THE ROLE OF THE NUCLEUS IN PROTEIN SYNTHESIS IN *AMOEBA**

by

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The hypothesis that the nucleus is the site of synthesis of proteins by the cell is an attractive one because it would provide an immediate association between the protein specificity and the genetic system, but it has found little experimental support. The results of cell-fractionation studies suggest that a large part of the protein-synthetic activity may reside in the RNA-containing "microsomes" or "basophilic network"[1,2]. An impressive experiment by BRACHET AND CHANTRENNE[3] showed that when the nucleus was removed from the algal cell, *Acetabularia*, protein synthesis as they measured it remained unaffected for several weeks.

Such a gradual decline, indicative of an indirect long-term action of the nucleus[4] was anticipated in the present experiments, which deal with the effects of enucleation on protein synthesis in *Amoeba proteus*. The original aim of the study was to correlate this with other changes that were known to be taking place, since more information on enucleation effects is available for this cell than for any other[5,6,7,8]. Unexpectedly, however, we found a quantitatively striking impairment of protein synthesis, measured as incorporation of methionine, immediately upon removal of the nucleus. The results suggest that in some kinds of cells, at least some of the protein-synthetic mechanisms are directly associated with the nucleus, and this proposition is the subject of the following discussion.

METHODS

These experiments were performed on the common uninucleated *Amoeba proteus*[9]. The amebae were cultured in an inorganic medium (differing somewhat from that used in our earlier investigations[10]) of the following composition: KCl, 6.0 mg; $MgSO_4$, 2.0 mg; $CaHPO_4$, 4.0 mg per liter of pyrex distilled water. They were fed by periodic addition of *Tetrahymena geleii* which had been grown on a proteose-peptone medium and washed in the above ameba medium before addition to the cultures. The *Tetrahymena* cannot grow in the ameba medium; they survive and serve as fodder for the amebae.

Groups of amebae were washed free of other organisms and left in the inorganic medium for one day before the beginning of the experiment. During this period, food contained in the amebae was digested and assimilated. Then some of the organisms were cut into equal halves with the aid of a fine glass needle leaving a sample of intact

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cells. By comparing nucleated and enucleated half cells, misinterpretations due to the effects of the cutting operation may be eliminated from consideration. The importance of employing intact amebae as an additional control will be evident in the later discussion.

Immediately after cutting the cells, the first groups of enucleated halves, nucleated halves and whole cells were transferred to inorganic medium to which ^{35}S labelled methionine had been added. All of the cells were incubated together in the same methionine solution; thus the external methionine concentration is excluded as a variable. The activity of the methionine solution was 1.5 μc per ml. The cells remained in the methionine solution for three hours, and were then washed free from radioactivity by transfers through inorganic medium. The whole cells, enucleated halves and nucleated halves were separated and each group (containing 50–75 individuals) was placed on a plastic planchet and dried.

In a similar manner, samples of all three classes were removed from the inorganic medium at 24 hours, 48 hours, and 72 hours after cutting and placed in the ^{35}S methionine. In all cases, the incubation in methionine was of three hours duration. Thus, we are comparing methionine incorporation into whole amebae, enucleated halves and nucleated halves immediately after cutting the cells and one, two and three days after cutting, maintaining all under the same starvation conditions for the same time intervals.

Temperature was kept at 21° C throughout the experiments.

The ^{35}S activity was measured with the aid of a windowless flow counter. After total ^{35}S activity was measured the amebae, still mounted on the planchets, were extracted with 5% trichloroacetic acid for five minutes at 17° C. This treatment was found by experiment to be adequate to remove all the TCA-soluble activity (Table I).

TABLE I

EXTRACTION OF METHIONINE-^{35}S-LABELLED AMEBAE WITH 5% TRICHLOROACETIC ACID AT 17° C

Time (min)	*Radioactivity (counts min cell)*	*Percent TCA-insoluble*
0	4.15	
0.5	3.50	84
1	3.50	84
2	3.55	86
5	3.50	87
10	3.50	84
15	3.45	83
25	3.30	80

RESULTS

In summarizing the results, we shall generally use the term "uptake" to refer to the total amount of ^{35}S recovered from cells that have been washed until no further radioactivity can be removed. The term "incorporation" will refer to ^{35}S that is associated with material that is insoluble in trichloroacetic acid (TCA). It is presumed that incorporation of methionine into the TCA-insoluble fraction of the cell signifies its incorporation into proteins, but we have no independent proof of this.

In discussing the data we shall not distinguish between net synthesis and turnover,

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since we would not expect any appreciable net synthesis of protein to take place under the starvation conditions we imposed[6]. "Synthesis" will imply only incorporation of amino acid into protein, regardless of what is happening to pre-existing protein. It may be added that the existence of protein turnover in *Amoeba proteus* has been proved by A. I. COHEN, in an investigation that will be published elsewhere. Needless to say, different results, quantitatively, may be expected under other nutritional conditions, but these measurements of formation of protein under conditions where all but one amino acid must be derived endogenously should serve as a useful indication of the intensity of the synthetic processes.

All of the data are summarized in Table II. This will show that the consistency of the results is such that *averages* will be instructive in the comparison of the three classes of cells, where the differences are large, but will not be as meaningful in evaluating time-trends within some of the classes.

TABLE II

DATA ON UPTAKE AND INCORPORATION OF METHIONINE-^{35}S BY WHOLE AMEBAE, NUCLEATED HALF-AMEBAE, AND ENUCLEATED HALF-AMEBAE

Each horizontal column gives the results of a single experiment, and the values for the three classes of cells may be compared.

Uptake and incorporation of methionine-^{35}S

Time (hours after cutting during which cells were incubated in methionine-^{35}S)	*Intact cells*			*Nucleated halves*			*Enucleated halves*		
	Amt. methionine (counts/min/cell)		*Percent incorporation*	*Amt. methionine (counts min/cell)*		*Percent incorporation*	*Amt. methionine (counts min cell)*		*Percent incorporation*
	Total	*TCA-insoluble*	*TCA-insol. / Total × 100*	*Total*	*TCA-insoluble*	*TCA-insol. / Total × 100*	*Total*	*TCA-insoluble*	*TCA-insol. / Total × 100*
0–3	3.50	2.20	63	1.80	1.30	72	0.52	0.05	10
	4.40	3.70	84	1.70	1.55	90	0.79	0.25	32
	1.59	1.19	75	0.99	0.70	70	0.51	0.14	27
	3.01	2.60	84	1.80	1.20	67	0.71	0.31	45
	1.67	1.09	65	1.00	0.81	81	0.47	0.16	37
24–27	1.20	0.96	80	0.57	0.42	74	0.30	0.11	37
	1.68	1.02	61	1.11	0.70	63	0.49	0.19	39
	3.23	2.79	86	1.89	1.41	75	0.45	0.14	36
	4.01	3.42	83	2.11	1.61	76	0.62	0.23	37
48–51	1.39	0.85	61	1.15	0.84	73	0.15	0.05	33
	4.16	3.50	84	1.73	1.20	69	0.18	0.07	39
	4.02	2.85	71	1.92	1.50	78	0.12	0.05	42
	3.20	2.79	87	2.08	1.61	77	0.21	0.07	33
	4.29	3.16	74	2.15	1.42	66	0.35	0.13	37
72–75	3.05	2.29	75	1.67	1.29	77	0.26	0.09	35
	4.00	2.77	69	1.97	1.51	77	0.21	0.05	24
	3.01	2.16	72	1.29	0.88	68	0.14	0.04	29

The averaged values are given in Table III. Table III also gives the ratios of activities in the various classes. Such ratios are often misleading because of arithmetical distortion of insignificant differences, but will be of some use in the later discussion.

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TABLE III

AVERAGED VALUES OF UPTAKE AND INCORPORATION OF METHIONINE-^{35}S AT VARIOUS TIMES AFTER CUTTING CELLS

(W = intact cells; N = Nucleated halves; E = Enucleated halves)

		Hours after cutting when incubated in methionine-^{35}S			
		0–3	24–27	48–51	72–75
Total amount taken up	W	2.83	2.53	3.41	3.35
	N	1.46	1.42	1.80	1.64
	E	0.60	0.47	0.20	0.20
	W/N	1.9	1.8	1.9	2.0
	N/E	2.4	3.0	9.0	8.2
Amount incorporated into TCA-insoluble material	W	2.16	2.05	2.63	2.41
	N	1.11	1.04	1.31	1.23
	E	0.18	0.17	0.07	0.06
	W/N	1.9	2.0	2.0	1.9
	N/E	6.1	6.1	18.7	20.0
Percent incorporated	W	76	81	77	72
	N	76	73	73	75
	E	30	36	35	30
	W/N	1.0	1.1	1.0	0.96
	N/E	2.5	2.0	2.1	2.5

The experimental findings themselves are simply summarized:

1. *Immediate effects of enucleation on total uptake.* Effects observed during the first three hours after cutting—the shortest interval over which it is practicable to carry out a test—will be referred to as "immediate". During this period, the total methionine uptake of the enucleated halves was lower than that of the nucleated halves by a factor of 2.4.

2. *Immediate effects of enucleation on incorporation.* During the first three hours, the *amount* of methionine ^{35}S incorporated into TCA-insoluble material by the enucleated half was only one sixth that incorporated by the nucleated half. Comparing uptake with incorporation, it is seen that the enucleated half incorporated only 30% of the methionine it had taken up, while the nucleated half incorporated 75% of the methionine it had taken up.

3. *Time-dependence of uptake.* During the three day period over which the tests were made, during which all of the cells were under starvation conditions, there was certainly no significant change in the rate of total methionine uptake by nucleated half cells or whole cells. The averaged figures in Table III suggest a *rise* in the rate of uptake at 48–51 hours and 72–75 hours, but we are uncertain as to whether it is significant.

The enucleated fragments showed a definite decline of total methionine uptake during the three day period.

4. *Time-dependence of incorporation.* The *percentage* of the methionine taken up that was incorporated did not change significantly with time in either the nucleated half (where it remained at 75) or the enucleated half (where it remained about 30). It is possible that the enucleated halves are showing a trend toward a decline in percentage incorporation after three days, but the data are not numerous enough to establish this. The decline in the *amount* of methionine incorporated by the enucleated half is almost

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entirely attributable to the decline in uptake. The latter was the only time-dependent process detected in this study.

5. *Comparison of nucleated half-cells with intact cells.* The nucleated half amebae, throughout the experiment, took up just half as much methionine in a given period as did the whole amebae and incorporated just half as much. The percent incorporated was identical in both groups and constant with time.

6. *Amount of TCA-soluble fraction.* The amount of TCA-soluble methionine ^{35}S— or its low molecular weight derivatives—is computed from the difference between the total activity in the cell and the activity remaining after TCA extraction. These figures are given in Table IV. It is evident that initial value for the TCA soluble fraction in the enucleated halves is not significantly different from the values observed in the nucleated halves. The amounts in the nucleated halves tend to be just 50% of those in the whole amebae.

TABLE IV

AVERAGED AMOUNTS OF TCA-SOLUBLE ACTIVITY

(in counts/minute/cell)

	Hours after cutting when incubated in methionine-^{35}S			
	0–3	24–27	48–51	72–75
Intact cells	0.67	0.48	0.78	0.94
Nucleated halves	0.35	0.38	0.49	0.41
Enucleated halves	0.42	0.30	0.13	0.14

INTERPRETATION OF RESULTS

The relationships between total uptake, total incorporation, and relative incorporation require some further analysis before the conclusions can be drawn concerning the role of the nucleus.

1. *Relation between uptake and incorporation.* The utilization of external methionine for the formation of protein must involve two stages: (1) the transport of methionine across the cell surface and possible conversion to TCA-soluble precursors and (2) synthesis of protein from amino acids available *inside* the cell. *The percent incorporation, as given in the tables, will be the best basis for comparing the synthetic capacities of two groups of cells, being a measure of their utilization of methionine that has entered.* The data show that enucleation reduces percent incorporation by a factor of about 2.5.

2. *Effect of enucleation on uptake. Time-dependence.* If initial uptake is limiting, then total incorporation—which has usually been measured in experiments of this type[5]—will not necessarily yield an accurate picture of synthetic capacity. This shows clearly in the present case. The total incorporation of methionine into protein declines rapidly with time in the enucleated cells suggesting a progressive deterioration in the synthetic mechanisms. But we see that the *percent incorporation* does not decline significantly in the same three day period. The decline in total incorporation may, therefore, be considered to reflect a reduced ability to supply methionine to the synthetic machinery, and not a depression of the machinery itself.

The time-dependence of uptake is readily interpreted in the same way as other time-

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dependent effects[4,7]. It may be assumed that it is governed by a process outside the nucleus that requires a contribution from the nucleus for its maintenance. In this case, the reasoning is obvious, since the activities of the cell surface will partly determine uptake. Since there is no evidence that the surface undergoes a physical change upon enucleation[8], it seems more likely that the decline in uptake reflects the decreasing activity of one or more enzymes that are involved in an active transport mechanism.

3. *Effect of incorporation on uptake.* We observe that the total uptake shows an apparent immediate decrease upon enucleation, by a factor of 2.4. Is this to be interpreted as a direct coupling of nuclear function with the uptake mechanism? We would, if the nucleus were controlling the uptake mechanism directly, expect to see this reflected in the TCA-soluble fraction, but such is not the case. Table IV shows the soluble fraction to be about the same in the nucleated and enucleated halves during the first three hours. The immediate (as opposed to the time-dependent) effect of enucleation on total uptake is reflected only in the amount of methionine *incorporated*. This is shown in another way, by the fact that the N/E ratio for percent incorporation (2.5) is virtually identical with that for total uptake (2.4). From this it is permissible to conclude that *incorporation governs uptake.*

This result is not difficult to explain if we assume that the entrance of methionine against a concentration gradient approaches a limit determined by the internal concentration of the "free" form. Therefore, continued entrance will be governed by the rate at which the "free" form is removed by incorporation into protein.

If incorporation governs penetration, it is not necessary to postulate any immediate effect of enucleation on penetration. Moreover, the six-fold difference in the total amount of methionine incorporated by the nucleated and enucleated halves would be a spurious indicator of an effect of enucleation on synthesis (where there is a 2.5-fold effect).

4. *Nucleated halves* vs. *intact cells.* The finding that the total incorporation by intact cells is twice that by nucleated halves is one that would be predicted from a *cytoplasmic* localization of the incorporation mechanism. To explain these results in such a way as to be compatible with nuclear localization of a considerable part of the synthetic mechanism requires an additional assumption. One possible assumption is that the synthetic mechanism is sensitive to *concentration* of precursor. In this case, the amount that could be incorporated over an appreciable time would depend on the *amount* of precursor replenishing the reservoir, represented by the TCA-soluble fraction. We have given reasons for assuming that there is an upper limit to the concentration of "free" methionine. Applying such a model, the *volume* of the cell would determine the *amount incorporated*, and the *percent incorporation* would not vary significantly.

Perhaps a more reasonable assumption is that methionine does not enter the cell in the form of a suitable immediate precursor for synthesis. If a cytoplasmic system were required to convert it to a precursor, then the total activity of a nucleus-centered synthesis would be limited by a cytoplasmic system, and hence be proportional to the amount of cytoplasm.

The introduction of assumptions such as these is justified only if all of the other evidence except the comparison of nucleated halves and whole cells requires the conclusion that the nucleus participates directly in protein synthesis. This we shall now examine.

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DISCUSSION

Enucleation experiments can yield results of four kinds: (1) no differences may be observed that can be related to the presence or absence of the nucleus, (2) there will be an immediate effect of the absence of the nucleus, (3) there will be no immediate effect but one that appears in time, or (4) there will be an immediate effect which is intensified with time. In attempting to assess the actual localization of processes in the cell nucleus, we are particularly interested in *immediate* effects, for an immediate effect of enucleation means either that the process under study is itself situated in the nucleus or can proceed only in conjunction with a process located there.

In the present study, an immediate effect of enucleation on the incorporation of methionine into protein has been found. During the first three hours after cutting the ameba in half, the enucleated half incorporates only 30% of the methionine that it has taken up from the medium; the nucleated half 75%.

It might seem unjustified to consider an effect observed during the first three hours to be immediate, but we see in this case that the percent incorporation does not change significantly during three days after cutting. Whether the absence of the nucleus would assert itself later in a more conspicuous decline in incorporation as the enucleated ameba degenerates remains to be tested.

Such evidence of an immediate, relatively time-independent effect of enucleation in reducing the capacity of a cell to incorporate an amino acid into proteins, makes it difficult to reject the conclusion that protein synthesis in the nucleus itself accounts for the results. We have already seen, in a detailed discussion, that the two-fold difference in total incorporation between the whole ameba and the nucleated half can be accounted for. If the nucleus is not a center of protein synthesis, the only alternative is that a part of the protein synthesis in the cytoplasm would be directly coupled with some process centered in the nucleus, while another part, as we shall see, would not.

Protein synthesis in the cytoplasm. The capacity of the enucleated half ameba to incorporate the methionine that it has taken up is not negligible. This leaves no doubt that the cytoplasm of *Amoeba* contains protein-synthetic systems. What is more surprising is that the capacity of enucleated cytoplasm to incorporate into protein methionine that has entered the cell does not change conspicuously over a period of three days.

The evidence is accounted for best by the hypothesis—one that already has much support—that the cytoplasm is capable of protein synthesis, and further that nucleus-associated synthesis and cytoplasmic synthesis are independent. The latter proposition must be qualified by the likelihood—to be discussed later—that the synthetic machinery in the cytoplasm is ultimately, on a long term basis, dependent on nuclear activity.

The short-term independence of cytoplasmic and nuclear protein synthesis may apply only to the basal conditions of this experiment. For reasons that will be treated in the concluding section of this paper, a nucleus-dependence of cytoplasmic synthesis might be very evident during growth or in any situation that called for an increase of synthetic *capacity*.

The N/E ratio of 2.5 for percent incorporation in nucleated and enucleated half-amebae may be superficially misleading as to the relative contributions of nucleus and cytoplasm in the ameba. A simple calculation shows that it implies that the nucleus is contributing about 40% of the methionine incorporation in the whole cell under the basal conditions of the experiment.

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The *amount* of methionine incorporated into the enucleated half, in contrast with percent incorporated, does decline with time, and we have, earlier in this paper, given reasons to conclude that this is attributable to a declining rate of entrance into the cell. This distinction is worthy of emphasis from the standpoint of experimental design. If, as we have done in other cases in the past, we had measured only total uptake or total incorporation, we would have come out with the conclusions that the incorporation mechanism declined with time and that there was an immediate effect on uptake as such. The former conclusion is definitely not true over the three day period. The latter may possibly be true, but as we have shown, it is more likely that the apparent immediate effect on uptake is merely a reflection of the established effect on incorporation.

In view of the supposed association between RNA and protein formation, it is interesting to note that, while the RNA level in the enucleated ameba is declining steadily,[11,12] it is declining no more rapidly than is the *amount* of methionine incorporated in a given time.

Comparison with data on other cells. The earlier work on the localization of protein synthesis has been discussed[1] chiefly in terms of the appealing generalization that the cell's proteins were of nuclear origin. The generalization has been rejected because of two lines of evidence. (1) Tracer experiments show that when labelled precursors are fed to a cell, the nucleus is not necessarily the site of the highest specific activity in the protein fraction[13,14]. Moreover, protein synthesis has been obtained in nucleus-free homogenates[1,15]. (2) In *Acetabularia*, a type of cell very convenient for such studies, enucleated fragments were found to incorporate CO_2 into proteins as effectively as nucleated fragments[3]. The present work on *Amoeba* certainly adds to the evidence that the nucleus is not an exclusive center of protein synthesis in the cell.

It is now necessary, however, to put the question somewhat differently. Is the nucleus a center of protein synthesis at all? What contribution does it make, quantitatively and qualitatively, to the cell's proteins? If we review the tracer studies already referred to, especially those in which the tracer was fed to the intact cell, and the cell fractionated later, they inform us that the nucleus *could* be a significant center of protein synthesis. Such experiments are designed to trace possible sources of protein. It is reasoned that a site where the protein has a lower specific activity cannot be the source of protein at a site where specific activity is higher. It is not concluded that the less active protein necessarily derives from the site where the activity is higher. The likelihood of independent origin is always present. In the case of *Amoeba*, we now have good reason to believe that nucleus-associated and cytoplasmic protein synthesis are independent. If we examine data of some of the tracer experiments, we find that the activity recovered with the nucleus is never negligible and in some cases the specific activity in the nuclear fraction is high as[14] and in one case higher[15] than that in the cytoplasm.

It is of interest that both of the studies just cited concerned themselves with the effects of growth variables, and show that the activities in the nucleus and the cytoplasm vary independently from each other. In short, a review of the tracer data suggests that protein synthesis by the nucleus need not be insignificant.

The case where the contribution of the nucleus to protein synthesis has appeared to be undetectable is that of *Acetabularia*. No difference was found between the capacity of nucleated and enucleated fragments to incorporate CO_2 into protein carboxyl groups, under illumination, until a long time after enucleation. Assuming that the same relationship holds for the incorporation of preformed amino acids[16], the results are in seeming contradiction to those we have obtained with *Amoeba*.

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Enucleation experiments, along with certain limitations, are free from certain artifacts that are always a possibility in cell-fractionation studies. The case of *Acetabularia*, already cited, has provided the strongest reason for thinking that protein synthesis by the nucleus is quantitatively insignificant. The present data on *Amoeba* contradict this. While the easiest escape from the contradiction is in platitudes regarding species differences, the fact is that we are interested in neither *Acetabularia* nor *Amoeba* for its own sake, but in the possibility of extending the conclusions to other kinds of cells. It is obvious in every investigation that the material under study is to some extent a "special case". In this instance, the differences between the two forms is worthy of closer examination. Unfortunately, no other kinds of cells have been used at all intensively for studies of the physiological effects of enucleation.

In the first place, the amebae were studied under conditions where no growth was taking place, while the *Acetabularia* were kept in the light and could grow even without a nucleus. Since growth would imply increase in cytoplasmic protein, the *relative* contribution of the nucleus might be less conspicuous. This is shown even in the data on liver[14] where the apparent intensity of protein synthesis in nucleus and cytoplasm is equal, in the non-growing state, but where growth leads to a pronounced increase in specific activity in the cytoplasm without affecting the nuclear values. In other words, evidence of nuclear activity may be swamped out by the growing *Acetabularia*.

There are other inconsistencies between data on nuclear function in *Acetabularia* and in *Amoeba*, especially if we consider the effects of illumination on the former. In *Amoeba*, enucleation leads to an immediate drop in P32 uptake. In *Acetabularia*, according to HÄMMERLING[17], there is no effect of enucleation on P32 uptake in the light, and the effect observed in darkness is reversed by light. In *Amoeba*, LINET AND BRACHET[11] and also JAMES[12] have found that enucleation renders the cell incapable of maintaining an RNA level equal to that of the nucleated half. Recently, BRACHET AND SZAFARZ[18] have reported that there is no detectable effect of enucleation on RNA turnover in *Acetabularia*. Whether the differences are intrinsic differences in the localization of function or consequences of different metabolic conditions under which enucleation studies are made in the two cases, the differences between the two are consistent. In every instance, *Acetabularia* seems to be relatively less sensitive to the effects of removing the nucleus.

No immediate effects of enucleation have been reported, while in *Amoeba* at least two metabolic effects—on P32 uptake and methionine incorporation—have been found, as well as a number of less easily measured early effects on locomotion, food capture, *etc.*[19]. Our inability to provide adequate nourishment to the enucleated cell renders *Amoeba* much less suitable for long-term studies than *Acetabularia*, but in short-term studies basal metabolic conditions may make nuclear contributions more conspicuous.

Obviously, *the question of nutritional capacities of nucleated and enucleated cells will not be decisive when we are studying immediate effects of enucleation, as is the case in the present study.*

The physiological capabilities of the enucleated *Acetabularia* are so remarkable as to raise the question whether they can be accounted for solely by the nutritional independence that goes with photosynthesis. Another possibility, first suggested to us by Dr. JACQUES MONOD, may deserve consideration. This is that the plastids may have acquired a number of functions ordinarily restricted to the nucleus just as they may possess a high degree of genetic autonomy[20]. If a cell contains semi-autonomous cyto-

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plasmic units that perform syntheses that otherwise would be carried out by the nuclear genetic apparatus, the physiological effects of enucleation will be more difficult to detect.

Amoeba proteus, however, also has some specialized properties which might influence the results of enucleation experiments. The nucleus is not only large, but very rich in RNA and protein. In fact (by staining criteria) the DNA is so diluted by these other constituents that Feulgen staining is faint and unsatisfactory. It is not inconceivable that the nucleus of ameba (and of other protozoa with large nuclei high in RNA and protein) represent cases where the nucleus contains within its boundaries active centers that in other cells are located in the cytoplasm.

CONCLUSIONS

We interpret the present experiments to mean that the nucleus is directly involved in the synthesis of part of the cell's proteins, while the cytoplasm possesses independent capabilities for protein synthesis. The results of tracer experiments on animal tissues are consistent with this conclusion. There is some evidence[21, 21a] to the effect that isolated nuclei may retain some capacity for incorporating amino acids into proteins. The valuable autoradiographic study of FICQ[22] demonstrates that in the starfish oocyte the nucleus and especially the nucleolus is the most active center for incorporating glycine into proteins. The question of the *existence* of nucleus-associated protein synthesis is at the moment as important as the question of how much of the protein made by the cell in a given situation originates in the nuclear mechanism. The quantitative contribution will be expected to vary, if we consider it in the light of MIRSKY's[23] hypothesis that quantitative physiological differences between nuclei may serve as a basis for differentaiation. Moreover, as has been stated, we shall expect that the quantitative contribution of the cytoplasm will depend on the growth status of the cell.

How shall we relate these findings to the general theory of nuclear function? What meaning can be attached to the existence of two sources of proteins, nuclear and cytoplasmic? One obvious possibility is that the nucleus is synthesizing those proteins which remain in the nucleus. Another is that it is forming some proteins which enter the cytoplasm but differ in function from those originating in the cytoplasm. There is powerful evidence that the "working proteins" of the cytoplasm are formed through the activity of *ribonucleoprotein* particles characterized as microsomes or parts of a basophilic network[24]. Where does this ribonucleoprotein—the protein-synthesizing protein—originate? It is now a widely-held opinion[25] that RNA of nuclear origin is involved in the transmission of genetic information from the chromosomes to the cytoplasm. But we do not encounter it biochemically as free RNA, but as ribonucleoprotein. The function of the protein moiety is unknown; indeed, the actual function of the RNA is only surmised. If the cytoplasmic RNA of nuclear origin is associated with protein synthesis, it is by no means improbable that it comes from the nucleus as ribonucleoprotein. In short we may propose that the nucleus-associated synthesis of protein is a reflection of the synthesis there of ribonucleoproteins, which in turn serve in the cytoplasm as the centers of formation of the normal working proteins of the cell.

This hypothesis is subject to experimental test. If it is true, there should be a parallelism between the effects of enucleation on the synthesis of protein and of RNA.

In the present study, the "protein synthesis" that we have dealt with is an endo-

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genous turnover process involving re-utilization of amino acids present in the cell. Unless there is some other explanation of the incorporation of methionine-^{35}S into TCA insoluble material, it is a measure of the activity of the protein synthetic mechanism under these conditions. It is of interest to predict, on the basis of our conclusions, what would happen under other conditions. It was first shown by CASPERSSON AND SCHULTZ[26] that when a cell that had been under basal conditions was stimulated to growth, there was an increase of ribonucleoprotein in the cytoplasm. An experimental test might show that much of the new ribonucleoprotein originated in the nucleus. Once an appropriate level of cytoplasmic ribonucleoprotein had been reached and the appropriate *species* of ribonucleoprotein were present this could continue to function in the synthesis of working proteins. There is evidence that protein synthesis, while associated with the *presence* of RNA, need not be continuously associated with the *synthesis* of RNA[24,27]. But there is at least one case where the formation of a protein seems to require the concomitant synthesis of RNA: the case of enzyme induction[28]. The apparent paradox can be accounted for. If the situation calls for the establishment of *new* protein-forming centers in the cytoplasm, it will call for the synthesis of new specific ribonucleoprotein either by the nucleus or by semi-autonomous cytoplasmic units which share some of the properties of the nucleus.

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SUMMARY

The presence or absence of the nucleus influences the incorporation of ^{35}S labelled methionine into the proteins of *Amoeba proteus* kept under basal conditions. The percent incorporation (relation between total amount of methionine taken up and the amount incorporated into TCA-insoluble material in a given time) is lower by a factor of 2.5 in the enucleated half immediately after the cell has been cut into two. The percent incorporation does not change in either the nucleated half or the enucleated half for three days after cutting. The *amount* of methionine that the enucleated half can incorporate in a given time-interval does decline with time, but this is shown to be attributable to the *uptake* mechanism and not to the incorporation mechanism. All of the evidence is consistent with the hypothesis that the nucleus is either the seat of a considerable proportion of the protein synthesis in the amebae under the conditions employed or that this nucleus-linked synthesis is very closely coupled with processes that are localized in the nucleus. On a short-term basis, synthesis of protein in the cytoplasm and the nucleus-linked synthesis seem to be independent of each other.

RÉSUMÉ

La présence ou l'absence de noyau influence l'incorporation de la méthionine marquée par ^{35}S dans les protéines d'*Amoeba proteus* maintenue dans des conditions standards. Le pourcentage d'incorporation (rapport entre la quantité totale de méthionine absorbée et la quantité incorporée dans les produits insolubles dans l'acide trichloracétique, dans un temps donné) est 2.5 fois plus faible dans la moitié anuclée immédiatement après sectionnement en deux de la cellule. Le pourcentage d'incorporation dans les deux moitiés ne change pas pendant les trois jours qui suivent la section. En fait la *quantité* de méthionine que la moitié anucléée peut incorporer dans un temps donné diminue avec le temps, mais ceci peut être attribué au mécanisme de l'*absorption* et non au mécanisme de l'incorporation. Toutes les observations sont en accord avec l'hypothèse selon laquelle le noyau est le siège soit d'une proportion considérable de la synthèse des protéines chez les amibes dans les conditions employées, soit de processus qui sont étroitement liés avec cette synthèse. Pendant des temps courts, la synthèse des protéines dans le cytoplasme et la synthèse liée au noyau semblent être indépendantes l'une de l'autre.

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ZUSAMMENFASSUNG

Die Gegenwart oder Abwesenheit des Zellkernes beeinflusst die Einverleibung von mit ^{35}S markiertem Methionin in die Proteine von unter Grundumsatzbedingungen stehenden Zellen der *Amoeba proteus* merklich. Die prozentuale Einverleibung (d.h. das Verhältnis zwischen der in einer gewissen Zeitspanne aufgenommenen Methioningesamtmenge und der Methioninmenge, die im selben Zeitabschnitt in durch Trichloressigsäure gefälltes Material einverleibt wurde), ist in der kernlosen Zellhälfte sofort nach Zerschneiden der Zelle 2.5 mal schwächer, als in der anderen Zellhälfte. Die prozentuale Einverleibung untergeht in beiden Zellhälften auch drei Tage nach Zerschneiden der Zelle keiner merklichen Änderung. Die *Gesamtmenge* des in die kernlose Zellhälfte in einem gewissen Zeitabschnitt einverleibten Methionins fällt mit der Zeit ab, doch wurde bewiesen dass dies dem Aufnahmemechanismus und nicht dem Einverleibungsmechanismus zuzuschreiben ist. Diese Tatsachen stimmen mit der Hypothese, wonach entweder der Zellkern selbst unter den gegebenen Bedingungen der Sitz eines Grossteils der Proteinsynthese in der Amöbe ist, oder die vom Zellkern abhängige Proteinsynthese mit Vorgängen im Zellkern engstens verknüpft ist, völlig überein. Doch sind, unter kurzfristigen Bedingungen die cytoplasmische Proteinsynthese und die an den Zellkern gebundene Proteinsynthese voneinander unabhängig.

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