PROTEINS IN NUCLEOCYTOPLASMIC INTERACTIONS

III. Redistributions of Nuclear Proteins During and Following Mitosis in *Amoeba proteus*

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ABSTRACT

The behavior of nuclear proteins in *Amoeba proteus* was studied by tritiated amino acid labeling, nuclear transplantation, and cytoplasmic amputation. During prophase at least 77% (but probably over 95%) of the nuclear proteins is released to the cytoplasm. These same proteins return to the nucleus within the first 3 hr of interphase. When cytoplasm is amputated from an ameba in mitosis (shen the nuclear proteins are in the cytoplasm), the resultant daughter nuclei are depleted in the labeled nuclear proteins. The degree of depletion is less than proportional to the amount of cytoplasm removed because a portion of rapidly migrating protein (a nuclear protein that is normally shuttling between nucleus and cytoplasm and is thus also present in the cytoplasm) which would normally remain in the cytoplasm is taken up by the reconstituting daughter nuclei. Cytoplasmic fragments cut from mitotic cells are enriched in both major classes of nuclear proteins, i.e. rapidly migrating protein and slow turn-over protein. An interphase nucleus implanted into such an enucleated cell acquires from the cytoplasm essentially all of the excess nuclear proteins of both classes. The data indicate that there is a lack of binding sites in the cytoplasm for the rapidly migrating nuclear protein. The quantitative aspects of the distribution of rapidly migrating protein between the nucleus and the cytoplasm indicate that the distribution is governed primarily by factors within the nucleus.

At the onset of mitosis a major part of the proteins of the nucleus is rapidly released into the cytoplasm (1, 2, 9, 13). This release is closely coincidental with the abrupt disappearance of organized nucleoli, fragmentation or complete disappearance of the nuclear envelope, cessation of all RNA synthesis in the nucleus, and release of most or all of nuclear RNA to the cytoplasm. Isotope tracer experiments (2, 6, 9, 13), staining procedures (1, 3, 7, 8), and interference microscope measurements (10, 11) show that much of the released protein returns to the reconstituting daughter nuclei in late telophase and early interphase.

In *Amoeba proteus* the nuclear proteins are divisible into two general classes, on the basis of physiological behavior (2, 4). One class, designated as rapidly migrating protein (RMP) and constituting at any one time about 40% of the total protein content of the nucleus, is in rapid migration back and forth between nucleus and cytoplasm. The remaining 60% has been designated slow turn-over protein (STP), of which no more than 5–6% can be histone. From radioautographic observations we have concluded previously (2, 9) that most of the RMP and STP disperses into the cytoplasm during mitosis and returns to the nucleus after division.

With more quantitative methods now available

for measuring such protein redistributions in amebae, we have determined (a) the time course and extent of protein return after mitosis, (b) the consequences of amputating cytoplasm from mitotic cells on the content of protein in the postmitotic nucleus, and (c) some aspects of the manner in which a nucleus deals with an enrichment or depletion of RMP and STP in a cell.

Because the nuclear proteins are released to the cytoplasm during division, the cutting of mitotic cells provides an enucleate fragment enriched in both RMP and STP relative to normal interphase cytoplasm and two daughter cells (or one binucleated cell) depleted in both protein classes.

MATERIALS AND METHODS

Information on culturing of amebae, ^{3}H amino acid-labeling of the cells, measurement of radioactivity, nuclear transplantation, and nuclear isolation is given in the first paper of this series (4). In some cases in which nuclear transplants were involved, the nuclei were assayed for radioactivity without isolation (to obviate any possibility of loss of nuclear proteins as a result of isolation). Nuclei to be assayed were transplanted into nonradioactive cells, and the whole cell was immediately prepared for assay.

RESULTS AND INTERPRETATIONS

1. Time Course of Protein Return Following Mitosis

Amebae heavily labeled with ^{3}H amino acids were selected at division, and the daughter cells were fed on nonradioactive Tetrahymena continuously for one generation (in order to "chase" the ^{3}H amino acid pool). At the next division, groups of cells in early cytokinesis were selected within a period of several minutes and kept without food. The nuclei of a given synchronized group were isolated at a prescribed interval after cytokinesis, with the first group isolated 15 min after the completion of cell division. Before 15 min, nuclei were not sufficiently reconstituted to be readily detectable by the isolation procedure. All nuclei were isolated in spermidine-triton solution (4).

The two such experiments plotted in Fig. 1 establish that the uptake of radioactive protein is one-half maximum at 1 hr and is maximum by about 3 hr after cytokinesis (i.e. within the first 10% of interphase); the radioactive protein content did not change appreciably during the

next 18 hr of interphase. At the time of the first measurements (15 min after cytokinesis) the radioactive protein content is 23% of the maximum content reached at 3 hr.

2. Extent of Protein Return Following Mitosis

When it had been established that the return of radioactive protein to the nucleus is completed by 3 hr after cytokinesis, the extent of this return was measured by comparing the content of radioactive protein in late G_2 nuclei with the content in daughter nuclei at 3 hr after division.

30 dividing amebae heavily labeled with ^{3}H amino acids were selected, and the daughters were cultured as sister pairs (F_1 in Fig. 2) on nonradioactive Tetrahymena during the ensuing interphase. When one of the sister cells of a pair divided, the nuclei were isolated from the two resultant daughter cells (F_1 in Fig. 2) when the protein return was maximal, i.e. 3 hr after cytokinesis. The nucleus of the undivided sister of the particular divider (F_1) was isolated sometime during this 3-hr interval; but 11 out of the 30 entered division before their nuclei could be isolated, and these amebae had to be discarded. The latter amebae served to demonstrate that a good degree of synchrony between sisters was present. In the light of this synchrony, the remaining 19 undivided amebae were considered to have been in a late part of the G_2 stage at the time of nuclear isolations. All nuclei were isolated in a spermidine-triton solution (4). The mean radioactive protein content of these 19 G_2 nuclei was 413 cpm, and the mean for the corresponding pairs of daughter cell nuclei was 416 cpm. The application of Student's t test to detect the difference between paired samples (12) gave a probability value greater than 0.9 that the protein contents for the G_2 nuclei and the paired daughters were identical (t = 0.10, N = 19). If there is a significant difference, it must be quite small.

The data show that by 3 hr after division the amount of radioactive protein in the two daughter nuclei is essentially equivalent to the amount in the premitotic nucleus.

We have not yet been able to determine how much of the total nuclear protein is released to the cytoplasm, but the data show that it must be at least 77%, for the following reason. A newly divided pair of daughter nuclei has at most 23% of the amount that it will ultimately have at 3 hr postdivision. As already shown, this final amount
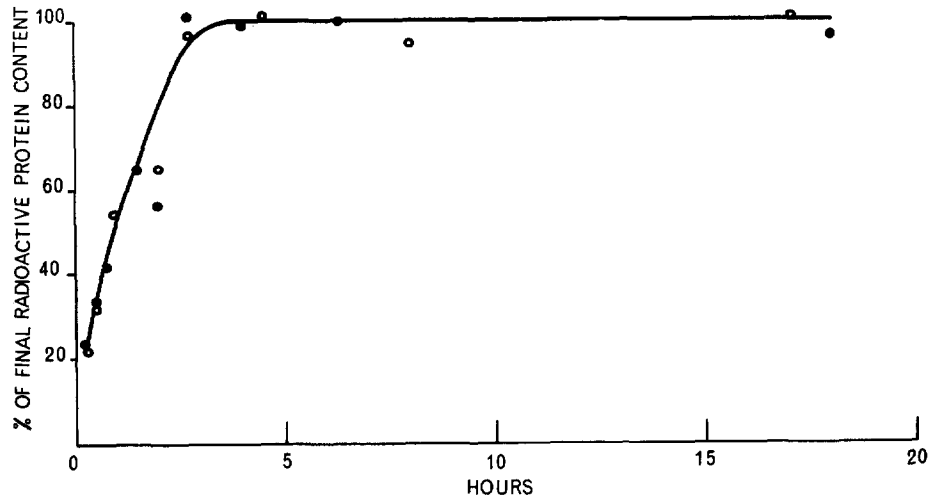
FIGURE 1 The two sets of points represent two experiments in which the return of protein from the cytoplasm to the nucleus during early interphase was followed. The returning protein is the same protein released in late prophase. The return is complete by 3 hr postdivision.
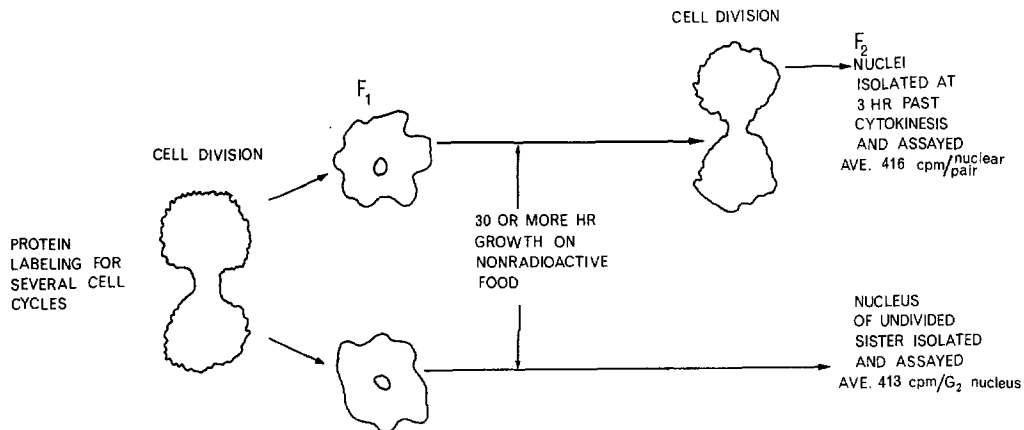

FIGURE 2 This scheme was used to determine how much of the protein released from the nucleus in late prophase was subsequently returned to the postdivision, daughter nuclei. The data are described in section 2 of Results and Interpretations.

in the pair of daughter nuclei is equal to the amount in the predivision nucleus (Fig. 2). Radioautographic studies have demonstrated that the returning protein is the same protein released in prophase (2, 9). According to the data in Fig. 1, the nuclei (chromosomes) could have retained during mitosis no more than 23% of the premitotic nuclear protein. In radioautographic analysis of such dividing cells, there was no concentration of radioactive protein with chromosomes, (2, 9) which indicates that the true percentage of protein

released at prophase must be considerably greater than 77%, probably more than 95%.

3. Depletion of Nuclear Protein by Amputation of Cytoplasm from Cells in Mitosis

Since it is known from radioautographic studies (2, 9) that the radioactive protein that returns to the nucleus following mitosis is the same radioactive protein released to the cytoplasm during

DIVIDING CELL
CUT INTO
HALVES

F_2
NUCLEI
ISOLATED AT
3 HR POST-
MITOSIS AND
ASSAYED
AVE. 194 cpm/nuclear pair

F_{1a}

CELL DIVISION

ENUCLEATE
ASSAYED TO
CALCULATE
PERCENTAGE
CUT FROM
MITOTIC CELL

PROTEIN
LABELING FOR
SEVERAL CELL
CYCLES

30 OR MORE HR
GROWTH ON
NONRADIOACTIVE
FOOD

F_{1b}

F_{1b}

F_{2a+b}

NUCLEUS (IN G_2)
OR
2 DAUGHTER NUCLEI
ISOLATED AT 3
HR POST-
MITOSIS AND
ASSAYED
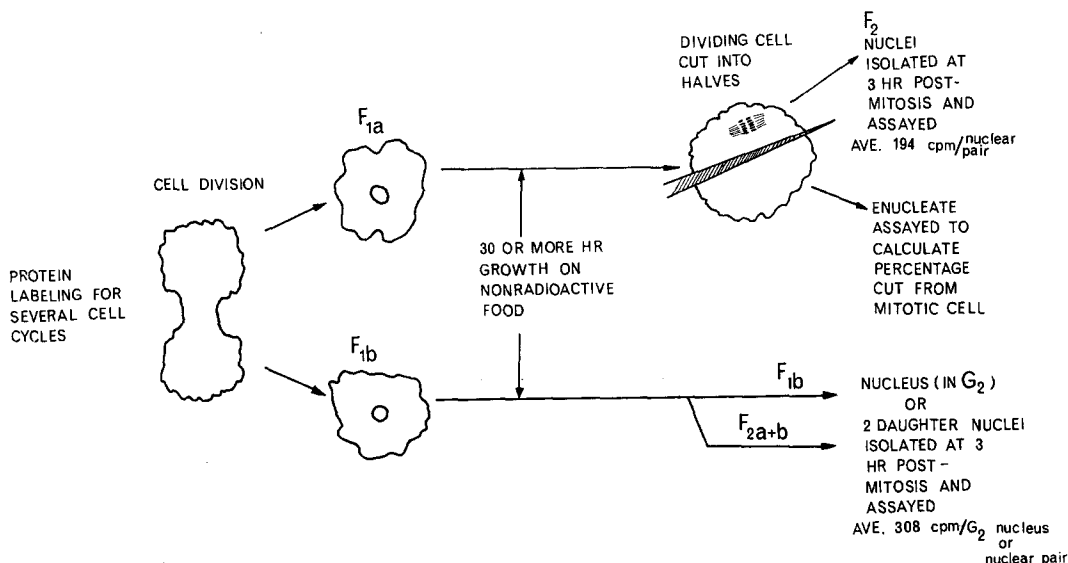AVE. 308 cpm/G_2 nucleus
or
nuclear pair

FIGURE 3 This scheme was used to determine the amount of reduction in nuclear proteins in daughter nuclei derived from a cell from which cytoplasm was amputated during mitosis. The experiments are described in section 3 of Results and Interpretations.

prophase, amputation of cytoplasm from mitotic cells was expected to cause a reduction in the amount of protein in the postmitotic nucleus. The expectation was tested in the following way. Dividing amebae heavily labeled with ^{3}H amino acids were selected, and the daughters were grown as sister pairs (F_1 in Fig. 3) on nonradioactive *Tetrahymena*. At the next division approximately one-half of the cytoplasm was removed from one of the sisters during division. 3–9 hr after completion of mitosis in the amputated cell, the two postmitotic nuclei (F_2 in Fig. 3) and the nucleus from the non-amputated sisters (F_1b in Fig. 3) were isolated and compared for radioactivity content. In about one-half of the cases (out of a total of 49) the non-amputated sisters (F_1b) had also divided, in which cases the two daughter nuclei (at 3 hr postcytokinesis) were considered equivalent to a single late G_2 nucleus from an undivided sister (see section 2).

According to our tentative hypothesis, the extent of the protein depletion in nuclei of amputated cells should depend on the amount of cytoplasm removed during mitosis. To obtain a measure of the amount of cytoplasm amputated, the radioactivity in the enucleated cytoplasmic fragments was measured and compared with the average radioactive content in a normal daughter cell

(F_2b in Fig. 3) If the mitotic cells had been cut exactly in half, the average for enucleate fragments should equal the average for daughter cells (the nuclear contribution being trivial). The average for the 49 enucleate fragments recovered was 7,025 cpm; the average counts per minute for 59 daughter cells was 7600. These values show that the average enucleate fragment cut from a mitotic cell was 92% of the size of the average daughter cell. This means that the amputations on mitotic cells removed an average of 46% of the cytoplasm.

The 49 pairs of daughter nuclei derived from amputated mitotic cells averaged 194 cpm/pair (isolated at 3 hr after mitosis). The corresponding average for nuclei from the 49 control G_2 cells or paired (unoperated) daughters was 308. All nuclei in this experiment were isolated in spermidine-triton solution (4). Mitotic cells deprived of an average of 46% of their cytoplasm yielded daughter nuclei with an average protein content decreased by 37%. According to Student's t test for the difference between paired samples (12), the probability that the radioactive protein contents (308 vs. 194) for the two sets of nuclei are the same is less than 0.001 (t = 11.75, N = 49). The amputation of cytoplasm during mitosis does deplete the amount of protein in the postmitotic nuclei.

From the hypothesis that there will be a 1:1 proportionality between the per cent of cytoplasm amputated during mitosis and the per cent of depletion of radioactive protein content of daughter nuclei, we predict that the daughter nuclei from cut mitotic cells will contain only half the protein of control nuclei, if exactly 50% of the mitotic cytoplasm is removed. Since only 46% of the cytoplasm was removed and the operated cells, therefore, retained 54% of their cytoplasm, the hypothesis predicts that paired daughter nuclei from operated cells will contain 54% of the amount in unoperated control nuclei. 54% of the unoperated control amount, 308 cpm, is 164 cpm, and this was compared to the actual experimental mean, 194 cpm. Student's t test of the difference between these two means (not paired samples) gives a probability of 0.01–0.001 (t = 3.11, N = 49 for each group) that the two means are the same. This shows that there is *not* a 1:1 proportionality between the amount of mitotic cytoplasm removed and the degree of depletion of protein in the daughter nuclei. Since daughter nuclei of operated cells contain more (194 cpm) than the predicted amount of radioactive protein (164 cpm), they must be capable of compensating to some degree by taking up protein that under normal circumstances would remain in the cytoplasm. Since a class of nuclear protein (RMP) has been shown always to be present in the cytoplasm in appreciable amounts (2, 4), it is possible that this cytoplasmic source of protein might be used to compensate for the depletion resulting from the amputation during mitosis.

A 10% carry-over of proteins with the chromosomes during mitosis would be sufficient to explain the degree by which the results vary from a 1:1 proportionality between the per cent of cytoplasm amputated from a mitotic cell and the resultant depletion of protein in the two daughter cell nuclei. Since, as discussed in section 2, as much as 23% of nuclear protein conceivably could be carried with the chromosomes through mitosis, this becomes a reasonable possibility. This appears not to be the correct explanation, however, since data given in section 5 indicate that nuclei do "compensate" by acquiring a disproportionate share of nuclear proteins availabe in the cytoplasm of an enucleate derived from a mitotic cell.

4. Uptake of Protein by a Nucleus in Cytoplasm Containing Excess Nuclear Protein

Since enucleate fragments obtained by amputation of cytoplasm from a mitotic cell contain an excess of nuclear protein, we compared the uptake of labeled protein by a nonradioactive nucleus which had been implanted into such an enucleate cell enriched with nuclear proteins with the uptake by its sister nucleus which had been implanted into a radioactive enucleate derived from an interphase cell (see Fig. 4). As subsequently determined, the 12 enucleates of mitotic cells contained a mean of 8725 cpm. 20 hr after transplantation, each nucleus was assayed. The mean counts per minute taken up by 12 interphase nuclei implanted into radioactive mitotic enucleates was 214. The comparable average value for the 12 sister nuclei implanted into interphase enucleates was 68. The 12 interphase enucleates contained an average of 8305 cpm. Thus, the nucleus implanted into the cytoplasm enriched with nuclear proteins acquired 2.45 ± 0.46% of the cytoplasmic total, and the nucleus implanted into normal interphase cytoplasm acquired 0.82 ± 0.22% of the cytoplasmic total, i.e. the interphase nucleus in mitotic enucleates acquired three times as much.

If the RMP distributes between the nucleus and cytoplasm in equal amounts regardless of the initial concentration in either compartment (2, 4, 5), then the nucleus in the mitotic enucleate would take up no more than twice as much as a nucleus in the interphase enucleate. If on the other hand, a nucleus took up all the cytoplasmic RMP that is present in excess of the normal concentration, then the nucleus in the mitotic enucleate would take up no more than 2.25 times more than a nucleus in the interphase enucleate. Since the nucleus implanted into the mitotic enucleate takes up three times as much radioactive protein as a nucleus implanted into an interphase enucleate, it seems that part of the excess STP of the mitotic cytoplasm contributes to the uptake by the interphase nucleus.

The calculations used in reaching these conclusions are as follows. Assume that there are 200 radioactive units of RMP in the nucleus and 200 radioactive units in the cytoplasm (we know from other experiments that the amount of RMP is normally about the same in the nucleus and cyto-

SISTER CELLS (NUCLEAR DONORS)

LABELED
MITOTIC
CELL

DISCARDED

ASSAYED
NUCLEI-214 cpm

CA.24 HR

CYTOPLASM-8725 cpm

LABELED
INTERPHASE
CELL

ASSAYED
NUCLEI- 68 cpm

CA.24 HR
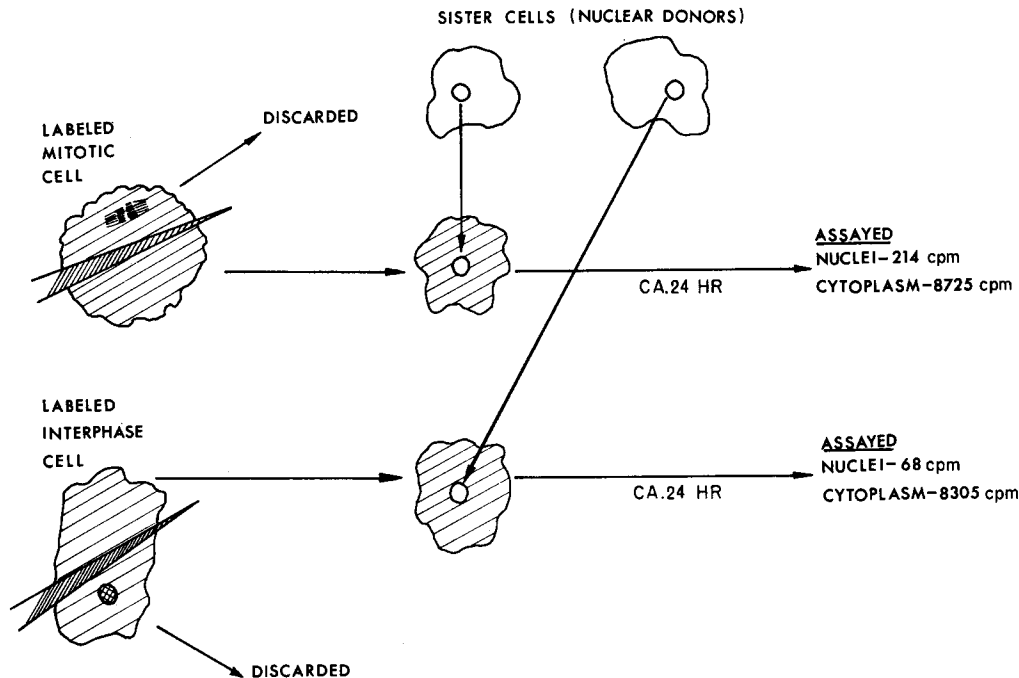
CYTOPLASM-8305 cpm

DISCARDED

FIGURE 4   This scheme was used to compare the uptake of radioactive protein by an interphase nucleus which had been implanted into cytoplasm cut from a labeled mitotic cell with the uptake by an interphase nucleus which had been implanted into cytoplasm cut from a labeled interphase cell. Cross-hatching of nucleus or cytoplasm indicates the presence of radioactive proteins. The experiments are described in section 4 of Results and Interpretations.

plasm). In the mitotic cell, assume that all 400 radioactive units of RMP are in the cytoplasm.

An enucleate fragment obtained by cutting a radioactive interphase cell into halves will contain 100 radioactive units of RMP. An interphase nucleus (in the G_2 state) implanted into this enucleate will bring 200 nonradioactive units of RMP.

If all of the RMP distributes equally between nucleus and cytoplasm, then the cytoplasm will contain 50 radioactive units of RMP and the nucleus will contain 50 radioactive units of RMP.

If, as our unpublished experiments show, the interphase nucleus retains 200 units of RMP regardless of the interphase cytoplasmic volume (in this case a half cell), then the cytoplasm will contain 33 radioactive units of RMP and the nucleus will contain 67 radioactive units of RMP.

An enucleate fragment obtained by cutting a radioactive mitotic cell will contain 200 radioactive units of RMP. An interphase nucleus (in the G_2 state) implanted into this enucleate will bring 200 nonradioactive units of RMP.

If all of the RMP distributes equally between nucleus and cytoplasm, then the cytoplasm will contain 100 radioactive units of RMP and the nucleus will contain 100 radioactive units of RMP.

Under these conditions the nucleus in the mitotic enucleate (100 radioactive units of RMP) will contain twice as many counts as a nucleus implanted into an interphase enucleate (50 radioactive units).

If all the excess RMP is taken up by the nucleus, then the cytoplasm will contain 50 radioactive units of RMP and the nucleus will contain 150 radioactive units of RMP.

Under these conditions, the nucleus in the mitotic enucleate (150 radioactive units) will contain 2.25 times as many counts as the nucleus in interphase enucleate (67 radioactive units).

5. *Distribution of STP and RMP under Conditions of Cellular Enrichment of Nuclear Proteins*

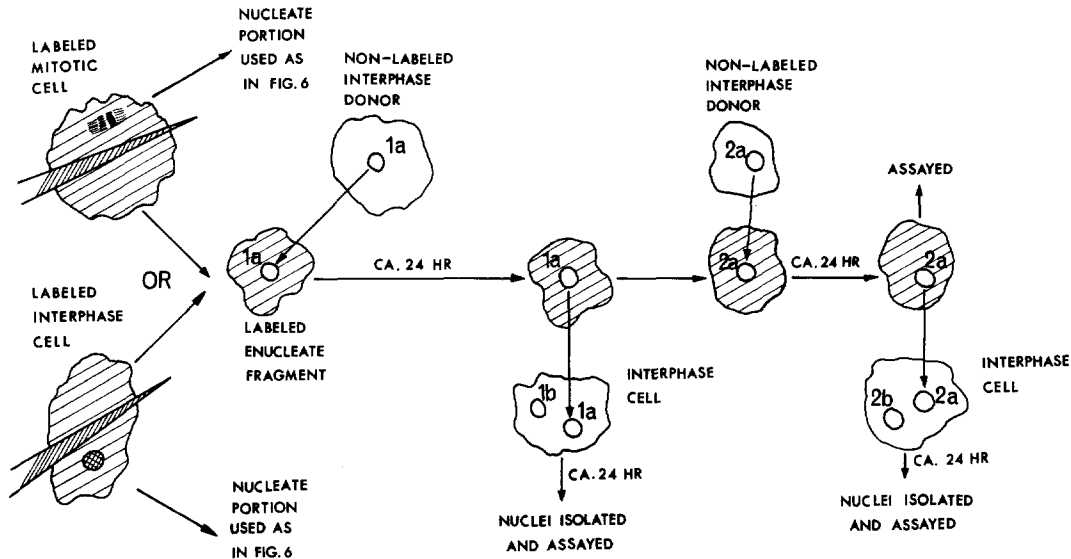In previous work, two methods (nuclear transplantation and repeated cytoplasmic ampu-

Figure 5   This scheme is a further elaboration of the scheme in Fig. 4 and was desiged to determine the uptake of STP and RMP by nuclei implanted into cytoplasm cut from a labeled mitotic cell or from a labeled interphase cell. For each nucleus which was implanted into cytoplasm cut from a mitotic cell, its sister nucleus was implanted into cytoplasm cut from an interphase cell. Cross-hatching indicates radioactive proteins. The experiments are described in section 5 of Results and Interpretations.

tations) were used to prepare cells in which nuclear proteins were radioactive and cytoplasmic proteins were nonradioactive (2, 4, 9). Radioauto-graphic studies of such cells demonstrated that at mitosis almost all of the radioactive nuclear proteins were released to the cytoplasm. Beginning in telophase and continuing into interphase, virtually all of the radioactive protein became relocalized in the nucleus. Because 60% of the radioactive proteins involved was of the STP class, this was the first evidence that after mitosis all STP returns to the nucleus. This conclusion is supported by the quantitative determinations on protein release and return in connection with mitosis given in section 2.

Proof that STP can be taken up by an interphase nucleus was provided by the implantation of a nucleus (nucleus *1a* in Fig. 5) into a nuclear protein-enriched, enucleated cell derived by cutting a protein-labeled cell in mitosis. Several hours later, following retransplantation of the nucleus into an interphase cell, the mean ratio of radioactive protein between the two nuclei (*1a* and *1b* in Fig. 5) of this binucleate was found to be 6.0:1 in one series of 12 transplants and 5.4:1 in another series of seven transplants.

A ratio of 6.0:1 means that in the transplanted

(radioactive) nucleus there must have been 5 parts of STP and 3 parts of RMP. When equilibrium in RMP distribution is reached, there will be 1 part in each of the two nuclei and 1 part in the cyto-plasm. When a ratio of 5.4:1 is obtained, a slightly lower proportion of the total protein of the nucleus was STP. To reach these close-to-normal ratios (4) the nucleus must have acquired both STP and RMP from the mitotic enucleate.

A second interphase nucleus (*2a* in Fig. 5) was implanted into the original mitotic enucleate and retransferred to an interphase cell in order to test for the nuclear proteins still remaining in the enucleate cytoplasm. The mean ratio for these nuclei (*2a* and *2b* in Fig. 5) when transplanted to interphase cells was 1.8:1 in each of two separate experiments. These ratios cannot be considered to be different from 1:1 (4), and it is concluded that the second nucleus, in contrast to the first, had acquired labeled RMP but not STP.

These transplants of nuclei into cytoplasm derived from cells in mitosis were compared with nuclei transplanted into comparably labeled enucleates derived from interphase cells. To minimize biological variation, each nucleus implanted into a labeled enucleate in these experiments was a sister to one of the nuclei implanted

into a mitotic cell enucleate in the experiment described in the preceding paragraph. Following implantation of a first nucleus (*1a*) into an interphase enucleate and subsequent retransplantation to an interphase cell (Fig. 5), the average ratios were 3.1:1 for a series of 10 such transfers and 3.3:1 (nuclei *1a* and *1b*) for a second series of seven transfers. These ratios indicate that the nuclei acquired some labeled STP from the enucleates cut from labeled interphase cells. Had the nuclei acquired only RMP, the ratio would have been closer to 1:1 (or actually under 2:1 as explained below). (This is fairly good evidence that under these circumstances there is a poll of STP—newly synthesized?—in the cytoplasm.) When the procedure was repeated with a second interphase nucleus (transplanted into each of the original enucleates), the average ratios were 2.0:1 and 1.7:1 (nuclei *2a* and *2b*). These latter ratios are obtained by arbitrarily considering the nucleus which showed more radioactive counts as the nucleus that was grafted into the last host cell (but there is actually no way of telling which nucleus is which unless one of the nuclei is considerably more radioactive than the other). Even if the two nuclei of this last host were truly equally labeled at equilibrium of RMP, biological as well as iostope decay variability would inevitably make it appear that one nucleus was more heavily labeled than the other, and thus the ratio for any pair of nuclei would rarely appear to be 1:1. Under such circumstances, we cannot consider that a ratio of less than 2:1 is truly different from 1:1. We conclude, therefore, that the first nucleus transplanted into interphase enucleate cytoplasm acquired RMP and some STP (ratios of 3.1:1 and 3.3:1) but that the second nucleus placed in the same cytoplasm obtained only RMP (ratios of 2.0:1 and 1.7:1). Since these latter ratios cannot be considered different from 1:1, we conclude that the first nucleus obtained RMP and some STP from the interphase enucleate but that the second nucleus obtained only RMP.

The counts per minute in these experiments bear out the conclusion that the first nucleus placed in cytoplasm derived from a mitotic cell takes up the excess RMP and STP. In the first experiment with mitotic enucleates, such nuclei took up an average 147 cpm out of a total of 8750 cpm in the average enucleate (11 cells) and 156 cpm out of a total of 14,920 cpm in the second experiment (seven cells). These numbers

represent an uptake of 1.68 and 1.06%, respectively. The second nuclei implanted into these enucleates took up an average of 20 cpm in both experiments, i.e. 0.23 and 0.14% of the cytoplasmic total. The first nucleus, therefore, obtained seven times more protein than did the second.

These numbers should be compared with those obtained with interphase enucleates. The first nucleus placed in an interphase enucleate took up an average of 30 cpm out of 8790 cpm in the average enucleate in one experiment (10 cells) and 29 cpm out of 10,010 cpm in the second (seven cells), i.e. uptakes of 0.34 and 0.29% by the nuclei of the cytoplasmic total. The second nuclei implanted into the *identical* cytoplasmic in this experiment took up an average of 17 and 11 cpm, respectively, or 0.20 and 0.11% of the total.

The amount of protein acquired by the first nucleus implanted into a labeled mitotic enucleate is over four times the amount acquired by the first nucleus implanted into labeled interphase enucleate (an average acquistion of 1.35% vs. 0.32%). This is really a repeat of the experiment reported in section 4 in which a three-fold difference was found. Whether the difference (three-fold vs. four-fold) is significant we cannot say, but the four-fold difference again supports strongly the conclusion that both excess STP and RMP of mitotic enucleates are acquired by the interphase nucleus.

The percentage uptake of labeled protein by the second nuclei implanted into the two types of enucleates was 0.23 and 0.14% with *an average of 0.16%* and 0.20 and 0.11 % with *an average of 0.15%*. These data indicate that the amount of RMP remaining in a mitotic enucleate (the ratios already discussed above indicate that only RMP is involved) after the sojourn of the first interphase nucleus is now normal, i.e. *all* excess RMP had been taken up by the first implanted nucleus.

6. Distribution of STP and RMP under Conditions of Cellular Depletion of Nuclear Proteins

As shown in section 3, daughter nuclei derived from mitotic cells from which cytoplasm has been removed are severely deplected in nuclear proteins, but the amount of depletion is not proportional to the amount of cytoplasm removed.

The nucleated fragments left over from the experiment diagrammed in Fig. 5 were used as shown in Fig. 6. The labeled daughter nuclei
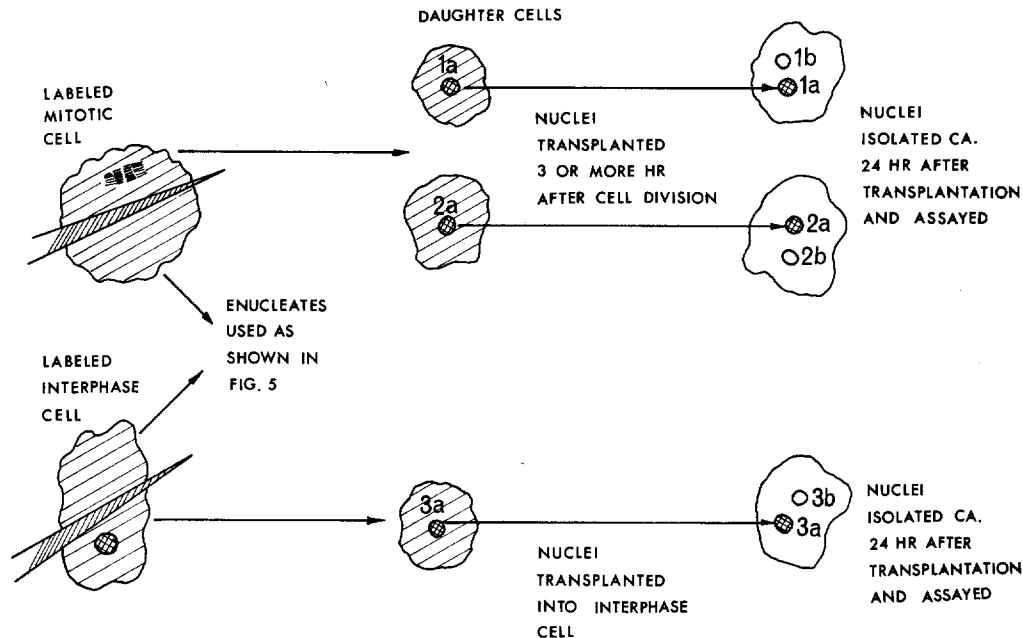
FIGURE 6 This scheme was designed to determine the amount of STP and RMP in daughter nuclei depleted in nuclear proteins. Cross-hatching indicates radioactive proteins. See section 6 of Results and Interpretations for experimental details and results.

depleted in nuclear proteins were explanted into interphase host cells in order to determine the proportions of RMP and STP in the remaining protein. Following equilibration of RMP between the two nuclei in the cell (*1a* and *1b*; *2a* and *2b*), these nuclear pairs were found to contain an average total of 148 cpm (11 pairs). The less radioactive nuclei (*1b* and *2b*) contained an average of 32 cpm (14 nuclei) and the more radioactive nuclei (*1a* and *2a*), 116 cpm (11 nuclei); the average ratio between these nuclei (e.g. *1a*:*1b*, *2a*:*2b*) was, therefore, 3.6:1. The corresponding values for the labeled nuclei (*3a*) from the interphase cells from which cytoplasm had been amputated (see Fig. 6) were as follows: the average total for both nuclei (*3a* and *3b*), 291 cpm; the less radioactive nuclei (*3b*), 46 cpm (average for 14 nuclei); the more radioactive nuclei (*3a*), 245 cpm (average for 13 nuclei); an average ratio between the two kinds of nuclei (*3a*:*3b*) of 5.3:1.

In other experiments not reported here, we have found that amputation of cytoplasm from interphase cells has no measurable effect on the content of radioactive proteins in the nucleus of *A. proteus*. We expected, therefore, that the total amount of radioactive protein in the nuclei of the

amputated interphase cells as well as the proportions of STP and RMP in such nuclei would be normal. The ratio of 5.3:1 is slightly less than the ratio for normal nuclei (6.0:1), but the number of nuclei in the experiment is too small to allow attachment of any significance to the small decrease in the ratio. We assume, therefore, that these nuclei possess the normal amount of RMP and STP.

The average ratio reached following implantation of protein depleted nuclei into interphase hosts was clearly below normal, i.e. 3.6:1. This shows that the depleted nuclei contained a higher proportion of RMP:STP than normal. To account for the increased proportion of RMP we must assume that the depleted nuclei had retained RMP with the chromosomes through mitosis or that such nuclei accumulated during the first 3 hr of interphase some of that RMP that would normally have remained in the cytoplasm. We cannot detect the concentration of radioactivity (with the mitotic chromosomes) necessary to support the idea of a significant carry-over of protein by the mitotic chromosomes. Therefore, we believe that it is extra return of RMP from the postmitotic cytoplasm that accounts for the lack of a 1:1

proportionality between the amount of cytoplasm removed from a mitotic cell and the degree of protein depletion in the daughter nuclei (see section 3).

This conclusion implies that the nucleus has a stronger priority than does the cytoplasm for establishing its quota of RMP.

CONCLUSIONS

(a) According to measurements on daughter nuclei isolated 15 min after cell division, at least 77% of the proteins of the ameba nucleus is released to the cytoplasm in prophase. Radioautographic analyses show that the amount of protein released during mitosis must be closer to 95%. All of these *same* proteins return to the nucleus in early interphase. The return is complete by 3 hr of interphase (roughly the first 10% of interphase).

(b) When cytoplasm is cut from a cell in mitosis, the resultant daughter nuclei have depleted protein contents. The depletion is less than proportional to the amount of cytoplasm removed, e.g. removal of 46% of the mitotic cytoplasm resulted in a 37% decrease in nuclear protein content. The lack of a 1:1 proportionality appears to be due to enhanced accumulation of rapidly migrating protein (RMP) from the cytoplasm, i.e. a major fraction of the RMP that would normally have remained in the cytoplasm is taken up by the nucleus. Accordingly, the nucleus has a stronger priority than the cytoplasm for establishment of its normal RMP content.

(c) Because most nuclear proteins are released from the ameba nucleus at late prophase, cytoplasmic fragments cut from dividing cells are enriched in nuclear proteins. An interphase nucleus implanted into such an enucleate acquires from the cytoplasm essentially all of the excess nuclear proteins of both the STP and RMP types.

In general, it appears that the nucleus will acquire most of the STP of the cell regardless of the amount available.

(d) An unusual mechanism of RMP accumulation by the nucleus is indicated by these data. If there is a reduced amount of cellular RMP, the nucleus acquires almost all available, until it has its "normal" quota. If an excess of RMP is present in the cell, the nucleus acquires all that is in excess of the normal total of the cell. Conversely, the cytoplasm relinquishes RMP to the nucleus if the total RMP of the cell is reduced in amount; when RMP is present in excess, the cytoplasm does not acquire more than its normal amount. These data may mean that there is a lack of binding sites for RMP in the cytoplasm. Such an interpretation agrees with an unpublished observation that the *volume* of cytoplasm is essentially without influence on the distribution of RMP between the nucleus and cytoplasm.

The finding that the distribution of RMP between the nucleus and cytoplasm is not the same, i.e. not a constant proportionality under different experimental conditions, suggests that the distribution is not governed by a (nuclear) membrane function. Since the RMP distribution apparently is not influenced by the cytoplasmic volume, it seems likely that distribution is goverened by factors within the nucleus. Whatever the governing factors, they permit the constant shuttling of RMP back and forth between the nucleus and cytoplasm.

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