NUCLEOCYTOPLASMIC EXCHANGES DURING CELL DIVISION

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INTRODUCTION

During interphase, macromolecular exchanges between the cytoplasm and nucleoplasm are regulated, to a large extent, by the nuclear envelope (1). When mitosis (nuclear division) occurs, generally accompanied by the breakdown of the envelope, an opportunity exists for nucleocytoplasmic exchanges to occur which perhaps could not take place during interphase. The present investigation is concerned with one aspect of this problem, that is, the extent to which cytoplasmic material can enter daughter nuclei during and shortly after mitosis.

The experimental method employed in this study involved microinjecting colloidal gold into the cytoplasm of both interphase and dividing amebas. At specific times after injection the cells were fixed and subsequently examined with the electron microscope, thus permitting visualization of individual colloidal particles. The general distribution of the gold and also the size of particles in the nucleoplasm were then determined. The results indicate that cytoplasmic substances can enter newly forming nuclei to a significantly greater extent than interphase nuclei.

MATERIALS AND METHODS

All experiments were performed on multinucleated amebas (*Chaos chaos*) which were cultured in an inorganic salt solution and fed *Paramecium aurelia* (2).

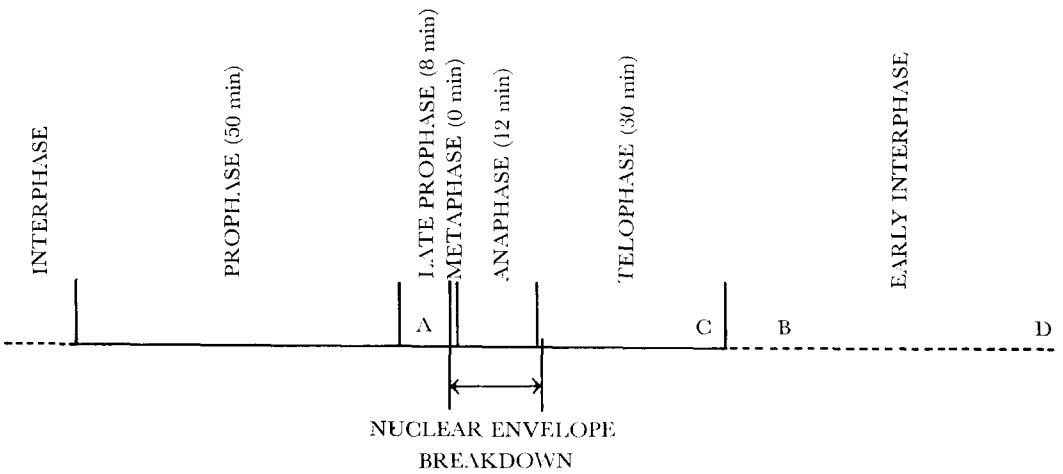

FIGURE 1 Stages of nuclear division in *C. chaos* as described by Kudo (4). The numbers in parenthesis indicate the approximate time of each phase. The letters (*A* to *D*) along the bottom of the outline refer to specific experimental periods (see Results).

Nuclear division in *C. chaos* has been studied by Roth and Daniels (3) and by Kudo (4). An outline of the process is shown in Fig. 1. Each ameba contains several hundred nuclei, all of which undergo mitosis synchronously. During cell division there is a correlation between the general appearance of the amebas and the state of the nuclei; thus, organisms containing nuclei in specific phases of mitosis can be readily identified in mass cultures.

Colloidal Gold, Microinjection, and Electron Microscopy

These techniques have been described in earlier reports (1, 2) and will be considered only briefly.

The colloidal preparations used in this investigation contained polyvinylpyrrolidone (PVP)-coated gold particles, ranging in diameter from approximately 30 to 170 A (L-fraction, reference 1). Before injection, the preparations were dialyzed against a medium containing 0.0127 M KCl, 0.0016 M K_2HPO_4, and 0.0011 M KH_2PO_4 (pH 7.0–7.1). The sols had apparent optical densities ranging from 29 to 34 (λ = 500 mμ, 1-cm light path).

The microinjection techniques were essentially the same as those described by Chambers and Kopac (5). In this study, no effort was made to inject a specific volume of sol into each ameba. It has already been demonstrated (1, 2) that the injection of PVP-coated gold particles does not affect the normal functioning of the amebas with regard to movement, feeding, and division.

In preparation for electron microscopy, the amebas were fixed for 10 min in 1% OsO_4 (pH 7.4), dehydrated in alcohol, and embedded in Araldite. Sections, approximately 1000 A in thickness, were then cut and examined with an RCA-EMU-3C. It has been shown that gold particles do not redistribute between the nucleus and cytoplasm during fixation (6). Thus, the distributions observed with the electron microscope are the result of nucleocytoplasmic exchanges which occurred while the cells were in the living state.

Particle Counts and Measurements

Amebas were injected at different times in the cell cycle and fixed after about 50 min. The relative rates at which the gold particles were incorporated into the nucleoplasm under different experimental conditions were determined by making particle counts. To compensate for the fact that different amounts of gold were injected into each ameba, counts were obtained for both the nucleoplasm and cytoplasm, and the results were expressed as the ratio of gold particles in the two regions of the cell.

In the first step of the counting procedure, electron micrographs were prepared (× 70,000) showing a region of nucleoplasm and a region of cytoplasm from the same section. Next, the nucleoli, mitochondria, and food vacuoles were cut out of the prints. These organelles, if not removed, could introduce a source of error since they tend either to exclude (nucleoli and mitochondria) or concentrate gold particles (food vacuoles). The remaining portions of the prints were adjusted so that equal areas of nucleoplasm and cytoplasm were obtained. This was done by randomly cutting off pieces of the heavier print until the weights of the two micrographs were equal. All of the gold particles in both the nucleoplasm and

TABLE I

*Particle Counts and Measurements**

Ameba	No. of nuclei examined	Particle Counts: Tot. volume examined (μ^3)	Particle Counts: No. of Particles in nucleoplasm	Particle Counts: No. of particles in cytoplasm	Particle Counts: Ratio nuc:cyto.	Size Distribution in Nuclei: 0–35 A	35–70 A	70–105 A	105–140 A	>140 A
A. Interphase										
1	3	1.9	81	233	1:2.9	11	29	21	1	0
2	6	3.1	531	1,015	1:1.9	7	111	77	5	0
3	7	4.7	525	668	1:1.3	1	60	56	2	0
4	7	4.8	690	1,056	1:1.5	52	313	77	2	0
5	4	6.7	240	893	1:3.7	2	77	62	4	0
6	6	9.8	590	3,017	1:5.1	5	212	128	5	0
7	4	6.7	252	699	1:2.8	4	107	60	8	1
Total	37	37.7	2,909	7,581	1:2.6	82	909	481	27	1
B. Early division										
1	6	3.9	3,439	517	6.6:1	1	333	192	22	3
2	5	3.5	3,169	358	8.9:1	5	184	135	16	0
3	10	7.4	2,843	347	8.2:1	0	101	211	26	2
4	2	2.4	1,699	298	5.7:1	1	105	131	30	2
Total	23	17.2	11,150	1,520	7.3:1	7	723	669	94	7
C. Cytokinesis										
1	3	4.6	3,853	938	4.1:1	3	83	142	36	6
2	6	9.3	2,408	579	4.2:1	0	222	167	26	3
3	6	9.6	4,421	978	4.5:1	0	108	149	16	0
4	6	9.9	1,549	767	2:1	0	124	118	17	1
5	6	6.9	10,539	1,645	6.4:1	1	120	133	22	3
Total	27	40.3	22,770	4,907	4.6:1	4	657	709	117	13

* Fifteen preliminary experiments were also performed in which amebas were injected at different times in the cell cycle. The results, although not analyzed in detail, were consistent with those presented here.

cytoplasm were then counted and the results recorded separately.

The particle counts given for each ameba (see Table I) were obtained by repeating the above procedure 2 to 10 times and combining the results. The values for "Total volume examined," shown in Table I, are estimates arrived at by adding the weights of all the prints used in the analysis of a particular ameba and dividing by the weight which corresponds to 1 μ^3. Using a final magnification of 70,000 and a value of 1000 A for the average section thickness, a volume of one cubic micron corresponds to a 490 cm^2 print.

The sizes of gold particles in the nuclei were obtained directly from the electron micrographs. For each series of experiments (see Results) 1500 randomly selected particles were measured. The measurements which are reported refer only to the dimensions of the colloidal particles; to correct for the thickness of the PVP coat it would be necessary to add 20 to 40 A to the given values (1).

RESULTS

In the initial series of experiments, amebas were injected during interphase and fixed after 45 to 55 min. The results, with regard to particle size and distribution, are shown in Table IA and Fig. 2. It can be seen that in every instance the particles were more numerous in the cytoplasm, the average concentration being 2.6 times greater than in the nucleoplasm.

In a second series of experiments, amebas were injected during late prophase, that is, just before the breakdown of the nuclear envelope (point *A*, Fig. 1). These amebas continued to divide and underwent cytokinesis 35 to 45 min after injection. The cells were fixed 2 to 20 min after the com-

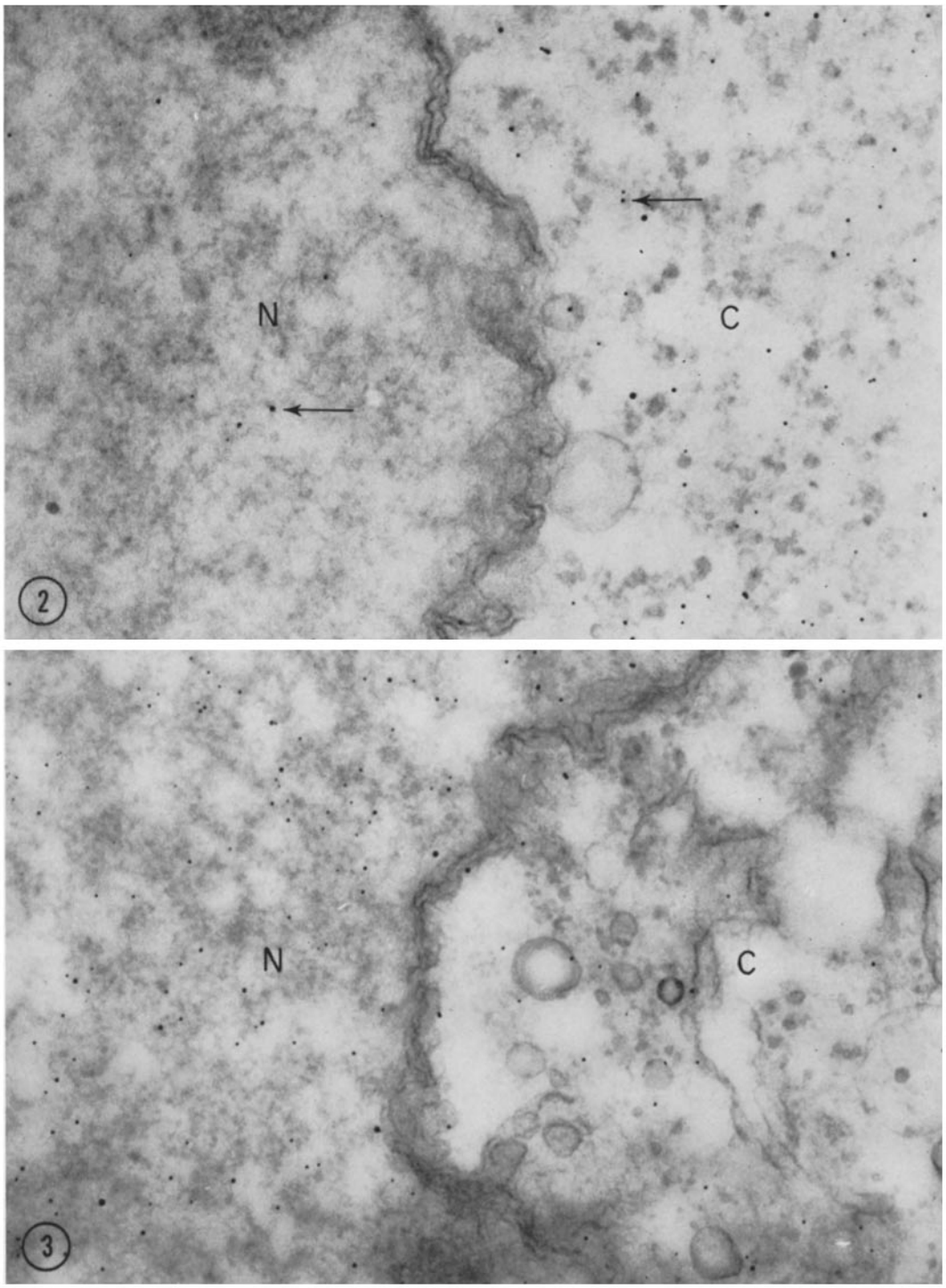

FIGURE 2 An electron micrograph of an ameba injected during interphase and fixed after approximately 50 min. Gold particles (arrows) are present in both the nucleoplasm (*N*) and cytoplasm (*C*) but are concentrated in the cytoplasm. × 70,000.

FIGURE 3 A section through an ameba injected during cytokinesis and fixed after 50 min. In this instance the gold particles are concentrated in the nucleoplasm (*N*). *C*, cytoplasm. × 70,000.

pletion of cytokinesis (point *B*, Fig. 1); thus, the total time between injection and fixation was approximately the same as in the interphase experiments. The results are given in Table IB. In all cases the concentration of gold in the nucleoplasm was considerably higher than in the cytoplasm, the average ratio being 7.3:1. When the over-all values are compared to the interphase results, it becomes apparent that (1) during or shortly after mitosis gold particles enter the nucleoplasm at a much greater rate; and (2) particles larger than about 70 A enter the nuclei more readily during division. These differences in the size distributions are significant ($P < 0.001$ using the $2 \times j$ table).

Experiments were then performed to determine whether the increase in the uptake of material by dividing nuclei is restricted to the period of nuclear envelope breakdown (metaphase to early telophase). Amebas were injected during late cytokinesis (point *C*, Fig. 1), by which time the nuclear envelopes have completely reformed. The cells were fixed 50 min after injection (point *D*, Fig. 1) and the size and distribution of the gold determined. The results of these experiments, shown in Table IC and Fig. 3, are comparable to those obtained for cells injected during late prophase. In both cases, gold particles were concentrated in the nuclei and, in addition, the size distributions were not significantly different ($0.05 < P < 0.1$). Therefore, the exchanges which occur after reformation of the envelopes can account for the increase in the number and size of the particles found in dividing nuclei. Thus, there is no evidence to indicate that there are specific exchanges which occur only while the nuclear envelope is broken down.

DISCUSSION

It has been found that during and shortly after cell division the nuclei are capable of incorporating cytoplasmic material at a much greater rate than the nuclei of interphase cells. Accompanying the increase in rate, there also appears to be an increase in the size of particles which can enter newly forming nuclei.

The present experiments indicate that the period of nuclear envelope breakdown is not of special importance with regard to the uptake of material by the nucleoplasm. This is consistent with electron microscope studies of dividing cells. Such studies have shown that the nuclear envelope reforms at the surface of the chromosomes (e.g., 7–9), suggesting that cytoplasmic substances are excluded from the daughter nuclei at the time of reconstruction.

There are several explanations which could account for the differences in the nucleocytoplasmic exchanges which occur shortly after mitosis and later in interphase. The most likely explanation is that the newly formed envelopes are more permeable than those of older cells. If the permeability of the envelopes does vary at different times in the cell cycle, one might expect to find corresponding structural changes. Efforts to detect such changes have so far been unsuccessful. These studies are being continued, however, particularly with reference to the electron-opaque pore material which is probably of special importance in regulating the nucleocytoplasmic exchanges of macromolecules (1).

At present, there is no satisfactory explanation to account for the fact that gold particles accumulate in the nuclei of *C. chaos*. It should be pointed out, however, that the ability to concentrate gold is not restricted to the nuclei of dividing cells. In long-term experiments (24 hr), similar concentrations have been found in interphase nuclei (2).

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