ON THE OCTAGONALITY OF THE NUCLEAR PORE COMPLEX

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INTRODUCTION

Gall (6) demonstrated an eightfold symmetry in nuclear pores of isolated, negatively stained preparations of nuclear envelopes. Other authors have made similar observations in sectioned material (1, 8), but Franke (4) in his review article distinguishes between an eight-sided symmetry of the pore proper and an eight-point symmetry which comes about by the presence of eight annular granules (see reference 4) or a symmetrical arrangement of eight "microcylinders" or "minitubules" (1) extending lengthwise through the peripheral part of the essentially round nuclear pore lumen (17–19). It is believed that the octagonality of the nuclear pore "is easily explained by the structural stability which, during the distortion or shrinkage, is affected by the symmetrically distributed eight-annular granules" (5: see also reference 14). I have reinvestigated the nuclear pore complex using freeze-etching and sectioning techniques and can demonstrate that both the membranous as well as the nonmembranous parts of the nuclear pore complex show eightfold symmetry. There is indication that the attachment of eight chromatin fibers to the rim of the nuclear pore results in the distortion of the membranous component of the pore.

MATERIALS

Human melanoma cells in vitro were treated in modified McCoy's medium containing 20% (v/v) glycerol for 1 hr at 0°C before freezing in liquid nitrogen-cooled Freon 22. Some cell suspensions were prefixed before glycerol treatment for 1 hr in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) which contained $CaCl_2$ (3 mM) before freezing. The specimens were freeze-etched in a Balzers freeze-etch device (Balzers AG, Ft. Liechtenstein), etched 2 min at 100°C, and subsequently shadowed with platinum-carbon (15). The replicas were examined in a Siemens 1A or 101 electron microscope at 80 kv. Some cells of the same type were fixed *in situ* for 1 hr at room temperature in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) which contained $CaCl_2$ (3 mM). The cells were postfixed in OsO_4 in phosphate buffer for 1 hr and prestained in 0.5% uranyl acetate (7) in H_2O for an additional hour before dehydration. They were flat embedded according to Brinkley et al. (2) and sectioned with an LKB ultratome. Serial sections were then mounted on collodion-coated single-hole grids and stained with uranyl acetate and lead citrate. Selected images of nuclear pores of freeze-etched preparations and conventionally fixed and sectioned specimens were subjected to the rotation analysis (9).

RESULTS

In freeze-etched preparations an angular outline of the membranous part of the nuclear pore complex could be observed if the inner nuclear membrane was exposed and viewed from the nucleoplasmic side. An angular outline could not be seen if the outer or inner nuclear membrane was viewed from the intracisternal aspect of the nuclear envelope. The cytoplasmic aspect of the outer nuclear membrane was found only very seldom and the areas were small. Most pores observed at such membranes appeared to be plugged. At the intranuclear aspect of the inner nuclear membrane in Fig. 1, nuclear pores show angularity which suggests octagonality. However, the material within the pores is seldom fractured out to the waist of the pore. Therefore octagonality was not readily recognizable. Also, the true pore diameter could not be measured because of the presence of a cytoplasmic plug.

Despite the asymmetric density-distribution due to the shadowing technique, symmetry enhancement at $n = 8$ was found (Fig. 2) using the method of Markham et al. (9), thus confirming the octagonal appearance of the original. The diameter of this octagonal pore from side to side is 975 A and diagonally from corner to corner is 1100 A. This may not be the true diameter since the material filling the pore has not been fractured out completely. There was no increase in the proportion of pores which appeared angular in preparations fixed in glutaraldehyde before freezing. In face-on views of sectioned nuclear envelopes, eight-sided symmetry was also found and confirmed using the rotation printing technique. These data are comparable with those found previously in annulate lamellae of human melanoma cells (11) and are not shown since they only confirm results of others (1, 8).

Cross-sections (Fig. 3) of nuclear pore complexes reveal fibrous material traversing the pore. They can be traced over more than 2000 A into the nucleoplasm. Also, these fibers seem to be continuous with chromatin (arrows). At the cytoplasmic side polysomes are often seen to be attached to the transversing fibers. The projection of several of these fibers can be seen in Fig. 4 within the pore proper (arrows) of the upper two pores, but the resolution of our technique does not allow us to determine if the traversing fibers attach to the membrane. The pore complex in the center of Fig. 3 is positioned obliquely to the electron beam. It shows the traversing fibers as short rods seemingly attached to the membrane of the pore waist. No evidence for annular granules around the pore margins was found. In sections which are approximately parallel to the nuclear membrane but include neither the membrane itself nor the heterochromatin, one can still find the projections of the traversing fibers. These projections often appear octagonally arranged (Fig. 5). They radiate to the sides and are seemingly in continuity with the chromatin. In fact, they are not distinguishable from chromatin. The traversing fibers themselves also seem to be interconnected. The diameter of the so-formed ring is slightly larger than the pore diameter. The distance between two opposing traversing fibers at the level of the pore is only two-thirds of the distance of these fibers below the heterochromatin level. The left side of Fig. 5 was included in order to judge the angle with which the section enters the nuclear envelope. Fig. 6 shows another arrangement of chromatin fibers which is octagonal in outline and continuous with surrounding chromatin. It is situated at approximately the same level relative to the nuclear membrane as the octagonally arranged fibers in Fig. 5.

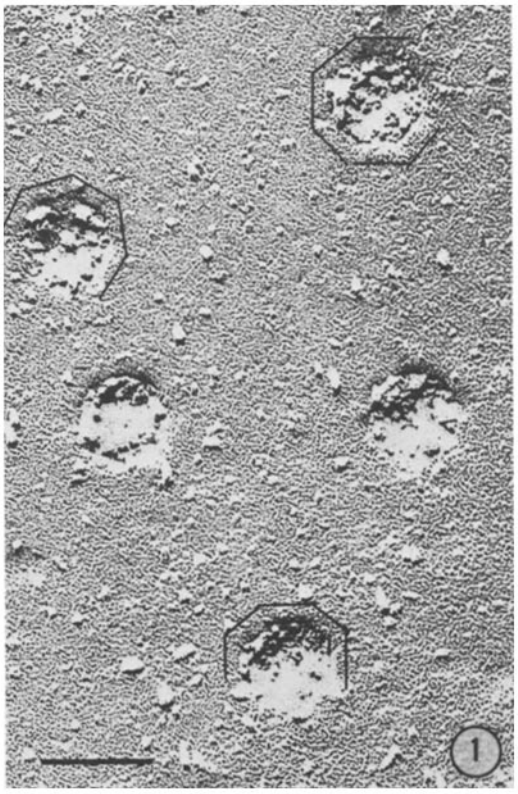
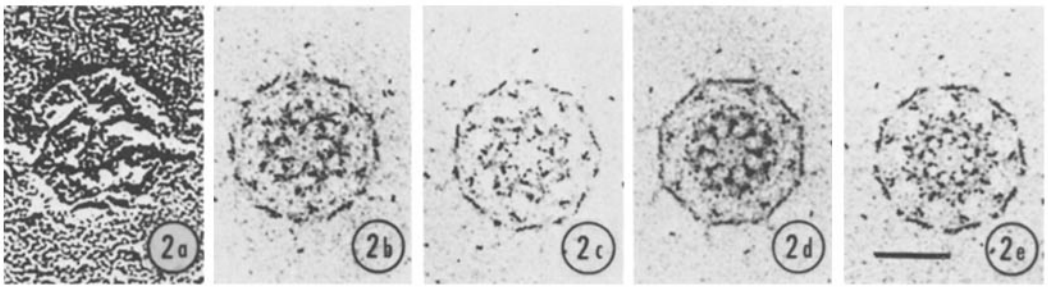


FIGURE 1 The inner nuclear membrane as seen from the nucleoplasm of a human melanoma cell line in vitro is exposed by the freeze-etching technique. The angularity of nuclear pores is indicated by black bars. Scale marker, 1000 A. × 150,000.

FIGURE 2 Rotation analysis (11) of an angular pore (*a*). Enhancement at $n = 8$ (*d*) confirms the octagonal appearance of the original. None of the other patterns at $n = 6$, 7, 8, or 9 have an equivalent in the original and are therefore not significant. Scale marker, 500 A. × 200,000.



DISCUSSION

Gall's (6) observation that the membranous part of the pore complex has an eight-sided symmetry has now been confirmed with three different preparatory techniques, i.e., with isolated, negatively stained nuclear envelope fragments (6), with fixed and sectioned material (1, 8, 10), and, in this report, with freeze-etched preparations (see also reference 16). Therefore, the angular outline of the pore rim seems not to be a preparatory artifact of one technique. Each of the utilized preparatory techniques has disadvantages with regard to the investigation of nuclear pores. During the isolating procedure the nuclear envelope fragments are traumatized and may be distorted and shrunk during application to the grids and subsequent drying with phosphotungstic acid. In sectioned material, shrinkage occurs during the dehydration steps in alcohol. During the freeze-fracture process the actual membranous structure and outline of the pore may be preserved, but the cytoplasmic material may not be fractured out to the waist of the nuclear pore. This obscures any angular outline of the pore and gives a wide range of apparent pore diameters, depending on how deep the nucleoplasmic or

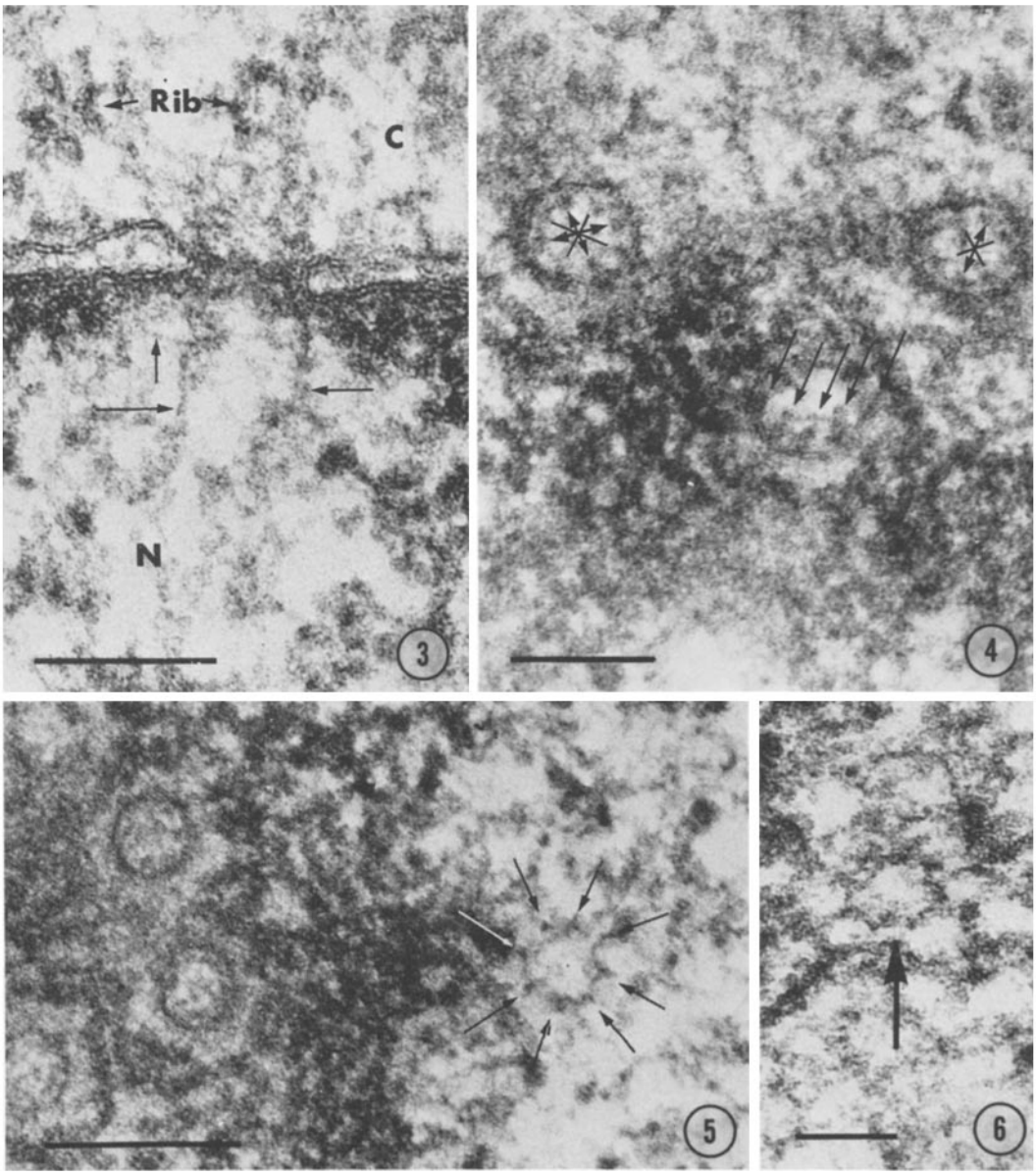


FIGURE 3 Cross-section of a nuclear pore complex. Fibrous material traverses the pore enhanced on both sides because of the superimposition of two or three fibers. At the nucleoplasmic side (*N*), these fibers seem to be continuous with chromatin (arrows). A polysome (*Rib*) seems to be attached at the traversing fibers at the cytoplasmic side (*C*). Scale marker, 1000 A. × 175,000.

FIGURE 4 The face-on view of nuclear pore complexes reveals the projection of the traversing fibers in the corners of the angular pore (upper left and right). The pore complex in the center (arrows) is situated obliquely with respect to the electron beam. Therefore the traversing fibers appear as short rods. Scale marker, 1000 A. × 190,000.

FIGURE 5 Tangentially sectioned nuclear envelope. At the left two pores are seen. Nuclear membrane and heterochromatin are present. At the center mainly heterochromatin is present and possibly the inner nuclear membrane. At the right of the section includes the space just below the heterochromatin. The thickness of the heterochromatin may be judged from the cross-section in Fig. 3. Arrows point towards the projection of eight traversing fibers and their connection to chromatin. It can also be assumed that the traversing fibers bend outward within the section. They seem to be interconnected, forming a ring slightly larger than the pore proper. Scale marker, 2000 A. × 130,000.

FIGURE 6 Tangentially sectioned nuclear envelope. The section plane does not include the heterochromatin attached to the inner nuclear membrane. Octagonally arranged chromatin in the center (arrow) seems to be the equivalent of the eight traverse fibers in Fig. 5. Some of the tips are clearly in continuity with surrounding chromatin. Scale marker, 1000 A. × 130,000.

cytoplasmic material was fractured out. This disadvantage, however, is minimized at the ascending side of the scooped-out nucleus as seen in the direction of the cut. In such areas of a nuclear envelope, angular pores were found most often.

In the first two preparatory techniques, shrinkage in relation to annular granules is said to be responsible for the eightfold symmetry (5, 14). If we consider an eight-pointed symmetry, one might explain it simply by the presence of eight nonmembranous components of the nuclear pore complex, which are more or less equally spaced around the pore. That means that this type of symmetry is present with or without assuming artifactual shrinkage due to drying in round or eight-sided pores. Eight-sided pores are either genuine or, if due to shrinkage, the shrinkage should occur around a rather sturdy structure. According to the model of Franke and Scheer (5), annular granules are present on the nucleoplasmic and cytoplasmic sides of the membranous part of the pore complex, but they are not present at the waist of the pore. The stabilizing effect of such an arrangement (5, 14) to deform a round, membranous pore into an eight-sided one during shrinkage is therefore not clear.

The eight-sided symmetry of the membranous part of the nuclear pore complex is accompanied by an eight-pointed symmetry of part of the nonmembranous nuclear pore complex. It consists of fibers traversing the pore at its corner (microcylinders [18], minitubules [1]). These fibers are seemingly continuous with chromatin at the nucleoplasmic side. Most often the traversing fibers radiate toward the sides and, in our material, farther into the nucleoplasm than the heterochromatin lining of the nuclear membrane. In fact, these fibers cannot be distinguished from chromatin (12). Such annulus-associated fibrils were also observed by Franke and Scheer (5) in isolated nuclear-envelope fragments, and in a different preparatory approach by Comings and Okada (3). The latter investigated water-spread preparations of interphase cells and found images which correspond to the ones obtained in our fixed and sectioned material. Their findings seem to confirm, on a purely structural basis, the chromatin nature of the fibers.

If one argues that eight-sided symmetry is caused by shrinkage, one could assume that the arrangement of the traversing fibers, provided they are attached to the pore rim, would account for this symmetry. If they are continuous with chromatin or attached to heterochromatin, a shrinkage of these fibers with or without a concomitant shrinkage of the membrane would result in the formation of an eight-sided membrane rim. Eight-sided symmetry could also result if the traversing fibers were under tension in the in vivo state. This seems possible in view of the fact that in freeze-etched preparations octagonal pores can be found. However, it cannot be excluded that the glycerol treatment induces some shrinkage which is not compensated after 1 hr of incubation. As previously mentioned, Franke (4) points out that one should not confuse the eight-sided nuclear pores (6) with either the eight-point radial symmetry of annular particles or with the symmetrical arrangement of eight "microcylinders" extending lengthwise through the peripheral part of the pore lumen (1, 17–19). If traversing fibers are under stress in vivo or during shrinkage, they may tear during processing, collapse and rest on the nuclear envelope, and then appear as annular granules. Such an assumption would unify the three types of eightfold symmetries, that is, the eight traversing fibers may appear as annular granules or as microcylinders which by tension in vivo or by shrinkage during dehydration determine the octagonal shape of the pore proper. The significance of the octagonal symmetry of nuclear pore complexes remains to be demonstrated.

The regular and ordered attachment of what could be chromatin to the rim of the membranous part of the pore should receive considerable attention in view of the fact that nuclear pores can be formed during interphase, a process which seems to be dependent on the metabolic activity of the cell (13).

Freeze-etching was carried out in the laboratory of Dr. Daniel Branton, University of California at Berkeley. I thank him for the opportunity of working in his laboratory, and would like to thank Miss Susan Whittock for her excellent technical assistance.

This investigation was supported by United States Public Health Service Research Grants CA 11654-02, CA 12680-1, and CA 11511-02 from the National Cancer Institute; an American Cancer Society Grant No. JN-88; and grant GB 13646 from the National Science Foundation to Dr. Daniel Branton.

Received for publication 9 March 1971, and in revised form 20 May 1971.

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