DEMONSTRATION OF CELLULAR UPTAKE OF POLYMERIZED DNA IN MAMMALIAN CELL CULTURES*

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Extracted nucleic acid polymers are known to contain and transfer genetic information in microbial systems. A prerequisite to the possibility of such information transfer in mammalian cell cultures would presumably be the cellular uptake of polymerized DNA. Therefore, the question of whether mammalian cells can take up polymerized DNA is of considerable interest. Suggestive evidence for DNA uptake in mammalian cell cultures has recently appeared (Borenfreund, Rosenkranz, and Bendich, 1959; Chorazy, Baldwin, and Boutwell, 1960; Gartler, 1959; King and Bensch, 1960; Sirotnak and Hutchison, 1959) and in this paper further evidence for such uptake is reported.

A critical demonstration of cellular uptake of polymerized DNA requires that any DNA taken up by host cells be identifiable as unchanged donor molecules. It is necessary, therefore, that the donor DNA be labeled in such a way that any changes occurring in the process of uptake be detectable. Substitution of a heavy atom into donor molecules in conjunction with equilibrium centrifugation in a CsCl density gradient meets this requirement. A molecule in a density gradient is characterized by its density (position in the gradient) and its molecular weight (width of the band formed in the gradient) (Meselson, Stahl, and Vinograd, 1957). Thus simple degradative changes can

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be detected by changes in band width, while dilution of donor label will result in a decrease in density and change in position of the band itself. If cells are exposed to heavy donor DNA and the DNA of the exposed cells then extracted and subjected to equilibrium centrifugation, the fate of any donor DNA taken up by the cells may be followed.

Methods and Materials

A strain of Earle's L cells was used in these experiments. They were grown on glass in 1 liter Roux bottles in medium 199 plus 10% horse serum and antibiotics. DNA of donor cells was labeled with a heavy atom by adding 5-bromodeoxyuridine (5BDU) to the medium for 48 hours at a concentration of 5 mg./L. Incorporation of 5BDU into cellular DNA was demonstrated by ultra-violet analyses of chromatograms of hydrolyzed DNA extracted from cells grown in the presence of 5BDU. 5-bromouracil was identified on such chromatograms by its position and characteristic ultra-violet spectra. The DNA of donor cells was also labeled with thymidine-C^{14}. This second label permitted detection of smaller amounts of donor DNA than would otherwise have been possible and afforded a second means by which the donor DNA could be characterized.

Unlabeled L cells were exposed to crude extracts of this doubly labeled DNA (first alcohol precipitation of dupanol lysed cells) for 1 to 2 hours at 37° C. Fresh medium was placed in the culture bottles and the labeled DNA was added to a concentration of about 0.1 μg./ml.. After exposure, the still completely viable cells were harvested, washed repeatedly with saline citrate, and the nuclei extracted with citric acid (Mirsky and Pollister, 1946). The nuclei were lysed in dupanol and an aliquot subjected to equilibrium centrifugation in a CsCl density gradient in the Spinco preparative centrifuge (Weigle, Meselson, and Paigen, 1959). After centrifugation, the contents of the tubes were carefully fractionated and the fractions assayed for radioactivity (liquid scintillation counting) and ultra-violet absorption at 260 mμ.

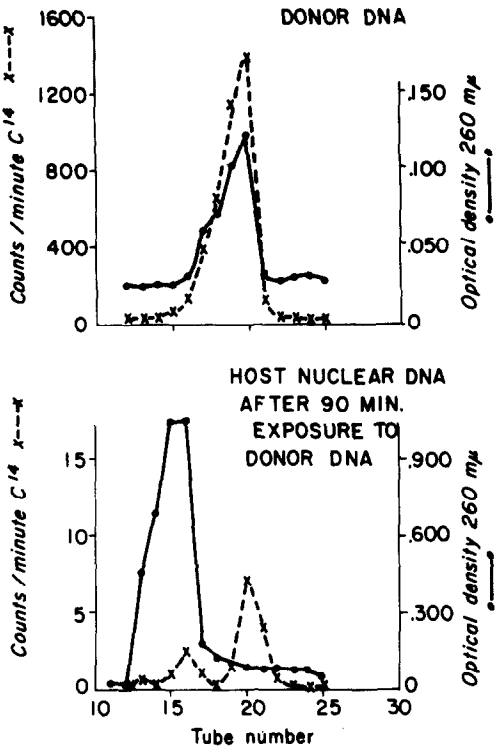

Fig. 1

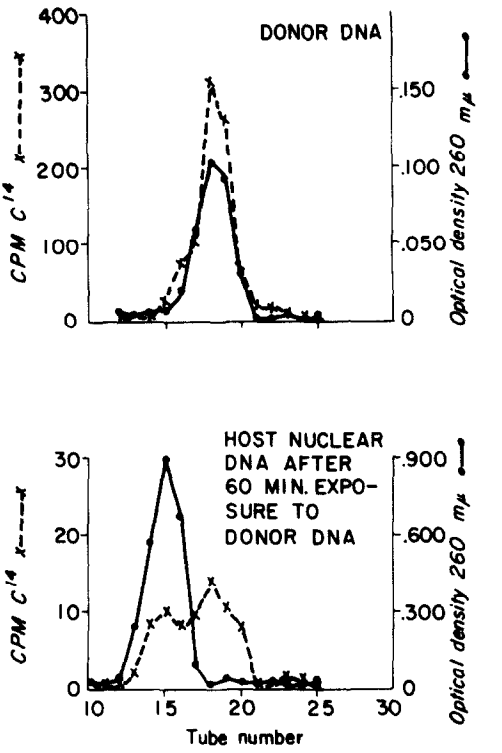

Fig. 2

Fig. 1 and 2. Density gradient pattern of donor DNA (above) and host DNA after exposure of host cells to donor DNA (below).

Results

In figures 1 and 2 are shown the results of two uptake experiments with doubly labeled DNA. The upper curve in each figure is the density gradient analysis of the doubly labeled donor DNA, while the lower curves show the density gradient analyses of the uptake experiments. In the lower curve, a single 260 mμ absorbing peak is visible in each of the experiments at a density position equal to that of unlabeled host cell DNA. Two small radioactive peaks are visible, the larger one at a density equal to that of the doubly labeled DNA used in the experiment, and the smaller one coincident with the main 260 mμ peak. The radioactivity is derived from donor DNA and represents a total uptake of approximately 0.04 μg. and 0.03 μg. in the two experiments respectively. This is approximately 0.5% and 3.0% of the administered donor DNA and approximately 0.1% and 0.9% of the host DNA in each experiment.

Discussion

The use of nuclear preparations minimized the possibility that the donor DNA analyzed in these experiments was merely absorbed onto the cell surface. Comparisons of total uptake levels between intact cells and nuclear preparations showed a proportionately much smaller loss in radioactivity than the corresponding loss of cytoplasmic material. For example, in one such experiment the loss in radioactivity was approximately 2-fold, whereas, the loss in cytoplasmic material was over 10-fold. Such data indicate that at least most of the donor label analyzed in these experiments represents intracellular uptake of DNA.

The larger donor radioactive peak in each experiment has the same density and approximately the same peak width as the original donor DNA from which it was derived, and therefore, must represent cellular uptake of unchanged donor DNA.

The smaller radioactive peaks, on the other hand, must represent incorporation of donor DNA fragments into unlabeled host DNA. A decrease in density such as these peaks exhibit indicates dilution of donor heavy label by incorporation into unlabeled host DNA. The smaller the donor fragment incorporated relative to the unlabeled host component, the greater will be the change in density of the resultant molecules. Under the present experimental conditions, it was only possible to detect a density difference of .010 gm./cm^3. The density difference between host DNA and labeled donor DNA was .050 gm./cm^3, which means that incorporation of less than 20% donor label will not lead to detectable density changes. Consequently, the pattern of the small radioactive peaks is compatible with either complete degradation of donor DNA and incorporation of constituent nucleotides, or partial degradation and incorporation of donor fragments representing as much as 20% of the new molecules.

The marked difference in total uptake between the two experiments (nearly 10-fold) was not completely unexpected in view of the fluctuations in DNA uptake known to occur in microbial forms (Goodgal and Herriott, 1957; Lerman and Tolmach, 1957). Accordingly this difference might reflect the existence of

factors affecting the uptake of large molecules by mammalian cells.

In conclusion, it has been shown that when mammalian cells in culture are exposed to polymerized DNA, 1) a small amount of intact DNA is taken up, and 2) a smaller amount is degraded and the fragments incorporated into cellular DNA.

References

Borenfreund, E., Rosenkranz, H. S., and Bendich, A., In: The Kinetics of Cellular Proliferation, F. Stohlman, ed. Greene and Stratton, New York and London, (1959).

Chorazy, M. R., Baldwin, H. H., and Boutwell, R. K., Fed. Proc., 19, 307 (1960).

Gartler, S. M., Nature, 184, 1505 (1959).

Goodgal, S. H. and Herriott, R. M., In: The Chemical Basis of Heredity, W. D. McElroy and B. Glass, eds., The Johns Hopkins Press, Baltimore, (1957).

King, D. W. and Bensch, K. G., Fed. Proc., 19, 308 (1960).

Lerman, L. S. and Tolmach, L. J., Biochim. Biophys. Acta, 26, 67 (1957).

Meselson, M., Stahl, F. W., and Vinograd, J., Proc. Natl. Acad. Sci., 43, 581 (1957).

Mirsky, A. E. and Pollister, A. W., J. Gen. Physiol., 30, 117 (1946).

Sirotnak, F. M. and Hutchison, D. J., Biochim. Biophys. Acta, 36, 246 (1959).

Weigle, J., Meselson, M., and Paigen, K., Brookhaven Symposium in Biology, 12, 125 (1959).