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Short Communication

Ribosomes during Development of Root Cells of *Zea mays*[1]

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Current theories on mechanisms of protein synthesis assign no specificity to the ribosomes for the type of protein synthesized. Hence, these particles may be expected to remain relatively stable during cell ontogeny although the type of protein synthesized and the rate of synthesis vary with cell age. Subunits of ribosomes of *Escherichia coli* are known to be conserved through several generations (5), but the state of ribosomes in higher plants during cell ontogeny is not at all clear. According to Nicolson and Flamm (6), senescence of cultured tobacco cells caused a decrease in total ribosomes as well as a degradation of the RNA in the ribosomes. Aging of barley leaves was reported (9) to be associated with a loss of total ribosomes and a lowering of protein content of the ribosomes. In roots, microsomal and total RNA showed apparent qualitative changes (1, 2) while total RNA per cell usually increased during cell differentiation and expansion, but ribosomes have not been examined in this regard. This study compares ribosomes of corn (*Zea mays* L.) root cells of different ages, including those in fully mature cells.

MATERIALS AND METHODS

Ribosomes were isolated (3, 4) at ice temperature in 0.01 M potassium phosphate (pH 6.5) containing 0.5 mM $MgCl_2$ by differential centrifugation, from either apices (0–3 mm) or basal segments (12–40 mm) of seminal roots of 3-day-old etiolated corn seedlings (var WF9 x M14) grown as described previously (3). As before (3), ribosomes obtained after one and two cycles of differential centrifugation are respectively designated DC-1 and DC-2. Ribosomal concentration was calculated from absorbancy at 260 mμ, using $E^{1\%}_{1cm} = 110$, as previously determined (3). Schlieren patterns of sedimenting ribosomes and RNA were obtained in 1.2-cm cells with a Spinco ultracentrifuge, model E. The base composition of RNA in ribosomes was determined by alkaline hydrolysis of ribosomes and column chromatography (3). Ribosomal RNA was extracted at about 4° from ribosomes in the phosphate-$MgCl_2$ buffer with phenol and sodium lauryl sulfate (0.1% final concentration). The aqueous layer was further extracted 3 times with phenol, and then several times with absolute ether, to yield RNA. When total RNA was extracted directly from roots, the RNA in the aqueous layer was precipitated and washed in 66% (v/v) ethanol and then dissolved.

Root segments were macerated for cell count by placing them in 2 N HCL-5% acetic acid (11), heating in boiling water for 15 min, and then pressing the cells with a glass rod plus vigorous agitation to separate them. Cells were counted in a hemocytometer with a chamber depth of 0.2 mm. Cell volume was calculated from root weight, assuming cell density to be that of water.

RESULTS

Elongation of cells, as determined by marking the root at various points with India ink and measuring the marked intervals after a time lapse, was very slight at 6 to 7 mm from the root apex and was completed at 8 mm. Cell volume increased markedly along the first 6 mm of the root (Table I) and remained essentially constant thereafter. For most of the experiments, ribosomes of apical (0–3 mm) segment of the root were compared with those of the basal segment (12–40 mm) containing only fully expanded and mature or maturing cells.

Ribosomes were isolated in good yields from both segments and had identical sedimentation coefficients and very similar patterns (Fig. 1). The mean numbers of ribosomes (DC-1) in cells of different ages (Table I) were calculated assuming the molecular weight of the ribosome to be 4×10^6. In spite of the great increase in cell volume, ribosomal content per cell only increased from 17×10^6 in the apical segment to 26×10^6 in the 6 to 12-mm segment. Ribosomal content per cell did not change with cell age once elongation ceased (Table I). Even the 40 to 60-mm segment, which had not been previously examined for its RNA, contained as many ribosomes per length of root as the 6 to 12-mm segment (Fig. 2).

Since varying amounts of ribosomes are lost when isolated by differential centrifugation due to aggregation resulting from pelleting (3), the ribosomal contents in Table I are based on only selected experiments in which ribosome yields were high. The data were verified by an experiment in which ribosomal contents were calculated from absorbancies of ribosomes sedimented into sucrose gradients from clarified homogenates without pelleting.

Deoxycholate was essential for the isolation of ribosomes from basal segments; without it, very few ribosomes were obtained. Apparently the association of ribosomes with the proliferating endoplasmic reticulum in the older cells yielded pieces large enough to sediment with mitochondiia and heavier particles.

There were no difference in base composition between ribosomes from apical and basal segments (Table II). This is contrary to the change in composition of microsomal RNA with cell age reported by Cherry *et al.* for corn roots (1). However, the base composition they reported was atypical of either total RNA or ribosomal RNA.

With the use of bentonite and special care, intact heavy and light RNA were isolated from apical and basal ribosomes most of the time (Fig. 3). Total nucleic acids containing intact ribosomal RNA were also obtained from basal segments by direct extraction of roots with phenol and bentonite. This demonstrates that the RNA of ribosomes in older cells was intact *in vivo*,

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although total ribonuclease and ribonuclease associated with isolated ribosomes from corn roots increase manyfold with cell age (4). Without special precaution during isolation, however,

Table I. *Estimated Mean Cell Size and Ribosomal Content per Cell in Corn Root Segments of Different Ages*

Data are composited from several experiments. Errors in cell counts may be as high as 25% because of the inherent difficulties in the method.

Segment	Number of Cells per mm of Root	Cell Volume	Number of Ribosomes per Cell
		$\mu^3 \times 10^{-4}$	$\times 10^{-6}$
0–3 mm, apical	36,000	1.3	17
6–12 mm	2,200	23	26
12–40 mm, basal	2,100	25	25

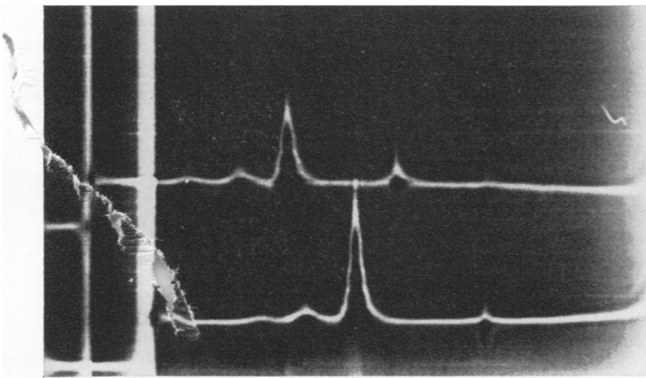

FIG. 1. Sedimentation of ribosomes (about 3.1 mg/ml) of corn isolated with deoxycholate from apical (top image) and basal (bottom image) segments. Sedimentation to the right. Photographed 14 min after reaching 42,040 rpm with phase plate at 60° and rotor temperature at 7°. The main peak is the 80-S component.

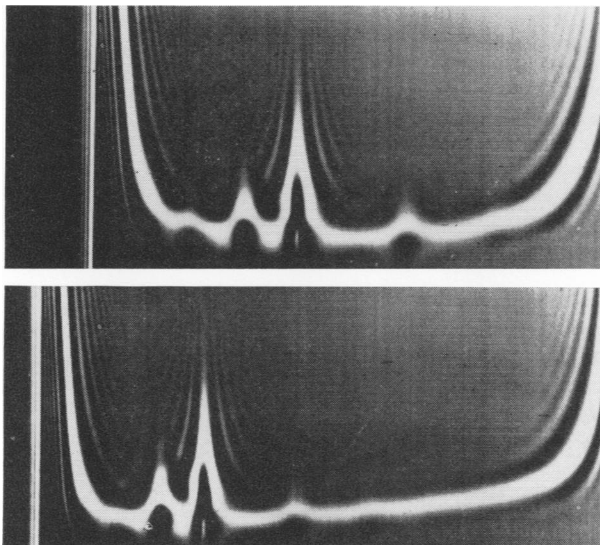

FIG. 2. Sedimentation of clarified (at 24,000 rpm for 15 min) homogenate of 6- to 12-mm (top) and 40- to 60-mm (bottom) root segments. Final deoxycholate concentrations were 0.24% (top) and 0.4% (bottom). One milliliter of either homogenate represents approximately 1050 mm of total root length. Photographed 10 min after reaching 42,040 rpm with phase plate at 40° and temperature at 9 and 1°, respectively. The main peak is the 80-S component. Boundaries near the meniscus represent the large amount of soluble material.

Table II. *Base Composition of Ribosomes from Apical and Basal Segments of Corn Roots*

Values are means of four DC-2 and one DC-1 preparations analyzed.

	Apical (0–3 mm)	Basal (12–40 mm)
	mole %	
Adenine	21.0	21.5
Guanine	34.4	34.4
Cytosine	24.7	24.3
Uracil	19.9	19.8

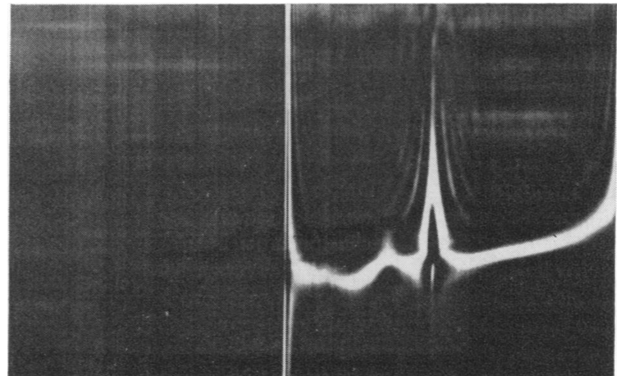

FIG. 3. Sedimentation of basal ribosomal RNA. Basal sections were homogenized in the presence of deoxycholate, bentonite (4.3 mg/g of wet tissue), and polyvinyl sulfate (0.2 mg/ml of homogenate). DC-1 ribosomes were isolated using a bottom layer of sucrose containing 0.1 mg of polyvinyl sufate per ml. RNA was extracted from isolated ribosomes. All operations were performed as fast as possible with the temperature kept close to 0°. Photographed 13 min after reaching 59,780 rpm with phase plate at 45° and temperature at 8°. The main peak is the 28-S (nominally) RNA, and the slow peak is the 18-S RNA. Polyvinyl sulfate was found not to be essential in subsequent experiments.

RNA obtained from apparently intact ribosomes was polydisperse. Usually more degradation occurred in RNA from basal than from apical ribosomes.

DISCUSSION

These data suggest that there is no qualitative and little quantitative change in ribosomes with cell maturation in corn roots. This is in apparent contrast to the effect of aging or physiological state in several other systems. In *Avena* coleoptile, according to the limited data of Setterfield (8), the number of ribosomes per cell declines sharply after elongation stops. Wright (11) also observed that the total RNA per cell declined sharply when elongation of wheat coleoptile ceased. In barley leaves, the amount of ribosomal material declines with leaf age (9). *E. coli* was estimated to contain a mean of 90,000 ribosomes per cell at the exponential phase of growth, but only about 20,000 at the stationary phase (10). Qualitative changes have also been reported. Senescence in cultured tobacco cells causes degradation of the RNA in the ribosomes (6). Aging of barley leaves supposedly causes a loss of protein from the ribosomes (9). With various microbes, the distribution of ribonucleoprotein among the ribosomal subunits and the monomer is altered markedly by changes in the rate and conditions of growth (7). The lack of changes in the ribosomes of corn roots with age may reflect the relatively permanent nature of the root cell, although the widely different metabolic activities of the root cells of different ages are well known and one may suspect that the mature cells would

have much lower rate of protein synthesis and therefore would need fewer ribosomes.

The fact that the number of ribosomes per cell did not change greatly with cell age suggests that most ribosomes are synthesized in the meristematic and elongating zones and possibly remain stable thereafter. Preliminary data on the incorporation of ^{32}P into RNA, however, indicate that the turnover of ribosomes in mature cells may be high. Roots were "pulse-labeled" (1 hr) with ^{32}P and then cut into apical and basal segments. Nucleic acids were extracted and separated on methylated albumin-kieselguhr columns. There was no marked difference between the two segments in the shapes of the profiles of acid-precipitable radioactivity.

Relative to the incorporation of ^{32}P into other RNA (soluble RNA and RNA which eludes off the column as a radioactive shoulder after ribosomal RNA) in the same cells, incorporation into ribosomal RNA was not markedly reduced upon cell maturation. This points to the possibility that the RNAs in ribosomes in mature cells are synthesized and degraded repeatedly. This possible interesting difference between ribosomes of higher plants and those of microorganisms, which are conserved through several generations of growth, warrants further study.

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