner. Samples of RNA treated as above were added to a column of Dowex-1-formate, and the component nucleotides were eluted by a formic acid gradient and collected in a Rinco collector. Each sample was read at 260 mμ, and from the known A_{260} values the quantity of each of the 4 nucleotides in the sample of RNA was determined. These quantities (based on 6 separate determinations) were referred to the initial 260 to 290 mμ absorption difference and used in calculating the conversion factor of 48: $(A_{260}-A_{290})$ (48) = μg RNA/ml of sample read.

After determining the RNA, each sample was neutralized with KOH to pH 4.5 to 5.5. After chilling, the $KClO_4$ precipitate was removed by centrifugation, and aliquots were plated, dried, and counted for determination of C^{14}-nucleotide incorporation into RNA. Counting was done with an automatic gas-flow counter equipped with a micromil window.

Table I

Effect of Auxin on Growth and RNA Metabolism in Excised Soybean Hypocotyl Tissue

0.75, 1.25, and 1.5 g of tissue section 1, 2, and 3, respectively, were incubated for 12 hours at 30° in a water bath shaker in a volume of 5 ml of solution containing 5×10^{-3} M KH_2PO_4 (neutralized to pH 6.0 with NH_4OH), 1 % sucrose, 20 μg/ml streptomycin, K salt of 2,4-D (pH 6.0), and 0.125 μC ADP-8-C^{14} (9.2 μc/mg).

μg/ml 2,4-D	% increase fr wt	mg RNA/ g fr wt*	cpm/g fr wt in RNA	cpm/mg RNA
		Tissue Section 1**		
0	45	2.84	5,330	1,875
5	71	2.98	7,155	2,405
25	67	2.95	7,020	2,380
100	57	2.87	5,200	1,810
500	24	2.87	2,660	925
		Tissue Section 2**		
0	32	1.07	2,300	2,145
5	102	1.21	3,950	3,260
25	95	1.22	3,680	3,020
100	73	1.14	3,175	2,780
500	20	1.01	1,310	1,095
		Tissue Section 3**		
0	9	0.58	2,270	3,915
5	26	0.76	4,925	6,480
25	25	0.75	5,600	6,800
100	20	0.72	4,815	6,700
500	7	0.63	1,355	2,150

* The RNA values compare to an initial RNA content of 3.02, 1.27, and 0.59 mg per g initial fresh weight of tissue sections 1, 2, and 3, respectively. All data are based on initial fresh weight. Data are averages of 3 closely duplicating experiments.

** Tissue section 1, 2, and 3 refer to distance from cotyledon where hypocotyl was sectioned and represent tissue of increasing mean cell age. Section 1, 0.0 to 0.5 cm below cotyledon; Section 2, 0.5 to 1.5 cm below cotyledon; Section 3, 1.5 to 3.5 cm below cotyledon.

ADP-8-C^{14} and ATP-8-C^{14} were purchased from Schwarz Bio-Research with a specific activity of 9.2 and 3.9 μc/mg, respectively. These compounds were used interchangeably based on their availability. The chemical form in which the C^{14} entered the tissue is not known.

Results

The incorporation of C^{14}-nucleotide into RNA of excised soybean hypocotyl tissue was enhanced by concentrations of auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), which promoted expansive growth of these cells (table I). Inhibition of C^{14}-nucleotide incorporation occurred at growth inhibitory levels of auxin. [During this investigation we discovered that previous results (8, table III) were complicated by bacterial contamination and are corrected by data in table I]. The magnitude of the auxin-induced changes in RNA synthesis depended upon the physiological age of the tissue as well as the auxin concentration (table I). No net increase in RNA occurred in meristematic and rapidly expanding cells (sections 1, 2) following auxin treatment even though incorporation of C^{14}-nucleotide was enhanced. In fully elongated cells (section 3), auxin induced a net increase in RNA (25 to 30% in most 12-hour experiments). Although higher concentrations are re-

Table II

Comparison of Effects of 2,4-D and IAA on RNA Synthesis in Mature Soybean Hypocotyl Tissue

1.5 g of tissue (section 3) were incubated in 5 ml solution containing 0.25 μc ATP-8-C^{14} (3.9 μc/mg) as described in table I.

Treatment	Mg RNA/g fr wt	cpm/g fr wt in RNA	cpm/mg RNA
None	0.49	3,620	7,400
25 μg/ml 2,4-D	0.66	8,770	13,280
25 μg/ml IAA	0.56	5,870	10,490
100 μg/ml IAA	0.63	8,900	14,100
Initial	0.49	...	...

Table III

Time Study on Auxin-induced Synthesis of RNA in Mature Soybean Hypocotyl Tissue

Incubation conditions same as in table I except that 0.25 μc of ADP-8-C^{14} (9.2 μc/mg) was used in a volume of 5 ml with 1.5 g of tissue. Initial RNA content was 0.61 mg/g initial fr wt. Tissue section 3 was used.

Incubation Interval (hr)	Mg RNA/ g fr wt	ADP-8-C^{14} in RNA cpm/g fr wt	cpm in RNA/ 3 hr interval	cpm/mg total RNA
		0 2,4-D		
0–3	0.60	1,105	1,105	1,840
0–6	0.61	2,473	1,368	4,050
0–9	0.60	3,612	1,139	6,020
0–12	0.59	4,332	920	7,650
		25 μg/ml 2,4-D		
0–3	0.61	1,290	1,290	2,115
0–6	0.66	4,406	3,116	6,680
0–9	0.70	7,535	3,129	10,750
0–12	0.75	10,698	3,163	14,250

Table IV

Cytoplasmic Distribution of RNA in Homogenates of Excised Soybean Hypocotyl Tissue

4 g of tissue (section 3) were incubated in 10 ml of solution containing 2 μc ADP-8-C^{14} (9.2 μc/mg) as described in table I.

Sub-cellular* Fraction	Medium 1**		Medium 2**	
	mg RNA/ g fr wt	cpm/mg RNA	mg RNA/ g fr wt	cpm/mg RNA
	0 2,4-D			
Nuclei rich	0.065	15,200	0.027	20,600
Mitochondria rich	0.060	8,390	0.024	6,350
Ribosome rich	0.284	6,650	0.302	6,350
Soluble	0.058	7,160	0.097	7,670
	25 μg/ml 2,4-D			
Nuclei rich	0.070	21,570	0.028	24,250
Mitochondria rich	0.077	15,940	0.026	15,400
Ribosome rich	0.389	13,320	0.419	13,020
Soluble	0.078	14,720	0.128	14,870

* Sub-cellular fraction: 1, 0–6,000 × *g* for 15 min (nuclei rich); 2, 6–20,000 × *g* for 20 min (mitochondria rich); 3, 20–100,000 × *g* for 120 min (ribosome rich); 4, 100,000 × *g* supernatant (soluble).

** Medium 1,-tissue homogenized in 0.5 M sucrose containing 0.01 Tris Cl buffer (pH 7.5). Medium 2,-tissue homogenized in 0.5 M sucrose containing 0.01 M Tris Cl buffer (pH 7.5), 0.001 M $MgCl_2$, and 0.5 % deoxycholate.

quired, IAA elicited the same general enhancement of RNA synthesis as 2,4-D (table II). The increase in fresh weight was associated primarily with cell elongation in sections 1 and 2 and with radial enlargement in section 3.

After an initial lag, the synthesis of RNA as measured by either C^{14}-nucleotide incorporation or by RNA determination proceeded at a linear rate for at least 9 hours in auxin-treated tissue (table III). However, the rate of RNA synthesis in untreated tissue as measured by C^{14}-nucleotide incorporation, sharply declined after 6 hours of incubation. A similar decline in the rate of C^{14}-nucleotide incorporation into RNA of expanding cells occurred concommitant with a decreased growth rate. At optimum concentration, auxin maintained higher rates of both growth and RNA synthesis.

The increase in RNA following auxin treatment occurred primarily in the microsome fraction of the cell (table IV). The same result was obtained when deoxycholate was included in the homogenization medium. Since the increase in RNA was primarily in fraction 3 in the presence of the detergent, it is assumed that there was a net synthesis of ribosomes.

Time-course studies during the linear phase of RNA synthesis showed that at early times a major portion of the C^{14}-RNA was associated with the nuclei-rich fraction (table V). The relative amount of C^{14}-RNA in this fraction progressively declined, although the total amount of C^{14}-RNA increased. The relative decrease in C^{14}-RNA in the nuclear fraction was similar to the increase in the proportion of C^{14}-RNA associated with the ribosomal fraction. The relative amount of C^{14}-RNA in fractions 2 and 4 thus remained essentially constant.

Actinomycin D, which effectively inhibits DNA-dependent RNA synthesis in bacteria (6), inhibited

Table V

Time Study on Cytoplasmic Distribution of C^{14}-RNA in Auxin-treated Soybean Hypocotyl Tissue

4 g of tissue (section 3) were incubated in 10 ml of solution containing 2 μc ADP-8-C^{14} (9.2 μc/mg) and 25 μg/ml 2,4-D. ADP-8-C^{14} was added at appropriate time after 3 hours of preincubation in 2,4-D with all tissue being harvested 12 hours after start of incubation. Homogenates were made in medium 1 and differentially centrifuged as described in table IV.

Exposure time to ADP-8-C^{14} (hr)	Subcellular Fraction				Total cpm in RNA
	Nuclei rich	Mitochondria rich	Ribosome rich	Soluble	
1.5	44	16	25	15	882
3	27	16	40	18	1,880
6	13	16	51	20	5,403
9	9	17	56	19	8,135

Table VI

Inhibition by Actinomycin D of RNA Synthesis in Excised Soybean Hypocotyl Tissue

1.5 g of tissue (section 3) were incubated in 5 ml of solution containing 0.25 μc ADP-8-C^{14} (9.2 μc/mg) and 25 μg/ml 2,4-D as described in table II. Initial RNA content was 0.56 mg/g fresh weight.

Actinomycin (μg/ml)	mg RNA/ g fr wt	ADP-8-C^{14} in tissue cpm/g fr wt	ADP-8-C^{14} in RNA cpm/g fr wt	% Inhibition by actinomycin of ADP-8-C^{14} incorporation into RNA
0.0	0.70	37,300	13,870	...
0.5	0.60	32,500	7,410	46.6
1.0	0.57	31,300	4,160	70.0
10.0	0.54	30,300	1,070	92.4

Table VII

Cellular Distribution of RNA and C^{14}-RNA in Soybean Hypocotyl Tissue as Affected by Actinomycin D

4.0 g of soybean hypocotyl tissue (section 3) incubated for 12 hours in 10 ml of solution containing 1 μc ATP-8-C^{14} (3.9 μc/mg), 25 μg/ml 2,4-D, and 10 μg/ml actinomycin D as described in table I. Tissue was homogenized in medium 2 and differentially centrifuged as described in table IV.

Cellular fraction	mg RNA/g fr wt		cpm/g fr wt in RNA		cpm/mg RNA		Ratio total C^{14} in RNA — Act. D / + Act. D
	— Actinomycin	+	— Actinomycin	+	— Actinomycin	+	
Nuclei rich	0.029	0.030	694	93	23,900	3,100	7.5
Mitochondria rich	0.026	0.020	414	36	15,900	1,800	11.5
Ribosome rich	0.414	0.309	5,540	742	13,400	2,400	7.5
Soluble	0.137	0.123	1,970	531	14,400	4,310	3.7
Total	0.606	0.482	8,618	1,402	14,200	2,910	6.1

C^{14}-nucleotide incorporation into RNA by 85 to 90 % at a concentration of 10 μg/ml (table VI). At a concentration of 0.5 μg/ml, actinomycin D inhibited RNA synthesis by 50 %. In the presence of actinomycin D, at a concentration which inhibited C^{14}-nucleotide incorporation by about 85 %, ribosomal RNA was the only cellular fraction of RNA which was significantly affected by the chemical (table VII). Also, the incorporation of C^{14}-nucleotide into supernatant RNA, although inhibited by actinomycin D, was relatively less affected than incorporation into RNA of other cellular fractions.

Discussion

Auxin (2,4-D) at growth-promoting concentrations enhanced C^{14}-nucleotide incorporation into RNA of the elongating zones of excised soybean hypocotyl tissue, whereas inhibitory concentrations decreased incorporation. The increase in incorporation was associated with a slightly higher RNA content than in comparable untreated tissue although somewhat lower than the initial RNA content.

In regions of the hypocotyl of fully elongated cells, auxin, at concentrations of 5 to 100 μg/ml, induced a 25 to 30 % net increase in RNA during 12 hours of excised incubation. IAA at higher concentrations caused a similar enhancement of RNA synthesis. After an initial lag, the synthesis of RNA in auxin-treated tissue proceeded at a linear rate for at least 9 hours. Differential centrifugation experiments showed that the major increase in RNA occurred in the ribosomal fraction. However, all RNA fractions showed about a twofold increase in specific activity relative to RNA from untreated tissue.

Many lines of evidence support the view that most cellular RNA is synthesized within the nucleus (15, 30), but there are reports that anucleated *Acetabularia crenulata* synthesize ribosomal RNA (20, 24). Ts'O and Sato (22) reported data from time course experiments suggestive of a transfer of labeled (P^{32}) RNA from the nucleus to the ribosomal fraction of the cell (however, no net increase in ribosomal RNA was realized). Results from time course experiments with our system (under conditions where there was a 30 to 40 % increase in ribosomal RNA) indicate a net transfer of C^{14}-RNA from the nuclear fraction into the ribosomal fraction. This net transfer occurred under such conditions that the other fractions of RNA maintained a constant proportion of C^{14}-RNA over the period. Thus, the proportion of C^{14}-RNA associated with the nuclear and ribosomal fractions showed a reciprocal relationship.

Actinomycin D, at concentrations as low as 10 μg/ml, inhibited the incorporation of C^{14}-nucleotide into RNA by 85 to 90 %. The actinomycin treatment also completely eliminated the net increase in ribosomal RNA following auxin treatment. There appears to be preferential labeling of supernatant RNA in the presence of actinomycin, presumably because of the end group addition of C^{14}-adenylate to s-RNA (21). Inasmuch as actinomycin D specifically blocks DNA-dependent RNA synthesis (6), most, if not all of the RNA synthesis in excised soybean hypocotyl tissue must occur on a DNA template, perhaps within the nucleus. A chromatin system as described for pea embryo nuclei (5) probably carries out the synthesis of RNA. Yankofsky and Spiegelman (29) recently presented evidence, based on specific hybridizations, for participation of DNA in ribosomal RNA formation.

Although it is clear that auxin has some pronounced effects on RNA metabolism, the significance of these responses are presently not fully understood. It is apparent, however, from data presented in the following paper (11), that functional RNA (and in turn protein) synthesis is essential for expansive growth of cells to proceed. The possibility that auxin enhances the growth rate by stimulating RNA synthesis seems reasonable, although more direct evidence is needed. The actual response to auxin varies somewhat with different tissues. In rapidly elongating sections of soybean hypocotyl there is only a slight decrease in RNA content during excised growth (table I) whereas in corn mesocotyl there is considerable RNA breakdown (10). Growth-promoting concentrations of auxin enhance RNA breakdown in corn mesocotyl (10) while causing the maintenance of a slightly higher level of RNA in soybean hypocotyl (table I). The net effect of auxin on C^{14}-nucleotide incorporation into RNA of these tissues is also quantitatively different. Again these differences are not fully understood, but they may well be related to differences in ribonuclease activity. Shannon and Hanson (17) have shown the ribonuclease content to increase about twofold during excised growth of corn mesocotyl tissue; with auxin the increase is about threefold. There is little or no change in ribonuclease activity in soybean hypocotyl tissue following excised

growth either in the presence or absence of auxin (unpublished). Also under the assay conditions used the specific activity of ribonuclease is initially much lower in soybean than in corn tissue (26). Thus these differences in the effects of auxin on RNA metabolism in excised corn and soybean tissues may be related to the differential responses obtained with ribonuclease, presumably the enzyme responsible for the degradation of RNA in these tissues.

Summary

Indoleacetic acid and 2,4-dichlorophenoxyacetic acid, at concentrations which promoted cell elongation, enhanced C^{14}-nucleotide incorporation into RNA of excised soybean hypocotyl tissue, whereas inhibitory levels decreased incorporation. In fully elongated cells, auxin induced a 25 to 30 % net increase in RNA, primarily ribosomal. Although different concentrations are required, 2,4-D and IAA produced similar effects on RNA metabolism. Results from time course studies on C^{14}-nucleotide incorporation into RNA, in conjunction with differential centrifugation of tissue homogenates, indicate that a net transfer of C^{14}-RNA from the nucleus to the ribosomes occurred. Moreover, the synthesis of RNA was inhibited by 85 to 90 % by actinomycin D. No increase in ribosomal RNA occurred in auxin treated tissue in the presence of the antibiotic. From the known action of actinomycin D, most of the RNA in soybean hypocotyl cells must be synthesized on a DNA template.

Acknowledgments

The authors are grateful to Mrs. Linda Rainford for technical assistance. The actinomycin D was kindly supplied by Merck, Sharpe, and Dohme, Rahway, New Jersey.

Literature Cited

1. Basler, E. and K. Nakazawa. 1961. Some effects of 2,4-D on nucleic acids of cotton cotyledon tissue. Botan. Gaz. 122: 228–32.
2. Biswas, B. B. and S. P. Sen. 1959. Relationships between auxins and nucleic acid synthesis in coleoptile tissue. Nature 183: 1824–25.
3. Cherry, J. H. 1962. Ribonucleic acid metabolism during growth of excised root tips from normal and X-irradiated corn seeds. Biochem. Biophys. Acta 55: 487–94.
4. Heyes, J. K. 1960. Nucleic acid changes during cell expansion in the root. Proc. Roy. Soc. London 152: 218–30.
5. Huang, Ru-chih C., N. Maheshrwari, and J. Bonner. 1960. Enzymatic synthesis of RNA. Biochem. Biophys. Res. Commun. 3: 689–94.
6. Hurwitz, J., J. J. Furth, M. Malamy, and M. Alexander. 1962. The inhibition of the enzymatic synthesis of RNA and DNA by actinomycin D and proflavin. Proc. Natl. Acad. Sci. 48: 1222–30.
7. Izawa, M. 1961. Effect of IAA on protein and ribonucleic synthesis in cultured bean germ-axes. Botan. Mag. Tokyo 74: 98–103.
8. Key, J. L. and J. B. Hanson. 1961. Some effects of 2,4-D on soluble nucleotides and nucleic acid of soybean seedlings. Plant Physiol. 36: 145–52.
9. Key, J. L., J. B. Hanson, H. A. Lund, and A. E. Vatter. 1961. Changes in cytoplasmic particulates accompanying growth in the mesocotyl of *Zea mays*. Crop Sci. 1: 5–8.
10. Key, J. L. 1963. Studies on 2,4-D-induced changes in RNA metabolism in excised corn mesocotyl tissue. Weeds 11: 177–81.
11. Key, J. L. 1964. RNA and protein synthesis as essential processes for cell elongation. Plant Physiol. 39: 365–70.
12. Masuda, Y. 1959. Role of cellular RNA in the growth response of Avena coleoptile to auxin. Physiol. Plantarum 12: 324–35.
13. Masuda, Y. 1960. Physiological significance of RNA in the growth promoting action of auxin. J. of Inst. Polytech. (Osaka City University) Series D, 11: 1–23.
14. Ormrod, D. P. and W. A. Williams. 1960. Phosphorus metabolism of *Trifolium hirtum* All. as affected by 2,4-D. Plant Physiol. 35: 81-87.
15. Prescott, D. M. 1962. Synthetic processes in the cell nucleus. II. Nucleic acid and protein metabolism in the macronucleus of 2 ciliated protozoa. J. Histochem. Cytochem. 10: 145–53.
16. Setterfield, G. 1961. Structure and composition of plant-cell organelles in relation to growth and development. Can. J. Botany 39: 469–89.
17. Shannon, J. C. and J. B. Hanson. 1962. Ribonuclease activity as a function of growth in the corn mesocotyl. Plant Physiol. suppl. 37: xx.
18. Skoog, F. 1954. Substances involved in normal growth and differentiation of plants. Brookhaven Symp. Biol. 6(BNL 258): 1–21.
19. Smillie, R. M. and G. Krotkov. 1960. The estimation of nucleic acids in some algae and higher plants. Can. J. Botany 38: 31–49.
20. Sutter, R. P., Sandra Whitman, and G. Webster. 1961. Cytoplasmic formation of the ribonucleic acid of ribosomes. Biochem. Biophys. Acta. 49: 233–34.
21. Tamaoki, L. and G. C. Mueller. 1962. Synthesis of nuclear and cytoplasmic RNA of Hela cells and the effects of actinomycin D. Biochem. Biophys. Res. Commun. 9: 451–54.
22. Ts'O, P. O. P. and C. S. Sato. 1959. Synthesis of RNA in plants. II. Phosphate^{-32} P incorporation into the RNA of the pea epicotyls. Exptl. Cell Res. 17: 237–45.
23. Ts'O, P. O. P. 1962. The ribosomes-ribonucleoprotein particles. Ann. Rev. Plant Physiol. 13: 45–80.
24. Webster, G., Sandra Whitman, and R. Heintz. 1962. Cytoplasmic formation of ribosomes. Plant Physiol. suppl. 37: xx.
25. West, S. H., J. B. Hanson, and J. L. Key. 1960. Effect of 2,4-D on the nucleic acid and protein content of seedling tissue. Weeds 8: 333–40.
26. Wilson, C. and J. C. Shannon. 1963. The distribution of ribonucleases in corn, cucumber, and soybean seedlings. Effects of isolation media. Biochem. Biophys. Acta. 68: 311–13.
27. Woodstock, L. W. and F. Skoog. 1960. Relationships between growth rates and nucleic acid contents in the roots of inbred lines of corn. Am. J. Botany 47: 713–16.
28. Woodstock, L. W. and F. Skoog. 1962. Distributions of growth, nucleic acids, and nucleic acid synthesis in seedling roots of *Zea mays*. Am. J. Botany 49: 623–33.
29. Yankofsky, S. A. and S. Spielgelman. 1963. Distinct cistrons for the 2 ribosomal RNA components. Proc. Natl. Acad. Sci. 49: 538–44.
30. Zalokar, M. 1960. Sites of protein and RNA synthesis in the cell. Exptl. Cell Res. 19: 559–76.