A RE-EVALUATION OF THE FRACTIONATION OF HIGH MOLECULAR WEIGHT RNA BY MAK CHROMATOGRAPHY[1]

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THIS COMMUNICATION DESCRIBES EXPERIMENTS IN WHICH POLYACRYLAMIDE GEL ELECTROPHORESIS (LOENING, 1967) WAS USED TO EVALUATE THE FRACTIONATION OF HIGH MOLECULAR WEIGHT RNA BY THE METHYLATED-ALBUMIN, KIESELGUHR COLUMN. THE RESULTS SHOW THAT CONSIDERABLE AGGREGATION OF BOTH 25S AND 18S RIBOSOMAL-RNA DOES OCCUR ON THE COLUMN. THE PRESENCE OF LARGER RNA MOLECULES WITHIN THE RIBOSOMAL-RNA REGION OF THE MAK COLUMN IS DEMONSTRATED, BOTH BY UV ABSORPTION AT 254 Mμ AND BY RADIOACTIVITY.

METHYLATED-ALBUMIN, KIESELGUHR COLUMN CHROMATOGRAPHY DOES NOT SEPARATE COMPLETELY THE HIGH MOLECULAR WEIGHT RNAs. RESOLUTION OF THE TWO RIBOSOMAL COMPONENTS IS PARTICULARLY POOR WITH PLANT RNA, WHERE THE RELATIVELY LARGE DIFFERENCE IN BASE COMPOSITION BETWEEN THE HEAVY AND LIGHT RIBOSOMAL-RNAs TENDS TO REDUCE THE SEPARATION WHICH SHOULD RESULT FROM THEIR SIZE DIFFERENCE (INGLE ET AL, 1965). THERE IS ALSO A SHOULDER FOLLOWING THE HEAVY RIBOSOMAL-RNA PEAK, THE SIZE OF THIS SHOULDER DEPENDING ON THE EXACT CONDITIONS USED IN HANDLING THE RNA AND THE COLUMN FRACTIONATION. RNA PREPARED FROM CERTAIN PLANTS, SUCH AS CORN OR BARLEY, FRACTIONATES TO GIVE A DISCRETE THIRD PEAK IN PLACE OF THIS SHOULDER (CHERRY AND LESSMAN, 1967). THE RATIO OF THE HEAVY TO LIGHT RIBOSOMAL-RNA, AS FRACTIONATED BY THE MAK COLUMN, IS USUALLY BETWEEN 3 AND 4 (ASANO, 1965; MARCOT-QUEIROZ AND MONIER, 1965; INGLE AND KEY, 1965;

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HAYES, ET AL, 1966), A VALUE CONSIDERABLY HIGHER THAN 2, THE APPROXIMATE RATIO PREDICTED FROM THE MOLECULAR WEIGHTS OF THE RNA SPECIES AND RIBOSOME STRUCTURE. THIS SUGGESTS THAT AGGREGATION OF THE RIBOSOMAL-RNAS MAY OCCUR DURING MAK CHROMATOGRAPHY AS A RESULT OF THE HIGH SALT SOLUTIONS EMPLOYED. ALTHOUGH THERE IS GOOD EVIDENCE FOR INTERACTIONS BETWEEN RIBOSOMAL-RNAS (MÖLLER, 1964; MARCOT-QUEIROZ AND MONIER, 1965), AND BETWEEN RIBOSOMAL-RNAS AND RAPIDLY-LABELLED-RNA (ASANO, 1965; HAYES, ET AL, 1966) IN THE PRESENCE OF HIGH IONIC CONDITIONS, THE NATURE OF THE POSSIBLE AGGREGATIONS OCCURRING ON THE MAK COLUMN HAVE BEEN LITTLE STUDIED. EXPERIMENTS WITH CORN RNA HAVE SUGGESTED THAT THE THIRD HIGH MOLECULAR WEIGHT PEAK MAY BE A 25S - 25S AGGREGATE, ALTHOUGH THERE WAS NO EVIDENCE OF 25S - 18S ASSOCIATION (HSIAO, 1967).

WHEN TOTAL RNA PREPARED FROM SOYBEAN HYPOCOTYL TISSUE (INGLE AND KEY, 1965) WAS FRACTIONATED BY MAK CHROMATOGRAPHY, THE RATIO OF HEAVY (25S; 1.3 x 10^6 MOL. WT. LOENING AND INGLE, 1967) TO LIGHT (18S; 0.7 x 10^6 MOL. WT.) RIBOSOMAL RNAS WAS 3.5 (FIG. 1). THE SAME SAMPLE OF RNA, FRACTIONATED BY GEL ELECTROPHORESIS HAD A RATIO OF 25S/18S EQUAL TO 1.5 (FIG. 2). THIS IS LOWER THAN THE THEORETICAL VALUE OF 1.85 BECAUSE OF NON-LINEARITY OF THE UV OPTICS

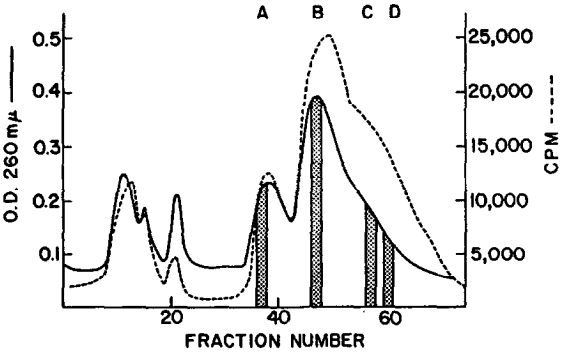

FIG. 1. MAK FRACTIONATION OF RNA. EXCISED SOYBEAN HYPOCOTYL WAS LABELED FOR 4 HR. WITH ^{32}P-ORTHOPHOSPHATE PRIOR TO PHENOL-SLS EXTRACTION AND PURIFICATION OF TOTAL NUCLEIC ACID AS DESCRIBED (INGLE, ET AL, 1965). THE TOTAL NUCLEIC ACID WAS FRACTIONATED ON AN MAK COLUMN USING A TWO-STAGE LINEAR GRADIENT: 120 ML EACH OF 0.35 M AND 0.8 M NACL AND 250 ML EACH OF 0.8 M AND 1.25 M NACL (ALL SALT SOLUTIONS CONTAINED 0.05 M SODIUM PHOSPHATE AT PH 6.7). SAMPLES WERE PLATED, DRIED, AND COUNTED IN A GAS FLOW COUNTER. LETTERS REFER TO SAMPLES POOLED FOR ANALYSIS BY GEL ELECTROPHORESIS (FIG. 3).

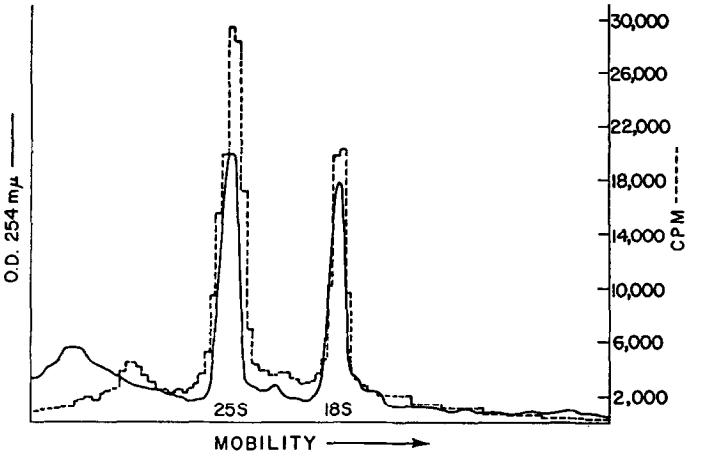

FIG. 2. ACRYLAMIDE GEL FRACTIONATION OF RNA. TOTAL NUCLEIC ACID (25 UG) DESCRIBED IN FIG. 1 WAS FRACTIONATED BY POLYACRYLAMIDE GEL ELECTROPHORESIS AS DESCRIBED BY LOENING AND INGLE (1967). THE RUNNING TIME WAS 3 HR AT 5 MA/GEL. THE GELS WERE SCANNED AT 254 MU, FROZEN, AND SLICED INTO 0.7 MM PIECES. THE GEL PIECES WERE PLACED ON STRIPS OF FILTER PAPER, DRIED, AND COUNTED IN A LIQUID SCINTILLATION SPECTROMETER.

OF THE CHROMOSCAN (JOYCE-LOEBL) UNDER THE EXPERIMENTAL CONDITIONS USED. A GEL LOADING OF 10 μG RNA, INSTEAD OF THE 25 μG USED, GIVES A 25S/18S RATIO OF 1.8 TO 1.9. RNA ELUTED FROM THE 18S REGION OF THE MAK COLUMN CONTAINED 18S AND 16S (0.56×10^6 MOL. WT.) COMPONENTS BY SUBSEQUENT GEL ELECTROPHORESIS (FIG. 3A). THE 16S RNA WAS ENRICHED RELATIVE TO THE 18S COMPONENT WHEN COMPARED WITH THE TOTAL RNA (FIG. 2). THE 25S REGION OF THE MAK CONTAINED NOT ONLY 25S RNA, BUT ALSO A CONSIDERABLE AMOUNT OF 18S, AND TRACES OF 23S (1.1×10^6 MOL. WT.) AND 16S RNAS (FIG. 3, BCD). THE "HEAVY" RIBOSOMAL-RNA FROM THE MAK COLUMN IN FACT CONTAINED 40% OF THE TOTAL 18S RNA, WHICH QUANTITATIVELY ACCOUNTED FOR THE HIGH 25S/18S RATIO OBTAINED FROM THE MAK FRACTIONATION. SIMILAR EXPERIMENTS WITH CORN RNA HAVE SHOWN THAT THE THIRD HIGH MOLECULAR WEIGHT COMPONENT, WHICH IS NOT PRESENT ON GEL ELECTROPHORESIS (LOENING AND INGLE, 1967), IS AN AGGREGATE OF THE RIBOSOMAL-RNAS RATHER THAN A LONG-LIVED MESSENGER-RNA SPECIFIC TO MONOCOTYLEDONOUS PLANTS AS SUGGESTED BY CHERRY AND LESSMAN (1967). FROM THESE EXPERIMENTS IT IS NOT POSSIBLE TO DECIDE WHETHER THERE IS SIMPLY 25S - 18S ASSOCIATION, OR WHETHER 25S - 25S AND 18S - 18S

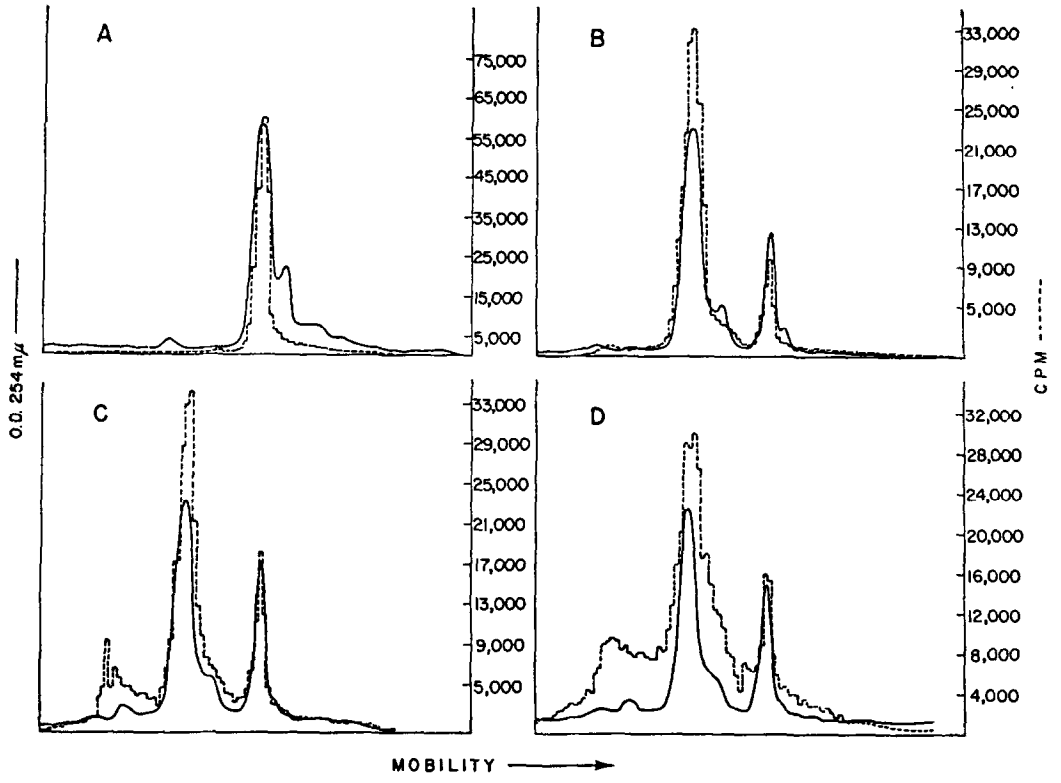

FIG. 3. ACRYLAMIDE GEL FRACTIONATION OF RNA FROM MAK COLUMNS. RNA FROM REGIONS A, B, C, AND D OF THE MAK COLUMN SHOWN IN FIG. 1 WAS PELLETED BY CENTRIFUGATION AT 36,000 RPM FOR 12 HR. THE RNA WAS THEN DISSOLVED IN 0.15 M SODIUM ACETATE CONTAINING 0.5% SODIUM LAURYL SULFATE. THE RNA WAS PRECIPITATED BY ADDITION OF 2 VOLUMES OF ETHANOL. FRACTIONATION WAS ACCOMPLISHED AS DESCRIBED IN FIG. 2. NOTE THAT THE CPM SCALE OF A IS DIFFERENT FROM B, C AND D.

AGGREGATES ARE ALSO PRESENT. THE SPECIFIC ACTIVITY OF THE 18S RNA ORIGINALLY ELUTED FROM THE 18S REGION OF THE MAK IS MUCH HIGHER THAN THAT OF THE 18S RNA ORIGINALLY PRESENT AS AN AGGREGATE IN THE 25S REGION OF THE COLUMN (COMPARE FIG. 3A WITH B,C,D). THIS SUGGESTS THAT THE MATURE 18S RNA IN THE TISSUE DIFFERS IN SOME PHYSICAL WAY FROM NEWLY SYNTHESISED 18S RNA. THE 16S RNA, PRESUMABLY PLASTID OR MITOCHONDRIAL RNA (LOENING AND INGLE, 1967), WHICH WAS PRESENT AS ONLY A TRACE IN THE TOTAL RNA FRACTIONATION, WAS CONSIDERABLY ENRICHED IN THE 18S REGION OF THE MAK COLUMN, INDICATING THAT THIS RNA DID NOT AGGREGATE TO THE SAME EXTENT AS 18S RNA.

GEL FRACTIONATION OF THE RNA ELUTED FROM THE SHOULDER REGION OF THE MAK COLUMN SHOWED TRACES OF ADDITIONAL RNAs, MUCH LARGER THAN RIBOSOMAL-RNA (FIG. 3, C AND D). THE SPECIFIC ACTIVITY OF THIS HIGH MOLECULAR WEIGHT RNA WAS MUCH HIGHER THAN THAT OF THE RIBOSOMAL-RNA. SINCE DNA-LIKE-RNA (D-RNA), WHICH IS DETECTED ONLY BY RADIOACTIVITY, IS ELUTED FROM THE MAK COLUMN IN THE SAME REGION AS THE SHOULDER 260 mμ O.D. (INGLE ET AL, 1965), IT WAS OF INTEREST TO SEE WHETHER THESE HIGH MOLECULAR WEIGHT COMPONENTS (AS MEASURED BY ABSORPTION AT 254 mμ) WERE ASSOCIATED WITH THE RADIOACTIVE D-RNA. PURIFIED D-RNA, OBTAINED BY THREE PASSAGES THROUGH MAK COLUMNS, TOGETHER WITH CARRIER RIBOSOMAL-RNA, HAVING A BASE COMPOSITION OF 20.9, 29.3, 23.4 AND 26.0 MOLE % CMP, AMP, GMP AND UMP, RESPECTIVELY, WAS FRACTIONATED BY GEL ELECTROPHORESIS (FIG. 4). THE TWO LARGE RNA COMPONENTS AS MEASURED BY ABSORBANCE AT 254 mμ, 32S (2.5 x 10^6 MOL. WT.) AND 30S (2.0 x 10^6 MOL. WT.), WERE CONSIDERABLY ENRICHED. THE RADIOACTIVE D-RNA MIGRATED IN THE SAME REGION AS THE 30 AND 32S COMPONENTS,

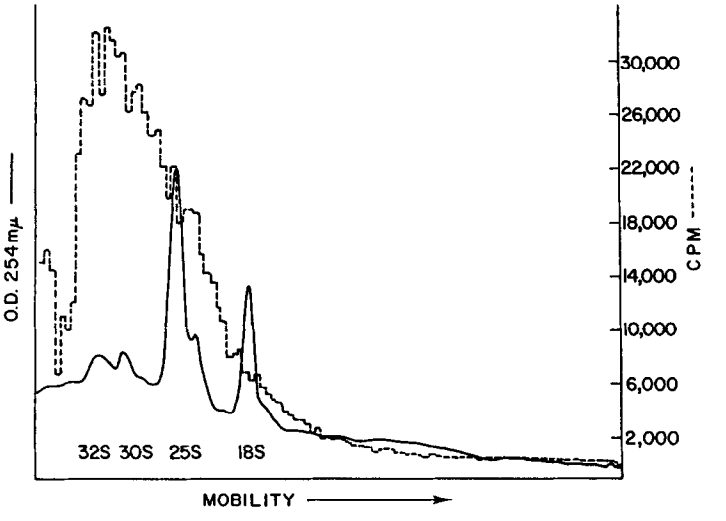

FIG. 4. ACRYLAMIDE GEL FRACTIONATION OF PURIFIED ^{32}P-D-RNA. ^{32}P-RNA TAKEN FROM REGIONS OF MAK COLUMNS CORRESPONDING TO C AND D IN FIG. 1 WAS PELLETED ALONG WITH CARRIER RNA AS DESCRIBED IN FIG. 3. THE RNA WAS THEN FRACTIONATED TOGETHER WITH CARRIER RNA ON A SECOND MAK COLUMN. THE ENTIRE PROCEDURE WAS REPEATED A SECOND TIME. AFTER THE THIRD CYCLE THROUGH THE MAK COLUMN THE ^{32}P-D-RNA REGION WAS THEN PREPARED AND ELECTROPHORESED AS DESCRIBED IN FIG. 3 EXCEPT THAT ELECTROPHORESIS WAS FOR 2 HR.

ALTHOUGH LEADING SOMEWHAT INTO THE RIBOSOMAL-RNA REGION. THE 25 AND 18S RIBOSOMAL RNAS, REPRESENTING CARRIER RNA ADDED DURING THE PURIFICATION OF D-RNA, HAD NO RADIOACTIVITY ASSOCIATED WITH THEM. SINCE THE CONDITIONS OF THE GEL ELECTROPHORESIS ARE UNFAVORABLE FOR AGGREGATION, IT SEEMS UNLIKELY THAT THE 32 AND 30S RNA COMPONENTS ARE ARTIFACTS OF AGGREGATION, BUT THE MIGRATION OF THE D-RNA RADIOACTIVITY WITH THEM MAY BE FORTUITOUS.

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