THE FATE AND ORIGIN OF THE NUCLEAR ENVELOPE DURING AND AFTER MITOSIS IN *AMOEBA PROTEUS*

I. Synthesis and Behavior of Phospholipids of the Nuclear Envelope During the Cell Life Cycle

HIROSHI MARUTA and LESTER GOLDSTEIN

From the Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80302. Dr. Maruta's present address is the Section on Cellular Biochemistry and Ultrastructure, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014.

ABSTRACT

The synthesis and behavior of *Amoeba proteus* nuclear envelope (NE) phospholipids were studied. Most NE phospholipid synthesis occurs during G2 and little during mitosis or S (*A. proteus* has no G1 phase). Autoradiographic observations after implantation of [3H]choline nuclei into unlabeled cells reveal little turnover of NE phospholipid during interphase but during mitosis all the label is dispersed through the cytoplasm. Beginning at telophase all the NE phospholipid label returns to the daughter NEs. This observation, along with the finding that no NE phospholipid synthesis occurs during mitosis or S, indicates that no *de novo* NE phospholipid production is required for newly forming NEs. Similarly emetine, at concentrations that inhibit 97% of protein synthesis, does not prevent the post mitotic formation of NEs, suggesting that previously manufactured proteins are used in making new NEs.

If a nucleus containing labeled NE phospholipids is transplanted into an unlabeled nucleate cell and the cell is allowed to grow and divide, the resultant four nuclei are equally labeled. This finding supports, but does not prove (see next paragraph), the conclusion that there probably is no continuity of the *A. proteus* NE during mitosis.

When a phospholipid-labeled nucleus is implanted into a cell in mitosis, the grafted nucleus is not induced to enter mitosis. There is, however, a marked increase in the turnover of that nucleus’s NE phospholipids with no apparent breakdown of the NE; this indicates that the mitotic cytoplasm possesses a factor that stimulates NE phospholipid exchange with the cytoplasm. That enhanced turnover is not accompanied by visible structural alteration makes less certain the earlier conclusion that no NE continuity exists during mitosis.

Perhaps the most important finding in this study is that there are present, at restricted times in the cell cycle, factors capable of inducing accelerated exchange of structural components without microscopically detectable disruptions of structure.
In most cells, the nuclear envelope (NE) undergoes a cyclic breakdown and reformation during mitosis (20, 27, 28), a process recognized a century ago. Its disappearance marks the end of prophase and the beginning of prometaphase in living cells. The process, as viewed in the electron microscope, seems not initially to involve an actual dissolution of the envelope, but rather a fragmentation and dispersal of small double membrane units. Once scattered in the cytoplasm the envelope fragments rapidly lose their identity and become indistinguishable from elements of the endoplasmic reticulum (ER). The fate of these fragments in unknown, but it is generally assumed that they do not retain any specificity (22) but become part of the “membrane pool” or the ER of the cell.

The degree of disruption of the envelope, however, can vary with the cell type. In Amoeba proteus, for example, the membranes of the complex NE are believed to be present in all stages but discontinuous in metaphase, while many of the fragments closely surround the mitotic spindle (26). The inner, thick, honeycomb layer of the NE completely disappears before metaphase, however, and reappears after telophase when nuclear reformation is in progress.

Reformation of the NE begins early in telophase. The process seems to be basically a reversal of the fragmentation process at the start of mitosis (9, 21). The origin of the reformed NE is unknown but it is generally speculated that it arises from elements of the cytoplasmic membrane system (21, 22). A strict de novo formation of the envelope cannot be excluded (16), however, and it is also possible that the vesicles initially associated with telophase chromosomes may act to “seed” the synthesis of new membrane along the chromosome surface.

This paper provides new kinds of evidence concerning the fate and origin of the NE during and after mitosis of A. proteus. Much of our evidence was obtained by transplanting nuclei, whose membrane phospholipids were specifically labeled with radioactive choline, into unlabeled cytoplasms and then following the fate of the label under different conditions.

MATERIALS AND METHODS

Organisms and Culture Methods

The experimental organism, A. proteus, used in this study was cultured according to the method of Prescott and Carrier (24), except that the culture medium contained: 3.7 \times 10^{-8} \text{ M CaHPO}_4, 8 \times 10^{-3} \text{ M KCl}, and 1.6 \times 10^{-4} \text{ M MgSO}_4.

Procedures for Labeling Amebas

Amebas were labeled with \(^{[3H]}\)choline (\(^{[methyl-3H]}\)choline chloride, 2.34 Ci/mmol, New England Nuclear, Boston, Mass.) by incubating the cells in a medium containing radioactive materials, usually at a concentration of 8 \(\mu\text{Ci/ml}\), in the presence of unlabeled Tetrahymena pyriformis added as a food organism. An ameba labeled in this way will be called a directly labeled ameba. (Amebas could not be satisfactorily labeled with radioactive choline by feeding them Tetrahymena that had been labeled by adding 25 \(\mu\text{Ci/ml}\) of \(^{[3H]}\)choline to the defined medium of Elliott et al. [5] in which the Tetrahymena were grown.) Before being used in experiments the labeled cells were usually “chased” for 2 days in ameba medium containing no radioactive materials.

To measure the effect of emetine on protein synthesis in amebas, starting in early mitosis amebas were labeled, in the presence or absence of emetine, by feeding them for 4 h with Tetrahymena that had been labeled by growth in a defined medium (5) containing \(^{[3H]}\)leucine (30.8 Ci/mmol, 60 \(\mu\text{Ci/ml}\)), \(^{[3H]}\)lysine (3 Ci/mmol, 20 \(\mu\text{Ci/ml}\)), \(^{[3H]}\)tryptophan (3.4 Ci/mmol, 20 \(\mu\text{Ci/ml}\)), \(^{[3H]}\)valine (2.88 Ci/mmol, 20 \(\mu\text{Ci/ml}\)), \(^{[3H]}\)alanine (30.3 Ci/mmol, 60 \(\mu\text{Ci/ml}\)), and \(^{[3H]}\)tyrosine (42.9 Ci/mmol, 60 \(\mu\text{Ci/ml}\)). All radioactive amino acids were furnished by New England Nuclear.

Nuclear Transplantation

Nuclei were transplanted according to the method described by Jeon and Lorch (13). The cells that survived the nuclear transplantation operations were maintained for various periods of time in ameba medium at 25°C with daily feedings of Tetrahymena until they were fixed for autoradiography.

Isolation of Nuclei

Nuclei were isolated according to the method described by Goldstein and Prescott (11).

Radioactive Assay

For radioactive assay, nuclei were placed on planchets which were then treated with 10% TCA and distilled H_2O for lipid-labeled nuclei or 10% TCA, 95% ethanol, and 100% ethanol for protein-labeled nuclei, followed by treatment with concentrated formic acid to achieve maximum spreading of the material on the planchet to minimize sample self-absorption of \(^{[3H]}\)beta particles. The radioactivity on the planchets was determined in an automatic, low-background (~3–5 cpm), gas-flow, windowless Geiger counter that assayed \(^{3H}\) with an efficiency of ~20%.
Synchronization of Amebas

In order to obtain synchronized amebas, dividing cells (from stock ameba cultures maintained at 16°C) were transferred with a braking pipette into a small culture dish, fed daily, and cultured with a daily change of medium containing "fresh" Tetrahymena. Within 30 min after the initial transfer all the cells finished their first division. Usually, the second division occurred (with poor synchrony) 4–5 days after the first division, a relatively long generation time under these culture conditions.

Cytological Fixation

In preparation for cytological examination, cells were fixed and flattened on a slide by dropping a cover slip with a small drop of 45% acetic acid on its underside onto cells on a slide and a few minutes later placing the slides on solid CO₂ (10). After the fixative was frozen, the cover slips were flicked off and the slides were placed in a mixture of 3 parts absolute ethanol and 1 part acetic acid for 5–10 min, in 95% ethanol for 5 min, in absolute ethanol for 5 min, and finally air-dried.

Autoradiographic Techniques

For most experiments, autoradiography was executed by dipping slides in Kodak liquid emulsion NTB-3 (Eastman Kodak Co., Rochester, N. Y.) and, after suitable exposure in the dark, developing by standard procedures. Slides were stained through the emulsion with Giemsa stain at pH 7.0 and permanently mounted with euparal.

 Autoradiographic Grain Counting

Autoradiographic grain counts were made at a magnification of about 400 with a squared grid in the eye piece. For assays of nuclei, grains were counted over 80–100% of the nuclear area. Cytoplasmic counts, which generally involved areas twice as large as the nuclear area, were made over randomly selected areas at least one nuclear diameter away from the nucleus. The area near the nucleus, which comprised 10–20% of the cytoplasm, was excluded because occasionally some "leakage" of radioactivity from the nucleus occurred; i.e., there were patches of radioactivity adjacent to the nucleus that suggested some (pre- or postfixation) irregularity about the appearance of this label in the cytoplasm. All counts were corrected for background.

Procedure for Lipid Isolation and Analysis

Lipids were isolated from the labeled nuclei, as well as the whole cells, as described by Bligh and Dyer (1). Analysis of radioactive lipid fractions was performed by Silica Gel-G (Van Waters & Rogers, Inc., San Francisco, Calif.) thin-layer chromatography with chloroform-acetic acid-methanol-water (75:25:5:2.2 by volume) as the developing solvent, using egg yolk lipids as markers for the individual phospholipids. For assay of radioactivity, the chromatographic spots were extracted with the solvent and placed on planchets. Phosphorous content of phospholipids was determined by the method of Chen et al. (3) for the extracted material of each chromatographic spot.

RESULTS

The Localization of Nuclear Phospholipid Labeling

When amebas are directly labeled with the lipid precursor [³H]choline, the radioactivity is found to be distributed generally throughout the cell (bottom of Table I). However, when nuclei from such cells are transplanted to unlabeled enucleated cytoplasms, for most of the cell cycle by far the highest concentration of label is associated with nuclei (Table I). (In fact, as will be noted later, this concentration differential persists for at least two cell generations.) Since in all subsequent experiments considered here only unlabeled cells possessing [³H]choline-labeled nuclei were employed, we

| TABLE I |
| Distribution of Radioactivity in Cells that had Received [³H]Choline-Labeled Nuclei |
| Mean grain count/135 μm² | Ratio of grain densities (nucleus:cytoplasm) |
| Nucleus | Cytoplasm | (n = 10) | (n = 10) |
| 18.1 | 1.1 | 16.4 |

Cells were labeled with [³H]choline (8 μCi/ml) for 3 days (the 3rd, 4th, and 5th days after cell division) and chased for 2 days. The labeled nuclei were then transplanted into unlabeled, enucleated cells, which were fixed 2 days after the operation.

| Distribution of Radioactivity in [³H]Choline-Labeled Cells* |
| Mean grain count/135 μm² | Ratio of grain densities (nucleus:cytoplasm) |
| Nucleus | Cytoplasm | (n = 5) | (n = 5) |
| 15.8 | 33.0 | 0.48 |

Cells were labeled with [³H]choline (8 μCi/ml) for 2 days (the 4th and 5th days after the first cell division) and chased for 4 days before fixation for autoradiographic study.

* These cells were equivalent to the nuclear donors of the above experiment.
assume that all the following observations are primarily of nuclear-specific lipids.

We believe that almost all of this nuclear-specific label is in the NE but have not demonstrated that conclusively. In fact, however, there is some controversy about the proportion of nuclear lipids that is membranous (17). Our attempts to localize the label by autoradiography of sectioned nuclei have been largely frustrated by the solubility of the label in media used for the processing of cells for embedding. Nevertheless, preliminary observations of Araldite-embedded material suggest that almost all the nuclear [SH]choline label is in the NE. Further evidence that at least a majority of this nuclear label is in the NE is the finding that, in a solution of 0.5 ml Triton X-100 and 4 mg spermidine-HCl per 100 ml, nuclei lose over half their [SH]choline label. Nuclei isolated in such a medium are known to have lost their outer but not their inner nuclear membranes (K. G. Murti, personal communication).

Of the acid insoluble-labeled material in [H]choline-labeled nuclei, 95% is present as lipid. Table II shows in what lipids this label is distributed and also shows that 82% of the total nuclear lipid labeled in this way is in phosphatidyl choline. Taken together with the above observations, these data demonstrate that the bulk of [H]choline labeling is in NE phospholipids, in agreement with earlier findings of Chlapowski and Band (4) for Acanthamoeba.

It is important to note, however, that the purpose of this study was not to investigate the metabolism of membrane lipids. [3H]Choline was used in these experiments as the most specific agent available for labeling the NE. This specificity was enhanced by transferring nuclei labeled in this way to unlabeled cells, thereby minimizing the amount of non-nuclear [H]choline label in the experimental cells.

**Time in the Cell Cycle that NE Phospholipids are Labeled**

Fig. 1 shows the pattern of nuclear incorporation of [H]choline during the A. proteus cell cycle. (The A. proteus cell cycle has no G1; S immediately follows mitosis and proceeds for 6–8 h, and the remainder of the cell cycle is G2.) Most nuclear incorporation obviously occurs in the latter part of G2; little incorporation is detectable during mitosis or S. It follows, then, that, if no NE phospholipid synthesis occurs during mitosis, preexisting phospholipids are used in postmitotic NE formation.

<table>
<thead>
<tr>
<th>Distribution in Acid-Insoluble Materials of Incorporated [H]Choline</th>
<th>cpm/100 acid-insoluble nuclei radioactivity % of total acid-insoluble radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid-insoluble fraction</td>
<td>1,407</td>
</tr>
<tr>
<td>Total lipid fraction</td>
<td>1,337</td>
</tr>
<tr>
<td>Phosphatidyl choline</td>
<td>1,090</td>
</tr>
<tr>
<td>Neutral lipids</td>
<td>139</td>
</tr>
<tr>
<td>Phosphatidyl ethanolamine</td>
<td>80</td>
</tr>
<tr>
<td>Unidentified lipids</td>
<td>28</td>
</tr>
</tbody>
</table>

Amebas were labeled with [H]choline (8 μCi/ml) for 2 days (4th and 5th days after division) and chased for 2 days. Labeled nuclei then were transplanted into unlabeled cells. 1 day later the lipids were extracted by the method of Bligh and Dyer (1) from the recipient cells. Separation of radioactive lipid fractions was performed by Silica Gel-G thin-layer chromatography.
FIGURES 2–7 Autoradiograms of cells that had been fixed and flattened as described in Materials and Methods. Differences in apparent sizes of nuclei are due in substantial part to differences in degree of flattening during fixation.

FIGURE 2 Autoradiograms of amebas which had received a [3H]choline-labeled nucleus 1 day (upper) and 4 days (lower) before fixation. × 700.
FIGURE 3  Autoradiograms of amebae fixed at various times during mitosis. In each case mitosis occurred 2 or 3 days after implantation of an [3H]choline nucleus. N, nucleus; C, chromosome; F, food vacuole. Fig. 3a, early prophase. Fig. 3b, late prophase. Fig. 3c, prometaphase. Fig. 3d, late anaphase. Fig. 3e, late telophase. Fig. 3f, early postdivision. × 700.
is seen to be still localized over the nuclei at a concentration much above that in the cytoplasm, indicative of only minor turnover and redistribution of NE phospholipids, at least as far as recycling within the same cell is concerned.

Redistribution of NE Phospholipids During and After Mitosis

[3H]Choline-labeled nuclei were grafted into enucleate, unlabeled cytoplasms and the postoper-
ative cells were fed unlabeled *Tetrahymena* until they entered division. At various stages of mitosis the amebas were fixed and processed for autoradiography; Fig. 3 shows the distribution of radioactivity at these stages. Most phospholipid radioactivity is seen to be concentrated in the nucleus during early prophase. In late prophase some radioactivity begins to appear in the cytoplasm, although the nucleus continues to be the most radioactive part of the cell. During metaphase and anaphase, when the NE has almost entirely disappeared, the radioactivity is dispersed evenly over
the cytoplasm. By telophase the radioactivity begins to be again significantly concentrated around chromosomes, although little NE reconstruction is as yet visible. Fig. 3f shows an autoradiogram of two daughter cells derived from a mother cell into which had been transplanted a \[^3H\]choline-labeled nucleus and reveals that labeled phospholipids, which were in the cytoplasm during mitosis, are almost exclusively in and evenly distributed between the two daughter nuclei, and that little labeled phospholipid remains in the cytoplasm.

These observations lead us to conclude that NE phospholipids are dispersed to the cytoplasm when apparent NE dissolution occurs during metaphase and anaphase and that the same phospholipids are reassembled into the NEs of the daughter nuclei from telophase on.

**Internuclear Exchange of NE Phospholipids as a Consequence of Mitosis**

In order to better understand the extent of NE phospholipid conservation from one cell generation to the next and to determine whether actual continuity of the NE structure is maintained through mitosis, \[^3H\]choline-labeled nuclei were transplanted into unlabeled interphase cells and such binucleate cells were allowed to grow and divide. After division the distribution of radioactivity among the four daughter nuclei per binucleate cell was ascertained by autoradiography. Fig. 4 and Table III show the typical finding, i.e., that the label is distributed essentially equally among the four daughter nuclei. Since in such a binucleate cell there are two separate mitotic apparatuses formed during division (L. Goldstein, unpublished experiments) (14) and since little exchange of labeled NE phospholipid between labeled and unlabeled nuclei occurs during interphase (Fig. 5 and Table IV), the above finding must mean that the premitotic NE phospholipids are completely dispersed during cell division and are randomly distributed to the NEs of the daughter nuclei. This also suggests that no structural continuity of the NE persists through mitosis and confirms the conclusion from the preceding experiment that NE phospholipids are preferentially reutilized in the formation of nuclear structures.

To examine another facet of possible exchanges of NE materials between nuclei, \[^3H\]choline-labeled nuclei were implanted into unlabeled cells already in mitosis, and 4 h later the cells were fixed and processed for autoradiography. First, we should note that as a consequence of this operation the completion of a cytokinesis was inhibited in all cases. Nevertheless, approximately half of the cells were found to have three nuclei (two small and one large), an indication that the host cell nucleus was able to complete mitosis. In the other half of the cells, the completion of mitosis apparently was prevented, since they contained only two (both large) nuclei. Most likely in the latter cases the labeled nuclei had been implanted at earlier stages than was the case in the cells that were able to complete mitosis.

The radioactivity distribution in both kinds of cells that had received labeled nuclei during mitosis was something of a surprise. Although there is no indication of any NE breakdown in the grafted nuclei (also observed by Feldherr [6] earlier), there occurred a substantial turnover of NE phospholipids as evidenced by the acquisition of label by the host nuclei (Fig. 6), whether they completed mitosis or not. This is in marked contrast to the minor exchange that occurs between two interphase nuclei (Fig. 5). The exchange of \[^3H\]choline-labeled material is generally less than that which is observed when the grafted radioactive nucleus enters mitosis at the same time as the unlabeled host cell nucleus. However, occasionally in those cells that have three nuclei the exchange approximates that of the latter situation, in that the two obviously postmitotic nuclei are as radioactive as the grafted nucleus. Since these observations imply that the state of the mitotic cytoplasm is responsible for this heightened turnover of NEs, it is likely that if mitosis had persisted longer, a complete exchange of NE phospholipids between the grafted nucleus and postmitotic nuclei would have occurred in most cases.

**Effect of a Protein Synthesis Inhibitor on NE Reformation**

Amebas in division were placed in emetine, at concentrations up to \(10^{-3} M\), and the effect on mitotic processes was observed. Although cleavage furrow formation was largely normal, the separation into two daughter cells could not be completed, and the furrow subsequently regressed. These cells, however, were found to be binucleate, an indication that emetine did not inhibit mitosis. Since the NEs of the daughter nuclei seemed essentially normal (Fig. 7) (12) (although electron microscope studies would be more useful in such a

**Hiroshi Maruta and Lester Goldstein**

**Nuclear Envelope in Amoeba proteus** 639
TABLE III
Distribution of Radioactivity Among the Four Progeny Nuclei of a Binucleate Cell that had Received an [3H]Choline-Labeled Nucleus Before Mitosis

<table>
<thead>
<tr>
<th>Daughter nuclei</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Cytoplasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean grain count/135 μm²</td>
<td>18.1</td>
<td>16.8</td>
<td>16.5</td>
<td>14.2</td>
<td>4.4</td>
</tr>
<tr>
<td>Ratio of mean nuclear grain densities</td>
<td>A:B:C:D</td>
<td>1.3:1.2:1.2:1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[3H]Choline-labeled nuclei were transplanted into unlabeled nucleate cells and each binucleate cell was separately incubated until it divided. Shortly thereafter all the progeny cells were fixed and subjected to autoradiography. (For each set of four nuclei, the nuclei were arbitrarily placed in the order A, B, C, D, in descending order of radioactivity.)

That emetine is an effective inhibitor of protein synthesis is shown by our finding that a concentration of 10^{-3} M inhibits at least 97% of the incorporation of labeled amino acids in acid-insoluble material (Table V). This experiment is not decisive, however, since cells in mitosis do not feed and hence would not be expected to incorporate [3H]amino acids even in the absence of emetine. Since this means that we may have been determination), it appears that emetine had little effect on NE formation. Thus, if emetine blocks ameba protein synthesis, we might conclude that newly forming NEs reutilize proteins of the parental NE, as is the case for phospholipids. Lacking the equivalent autoradiographic evidence showing a reutilization of NE proteins, however, the possibility exists that the material came from a predivision cytoplasmic pool of NE proteins.

FIGURE 4 A [3H]choline-labeled nucleus was grafted into an unlabeled nucleate cell, which 5 days later divided into one binucleate and two mononucleate cells that were fixed shortly thereafter. (Binucleate cells divide into two, three, or four cells but the progeny always possess a total of four nuclei.) Upper and middle photos show parts of the two mononucleate cells containing the nuclei. Lower photo shows the binucleate cell, but one of the nuclei was obscured and is not seen. Note the similarity in autoradiographic grain density over all nuclei. × 700.

FIGURE 5 Autoradiogram of an (now binucleate) interphase cell into which a [3H]choline-labeled nucleus had been grafted 24 h before fixation. × 700.
TABLE IV
Distribution of Radioactivity in Cells that had Received [3H]Choline-Labeled Nuclei

<table>
<thead>
<tr>
<th>Mean grain counts/135 μm²</th>
<th>Ratio of mean nuclear densities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor nucleus Host nucleus Cytoplasm</td>
<td>Donor nucleus:Host nucleus</td>
</tr>
<tr>
<td>25.3 (n = 10) 1.8 (n = 10) 1.2 (n = 10)</td>
<td>14.1:1</td>
</tr>
</tbody>
</table>

[3H]Choline-labeled nuclei were transplanted into unlabeled cells and each binucleate was separately incubated until the cells cited in Table III had divided, at which time they were fixed; the above cells had not divided after the operations.

observing only the effects on protein synthesis after telophase, the possibility cannot be excluded that some (undetectable) newly synthesized proteins are being incorporated into newly forming NEs.

DISCUSSION

Since phospholipids are not incorporated into amoeba NEs during mitosis or shortly thereafter, apparently previously synthesized materials are used in the formation of NEs during telophase. A similar conclusion about proteins is suggested by the finding that emetine, at concentrations that inhibit nuclear protein synthesis by at least 97%, does not prevent the formation of NEs at the end of mitosis, but, as noted above, the emetine experiments are not unambiguous.

The various experiments on the fate of labeled NE phospholipids of transplanted nuclei not only show that previously synthesized material is utilized by newly forming NEs, but they decisively demonstrate that the old NE phospholipids are used by the new NEs. Unfortunately, equivalent experiments on the behavior of NE proteins cannot be done at present because we lack the ability to label specifically the NE proteins and no other components of the nucleus. Until that is achieved, we will not know whether NE lipid-protein complexes remain intact through mitosis, but that seems to be a reasonable possibility. Since almost all the proteins of the A. proteus nucleus are released to the cytoplasm during mitosis and return, apparently in their entirety, to the postdivision nuclei (2, 23, 25), it is reasonable to infer that the NE proteins are a part of these returning nuclear proteins.

Our observations do not exclude the possibility that cytoplasmic membrane systems like the ER contribute to some extent to the newly forming NEs. Such a possibility has been indicated by electron microscope observations of the role of the ER in the formation of new NEs (22) and in the repair of microsurgically damaged nuclei (7, 8). If new NEs arise only from the ER, our results would have to be interpreted as revealing that old NE components are first taken up by the ER (and probably a very select part of it) and then transmitted in their entirety to the new NEs.

Our results seem to refute earlier notions about the structural continuity of the NE through mitosis. In electron microscope studies of dividing A. proteus, Roth et al. (26) observed that during most of mitosis the inner NE honeycomb layer is absent and that gaps are present in the NE double membrane. These workers believed that little further NE dissolution occurs and suggested that some continuity of outer membranes persists throughout mitosis (perhaps via submicroscopic connections where the gaps are observed), and this would certainly account for our findings that "parental" NE material is conserved in the progeny NEs. However, Roth et al. (26) report no observations between metaphase and late anaphase (which appears to us from Fig. 13 to be telophase), and so it cannot be excluded that the NE disappears entirely during anaphase.

We suspect that no continuity of the NE persists through mitosis, for the following reasons. Our autoradiographic studies of mitotic cells that had earlier received [3H]choline-labeled nuclei show no localized phospholipid between metaphase and late anaphase. One might argue that [3H]choline is incorporated only into the honeycomb layer (which does disappear entirely during mitosis) and, thus, that the absence of localized radioactivity says nothing about the continuity of the outer membranes. This seems improbable to us, but more importantly recent preliminary experiments show that when [3H]choline-labeled nuclei are placed in Triton X-100, which removes the outer nuclear membrane (K. G. Murty, personal communication), more than half of the radioactivity is lost. Loss of NE continuity is more convincingly demonstrated by the finding that when a cell that possesses a [3H]choline-labeled nucleus and an unlabeled nucleus goes through mitosis, the result-
FIGURE 6 Autoradiograms of cells that were fixed 4 h after implantation of [3H]choline-labeled nuclei. The (unlabeled) recipients were in mitosis at the time they received the labeled nuclei. H, host nuclei; D, donor nuclei. Fig. 6 a shows a binucleate cell, which apparently did not complete mitosis after implantation of the nucleus, and Fig. 6 b shows a trinucleate cell, which did complete mitosis after implantation of the nucleus. × 900.
FIGURE 7 Binucleate cell created by emetine treatment. The cells in early mitosis were treated with emetine (2 x 10^{-3} M) and fixed 1 h later. The two daughter nuclei were formed normally but cytokinesis was inhibited. × 700.

### TABLE V

<table>
<thead>
<tr>
<th>Emetine concentration</th>
<th>Experiment No.</th>
<th>Mean cpm per nucleus</th>
<th>Inhibition of amino acid incorporation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 M</td>
<td>1 (n = 80)</td>
<td>577</td>
<td>--</td>
</tr>
<tr>
<td>10^{-4} M</td>
<td>1 (n = 40)</td>
<td>12</td>
<td>98</td>
</tr>
<tr>
<td>0 M</td>
<td>2 (n = 80)</td>
<td>777</td>
<td>--</td>
</tr>
<tr>
<td>10^{-5} M</td>
<td>2 (n = 40)</td>
<td>21</td>
<td>97</td>
</tr>
</tbody>
</table>

Amebas in early mitosis were fed [3H]amino-acid-labeled *Tetrahymena* (see Materials and Methods) in the presence or absence of emetine. 4 h later the nuclei were isolated in a Triton X-100 and spermidine solution, placed on planchets, extracted with cold 10% TCA, rinsed in 95% and 100% ethanol, and assayed for radioactivity. Nuclei, rather than whole cells, were assayed because the cytoplasm obviously contained undigested radioactive material in food vacuoles, whereas the nuclei were “clean” of any such contamination.

* In acid-insoluble material.

Since there was little mixing of the radioactivity between the two predivision nuclei, this must mean that all the NE-labeled material is dispersed to the cytoplasm during mitosis and is incorporated by any and all telophase nuclei, a seemingly compelling indication that there is no persistent structural continuity of the NE.

But such a conclusion cannot be decisive because we have found that mitotic cytoplasm can induce a marked turnover of NE phospholipid of an implanted interphase nucleus without any microscopically perceptible breakdown of that nucleus’ NE. That mitotic cytoplasm stimulates a marked turnover of NE phospholipids of an interphase nucleus implanted into a dividing cell, even though there is no effect on the fine structure of nuclei’ NE (6), is surprising. Thus, Feldherr’s conclusion that “the cytoplasm contains no factor capable of disrupting the nuclear envelope” cannot be applied to events that occur at the molecular level (6). That certain cellular factors, present discontinuously through the cell cycle, can stimulate the replacement of structural subunits without causing a breakdown of the structure is a most unexpected finding. It suggests, beside the need for this cytoplasmic factor, that the NE may have to reach a certain “maturity” before that factor can effect substantial NE dissolution. Several other studies have demonstrated the existence of cytoplasmic factors...
responsible for NE disruption (cf., 15, 18, 19) but all have been concerned with effects on structure and none have dealt with effects on the molecular subunits of NE structure.

The authors thank Christine Ko and Eunice Wang for their advice and help throughout this investigation. Drs. David M. Prescott and Andrew Staehein also provided valuable advice during the course of this study.

This investigation was supported by a United States Public Health Service International Postdoctoral Research Fellowship (I-F05-TW-2072-01) to Hiroshi Maruta and by National Institutes of Health grant GM 15156 to Lester Goldstein.

Received for publication 15 October 1974, and in revised form 24 February 1975.

REFERENCES