PROTEINS IN NUCLEOCYTOPLASMIC INTERACTIONS

II. Turnover and Changes in Nuclear Protein Distribution with Time and Growth

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ABSTRACT

In previous studies, we showed that essentially all the proteins of the *Amoeba proteus* nucleus could be classified either as Rapidly Migrating Proteins (RMP), which shuttle between nucleus and cytoplasm continuously at a relatively rapid rate during interphase, or as Slow Turnover Proteins (STP), which seem to move hardly at all during interphase. In this paper, we report on the kinetics and direction of the movement of *both* classes of protein, as well as on aspects of their localization, with and without growth. The effects of growth were observed with and without cell division. These nuclear proteins have been studied in several ways: by transplantation of labeled nuclei into unlabeled cells and noting the rate of distribution to cytoplasm and host cell nuclei; by repeated amputation of cytoplasm from labeled cells—with and without initially labeled cytoplasm—each amputation being followed by refeeding on unlabeled food; by noting the redistribution of the various protein classes following growth and cell division. The data show (*a*) labeled RMP equilibrate between a grafted labeled nucleus and an unlabeled host nucleus in ca. 3 hr, but are detectable in the latter less than 30 min after the operation; (*b*) STP label does, indeed, leave the nucleus and does so at a rate of ca. 25% of the nuclear total per cell generation (ca. 36–40 hr at 23°C); (*c*) the cytoplasm appears to have a reserve of material that is converted to RMP; (*d*) when labeled cells are amputated just before they would have divided and are refed unlabeled food after each amputation, there is a loss of 20–25% of the nuclear protein label with each amputation; (*e*) under the latter circumstances, an essentially complete turnover of all nuclear protein can be demonstrated.

All the nuclear proteins of *Amoeba proteus* can be classified into two major groups (6): (*a*) rapidly migrating proteins (RMP), which are in constant movement back and forth between nucleus and cytoplasm but with no apparent *net* shift in amount in either direction during interphase; and (*b*) slow turnover proteins (STP), which either break down slowly by some metabolic activity in the nucleus (with an accompanying loss from the nucleus of the products), move at a relatively slow rate out of the nucleus as intact protein molecules, or go through both kinds of nuclear "turnover."

The roles of RMP and STP in the life of the cell remain largely unknown, although the completely unpredicted shuttling behavior of RMP that led to their discovery (3) suggests several interesting possible roles in genetic control mechanisms. In a search for additional clues to the functions of the nuclear proteins, the long-term

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behavior of these proteins under varying growth conditions was examined with special emphasis on metabolic stability and cellular localization. Preliminary studies had suggested that these proteins do not behave predictably in these respects.

The experiments described here provide a sufficient understanding of the behavior of STP to permit some elementary speculation on their function in the cell. Our data clearly show that, under appropriate conditions, STP are lost from the nucleus and that STP probably move as intact protein from cytoplasm to nucleus during all of interphase. (The actual pattern of these movements, however, requires additional experimental analysis that will be discussed later.) Furthermore, the rate at which STP leave the nucleus under different conditions implies that there are at least two subclasses in this group. Related data also hint that there is a conversion of some cytoplasmic material to RMP as part of a continuous cell growth cycle. The data confirm that RMP shuttle between nucleus and cytoplasm in a fashion predicted from earlier experiments, and they give some idea of the kinetics of the shuttling process.

Finally, it is established that *no* nuclear proteins have the metabolic stability and permanent nuclear localization that are characteristic of DNA.

MATERIALS AND METHODS

Description of the *Amoeba proteus* strain used in these experiments, the ameba culture methods, the amino acid-^{3}H labeling procedures, nuclear isolation technique, method of transplantation of nuclei between cells, and radioactivity-counting procedures can be found in an earlier paper (6).

All amputations into nucleate and enucleate fragments were performed as described by Prescott and Carrier (9).

Radioautography with NTB-3 was performed as described by Prescott (7).

RESULTS

Effect of Starvation on the Protein Content of the Nucleus

To obtain a first approximation of the metabolic stability and/or the permanence of nuclear protein localization, we studied the effect of starvation on the retention of radioactive proteins by the nucleus. We expected to obtain from this a basis for evaluating the effect of other conditons on the protein content of nuclei.

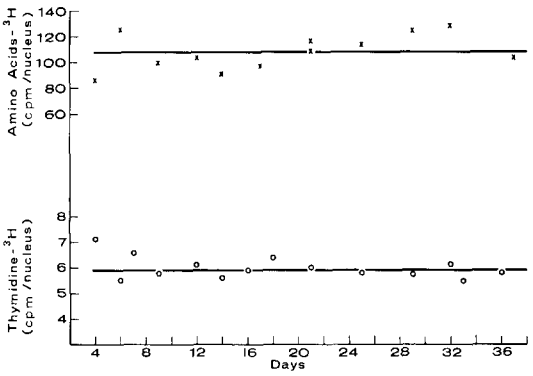

FIGURE 1 Data showing the change in amount of radioactivity (amino acid-^{3}H or thymidine-^{3}H) with time during starvation. Upper curve shows pattern of change of protein-^{3}H, and lower curve shows pattern of change of DNA-^{3}H. Between 25 and 50 nuclei were isolated, pooled, and assayed for each point. Abscissa indicates number of days cells were without food. DNA labeling was achieved by feeding amebae with *Tetrahymena* that had grown in a medium containing 10 μc thymidine-^{3}H/ml. Curves drawn by eye.

Fig. 1 shows the pattern of change in the protein-^{3}H content of nuclei (following isolation) as a function of the number of days without food. (Included in Fig. 1 as a "standard" of measurement are similar data for the retention of DNA-^{3}H by nuclei undergoing parallel starvation.) Measurements are not given prior to 4 days because cell divisions were still observed in the populations during the first 3 days without food.

Despite some scatter in the data, we conclude that in the absence of growth there is no substantial loss (by metabolic breakdown or *net* movement to the cytoplasm) of protein from the ameba nucleus. However, the possibility that there is reutilization of products from protein breakdown cannot be excluded.

Change in the Distribution of RMP and STP with Time

RATE OF EQUILIBRATION BETWEEN NUCLEUS AND CYTOPLASM OF LABELED RMP: The rate at which labeled RMP equilibrate as a result of their shuttling activity between a transplanted protein-^{3}H nucleus and the nucleus and cytoplasm of an unlabeled recipient cell was found by determining the ratio of radioactivity between

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the nuclei at intervals following such a transplantation. Fig. 2 shows that the ratio of radioactivity between grafted and nongrafted nuclei falls rapidly and almost levels off at ca. 3 hr after the operation. This time compares with a reported time of 4–5 hr based on analyses of radioautographs (1). Since the values shown are for the distribution of added radioactive proteins that are moving back and forth along with unlabeled proteins already present, and since the time is the length of time required to reach *equilibrium*, under normal circumstances an RMP molecule probably goes from nucleus to cytoplasm and back into the nucleus in much less than 2 hr, on the average.

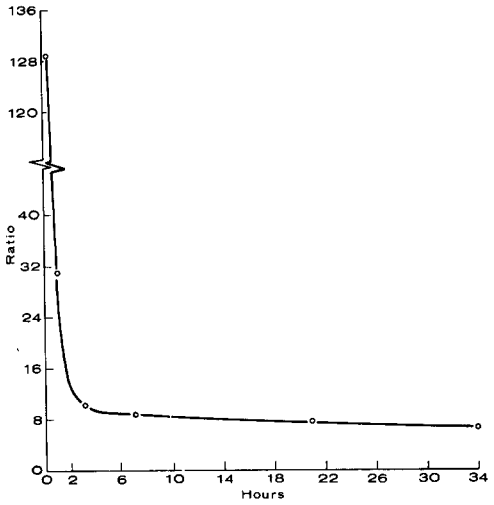

FIGURE 2 Change in ratio (the number of ordinate to 1) of protein-^{3}H radioactivity between grafted nucleus and host cell nucleus with time during the 1st several hours after the transplantation of labeled nuclei into unlabeled nucleate hosts. Each point represents the mean of the ratios determined for each of 10–20 pairs of nuclei. The ordinate represents the number of times more radioactivity that is present in the donor than in the host nuclei, and the abscissa shows the time after nuclear transplantation.

RATE OF LOSS OF STP LABEL FROM NUCLEUS: Close examination of Fig. 2 reveals that the ratio of activity between the two nuclei does not, in fact, level off at 3 hr after the implantation of the labeled nucleus; there is, however, a dramatic alteration in the rate at which the ratio changes. This is seen more clearly in Fig. 3, which is a plot of data from an experiment similar to the one described above, but extended for a longer time period. The figure shows that the ratio of activity between the nuclei continues to fall for at least 102 hr, and presumably longer.

Examination of the data we used to compute the ratios plotted in Fig. 3 reveals that the slow rate of ratio change after the first 3 hr probably is due largely to a loss of STP label from the grafted nucleus. In at least one experiment, however, the amount of radioactivity in the recipient cell nuclei appeared to be increasing slowly, suggesting (as one possibility) that STP were moving from cytoplasm to nucleus also—but, of course, this would be at a rate much slower than that for RMP movement from cytoplasm to nucleus. Although more work needs to be done to establish that such movement is truly occurring under these conditions, other experiments to be described below support that idea.

Three experiments dealing with the change in ratio with time were carried out, each under starvation conditions. However, for one point in each of two experiments, some of the cells were

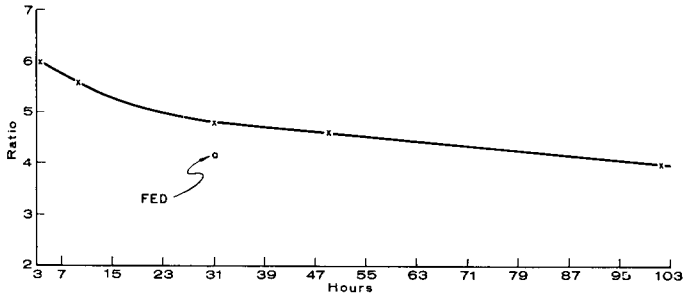

FIGURE 3 Similar to experiment described in Fig. 2, but extending over a period in excess of 4 days. Each point represents the mean of the ratios determined for each of 10–15 pairs of nuclei. All nuclei were starved from 0 hr, except those marked *FED*; these were fed unlabeled *Tetrahymena* continuously following the transplantation operations.

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TABLE I

The Effect of Growth and Cell Division on Nuclear Protein-^3H Distribution.

1 Division*	2 No. of cells	3 $\bar{X}$ Ratio‡	4 $\bar{X}$ Cpm in RMP§	5 $\bar{X}$ Cpm in STP‖	6 $\bar{X}$ Cpm in Enucleate donor
Exp. 16/5					
1st	19	6.4:1	18	98	9,300
2nd	14	4.4:1	12 (67)¶	35 (36)¶	3,950 (42)¶
3rd	11	3.6:1	6.5 (36)	15 (15)	1,650 (18)
Exp. 7/7					
1st	48	6.4:1	24.0	120.4	14,080
2nd	39	5.9:1	10.7 (45)¶	50.2 (42)¶	6,420 (46)¶
3rd	28	4.4:1	7.8 (32)	23.7 (20)	3,130 (22)
4th	30	3.6:1	4.9 (20)	12.2 (10)	1,570 (11)

* The division after cells were placed on unlabeled food. Transplantation of one daughter cell nucleus into unlabeled, nucleated host cell performed after indicated division.

‡ The ratio of radioactivity in the more radioactive nucleus of a pair (from host cell described above) to the amount of radioactivity in the less radioactive nucleus of the pair.

§ The amount of radioactivity in the less radioactive nucleus of the pair described above. It is assumed that the other nucleus has an equal amount of radioactivity in RMP (6).

‖ Amount found only in more radioactive nucleus as estimated from the radioactivity difference between the two nuclei of the pair as described above.

¶ The figures in parenthesis show % of the values given in the 1st Division row.

fed continuously from the time of the nuclear transplantations. Both cases indicate that feeding causes the ratio to fall more rapidly than it does under fasting conditions (cf. Fig. 3). It appears that, under these conditions also, the fall in ratio is primarily due to the loss (at an increased rate) of STP label from the grafted nucleus.

The Effect of Growth and Cell Division on the Distribution of Labeled Nuclear Protein

What happens to the distribution of labeled nuclear proteins as the cell grows on *unlabeled* nutrients and proceeds through cellular reproduction? If the nuclear proteins behave like what are often considered to be "typical" protein molecules, the number of labeled molecules per cell compartment should be approximately halved with each division on nonradioactive food. Furthermore, if the "typical" situation obtains for the ameba nuclear proteins, the radioactivity of each of the two major nuclear protein classes would be expected to be diluted in the same fashion. The data given below show not only that neither of the two classes behaves typically but also that the two classes behave differently from one another.

The general pattern of the results for the four experiments of this kind was similar, but only the data of the two most comprehensive and clearest experiments will be considered (Table I). The following procedure was used. After amebae had grown for two or three generations on amino acid-^3H-labeled *Tetrahymena*, they were removed to a nonnutrient medium for several hours so that extensive digestion of the labeled contents of the ameba food vacuoles could take place. Many dividing amebae were selected from this population and placed in a fresh medium containing unlabeled *Tetrahymena*. Approximately 36 hr later, dividing amebae were selected from this subculture. One sister of each pair was again fed on unlabeled food and permitted to grow while the other sister was used as a donor of a nucleus grafted into an unlabeled cell, for obtaining the "1st Division" data of Table I. At each subsequent division, the same plan was followed: one sister was fed unlabeled food for further growth and the other sister was used as a nuclear donor, for obtaining data on labeled nuclear protein distribution. The Table I data, which are somewhat complex, have been simplified by furnishing mean counts per minute for RMP and STP as a percentage (in parenthesis) of the 1st Division value.

For all divisions in those experiments, except the 2nd Division of Exp. 7/7, the amount of label in RMP is reduced by *less* than half with each

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division. For STP, there is perhaps a reduction by *more* than half between the pre- and post-2nd Division cells, and, thereafter, there is an approximate halving of the amount of label at each division. (In view of these data, we should like to know what happened as a consequence of the 1st Division, but the information is not accessible at present.) The ratio of radioactivity between the grafted and host nuclei declines after each division, reflecting the changes in both labeled nuclear RMP and STP. Information about events after further divisions would be useful, but insufficient radioactivity remains in the nuclei to permit accurate analyses after four divisions.

The Distribution of Nuclear Protein Label as a Consequence of Growth without Cell Division

By the device of appropriately timed cytoplasmic amputation (8), one can allow a cell to grow apparently normally while simultaneously preventing any cell or nuclear divisions. The distribution of nuclear protein under conditions of continuous growth without the interference of activities associated with mitosis and cell division was thus studied in five separate experiments. The results of only two experiments will be described since there were no important differences from one experiment to another; a few minor differences will be noted.

In order that we might start with all of the cell's initial radioactivity in the proteins of the nucleus (as a means of simplifying the analysis of the results), a nucleus from an ameba well labeled with amino acids-^{3}H (that had been "chased" by feeding unlabeled *Tetrahymena* for at least 15 hr) was grafted into an unlabeled enucleate cell. Between 100 and 150 such cells were prepared at the start of each experiment. (As shown in Fig. 2, the distribution between nucleus and cytoplasm of labeled RMP reaches equilibrium approximately 3 hr after implantation of the labeled nucleus. At equilibrium, ca. 50% of the cell's RMP label is in the cytoplasm (6).) The following day, approximately 50% of the cytoplasm was amputated from all cells, and all enucleate fragments were saved for radioactivity assay. The nucleate fragments were fed unlabeled *Tetrahymena* until the amebae attained a size approximately double that of a freshly amputated cell, at which time another approximately 50% of the cell volume was again cut away. This sequence was repeated until the

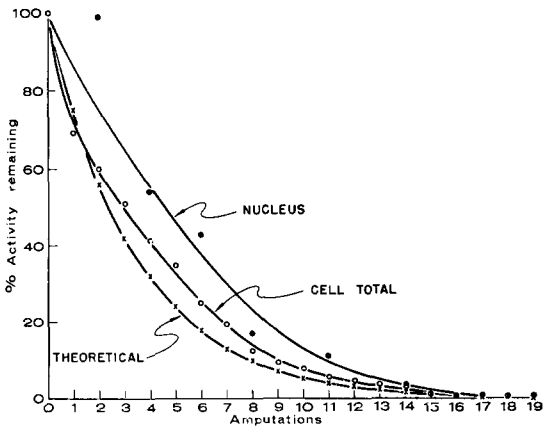

FIGURE 4 Experiment showing the pattern of loss of proteins-^{3}H from nucleus and from whole cell during a series of repeated cytoplasmic amputations in which approximately half the cytoplasm was cut every time the cell volume was estimated to have doubled as a consequence of growth on unlabeled food. At the start of the experiment, we prepared approximately 150 cells by grafting a protein-^{3}H nucleus into an unlabeled enucleate cell. Each point for the *Nucleus* curve is based on the assay of approximately 15 nuclei; each point for the *Cell Total* curve is based on extrapolations from assays of the cytoplasmic fragments cut away or from assays of removed cytoplasmic fragments plus isolated nuclei when available. The *Theoretical* curve is the expected pattern if the amount of labeled RMP is distributed equally between nucleus and cytoplasm essentially at all times and if 25% of the remaining labeled nuclear STP leaves the nucleus with each doubling in cell mass. This would result in a 20–25% loss of protein-^{3}H from the cell with each amputation—after the first one or two amputations.

radioactivity remaining in the cells was too low to be measured with reasonable accuracy.

Fig. 4 shows the rate of decline in protein-^{3}H content as a function of the number of amputations. This figure illustrates the decline for the nucleus, the decline for the whole cell (as calculated from the data of the enucleate fragments amputated at each point and of the isolated nuclei where available), and a theoretical decline. The latter is based on the conclusion that there are equal amounts of RMP in nucleus and cytoplasm (6), that the equilibrium distribution is achieved rapidly, that ca. 25% of STP label leaves the nucleus per doubling in cell volume (see below), and that approximately 50% of the labeled material in the cytoplasm is removed with each amputation. This leads (as depicted in Fig. 4) to

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a prediction that from 20 to 25% of the total cell label is lost with each amputation after the first.

The data show that the actual rate of decline is close to the theoretical, thereby providing confidence in our assumptions. (Any deviation from theoretical could reflect a number of imprecisely determined variables, such as some uncertainty in our estimate that the amount of cytoplasm amputated was 50% of the total, but the precision, in general, seems sufficient to justify considering the correspondence between theoretical and observed as more than coincidence.) The data also show that essentially all the label is eventually lost. Thus, the protein label declines from a mean of 154 cpm/nucleus[1] to less than 0.5 cpm/nucleus (which is at the limit of the assay method) and from 128 cpm/cytoplasm to less than 0.5 cpm/cytoplasm. This means that no more than 0.3% of the nuclear protein can be metabolically stable and permanently localized in the nucleus. More recent experiments extended for more amputations and employing more sensitive quantitative methods[2] show that any protein stably localized in the nucleus would have to be less than 0.3% by *at least* an order of magnitude. It thus seems reasonable to expect that *no* nuclear protein is irreversibly associated with the nucleus in the way that chromosomal DNA is.

In a related experiment, we compared the decline of RMP and STP—starting with uniformly labeled protein-^{3}H cells rather than cells composed of labeled nuclei in unlabeled cytoplasm. The amputations and feedings were performed as in the above experiments but, after every third amputation, nuclei were transferred from a sample of the amputated cells to unlabeled nucleate cells. From such cells we obtained a ratio of activity between nuclei, which provided us with a measure of the relative proportions of labeled nuclear RMP and STP. We also assayed the activity of the enucleate protein-^{3}H donors after the nuclear transfers, thereby determining how much cytoplasm had been removed by the preceding amputations.

Fig. 5 shows the somewhat scattered data with curves that, although drawn by eye, are probably reasonable representations of the true kinetics; the data permit the following conclusions. As expected from the data already given and from the radioautographic studies of Prescott and Bender (8), the loss of protein label from the nucleus proceeds at a much slower rate than the loss of cytoplasmic protein label; this is reflected in the curve showing that the percentage of the total cell activity in the nucleus rises from 2.6 to 54%. The rate of decline in cytoplasmic label (curve for enucleate activity) is only slightly slower than the theoretical expectation (50% of the remaining activity lost with each amputation)—the deviation from theoretical reflects the continuous, relatively small contribution of labeled protein of the nucleus to the cytoplasm.

Unexpectedly, since data such as that illustrated in Fig. 2 show that there is a marked difference in the rate of movement of RMP and STP from the nucleus, we find that the proportions of radioactivity in RMP and STP remain the same over the course of many amputations. That these proportions remain the same is evident from the fact that, at any stage in the course of the amputation series, when the nucleus from a labeled cell is grafted into an unlabeled host cell, the ratio of radioactivity between host and donor nuclei is essentially the same as at any other stage (top curve in Fig. 5). This must mean, of course, that RMP and STP labels are depleted from the nucleus at approximately the same rate. A hint of this is provided by the results shown in Figs. 2 and 3, in which it is seen that RMP do migrate rapidly, but *reach a steady-state equilibrium* with no further *net* movement of radioactivity from the nucleus at about 3 hr; whereas STP seem to show a *continued net movement* for at least 4 days—and perhaps indefinitely. This realization leads us to interpret the results as follows (accepting that 50% of the cell's RMP is in the nucleus and that 50% is in the cytoplasm (6)). At every amputation of 50% of the cytoplasm, 25% of the RMP label is removed from the cell and—since RMP equilibrate rapidly between nucleus and cytoplasm—the loss is effectively 25% from each compartment. Since the radioactivity ratio between the nuclei seems not to change over the course of 12 amputations (top curve in Fig. 5), it follows that ca. 25% of nuclear STP label is also lost (and being replaced by unlabeled STP) between amputations. Thus, since the cell growth between amputations is approximately equal to that for one normal cell generation

[1] There are several reasons for suspecting that this is lower than the true value, owing to technical factors we have not yet determined.

[2] D. M. Prescott and L. Goldstein. 1967. Proteins in nucleocytoplasmic interactions. IV. The turnover of acid-soluble nuclear proteins, acid-insoluble nuclear proteins, and histone in *Amoeba proteus*. Data in preparation.

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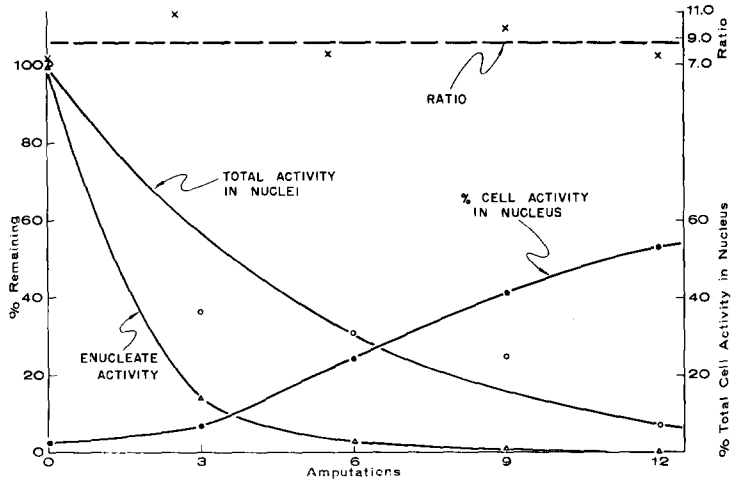

FIGURE 5 Experiment showing the change in the distribution of protein-^{3}H during the course of repeated cytoplasmic amputations performed every time a cell's volume was estimated to have doubled since the prior amputation. At the outset, the cells were uniformly labeled (i.e., nucleus and cytoplasm) with amino acids-^{3}H and were fed unlabeled food during the course of the amputations. The procedure was much like that for the experiment described in Fig. 4, except that to obtain data for *Ratio* curve, we transplanted nuclei from amputated cells to unlabeled cells after every third amputation. Pairs of nuclei from 10–20 such cells were isolated for each point, and means for the ratios of all are plotted. The *Enucleate Activity* curve is the mean activity per cell remaining in the cytoplasm of the donors in the above transplantations; the *Total Activity In Nuclei* curve is the same for the nuclei; and the *% Cell Activity In Nucleus* curve is derived from the data for both preceding curves. All curves drawn by eye.

(36–40 hr under these conditions), we estimate that about 25% of the nuclear STP move out of the nucleus (by migration of macromolecules or metabolic turnover) during the interphase between cell divisions. (It was actually this argument that led to the assumption resulting in the *Theoretical Cell Total* curve in Fig. 4.)

DISCUSSION

The behavior of the *A. proteus* nuclear proteins over the course of time and growth seems to be unusual and unexpected. In the light of earlier work (1), however, it is not surprising to find that RMP equilibrate between transplanted protein-^{3}H nucleus and unlabeled host cell nucleus in about 3 hr or that radioactivity can be detected in the host cell nucleus in less than 30 min. These are, of course, only crude approximations of the rate of movement between the two compartments and establish only a lower limit on the rate of the migration. If we assume that the labeled molecules that appear first in the host cell nucleus are those that were on the verge of escaping from the grafted nucleus at the time of the transplantation, we are still faced with ignorance of a large—and very important—time factor: how long a time is taken between the time an RMP molecule enters a nucleus and the *next* time it enters?

That the amount of radioactivity in a transplanted nucleus continues to fall after 3 hr is a surprise, in view of evidence to the contrary (1). It is now clear (6) that the methods used in the earlier work were inadequate for the detection of changes of this magnitude. The label that moves out of the nucleus at the slower rate (ca. 25% of the nuclear amount per cell generation) seems to be largely of the STP kind. Is the movement of STP label due to metabolic turnover or migration of intact protein molecules? Because there is no *net* loss of nuclear STP label during starvation and because in some experiments there is a suggestion that STP molecules return to the nucleus, we favor the view that STP leave the nucleus as intact proteins; but this suggestion cannot be strongly supported at this time. In fact, if the metabolic breakdown products are not lost from the cell (a condition yet to be investigated for *A. proteus*), it will be very difficult to distinguish between no turnover and metabolic turnover accompanied by reutilization of the products.

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Assuming that intact STP molecules move from nucleus to cytoplasm, we can ask whether these same molecules return from the cyoplasm to the nucleus. What evidence there is available on this point suggests that some STP molecules do move from cytoplasm to nucleus. In another paper[3] we will show that STP goes from cytoplasm to nucleus under two circumstances: (*a*) all, or almost all, STP are liberated from the nucleus during mitosis but return relatively rapidly immediately after cytokinesis; and (*b*) STP move from cytoplasm to nucleus at any time during interphase, but it is not clear whether those STP had been in the nucleus earlier or whether they were newly synthesized STP. Byers et al. (1) have shown that at least some STP are made in the cytoplasm of amebae and migrate to the nucleus, and Zetterberg (11) has demonstrated that some protein newly synthesized in the cytoplasm migrates to the nucleus during interphase of in vitro mouse fibroblasts. Robbins and Borun (10) have shown that the histones of the HeLa cell nucleus are made in the cytoplasm, and hence these proteins, too, must migrate from cytoplasm to nucleus.

Not only do RMP molecules migrate between nucleus and cytoplasm in a manner much different from that of STP, but their "turnover" during growth reflects what probably is a marked difference in metabolism. In Table I, we see that the reduction in labeled STP with growth parallels fairly closely that of the reduction in total protein label (column *5* vs. column *6*). For RMP, the reduction in the amount of label is as much as 50% per generation in only one instance (column *4*, Table I). Thus, in these data there is little indication of metabolic turnover of RMP during growth, supporting earlier evidence (1) on the same point. That there is even *less* than 50% "growth dilution" per generation is surprising and suggests that there is a cytoplasmic reserve of labeled RMP-specific precursors which—under conditions of growth on unlabeled nutrients—appears to be converted to migratory labeled RPM molecules.

There has been some speculation (cf. 4) regarding the physiological role of RMP, but little about that of STP. It is easy enough to imagine that STP consist of such components as histone, nuclear envelope protein, "residual" chromosomal protein, structural proteins of the nucleoli, proteins of nascent ribosomes, etc., but what does the newly observed migratory behavior tell us about these or any other proteins? In the light of current information, most workers would agree that only ribosomal protein is a certain candidate for a role in movement from nucleus to cytoplasm—but our data demonstrate that *all* nuclear proteins move out of the nucleus (unless, of course, the loss of nuclear label is by metabolic breakdown). But ribosomal proteins probably do not migrate from nucleus to cytoplasm. Recent work of Craig (2) shows that the cytoplasm of unlabeled cells that had received well labeled protein-^{3}H nuclei many hours earlier had no, or very little, ribosomal protein-^{3}H that could be detected by sucrose gradient analyses, whereas parallel experiments were able to show that ribosomal RNA is derived from the nucleus. Thus, the large amount of nucleolus-associated STP (5), which appeared to be a likely reservoir of ribosomal proteins, must have other functions.

If we speculate that RMP serve in the regulation of genetic activity and enter the cytoplasm for the purpose of detecting environmental signals, what other roles can we envision for STP molecules that perhaps shuttle between nucleus and cytoplasm at a slower rate? A likely role is as carriers of messenger RNA. Those two functions—regulation and transportation—seem the most reasonable to be considered at the moment.

In which of these groups are the histones to be found? That is difficult to say. (It seemed most reasonable to look for histones in the STP fraction, but other experiments[2] show that it is difficult to exclude histones from the RMP group.) What can be said with certainty is that neither histones nor any other proteins have a nuclear stability comparable to that of DNA, and hence it is unlikely that any protein has a genetic function, a structural role, or a metabolic stability which is in any way like that of DNA. The data given here only establish that not more than 0.3% of the original nuclear protein can be stably localized in the nucleus; in a forthcoming paper, data obtained by more sensitive methods will show that the maximum amount of "stable"nuclear protein, if any, is less than 0.3% by an order of magnitude.

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[3] D. M. Prescott and L. Goldstein. 1967. Proteins in nucleocytoplasmic interactions. III. Redistribution of nuclear proteins during and following mitosis. Data in preparation.

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