Plant Physiol. (1973) 51, 412–414

Short Communication

The Interpretation of Studies on Rapidly Labeled Ribonucleic Acid in Higher Plants

Received for publication July 14, 1972

M. JACKSON[1] AND J. INGLE
Department of Botany, University of Edinburgh, Edinburgh, Scotland

The properties of at least one class of rapidly labeled RNA, the precursor rRNA, have been well characterized in eucaryotes, particularly in HeLa cells. The initial transcription product of the gene is a large molecule containing one sequence of each of the two stable rRNAs plus additional nonribosomal RNA, the latter being removed during the processing or maturation of the initial transcription unit (1, 9). In higher plants, as in cold blooded animals, the initial transcription unit is smaller than in HeLa, and there is some evidence for two or more initial transcription products in carrot (6) and mungbean (2), although their relationship is not clear. In plants such as pea or artichoke the initial transcription unit is 2.3×10^6 daltons, and this is cleaved to produce 1.4×10^6 and 0.9×10^6 dalton components which finally yield the stable 1.3×10^6 and 0.7×10^6 r-RNAs respectively (10).

It is, however, more difficult to consider the synthesis and processing of rRNA in the context of normal growth and development. Many studies on RNA synthesis, both of rRNA (6, 10) and nonribosomal RNA (4), have utilized excised plant tissue. Unfortunately, one of the major effects of excision is an alteration of RNA metabolism; for example, growth of intact soybean hypocotyl is accompanied by an accumulation of RNA, whereas no accumulation occurs during growth of the excised tissue (5). Excision of the hypocotyl results in a gross shift of RNA metabolism with a reduction in rRNA synthesis relative to DNA-like, polydisperse RNA (3). The study of RNA synthesis in intact tissue is therefore more relevant to the understanding of normal growth and development. The technical difficulties of working with intact plants (such as maintaining sterility) are greater, and the need to minimize the amount of radioisotope employed usually necessitates the selection of uniform material after a period of growth. The conditions of the experimental incubation must not differ greatly from the preselection growth conditions, and any disturbances caused by the transfer of material at the selection stage must be minimal, since the rapid loss of polyribosomes during such manipulations (7) demonstrates the sensitivity of protein and nucleic acid metabolism towards such changes.

The synthesis of RNA was therefore studied in intact pea roots grown under conditions of minimal disturbance. Under such conditions the amount of the rRNA initial transcription product (2.5×10^6 daltons) present in the gel fractionation was very low compared to published studies (2, 6, 10). In some experiments much more 2.5×10^6 precursor rRNA was

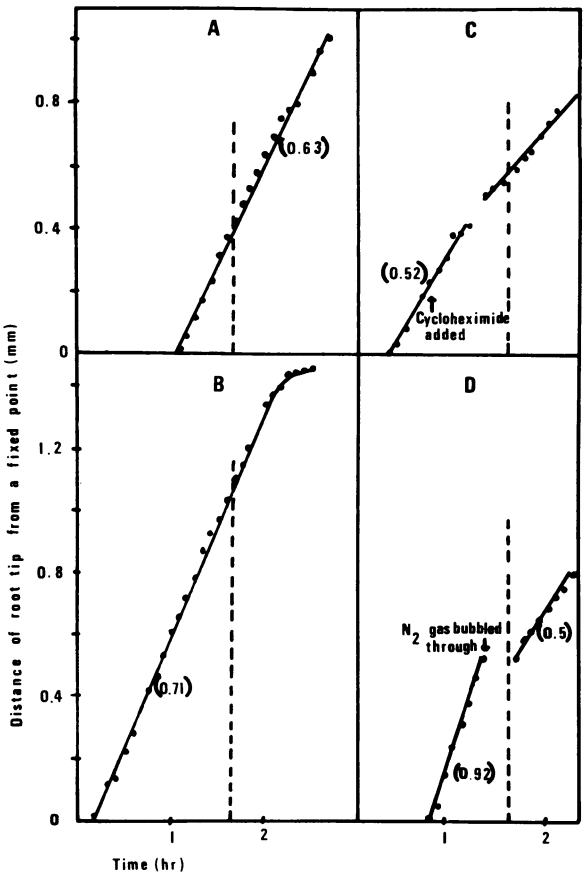

FIG. 1. Seeds were surface sterilized by rinsing with ethanol, soaking in sodium hypochlorite (2% free chlorine) for 10 min and rinsing thoroughly with sterile water. The seeds were germinated in water under sterile conditions for 72 hr and 20 seedlings with roots 3.5 to 4.0 cm long were selected and transferred to a small, sterile incubation container. The linear growth rate of roots was determined by measuring, with a traveling microscope, the position of the root tip relative to a fixed point at 10-min intervals. All of the incubations contained 0.4 μM carrier phosphate. A: Control, 60 μc/ml ^{32}P orthophosphate; B: 160 μc/ml ^{32}P orthophosphate; C: 60 μc/ml ^{32}P orthophosphate plus 0.2 μg/ml cycloheximide; D: incubation gassed with N_2 for 10 min prior, and 5 min subsequent, to the addition of 60 μc/ml ^{32}P orthophosphate. The time of addition of cycloheximide and of N_2 gassing is indicated by the arrow. The broken vertical line indicates the time of addition of the ^{32}P orthophosphate. The absolute growth rates (mm/hr) of individual roots are shown in parentheses.

[1] Present address: P.E.S. (Plant Physiology), School of Agriculture, University of Nottingham, Sutton Bonington, Loughborough, Leics, England.

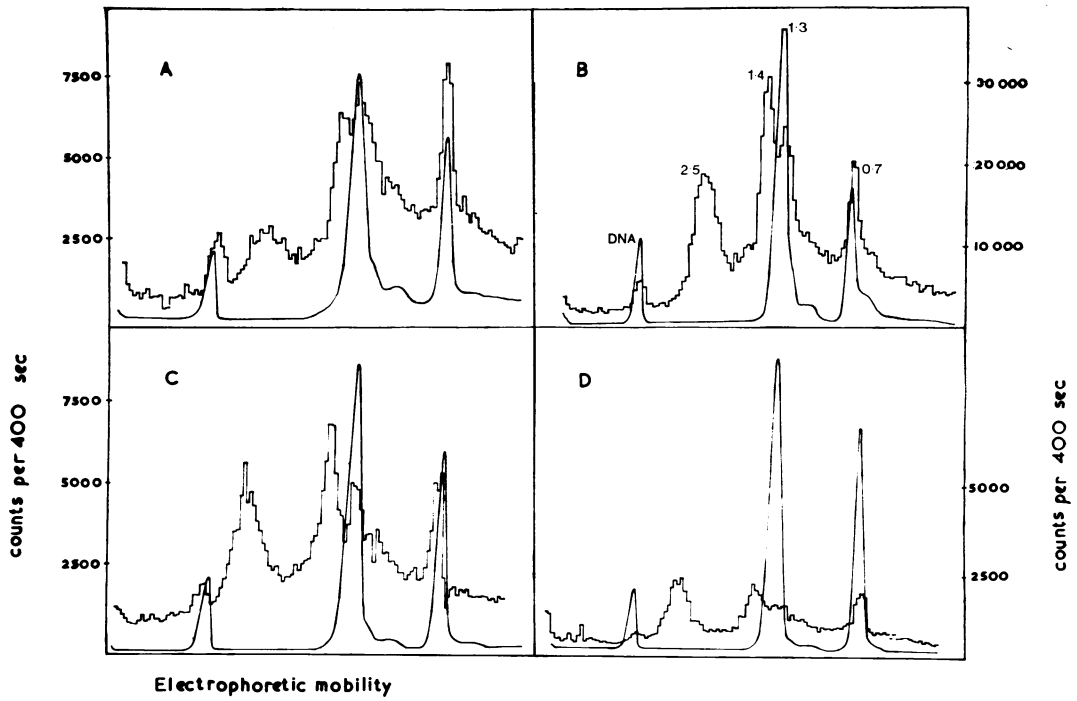

FIG. 2. Total nucleic acid was prepared 1 hr after the addition of ^{32}P orthophosphate to the samples described in Figure 1. Root tips (1 cm) were homogenized with a pestle and mortar in 4 ml of detergent medium containing 1% tri-isopropylnaphthalene sulphonate, 6% *p*-aminosalicylate, 50 mM NaCl, 10 mM tris HCl, pH 7.4, 6% phenol. The homogenate was shaken with a phenol mixture (phenol containing 10% *m*-cresol and 0.1% 8-hydroxyquinoline, saturated with 10 mM Tris HCl, pH 7.4), and the phases were separated by centrifugation. The lower phenol phase was removed, the aqueous phase made up to 0.5 M NaCl and shaken with fresh phenol mixture. After centrifugation the aqueous phase was removed, futher extracted with phenol mixture, and then the nucleic acids were precipitated by the addition of 2 volumes of ethanol. The nucleic acid was reprecipitated from 0.5% sodium dodecyl sulfate, 0.15 M sodium acetate, washed with 80% ethanol containing 0.5% sodium dodecyl sulfate, and fractionated on 2.2% polyacrylamide gels (9) for 3 hr at 50 v. The continuous scan shows the $E_{265\ nm}$, and the histogram the radioactivity per 0.5 mm slice.

present, and this appeared to be correlated with disturbance of growth. The linear growth rate of the root was therefore monitored continuously throughout the course of experiments in which growth was disturbed by various treatments. Under the experimental conditions, in the absence of radioisotope, roots grew at a constant rate for at least 16 hr. In the control sample, which contained 60 μc/ml ^{32}P orthophosphate, growth continued at the normal rate during the 1-hr experimental period (Fig. 1A), although the roots did stop growing quite abruptly approximately 5 hr after the addition of the ^{32}P orthophosphate. The period of normal growth prior to cessation decreased as the concentration of ^{32}P orthophosphate was increased. Growth stopped after 1.5 hr in 120 μc/ml and after 0.5 hr in 160 μc/ml ^{32}P orthophosphate (Fig. 1B). A low concentration of cycloheximide (0.2 μg/ml) and partial anaerobiosis caused by nitrogen gassing both reduced the linear growth rate by about 50% (Fig. 1, C and D).

The distribution of RNA molecules synthesized during these treatments varied greatly (Fig. 2). The most obvious difference was the relative amount of the 2.5×10^6 precursor rRNA, which was increased from 3.4% of the newly synthesized RNA in the control roots to 12 to 13% in the inhibited samples (Table I). This increase in the percentage of precursor in the inhibited roots was not simply the result of an apparent shorter pulse due to the reduced growth rate, (1 hr of inhibited growth may be considered to be equivalent to 0.5 hr of control growth), since the percentage of precursor was no greater in the control after incubations of less than 1 hr. Furthermore, with both cycloheximide and high ^{32}P orthophosphate, RNA synthesis as measured by specific activity (Table I) did not simply reflect the reduced growth. The inhibitory growth conditions had a relatively greater effect on the processing of the initial transcription product than on the actual rate of transcription, as exemplified by the ratio of precursor to stable rRNA ($2.5 \times 10^6/0.7 \times 10^6$, Table I). Although the inhibition of growth by these three quite different means—disruption by radiation damage, inhibition of protein synthesis by cycloheximide, and reduction of available energy by anaerobiosis—all resulted in

Table I. *Effect of Growth Disturbance on RNA Synthesis*

Values were derived from the gel fractionations shown in Figure 2.

Growth Conditions	Specific Activity RNA	Distribution of Newly Synthesised RNA				Ratio $\frac{2.5 \times 10^6}{0.7 \times 10^6}$
		2.5×10^6	$1.4 + 1.3 \times 10^6$	0.7×10^6	Polydisperse	
	cpm/µg	*% of total*				
^{32}P orthophosphate (60 µc/ml)	3900	3.4	23.0	10.7	63.0	0.3
^{32}P orthophosphate (160 µc/ml)	9000	13.3	26.0	7.9	51.0	1.7
^{32}P orthophosphate plus 0.2 µg/ml cycloheximide (60 µc/ml)	4400	11.7	18.6	5.3	64.0	2.2
^{32}P orthophosphate after N_2 gassing (60 µc/ml)	930	12.8	11.7	5.4	70.5	2.4

the accumulation of the precursor rRNA, the balance between rRNA and nonribosomal RNA synthesis responded rather differently, with high radioactivity favoring rRNA synthesis (49% rRNA) and anaerobiosis reducing the proportion of rRNA synthesized (30% rRNA).

The large, sharp peak of the precursor rRNA appears therefore to be the result of tissue disturbance, probably caused by the very high levels of radioisotope used. The accumulation of the precursor rRNA under these conditions has possibly facilitated the identification and characterisation of the precursor molecules, but such results may have limited significance in the study of rRNA synthesis during normal growth. It must be emphasized that although the control roots, incubated in 60 μc/ml ^{32}P orthophosphate, grew normally during the experimental period, they were affected by the radioisotope, resulting in cessation of growth 4 hr after the end of the experiment. The validity of using this material as the "normal" is obvious, but further reduction of the isotope concentration resulted in an RNA preparation with specific activity too low to allow subsequent detailed fractionation. These results suggest that perhaps the synthesis of nonribosomal polydisperse RNA may be similarly affected by the high levels of radioisotope frequently employed, a situation which would be extremely difficult to detect. It is therefore necessary to establish that the RNA synthesis studied by means of radioisotope incorporation is not simply a response to growth disturbances caused by the radioisotope.

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