Study of messenger like RNA extracted from HeLa cells polysomes. I. - Sedimentation and chromatographic data.

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Short exposures of animal cells to a radioactive precursor of RNA and subsequent analysis of the whole cellular RNA on methylated bovine serum albumin kieselguhr columns (MAK) has revealed the presence of three RNA species : Q_1RNA, Q_2RNA (Yoshikawa, 1964 ; Yoshikawa-Fukada, 1965) and TDRNA (Ellem, 1966). Q_1RNA was identified as the 45 S nuclear precursor of ribosomal RNA (Yoshikawa-Fukada, 1965 ; Yoshikawa-Fukada, 1967). Q_2RNA and the tenaciously bound DNA like RNA therefore called TDRNA having mean sedimentation coefficients of 50 S and 16 S respectively were tentatively identified as the part of the cellular mRNA that is present in the nucleus.

The present communication shows that the heterogeneously sedimenting RNA associated with polyribosomes present in the cytoplasm of HeLa cells, when fractionated on MAK columns, yields three peaks, two of them being eluted together respectively with Q_2RNA and TDRNA. Sucrose gradient analysis of the whole heterogeneously sedimenting polysomal RNA reveals the polysomes to contain more rapidly labelled RNA than inferred from the actually known structure of polysomes.

Methods. -

Growth of HeLa cells in spinner cultures, preparation of the cyto-

plasmic extracts, pelleting the polysomes and subsequent analysis by sucrose gradient centrifugation have been previously described (Miller, 1967). Extraction and purification of the polysomal RNA were conducted according to previously published data (Bramwell et al., 1966). RNA was eluted at 35° from the MAK columns by a 200 ml linear NaCl gradient (0.6 to 1.1M NaCl buffered by 5.10^{-2}M phosphate pH 6.8). The tenaciously bound RNA was eluted by bringing the salt molarity to 2M, imposing simultaneously a temperature gradient from 35 to 97°.

Counting the activity from both sucrose gradient and MAK column fraction was done by a new method (Miller, 1968a).

Results and Discussion. -

RNA samples from polysomes were simultaneously analyzed by sucrose gradient sedimentation and by chromatography on MAK columns. We shall first examine the sedimentation data.

Evaluation on the sucrose gradient patterns of the activity pertaining to ribosomal RNA (16 S and 28 S) by a method described in detail earlier (Girard et al., 1963 ; Miller, 1967) allowed us to evaluate the activity contained within the polysomal heterogeneously sedimenting RNA molecules (fig. 1).

These data show the progressive appearance of the label within 16 S (30 min) and 28 S (45 min) polysomal rRNA. After 24 h continuous labelling (one generation time) the contribution of heterogeneously sedimenting polysomal RNA to total polysomal activity is still high (50 %). Extending the incubation up to 48 h and even to 72 h (three generation times) lowers that contribution to only about 30 % (Miller, 1968b). This is at variance with the 5 % mean contribution of mRNA to total polysomal RNA calculated by others (Latham et al., 1965). These data lead us to suspect that polysomes might contain

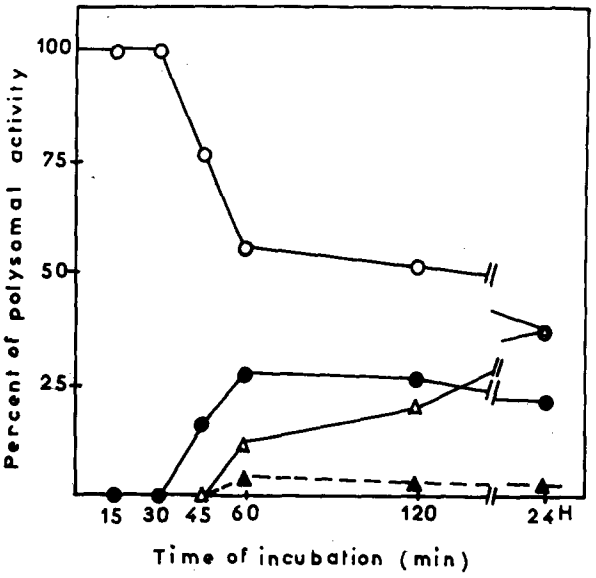

Fig. 1 - Distribution of incorporated ^{3}H adenosine between the RNAs extracted from HeLa cell polysomes, as determined by sucrose gradient centrifugation. (The extracted polysomal RNA was not submitted to the purification step with 2M NaCl).
▲——▲ 4S transfer RNA
●——● 18S ribosomal RNA
△——△ 28S ribosomal RNA
○——○ heterogeneous sedimenting RNA

or be contaminated by some other rapidly labelled RNA molecules, distinct from the mRNA which is thought to constitute the "classical" polysome backbone itself.

Chromatography on MAK columns was simultaneously done as a trial to further fractionate these supplementary rapidly labelled polysomal RNAs. The results of such analysis are given in fig. 2 and 3.

Fig. 2 exemplifies the fractionation of the rapidly labelled polysomal RNA within three peaks. To avoid multiplicity of terms the two peaks which were eluted at the same place as Q_2RNA and TDRNA (see fig. 3) were called Q_2^p RNA and TD^pRNA, the suffix "p" (serving as a reminder of) their polysomal origin. If the RNA labelled by ^{14}C adenosine (30 min pulse) extracted

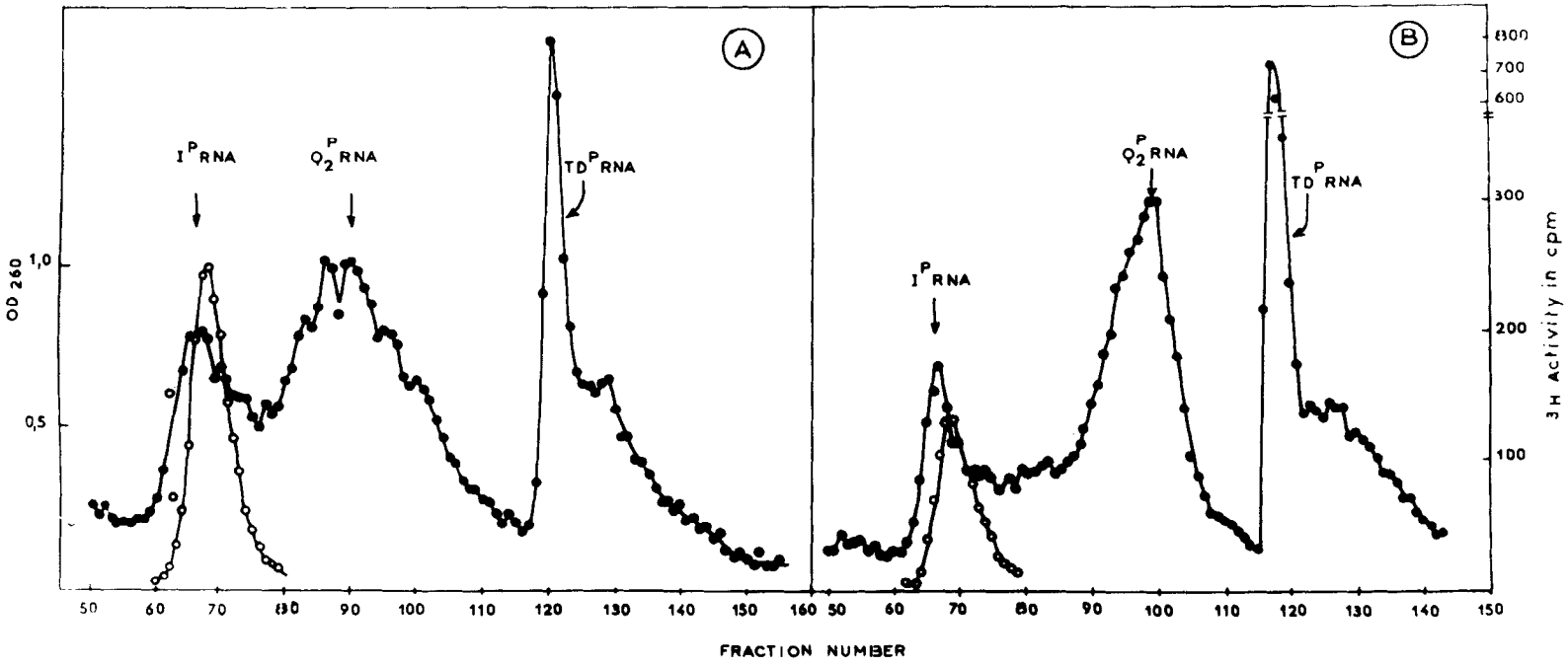

Fig. 2 - Chromatographic analysis of HeLa cell polysomal RNA labelled by ^{3}H adenosine.
$4.3\ 10^8$ cells in 200 ml BME 0405 SP were pulsed with ^{3}H (2,770 mc/mM ; 2 µC/ml final activity). The polysomal RNA is then extracted, purified and analyzed on MAK column. A : 15 min pulse O——O OD_{260}
B : 30 min pulse ●——● ^{3}H activity.

from nuclei prepared by the Tween method (Fisher, 1962) is mixed with the 30 min (^{3}H adenosine) labelled polysomal RNA (fig. 3) the coelution of Q_2RNA (^{14}C label) and Q_2^P RNA (^{3}H label) on the one hand, and of TDRNA (^{14}C label) with TD^{P}RNA (^{3}H label) on the other is clearly visible.

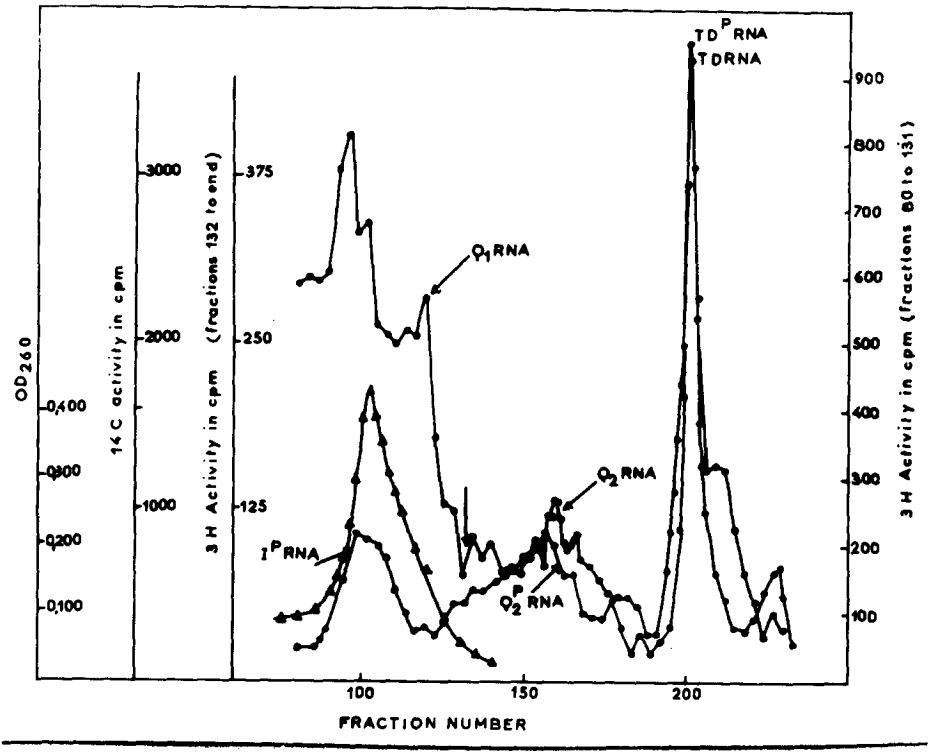

Fig. 3 - Chromatographic analysis of a mixture of 30 min pulsed nuclear and polysomal RNA. 4×10^8 cells are pulsed in 200 ml BME 0405 SP with ^{3}H adenosine (500 mc/mM ; 2 µc/ml final activity) and their polysomal RNA extracted as described in fig. 1.

3×10^8 cells are pulsed under the same experimental conditions with ^{14}C adenosine (523 mc/mM ; 0.1 µc/ml final activity) and their nuclear RNA extracted as described by Fisher (Fisher et al., 1962). Both RNA are mixed and analyzed on MAK column with an extended gradient of 0.6 to 1.1 M NaCl (400 ml). Note the change in ^{3}H activity scale (arrow) at fraction 131.

●—● ^{14}C activity ○—○ ^{3}H activity Δ—Δ $^{OD}_{260}$

The 4 to 30 S spread (with a mean at about 16 S) of sedimentation coefficients for polysomal RNA was shown to exist also within each of the three peaks I^P, Q_2^P and TD^P (Miller, 1968b). The factor critical for the elution of Q_2^P RNA (16 S) and Q_2RNA (50 S) (fig. 3) therefore cannot be the size but should be base composition (or sequence) and/or secondary structure. Although much care has to be exercised concerning the interpretation of these data, a possible interpretation would be that Q_2^P RNA represents the cytoplasmic fragmented counterpart of messenger RNA present in the nucleus as giant polycistronic molecules (Scherrer et al., 1965).

Experiments are under way to link all three RNA species isolated by MAK chromatography to the RNAs found in ribonucleoprotein particles associated with polysomes (Perry, 1968 ; Penman, 1968).

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