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Nuclear Electrophysiology

J. O. Bustamante

University of Maryland School of Medicine, Departments of Medicine and Physiology, Division of Cardiology-Heart Center, 655 West Baltimore Street, Baltimore, Maryland 21201-1559

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Introduction

Cell growth, proliferation and senescence are regulated by complex nuclear processes (e.g., Hoffman, 1993) which, in turn, are influenced by cytosolic signals of intra- and extracellular origin (e.g., cyclic nucleotides, hormones and mechanical load). How these signals induce biochemical cascades that result in modification of gene expression has been a difficult and elusive subject (e.g., Marx, 1993; Whiteside & Goodburn, 1993). Therefore, the recent patch-clamp detection of ion channel activity at the nuclear envelope (e.g., Matzke, Weiger & Matzke, 1990; Mazzanti, et al., 1990, Mazzanti, DeFelice & Smith, 1991; Bustamante, 1992, 1993, 1994; Matzke et al., 1992) puts into focus the potential regulation of nuclear ion signaling by one or more envelope transport systems. Nuclear ion channels (NICs) have a conspicuously large conductance (up to 1,000 pS, e.g., Matzke et al., 1990, 1992; Mazzanti et al., 1990, 1991; Tabares, Mazzanti & Clapham, 1991; Bustamante, 1992, 1993, 1994). Consequently, they exert a sizeable influence on nuclear ion activities (*see Appendix* in Bustamante, 1992) known to modulate nuclear structure and function (e.g., Kroeger, 1963; Lezzi & Gilbert, 1970; Leake, Trench & Barry; 1972; Morgan & Curran, 1986; Li & Rokita, 1991; Lippard, 1993). This paper reviews electrophysiological evidence, gathered for over three decades, which indicates that the nuclear envelope of certain cell types possesses a restrictive barrier to the flow of small physiological ions (Na^+, K^+, etc.). Due to space restrictions, references appearing earlier than 1990 have been curtailed when possible. The reader is also referred to a previous review on the subject (Matzke & Matzke, 1991).

Key words: Nucleus — Nuclear membrane — Nuclear envelope — Nucleocytoplasmic transport — Nuclear pore complex — Ions

Nuclear Envelope, Nuclear Pore Complexes and Nucleocytoplasmic Transport Theory

The envelope is made up of two concentric membranes referred to as the inner and outer nuclear envelope membranes (e.g., Burke, 1990; Dessev, 1992; Dingwall & Laskey, 1992). The two membranes are separated by the perinuclear space but fuse at specific, discretely distributed points: the nuclear pore complexes (NPCs), through which macromolecular transport is thought to occur (Dingwall, 1991; Jarnik & Aebi, 1991; Hanover, 1992; Hinshaw, Carragher & Milligan, 1992; Akey & Radermacher, 1993; Hurt, 1993; Panté & Aebi, 1993). The outer nuclear membrane is in contact with the cytosol and is structurally continuous to the endoplasmic reticulum (ER). This architecture of the nuclear envelope complicates interpretation of NIC data (Matzke et al., 1990, 1991; Mazzanti et al., 1990, 1991; Tabares et al., 1991; Bustamante, 1992, 1993, 1994; DeFelice et al., 1993; Innocenti & Mazzanti, 1993) for the argument may be raised that the recorded NIC activity (in the form of ion current) is localized at the outer nuclear membrane or results from ER remains. These arguments are dispelled, however, by *in situ* microelectrode measurements of high envelope electrical resistance (Loewenstein & Kanno, 1962, 1963*a,b;* Kanno & Loewenstein, 1963; Loewenstein, 1964; Ito & Loewenstein, 1965; Kanno, Ashman & Loewenstein, 1965; Loewenstein, Kanno & Ito, 1966; Palmer & Civan, 1977) and by experiments carried out in preparations lacking ER, Gol-

gi and other endomembranes (Matzke et al., 1990, 1992). Arguments favoring the concept of a nucleocytoplasmic ion pathway have also been given on the basis of nuclear volume response to osmotic changes (Innocenti & Mazzanti, 1993). The existence of a high envelope resistance (implying a restrictive barrier to monoatomic ion flow), despite the large diameter of the central NPC channel, and despite the high density of NPCs per unit area, may be explained by the large (MD) plug of the central channel (e.g., Dingwall, 1990; Reichelt et al., 1990; Jarnik & Aebi, 1991; Akey & Radermacher, 1993; Hurt, 1993; Panté & Aebi, 1993). That such a plug or luminal material restricts monoatomic ion flow was proposed in electron microscopy (EM) investigations of classical electrophysiological preparations (Wiener, Spiro & Loewenstein, 1965). It was recently suggested by high-resolution EM that the large central channel of NPCs is surrounded by eight smaller channels with a putative function for the transport of small ions and solutes (Hinshaw et al., 1992). This interesting observation, however, could not be confirmed (Akey & Radermacher, 1993; *see also* Panté & Aebi, 1993).

Import and export of material in the nucleus is thought to occur exclusively through the NPCs (e.g., Dingwall, 1990). Nucleocytoplasmic transport of macromolecules has several known requirements (Burke, 1990; Maquat, 1991; Miller, Park & Hanover, 1991; Nigg, Baeverle & Lührmann, 1991; Silver, 1991; Davis, 1992; Dingwall & Laskey, 1992; Gerace, 1992; Hanover, 1992; Stochaj & Silver, 1992). Transported molecules must contain one or more nuclear localization signals. Cytosolic "factors" must be present. A molecule to be transported must be recognized, through its localization signal(s), and bound by a cytosolic receptor for the molecule. The complex thus formed is thought to travel to and dock on a "docking site" or receptor at the cytoplasmic side of the envelope. Translocation to the nuclear interior then takes place through the nuclear pore. Macromolecular transport depends on ATP-dependent phosphorylation and, on the basis of similarities between macromolecular transport of the nuclear and ER membranes, GTP-hydrolysis has been proposed to intervene (e.g., Goldfarb, 1992) since GTP-hydrolysis is known to affect the interaction between the signal recognition particle and the docking site in the ER membrane (e.g., Connolly, Rapiejko & Gilmore, 1991).

Fluorescence Microscopy Suggests Nuclear Envelope Restricts Ion Flow

Fluorescent ratio imaging of free ion concentrations has gained wide acceptance. The ratiometric approach (utilizing the ratio of the responses at two wavelengths) eliminates, in principle, artifactual values resulting from heterogeneous distribution of fluorescent probes (e.g., Poenie, 1990). Furthermore, when images are restricted to the focal plane (confocal microscopy), the overlapping fluorescence from other vertical planes is eliminated and, therefore, it is possible to distinguish how the target is distributed across the nuclear envelope. This is terribly important in the case of the nucleus because the lumen of the envelope, the perinuclear space, makes it difficult, if not impossible, to identify whether the probe has localized in the envelope or in the nuclear interior (*see* Papageorgiou & Morgan, 1990). In addition to this problem, potential technical artifacts from other sources do exist. For example, dye loading into the nucleus may depend on the cleavage of the AM-form or on passive diffusion of the free form (*reviewed in* pp. 215–217 of Missiaen et al., 1992). Thus, loading of hepatocyte nuclei only occurred with Fura-2 AM and not with Fura-2 free acid (Malviya, Rogue & Vincedon, 1990), whereas smooth muscle nuclei loaded equally well with Fura-2 AM or Fura-2 free acid during reversible permeabilization (Goldman, 1991).

Under conditions that take care of these potential artifacts, fluorescent microscopy shows that the concentration of free ions and of components of signal transduction cascades (e.g., cAMP-dependent protein kinase) does not change simultaneously in the cytoplasm and nucleus (e.g., Hernández-Cruz, Sala & Adams, 1990; Takamatsu & Wier, 1990; Adams et al., 1991; Goldman, 1991; Przywara et al., 1991; Waybill et al., 1991; Himpens, De Smedt & Casteels, 1992*a;* Himpens et al., 1992*b;* Yamaguchi, 1992; Bacskai et al., 1993; Himpens et al., 1993; *see* pp. 215–217 of Missiaen et al., 1992). Since there is potential UV light-induced modifications during these Fura-2 and indo-1 experiments (e.g., Devary et al., 1993), new approaches have been implemented with visible light probes. Confocal microscopy with a mixture of fluo-3 and fura-red (visible light excitation) confirms that cardiac myocyte nuclei behave like a barrier to the propagation of intracellular-free Ca^{2+} waves (Lipp & Niggli, 1993*a,b*).

Diffusional barriers to the movement of Ca^{2+} ions have been postulated (e.g., Hernández-Cruz, Sala & Connor, 1991; *reviewed in* Bachs, Agell & Carafoli, 1992; Missiaen et al., 1992). Transport of Ca^{2+} across the nuclear envelope is thought to occur not through the nuclear pores but through pumps and inositol 1,4,5 trisphosphate (IP_3) ion channels (e.g., Bachs et al., 1992). The existence of Ca^{2+} pump is suggested by the inhibition of nucleocytoplasmic gradient by thapsigargin (Thastrup et al., 1989; *see also review by* Bachs et al., 1992) while IP_3-gated Ca^{2+} channels have been suggested by the ER-like signals recorded after IP_3 action (*see* Bachs et al., 1992). The idea of Ca^{2+} moving in and out of the nucleoplasm through the lumen of the envelope is consistent with the observation that Ca^{2+} did not affect NIC activity in patch-clamp experiments

(Bustamante, 1992). Recent experiments by Urs Gerber and Larry Gerace (*personal communication, in preparation*) suggest that Ca^{2+} stored in the ER-nuclear envelope lumen plays a role in macromolecular transport mediated by nuclear localization signal. In these experiments, *in situ* depletion of Ca^{2+} from the ER-envelope lumen in NRK cells, using either Ca^{2+} ionophores (A23187 or ionomycin) or thapsigargin, resulted in strong inhibition of nucleoplasmin-mediated import and passive diffusion of 10 kD dextran into the nucleus. The effect was rapid (15 min) and reversible, and is apparently specific for loss of Ca^{2+} from the ER-envelope lumen, rather than entry into the cytoplasm, because cytoplasmically injected BAPTA has no effect on nuclear import. These results suggest that control of nuclear pore gating may be exerted from the perinuclear space.

Nuclear molecules with high-binding affinity (e.g., calmodulin, calbindin, calpactin, calpains, calcineurin) have a role in determining total nuclear $[Ca^{2+}]$ and may influence Ca^{2+} flow. A number of Ca^{2+}-binding proteins have been identified. Nuclear calmodulin level is modulated by hormones and growth factors and may play a role in DNA replication and mitosis (*see* Bachs et al., 1992, page 263). Other important Ca^{2+}-binding proteins identified in the nucleus are protein kinase C, calbindin, calpactin, calpains and calcineurin (*see* Bachs et al., 1992). The existence of these Ca^{2+}-binding proteins may be used to explain trans-envelope $[Ca^{2+}]$ gradients, under the paradigm that nuclear pores are open aqueous channels of $\approx$10 nm diameter. However, lack of detection of free Ca^{2+} movement across the envelope cannot be explained by compartmentalized molecules with high Ca^{2+}-binding capacity, since for molecules with sufficiently high binding affinity to eliminate free Ca^{2+} in the nucleus (effectively making the nucleus an infinite sink or "black hole" for this ion species), the binding sites would not be saturable and the sites should act with relatively infinite speed (infinite cytoplasmic source of free ions since cytoplasmic volume is much larger than that of the nucleus—e.g., cardiac myocytes). This is clearly not the case (*see* Hernández-Cruz et al., 1991). Alternatively, an equally effective counter-transport system (which returns ions to the cytoplasm and which somehow also binds the internalized ions so as to mask their detection by the fluorescent probe) would have to be postulated to compensate for the large quantity of free ions entering into the nucleus. Therefore, a straightforward mechanism based on high-affinity binding sites seems unable to explain $[Ca^{2+}]$ gradients. Nevertheless, the possibility of a role for high-affinity binding sites seems unable to explain $[Ca^{2+}]$ gradients. Nevertheless, the possibility of a role for high-affinity binding sites has been suggested in regard to transport of Zn^{2+}, because of its lower abundance (Hechtenberg & Beyersman, 1993). In summary, fluorescence microscopy supports the idea of a nuclear envelope restrictive to ion flow, with Ca^{2+} moving not through the pores but through pumps, envelope lumen, and ligand-gated channels. Due to space limitations, the reader is referred to Bachs et al. (1992) and pp. 215–217 of Missiaen et al. (1992).

Nuclear Resting Potential and Nuclear Envelope Resistance

Classical microelectrode experiments demonstrating the existence of a diffusional resting potential and a nuclear envelope resistive to ion flow (Loewenstein & Kanno, 1962, 1963*a,b;* Kanno & Loewenstein, 1963; Loewenstein, 1964; Ito & Loewenstein, 1965; Kanno et al., 1965; Loewenstein et al., 1966) came to a standstill for over two decades (with few exceptions: Giulian & Diacumakos, 1977; Palmer & Civa, 1977; *see also* Matzke & Matzke, 1986; Matzke, Matzke & Neuhaus, 1988). This standstill resulted from the conflicting conclusions reached from experiments on macromolecular transport (e.g., Century, Fenichel & Horowitz, 1970; Horowitz & Fenichel, 1970; Paine, 1975; Paine, Moore & Horowitz, 1975; Horowitz & Paine, 1976) inspired by the "association-induction theory," explaining the cell resting potential on the sole basis of water properties—without the need of ATP-dependent membrane pumps (e.g., Ling, Ochsenfeld & Karreman, 1967; Ling, 1992). Those investigations concluded that ions distribute according to the water-in-gel state and, thus, that the nuclear envelope is transparent to small physiological ions. Therefore, electrophysiological data (including loss of resting potential upon puncture of the envelope) have been discarded as resulting from microelectrode artifacts (e.g., Paine & Horowitz, 1980, pp. 320–321). As a result, interpretations of nuclear electrophysiology in terms of the association-induction theory or fixed electrical charges (e.g., Donnan-like effect, Paine & Horowitz, 1980, p. 304) have prevailed to explain away restrictive flow of monoatomic ions across the nuclear envelope and to maintain the paradigm that the nuclear pores have a 10-nm diameter channel. However, the applicability of these theories (not the theories themselves) to nuclear electrophysiology has been challenged again by the recent patch-clamp data (Matzke et al., 1990, 1991; Mazzanti et al., 1990, 1991; Tabares et al., 1991; Bustamante, 1992, 1993, 1994; DeFelice et al., 1993; Innocenti & Mazzanti, 1993). Consistent with classical microelectrode data (e.g., Loewenstein & Kanno, 1962), patch-clamp investigations have shown the existence of a diffusional resting potential across the nuclear envelope (e.g., Mazzanti et al., 1990) and a nuclear envelope resistive to ion flow (Matzke et al., 1990, 1991; Mazzanti et al., 1990, 1991; Tabares et al., 1991; Bustamante, 1992, 1993, 1994; DeFelice et al., 1993; Inno-

centi & Mazzanti, 1993). Just as it occurs with whole-cell resting potentials, the existence of a diffusional potential does not deny a role for fixed charges but says, instead, that a restrictive diffusional barrier must exist to maintain a steady-state electrochemical potential.

Oocytes from the newt *Triturus viridescens,* the frog *Xenopus laevis,* and the marine species *Asterias forbesi, Nereis limbata, Spisula solidissima,* and *Hydractinia echinata,* were also studied because their transparent surface facilitated microelectrode impalements (Loewenstein & Kanno, 1963*a,b* and Kanno et al., 1965, respectively). In these preparations, only negligible nuclear resting potential and envelope resistance were detected (*see* Table 1 in Kanno et al., 1965). Therefore, it was concluded that a feature of the nuclei of gamete cells (in their germinal vesicle stage) is their low envelope electrical resistance (implying high ion permeability). This was in sharp contrast with the observations from experiments with nuclei of somatic cells (Kanno et al., 1965; *see also* Table 1 in Loewenstein et al., 1966). These results are, at first sight, different from recent patch-clamp data on isolated nuclei from starfish oocytes (DeFelice et al., 1993). However, the differences can be explained by the fact that the recent data were obtained in saline solution (i.e., containing no substrate for transport) and, therefore, the normally large effective diameter of the nuclear pores may have been reduced by the substrate-free conditions (*see* above discussion on the importance of cytosolic factors).

During the course of the pioneering investigations on nuclear electrophysiology (e.g., Loewenstein & Kanno, 1962; Loewenstein et al., 1966) there was an apparent relation between resting potential, envelope resistance and cell development (Kanno & Loewenstein, 1963). Hormonal action was also shown to affect these magnitudes in the midge *Chironomus thummi* salivary gland nuclei (Ito & Loewenstein, 1965, *reviewed in* Loewenstein et al., 1966). Following the early fourth instar stage, envelope resistance increased to about one-fifth its basal value over 3–5 days of development, decreasing again over the next 2–4 days. Increased envelope resistance was observed within 1 hr of injection of ecdyson, a steroid growth hormone (Ito & Loewenstein, 1965). Thus, from these experiments it was obvious that envelope ion currents were sensitive to components of biochemical signaling pathways associated with hormonal action. This observation compares favorably with patch-clamp data demonstrating upregulation of NIC activity (currents) by cAMP-dependent protein kinase (Bustamante, 1992), data that suggest a connection of NICs with gene control by signaling pathways (e.g., Karin, 1991; Meek & Street, 1992).

That the issue of the resting potential nature remains to be settled is demonstrated by a recent study with epithelial cell nuclei, bathed in saline (Oberleithner et al., 1993). In this study, the conclusion was reached that nuclear resting potential depends only on the negative charges of the chromatin and is independent of the nuclear envelope (Oberleithner et al., 1993). However, absence of resting potentials in oocyte nuclei or high nuclear envelope resistance in differentiated cells (e.g., Loewenstein et al., 1966) can only be explained by restricted diffusion of monoatomic ions.

Properties of Known Nuclear Ion Channels

NIC current, a measure of NIC gating activity, was recorded from murine pronuclei and shown to be K^+-selective (on the basis of their response to ion species substitution), with a maximal conductance of 200 pS at room temperature (Mazzanti et al., 1990). Large conductance channels were also seen in murine nuclei, oocyte germinal vesicles, zygote pronuclei, two-cell blastomeres, and hepatocytes (Mazzanti et al., 1991). Cation-selective NICs, of 800 pS maximal conductance at room temperature, were found in avian erythrocytes (Matzke et al., 1990). EM of the avian erythrocytes did show absence of endomembranes (e.g., ER and Golgi—Matzke et al. 1990). Large conductance ion channel activity (*ca.* 1,000 pS) has also been demonstrated in the nuclear envelope of immature coconut endosperm and giant liposomes containing fragments of this preparation (Matzke et al., 1992). These NICs were thought to be slightly cation selective (Matzke et al., 1992). EM studies demonstrated that the nuclear envelope of the isolated coconut nuclei retained its outer and inner membranes (Fig. 1*D* in Matzke et al., 1992). The identification of NIC activity in preparations lacking endomembranes (Matzke et al., 1990, 1992) underlines their suitability for nuclear electrophysiology, and demonstrates that NICs are intrinsic to the nuclear envelope.

Potassium-selective NICs, of 100–550 pS (22–36°C), have also been observed in nuclei isolated from adult cardiac myocytes (Bustamante, 1992, 1993, 1994). Cardiac myocyte NICs were insensitive to tetrodotoxin and diltiazem, known blockers of cell surface of Na^+ and Ca^{2+} channels, and to Cs^+ and TEA^+, known blockers of some of the many sarcolemmal K^+ channels (Bustamante, 1993). These results indicate not only the distinct nature of NICs but also that NICs do not result from fusion of newly synthesized channels, in transit to the cell surface membrane (Wonderlin & French, 1991). Cardiac NICs were depressed by Zn^{2+} and lanthanide ions, but were stimulated (transiently) by deoxynucleotides (Bustamante, 1993), observations that may be relevant to certain nuclear processes. In addition, GTP-γ-S, a nonhydrolyzable analogue of GTP, depressed NIC activity of adult cardiac myocytes (Bustamante, 1993). As at the time no report had appeared

on the role of GTP hydrolysis in nucleocytoplasmic transport, one could only extrapolate from the similarities between nuclear and ER protein transport (Connolly et al., 1991; Goldfarb, 1992) that this role of GTP hydrolysis on NIC function may be an indication of the dependence of macromolecular transport on this mechanism. Indeed, recent studies published during the revision of this review demonstrated modulation of macromolecular transport by the cytosolic Ras analogue Ran/TC4 (Moore & Blobel, 1993). Chloride-selective channels with electrical conductances of 150 and 58 pS were identified in isolated rat liver nuclei (Tabares et al., 1991) on the basis of NIC sensitivity to ion replacement of Cl^- and their blockade by agents known to block Cl^- channels (niflumic acid and DIDS: 4,4′ diisothiocyanotostilbene. 2,2′ disulfonic acid). In contrast to the large, 150 pS channel, the 58 pS channel was blocked by ATP. More recently, starfish oocytes in the germinal-vesicle stage were studied and found to display NIC activity similar to that of murine nuclei from oocytes in the same stage (DeFelice et al., 1993).

In all NIC studies (Matzke et al., 1990, 1991; Mazzanti et al., 1990, 1991; Tabares et al., 1991; Bustamante, 1992, 1993, 1994; DeFelice et al., 1993; Innocenti & Mazzanti, 1993), the recorded channel current activity was voltage dependent only in the sense that NIC current was directly proportional to the electrical potential across the nuclear envelope (Ohm's law with the constant of proportionally being the conductance). Voltage-gated NIC activity displaying inactivation (time-dependent closing after voltage-gated opening) was reported for cardiac myocytes (Bustamante, 1992). Envelope patches containing both inactivating and noninactivating NICs have been identified (Bustamante, 1992, 1993, 1994), suggesting a different population of channels, some of which may be ligand gated and some of which may be voltage gated. For either type of activity, single channel conductance was constant with electrical potential across the nuclear envelope (Bustamante, 1992). Noninactivating channels may represent ligand-gating mechanisms and/or loss of inactivating gate(s) through biochemical action. Cytosolic signals known to affect nucleocytoplasmic transport and gene activity (e.g., Karin, 1991; Davis, 1992) have been shown to affect NIC activity (e.g., cAMP-dependent protein kinase, Bustamante, 1992, 1993), strengthening the view of a correlation between NICs and NPCs and underlining the potential relevance of NICs to gene control by cellular and nuclear stimuli (Bustamante, 1993).

Estimates of NPC conductance based on a rigid cylindrical geometry with 9 nm diameter and 80 nm length, and a conductivity of 100 $\Omega \cdot$ cm, gives expected values of 1,000 pS (Mazzanti et al., 1990). However, the reported material inside, and surrounding, the lumen of the NPC effectively reduces the calculated conductance (e.g., Reichelt et al., 1990; Jarnik & Aebi, 1991; Akey & Radermacher, 1993; Hurt, 1993; Panté & Aebi, 1993). Arguments favoring identity of NICs with structural features of NPCs have been given (e.g., Mazzanti et al., 1990, 1991; Bustamante, 1992, 1993, 1994,—*see also review by* Matzke & Matzke, 1991). Briefly, NPCs are fusion points of the nuclear envelope membranes and NPC density per unit area ensures that many NPCs are found in envelope patches under patch-clamp pipettes. Assuming that NPCs were functional in the preparations in which the experiments were performed, it is impossible to argue that NICs are not NPCs or part thereof.

Open channel substates have been reported (e.g., Mazzanti et al., 1990, 1991; Bustamante, 1992, 1993). A wide range of transition times (from submilliseconds to hundreds of milliseconds) between closed and open states has been recorded (Bustamante, 1994). The magnitude of the transition time may be associated with the size of the gating structure, with the larger transition times (tens of milliseconds) being potentially related to the massive NPC plug. This broad range of transition and lifetimes for both open and closed channel states may be useful in explaining various nuclear transport phenomena, as nuclear processes are known to take from a few to many minutes (e.g., nuclear signaling preceding transcription).

The working paradigm that NPCs are NICs may lead to arguments against the physiological significance of patch-clamp data because the experiments were carried out with pipettes filled with saline solutions and with isolated nuclei bathed in saline. That is, due to the known cytosolic requirements for proper NPC operation (Adam, Sterne Marr & Gerace, 1991*a,b*), one should expect that NPCs were closed in these experiments. However, a high resistance is measured in several preparations with intact, *in situ,* envelopes (e.g., Loewenstein & Kanno, 1962). Our initial observations correlating NIC detection with cytosolic factors (Discussion in Bustamante, 1992) led to the design of new experiments, one of which is illustrated in Fig. 1. These experiments demonstrate that NICs cannot operate when deprived of cytosolic factors, yet to be identified but present in the cell lysates used. Since the lysates contain factors and substrates for sustained macromolecular transport (Adam et al., 1991*a,b)* it is likely that NPCs were functional. Indeed, NIC activity under lysate conditions was concurrent to transport of b-phycoerythrin (240 kD) conjugated to the nuclear localization signal of the simian virus 40 large T antigen, and channel activity and macromolecular transport were blocked by wheat germ agglutinin (J.O. Bustamante et al., *in preparation*), a lectin known to block macromolecular transport through NPCs (e.g., Miller et al., 1991; Nigg et al., 1991). These observations can be interpreted to mean that NICs are protein-conducting channels by using the rationale used

in recent studies of putative protein-conducting channels of ER (Simon & Blobel, 1991, 1992).

Conclusions

Patch-clamp, fluorescence microscopy and high-resolution EM have yielded new data which question current concepts of ion transport across the nuclear envelope. The current challenge is to prove that NICs play an important role in nuclear function either through their identity with NPCs or parts thereof. Electrophysiological designs must incorporate cell biology approaches as done for putative protein-conducting channels of the ER (Simon & Blobel, 1991, 1992).

Preliminary studies (J.O. Bustamante et al., *in preparation*), illustrated in Fig. 1, confirm that, as is the case of NPCs, NICs cannot function in an extracellular environment deprived of cytosolic factors. Our current efforts aim at clarifying if the lysate factors required for macromolecular transport through NPCs (e.g., Adam et al., 1991*a*,*b*) are those required for NIC open-shut gating. Monoclonal antibodies to identified NPC proteins should be helpful in furthering the identification of NICs with NPCs. Our observation of blockade of NIC activity with wheat germ agglutinin, discussed above, supports the idea that NPCs are the structural foundation for NICs. Should NICs be identified with NPCs or otherwise proven essential to nucleocytoplasmic transport, NIC response to cytoplasmic signals would suggest that they are relevant to mediating gene control by transduction and other cytosolic signals (Karin, 1991; Davis, 1992). NIC influence on intranuclear free ion concentrations is potentially important to controlling gene activation, repression, as well as the efficiency and fidelity of gene expression (e.g., Kroeger, 1963; Lezzi & Gilbert, 1970; Leake et al., 1972; Morgan & Curran, 1986; Li & Rokita, 1991; Lippard, 1993). As electrophysiological and cell/molecular biology approaches merge, the prospects improve for the field of nuclear electrophysiology.

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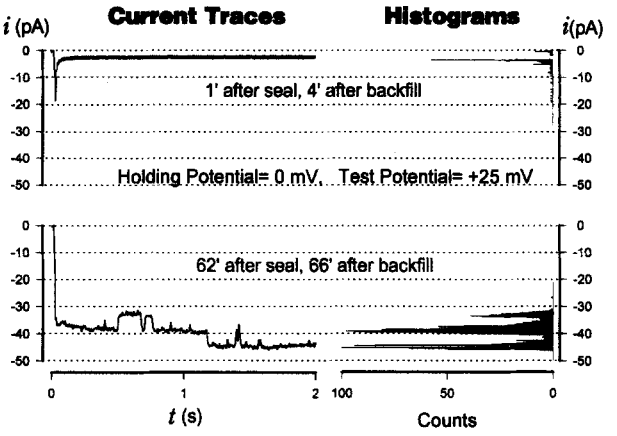

Fig. 1. Nuclear ion channels require cytosolic factors to operate. NIC activity, in the form of electrical current (*i*), was recorded with pipette attached to the cytoplasmic side of the nuclear envelope of a ventricular cardiomyocyte nucleus (Bustamante, 1992,1993,1994). The current traces (*i vs. t*) on the left were elicited with a +25-mV pulse applied to the pipette solution from a holding potential of 0 mV. Downward current deflections correspond to inward flow of positive ions, attributed to movement of K^+ (Bustamante, 1992, 1993). Plots on the right show the respective current amplitude histograms (*i vs.* counts) for traces on the left. The horizontal discontinuous lines join identical levels of current. Prior to attaching the pipette to the nucleus, the initial 4 mm length of pipette tip was filled, from its opening to the shank, with high-K saline solution (mM: 150 KCl, 5 $MgCl_2$, 10 HEPES, and 5 KOH; pH 7.2–7.3) and the contiguous 3 mm length of the pipette lumen was backfilled with commercial reticulocyte lysate containing all the substrates and optimized for transcription, mRNA export, and translation (Promega, Madison, WI). This lysate is known to support macromolecular transport (Adam et al., 1991*a*). The nuclear suspension was placed on the experimental chamber. Nuclei were allowed to settle and attach to the glass bottom of the chamber for 5 min, and then washed out with high-K saline for 15 min. In this experiment, it took about 3 min from the moment of pipette backfilling to the moment of attaining the high-resistance (GΩ) seal between the rim of the pipette tip and the cytosolic surface of the nuclear envelope. The top record on the left clearly shows no activity under saline conditions. The bottom record on the left shows NIC activity appearing immediately after the required, unknown factor(s) reached the nuclear surface. Note that the cytosolic factor(s) required for NIC activity must have had a high molecular weight due to the long time it took to reach the pipette tip. This phenomenon was seen in 100% of the 32 experiments carried out (J.O. Bustamante et al., *in preparation*). Experiment 93090802.

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