SYNTHESIS OF NUCLEAR AND CYTOPLASMIC RNA OF HELA CELLS AND THE EFFECT OF ACTINOMYCIN D.*

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Sedimentation of phenol-extracted nucleic acids from HeLa cells through sucrose gradients has revealed five major fractions of RNA in this mammalian cell: a rapidly labeling 45s, an intermediate labeling 33s, the slow labeling 28 and 16s (ribosomal) and a 4s (transfer) RNA (Scherrer and Darnell, 1962). In the present study a similar RNA analysis has been carried out on isolated nuclei and cytoplasm. The data illustrate that the nucleus plays the major role in RNA synthesis of the HeLa cell for all fractions except the 4s RNA. In agreement with a proposed nuclear action of actinomycin D, low levels of this agent block the incorporation of P^{32} orthophosphate into all fractions of RNA except the 4s RNA which is inhibited to a lesser extent.

HeLa cells were grown in spinner flasks in a modified Eagle's medium as previously described (Rueckert and Mueller, 1960). Labeling of exponentially growing cultures was accomplished by the addition of 0.5 μc./ml. of P^{32} orthophosphate. In the inhibition studies 0.005 to 0.25 μg./ml. of actinomycin D was added 15 minutes prior to the P^{32}. At indicated times the cells were centrifuged down and lysed in distilled water (Stubblefield, 1961). After sedimentation at low speed for 5 minutes the nuclei were washed once by resuspension in 0.002 M $CaCl_2$ in 0.002 M tris buffer at pH 7.4; the wash solution was combined with the cytoplasmic fraction.

For extraction of the nucleic acids the cytoplasmic and nuclear fractions were adjusted to 0.5 M NaCl, 0.02 M trisodium citrate and 0.2% sodium

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dodecyl sulfate with the equivalent of 10 x 10^6 cells per ml. Each fraction was shaken with an equal volume of water-saturated phenol at room temperature. The aqueous layer was re-extracted with phenol twice more. After dialysis overnight at 4°C. against a solution of 0.001 M $MgCl_2$ and 0.01 M tris buffer at pH 7.4 the resulting nucleic acid solution was used directly in the centrifugation studies. Over 80% of both DNA and RNA of the HeLa cell was recovered in the extract by this procedure; all DNA was contained in the extracts from the nuclei.

Centrifugation was carried out in a SW-39 rotor at 4°C. in a linear gradient from 5 to 30% sucrose in a solution of 0.001 M $MgCl_2$ and 0.01 M tris buffer, pH 7.4, at 35,000 RPM for 5 hours.

After measurement of the total O.D. (260 mμ) the successive fractions were precipitated in the presence of 1 mg. serum protein by 2.5% perchloric acid (PCA); residues were suspended in 0.04 M $KHCO_3$ and incubated with 20 μg. RNAse for 30 minutes at 37°C. The released material which was soluble in 2.5% PCA was measured for U.V. absorption at 260 mμ and for P^{32} activity.

Results

As previously shown by Scherrer and Darnell (1962) the bulk of the RNA extracted from whole cells was contained in 3 O.D. peaks of the centrifuge pattern which correspond to the 28 and 16s ribosomal RNA fractions and the 4s transfer RNA. In the present study comparison of the RNA profiles from the nuclei and cytoplasm revealed the O.D. patterns to be very similar (Fig. 1A-1D). The quantity of transfer RNA (4s), however, was relatively low in the nucleus.

Incubation of the cells with P^{32} for 1 hour revealed the rapidly labeling, heavy RNA in the areas of the 45 and 33s types described by Scherrer and Darnell. This material was completely confined to the nuclei. In addition the 28 and 16s RNA of the nucleus was labeled significantly at 1 hour whereas the corresponding peaks from the cytoplasm were not (Figs. 1A, 1B). Only the 4s type of the cytoplasmic RNA showed significant labeling in this period.

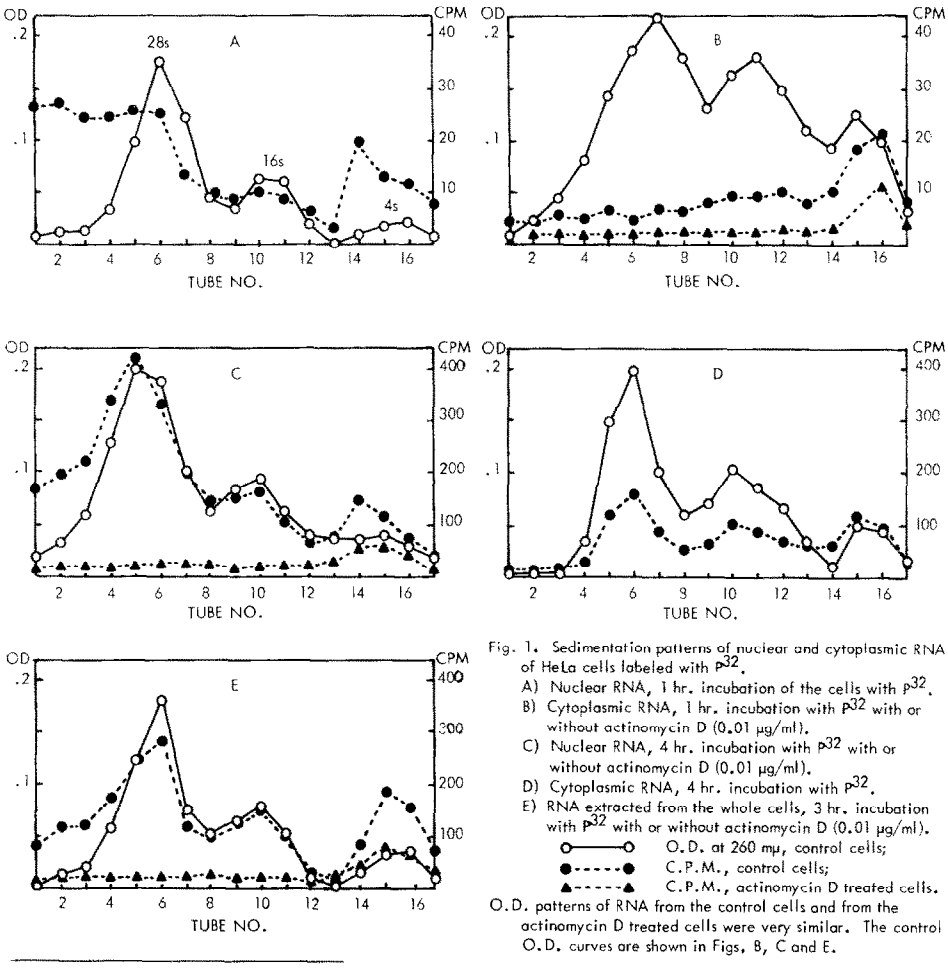

Fig. 1. Sedimentation patterns of nuclear and cytoplasmic RNA of HeLa cells labeled with P^{32}.
A) Nuclear RNA, 1 hr. incubation of the cells with P^{32}.
B) Cytoplasmic RNA, 1 hr. incubation with P^{32} with or without actinomycin D (0.01 µg/ml).
C) Nuclear RNA, 4 hr. incubation with P^{32} with or without actinomycin D (0.01 µg/ml).
D) Cytoplasmic RNA, 4 hr. incubation with P^{32}.
E) RNA extracted from the whole cells, 3 hr. incubation with P^{32} with or without actinomycin D (0.01 µg/ml).
O——O O.D. at 260 mµ, control cells;
●- - - -● C.P.M., control cells;
▲- - - -▲ C.P.M., actinomycin D treated cells.
O.D. patterns of RNA from the control cells and from the actinomycin D treated cells were very similar. The control O.D. curves are shown in Figs. B, C and E.

In experiments in which the labeling was allowed to continue for 4 hours the labeling of the other cytoplasmic RNA peaks became demonstrable (Fig. 1D). The specific activity of the corresponding peaks was, however, still higher in the RNA extracted from the nuclei (Fig. 1C). In fact, the progressive labeling of the bulk of the nuclear RNA tended to obscure the presence of the RNA sedimenting in the 45 and 33s area, which rapidly equilibrated with the labeled precursors but was present in relatively small amounts. The finding that this rapidly labeling, heavy RNA was present only in the nucleus may be indicative of a functional relationship to the "messenger RNA" described by Jacob and Monod (1961). This rapidly labeling RNA from the nuclei was isolated and degraded with 1M KOH. The radioactivity was accounted for chromatographically in AMP, CMP, UMP and GMP (2',3' nucleotides).

The incorporation of P^{32} into RNA is markedly suppressed by low levels of actinomycin D: 0.005, 0.01 and 0.25 µg./ml. produced 60, 75, and 90% inhibition of the incorporation of P^{32} into RNA over a 1 hour period. This inhibition prevailed throughout a 4 hour incorporation period. Analysis of the labeling with respect to RNA classes revealed that actinomycin D blocked the incorporation into all RNA fractions except the 4s class which was inhibited but to a lesser extent (Figs. 1B, 1C, 1E). In studies to be reported the synthesis of DNA which is already in progress is not affected by actinomycin D.

Since actinomycin D appears to act by combining with the DNA template (Reich et al., 1961 and 1962) and preventing the action of the DNA-dependent RNA polymerase (Goldberg and Rabinowitz, 1962; Hurwitz et al., 1962), the present results suggest that all RNA synthesis in the HeLa cell except the 4s variety is highly dependent on this system. Even the major synthesis of the 4s RNA could depend on this system since it is well known that the terminal 3 nucleotides of the transfer RNA turn over independently from the rest of the RNA chain (Herbert, 1959) and could thus explain the present results.

In conclusion the present data support the concept that the nucleus is the seat of RNA synthesis in the HeLa cell and that the synthesis of all classes of RNA in the HeLa cell is inhibited by actinomycin D.

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