THE SPECIFICITY OF RIBOSOMAL RIBONUCLEIC ACIDS OF PLANTS

Clifford J. Pollard
Department of Botany and Plant Pathology
Michigan State University
East Lansing, Michigan

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The similarities in composition of whole ribosomal RNA from pea and clover (Ts'o, 1961) and whole-cell RNA from related and unrelated varieites of cotton (Ergle et. al., 1964) have been commented on by these workers. Midgeley (1962) demonstrated differences in base compositions between the 50S and 30S ribosomes from bacteria but there was a surprising constancy among species. Other reports indicate that 18S and 28S ribosomal ribonucleic acids differ in base composition (Lerner et. al., 1963, Brown and Gurdon, 1964 and Doi and Igarashi, 1964). They also appear to be formed from different loci on the DNA molecule (Yankofsky and Spiegelman, 1963, Chipchase and Birnstiel, 1963). Since an anucleolate mutant of Xenophus laevis was unable to synthesize neither 28S nor 18S ribosomal RNA, Brown and Gurdon (1964) suggested a nucleolar site for these cistrons; however, the annealing experiments of Chipchase and Birnstiel (1963) on pea ribosomal RNA suggested that only a minority of ribosomal RNA cistrons occur in the nucleolar DNA.

This note presents evidence that both the 28S and 18S ribosomal ribonucleic acids from plants have distinct base compositions which are apparently characteristic of the species. The data also suggest that in higher plants the precision of formation of the nucleic acids is preserved during certain mutations since the base compositions of both RNA particles from varieties showing distinct phenotypic expressions are similar.

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Materials and Methods

Zea mays 1, obtained from Dr. R. S. Bandurski, was a mutant characterized by having a purple endosperm. Zea mays 2 was a yellow sweet variety and Zea mays 3 was a dwarf variety (Popcorn). Cabbage 1 was a green variety whereas Cabbage 2 was a red variety.

Tissue used from cauliflower, cabbage and parsnip were florets, inner leaves and roots, respectively. Celery petioles were trimmed so that chlorophyll-containing areas were largely removed. Etiolated shoots from 4-5 days old Zea mays and fruiting bodies of fungi were employed.

Homogenizing Medium: 0.05M Tris pH 7.8, 0.25M sucrose, 0.01M magnesium chloride.

Homogenizer: Waring Blendor, one-half to one-third line voltage, 2-3 minutes.

Microsomes: 104,000 x g residue from post-mitochondrial supernatant (15,000 x g).

Ribosomes: Once-sedimented after treatment of microsomes with 0.01M sodium acetate-0.0001M magnesium chloride, 0.5% sodium deoxycholate.

Ribosomal RNA: Extracted with 1% sodium dodecyl sulfate-80% phenol at 5°C, aqueous layer precipitated with equal volume ethanol.

Sucrose Density Gradients: Linear (4-20% sucrose) in solution of 0.1M sodium acetate pH 5.8, 0.0001M EDTA. Thirteen hour centrifugation at 23,000 rpm., Spinco 25.1 rotor. Hydrolysis, chromatography and estimation of bases as given by Bendich, (1957).

Results and Discussion

A perusal of the tables reveals that the nucleic acids are specific. Note, for example, that although the 28S ribonucleic acid from cabbage 1 and parsnip had similar base compositions, the 18S nucleic acids were significantly different. The guanine content of both species of RNA from higher plants was fairly constant; the uracil content was generally lowest. Exceptions were noted. The mushroom nucleic acids were characterized by low cytosine content. Invariably, the uracil content of the 28S RNA was lower

Table I
Base Composition of 28S Ribosomal RNA (moles percent)

Species	Guanine	Adenine	Cytosine	Uracil	Number of Analyses
Cauliflower	33.1 ± 0.7*	25.7 ± 0.5*	22.7 ± 0.4*	18.4 ± 0.9*	4
Cabbage 1	32.9	23.6	24.3	19.1	3
Cabbage 2	32.8	22.4	25.7	19.0	3
Parsnip	33.3	23.1	23.8	19.4	3
Celery	31.5	22.3	25.2	21.4	2
Zea mays 1	34.2	21.9	27.6	16.2	2
Zea mays 2	33.7	20.7	29.1	16.5	2
Zea mays 3	33.8	21.1	27.3	17.9	2
Psalliota campestris	27.8	27.7	20.1	24.3	2
Coprinus micaceus	30.3	26.6	21.3	21.9	3

*Standard Deviations

Table II
Base Composition of 18S Ribosomal RNA (moles percent)

Species	Guanine	Adenine	Cytosine	Uracil	Number of Analyses
Cauliflower	32.9 ± 0.1*	25.6 ± 0.4*	20.6 ± 0.2*	21.0 ± 0.8*	2
Cabbage 1	31.9	23.2	24.1	20.5	2
Cabbage 2	31.7	23.9	23.2	20.5	2
Parsnip	31.6	23.1	22.8	22.5	3
Celery	28.6	22.9	23.3	25.4	2
Zea mays 1	33.1	21.5	24.9	20.6	2
Zea mays 2	33.1	22.1	25.4	19.4	2
Zea mays 3	32.1	22.3	24.9	20.8	2
Psalliota campestris	30.1	24.9	18.0	27.1	2
Coprinus micaceus	30.8	25.1	20.6	23.6	2

*Standard Deviations

than its 18S counterpart. Note that there were no obvious similarities in the crucifers, cabbage and cauliflower, nor were there similarities in the parsnip and celery, members of the Parsley Family. Clearly, however, the two varieties of cabbage and the three varieties of corn resemble each other. This suggests that some precision exists in the formation of ribosomal RNA and that these cistrons are preserved through certain alterations of the DNA template. On the other hand, the technique used here would not detect extremely small differences in base compositions.

Criticism that the values from higher plants may not be directly comparable, since different tissues were used, is probably invalid since I have found that the 28S species from germinating mung bean cotyledons and roots were identical (unpublished). It was also demonstrated that both species of ribosomal ribonucleic acids from spores and vegetative cells from B. subtilis had identical base composition (Doi and Igarashi, 1964). Of equal concern was contamination from ribosomes contained in chloroplasts. The selection of tissue, grinding procedure and differential centrifugations have probably minimized this source of error.

In other experiments on whole ribosomal RNA from a variety of plants, I noted an apparent constancy in whole ribosomal RNA. This constancy disappeared upon fractionation of the nucleic acids. In view of the well-documented binding of messenger RNA to ribosomes and the difference in base composition of the two nucleic acids, assays of whole ribosomal RNA are probably of questionable merit.

If all ribosomal ribonucleic acids were identical there would be no reason to suspect that they contain information except perhaps that characteristic of all organisms. Indeed, the apparent similarity of ribosomal ribonucleic acids from different sources and the lack of similarity of the base composition of the nucleic acids to DNA have been potent arguments against a role for ribosomal RNA as a messenger. (Perutz 1962). Although Willson and Gros (1964) noted no stimulation of protein synthesis

by E. coli ribosomal RNA with a system from E. coli, Otaka et. al. (1964) presented evidence that 'premature' ribosomal RNA, which apparently has a secondary structure distinct from mature ribosomal RNA (Mitsui et. al 1963), was active. There still exists the possibility that ribosomal RNA imparts some degree of specificity in protein synthesis.

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