Evidence for Ca^{2+}- and ATP-sensitive peripheral channels in nuclear pore complexes

VICTOR SHAHIN, TIMM DANKER, KAROLINE ENSS, RAINER OSSIG, AND HANS OBERLEITHNER[1]

Department of Physiology, University of Münster, Germany

ABSTRACT **In eukaryotic cells the nuclear envelope (NE) serves as a functional barrier between cytosol and nucleoplasm perforated by nuclear pore complexes (NPCs). Both active and passive transport of ions and macromolecules are thought to be mediated by the centrally located large NPC channel. However, 3-dimensional imaging of NPCs based on electron microscopy indicates the existence of additional small channels of unknown function located in the NPC periphery. By means of the recently developed nuclear hourglass technique that measures NE electrical conductance, we evaluated passive electrically driven transport through NPCs. In isolated *Xenopus laevis* oocyte nuclei, we varied ambient Ca^{2+} and ATP in the cytosolic solution and/or chelated Ca^{2+} in the perinuclear stores in order to assess the role of Ca^{2+} in regulating passive ion transport. We noticed that NE electrical conductance is large under conditions where macromolecule permeability is known to be low. In addition, atomic force microscopy applied to native NPCs detects multiple small pores in the NPC periphery consistent with channel openings. Peripheral pores were detectable only in the presence of ATP. We conclude that NPC transport of ions and macromolecules occurs through different routes. We present a model in which NE ion flux does not occur through the central NPC channel but rather through Ca^{2+}- and ATP-activated peripheral channels of individual NPCs.—Shahin, V., Danker, T., Enss, K., Ossig, R., Oberleithner, H. Evidence for Ca^{2+}- and ATP-sensitive peripheral channels in nuclear pore complexes. *FASEB J.* 15, 1895–1901 (2001)**

Key Words: nuclear hourglass technique · Ca^{2+} stores · nuclear envelope · atomic force microscopy

Nuclear pore complexes (NPCs) span the inner and outer membranes of the nuclear envelope (NE), a functional barrier that mediates active and passive transport of inorganic ions and organic macromolecules between the nucleus and cytoplasm. The individual NPC has an estimated molecular mass of 124 MDa made up of more than 100 proteins, termed nucleoporins, that form a tripartite structure (1) with a centrally located channel of 8–12 nm in diameter (2). Three-dimensional reconstructions of electron microscopic data indicate the existence of eight small, so-called peripheral channels that are supposed to penetrate the individual NPC subunits around the large central channel (3, 4). Despite considerable progress toward a better understanding of the 3-dimensional architecture of the NPC in recent years, the functional evidence of the peripheral channels is still unknown. Although active transport of nuclear localization signal (NLS) -containing proteins into the nucleus has been widely studied, little is known about regulatory mechanisms of passive transport. It has been shown that molecules lacking an NLS, but small in size (<40 kDa), are passively transported into the nucleus (5). However, passive transport of ions and small macromolecules is also a regulated process that deserves attention. Passive transport of intermediate-sized macromolecules through NPCs is regulated by the filling state of the NE Ca^{2+} store (6, 7). After Ca^{2+} store depletion by inositol-1,4,5-triphosphate or Ca^{2+} chelators, the passive transport of intermediate-sized molecules (10 kDa) has been blocked (7). These findings were confirmed by studies using atomic force microscopy (AFM), where the internal diameter of the NPC declined by half after store depletion (8). Ca^{2+} store repletion through adding Ca^{2+} and ATP reversed this NPC shape change and relieved the transport blockade. Based on analysis of slow transport kinetics (movements of fluorescent labeled molecules) rather than on fast transport components (movement of ions using electrical patch clamp-techniques), small molecules (<1 kDa) and inorganic ions are supposed to freely diffuse into the nuclei even after depletion of Ca^{2+} stores (7).

Determination of NE electrical conductance, which, in a simplified view, corresponds to passive ion transport across the NE, has been confounded by the lack of an appropriate technical approach. The nuclear patch clamp technique is restricted to preparations with more or less electrically closed NPCs, whereas microelectrode techniques are of some use in small somatic nuclei (9) but fail to resolve the very low input resistance of the large oocyte nuclei (reviewed in ref 10). We recently developed the nuclear hourglass technique (NHT) (11) to address this problem and to measure the NE electrical conductance of *Xenopus laevis* oocyte nuclei.

[1] Correspondence: Department of Physiology, University of Münster, Robert-Koch Str. 27a, D-48149 Münster, Germany. E-mail: oberlei@uni-muenster.de

The NHT allows the measurement of the highly conductive NE in an isolated nucleus with great precision.

Several groups, including ours, used the patch clamp technique for transport studies indicating the existence of ion channels in the NE directly or indirectly associated with NPCs (12–17). A conclusion drawn from these studies was that during patch clamp experiments most, if not all, of the NPCs are in a closed state (18). The presence of ATP is necessary to maintain the channels in an open state, thus increasing nuclear ionic permeability (18). Furthermore, Ca^{2+} and ATP are directly involved in the modulation of NE passive ionic permeability (19). However, as pointed out by Bustamante and co-workers, the presence of macromolecule transport through the NPC central channel also determines (directly or indirectly) single NPC ionic conductance by promoting transient channel plugging during macromolecule translocation (20).

In contrast to patch clamp experiments, the NHT examines a wide area of the NE and thus provides information on mean NPC electrical properties. This approach has the disadvantage that a single NPC event cannot be detected. However, the NHT offers also an advantage over nuclear patch clamping that could be crucial for evaluation of the physiological relevance of the electrical NE conductance: NHT analyses NE conductance with minimal nuclear manipulations whereas patch clamping of the double-layered NE (including the Ca^{2+} stores) turned out to be invasive (21). Using NHT, we recorded large NE electrical conductances indicating that NPCs are usually permeable for inorganic ions. (15). These recent observations confirm early microelectrode experiments by Loewenstein and co-workers indicating that the extremely low electrical resistance of the NE barrier was due to electrically open NPCs (22, 23).

In a previous study using the NHT we realized that neither block of macromolecule transport through the NPC central channel nor stimulation of this pathway affected NE electrical conductance (11). We concluded that separate pathways must exist, one for macromolecules and another one for inorganic ions. In the present study, we tested whether Ca^{2+} and ATP, well-known modulators for both macromolecule and ion transport, change the conductive properties of the NE.

MATERIALS AND METHODS

Preparation of cell nuclei

Female *Xenopus laevis* were anesthetized with 0.1% ethyl m-aminobenzoate methanesulfonate (Serva, Heidelberg, Germany) and their ovaries were removed. Oocytes were dissected from ovary clusters and stored in modified Ringer's solution (87 mM NaCl, 6.3 mM KCl, 1 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM HEPES, 100 U/100 μg penicillin/streptomycin, pH 7.4) before use. For isolation of the cell nuclei, the oocytes were transferred into nuclear isolation medium (NIM) composed of (in mM): 90 KCl, 26 NaCl, 5.6 $MgCl_2$ (corresponds to free Mg^{2+} of 2 mM), 1.1 EGTA, 1.02 $CaCl_2$ (corresponds to free Ca^{2+} of 10^{-6} M), ATP regenerating system (3 ATP, 3 creatine phosphokinase, 10 phosphocreatine; Sigma, St. Louis, MO), 10 HEPES, titrated to pH 7.4. We added 1.5% polyvinylpyrrolidone (PVP, Mr=40,000; Sigma) to compensate for the lack of macromolecules in the NIM mimicking the intact cytosol. The presence of PVP is crucial in order to prevent the swelling (more than 100% in the absence of PVP) of total nuclear volume that instantaneously occurs during isolation in pure electrolyte solution. Nuclear Ca^{2+} stores were depleted by incubating the isolated nuclei for 10 min either in NIM containing 1 μM thapsigargin, an inhibitor of NE active Ca^{2+} uptake, and 50 μM BAPTA-AM (BAPTA-AM/DMSO-stock solution with a concentration of 50 mM; Sigma), a membrane permeable Ca^{2+} chelator, or by removing ambient Ca^{2+} and/or ATP from the NIM. We added adequate concentrations of DMSO to the respective control experiments. Small changes in pH and electrical fluid conductivity induced by adding BAPTA-AM were corrected with 1 M HCl and 1 M KCl, respectively.

Nuclear hourglass technique

The technical aspects of the method and its application in isolated cell nuclei have been described in detail (11). In short, the method uses a tapered glass tube that narrows in the middle to two-thirds the diameter of the nucleus. A current of up to 1 mA is injected via two Ag/AgCl electrodes through either end of the glass tube. The voltage drops across the cell nucleus are measured with two conventional microelectrodes. The tips of these microelectrodes are placed near the narrow part of the capillary opposite each other to measure the electrical resistance of the fluid column between them. Since current and voltage are simultaneously measured, the resistance can be calculated online and monitored during the measurements (**Fig. 1**). The nucleus is sucked into the narrow part of the capillary by gentle fluid movement. Thus, the current flows through the nucleus. The resulting rise in total electrical resistance indicates the specific electrical resistance of the cell nucleus.

Evaluation of the leak resistance between glass wall and nucleus (R_{Shunt}) was published elsewhere (11). The maximum estimate for the error introduced by shunt leakage was less than 6%. Thus, we made no attempt to make corrections for R_{Shunt}.

Preparation of nuclear envelopes

After placing the oocytes in NIM, nuclei were manually isolated by piercing the oocyte with two pincers. Individual intact nuclei were picked up with a Pasteur pipette and transferred to a glass coverslip placed under a stereo microscope. Then the chromatin was carefully removed using sharp needles and the nuclear envelope was spread on poly-L-lysine coated glass, with the nucleoplasmic side facing downward. Finally, the specimen were washed with deionized water and dried.

Atomic force microscopy (AFM)

Application of AFM to nuclear envelopes has been described in detail previously (21). We used a Multimode™ (with a NanoScope IIIa controller; Digital Instruments, Santa Barbara, CA) equipped with an optical microscope, a videocamera, and a monitor to visualize the NE and the AFM tip on the AFM head stage. We used standard V-shaped 200 μm long silizium nitride cantilevers with spring constants of 0.06 N/m and pyramidal tips with an estimated tip diameter of 10 nm (Digital Instruments). The images were recorded with 512

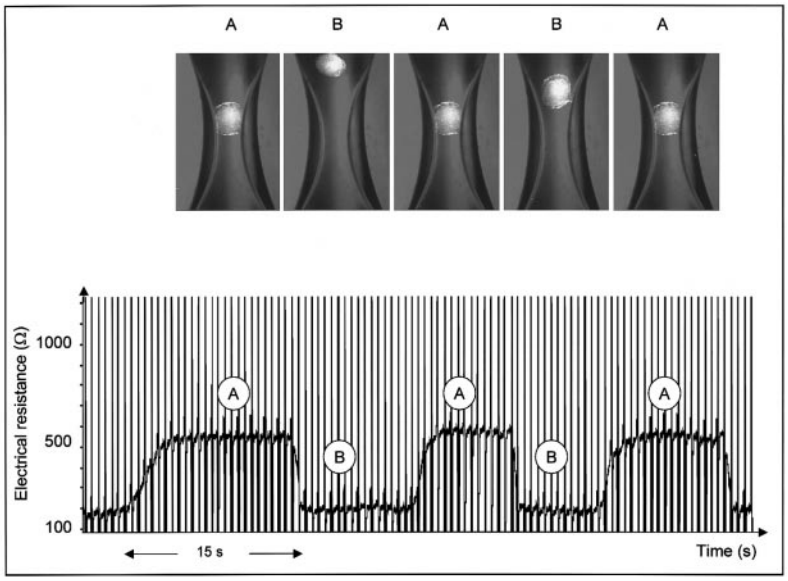

Figure 1. Example of three successive electrical measurements in the same nucleus using the nuclear hourglass technique. The method uses a tapered glass tube, which narrows in its middle part. Electrical current pulses are injected and the resulting voltage changes are measured and monitored as electrical resistance. The isolated nucleus is moved into (*A*) and out (*B*) of the tapered part of the capillary. Electrical resistance increases when the nucleus is moved into the tapered part. The change in resistance corresponds to nuclear envelope electrical resistance, since the current flows through the nucleus. The polarity of the electrical current is reversed every 1–2 s in order to avoid polarization in the nucleus.

lines per screen at constant force (height mode) in contact mode with a scan rate of 3 to 10 Hz. The forces applied during the scanning procedure were minimized by retracting the AFM-tip until it lost contact with the sample surface and reengaging the tip at a set point (i.e., force value) minimally above the lift-off value. We could usually obtain scanning forces below 3 nN with this approach. Experiments were performed using a so-called fluid cell. Although the AFM tip physically interacts with the nuclear envelope, we usually were able to perform multiple scans without damaging the preparation. Scanning at low forces (3 nN or less) left no visible marks in the preparation.

Statistics

Data are given either as individual paired measurements or are shown as mean values ± SE in % of the corresponding control values (n=number of nuclei). Each experimental group was compared with its own control. Significance of differences were tested by the paired Student's t test. An asterisk indicates a significant difference of $P < 0.05$ or less.

RESULTS

We applied the nuclear hourglass technique to investigate the role of Ca^{2+} stored in the perinuclear space of the NE and the role of ambient Ca^{2+} and ATP in regulating passive ion transport across the nuclear envelope.

Figure 1 shows the NHT. As indicated in the schematic microphotographs, the isolated cell nucleus can be positioned in the tapered part of the capillary (Fig. 1*A*) or displaced into the upper or lower wide part of the glass tube (Fig. 1*B*). Correspondingly, electrical resistance measured across the tapered part of the capillary changes. From the changes in electrical resistance, the nuclear envelope electrical resistance (NEER) can be calculated as outlined above (see Materials and Methods) and described in detail previously (11).

In a first series of experiments, we measured NEER in individual nuclei with intact perinuclear Ca^{2+} stores and then depleted the perinuclear Ca^{2+} stores by incubating them in NIM containing the Ca^{2+} pump inhibitor thapsigargin and the Ca^{2+} chelator BAPTA-AM. **Figure 2** (upper) shows the results. NE electrical resistance decreased in all 12 experiments. This was unexpected in view of previous experiments showing central channel macromolecule plugging by this maneuver (8). Therefore, we designed another series of experiments with intact NE Ca^{2+} stores but lacking ambient ATP (Fig. 2, lower). Under these conditions, NEER consistently increased, indicating that passive ion permeability of the NE is altered. Removal of both ambient ATP and ambient Ca^{2+} further increased NEER. Addition of ATP to the nuclei depleted of ambient (cytosolic) Ca^{2+} again lowered NEER (Fig. 2, middle).

Figure 3 summarizes the results. They are displayed as NE electrical conductance (NEEC), which is the reverse value of NEER. The largest NEEC was measured with empty perinuclear Ca^{2+} stores (NEEC increase vs. control: 7.3±0.02%; mean±SE; n=12). In contrast, the lowest NEEC was measured when both Ca^{2+} and ATP were absent in the ambient (cytosolic) fluid (NEEC decrease vs. control: 20.3±3.7%; mean±SE; n=10). Removal of either ambient Ca^{2+} or ATP also decreased NEEC, but to a lower extent. Corresponding control experiments applying 0.1% DMSO, the solvent used in the thapsigargin/BAPTA-AM experiments, revealed no significant changes in NEEC (NEEC increase vs. control: 0.45±2.16%; mean±SE; n=10).

We applied AFM to search for peripheral channels supposed to be holes in the range of a few nanometers visible on the cytoplasmic surface of NPCs. We used isolated NEs spread on glass and visualized the NPCs in air at native (unfixed) conditions. We tested two conditions: *1)* we incubated NEs with control solution containing both ambient Ca^{2+} and ATP for 10 min, then washed the preparation and imaged the NE

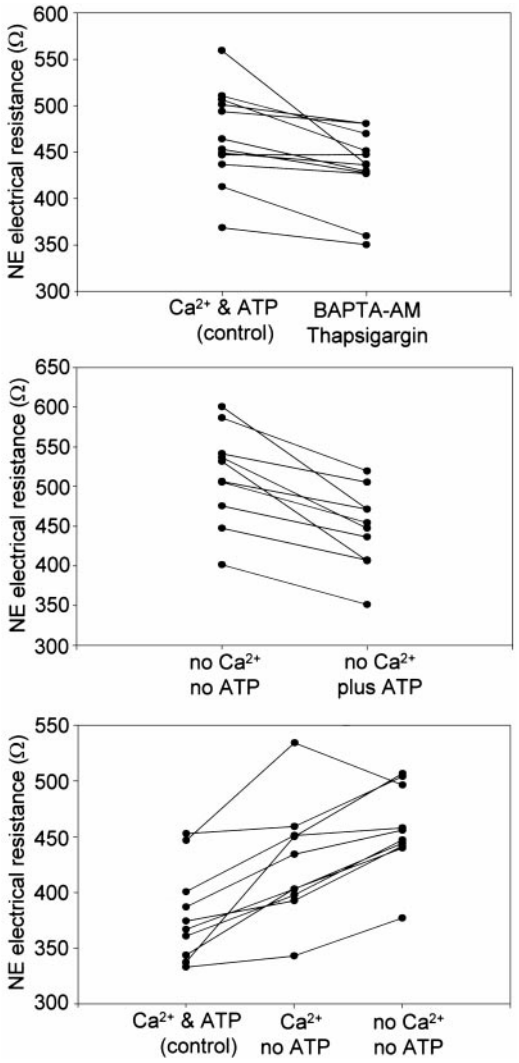

Figure 2. Upper panel: the nuclear envelope resistance (Ω) decreased after addition of thapsigargin/BAPTA-AM to the control buffer (Ca^{2+} store depletion in the presence of ambient Ca^{2+} and ATP). Middle panel: the nuclear envelope resistance (Ω) decreased after addition of ATP in the absence of ambient Ca^{2+}. Lower panel: the nuclear envelope resistance (Ω) increased after removal of ATP and again when both ATP and Ca^{2+} were absent in the ambient solution.

surface in air; in a second step, *2)* we reincubated the same NEs with a solution lacking ATP. Representative images are shown in **Fig. 4**. NPCs deprived of ATP are shown on the left. NPC surface is smooth and no channel openings are detected other than the central channel entrance. In contrast, ATP incubation led to a dramatic shift in NPC surface topography. Multiple small pores are detectable in the NPC periphery.

DISCUSSION

An individual NPC has a large central channel that mediates both active and passive transport of macromolecules across the NE. The existence of such a large central channel has been known since 1949 (24). More recently, 3-dimensional modeling of the NPC by analyzing electron microscopical data indicated the existence of eight small peripheral channels circularly arranged around the central channel (1, 4). The physiological relevance of these small channels is still unknown, although a role in inorganic ion exchange between cytosolic and nucleoplasmic compartments was suggested at the time of their discovery (4). Considerably more is known about passive transport of medium-sized macromolecules (~10 kDa) through the central NPC channel. It became obvious that passive macromolecule transport is regulated by the filling state of the NE Ca^{2+} store (7). The functional state of the NE Ca^{2+} store is supposed to determine NPC conformation (6, 25). The nucleoporin gp210, an integral transmembrane glycoprotein with high Ca^{2+} binding capacity, has been suspected to play a major role in regulating macromolecule transport through the NPC since a monoclonal antibody raised against its lumenal domain (i.e., the domain of the protein localized in the perinuclear space) reduced passive protein transport (26). The present study focused on inorganic ion transport across the nuclear envelope. We tested the role of the NE Ca^{2+} store in regulating the NE electrical conductance, a functional equivalent to the passive permeability of small electrically charged solutes. We found an increase in electrical conductance (i.e., passive ion permeability) when NE stores were Ca^{2+} depleted. This experimental condition is known to induce central channel plugging as previously shown by atomic force microscopy (8, 25). We conclude, therefore, that central channel plugging is the structural correlate of blocked macromolecule transport whereas inorganic ions travel through different routes between cytosol and cell nucleus. The results support also previous data indicating that neither physical block (i.e., application of antibodies) of the central channel NPC pathway for macromolecules nor stimulation of protein import (i.e., addition of karyophilic proteins) affected passive ion permeability (11).

Ambient ATP and Ca^{2+} seem to be necessary for normal function of the putative peripheral channels. This finding agrees with patch clamp experiments in nuclei of *Xenopus laevis* oocytes (18) and of mouse liver (19). From time-lapse experiments applying atomic force microscopy on nuclear envelopes of *Xenopus laevis* oocytes, we know that both ATP and Ca^{2+} contract NPCs (27, 28). We used high Ca^{2+} concentrations (10 μM) in our experiments. It can be assumed that Ca^{2+} is released into the nucleoplasm through inositol 1,3,4 trisphosphate-activated Ca^{2+} channels of the nuclear envelope accumulating for seconds at high concentrations near NPCs (14, 29, 30).

ATP and a sufficiently high concentration of Ca^{2+} trigger macromolecule transport through the central channel, probably by a peristaltic wave of contractions. It has been proposed that a contractile apparatus may be involved in the nucleocytoplasmic transport of macromolecules through the NPC (31). A model was developed proposing that the nuclear pore contains

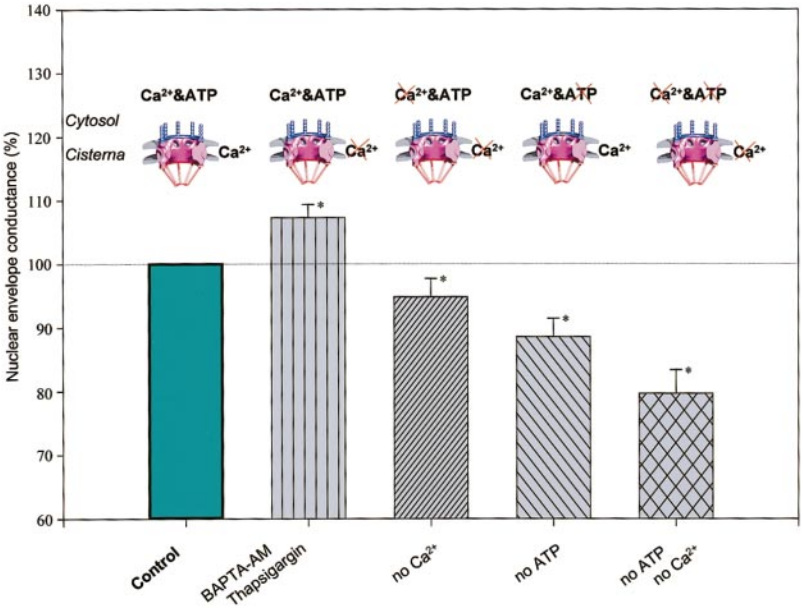

Figure 3. Nuclear envelope electrical conductance in % of control (Ca^{2+} and ATP present in solution). The schematics above each column show sections through individual NPCs perpendicular to the NE (schematics modified from ref 33) and indicate the experimental conditions. We assume empty cisternal Ca^{2+} stores after pretreatment with thapsigargin/BAPTA-AM despite the presence of ambient Ca^{2+}. We also assume empty cisternal Ca^{2+} stores in experiments where ambient Ca^{2+} was absent. *Mean values significantly different from the corresponding control values ($P<0.01$; paired experiments).

eight myosin molecules arranged in an octagonal array, with their heads facing the cytoplasm and their tails pointing toward the nucleus. These molecules are in contact with another eight myosin molecules arranged in an opposite direction, with their heads facing the nucleoplasm. According to this model, macromolecule transport through the pore would be accomplished by contractions generated by ATP hydrolysis in the myosin heads (32). In light of the present experiments, we want to modify the 'peristaltic transport' model by separating the route of the macromolecules from the route taken by ions. We assume that under physiological conditions, i.e., when karyophilic proteins are present for transport, the central channel is usually plugged by proteins in transit. This view is derived from the original model by Bustamante and co-workers (12). It means that passive electrical permeability of the plugged central channel must be very low. However, the passive NE electrical permeability is usually large. Therefore, we need to postulate peripheral channels that operate in parallel to the central NPC channel.

When the cytoplasmic aspects of NPCs are carefully analyzed by AFM using unfixed, native preparations, multiple dimples can be discovered on the NPC surface. These dimples are found only in NPCs challenged with ATP before scanning. They could represent the cytoplasmic openings of the putative peripheral channels described by electron microscopy several years ago (4). Due to limitations of the AFM technique, we cannot identify them in more detail. The limitation of the AFM used for imaging the rather complex native NE is based on the physical extension of the AFM stylus that interacts with the surface. Therefore, the smaller the lateral dimension of a hole is in an individual NPC

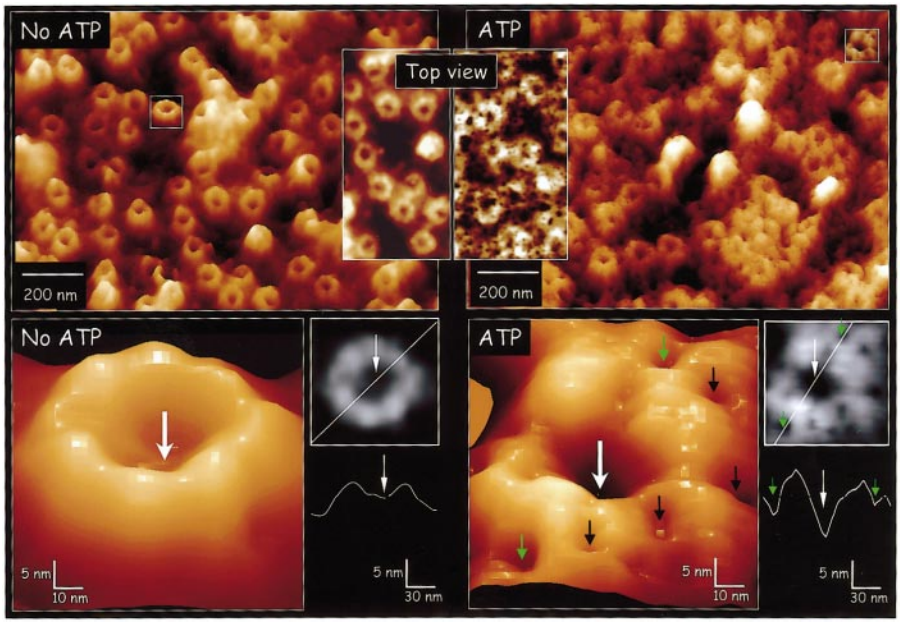

Figure 4. Cytoplasmic domains of nuclear pore complexes (NPCs) of *Xenopus laevis* oocyte are visualized in a native nuclear envelope by AFM. The same NE preparation was scanned before (left part of figure) and after (right) incubation with 3 mM MgATP. In the absence of ATP, NPCs appear smooth and regularly shaped. A single NPC, indicated by the white frame in the overview image, is shown in more detail. The eightfold symmetry of the cytoplasmic ring is detectable. The profile through the NPC shows the central channel (arrow). No other channels are visible. In contrast, multiple 'holes' are visible after ATP incubation. They are barely detectable in the 3-dimensional overview but visible in the top view position. High magnification (framed NPC of overview image) disclosed multiple dimples in the NPC ring periphery (small arrows). The profile shows the dimensions of the small channels compared with the central channel.

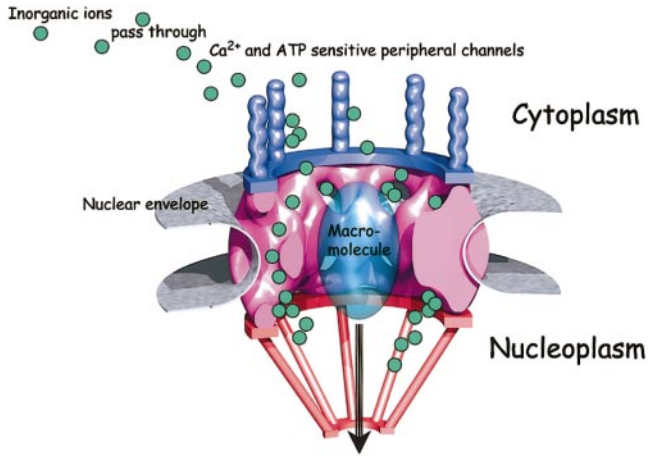

Figure 5. Hypothetical NPC model that shows two separate pathways: one for macromolecules (central channel) and one for ions (peripheral channels). ATP and Ca^{2+} maintain peripheral channels in an open state. The 3-dimensional architecture of the NPC was published previously (33). The major structural components include the basic framework (i.e., 'spoke' complex with the putative peripheral channels; shown in pink), the central plug (macromolecule; translucent light blue), the cytoplasmic ring and the cytoplasmic filaments (blue), and the nuclear ring and nuclear basket (orange).

the more the respective hole will be imaged as a shallow dimple. Taking this limitation into account, the dimples are likely to represent the entrances of the small peripheral channels. The dimples were spread over the whole NPC surface and it was not possible to count them. The main reason we cannot determine the exact number of dimples per NPC is the irregularity in NPC structure. This irregularity is the price we must pay for using a native, nonfixed NE preparation that still responds to physiological stimuli as ATP.

Figure 5 shows the model we propose. The NPC structure is based on a 3-dimensional model developed a few years ago (33) and includes the observations of the present paper:

1. The existence of small peripheral channels is derived from the observation that blockage of central channel transport by NE calcium store depletion is not paralleled by a comitant decrease of the passive electrical conductance. To the contrary, the electrical conductance is increased.

2. Ambient ATP and Ca^{2+} are important prerequisites for maintaining the peripheral channels in an open state. This is shown functionally by the increased electrical conductance and structurally by the appearance of small channels on the NPC surface.

3. The large NE electrical conductance is explained by the passive electrical permeability of peripheral channels, at least under conditions when karyophilic macromolecules are available for NPC central channel plugging.

4. The central channel itself does not explain the passive electrical NPC permeability. Obviously, inorganic ions do not pass through the central channel as long as the latter is occupied by macromolecules.

However, the functional state of the NPC central channel can modify peripheral channel function. Thus, macromolecule transport pathway and ion transport pathways can interfere with each other as previously shown (12). FJ

The excellent technical assistance of Hannelore Arnold and Marianne Wilhelmi is gratefully acknowledged. We thank our collaborators Andrea Schlune, Claudia Schäfer, and Ilsa Buchholz for many helpful discussions and Prof. Glasl, Department of Pharmaceutical Biology and Phytochemistry, University of Münster, for his constant support of our work. Experiments were performed by V.S. in partial fulfillment of his Ph.D. thesis. The study was supported by the Deutsche Forschungsgemeinschaft ('Graduiertenkolleg: Membranproteine: Signalerkennung, Signaltransfer und Stofftransport', AG Oberleithner) and the Interdisziplinäre Zentrum für Klinische Forschung (IZKF, TP I/90). We gratefully acknowledge the support of VEECO Instruments GmbH, Mannheim.

REFERENCES

1. Pante, N., and Aebi, U. (1996) Molecular dissection of the nuclear pore complex. *Crit. Rev. Biochem. Mol. Biol.* **31,** 153–199
2. Keminer, O., and Peters, R. (1999) Permeability of single nuclear pores. *Biophys. J.* **77,** 217–228
3. Aebi, U., Pante, N., and Jarnik, M. (1992) Structure and function of the nucleare pore complex, a supramolecular machine mediating molecular trafficking across the nuclear envelope. *Verh. Dtsch. Zool. Ges.* **85,** 285–296
4. Hinshaw, J. E., Carragher, B. O., and Milligan, R. A. (1992) Architecture and design of the nuclear pore complex. *Cell* **69,** 1133–1141
5. Gerace, L. (1988) Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* **4,** 335–374
6. Greber, U. F., and Gerace, L. (1995) Depletion of calcium from the lumen of endoplasmic reticulum reversibly inhibits passive diffusion and signal-mediated transport into the nucleus. *J. Cell Biol.* **128,** 5–14
7. Stehno-Bittel, L., Perez-Terzic, C., and Clapham, D. E. (1995) Diffusion across the nuclear envelope inhibited by depletion of the nuclear Ca^{2+} store. *Science* **270,** 1835–1838
8. Wang, H., and Clapham, D. E. (1999) Conformational changes of the in situ nuclear pore complex. *Biophys. J.* **77,** 241–247
9. Oberleithner, H., Brinckmann, E., Schwab, A., and Krohne, G. (1994) Imaging nuclear pores of aldosterone sensitive kidney cells by atomic force microscopy. *Proc. Natl. Acad. Sci. USA* **91,** 9784–9788
10. Mazzanti, M., Bustamante, J. O., and Oberleithner, H. (2001) Electrical dimension of nuclear envelope. *Physiol. Rev.* **81,** 1–19
11. Danker, T., Schillers, H., Storck, J., Shahin, V., Krämer, B., Wilhelmi, M., and Oberleithner, H. (1999) Nuclear hourglass technique: novel approach detects electrically open pores in *Xenopus laevis* oocyte. *Proc. Natl. Acad. Sci USA* **96,** 13531–13535
12. Bustamante, J. O., Liepins, A., and Hanover, J. A. (1995) The nuclear pore ion channel activity. *J. Membr. Biol.* **146,** 239–251
13. Draguhn, A., Boerner, G., Beckmann, R., Buchner, K., and Hucho, F. (1997) Large-conductance cation channels in the envelope of nuclei from rat cerebral cortex. *J. Membr. Biol.* **198,** 159–166
14. Mak, D. O., and Foskett, J. K. (1994) Single-channel inositol 1,4,5-trisphosphate receptor currents revealed by patch clamp of isolated *Xenopus* oocyte nuclei. *J. Biol. Chem.* **269,** 29375–29378
15. Mazzanti, M., DeFelice, L. J., and Cohen, J., Malter, H. (1990) Ion channels in the nuclear envelope. *Nature (London)* **343,** 764–767
16. Prat, A. G., and Cantiello, H. F. (1996) Nuclear ion channel activity is regulated by actin filaments. *Am. J. Physiol.* **270,** C1532–C1543
17. Tabares, L., Mazzanti, M., and Clapham, D. E. (1991) Chloride channels in the nuclear membrane. *J. Membr. Biol.* **123,** 49–54

18. Mazzanti, M., Innocenti, B., and Rigatelli, M. (1994) ATP-dependent ionic permeability on nuclear envelope in in situ nuclei of *Xenopus* oocytes. *FASEB J.* **8,** 231–236
19. Assandri, R., and Mazzanti, M. (1997) Ionic permeability on isolated mouse liver nuclei: influence on ATP and Ca^{2+}. *J. Membr. Biol.* **157,** 301–309
20. Bustamante, J. O., Michelette, E. R., Geibel, J. P., Dean, D. A., Hanover, J. A., and McDonnell, T. J. (2000) Calcium, ATP and nuclear pore channel gating. *Pfluegers Arch.* **439,** 433–444
21. Danker, T., Mazzanti, M., Tonini, R., Rakowska, A., and Oberleithner, H. (1997) Using atomic force microscopy to investigate patch clamped nuclear membrane. *Cell Biol. Int.* **2111,** 747–757
22. Loewenstein, W. R. (1964) Permeability of the nuclear membrane as determined with electrical methods. *Protoplasmatologia* **5,** 26–34
23. Loewenstein, W. R., and Kanno, Y. (1962) Some electrical properties of the membrane of a cell nucleus. *Nature (London)* **195,** 462–464
24. Callan, H. G., Randall, J. T., and Tomlin, S. G. (1949) An electron microscope study of the nuclear membrane. *Nature (London)* **163,** 280
25. Perez-Terzic, C., Pyle, J., Jaconi, M., Stehno-Bittel, L., and Clapham, D. E. (1996) Conformational states of the nuclear pore complex induced by depletion of nuclear Ca^{2+} stores. *Science* **273,** 1875–1877
26. Greber, U. F., and Gerace, L. (1992) Nuclear protein import is inhibited by an antibody to a lumenal epitope of a nuclear pore complex glycoprotein. *J. Cell Biol.* **116,** 15–30
27. Oberleithner, H. (1999) Aldosterone and nuclear signaling in kidney. *Steroids* **64,** 42–50
28. Rakowska, A., Danker, T., Schneider, S. W., and Oberleithner, H. (1998) ATP-induced shape change of nuclear pores visualized with the atomic force microscope. *J. Membr. Biol.* **163,** 129–136
29. Gerasimenko, O. V., Gerasimenko, J. V., Tepikin, A. V., and Petersen, O. H. (1995) ATP-dependent accumulation and inositol triphosphate- or cyclic ADP-ribose-mediated release of Ca^{2+} from the nuclear envelope. *Cell* **80,** 439–444
30. Petersen, O. H., Gerasimenko, O. V., Gerasimenko, J. V., Mogami, H., and Tepikin, A. V. (1998) The calcium store in the nuclear envelope. *Cell Calcium* **23,** 87–90
31. Schindler, M., and Jiang, L.-W. (1986) Nuclear actin and myosin as control elements in nucleocytoplasmic transport. *J. Cell Biol.* **102,** 859–862
32. Berrios, M., and Fisher, P. A. (1986) A myosin heavy chain-like polypeptides is associated with the nuclear envelope in higher eukaryotic cells. *J. Cell Biol.* **103,** 711–724
33. Pante, N., and Aebi, U. (1993) The nuclear pore complex. *J. Cell Biol.* **122,** 977–984

Received for publication November 28, 2000.
Revised for publication April 9, 2001.