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Electrophoretic Plugging of Nuclear Pores by Using the Nuclear Hourglass Technique

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Abstract. The nuclear hourglass technique (NHT) was recently introduced as a novel technique that measures the electrical nuclear envelope (NE) conductance of isolated *Xenopus laevis* oocyte nuclei. The main conclusion drawn from NHT work so far is that nuclear pore complexes (NPCs) of oocytes are in an electrically open state under physiological conditions, with a mean conductance of 1.7 nS per NPC. Since nuclear patch-clamp data indicate that usually NPCs are electrically closed, our work has been challenged by the notion that NHT cannot assure a high resistance seal ("gigaseal") between glass wall and NE like that required for patch-clamp experiments. Thus, NHT could have dramatically underestimated NE electrical resistance. Here we demonstrate that NHT does not require a gigaseal for accurate NE conductance measurements. In addition, we present experimental conditions where mean single NPC electrical conductance is reduced 26-fold due to electrophoretic plugging by negatively charged nucleoplasmic macromolecules. In addition, data indicate that under physiological conditions (i.e., when macromolecules are offered in the cytosolic solution) the nuclear surface is heavily folded, underestimating "true" NE surface by a factor of 2.6. When "true" NE surface area is taken into consideration, modified values of mean single NPC conductances of 654 pS for electrically open conditions and 25 pS for electrically plugged conditions can be calculated.

We conclude that the large overall NE conductance detected with the nuclear hourglass technique in intact *Xenopus laevis* oocyte nuclei can be explained by the sum of single NPC conductances in the pS range, as long as open probability is high. This confirms previous patch-clamp work concerning single NPC conductance, but disagrees with the view that mean open probability of NPC channels is usually low.

Key words: Nuclear envelope — *Xenopus laevis* oocyte — Macromolecular transport — Nuclear permeability — Nuclear electrophysiology

Introduction

The nuclear envelope (NE) shields the chromatin from the cytosol. Nuclear pore complexes (NPCs) provide the gates through which specific macromolecules enter or leave the nucleus. Additionally, NPCs provide a regulated permeability for inorganic ions and small molecules, thus establishing defined nuclear signaling between cytosol and nucleoplasm. This has been shown for Ca^{2+}, which participates in the control of gene expression (Hardingham et al., 1997; Bading et al., 1997). Measurement of NE electrical resistance (1/conductance) is a promising approach to obtain valuable information about this nucleocytoplasmic pathway (Matzke & Matzke, 1991). In this context, it appears useful to focus on the overall electrical NE resistance rather than on open and closed states of single NPCs. The main question is whether the majority of NPCs is electrically open or closed under physiological conditions. Literature on nuclear electrophysiology is still controversial at this point, dependent on the methods used in the various studies (Mazzanti et al., 2001).

Patch clamp investigators usually find that NE electrical resistance is high enough to allow single-channel recordings, which implies that a majority of NPCs in the patch is electrically closed (Mazzanti et al., 1990; Bustamante et al., 1995b). They have described a number of small-conductance nuclear ion channels probably unrelated to NPCs (Stehno-Bittel, Lückhoff & Clapham, 1995; Rousseau et al., 1996; Longin et al., 1997; Guihard et al., 2000; Franco-Obregon, Wang & Clapham, 2000;

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Tonini et al., 2000). In addition, large-conductance channels have been described, ranging from 200 pS to 1 nS, possibly related to NPCs (Mazzanti et al., 1990; Matzke et al., 1992; Bustamante Liepins & Hanover, 1994; Bustamante, Hanover & Liepins, 1995a; Tonini et al., 1999). However, despite of the high density of NPCs in the investigated NE (about 40 NPCs per μm^2 of NE), only a small fraction of NPCs appeared electrically conductive in patch-clamp experiments. The number of electrically active NPCs was shown to increase dramatically in presence of ATP (Mazzanti, Innocenti & Rigotelli, 1994; Assandri & Mazzanti, 1997) and to decrease during macromolecular transport (Bustamante et al., 1995b; Bustamante and Veranda, 1998; Bustamante et al., 2000). From these findings it was concluded that the NPC can switch between three functional configurations, an open, a closed, and a transporting, nonconductive "plugged" configuration, and thus is able to maintain a high NE electrical resistance (Bustamante et al., 1995a; Bustamante et al., 2000). In consequence, the NE might serve as a barrier for small messenger ions like Ca^{2+} while, at the same time, allowing (electrically silent) diffusion of large macromolecules (Santella, 1996). Patch-clamp experiments are reliable only in high-resistance patches, while low-resistance patches can be due to either artificial leakage between glass and patch and/or due to open NPCs. Therefore, the patch-clamp researcher unconsciously selects conditions that do not neccessarily reflect physiological conditions.

Microelectrode studies, some of which even date back to the late sixties, came to a fundamentally different conclusion. According to these studies, NE has an extremely low electrical resistance (Loewenstein & Kanno, 1962; Loewenstein, 1964; Reynolds & Tedeschi, 1984; Oberleithner et al., 1994) due to electrically open NPCs. In the case of large oocyte nuclei, where high density of NPCs combines with unfavourable surface to volume ratios, electrical NE resistances turned out to be too low for investigating them with microelectrode techniques (Kanno, Ashman & Loewenstein, 1965).

We recently filled this methodical gap with the development of a new method for NE resistance measurements on oocyte nuclei, the nuclear hourglass technique (NHT) (Danker et al., 1999). In this method, the isolated cell nucleus occludes a glass capillary through which an electrical current is driven, so that measurements can be performed without electrode impalements of the NE. The results of our work with NHT forced us to take side in favor of the classical microelectrode studies, showing that the *Xenopus* oocyte NE has a low electrical resistance. The studies indicated that the majority of NPCs is electrically open, with a large single NPC electrical conductance of 1.7 nS (Danker et al., 1999). Obviously, this finding is in conflict with the nuclear patch-clamp work, where NPCs exhibited a low open probability, resulting in a much higher NE electrical resistance. In the ensuing discussion on how to reconcile our data with those from patch-clamp studies, the NHT has been suspected to dramatically underestimate the NE electrical resistance. In fact, the measurement principle of the NHT does not rely on a patch clamp-like "gigaseal", so it is doubtful that a low shunt resistance (i.e., resistance pathway between glass wall and cell nucleus) invalidated the measurement and produced the low NE electrical resistance that we observed. In the present paper, we will throw light on this matter from two sides: First, we will present a quantitative evaluation of an electrical circuit analysis of the NHT method. On the basis of known parameters like buffer fluid conductivity and capillary geometry, this inspection will allow us to judge under which basic conditions our NHT results are correct. Second, besides this mathematical examination, we will describe an experimental protocol that leads to a dramatic increase of the electrical NE resistance. This is done by the application of constant current, which causes electrophoretic plugging and thus electrical closure of the NPCs. Finally, we realized that the NE surface is usually folded under physiological condition, i.e., when macromolecules are present in the cytosol. This observation has been taken into consideration for the calculation of mean single NPC conductance.

Materials and Methods

Preparation of Oocyte Nuclei

Female *Xenopus laevis* were anesthesized with 0.1% ethyl *m*-aminobenzoate methane sulfonate (Serva, Heidelberg, FRG), and their ovaries were removed. Oocytes (stage VI) were dissected from ovary clusters and stored in modified Ringer solution (in mM: NaCl 87, KCl 6.3, $MgCl_2$ 1, $CaCl_2$ 1.5, HEPES 10, glucose 5.5, pH 7.8) before use. For isolation, oocytes were transferred into nuclear isolation medium, NIM (in mM: KCl 90, NaCl 10, $MgCl_2$ 2, EGTA 1.1, HEPES 10, Ca^{2+} 0.16 (corresponding to free Ca^{2+} of 10^{-8} M due to the presence of EGTA), polyvinylpyrrolidone (PVP, Mr 40,000, 1.5%), pH 7.32. Nuclei were isolated by piercing the oocyte with two pincers. PVP was added to compensate for the lack of macromolecules in the isolation medium as compared to the intact cytosol. The presence of PVP is important to prevent the dramatic swelling of the cell nucleus, which occurs in pure electrolyte solution. This was shown in a separate series of experiments. There, nuclei were isolated in NIM either in presence or absence of 1.5% PVP. NE surface area was calculated from images stored on the computer at different time periods after isolation. Nuclei were treated as perfect spheres.

Principle of the Measurement

For a detailed description of the setup and standard experimental procedures *see* Danker et al. (1999). The main part of the setup is the buffer-filled capillary with the tapered part in its middle. At this site, the inner diameter of the capillary is about 2/3 of the diameter of the nucleus. A current of up to 1mA is injected via two massive Ag/AgCl

electrodes placed at the ends of the capillary. The resulting voltage drop is measured with two conventional Ag/AgCl electrodes. Since current and voltage are measured at the same time, the resistance can be calculated online and displayed during the measurements. By gravity and a gentle downward flow of the buffered fluid, the nucleus is maneuvered into the tapered part of the capillary. Thus, the current now flows through the nucleus. The resulting rise in electrical resistance indicates the electrical resistance of the nucleus.

Tapered capillaries are made from glass capillaries with 2 mm outer and 1.7 mm inner diameter (Servoprax, Wesel, FRG). The narrowing is introduced using a vertical patch-pipette puller (LM-3P-A, List Elektronik, Darmstadt, FRG) and a modified pulling protocol. In a first step, heat and pulling force is applied to introduce an initial narrowing to about 50% of the initial outer diameter. In the second step, only heat and no pulling force is applied. Under these conditions, the walls of the narrowed part of the glass capillary become thickened, so that the inner diameter is reduced but the outer diameter remains essentially unchanged.

NHT basically measures the change in resistance (ΔR) when a nucleus is introduced in the central part of the capillary. Thus, the electrical resistance of the buffer fluid in this central part is replaced ($R_{replaced}$) by a system of intranuclear resistance ($R_{intranuclear}$) and lumped NE electrical resistance (R_{NE}):

$$\Delta R = R_{NE} + R_{intranuclear} - R_{replaced} \quad (1)$$

To obtain R_{NE} from ΔR, we need information on $R_{intranuclear}$ and $R_{replaced}$. Previously, we performed measurements showing that $R_{intranuclear} \cong R_{replaced}$ (Danker et al., 1999). Therefore, we could use the simple equation

$$R_{NE} = \Delta R \quad (2)$$

Of course, this approach does not consider the possible influence of R_{shunt} on our measurements. R_{shunt} is the electrical resistance pathway between the nucleus and the wall of the glass capillary. Thus, the following equation is used to include R_{shunt}:

$$\Delta R = \frac{1}{\frac{1}{R_{NE} + R_{intranuclear}} + \frac{1}{R_{shunt}}} - R_{replaced} \quad (3)$$

$$R_{NE} = \frac{1}{\frac{1}{\Delta R + R_{replaced}} - \frac{1}{R_{shunt}}} - R_{intranuclear} \quad (4)$$

We have now to prove that $R_{shunt} \gg R_{NE} + R_{intranuclear}$. As long as this is the case, we may use Equ. 2 instead of Equ. 4 without introducing too much error. Proofs are shown in the first section of results.

Electrophoretic Effects

The flow of electrical current through the NE, which is a necessary requirement of every NHT measurement, can also be used to induce movement of charged nucleoplasmic macromolecules in the electric field (i.e., electrophoresis). Since this is usually an unwanted side effect, we effectively avoid electrophoretic polarization of the nucleoplasm in our standard experimental procedures by reversing current direction every second. However, in this study we focused on electrophoretic effects. Therefore, the alternating current was switched to a constant current after the nucleus was appropriately placed inside the glass capillary. During the experiments shown here, a constant current of up to 200 μA was applied for a prolonged period of up to 2 minutes. Another feature different to standard experimental procedures was that, in a special series of experiments, we used a glass capillary tapered to a diameter too narrow to let the nucleus pass through. Hence, during the measurement, the nucleus will have an asymmetric shape, and thus the two NE surface poles that are exposed to current flow will have different sizes (i.e., a large pole and a small pole). The smaller of the two NE surface poles will contribute most to the lumped NE electrical resistance measured. When a constant electrical field is applied, charged nuclear particles are pulled off one NE surface pole and, subsequently, become attached at the opposite NE pole. Therefore, if such particles alter NE electrical resistance once arrived at the nucleoplasmic NE surface, then we expect a strong resistance change when particles arrive at the small NE pole and expect a weak resistance change when paticles arrive at the large NE pole. This asymmetry provides a pragmatical approach to determine whether negatively or positively charged molecules are responsible for the NE resistance changes.

Atomic Force Microscopy (AFM) on Isolated Oocyte Nuclei

For AFM we used a multimode NanoScope III (Digital Instruments, Santa Barbara, CA) for isolated nuclei, applying techniques described in detail previously (Rakowska et al., 1998). In short, oocyte nuclei were isolated as described above and then, instead of using them for electrical experiments, transferred onto glass and subsequently fixed with glutaraldehyde (2%). The preparation was then mounted on the AFM stage and the cytoplasmic NE surface was scanned with conventional AFM tips (silicium nitride; cantilever spring constant: 0.06 N/m), applying the contact mode. Surface topography was analyzed by using appropriate software supplied by the manufacturer (Digital Instruments).

Results

The Influence of R_{shunt} Can Be Neglected

Despite our unit of measure, ΔR, and our unknown variable, R_{NE}, all parts of Eq. 4 can be calculated based on the electrical conductivities of the buffer fluid (κ), the nucleoplasm (κ') and the geometrical parameters of the capillary, the nucleus and the buffer-filled cleft in between. We simplify the geometry of the capillary to that of a buffer-filled cylinder. We also simplify the nucleus to a short cylinder. Then, the cleft is a hollow cylinder with length L and width w (*see* Fig. 1).

While κ, r and L can easily be measured with fair resolution, the most critical and, at the same time, the most uncertain parameter in this scenario is the width of the buffer-filled cleft, w. In a good NHT experiment, the nucleus is somewhat larger in diameter than the narrow part of the capillary and therefore is slightly deformed during its passage. Thus, the NE is pressed against the capillary wall by the inner hydrostatic pressure of the nucleus. From the ultrastructural point of view, the height of the largest protrusions found on the outer surface of the nuclear membrane should be taken as a minimum estimate for w. Data for this are readily available from our AFM studies on the oocyte NE (Oberleithner et

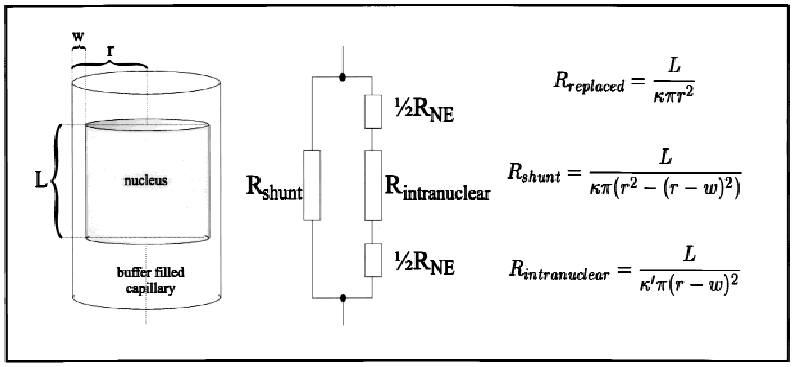

Fig. 1. Simplified model of the nuclear hourglass technique (NHT) used for electrical circuit analysis. Assuming a cylindrical shape for capillary and nucleus, R_{shunt} can be calculated from the geometrical parameters L, r and w and the electrical conductivity of the buffer fluid, κ. The electrical conductivity of the nucleoplasm is κ'. Buffer fluid conductivity and nucleoplasmic conductivity do not differ from each other (13.2 mS/cm^2). $R_{replaced}$ is the resistance of the buffer-filled capillary space (= volume) that has been replaced by the nucleus and the shunt.

al., 1994; Danker et al., 1997; Rakowska et al., 1998; Oberleithner et al., 1999) showing that w is likely to be smaller than 1 μm. In a separate series of experiments we directly evaluated the surface topography of an isolated oocyte nucleus by using AFM (Fig. 2). We prepared the nuclei identically as for electrical measurements but, in addition, fixed them with glutaraldehyde to maintain overall nuclear shape. We measured maximal height differences across the NE in the range of about 300 nm. They were most likely caused by locally active chromatin spots near the inner NE surface. The inset in Fig. 2 shows a small area of the NE surface. Individual NPCs are made visible by contrast enhancement. NPCs protrude from the cytoplasmic NE surface by about 20 nm.

As an example, we calculated the different electrical resistance components contributing to the NHT measurement. Let us first assume that no shunt-related error occurs. In this example, $R_{nucleus}$ is 4090 Ω. Most of this resistance is due to the resistance of the nucleoplasm, which, based on the its conductivity, is 3574 Ω. Then, R_{NE} is assumed to be 516 Ω. Since the nucleus physically replaces an amount of buffer fluid which also has a resistance of 3574 Ω, we should measure an increase in resistance $\Delta R = R_{NE}$ of 516 Ω if no shunt-related error occurs. If we now introduce R_{shunt} calculated for a cleft width of 1 μm, R_{shunt} turns out to be more than 50 times larger than $R_{nucleus}$. As a result, the difference between ΔR and R_{NE}, and hence the impact of R_{shunt} on our measurement, is as small as 3.1%. Although AFM measurements show that the distance between NE and glass can be expected to be much less than 1 μm, we simulated R_{shunt} for values of w ranging from less than 0.1 to 10 μm in order to see the impact of any shunt leakage (Fig. 3). Within this range, R_{shunt} remains high compared to R_{NE}.

Electrophoretic Plugging of Nuclear Pores with Negatively Charged Nucleoplasmic Particles

Application of constant current reproducibly leads to an increase in electrical resistance (Fig. 4). At the onset of the measurement (i.e., with the insertion of the cell nucleus into the tapered part of the glass capillary), the initial value, ΔR, is measured under alternating current. ΔR is about 500 Ω. At constant current, however, ΔR starts to increase and reaches a new steady-state value (ΔR_{max}) of 6400 ± 500 Ω after 90 ± 9 seconds ($n = 6$). The increase in electrical resistance is accompanied by a "visible polarization" of the nucleus. Two minutes after constant application of 100–200 μA, a white precipitate appears near the nucleoplasmic NE surface adjacent to the positive electrode. Sometimes, the bulk chromatin moves into the same direction, although this is not clearly visible in all cases.

To define whether negatively or positively charged macromolecules are responsible for R_{max}, we asymmetrically squeezed the isolated cell nucleus into the tapered glass tube. In this asymmetrical configuration, one of the two NE surface poles exposed to electrical current is considerably smaller. Now we observed a strong dependence of R_{max} on the polarity of the electric field. That is, whenever the positively charged electrode was adjacent to the small NE surface pole, we observed a drastic increase in NE electrical resistance. In contrast, if the negatively charged electrode was adjacent to the small NE surface pole, only very weak effects were observed (*data not shown*).

This observation can be explained by the fact that the small NE surface pole of the cell nucleus contributes the most to the overall nuclear resistance, while the contribution of the large NE surface pole is much smaller. In other words, the major effect takes place at the NE surface pole adjacent to the positively charged electrode. We therefore conclude that negatively charged nucleoplasmic macromolecules are drawn towards the positive electrode causing macromolecule crowding at the inner NE surface combined with a marked increase of electrical resistance. Obviously, some molecules have just the right size to be drawn into the NPC channels by the electrical field. Thus, NPCs will become plugged with nonconducting material. This could explain the dramatic increase in electrical resistance. We call this concept

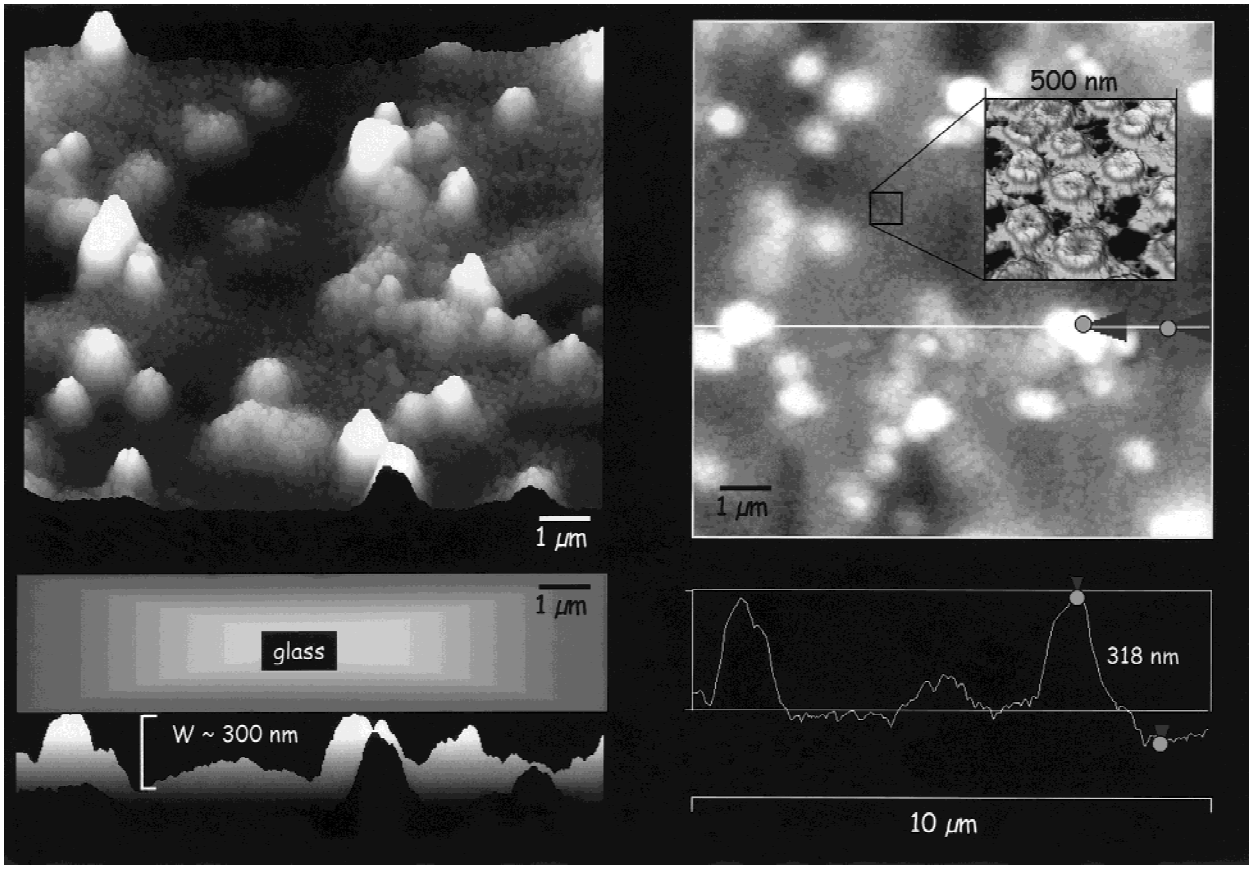

Fig. 2. Nuclear envelope (NE) topography of an isolated oocyte nucleus imaged by atomic force microscopy (AFM). (*Upper left*) 10 × 10 μm NE area shows multiple protusions caused by active chromatin spots near the NE. (*Lower left*) NE shown as profile (zero tilt) attached to glass. The model indicates the space expected between NE and glass wall of the capillary. (*Upper right*) Top view of the same NE surface shown at left. The line corresponds to the profile shown in detail below. The insert highlights a small area (500 × 500 nm) of the NE surface. Nuclear pore complexes (NPCs) are made visible by contrast enhancement. (*Lower right*) NE profile indicating a maximal height difference of 318 nm.

"electrophoretic plugging," a term which has been chosen to stress its analogy to the concept of "macromolecular plugging" for actively transporting NPCs (Bustamante & Veranda, 1998).

Nuclei Swell Dramatically when Isolated in Absence of Macromolecules

When oocyte nuclei are isolated in buffered electrolyte solution, mimicking the cytosol but lacking macromolecules, dramatic swelling occurs within minutes. We tested different synthetic macromolecules and found out that 40-kD PVP at a concentration of 1.5% prevents swelling of nuclei after isolation (Fig. 5). In time-lapse experiments we followed the nuclear shape changes in absence and presence of PVP. Since nuclei were usually spherical in shape we could easily calculate the nuclear NE surface area. Experiments are summarized in Fig. 6. In presence of 1.5% PVP, nuclei maintain shape and volume over at least 60 minutes. In absence of PVP, nuclei swell, exhibiting a steady-state nuclear surface area 2.6 times larger than the original (physiological) NE surface. Clearly, salt and water move into the nucleoplasm when the nucleus is transferred from its natural cytosol, rich in macromolecules, into the electrolyte solution lacking colloid-osmotic (oncotic) pressure. This finding must be included in the calculation of mean single NPC conductance derived from the "apparent" NE surface.

Discussion

The finding that a 1-μm wide, buffer-filled cleft between capillary wall and nucleus would not invalidate NHT measurements may be stunning, at least for the electrophysiologist who is used to situations where proper recording requires tight gigaseals. In fact, a mathematical evaluation as presented in this paper would be much less applicable to a patch-clamp experiment on plasma membrane. What makes the difference here is that the resis-

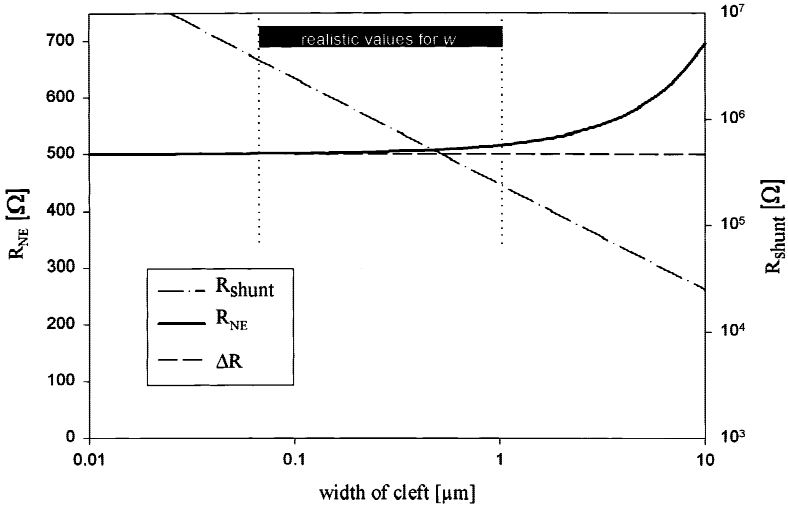

Fig. 3. Dependence of R_{shunt} on the width w of the buffer-filled cleft between capillary wall and nucleus. (L = 270 μm, r = 135 μm, $\kappa' = \kappa$ = 13.2mS/cm^2). Within a realistic range for w, R_{shunt} does not fall below $10^5\Omega$ and the difference between ΔR (as calculated with equation (4)) and R_{NE} is small (<3.5%).

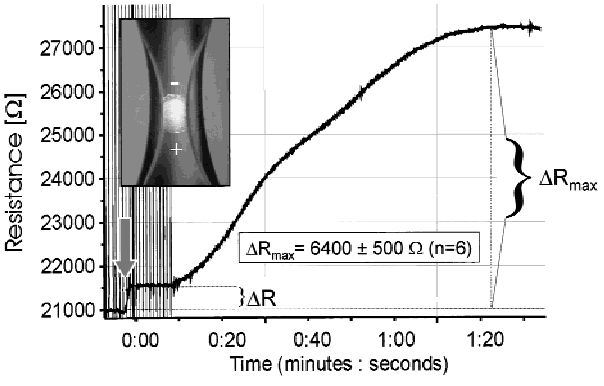

Fig. 4. Effect of constant current on nuclear electrical resistance. In the beginning of this experiment, alternating current is applied (where each change in current direction is indicated by a dark vertical line). In this mode, the introduction of the nucleus at t = 0 increases the electrical capillary resistance by ΔR = 500 Ω (tracing modified to match mean value). Once the nucleus is in position, the resistance stays constant as long as alternating current is applied. After switching to constant current, the electrical field has the polarity indicated in the inset. The electrical resistance begins to increase and reaches a mean ΔR_{max} of 6400 Ω after one minute.

tance of the nuclear membrane is much smaller than that of the plasma membrane. Thus, the nucleoplasm makes a sometimes larger contribution to the whole nuclear resistance than its surrounding membrane, the NE. This is particularly true for the *Xenopus* oocyte nucleus, where NPC density is about 10 times larger than in somatic cells (Mazzanti et al., 2001). Here, the NE contributes just a fraction of the overall nuclear electrical resistance. These conditions are far from those usually present in plasma membrane. As a result, the shunt resistances we calculated for NHT do not allow similar measurements on high-resistance plasma membranes, but cause no problems in low-resistance oocyte NE. It must be stressed that this is not a circular argument in the sense that we had to assume a low NE electrical resistance in order to justify our neglect of R_{shunt}. The shunt-related error we calculated strongly depends on the measured ΔR. In cases where a membrane with higher resistance is present, we would measure an increased ΔR, which would notify us about both the increased membrane resistance and the increased shunt-related error.

A confirmation that these rather theoretical investigations on R_{shunt} also prove true in practice is given with the constant-current experiments. The increase in resistance observed under these conditions is accompanied with an electrophoretic movement of chromatin and a visible accumulation of nucleoplasmic material at the inner surface of the NE. This physical polarization of nucleoplasmic macromolecules could not be observed if the electrical current would have used the extranuclear shunt-pathway rather than the intranuclear NPC-pathway. Furthermore, this polarization is obviously the cause of the observed increase in resistance. Some of the charged macromolecules will have the appropriate size to be drawn into or even through the channels of the NPCs. Eventually, the electrically conducting channels of the NPCs will become completely occupied by these macromolecules, which are electrically "silent" (nonconducting) and thus increase the NPC resistance. This effect is reminiscent of "macromolecular plugging" reported for actively transporting NPCs (Bustamante & Veranda, 1998). Therefore, we suggest the term "electrophoretic plugging" for the phenomenon described here. In light of these observations it is highly unlikely that the increase in resistance is caused by an increase of R_{shunt} due to better sealing in the presence of an electrical field. Such effects have been reported from patch-clamp experiments but can not explain the details of our observations. Since the measured ΔR can never be greater than

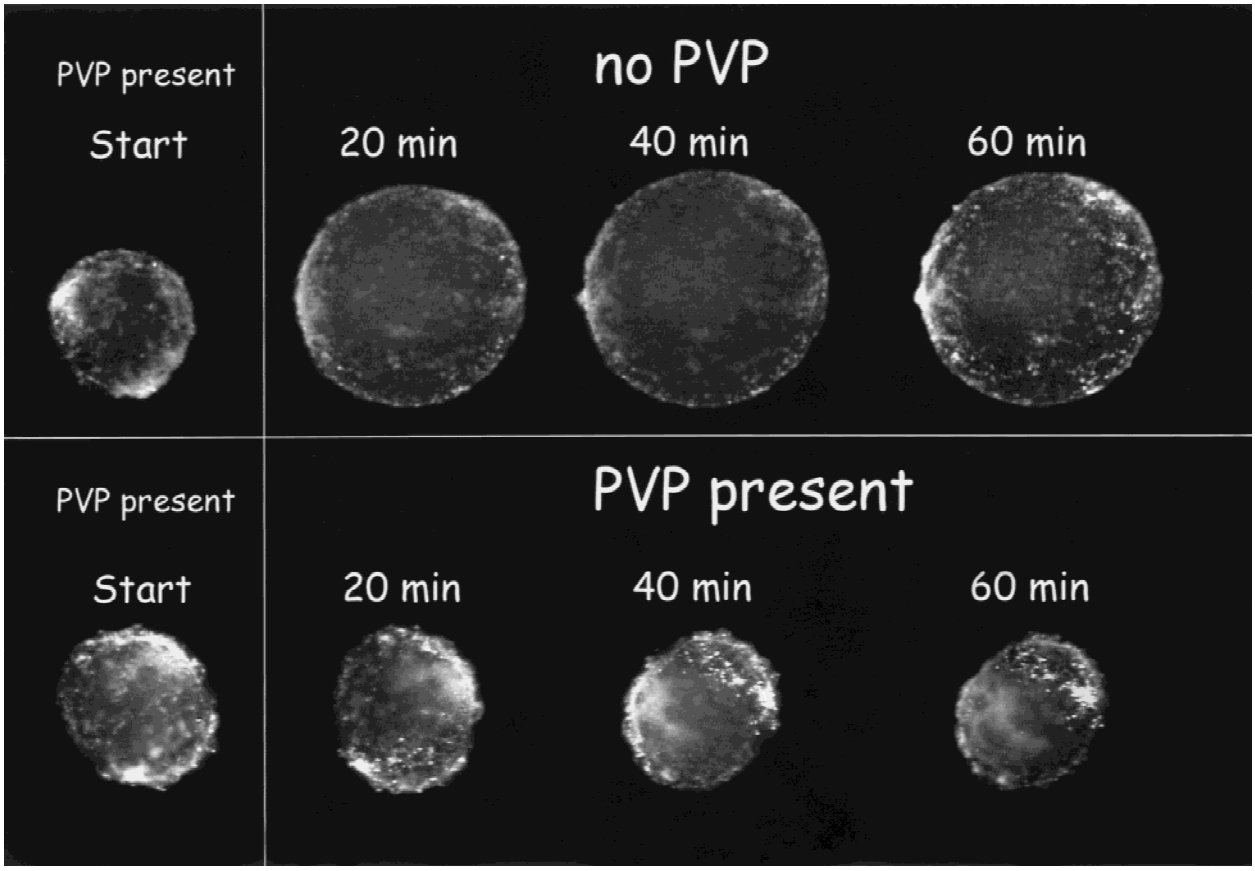

Fig. 5. Time-lapse experiments of isolated *Xenopus laevis* oocyte nuclei bathed in absence or presence of 1.5 % PVP. (*Upper row*) The cell nucleus remains at its physiological size when isolated from a Stage VI oocyte in electrolyte solution mimicking the cytosol plus 1.5 % PVP. The nucleus dramatically swells when PVP is removed. (*Lower row*) The cell nucleus retains its original size over at least an hour when PVP is present in the electrolyte solution.

R_{shunt}, this also means that the shunt resistance is at least as high as the maximum value observed in these experiments.

Compared to nuclear patch-clamp data, our results emphasize the need of overall NE conductance measurements in addition to single-channel analysis. This is especially important when the barrier function of the NE is discussed. It has already been realized that nuclear patch clamp is likely to underestimate the number of NPCs in the NE (Mazzanti et al., 1990), probably due to NPC closure under patch-clamp conditions. NPCs are huge, fragile macromolecular assemblies spaning a double-layered membrane system. Compared to classical ion channels, they may be much less resistant to mechanical stress during patch clamp. Analysis of NHT data has shown that the majority of NPC is electrically open, with an average NPC conductance of about 1.7 nS (Danker et al., 1999). As we have shown here, this conclusion is not weakened by the negligibly small shunt-related error. However, this NPC conductance value was measured in presence of PVP, a synthetic macromolecule, in the solution. Under these conditions, nuclei maintain their physiological shape exhibiting interdigitations, undetectable with light microscopy. AFM showed such NE surface irregularities, and removal of macromolecules functionally proved their existence. As a consequence we have to consider the "true" NE surface area when a mean NPC conductance is calculated. As pointed out above, cell nuclei swell in absence of macromolecules by a factor of 2.6. Thus the most realistic value for mean NPC conductance is 654 pS (i.e., 1.7 nS divided by 2.6).

This value refers to the "open" pore. As shown in the present study electrophoretic plugging increases overall NE resistance by a factor of about 13 (from $\Delta R = 500$ Ω to $\Delta R_{max} = 6400$ Ω). Since electrophoretic plugging occurs in a polarized fashion affecting only one NE surface pole, namely the one next to the electrically positive electrode, we have—with symmetrical surface poles—a 26-fold increase in mean NPC resistance (or decrease in mean NPC conductance). Thus, an electrophoretically plugged NPC has a mean conductance of about 25 pS (654 pS divided by 26). Although this value matches the

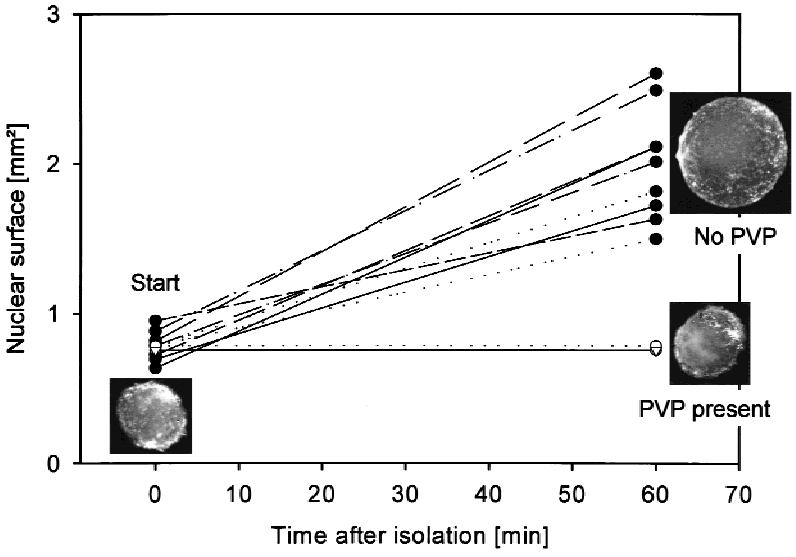

Fig. 6. Apparent changes in nuclear surface area induced by the omission of 1.5 % PVP. On average the apparent surface increases by a factor of 2.6.

low electrical conductances of individual plasma membrane ion channels, it should be kept in mind that usually plasma membrane channels are closed for long periods and open (i.e., electrically conductive) only in the millisecond range, while our calculations are based on permanently open pores.

In summary, we have demonstrated in theory and practice that NHT is well suited to accurately measure the electrical resistance of oocyte NE. Shunt-related errors will play a negligible role as long as the nucleus is gently squeezed into the capillary. We demonstrated electrophoretic NPC-plugging and showed that these effects are caused by negatively charged nucleoplasmic macromolecules.

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