NUCLEAR-CYTOPLASMIC RELATIONSHIPS IN HUMAN CELLS IN TISSUE CULTURE

IV. A STUDY OF SOME ASPECTS OF NUCLEIC ACID AND PROTEIN METABOLISM IN ENUCLEATE CELLS

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SUMMARY

Enucleate pieces of cytoplasm were prepared from human amnion cells in tissue culture by a method of microsurgery. Vital activities of the fragments were retained for 10–30 h. One of several amino acids or RNA precursors labeled with ^{14}C was added to the culture medium just before or just after preparation of a group of enucleate cytoplasmic fragments. When about half the fragments had "died", the surviving enucleates were fixed and examined by autoradiography for evidence of incorporation of the labeled compound. Amino acids were incorporated as actively by enucleate cytoplasm as by cytoplasm of complete cells. This incorporation could be blocked by excess label-free L-isomers of the corresponding labeled amino acid but not by the label-free D-isomer. RNA precursors were not incorporated by enucleate cytoplasmic fragments in detectable amount, although neighboring intact cells incorporated the precursors actively into cytoplasm.

The conclusions reached are (a) that protein synthesis continues in enucleate cytoplasm of mammalian cells, but (b) that RNA synthesis stops or is so slight as to escape detection by these methods. These data add to the evidence that cytoplasmic RNA is derived chiefly from the nucleus.

The labeled precursors used were: [^{14}C]adenine, [^{14}C]uridine, L-[^{14}C]glutamic acid, L-[^{14}C]leucine, L-[^{14}C]phenylalanine and DL-[^{14}C]tryptophan; [8-^{14}C] adenylic acid was also used.

INTRODUCTION

The importance and necessity of studying the metabolism of enucleate cell fragments have been emphasized on numerous occasions[1,2]. We would like briefly to reiterate the importance of determining conclusively whether a cell without a nucleus is capable of RNA synthesis.

Abbreviation: RNA, ribonucleic acid.

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There is abundant cytological evidence[3-6] that a substantial fraction of cytoplasmic RNA probably is of nuclear origin. This suggests that RNA (which is concerned with the synthesis of cytoplasmic protein) may be the agent for the transmission of genetic information from the chromosome. If this hypothesis is correct, one would expect no independent synthesis of RNA in the cytoplasm. The most conclusive approach presently available for establishing whether this is the case is to study the metabolism of RNA in enucleate cells. If it should be shown that RNA synthesis is possible in the absence of a nucleus, it would then be necessary seriously to reconsider the nature of the biological role of RNA.

In earlier studies on the metabolism of enucleate cells of lower form, some doubt as to the importance or validity of the observed results arose because the cells obviously manifested overt abnormality as an immediate result of enucleation. Thus, in the case of amoeba[1], locomotion either ceases at once or becomes decidedly abnormal. In sea urchin and amphibian eggs, cell division, if it proceeds at all, is also decidedly abnormal in the absence of a nucleus[7]. In the case of human tissue culture cells, however, the removal of the nucleus appears to have little immediate effect on the behavior of the cell[8]. For this reason, a study of the ability of enucleate mammalian tissue culture cells to incorporate amino acids and nucleic acid precursors was felt to hold more promise for an understanding of some aspects of the relative role of nucleus and cytoplasm. Similar studies had been made earlier with the so-called more "primitive" forms, and we have executed essentially identical experiments.

MATERIALS AND METHODS

The cells used throughout the course of these experiments were human amnion cells established in culture[9]. The culture methods, equipment and techniques employed to enucleate the cells were similar to those described earlier[8]. Fixation of cells, enzyme digestions and autoradiography have been described previously[6].

The labeled precursors used in these experiments were: [^{14}C]adenine, Isotopes Specialties Co., specific activity, 3.3 mC/mmole; [8-^{14}C]adenylic acid, Schwarz Laboratories Inc., specific activity, 1.64 μC/mg; [^{14}C]uridine, Schwarz laboratories Inc., specific activity, 0.52 μC/mg; L-[^{14}C]glutamic acid, Nuclear-Chicago Corp., specific activity, 4.67 mC/mmole; L-[^{14}C]phenylalanine, Nuclear-Chicago Corp., specific activity, 11.35 mC/mmole; DL-[^{14}C]tryptophan, Nuclear-Chicago Corp., specific activity, 4.67 mC/mmole; L-[^{14}C]leucine Nuclear-Chicago Corp., specific activity, 7.95 mC/mmole.

EXPERIMENTAL PROCEDURE

The cell-cutting operation[8], which was the method employed here for obtaining enucleate cells, required 1–2 h per chamber during which time as many as 21 enucleate fragments were produced. Labeled precursor was added either before beginning the operation or after it was completed.

When label was added after micrurgy, the medium was not covered with oil before the operation, but was covered after the labeled medium was added.

Approx. 10–20 enucleates were prepared in one operating chamber. Observations were made every 2.5 h to determine the proportion of surviving enucleates, using

criteria described previously[8]. An attempt was made to fix the cells at a time when approximately one-half the enucleates had died. This was not always possible, but in all cases some enucleates had died before fixation was effected. An attempt was made to adjust the length of the period the cells were under autoradiographic film according to the length of exposure to the radioactive precursor.

The controls, with which the enucleates were compared, were nearby normal nucleate cells in the same chamber. A subjective estimate of the relative radioactivity was possible by comparing autoradiographs of the enucleates and of the intact cells.

RESULTS

Incorporation of RNA precursors

A series of [^{14}C] RNA precursors (Table I) was administered to enucleated cells, but significant incorporation was not detectable over background. In all cases, the nearby normal nucleate cells showed considerable incorporation. It is clear that if the enucleates are incorporating any label, the extent of the incorporation must be considerably less than 1 % of that in nucleate cells[§].

TABLE I

SUMMARY OF EXPERIMENTS IN WHICH LABELED RNA PRECURSORS WERE ADMINISTERED TO ENUCLEATE AMNION CELLS

Experiment No.	*Precursor*	*Activity in medium (μC/ml)*	*Approx. time between operation and fixation (h)*	*No. of living enucleates at time of fixation over No. of living enucleates at start*
1*	[^{14}C]adenine	0.5	22	3/10
2a	[^{14}C]adenine	0.5	25	6/14
2b*	[^{14}C]adenine	0.5	21	7/13
3a	[^{14}C]adenine	2.0	25	13/16
3b*	[^{14}C]adenine	2.0	20	9/14
4a	[^{14}C]adenine	2.0	26	13/18
4b	[^{14}C]adenine	2.0	21	7/17
5a	[^{14}C]adenine	2.0	25	6/9
5b*	[^{14}C]adenine	2.0	23	11/13
6a*	[^{14}C]adenine	2.0	22	7/18
6b	[^{14}C]adenine	2.0	22	9/19
7a	[^{14}C]adenine	2.0	24	6/21
7b	[^{14}C]adenine	2.0	24	8/19
8a	[8-^{14}C]adenylic acid	1.0	29	6/14
8b	[8-^{14}C]adenylic acid	1.0	26	2/7
9a	[8-^{14}C]adenylic acid	1.0	30	13/18
9b	[8-^{14}C]adenylic acid	1.0	29	8/14
10a	[8-^{14}C]adenylic acid	1.0	24	3/12
10b	[8-^{14}C]adenylic acid	1.0	27	6/11
11a	[^{14}C]uridine	1.0	24	5/14
11b	[^{14}C]uridine	1.0	24	4/6
12a	[^{14}C]uridine	1.0	24	7/13
12b	[^{14}C]uridine	1.0	24	4/13

* Experiments designated thus were labeled after enucleation.

§ [^{3}H]cytidine was employed in similar experiments. Variably high background and evidence that nonspecific adsorption may give autoradiographs over areas where true incorporation has not occurred indicate need for further study. Preliminary interpretations are, however, not incompatible with those from ^{14}C precursors.

It should be noted that in many instances the cells were in the presence of label for as long as 1–2 h before all the enucleation operations in one chamber were completed. As a result the label was available for the enucleate metabolic pool, since the precursor was able to penetrate the cell membrane prior to enucleation. Thus, any possible permeability barriers resulting from the absence of the nucleus would be circumvented. Furthermore, our earlier studies[8] indicated that the enucleate cell does retain the ability to take up substances from the environment by pinocytosis.

Incorporation of protein precursors

All 4 labeled amino acids (L-[^{14}C]glutamic acid, DL-[^{14}C]tryptophan, L-[^{14}C]leucine and L-[^{14}C]phenylalanine) administered to enucleate cells, at concentrations of 1–2 μC/ml, were incorporated in significant amounts. In fact, incorporation into enucleate cells appeared to be of the same order of magnitude as the incorporation into the non-nuclear cytoplasmic areas of neighboring normal cells.

Incorporation of labeled tryptophan and labeled phenylalanine into enucleate cells in the presence of excess amounts of either the unlabeled D-isomer or the unlabeled L-isomer of the labeled amino acid* was studied in greater detail. With the unlabeled L-isomer in the medium, incorporation of the label was greatly reduced in the enucleate cell fragments. (Labeling in normal cells was, of course, also greatly inhibited.) On the other hand, with the unlabeled D-isomer there was essentially no effect on the amount of incorporation. These results clearly suggest that the labeling is truly physiological in nature and that it probably is related to protein synthesis.

DISCUSSION

In view of the fact that enucleate amnion cells retain all normal visible dynamic activity for an average of about 20 h and that they are apparently capable of incorporating labeled amino acids into protein in a normal fashion, the observation that such cell fragments cannot incorporate a variety of labeled RNA precursors leads to the conclusion that no independent RNA synthesis takes place in the absence of the nucleus under otherwise normal conditions. Moreover, these experiments may be taken to mean that all the RNA of the cell is of nuclear origin. They are not conclusive experiments of course, since it is possible, for example, to imagine that the nucleus furnishes to the cytoplasm a labile cofactor which is necessary for the synthetic processes involved in independent cytoplasmic synthesis of RNA.

Our results are in excellent agreement with recent work of PRESCOTT[10], who studied the incorporation of several radioactive RNA precursors in enucleate cells of *Acanthamoeba* grown in sterile culture. PRESCOTT was unable to detect any incorporation of these precursors into enucleate cells but readily demonstrated incorporation into nucleate parts. In discussing the earlier conflicting results dealing with this problem, he concludes that a good deal of the disagreement was due to lack of equivalent control of contaminating organisms. (PRESCOTT's paper[10] should be consulted for a review of the issues.) In our experiments, as in PRESCOTT's, the cells were maintained under aseptic conditions.

* Carrier phenylalanine was present at 0.2 mg/ml, an excess of 13.3 times the concentration of labeled precursor. Carrier tryptophan was present at 0.2 mg/ml, an excess of 8.7 times the concentration of labeled precursor.

It thus appears, on the basis of the most careful experiments, that the nucleus must be present in order for a cell to synthesize RNA.

Whether the cytoplasm with a nucleus present is capable of independent RNA synthesis is still unestablished. It is our belief that only the recent work of HARRIS[11] can be taken as support for the view that cytoplasm is capable of such independent synthesis—without any direct intervention of the nucleus. On the basis of his autoradiographic studies of incorporation of labeled precursors into tissue culture cells he believes that, although the same precursors are used to manufacture both nuclear RNA and cytoplasmic RNA, there is a separate pool of intermediates for each and that it takes the label longer to move through the cytoplasmic RNA intermediate pool. His conclusion is based on the fact that, although after a brief exposure of cells to radioactive precursors all the acid-insoluble label is in nuclear RNA, the later appearance of label in cytoplasmic RNA occurs at a rate and a magnitude incompatible with the view that nuclear RNA is a precursor of cytoplasmic RNA. He believes that the loss of label from the nucleus is due to independent turnover and that the labeling in the cytoplasm comes from an acid-soluble intermediate pool, which ordinarily is undetected in this type of study due to the methods of cell fixation employed.

WOODS[12] has carried out very similar experiments with plant root cells and his data are comparable to HARRIS'—or perhaps even more dramatic in the difference observed between the initial amount of label in the nucleus and the ultimate amount of label found in the cytoplasm. While WOODS also believes that an acid-soluble pool of label (remaining after removal of the administered label from the external medium) is responsible for the marked increase in the degree of labeled RNA in the cytoplasm, he is of the opinion that the components of the acid-soluble pool are nevertheless going through the RNA-synthesizing machinery of the nucleus.

Apparently neither point of view can be firmly established on the evidence now available. The resolution of this problem will not be simple. Nevertheless, experiments are being designed that we hope will unequivocably settle the issue one way or the other.

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