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Electrical Dimension of the Nuclear Envelope

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Mazzanti, Michele, José Omar Bustamante, and Hans Oberleithner. Electrical Dimension of the Nuclear Envelope. *Physiol Rev* 81: 1–19, 2001.—Eukaryotic chromosomes are confined to the nucleus, which is separated from the rest of the cell by two concentric membranes known as the nuclear envelope (NE). The NE is punctuated by holes known as nuclear pore complexes (NPCs), which provide the main pathway for transport of cellular material across the nuclear-cytoplasmic boundary. The single NPC is a complicated octameric structure containing more than 100 proteins called nucleoporins. NPCs function as transport machineries for inorganic ions and macromolecules. The most prominent feature of an individual NPC is a large central channel, ~7 nm in width and 50 nm in length. NPCs exhibit high morphological and functional plasticity, adjusting shape to function. Macromolecules ranging from 1 to >100 kDa travel through the central channel into (and out of) the nucleoplasm. Inorganic ions have additional pathways for communication between cytosol and nucleus. NE can turn from a simple sieve that separates two compartments by a given pore size to a smart barrier that adjusts its permeabiltiy to the metabolic demands of the cell. Early microelectrode work characterizes the NE as a membrane barrier of highly variable permeability, indicating that NPCs are under regulatory control. Electrical voltage across the NE is explained as the result of electrical charge separation due to selective barrier permeability and unequal distribution of charged macromolecules across the NE. Patch-clamp work discovers NE ion channel activity associated with NPC function. From comparison of early microelectrode work with patch-clamp data and late results obtained by the nuclear hourglass technique, it is concluded that NPCs are well-controlled supramolecular structures that mediate transport of macromolecules and small ions by separate physical pathways, the large central channel and the small peripheral channels, respectively. Electrical properties of the two pathways are still unclear but could have great impact on the understanding of signal transfer across NE and gene expression.

I. INTRODUCTION

A. Biophysical View of the Cell Nucleus

Membranes of living cells play two fundamental roles: structural and functional. The membranes delimit the various cellular environments, and in doing so, they help to segregate, restrict, and concentrate cellular material. This function creates a specialized space for molecular interaction. The lipid bilayer forming the membranes contains specialized molecules and macromolecular complexes that detect and discriminate the passage of solutes, including monoatomic ions and small molecules. In principle, all biological membranes retain these characteristics whether confined to the cell surface or located in the cytoplasm. However, although it is commonly accepted that the plasma membrane, the endoplasmic reticulum (ER), and the mitochondrial membranes are semipermeable barriers, the opinions concerning the properties of nuclear envelope (NE) permeability are not unambiguous. Most cell biologists agree that only large proteins are selectively transported into and out of the cell nucleus. Small molecules and ions seem to have free access through the NE, independent from species and electrical charge. However, there is increasing evidence suggesting that the NE is a dynamic ion-selective membrane rather than the more popular view that it is a crystallized molecular sieve. More than 30 years ago, membrane physiologists and biophysicists began to investigate the nucleus with electrophysiological techniques (Fig. 1). At that time, work with microelectrodes suggested that the NE behaved as a membrane barrier, limiting the passage of ions while shielding the genetic material from the rest of the cell. During the ensuing time, cell and molecular biologists and membrane physiologists have described with the aid of more sophisticated techniques the finer details of the NE structure and function. The picture that has emerged is one of an active, rather than passive, envelope. This review focuses on the electrical properties of the NE. Because the nucleus contains a large number of charged particles, one of which is DNA, it seems likely that nucleocytoplasmic transport can be influenced, and even driven, by electrical forces and that their electrical charge carriers, the monoatomic ions, play a significant role in NE function. We hope that this review sheds light on some of the current concepts in the field of the electrophysiology of the cell nucleus.

B. Nuclear Envelope Structure

In all eukaryotic cells the NE is formed by two concentric, lipid bilayer membranes encircling the genetic material (165). While the outer nuclear membrane (ONM) is in continuity with the ER, the inner nuclear membrane (INM) is in contact with the nuclear lamina, a fibrous network located just below the envelope (Fig. 2). The inner and the outer membranes make contact at several points forming aqueous apertures through the two membranes: the nuclear pore complexes (NPCs). A single NPC, a supramolecular structure formed by hundreds of proteins, occupies the cylindrical invagination formed by the junction of the two membranes. This structure is the only known route for direct nucleocytoplasmic exchange of matter. The NE can be considered as formed by two distinct parts: one consisting of "structural" components and one consisting of "functional" components conferring the permeability barrier properties.

C. Nuclear Pore Complex

The NPC is one of the most conspicuous structural features of the NE (104, 127) (Fig. 2). NPCs stand out as the most prominent structures in topological imaging of the NE detectable with scanning electron microscopy (SEM; Ref. 141) and atomic force microscopy (AFM; Refs. 114, 134). They are supramolecular structures consisting of more than 100 individual proteins with a total mass of ~124 MDa (127). Electron and fluorescence microscopy indicate that small molecules, ions, and particles cross the NE (104, 109, 122). These observations have been interpreted to mean that NPCs have a diameter on the order of 10 nm and that this diameter may vary from a few nanometers to several tens of nanometers according to cell type and cell cycle (104, 109). This large diameter leads to the inescapable conclusion that monoatomic ions must flow unrestricted through NPCs. Consequently, any observed heterogeneous concentration distribution of small ions and molecules cannot result from the presence of a selective membrane. "Cytoplasmic exclusion" (68) caused by the compartmentalized distribution of water and molecular binding sites is the most accepted hypothesis to explain unequal distribution of small ions between the cytoplasm and cell nucleus (104, 122, 123).

A frequently discussed question over the past years was whether it is possible that the NE, densely packed with NPCs, can serve as a semipermeable barrier and, as a consequence, can electrically separate two different environments (i.e., cyto- and nucleoplasm). The presence of a resting electrical potential is not, per se, a guarantee that the envelope is semipermeable. There are several ways to create "solute inhomogeneities" within the same biological environment (35). The cytoplasmic exclusion principle affirms that because the state of water is different in the different cellular compartments, the solubility, and thus the concentration, of a solute is compartmentalized (in our case, between nucleus and cytoplasm). The principle resembles the

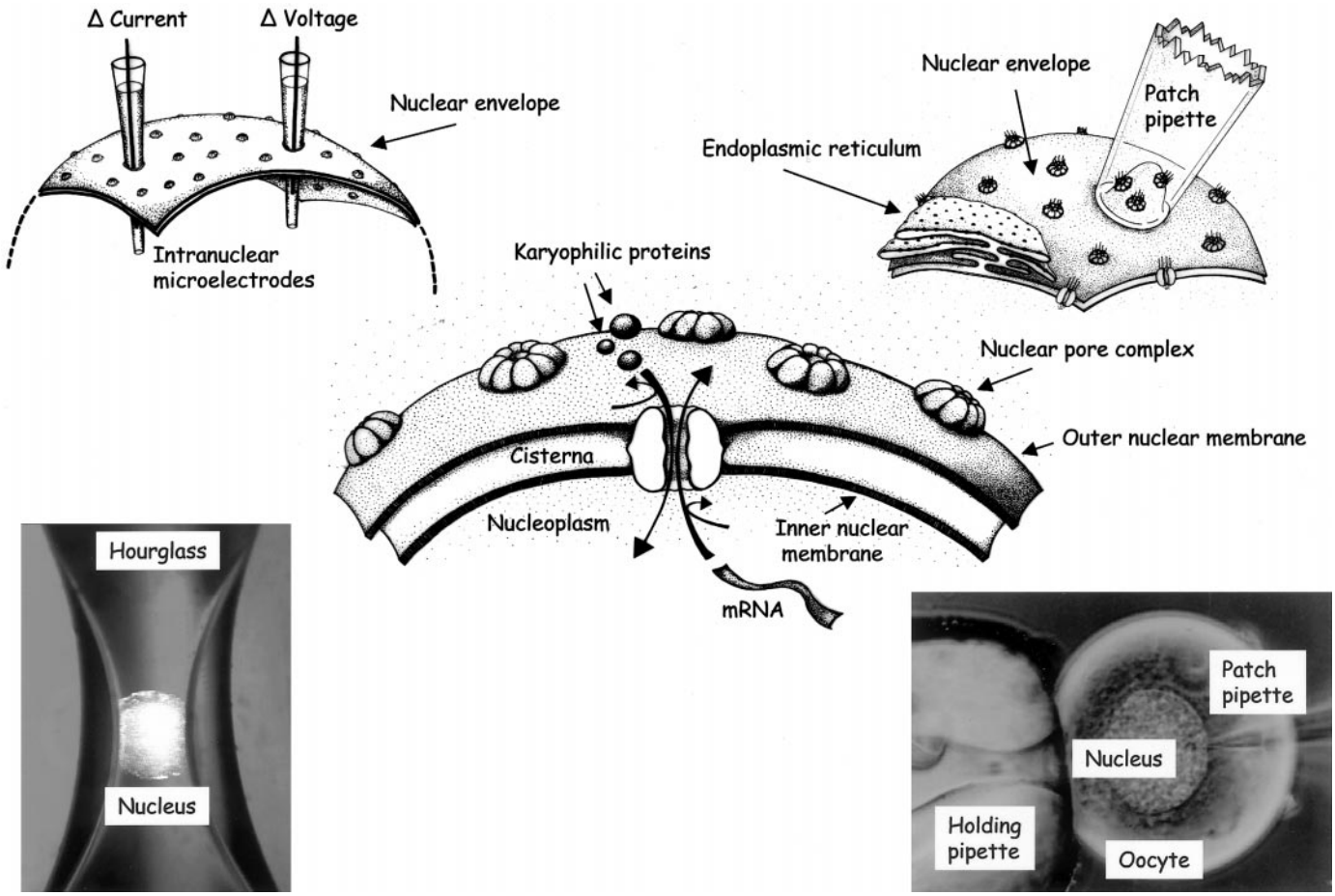

FIG. 1. Three electrophysiological approaches for investigating passive permeability of inorganic ions of the nuclear envelope (NE). *Center*: schematic diagram of the NE with a cut-open view of the nuclear pore complex (NPC). Macromolecular transport through the central channel of the NPC is indicated by the bent arrows. *Top left*: 2-microelectrode technique used to measure the NE resistance in intact cell nuclei. *Top right*: patch-clamp experiment in the "nucleus-attached" configuration. The presence of the endoplasmic reticulum represents a potential danger in patch-clamping the "wrong" membrane. [Modified from Danker et al. (31).] *Bottom right*: nucleus-attached patch-clamp experiment in intact *Xenopus laevis* oocyte. The oocyte is maintained in position at the tip of the holding pipette via light suction. After perforating the plasma membrane, the tip of the patch pipette is gently pushed against the nuclear surface. [Modified from Mazzanti et al. (108).] *Bottom left*: nuclear hourglass technique. An isolated nucleus of *X. laevis* oocyte is moved into the tapered part of a glass capillary by a gentle fluid movement. Electrical current is injected via massive Ag/AgCl electrodes placed at the ends of the capillary. The voltage drop across the nucleus is measured with 2 conventional microelectrodes placed near the nuclear surface. The nuclear hourglass technique measures the absolute NE electrical resistance in large nuclei of *X. laevis* oocytes. [Modified from Danker et al. (32).]

"association-induction" hypothesis that explains cellular resting potentials without the need for plasmalemma Na^+-K^+ pumps (85–88). If it is accepted that the cell membrane plays a fundamental role in the definition of cytoplasmic molecules and ion concentrations, the same principle should be valid also for the NE. The differences concern the dynamics of the system. Nuclear envelopes disappear and reconstitute at each cell division. However, in quiescent cells, nucleocytoplasmic concentration gradients exist for macromolecules and inorganic ions. Most likely, the semipermeability of the NE is restored under such conditions and used for important cell functions. In a review about cell electricity, De Loof (36) termed a cell as functioning as a "miniature electrophoresis" chamber. Furthermore, the role of plasma membranes as "privileged sites of electric fields" was discussed by Olivotto et al. (120), who also described membrane proteins as "electrical devices" that respond to electric fields. Matzke and Matzke (103) extended this view to the NE. They discussed the regulation of eukaryotic gene expression from a bioelectrochemical perspective, giving evidence for the close association of DNA and proteins with the NE (101). With the above-mentioned exception of the oocyte preparation (77), intranuclear voltages (intranuclear minus extranuclear voltage, with extranuclear

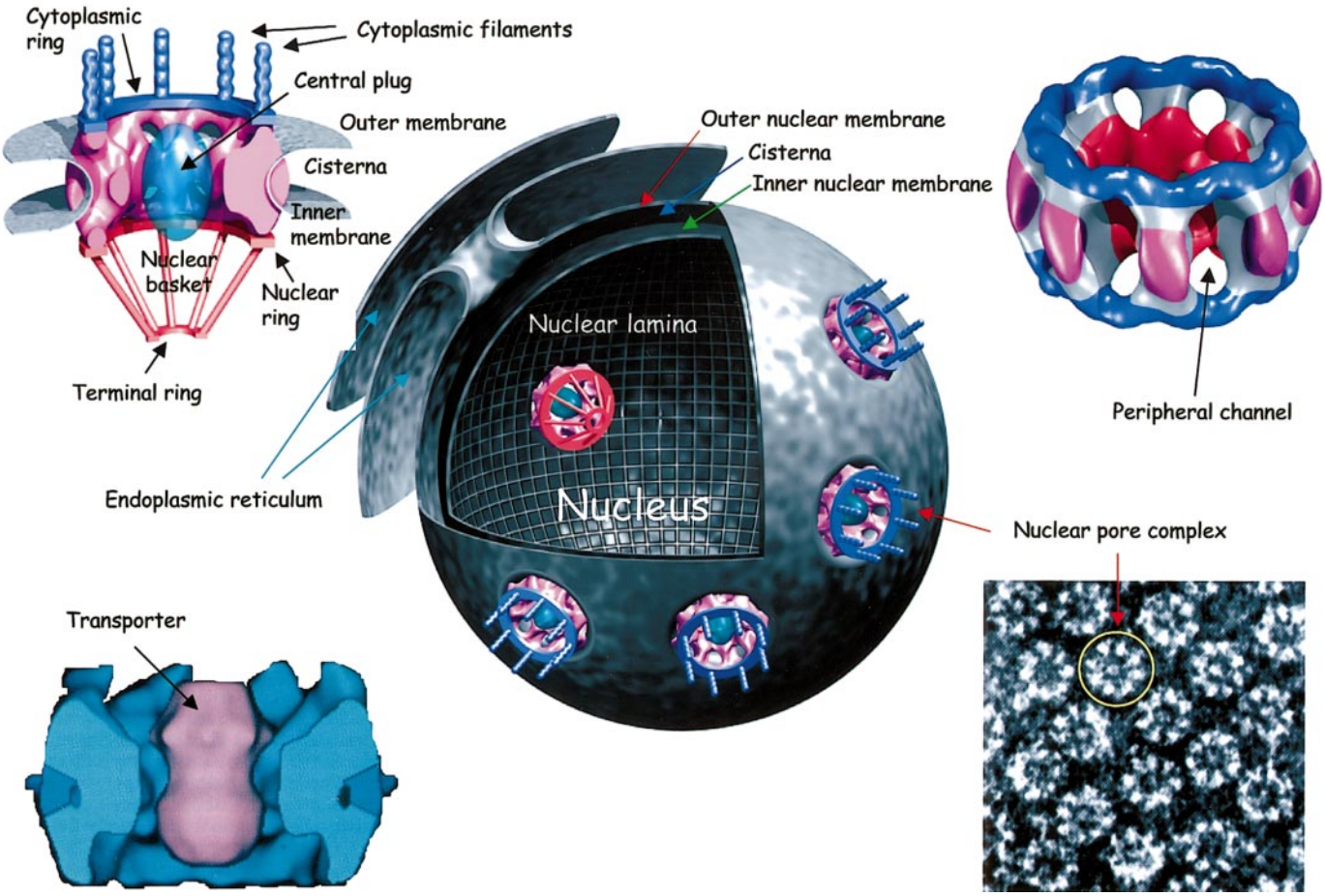

FIG. 2. Structural basis of NE and NPC. *Middle*: the NE consists of an inner and outer nuclear membrane that are joined at the nuclear pores. The outer nuclear membrane is continuous with the endoplasmic reticulum so that the perinuclear space (cisterna) of the NE is contiguous with the lumen of the endoplasmic reticulum. The inner nuclear membrane is lined by the nuclear lamina, a near-tetragonal meshwork made of intermediate-type filaments. [Modified from Aebi et al. (2).] *Bottom right*: transmission electron microscopy image of densely packed, negatively stained NPC prepared from spread nuclear envelopes manually isolated from *X. laevis* oocytes. *Top left*: 3-dimensional architecture of the NPC. The major structural components include the basic framework (i.e., "spoke" complex; shown in pink), the central plug or channel complex (shown in translucent light blue), the cytoplasmic ring and the cytoplasmic filaments (shown in blue), and the nuclear ring and nuclear basket (shown in orange). [Modified from Pante and Aebi (126).] *Bottom left*: a cut-away side view of the detergent-extracted NPC, showing the spoke-ring assembly with the so-called central transporter. Internal channels located between the hourglass-shaped transporter and the inner spoke ring are revealed. [Modified from Akey et al. (4).] *Top right*: 3-dimensional map of the NPC based on electron microscopy of detergent-released NPC. Different subunits are color coded. Anular subunits are red, rings are blue, and luminal subunits are pink. Some of the 8 peripheral channels are visible. [Modified from Hinshaw et al. (64).]

voltage used as zero reference) were found more or less electrically negative (Table 1). The largest values were reported from nuclei of HeLa cells (47), and the lowest values were found in *Chironomus* gland preparations (125). It should be emphasized that no correlation was found between NPC density and intranuclear voltage. One paper reported a shift in intranuclear voltage toward more negative values when HeLa cells changed from the resting state into mitosis (47). A similar observation was made when kidney cells were stimulated by the mineralocorticoid hormone aldosterone (114).

II. INTRANUCLEAR VOLTAGE

A. Intranuclear Voltage Modeled as a *Trans*-Envelope Diffusion Potential

There is experimental evidence that the mechanisms of ion transport are qualitatively and quantitatively different in the two NE membranes (45, 98). This structural (and thus functional difference) is the prerequisite for an electrical polarization of the NE. The concept is similar as known for epithelia. There the magnitude and the polarity

TABLE 1. *Microelectrode data obtained in intact cell nuclei of various species*

V_{NE}, mV	R_{NE}, $\Omega \cdot cm^2$	G_{NE}, $S \cdot cm^{-2}$	G_{pore}, pS	$\varnothing_{pore}$, nm	$\varnothing_{nuc}$, μm	Pores, μm^{-2}	A_{NE}, μm^2	Pores/Nucleus	Cell Type	Condition	Reference No.
−13	1.5	0.7	200	3.2 (1.5)	30	40	2,827	113,080	Salivary gland	In situ	92
≈0	<0.2	>5.8	>1,000	>6.0 (3.0)	80	58	20,106	1,166,148	Oocyte	In situ	77
−13	1.5	0.7	200	3.2 (1.4)	30	40	2,827	113,080	Salivary gland	In situ	94
−15	1.0	1.0	286	3.8 (1.7)	30	40	2,827	113,080	Salivary gland	In situ	93
−2 to −5	0.7[a]	1.4	428	4.0 (1.8)	30	40	2,827	113,080	Salivary gland	In situ	71
−2 to −5	1.4[b]	0.7	214	2.8 (1.3)	30	40	2,827	113,080	Salivary gland	In situ	71
−1.2 to −0.3	2.0	0.5	150	2.3 (1.0)	30	40	2,827	113,080	Salivary gland	In situ	125
−33	ND	ND	ND	ND	12	11	452	4,972	HeLa cells	In situ	47
ND	0.3	3.3	2,100	10.3 (5.2)	9	16	254	4,064	Liver cells	In vitro	135
−10	<8.0	>0.1	>300	>3.9 (1.8)	16	3	804	2,412	Pronucleus	In vitro	106
−4	ND	ND	ND	ND	9	7	254	1,778	MDCK cells	In situ	119
−6	ND	ND	ND	ND	9	7	254	1,778	MDCK cells	In vitro	117
−3	1.4[c]	0.7	932	7.5 (3.5)	9	7	254	1,778	MDCK cells	In vitro	114
−6	1.0[d]	1.0	980	7.7 (3.6)	9	10	254	2,540	MDCK cells	In vitro	114
ND	1.7[e]	0.6	798	6.4 (2.9)	9	7	254	1,778	MDCK cells	In vitro	116
ND	1.1[f]	0.9	1,198	7.8 (3.6)	9	7	254	1,778	MDCK cells	In vitro	116

[a] No ecdysone. [b] 1–5 h of ecdysone. [c] Aldosterone-depleted cells. [d] Aldosterone-supplemented cells. [e] No TATA-binding protein (TBP). [f] 10 Min post-TBP. ND, no data available; V_{NE}, nuclear envelope (NE) potential; R_{NE}, total NE resistance; G_{NE}, total NE conductance; A_{NE}, total nuclear envelope area; G_{pore}, single nuclear pore complex (NPC) conductance, estimated from G_{NE} and NPC density; $\varnothing_{nuc}$, nuclear diameter; $\varnothing_{pore}$, electrical pore diameter (d) estimated from the equation $d = 2[(\rho_x l_x G_{pore})/\pi]^{1/2}$. In this equation ρ is the resistivity of the cytosolic solution (70–100 $\Omega \cdot$ cm depending on the preparation) and l is the estimated pore length (40 nm based on measurements of the total height of native NPCs with atomic force microscopy). The $\varnothing_{pore}$ values in parentheses were calculated for a pore length of 10 nm, assuming that the central part of the nuclear pore channel has only the length of the thickness of a double lipid bilayer, whereas the channel entrances at both sides of the nuclear pore complex were assumed to be wide and, thus, were electrically neglected. [Nuclear pore density was taken from the indicated papers or from Maul (104).]

of the transepithelial potential difference depends on the individual potential differences of the two cell poles (i.e., the apical and basolateral cell membrane) and on the electrical "leakage" of the tight junctions. In analogy, the *trans*-envelope potential difference depends on the individual potential differences of the ONM and INM and on the electrical leakage of the NPCs. The greater this leakage, the less the ONM and INM potentials will affect the nucleocytoplasmic electrical gradient. Although the origin and role of the potential difference across epithelia has been well established over the past 30 years, the potential difference across the NE seems to resist explanation on the basis of ionic electrochemical gradients alone. Investigations in cell-free murine pronuclei attributed the origin of the negative intranuclear potential to the existence of K^+-selective channels (106). In contrast, studies in nuclei isolated from cultured kidney cells revealed that the intranuclear potential remained rather undisturbed when the Na^+-to-K^+ ratio of the bath was dramatically changed (117). This observation was a strong argument against the view that the *trans*-envelope potential was caused by a K^+ diffusion potential. Taken together, it is not yet possible to predict the intranuclear potential because most parameters necessary for such a prediction are still unknown. We do not know the ion activities inside the NE cisternae and thus cannot evaluate the chemical gradients across the ONM and INM and, therefore, across the NE. We also do not know the passive ionic permeabilities of either of the two NE membranes, and thus we cannot apply the Goldman-Hodgkin-Katz equation that could predict the voltage across the individual membranes.

B. Intranuclear Voltage Modeled as a Gibbs-Donnan Potential

Studies performed in isolated epithelial cell nuclei do not support the view that the NE is, like the plasma membrane, a selective ion barrier (117). In this study, the intranuclear potential remained negative despite rigorous detergent treatment of the isolated nuclei. Moreover, microelectrode measurements showed that DNA has an inherent negative potential similar to that found in intact cell nuclei. This "intra-DNA" potential was pH sensitive, being more negative with more alkaline pH. These observations support the view that the intranuclear potential originates from the composition and the functional state of the nuclear chromatin rather than from the permeability properties of the NE itself. About one-half of the nuclear mass in eukaryotic cells is due to DNA. These molecules are characterized by negative electrical charges due to the phosphate residues of the DNA backbone. They are partially neutralized by histones, basic proteins allowing adequate DNA packing in interphase nuclei. DNA molecules can be considered polyanions tightly organized in the cell nucleus and giving rise to a Gibbs-Donnan potential. The NE could serve as a more or less permeable barrier for inorganic ions and macromolecules. Thus the cell nucleus could be considered as a

"negatively charged sink" that is in continuous interaction with the positive charges originating either from macromolecules imported into the cell nucleus by specific mechanisms through the NPCs or from inorganic cations (such as Ca^{2+} and K^+) that could diffuse across the NE via ion channels. The amino acid sequence Pro-Lys-Lys-Lys-Arg-Lys-Val was shown to be necessary and sufficient for the nuclear localization of proteins (76). This nuclear localization sequence or signal (NLS) is positively charged and thus is attracted by the electrical negativity of DNA. Thus transport of positively charged karyophilic proteins directed from the cytosol into the cell nucleus could be regulated by the electrical field that originates from the separation of electrical charges by the NE.

In conclusion, the electrical potential difference across the NE is described as the combined result of *1*) intranuclear fixed electrical charges, *2*) diffusion across both membranes of the NE (i.e., INM and ONM), and *3*) diffusion along the NPCs. Both diffusion potentials and fixed charges could be altered by various types of stimuli common to normal nuclear function: acute (e.g., Ca^{2+} release from the NE cisternae), subacute (e.g., shift in intracellular pH), and chronic stimuli (e.g., late response of steroid hormones).

III. ION TRANSPORT ACROSS THE NUCLEAR ENVELOPE

A. Nuclear Envelope: a Dynamic Ion Barrier

The idea of an active role of the NE in the modulation of nucleocytoplasmic ion movement has received considerable experimental support over the past 10 years. Na^+, K^+, and Cl^- could be used to electrically balance highly charged proteins or mRNA molecules when they cross the envelope. In the past few years the main interest focused on Ca^{2+} (23, 142). This divalent cation is an important second messenger, and its intracellular fluctuations are responsible for a wide variety of cytoplasmic reactions. Recently, attempts were made to differentiate the modulatory action of Ca^{2+} on gene expression (10, 39, 57). The modulation could be dose and/or time dependent. The NE could be a crucial regulatory site for the control of free [Ca^{2+}] in the cell nucleus. This topic has been recently reviewed (23, 130, 142). Reports on the role of the envelope as a Ca^{2+} barrier can be divided essentially into two apparently opposing groups. In the first group, studies demonstrate that, in response to an external stimulus, [Ca^{2+}] rises almost equally and simultaneously in both the nucleus and cytosol (5, 13, 27). In the other group, the investigations show attenuated Ca^{2+} diffusion into the nucleus (6) or nucleocytoplasmic Ca^{2+} concentrations gradients, (46, 133) or a total lack of diffusion across the NE (143). Therefore, it is reasonable to state that, under certain conditions (e.g., high cytosolic [Ca^{2+}]), the nucleus is not freely permeable to Ca^{2+} (142). To keep Ca^{2+} out of the nucleus, there must be mechanisms more flexible than what we currently think the NPC is: a fixed, crystallized 9-nm-wide aqueous pore spanning the NE (38). Indeed, if Ca^{2+} is required for gene expression inside the cell nucleus, then long exposure of the chromatin to divalent cations could trigger the activity of specific endonucleases and thus lead to DNA fragmentation (121). A direct link between Ca^{2+} release from internal stores and inhibition of nuclear ion diffusion has been recently demonstrated (53, 154).

Although the first hypothesis on ion selectivity of the NE was postulated many years ago (77, 93, 94), suitable techniques for testing this hypothesis (e.g., patch clamp, specific transport blockers) were not then available. The possibility of electrically isolating the nucleus from the cytoplasm required the existence of not only a barrier but also one with a gated ion channel spanning both membranes of the NE. Therefore, on the basis of patch-clamp data, it is legitimate to hypothesize a dynamic ion-barrier function for the NPC. Clearly, this hypothesis opposes the classical view of the pore as a static aqueous channel. The introduction of the patch-clamp technique and the possibility of obtaining functional cell-free nuclei have opened the possibility of studying directly NE ion permeability at the single-channel level (102, 106).

B. "Gigaseals" in the Presence of Numerous Nuclear Pore Complexes

The calculated electrical conductance of an open nuclear pore, reduced for simplicity to an aqueous channel, is ~1 nS (nanoSiemens = 10^{-9} Siemens) (102, 106). In view of the high NPC density in many cell types with up to 60 NPCs per square micron (104), it is reasonable to expect that the envelope does not offer significant electrical resistance to allow the rise of a voltage difference. Even when recent studies suggested a value of 300–400 pS for channels associated with NPCs (18), the high NPC density and the high NPC electrical conductance must result in a leaky and poorly selective barrier. From this point of view, the nucleus-attached patch-clamp approach would be of only little use; with a pore density of 15–20 NPCs/μm^2 (adult mouse liver nuclei, Ref. 70), the electrical conductance of a patch of NE (estimated area ~1 μm^2) investigated in a patch pipette would be expected to be at least 10 nS, making it difficult to observe channel events with low single-channel conductances. However, this was not the case; virtually every NE patch showed single-channel events. Because the NE patch, sealed to the pipette tip, was expected to contain many NPCs (Fig. 3) there were only two possibilities that could explain the recording suggesting a gigaseal: either the pores were

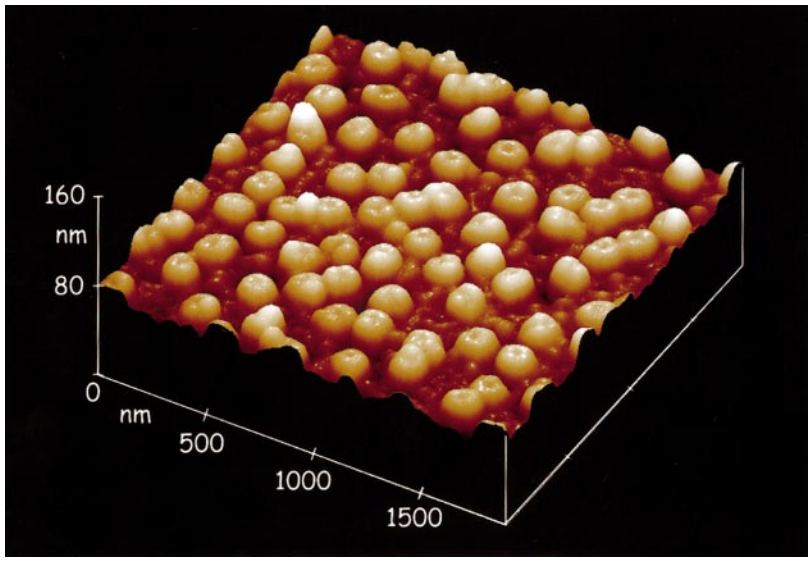

FIG. 3. The cytoplasmic surface of a native nuclear envelope of *X. laevis* oocyte is viewed with the atomic force microscope. NPCs protrude by some 20 nm from the cytoplasmic face of the nuclear envelope. The density of nuclear pore complexes is 36 pores/μm^2. [Modified from Danker et al. (32).]

plugged and/or they acted like gated ion channels. The experimental evidence suggested that NPCs can indeed function as ion channels. If the recording pipette contained simple saline solution, the ion channels observed appeared as a homogeneous population of ion pathways that could exhibit one or several levels of electrical conductance up to ~1,000 pS (100, 102, 108). In addition, higher conductances appeared to be based on multiples of single-current sublevels, consistent with the presence of populations of ion channels with high density (15, 107). Furthermore, the kinetic properties of the single-channel currents were comparable to those of whole nucleus macrocurrent recordings. The average of single-channel recordings, obtained from several current traces at the same test potential, showed a slow decay or "inactivation" (8, 15, 105, 108). In two of the studies, the inactivation was seen only for the outward current (from the nucleus to the cytoplasm) but not for the inward current (8, 35), whereas in the other study (108) inactivation was shown for both polarities (15). The asymmetry between outward and inward currents was also found in whole nucleus voltage-clamp experiments (30). This similarity in the kinetics of the whole NE and the averaged single NE channel ion flow suggested that both measurements originated from the same ion pathway. The total patch conductance was found to be much less than what was expected if all NPCs were open. If the recorded channel activity was taken to correspond to NPCs, then NPCs were supposed to behave as highly dynamic structures able to reduce NE permeability to extremely low values and to transform the NE into a charge separator. In the absence of ATP and other cytoplasmic components, NE ion permeability could remain low (17).

Attention should be drawn also to a recent study identifying ER instead of NE as the membrane patch residing in the tip of the patch pipette (31). This problem occurred despite the fact that isolation procedures were used that should have completely removed the ER from the cell nuclei (157). The authors concluded that ER cannot be excluded a priori in patch-clamp experiments as a potential membrane source. It was strongly suggested that putative NPC currents must be identified by specific electrical criteria as typical current inactivation characteristics or by stimuli directly affecting NPC function (19). Also, the question was raised whether NPC ion-gating mechanisms contributed to specific cell functions or whether they were simply redundant (or artificially induced) epiphenomena during nucleocytoplasmic macromolecule transport. Several publications addressed this problem, and a clearer (yet incomplete) picture of nuclear ion transport emerged (15, 18, 41, 101). Numerous external factors modifying the physiological function of a cell are now known to modulate NE permeability. Osmotic pressure (70, 159), pH (52, 117, 149), hormones (43, 71, 114), NE cisternal calcium (53, 153), ATP (8, 108, 131), intracellular calcium ions (8), cytoskeleton assembly/disassembly (132), and specific cytoplasmic proteins (17, 21) are factors that determine the dynamic role of the envelope. Some of these modulatory agents mediate normal physiological processes, whereas others may mediate pathophysiological mechanisms in the cell nucleus. The NE could be used by the nucleus as a protective shield against cytosolic damaging signals. The development of a nucleocytoplasmic electrical potential difference to accelerate (or slow down) macromolecule transport could be thought of as another NE ion channel function.

C. Ion Channels in the Nuclear Envelope

Patch clamp measures single-channel events of biological membranes. For this technique to be effective, the

membrane to be investigated has to be accessible to the pipette tip and has to be clean. Both of these prerequisites are virtually absent when the measurement target is the cell nucleus. Thus gigaseals on the NE surface are easier to be obtained in isolated cell nuclei. The most common isolation procedure uses sucrose gradient-centrifugation of cell homogenates (11; modified by Ref. 15). Isolated nuclei can also be obtained by microdissection of single cells with glass needles (30, 98, 106, 107). NE fractions can be fused with liposomes (102) or with lipid bilayers (140). Fusion offers the possibility of separating the INM from the ONM. A different approach for the study of NE ion channels incorporates isolated NE proteins into lipid vesicles or planar bilayer (110). A novel procedure, adopted recently to study the expression of new proteins after a physiological stimulation, took advantage of a RNA subtraction library using nontreated and treated cells. One of the proteins expressed during macrophage activation was a Cl^- channel localized in the nucleus. The cell line transfected with the appropriate cDNA expressed this particular ion pathway that could be identified with patch clamp (161). Although difficult, in situ (nucleus-attached) patch-clamp measurements have been carried out in first-stage *Xenopus laevis* oocytes (108). Table 2 lists the characteristics of NE channels in different cell types and in artificial lipid bilayers. The wide range of values for the electrical conductances in nucleus-attached patches could reflect the functioning of many channels of the same kind, different ion pathways, many substates of large conductances, or any combination of these possibilities. In some reports, the channels were identified as ER-associated pathways. Because of their similarities with endomembrane channels, Cl^- channels (140, 157, 161), Ca^{2+}-activated K^+ channels (98), inositol trisphosphate (IP_3)-activated channels (96), and Bcl-x_L protein channels (110) are probably located in the ONM and, less likely, in the INM. It is tempting to associate a function to these channels. Ca^{2+}-activated K^+ channels could influence the release of Ca^{2+} from IP_3-sensitive channels (45, 69). Cl^- channels could be probably involved in osmotic volume regulation of the nucleus (70). Bcl-x_L ion-conducting proteins, due to their dependence on cytoplasmic pH, could be probably involved in the regulation of nuclear pH (117). However, the most common channel feature is a multiple conductive state. Under different experimental conditions, different nuclei display NE channel conductances from 100 pS to 2 nS (15, 30, 41, 102, 106, 132). The large-conductance channel responds as a K^+-selective pore during drastic changes in test solutions (70). Its activity is blocked by Zn^{2+} and guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) (16) and minimized by wheat germ agglutinin (WGA), a protein transport blocker, acting on nucleocytoplasmic protein import (29, 42, 168). It was shown that ATP, essential for NPC function, and the

TABLE 2. *Nuclear ion channels in different species and different preparations*

Cell Type	Putative Selectivity	Maximal Conductance, pS	Pipette Contact Site	Stimulus	Blocker	Reference No.
Mouse zygote	K^+	200	ONM	El/chem	None	106
Avian erythrocyte	Cations	800	ONM,INM	El/chem	None	102
Mouse liver	K^+	220	ONM	El/chem	None	107
Rat liver	Cl^-	150	ONM	El/chem	DIDS	157
Rat liver	Cl^-	58	ONM	El/chem	ATP	157
Higher plant	Cations	1,000	ONM	El/chem	None	100
Mouse myocyte	K^+	550	ONM	El/chem, PKA	None	15
Mouse liver	K^+	250	ONM	El/chem	None	70
Mouse myocyte	K^+	550	ONM	El/chem, dNTP	Zn^{2+}/GTPγS	16
Starfish oocyte	Cations	120	ONM	El/chem	None	30
Frog oocyte	K^+	1,000	ONM	El/chem, ATP	None	108
Frog oocyte	Ca^{2+}	113	ONM	El/chem, IP_3	Heparin	96
Mouse myocyte	K^+	420	ONM	El/chem	WGA/MAb414	17
Rat pancreas	K^+	200	ONM	El/chem, Ca^{2+}	Low Ca^{2+}	98
Frog kidney	Cations	420	ONM	El/chem, actin	MAb414	132
Lipid bilayer	Cl^-	180	ONM	El/chem	None	140
Lipid bilayer	Cl^-	30	ONM	El/chem	None	140
Lipid bilayer	Cations	180	ONM	El/chem	None	140
Lipid bilayer	Cations	75	INM	El/chem	None	140
Lipid bilayer	Cations	270	ONM	El/chem	Low pH	110
Rat brain	Cations	166	ONM	El/chem	None	41
Mouse liver	K^+	1,700	ONM	El/chem, ATP	Ca^{2+}	8
Transformed cell	Cl^-	22	ONM	El/chem	None	161
Mouse liver	K^+	300	ONM	El/chem, cytochalasin	None	160

ONM and INM, outer nuclear membrane and the inner nuclear membrane, respectively; el/chem, electrochemical gradient applied across the nuclear membrane patch during the patch-clamp experiment; PKA, protein kinase A; MAb414, monoclonal antibody (see text for details); GTPγS, nonhydrolyzable GTP; DIDS, stilbene derivative (anion channel blocker); dNTP, nucleotide triphosphates.

catalytic subunit of the cAMP-dependent protein kinase, can increase the activity of the channel by multiplying its current levels (15, 108). A close association of this large ionic pathway with the NPC is likely (15, 16, 30, 41, 70, 100, 102, 106–108, 132).

IV. MACROMOLECULAR TRANSPORT ACROSS THE NUCLEAR ENVELOPE

A. Macromolecular Transport Capacity of Nuclear Pore Complexes

Nuclei used in some of the patch-clamp investigations showed the expected properties of macromolecule transport. It was demonstrated by fluorescence microscopy that <10-kDa dextran molecules translocate across the NE under simple saline conditions that normally prevent macromolecular transport (MMT; Ref. 154). These results demonstrated that the nuclei utilized in those electrophysiological studies conformed to the prevalent concept that NPCs allow translocation of not only monoatomic ions but also of small to medium-sized molecules and particles. More importantly, nuclei were shown to retain their macromolecular transport capacity (17). This capacity was demonstrated by constructing a fluorescent probe that localizes to the nucleus. This probe was a conjugate of the fluorescent phycobiliprotein B-phycoerythrin (B-PE, 240 kDa) conjugated to the nuclear localization signal (NLS) of the simian virus 40 (SV40) large-T antigen (17, 168). Similar fluorescent probes for the study of MMT have been used (1). There are several specific reviews on the mechanisms required for MMT (40, 48, 51, 83, 111–113). The proteins must first bind to a cytosolic receptor, then bind to a docking site at the NPC, and then translocate in an ATP-dependent process. The process depends on other factors, including Ran/TC4 and phosphorylation of some of the substrates involved. In this context, the conjugation of B-PE to NLS was required to make the complex recognizable by the NPC-mediated mechanism for MMT (17).

B. Macromolecules and Single Nuclear Pore Conductance

Nuclear proteins, mRNA, native and foreign nucleic acids, and other macromolecules utilize the NPCs to cross the NE (40, 48, 51, 83, 111–113). Because these macromolecules are poor electrical charge carriers and because they take over the space within the NPC channel otherwise occupied by monoatomic ions, MMT reduces the ion conductance of a single NPC (G_{pore}). Nuclear-targeted B-PE silenced the NPC ion channel activity (17). However, the activity was progressively and spontaneously relieved with time, in a time course that paralleled the time course required for the complete nuclear transport of the nuclear-targeted B-PE probe in fluorescence microscopy experiments. It must, therefore, be concluded that while the channel activity was silent, the B-PE macromolecules were continuously transported into the nucleus. Experiments with transcription factors, an important group of naturally occurring nuclear-targeted macromolecules involved in the regulation of gene activity, also transiently silenced ion channel activity (17, 20, 21). These experiments demonstrated that when transcription factors of ~40 kDa (AP-1/c-jun, NFκB, SP1, and TATA-binding protein) and other nuclear molecules were added, the ion channel activity was silenced for a period of time that ranged from a few seconds (with substrates) to hours (without substrates). Both cases correlate with the EM observation that without substrate the NPC is plugged (127). Because transcription factors enter the nucleus exclusively through the NPC, it was concluded that the recorded ion channel activity in these MMT-competent nuclei resulted from gated ion flow along the NPC rather than from an indirect action of the transcription factors (17). When comparing the translocation times in these nuclei with the times in situ, it should be noted that the electrophysiological experiments were conducted under conditions that are far from optimal for normal MMT (40, 48, 51, 83, 111–113). For example, cytosolic factors such as Ran/TC4 were not added. Furthermore, the rate of nucleocytoplasmic protein transport was shown to be determined by the casein kinase II phosphorylation site flanking the NLS of the SV40 T antigen (136). Therefore, it is likely that protein kinases and phosphatases play a role in nuclear transport by controlling the phosphorylation levels of both transport substrates and NPCs. In other words, even when the cargo molecules (e.g., transcription factors) are loaded in the recording patch-clamp pipette, they cannot enter the nucleus once the MMT substrates are exhausted.

C. Macromolecular Transport and "Plugging"

It has recently been recognized that $[Ca^{2+}]$ in the NE cisterna ($[Ca^{2+}]_{NE}$) plays a major role in the NPC-mediated translocation of macromolecules (53) and medium-sized (>10 kDa) molecules (129, 131, 154). When $[Ca^{2+}]_{NE}$ is low, conformational changes of the NPC occur (164), and translocation of macromolecules is inhibited. Although active macromolecule import was shown to be independent of $[Ca^{2+}]_{NE}$ (156), it is likely that in cells where there is a great deal of Ca^{2+} release from the NE cisterna, coupled with a faulty Ca^{2+}-loading machinery, at least passive transport of macromolecules will be adversely affected. The impaired MMT machinery, in turn, should cause the macromolecules to be caught in the

middle of the NPC hole. Therefore, like a plug, they should obstruct the pathway for ion flow and, consequently, result in an impaired translocation of small molecules and monoatomic ions (Fig. 4). Structural support for the electrophysiological idea of the translocating macromolecule behaving like a plug came from EM observations that, when MMT substrates are supplied, most NPCs are unplugged (i.e., lack of electron-dense material inside the NPC), whereas when the substrates are absent, most of the NPCs are plugged (i.e., the NPC is filled with electron-dense material) (127). Because both transcription factors and mRNA use the NPCs for their translocation, it can be concluded that cellular events affecting $[Ca^{2+}]_{NE}$ should be reflected on the level of gene activity and expression.

The concept of channel plugging by molecules is not new. Indeed, the concept of a plugged channel state distinct from the open, closed, and inactivated states was proposed for other organellar preparations. In mitochondrial outer membranes, the peptide pCyt OX IV (1–12)Y silences a large-conductance cation channel (59–61). This channel, known as the peptide-sensitive channel (PSC), was detected in mutant porin-less preparations (9, 24, 37, 61, 73, 74, 128, 158, 162). The open probability of the voltage-dependent anion channel (VDAC) of the outer mitochondrial membrane (porin type 1; which also is expressed in sarcoplasmic reticulum; Ref. 75) was eliminated with a soluble 54-kDa mitochondrial protein (25, 65, 89–91). A 10-kDa, synthetic polyanion (a copolymer of methacrylate, maleate, and styrene) interrupted VDAC currents (26). Despite its large aqueous diameter of 3 nm, the VDAC was recently shown to gate the transport of ATP, even though the putative VDAC closed state had 50% of the maximal channel conductance (139). The capacity of VDAC to regulate ATP translocation was explained in terms of an electrostatic barrier that results in inversion of the selectivity of the channel (139). Furthermore, type 1 porin channels were inhibited by proteins of cytosol and amniotic fluid (58). Mitochondria-targeting peptides plug the mitochondrial inner membrane channel whose properties resemble those of the PSC (72, 79, 95). Finally, protein translocation through an aqueous pore of the ER, the translocon, eliminated channel activity (7, 28, 56, 63, 97, 146, 150, 151). Independent fluorescence accessibility studies showed that the aqueous pore in a functioning translocon is 4–6 nm in diameter, making it the largest membrane channel, after that of the NPC, that maintains a permeability barrier (55).

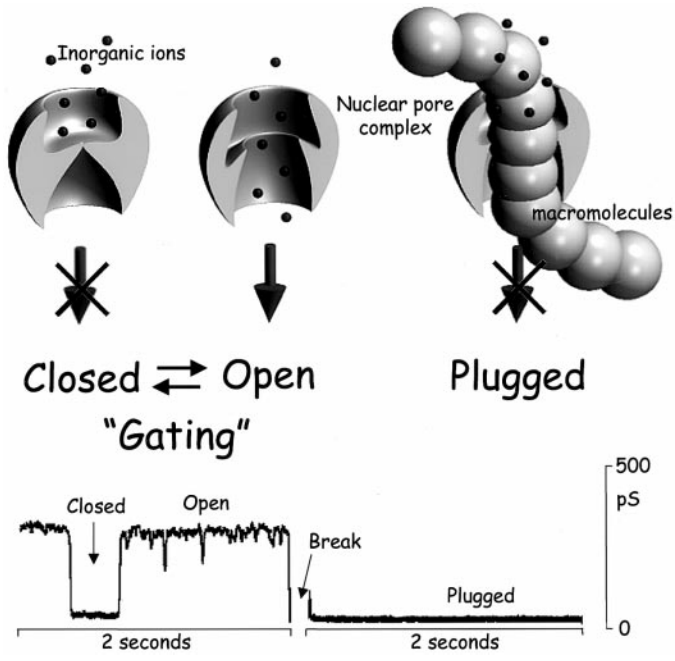

FIG. 4. Transport model for inorganic ions and macromolecules derived from patch-clamp experiments. In the absence of macromolecular transport, the electrical conductance (in pS) of the NPC is variable, shifting between open and closed states. In the presence of macromolecule transport, the central channel of the NPC is filled with electrically isolating material that plugs the tunnel. This results in a low passive permeability. In the closed state (closed NPC without transport cargo inside) and in the plugged state (open NPC with electrically isolating transport cargo inside), the patch resistance is high (in GΩ). To measure such high membrane resistances a "gigaseal" (high-resistance connection between glass pipette and NE) is a prerequisite.

V. NUCLEAR ENVELOPE CONDUCTANCE

A. Electrical Cross Talk

Changes in NE ion conductance were shown to be associated with DNA replication (12). This could imply a role for the cell membrane potential, which changes with DNA synthesis and cell-cycle control (34, 155). Cytoplasmic Ca^{2+} and osmotic pressure changed gene expression (14, 57). In addition to biochemical events triggered by a dynamic change in cytoplasmic ionic milieu, there is also increasing evidence for direct electrical interaction between plasma membrane and NE (49, 50, 101). The main advantage of an electrical transduction system able to communicate signals from the extracellular compartment directly to the cell nucleus, in which NE ion channels would play a crucial role, could be the high speed of this process. Two mechanisms could be envisioned involving external stimuli in the plasma membrane, NE ion channels, and electricity as a cytoplasmic "communication carrier": *1*) direct electrical interaction and/or *2*) fluctuation of cytoplasmic factors. Both of them could control NE permeability. These mechanisms require the ability of the nucleus to become a separate structure, a sort of "cell within a cell" (105). The critical point of the suggested signal transduction mechanism concerns the cytoplasmic elements responsible for such a pathway.

It was recently proposed that the cytosolic element in charge of electric signal transfer from plasma membrane

to NE could be the ER (101). In eukaryotic cells, the ER is in physical continuity with the ONM and in close contact with the plasma membrane. In some cases, such as in myotubes or in adipocytes, the cell nucleus is located particularly close to the plasmalemma. This close apposition of structures could be useful for transferring membrane voltage oscillations to the nucleus. The NE could receive the signal and respond. In cooperation with substances evoked by the same stimulus (i.e., Ca^{2+} and H^+ influx), the permeability of the envelope could be modulated or other molecules could be released by the NE cisternae. A second effect could be a direct interaction with the DNA anchored to the INM or to the lamina (101). Furthermore, there are other structures that could be involved in linking the plasma membrane with the nucleus. Lin and Cantiello (84) demonstrated that actin filaments are able to pass current more efficient than a physiological solution. The actin network runs single filament units across the cytoplasm, in contact with virtually all cellular organelles including the nucleus. The direct regulation of NE ion channels by actin filaments has been recently shown (132). On the basis of the cable properties of actin, a role of the "actin wires" in the modulation of NE conductance appears likely (84). The involvement of electrical signals in intracellular communication would be like a "cytoplasmic nervous system" appropriate for fast responses, coexisting in parallel with a "hormonal signaling network" represented by the second messenger cascade.

B. Total Nuclear Envelope Conductance

In Table 1 we summarized microelectrode experiments performed in intact cell nuclei of various animal species. The fact that only a few papers were published on this topic between 1962 and 1990 reflects the general view that microelectrodes were not suitable for detailed characterization of the function of the NE barrier. In particular, because electrophysiological techniques were thought to detect exclusively the route of inorganic ions (and not macromolecules) traveling between cytosol and nucleoplasm, there was considerable doubt about the usefulness of applying such techniques to pathways that mainly transport proteins and ribonucleic acids two orders of magnitude larger than ions. Horowitz and colleagues (66, 67, 124), using their favorite preparation, the nucleus of amphibian oocytes, performed experiments taking into account the large dimensions of the NPCs (22, 104, 165, 167), together with the high passive permeability of the NE for ions and macromolecules. Their data indicated conclusively that the NE was a significant barrier only for macromolecules larger than ~7 nm in diameter (124). This early conclusion should now, however, be reevaluated in the light of more recent investigations using the patch-clamp technique.

Close inspection of Table 1 shows a large scatter in NE permeability values, expressed in terms of total NE electrical conductance (G_{NE}). This parameter, derived from the input electrical resistance of the cell nucleus (R_{NE}) and calculated from the current-voltage relation for the NE, stands for the passive electrical leak pathway of the envelope. The G_{NE} values collected from various papers were normalized for the difference in nuclear surface area among the different preparations and thus expressed as G_{NE} per cm^2. Direct comparison of the data for the different preparations reveals that G_{NE} varied by at least a factor of 10. At first sight this is not very surprising since NPC density per unit area can vary among different species to a similar extent (104, 144, 145). However, variations in G_{NE} occurred virtually independent of NPC density. The NE of *Chironomus* salivary gland, for example, showed a similar G_{NE} per cm^2 (40 pores/μm^2) (71, 125) compared with G_{NE} of cultured kidney cells (7 pores/μm^2) (114, 116). This indicates that NPCs are regulated structures that can control their inherent tunnel function. Already these early studies suggested that the nuclear pores could function as a dynamic barrier for inorganic ions (71, 92–94, 125).

C. Single Nuclear Pore Conductance

Because the NPC density in the NE is known for all preparations, a mean value of the G_{pore} can be calculated (114, 116). Such a calculation is based on several assumptions that should be kept in mind. First, it is assumed that the electrical conductance is caused entirely and exclusively by the leak pathway of the NPCs. Any other ion channels located in the ONM or INM and functioning independently of the NPCs are not taken into consideration. Second, it is assumed that all NPCs in the NE of an individual cell nucleus function similarly, i.e., exhibit the same electrical conductance. Inspection of Table 1 shows that G_{pore} can vary from 150 to >1,000 pS. There is no correlation between NPC density (number of NPCs per area of NE) and G_{pore} values. Oocyte nuclei exhibit very high NPC densities and large G_{pore}, actually too large to be reliably measured with microelectrodes. Recent studies in isolated oocyte nuclei using a different experimental approach gave a more reliable mean G_{pore} value of 1,700 pS (32). Nuclei of liver cells exhibit low NPC densities compared with oocytes but exhibit G_{pore} values similar to oocyte. Nuclei of *Drosophila* salivary gland cells exhibit high NPC densities but comparably low G_{pore} values. Finally, cultured kidney cells exhibit low NPC densities but rather high G_{pore} values. In conclusion, passive ion permeability of NPCs reflected by the individual G_{pore} values is strongly dependent on cell type and metabolic state.

D. Envelope Conductance in Large Nuclei With High Pore Density

As reviewed by Maul (104), oocyte nuclei from various species express up to 40 millions of NPCs, depending on the state of maturation and thus nuclear size. Nuclei of mature oocytes have sizes in the range of 1,000 μm in diameter (3,000,000 μm^2 of surface area!). Although these nuclei facilitate microelectrode impalement, they are not suitable for conventional electrophysiological measurements. Because this is crucial for the understanding of the microelectrode data in intact nuclei, we calculated the relationship between nuclear diameter and nuclear input electrical resistance for five different G_{NE} values. This relationship is shown in Figure 5. Under typical experimental conditions, a suitable microelectrode cannot pass much more than ~100 nA of current into the nucleus. Therefore, the input resistance of the NE must be at least in the range of 500 Ω to obtain a measurable voltage (induced by the injected current pulses) of ~50 μV. This voltage deflection represented (and still represents) the lower detection limit of a well-shielded and well-arranged experimental setup. The curves shown in Figure 5 correspond to the range of values obtained in the published studies. Each curve represents a G_{pore} value. There are several conclusions drawn from this figure and related to the studies summarized in Table 1.

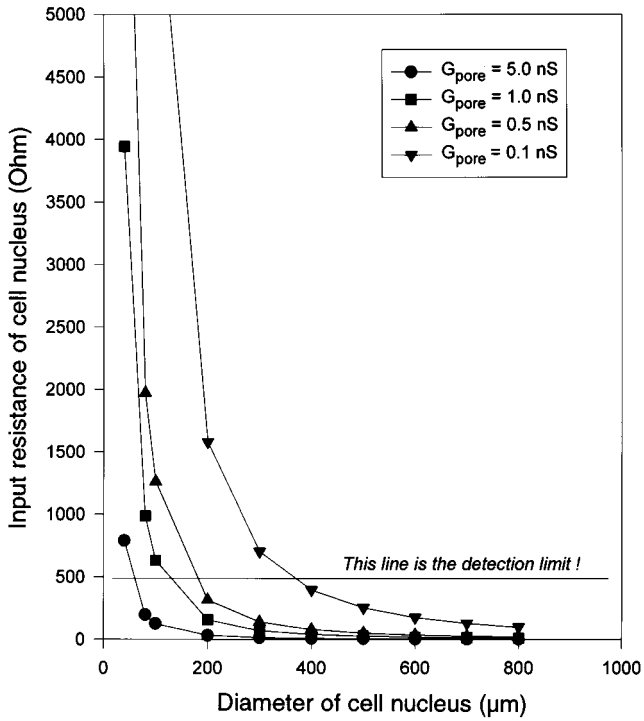

FIG. 5. Modeling of the nuclear electrical input resistance (given in Ω) as a function of the diameters of the cell nuclei. For calculations we modeled the *X. laevis* oocyte nucleus as a sphere with 36 NPCs/μm^2. The different curves were calculated on the basis of different "single nuclear pore conductances" (G_{pore}) ranging from 5 to 0.1 nS. The technical detection limit for microelectrode measurements is 500 Ω (i.e., only input resistances larger than 500 Ω can be detected). This figure indicates that oocyte nuclei are usually too large to allow electrical resistance measurements even when G_{pore} is as low as 0.1 nS. This technical limitation was recently overcome by a novel electrical approach (32).

1) A measurable nuclear input resistance could be expected only when nuclei were well below 300 μm in diameter even when an extremely low G_{pore} value of 100 pS was assumed. Loewenstein's group (77) used oocyte nuclei with diameters of ~80 μm but could not detect a significant input resistance. According to Figure 4, this only indicated that the apparent G_{pore} was larger than 1,000 pS. Technical limitations restricted a more precise determination of G_{pore}. Data from more recent microelectrode experiments in isolated somatic cell nuclei (114, 116) suggest that G_{pore} in the oocyte was not much larger than 1,000 pS (77). Recent patch-clamp data point in a similar direction (19–21, 99, 100, 102, 106).

2) Oocyte passive NE permeability is somewhat larger than that of other species but still could be in a similar range. Only the large surface area of oocyte nuclei combined with the high pore density led to the general view that this preparation was much more permeable than any other species. A rather small and immature *X. laevis* oocyte nucleus has ~1 million NPCs (104), whereas a cultured kidney cell nucleus has ~2,000 pores. Therefore, G_{pore} could be successfully measured in kidney cell nuclei (114, 116), whereas it was not measurable in oocyte nuclei (77). Thus, although overall NE permeability is larger in the oocyte, individual NPCs are not necessarily different from any other preparations.

To test such conclusions, it would have been necessary to block the nuclear pore pathways and thus to increase NE resistance to measurable values. Such nuclear pore blockers were not available in the past but are available now. Two recent patch-clamp studies showed (19, 132) that both WGA, a known blocker of NPC-MMT, and the monoclonal antibody MAb414 (33) reduce the electrical conductance of NE patches (29, 42).

E. Structure-Function Relationship of Nuclear Pore Complexes

The electrical conductance of individual NPCs can be used to calculate the diameter of the transport channel. In a simplified view, the channel can be modeled as a cylinder of specific length filled with electrolyte solution mimicking the cytosolic composition. We calculated the pore diameters on the basis of two different pore lengths. One length, 40 nm, was derived from the transporter length imaged by EM (3), optical single transporter recordings (78), and AFM measurements (134). The other, 10 nm, was based on the assumption

that the input resistance of a single NPC was mainly generated by the inner segment of the channel that spans the two NE membranes (i.e., ONM and INM) and that has the dimension of the thickness of a double-layered lipid bilayer (i.e., 2 × 5 nm). The estimated nuclear pore dimensions calculated from the data of the microelectrode papers are summarized in Table 1. The 40-nm channels range from 2.3 to >6.0 nm in diameter. The 10-nm channels range from 1.0 to >3.0 nm. Although such calculations are based on simplified assumptions (e.g., they do not consider an assembly of channels within an individual NPC), they may be useful in predicting the effects of molecules that traverse this barrier. It has been known for a long time that ions and globular solutes of 4 kDa can diffuse into the nucleus while globular solutes with molecular masses of ~10–70 kDa will diffuse through the nuclear barrier with increasing difficulty as their size increases (124). Let us take the universal transcription factor, the TATA-binding protein TBP (molecular mass 40 kDa), as a representative example for a karyophilic protein that is readily transported into the nucleus (19–21, 62, 116). This protein has a globular diameter of 2.6 nm (116). At least from the physical point of view, this transcription factor may or may not enter the nucleus, depending on the actual conformational state of the NPC.

F. Ecdysteroids and Envelope Conductance

In 1963, the so-called "ion hypothesis of gene activation" was proposed based on experiments carried out in insect cells (80). According to this hypothesis, specific genes were activated by a specific ion concentration pattern in the cell nucleus. Changes in ion concentration were inducible by hormones such as ecdysone, an important ecdysteroid hormone in the larval moulting process of insects (80–82). Based on nuclear NPC density and nuclear electrical input resistance, we calculated G_{pore} for the two conditions (i.e., presence and absence of hormone). As shown in Table 1, G_{pore} was in the picoSiemens range and dropped by ~50% (from 428 to 214 pS) in response to the hormone. Such a low NPC conductance could indeed serve as an effective ion barrier as previously assumed (71).

In 1966, Kroeger (82) showed an effect of 20-hydroxyecdysone on the resting membrane potential of insect salivary gland cells. The hormone increased the electrical potential within 1 min, whereas no effects on the cell nucleus were observed even 20 min after application of the ecdysteroid. This observation was reproduced 30 years later and explained as being a hormone-induced activation of plasma membrane K^+ channels (147). The early observation (82) led to the hypothesis that the steroid hormone interacts first with the plasma membrane ("early" response) before its well-known receptor-mediated action takes place in the cell nucleus ("late" response). As shown recently (147), the early response of ecdysteroids in salivary gland of *Drosophila* resulted from the instantaneous activation of a Ca^{2+}-sensitive Na^+/H^+ exchange protein in the plasma membrane (Fig. 6). The activation of this integral plasma membrane protein led to a sustained intracellular alkalinization (147), which turned out to be a prerequisite for nuclear swelling and gene activation (52, 54, 117, 137, 170). Nuclei of *Drosophila* salivary gland cells dramatically increased in volume (170) caused by the induction of specific "puffs" in the polytene chromosomes. In 1979, Wuhrmann et al. (169) measured an increase of intranuclear K^+ activity by a factor of 2.6 as oligopausing larvae of *Chironomus* developed into prepupae. With the combination of early and recent data, it is concluded that the nuclear envelope plays an important regulatory role in ecdysteroid-induced gene expression.

G. Aldosterone and Envelope Conductance

The mineralocorticoid hormone aldosterone regulates transepithelial electrolyte transport in kidney. Similar to ecdysone, an early response (nongenomic; reviewed in Ref. 148) was detected at the level of the plasma membrane involving increase in intracellular Ca^{2+} concentration (44), activation of Na^+/H^+ exchange and intracellular alkalosis (118, 163, 166), and stimulation of plasma membrane K^+ conductance (115). As one of the late (possibly genomic) responses of a kidney cell, the number of NPCs increased within hours of hormone treatment by almost 25% (114). This led to a proportional increase in the total NE conductance consistent with the fact that aldosterone served as a proliferative hormone in kidney cells, thus facilitating nucleocytoplasmic exchange of macromolecules. Although G_{pore} derived from G_{NE} and NPC density turned out to be constant (Table 2), another study applying AFM showed marked differences in the surface topology of the NPCs (43). The cytoplasmic face of nuclear pores in hormone-treated cells was devoid of proteins (due to stimulated macromolecule import; pores in "transport mode"), whereas nuclear pores of hormone-depleted cells were covered with macromolecules (due to reduced macromolecule import; pores in "stand-by mode"). This indicated that not only the total number of NPCs per nucleus was increased by the steroid hormone but also protein import of individual NPCs.

VI. FUTURE PERSPECTIVES

Application of electrical tools to the cell nucleus can help us to understand one of the most interesting topics in

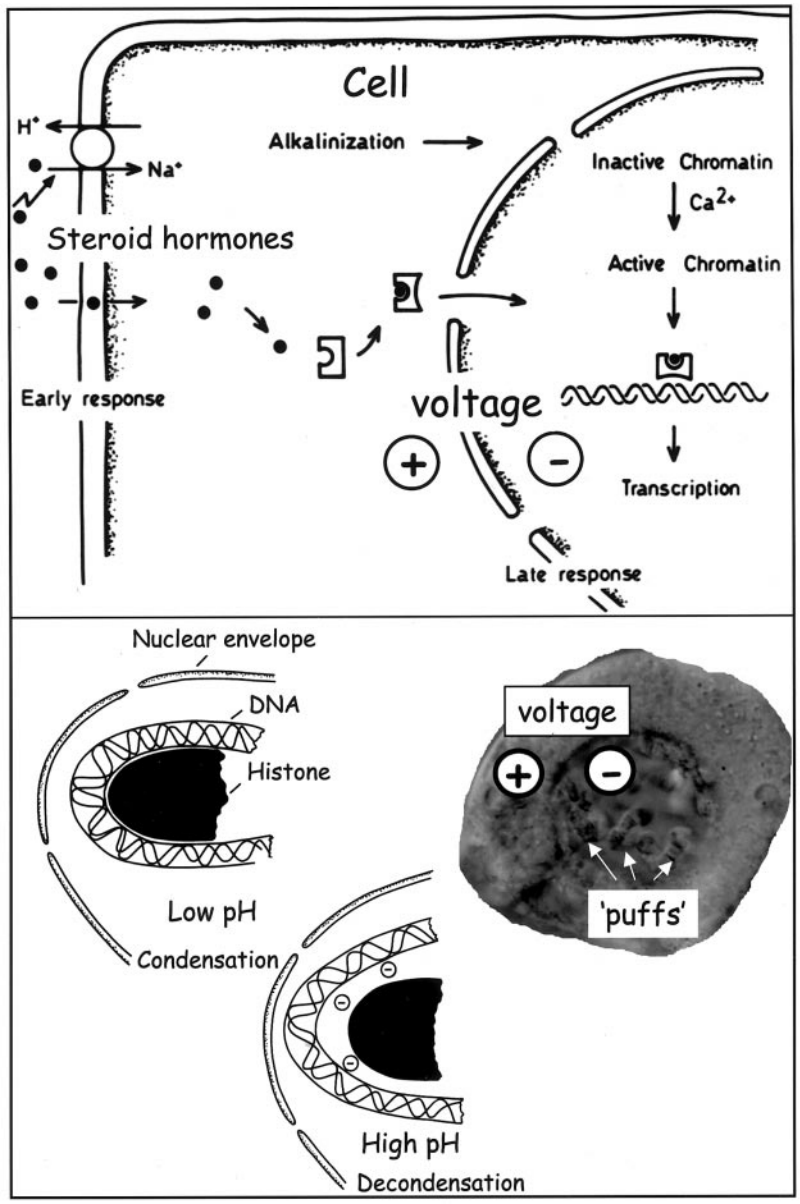

FIG. 6. Schematic model of early and late cellular response to steroid hormones (solid circles in top graph). Early response is the nongenomic activation of the plasma membrane Na^+/H^+ antiporter followed by the late genomic response. The latter involves the hormone-induced activation of an intracellular receptor (Ω-shaped symbol) that is translocated into the cell nucleus. The concomitant intracellular alkalinization, mediated by plasma membrane Na^+/H^+ exchange, leads to the decondensation of the chromatin ("puffing" in *Drosophila* salivary gland cells). Negatively charged DNA is exposed leading to negative intranuclear potentials. [Modified from Oberleithner et al. (117) and Wünsch et al. (170).]

current cell research, namely, the regulation of nucleocytoplasmic transport. Recently, a novel electrophysiological approach, the so-called "nuclear hourglass technique," was described for studying NE electrical conductance of large oocyte nuclei (32). This method circumvented the limitations inherent to patch clamping or intracellular microelectrodes and thus could prove useful for future research. It led to the assumption that, in addition to the central channel, other transport pathways exist in NPCs. Ions obviously use the NPC peripheral channels rather than (or in addition to) the central NPC channels (32). These new functional data are supported by structural data postulating small channel-like structures that span an individual NPC but are physically separated from the large central channel (64). On the basis of the electrical data so far available on NPC function, we would suggest the following model (Fig. 7).

The major route for macromolecules (molecular mass >1,000 Da) is the central channel of the NPC. Usually, under physiological conditions, macromolecules travel through the central channel into (and out of) the nucleus. Because of the specific geometry of the central channel and the plasticity of the NPC structure, macromolecules line up at either side of an individual NPC and then sneak through the tunnel. There is virtually no space for inorganic ion movement in either direction due to the tight engagement of the macromolecules with the NPC tunnel surface. Inorganic ions such as K^+, Na^+, H^+, Ca^{2+}, and Cl^- use parallel peripheral channel pathways to enter or exit the cell nucleus. Although physically separated,

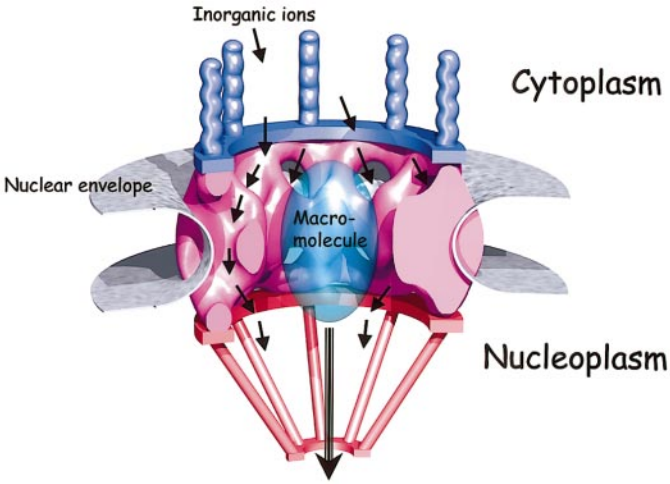

FIG. 7. Transport model of the NPC (for structural details, see legend to Fig. 2). Macromolecules are transported through the central channel pathway of the NPC. In addition to the central channel pathway, inorganic ions can pass through NPC peripheral channels. There is cross talk between the 2 pathways, although details are as yet unknown. Electrically silent macromolecule transport interferes with electrodiffusive ion transport. Therefore, electrical tools can be used for the investigation of NPC macromolecule transport. [Modified from Pante and Aebi (126).]

cross talk could occur between these pathways. Thus macromolecule transport can be traced by patch clamping (i.e., by measuring electrical ion currents through the ion pathways that parallel the central channel) despite the fact that macromolecules themselves do not generate measurable electrical currents when transported. However, cross talk between central and peripheral channels is likely to be complex and under regulatory control of yet unknown mechanisms. These new exciting aspects on mechanisms of NPC function should be addressed in future experiments.

1) Better lateral resolution using AFM should make it possible to directly visualize the putative peripheral channels located in the NPC rings.

2) Biochemical and pharmacological stimuli and blockers should disclose the specific function of the NPC ring permeability.

3) Substrates, either physiological or pharmacological, should be defined that clearly interfere with NPC central channel macromolecule transport.

4) Electrical measurements (similar to cable analysis in tubular structures) may help characterize the interaction among NPCs.

5) Nucleocytoplasmic ion movement should be directly related to different states of the perinuclear space.

A concept of the membrane should thus evolve that embraces the major ion pathways involved in nucleocytoplasmic transport under different metabolic conditions.

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