BINDING WITHIN THE NUCLEAR ANNULI AND ITS POSSIBLE EFFECT ON NUCLEOCYTOPLASMIC EXCHANGES

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INTRODUCTION

Evidence exists which indicates that the pores of the nuclear envelope are important pathways for macromolecular exchanges between the nucleoplasm and cytoplasm (1–4). The pores as seen with the electron microscope often contain an electron-opaque material (5–7), and it has been suggested that the presence of such pore material could affect nucleocytoplasmic exchanges (8). Investigations in which the passage of colloidal particles through the nuclear annuli of intact amebas was studied support this hypothesis, and further suggest that the colloidal particles bind to the material within the pores (3). If such binding does occur, it could be an important factor in controlling macromolecular exchanges between the nucleus and cytoplasm.

To determine the extent to which binding to the pore material can occur, experiments were conducted in which isolated nuclei or isolated nuclear envelopes were treated with protected colloidal gold particles and subsequently examined with the electron microscope.

MATERIALS AND METHODS

All experiments were performed on the nuclei of mature oocytes obtained from *Rana pipiens*. The nuclei were isolated by hand in a medium containing 0.102 M KCl, 0.011 M NaCl and 0.012 M potassium phosphate buffer. The pH of the medium, which will be designated as intracellular medium, was 6.9 to 7.1.

The composition of this medium is based on an analysis of the salt content of oocyte protoplasm (9).

Colloidal gold was prepared by reducing chlorauric acid with phosphorus (3). The gold sols were stabilized by adding, to each 10 ml of colloid, either 1 mg of polyvinylpyrrolidone (PVP) (average mol wt 15,000 or 40,000), 1 mg of poly-l-proline (mol wt 25,000 to 50,000) or 30 mg of poly-l-lysine (mol wt 100,000 to 200,000). Since stabilization involves the adsorption of material to the gold, the gold particles take on the characteristics of the protecting agents.

After stabilization, the gold sols were fractionated by means of differential centrifugation. This process also served to remove excess protective agent (3). In all fractions prepared, the diameters of the gold particles fell within a range of 25 to 100 A, as determined with the electron microscope. The actual size of the stabilized colloidal particles, which depends not only upon the size of the gold particle, but also upon the thickness of the protective coat, was not determined.

Following centrifugation, the protected gold sols were dialyzed against intracellular medium and finally adjusted to an optical density of 1.1 to 3.5 (λ = 500 mμ, 1 cm light path).

Two types of experiments were performed. The first study was carried out on intact isolated nuclei, in which case only the cytoplasmic side of the nuclear envelope was exposed to the gold. In the second series of experiments, the nuclei were intentionally ruptured so that the nuclear side, as well as the cytoplasmic side, of the envelope would be exposed to the colloidal particles. In both cases the experimental material was treated with the colloid for 5 minutes and then the unbound gold was removed by a 5-minute wash in intracellular medium. Next, the material was fixed in OsO_4 for 8 minutes, dehydrated, embedded in epoxy resin, and examined with a Siemens Elmiskop I. In all, 21 nuclei were used.

RESULTS

INTACT NUCLEI: In perpendicular sections through nuclei treated with either PVP– or poly-l-proline–coated gold, the colloidal particles were concentrated within the pores and just adjacent to the pores on the cytoplasmic side of the nuclear envelope (Fig. 1). Although the gold was most concentrated in the vicinity of the pores, colloidal particles were also present on or adjacent to the outer membrane of the nuclear envelope. Only occasionally were gold particles found on the nuclear side of the envelope.

In tangential sections, it was found, for both PVP– and poly-l-proline–coated gold, that the colloidal particles associated with the annuli were often most numerous at the margins of these structures (Fig. 5).

Colloidal particles were observed along the cytoplasmic surface of nuclei treated with poly-l-lysine–coated gold, although not so consistently as when PVP or poly-l-proline were used as protective agents. Furthermore, the poly-l-lysine–coated particles were less frequently located within the pores, but instead accumulated preferentially adjacent to the pores and along the outer membrane of the envelope (Fig. 2). A different binding pattern can likewise be seen in tangential sections, where the poly-l-lysine–coated particles, presumably corresponding to those located adjacent to the pores in perpendicular sections were often superimposed over the central regions of the annuli rather than over their peripheries (Fig. 6). It can also be noted in the tangential view that the accumulation of poly-l-lysine–coated gold is not extensive.

ISOLATED NUCLEAR ENVELOPES: When ruptured nuclei were treated with PVP- or poly-l-proline–coated gold, the distribution of the colloidal particles on the cytoplasmic side of the envelope was the same as that described above. On the nuclear side, gold particles were generally observed all along the surface of the envelope, but in the areas adjacent to the pores the gold extended farther from the nuclear envelope (Fig. 3).

When the nuclear surface of the envelope was exposed to poly-l-lysine–coated gold, accumulations of particles also occurred. The particles, however, were often arranged in aggregates, and, rather than being in direct contact with the envelope, they were usually slightly removed from the inner membrane (Fig. 4).

DISCUSSION AND CONCLUSIONS

The fact that few gold particles penetrated the pores of intact nuclei indicates, as do other studies (10, 11), that the pores are not areas which permit free exchange between the nucleus and cytoplasm. Presumably the substance which prevents free exchange corresponds to the electron-opaque pore material seen with the electron microscope. It should be kept in mind, however, that in amebas colloidal gold particles are able, in long term experiments, to enter the nuclei. Thus, although the material within the pores may prevent the free diffusion of large particles, such substances may enter by some other means.

The accumulation of gold in the vicinity of the pores provides evidence that binding does occur in

these areas of the nuclear envelope. If the distribution of the gold particles associated with the pores is compared to the distribution of the electron-opaque pore material, as determined for isolated oocyte nuclei by Merriam (12), it can be concluded that the gold particles coated with negatively charged PVP or with poly-l-proline, which is neutral, bind to the pore material. The different pattern of binding obtained with positively charged poly-l-lysine may be explained in one of three ways: first, that specific binding sites exist on the pore material for positively charged particles; second, that the poly-l-lysine–coated gold does not actually bind to the pore material but rather to negatively charged substances previously bound at the pore area; or third, that the over-all size of the particles, including the coating material, may be such as to effect their ability to penetrate the pore material.

In considering the significance of binding to the pore material, it is of interest to examine the functions of binding in pinocytosis, a well established mechanism for the incorporation of macromolecules into the cell. In this process, binding to the cell surface not only stimulates uptake, but can also function in concentrating certain materials (13). Furthermore, the binding characteristics of the coat substance on the cell surface serves, to a limited extent, in selecting the molecules that are taken up (14). Binding to the pore material could function similarly in nucleocytoplasmic exchanges. Thus, the present results suggest that the pore material could be responsible for selecting and accumulating the substances to be transported across the nuclear envelope. Actual movement of the bound substance, a phenomenon which may take place only at the centers of the annuli (3), could then be accomplished either by a process involving adsorption and desorption, or by movement of the pore material, resulting also in movement of the bound substance. The latter mechanism may involve movement of the membranes of the nuclear envelope, as suggested by Bennett (15).

A discussion of the mechanisms is, of course, highly speculative at this time, and it may be found that binding to the pore material, although a means of preventing free diffusion, is not part of a transport mechanism. Even if this were the case, the amount and chemical composition of material bound within the pores at any particular phase of cellular activity could greatly influence the characteristics of the pores and, thus, the ability of molecules to penetrate the nuclear envelope.

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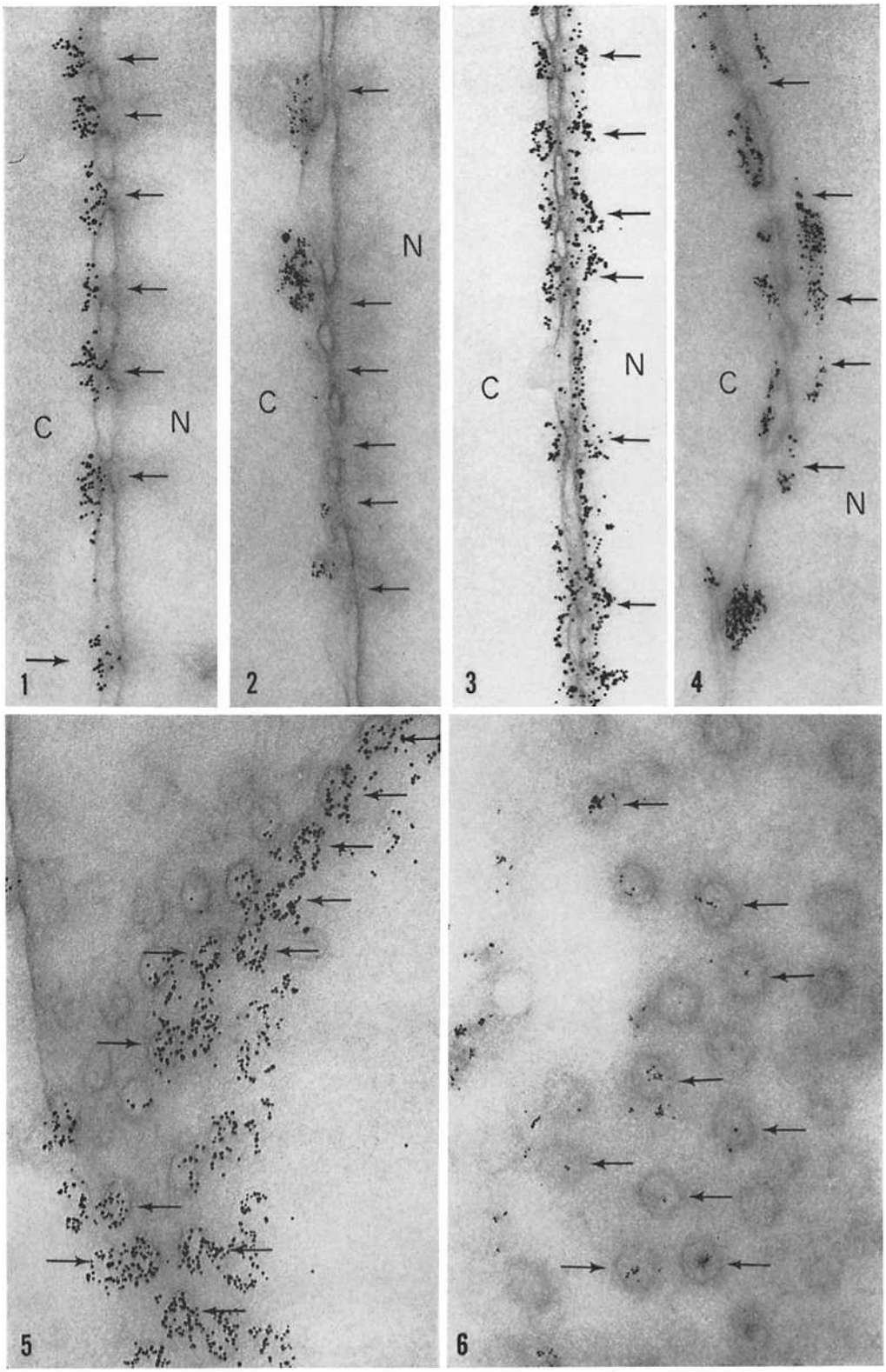
FIGURES 1 and 2 Sections through isolated nuclei treated with poly-l-proline–coated gold (Fig. 1) and poly-l-lysine–coated gold (Fig. 2). The distribution of the gold in relation to the pores (arrows) is shown. *C*, cytoplasmic side of the nuclear envelope; *N*, nuclear side of the envelope. × 84,000.

FIGURES 3 and 4 Perpendicular sections through isolated nuclear envelopes. The distribution of poly-l-proline–coated gold (Fig. 3) and poly-l-lysine–coated gold (Fig. 4) can be seen on both the cytoplasmic (*C*) and nuclear (*N*) sides of the envelope. The arrows indicate the pores. × 84,000.

FIGURES 5 and 6 Tangential sections through isolated nuclei, treated with poly-l-proline–coated gold (Fig. 5) and poly-l-lysine-coated gold (Fig. 6). The proline-coated gold appears to concentrate primarily around the periphery of the annuli (arrows), whereas the lysine-coated gold tends to accumulate adjacent to the central regions of the annuli. × 84,000.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