Ion Permeability of the Nuclear Envelope

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The nuclear envelope mediates nucleocytoplasmic communication. Nuclear pores transport proteins and RNA into and out of the nucleus. The pore is believed to allow free ion diffusion. Using an electrophysiological approach, we show the possible semipermeable properties of the envelope. To accomplish these functions we hypothesize a mechanism in which the pore complex acts as a molecular diaphragm.

In the last five years several laboratories have begun to explore possible new mechanisms of nucleocytoplasmic traffic. In particular, the patch-clamp technique applied to isolated and intact nuclei (1, 8, 9) has indicated the presence of ion channels on the external (cytoplasmic) face of the nuclear envelope that may participate in communication between the cytosol and nucleoplasm. The concept that the double membrane separating the nucleoplasm from the cytoplasm behaves not only as a passive barrier but also as a functional boundary against free diffusion was formulated several years ago (11). The work of many cell biologists has contributed to a scenario in which the nuclear envelope plays a critical role in selecting specific proteins and RNA molecules. With the use of techniques such as electron microscopy, photobleaching, fluorescent indicators, confocal imaging, and molecular biology (2), the identification of the nuclear pores as a selective nucleocytoplasmic pathway has become dogma. According to this hypothesis, nuclear pore selectivity is based on two factors: molecular weight and the presence of a signal sequence in the protein or RNA. Molecules with a molecular weight <30–40 kDa freely diffuse across the double membrane of the envelope; larger molecules that contain the correct signal sequence are transported by an ATP-or/and GTP-dependent process (2, 12). Using the patch-clamp technique, we have obtained electrophysiological data that challenge this model.

In light of the work mentioned above, it did not seem plausible that the nuclear envelope, and specifically the nuclear pores, could represent a selective barrier for ions. However, as observed in nuclear patch-clamp experiments, ion channels are present on the cytoplasmic face of the nuclear envelope (the outer membrane). In the cell, the nuclear outer membrane is continuous with the endoplasmic reticulum (Fig. 1A). After isolation of nuclei (Fig. 1B) the nuclear envelope loses all connections with the endoplasmic reticulum, but, with the patch-clamp technique, ion channels are observed when the nuclei are approached with the patch pipette (Fig. 1C).

From an electrical point of view, these channels must be located in parallel with nuclear pore complexes. The nuclear pores are thought to be large water-filled openings and would be expected to have a high conductance compared with an ion channel. If in the nuclear envelope ion channels are present in parallel with the nuclear pores, the large conductance of the pore would mask the proposed outer membrane ion channels, making their detection impossible with present techniques (Fig. 1D). However, on-nucleus patches (1, 8, 9) reveal current traces readily interpreted as single ion channel openings and closings, which are easy to record in several biological preparations. Are the nuclear pores and the ionic channel the same object?

There are two interpretations of the nucleus patch-clamp data. 1) Under our experimental conditions, using isolated nuclei, the pores are closed, and the observed single-channel currents originate from ion pathways present on the external membrane of the envelope; or 2) the ion channels on the external nuclear membrane are the nuclear pores. In the second case, even in the absence of agonists or cytoplasmic regulators, the pores behave as an ion channel with several conductance states.

There is an important difference between the two possibilities. In the case of total or partial pore closure, closure could perhaps be due to nuclear isolation or to the loss of important cytoplasmic factor(s). This, however, is not the case for experiments performed on intact Xenopus oocytes (10). If the pores regulate nucleocytoplasmic ion movement, they may play a role in...
the modulation of not only large molecules but also small solutes. Previous studies support the latter possibility and indicate that the nuclear envelope modulates the movement of charged particles. Before the patch-clamp technique became available, Loewenstein and colleagues (7) demonstrated that the nuclear envelope could function as an ion-selective interface under certain conditions. The data of the Loewenstein group demonstrated the presence of a potential difference between cytosol and nucleoplasm in some cells but not in others, and an increase in nuclear envelope electrical resistance on stimulation of cells with steroid hormones was observed. Another example concerns the permeability of intracellular Ca^{2+} through the envelope. A priori there is no reason to believe that a pathway in which molecules of 40 kDa passively diffuse restricts the passage of small ions such as Ca^{2+} unless we propose that the nuclear pore is not a static structure but, rather, has the ability to modify its diameter to modulate nucleocytoplasmic communication. Furthermore, the experiments that demonstrate an active role of nuclear cisternae in the regulation of Ca^{2+} around the nuclear envelope could represent an additional sophistication of the nucleocytoplasmic communication (4, 15).

**Characteristics of nuclear ion channels**

Figure 2 shows the first experiment realized on the nuclear envelope using the patch-clamp technique (9). The nuclei were extracted from a mouse zygote by mince of micromanipulation procedure (Fig. 2A). Enucleation is the way in
FIGURE 2. A: procedure adopted to enucleate mouse zygote. Mouse zygotes were collected from superovulated adult females 12 h after mating, enzymatically cleaned by the cumulus cells, and transferred to the micromanipulation chamber. Sequence of steps from holding the zygote by the zona pellucida with light suction (a), entering the zygote with a smaller suction pipette (b), attaching it to the pronucleus (c), and extracting and holding the pronucleus for patch clamping (d), takes <10 min. During the experiment pronucleus was immersed in a solution tonically similar to the cytoplasm: (in mM) 120 KCl, 2 MgCl₂, 1.1 EGTA, 0.1 CaCl₂, 5 glucose 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4. B: single-channel measurements from the nuclear envelope. In all on-nucleus experiments, patch pipette contained the solution reported above. a: Examples of current traces elicited by +30, +20, −20 and −30 mV. b: Example of amplitude histograms derived from current recordings on left. c: Current/voltage relation plotted, using values from histogram peaks and average nucleus potential measured with intranuclear electrodes (9). d: 3 examples of channel opening and closing at −10 mV on a faster time scale. [From Mazzanti et al. (9).]
which the nuclear surface becomes accessible to the patch pipette. In this manner we formed
nucleus-attached patches to investigate the presence of ion-selective channels. Figure 2B shows
electrophysiological recordings and analysis of a single-channel experiment. Figure 2Ba depicts
patch currents at a pipette voltage (V_p) of -30, -20, +20 and +30 mV. Examples of the single-
channel records at V_p = -10 mV are shown in Figure 2Bd on a faster time scale. From the current/voltage (i/V) curves in Fig. 2Bc, which are obtained by plotting the current at the peaks of the amplitude histograms (Fig. 2B), the channel conductances are 200 and 55 pS.

From more than 300 similar experiments performed on different types of isolated nuclei, we can delineate some of the main properties of such ionic permeabilities. Most of the patches show similar channel-like behavior. Current amplitudes appear randomly distributed within the same experiment when different current recordings obtained under the same conditions are compared. Up to now we have never identified a predominant conductance. The actual range is much wider than the one presented in Fig. 2Bc, and intermediate conductance values can be as low as 25 pS. Current traces often show either few or many sublevels whose amplitudes vary on a 10-fold scale from experiment to experiment.

Current amplitude could depend on many factors. Since we are working with isolated nuclei, the state of permeability of the nuclear pore proteins could be influenced by the unusual environment in which we perform electrophysiological measurements. In the cell-free nuclei experiments, artifacts may be introduced by isolation procedures or the solution in which the nuclei are immersed may lack cytoplasmic factors, both of which could alter nuclear pore functions. In the experiments presented in Fig. 3, we attempt to circumvent some of these problems by applying the patch-clamp technique on the nuclear membrane in situ, i.e., without cell nucleation (10). Figure 3A shows the sequence of events for obtaining pipette seals on the nuclear envelope. When the patch electrode contains a solution that ionically mimics the cytoplasm, it is possible to observe single-channel openings that are activated by different pipette voltages. Figure 3B depicts examples of such recordings from different experiments at various test potentials. From analysis of the current traces we obtained conductance values that ranged from 50 to 1,000 pS. Single-channel recordings with similar characteristics are commonly obtained in cell-free nuclei from other cell types (1, 8, 9).

Structural data are particularly relevant here. Analysis of nuclear pore density using freeze-fracture electron microscopic techniques (6) indicates that the density is between 10 and 30 pores per typical patch-size membrane isolated by the electrode. Taking the structural and electrophysiological data together, we suggest that we are observing nucleocytoplasmic current flow through the nuclear pores themselves.

On the basis of structural, electrophysiological, and fluorescent particle diffusion experiments performed in our laboratory and others, we propose that the nuclear pores behave as ion channels under certain experimental conditions. The intrinsic gating mechanism may be different from a conventional ion pathway, yet we propose that the nuclear envelope retains the ability to become a selective electrical barrier upon specific physiological stimulation.

A model for ionic pathways associated to nuclear pores

How can a large aqueous pore function as an ionic channel? The question is legitimate, because there is substantial evidence that small molecules freely diffuse between the nucleus and the cytosol. We see, however, no contradiction between our view of nucleocytoplasmic communication and previous views. A unifying hypothesis is that, under specific physiological conditions, the pores are open and molecules larger than 40 kDa are translocated into and out of the nucleus in an ATP- and/or GTP-dependent manner. In addition to this traditional pathway, morphological studies of the nuclear pores indicate possible sites for ion diffusion. Milligan's group (5) has described in their nuclear pore model a large central pore in the complex, usually associated with macromolecular transport, surrounded by eight smaller channels. More recent structural studies using atomic force microscopy (3) show the presence of a depression in the central granule of the pore that could represent either the protein/RNA pathway or an ion channel or both. In a recent paper Perez Terzic and colleagues (13) demonstrated that the protein subunits composing the nuclear pore are mobile and that they change configuration, depending on the cytoplasmic environment. The motor for a structural change could be provided by actin and myosin filaments that were detected in association with the pore complex (14). ATP, essential for the nuclear pore function (2), would maintain the open permeability of the envelope to small solutes. A decrease of the energetic molecules in the nucleoplasm could promote a conformational change in the pore structure, chemically and electrically isolating the nucleoplasm.

We propose four different possibilities in...
which ATP-dependent transporter and ion channels are present in the same protein complex (Fig. 4). The overall organization recalls the recent cytoplasmic arrangement of the pore complex presenting the usual eight particles encircling the central granule (3, 5, 13). In our view, the protein transporter could be physically the same object of the ion pathway or a separate pore in parallel with many aqueous channels.

In Fig. 4, A and B, the ion channel and transporter are depicted as the same object. In these two cases the eight subunits have a structural function. The central granule, by morphological changes, could open nucleocytoplasmic pathways in which ions are freely permeable and big molecules are transported through the central pore or through the openings created by the modified central plug and the surrounding pore complex subunits.

In the models in Fig. 4, C and D, the transporter is located in the center of the granule, moving proteins and RNA in and out of the nucleus without creating an aqueous pore. The diffusional pathways are located in the periphery.

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**FIGURE 3.** A: summary of procedure we used to perform nucleus-attached patches inside the *Xenopus* oocyte. Enzymatically isolated first-stage oocytes were transferred into the experimental solution (in mM: 140 NaCl, 5 KCl, 0.5 MgCl₂, 1.5 CaCl₂, 5 glucose, 10 HEPES, pH 7.4). a. Oocyte was maintained in position on the holding pipette (left) via light suction. After plasma membrane was perforated (b), patch pipette (right) was pushed gently against nuclear surface (d). A clear shadow in the oocyte cytoplasm is visible in c due to a temporary positive pressure applied in the pipette. Outflow of solution keeps tip of electrode clean (horizontal bar: 50 µm). B: single-channel recordings from nucleus-attached experiments inside the cell. Six different test voltages delivered into the pipette (value above each trace) triggered opening of ionic channels in different experiments. Channel conductances range from 50 (top left) to 1,000 pS (bottom left). [From Mazzanti et al. (10).]
of the complex either as classical ion channels (C) or formed by the centrifugal movement of the peripheral subunits (D). From the electrophysiological point of view each of these four models can explain our current recordings. If we observe the example of Fig. 4A, the central nucleocytoplasmic pathway can be totally closed (left) or totally open (right) with many intermediate positions (center). If we assume that such a big pore can open and close like a diaphragm in many steps, we can readily interpret electrophysiological data. If, in contrast, the ionic pathway is not placed on the central granule or is physically distinct from the big molecule transporter, it is possible that conformational changes of the central granule (B), contemporary opening of many channels (C) or movement of the peripheral subunits of the pore could form many nucleocytoplasmic ionic communications that behave electrophysiologically like the data we obtained in patch-clamp experiments on nuclei.

In conclusion, our hypothetical view of the ion
permeability of the nucleus is consistent with the pore complex as the site of nucleocytoplasmic exchange. The flow of charge could take place within the same opening that transports proteins and RNA or a structure parallel to this opening. The actual “ion channel” may come to complete closure. Kinetically, it appears more like a diaphragm with several intermediate states than a conventional ion channel with one or, at most, a few conducting states.

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