SEDIMENTATION CHARACTERISTICS OF RAPIDLY LABELLED RNA FROM HELA CELLS*

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The accumulation of evidence in support of the "messenger RNA (m-RNA) theory" (1) of protein synthesis in bacteria has been greatly facilitated by sucrose gradient sedimentation analysis of bacterial RNA (2,3). The RNA fraction thought to perform the function of information transfer from DNA base sequence to protein amino acid sequence has been found to have different sedimentation characteristics from the bulk of the RNA. This has aided in demonstrating its rate of labelling, its derivation by base-pair copying from DNA and its ability to attach to ribosomes and stimulate protein synthesis (3,4,5,6). This report describes the use of sucrose gradient analysis to define the kinetics of labelling of the RNA in a cultured animal cell.

The cells used for this study were suspension cultures of a strain of HeLa cells growing exponentially with a generation time of about 24 hours (7,8). Labelling of the RNA of the cells was accomplished by adding to the growth medium radioactive uridine of varying specific activities depending on the length of the labelling period. (Details of the experiments are presented with Figure 1.) To stop the incorporation of radioactivity, the cells were chilled by addition of frozen .14 M NaCl, centrifuged (1000g x 5 min.), washed in cold Earle's saline and frozen. Extraction of the RNA was accomplished by thawing and resuspending the cell pellet in cold .01 M acetate buffer, pH 5.1, containing 2 µg/ml polyvinyl sulfate (PVS); sodium dodecyl

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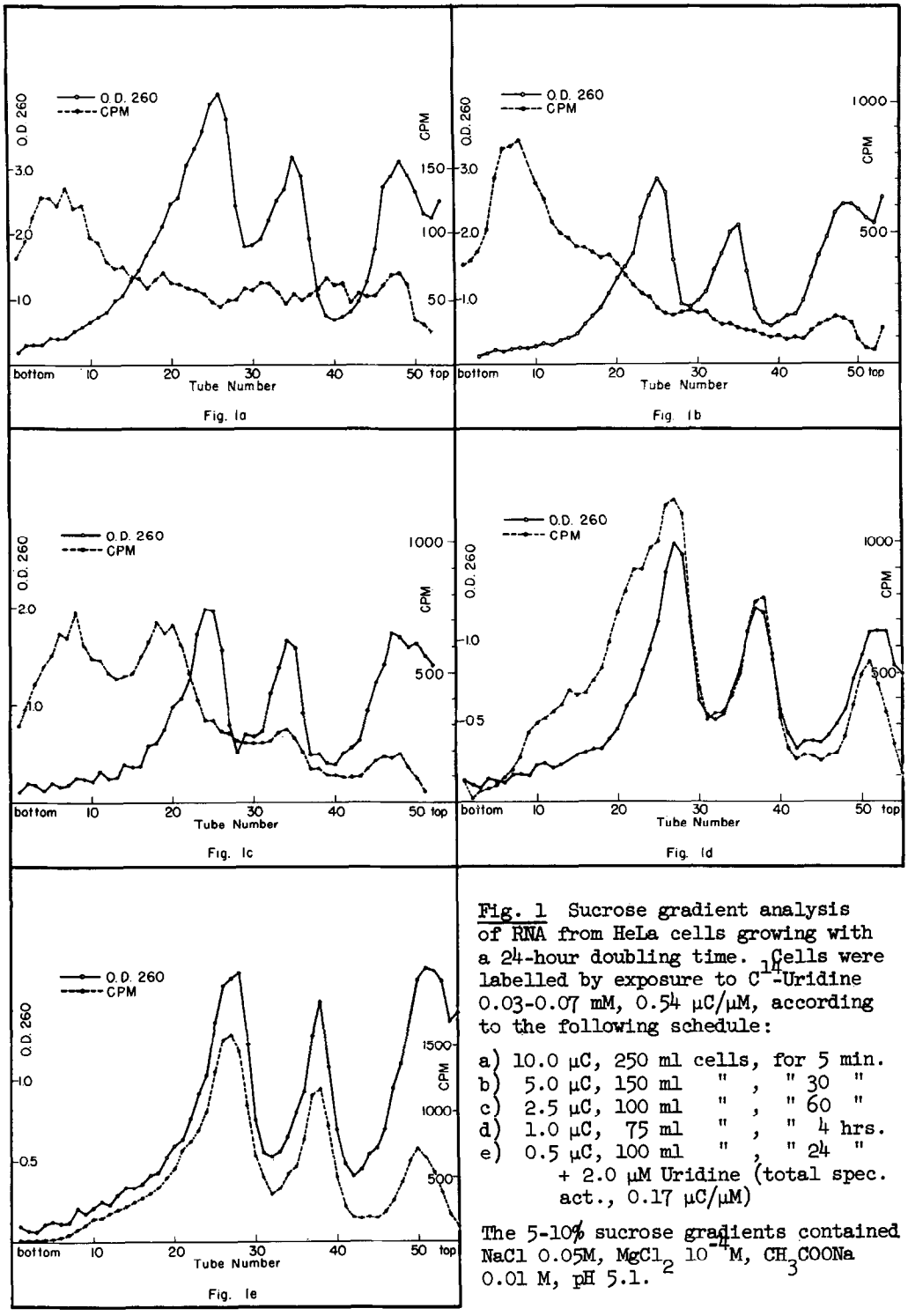

Fig. 1 Sucrose gradient analysis of RNA from HeLa cells growing with a 24-hour doubling time. Cells were labelled by exposure to C^{14}-Uridine 0.03-0.07 mM, 0.54 μC/μM, according to the following schedule:

a) 10.0 μC, 250 ml cells, for 5 min.
b) 5.0 μC, 150 ml " , " 30 "
c) 2.5 μC, 100 ml " , " 60 "
d) 1.0 μC, 75 ml " , " 4 hrs.
e) 0.5 μC, 100 ml " , " 24 "
+ 2.0 μM Uridine (total spec. act., 0.17 μC/μM)

The 5-10% sucrose gradients contained NaCl 0.05M, $MgCl_2$ 10^{-4}M, CH_3COONa 0.01 M, pH 5.1.

sulfate was then added to a final concentration of 0.5%. An equal volume of hot phenol was then mixed with the cells and shaken for 3 minutes in a 60° water bath (9). The temperature in the mixture was approximately 50° at the end of the extraction and was reduced to 4° by rapid chilling in a -20° ice bath. The emulsion was then broken by centrifugation at 20,000 g for 1 minute at 4° and the extraction repeated twice more on the aqueous layer. The majority (>95%) of the DNA remained either in the interface or in the phenol layer under these conditions. The RNA was precipitated from the aqueous layer by the addition of 2 vols. of cold ethanol and redissolved in the pH 5.1 acetate buffer containing 0.05 M NaCl and 10^{-4} M Mg^{++}. Additional PVS, which is reported to be an inhibitor of RNAse (10), was added at this time. The solution of RNA was layered onto 25 ml linear sucrose gradients of from 5-20% w/w concentration and spun for 8 hours at 25,000 RPM in the SW25 head of the Model L Spinco ultracentrifuge. The temperature in the gradient at the conclusion of the run was 6°. The gradients were then collected through a needle attached by polyethylene tubing to a finger pump. This allowed very accurate and reproducible drop sizes so that comparison between gradients could be made with assurance.

RESULTS

The main results to be presented here can be summarized as follows:

1) There are three classes of RNA from Hela cells (s_{20} values, approximately 28, 16, 4) which comprise the bulk of the cellular RNA.[1] This agrees with the report of Philipson (11).

2) There are two additional fractions of RNA which are labelled in advance of the bulk RNA. The most rapidly sedimenting fraction (approximately 45s) seems to be completely labelled within 30 minutes. Label begins to appear in a second fraction (approximately 33s) which also sediments more rapidly than the bulk RNA. Thus at 1 hour after the addition of label two peaks

[1] These S_{20} values were approximated by comparison with the ribosomal RNA from E. coli, the S values for which have been established (3).

are visible which sediment more rapidly than the bulk of the RNA. In addition there are a considerable number of counts scattered in smaller components including the S-RNA which are found higher up in the gradient.

3) By 4 hours after the addition of uridine 2-C^{14} to the medium, radioactivity has appeared in all of the RNA of the cell and the radioacitivity and UV absorption are very nearly parallel.

The nature of the various classes of RNA described above is being investigated at present. From the work of others with other animal cells and with HeLa cells it seems certain that the classes containing the majority of RNA (4s, 16S, 28s) will prove to be soluble RNA and the RNA from ribonucleoprotein particles respectively. Preliminary characterization of the rapidly labelled fractions indicates that they contain no DNA, that the counts are incorporated as ribonucleotide internally in an RNA chain, and that there is no protein or peptide material associated with this RNA.

It may be of interest to point out that the original reports of "pulse labelled" (3,4) bacterial RNA indicated that the s-value of the most rapidly labelled fraction was around 12 with very few counts in larger material. It has been found recently, however, that with different techniques, a significant portion of the "pulse label" RNA sediments faster than the larger ribosomal RNA (12).

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