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Patch Clamp Detection of Transcription Factor Translocation Along the Nuclear Pore Complex Channel

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Abstract. Transcription factors (TFs) are cytoplasmic proteins that play an essential role in gene expression. These proteins form multimers and this phenomenon is thought to be one of the mechanisms that regulate transcription. TF molecules reach their DNA binding sites through the large central channel of the nuclear pore complex (NPC). However, the NPC channel is known to restrict the translocation of molecules $\geq$20–70 kD. Therefore, during their translocation, TF molecules and/or their multimers may plug the NPC channel and thus, interrupt ion flow through the channel, with a concomitant reduction in the ion conductance of the channel (γ). Here we show with patch clamp that γ is reduced during translocation of three major TFs: c-Jun (40 kD), NF-κB (≈50 kD), and SP1 (≈100 kD). Within a minute, femtomolar concentrations of these proteins reduced γ suggesting a purely mechanical interaction between single TF molecules and the inner wall of the NPC channel. NPCs remained plugged for 0.5–3 hr in the absence of ATP but when ATP was added, channel plugging was shortened to <5 min. After unplugging, channel closures were rarely observed and the number of functional channels increased. The transcription factors also stabilized the NPCs as shown by the extended duration of the preparations which allowed recordings for up to 72 hr. These observations are the first direct demonstration of the important role of NPCs in mediating nuclear translocation of TFs and, therefore, in forming part of the mechanisms regulating gene expression. The studies also demonstrate the potential of the patch clamp technique in quantifying TF translocation to the nucleus, mRNA export, and other processes governing gene expression.

Key words: Nuclear pore complex — Nuclear ion channels — Gene activity — Control of gene expression — Transcription factors — Oncogenes — Proto-oncogenes — AP-1 — c-Jun — NF-κB — SP1 — Patch clamp — Cardiac myocytes — Cell nucleus

Introduction

In the preceding paper (Bustamante et al., 1995*a*), we demonstrated that the large ion conductance of the NPC channel, γ, is a potentially useful measurement of signal transduction to the nucleus, of mRNA export, and of other nuclear mechanisms mediated by NPCs. The basic mechanisms of NPC channel gating are given in Figure 1. Briefly, since NPCs selectively restrict translocation of molecules larger than 20–70 kD (depending on cell type, activity, cycle, etc., *see* Miller et al., 1991), during translocation, these large molecules oppose ion flow through the NPC channel which, in turn, results in reduced γ. Transcription factors contain nuclear localization signals which help direct these proteins toward the nuclear interior (e.g., Boulikas, 1994). Three of these DNA-binding proteins were tested because of their importance in many processes regulating gene expression: c-Jun, NF-κB and SP1. The transcription factor c-Jun, a member of the activator protein 1 (AP-1) family, was selected because it is an oncogene product that responds to a plethora of stimuli influencing cell cycle and cell transformation (e.g., Bohmann et al., 1987; Marx, 1988;

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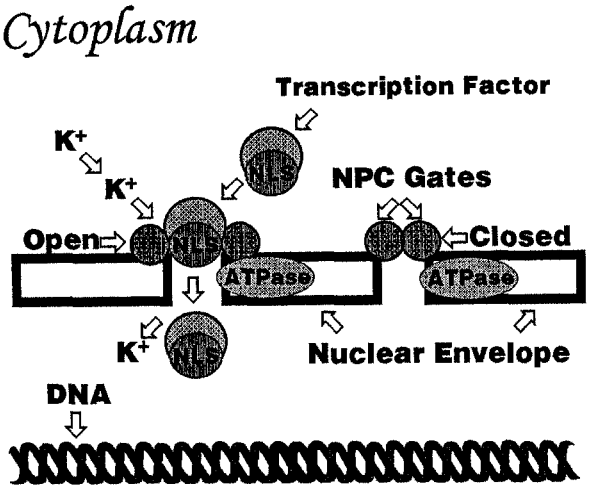

Fig. 1. Model of transcription factor-NPC interactions. NPC channels may assume one of two basic configurations: open or closed. Only when open, are the pores capable of allowing ion and transcription factor translocation. However, the ion conductance of the NPC channel, γ, is reduced when transcription factors, TFs, translocate along the NPC channel since TFs are poor electrical charge carriers. For simplicity, K^+ has been chosen as the sole electrical charge carrier because in the preparation used in these studies (cardiac myocyte nuclei) it was demonstrated that this ionic species is the predominant charge carrier (Bustamante 1992, 1993). For more details on the macromolecule-conducting channel model *see* Fig. 1 in the preceding companion paper (Bustamante et al., 1995*a*).

Gentz et al., 1989; Turner & Tjian, 1989; Abate et al., 1991; Angel & Karin, 1991; Lian et al., 1991; Webster et al., 1993; *reviewed in* Radler-Pohl et al., 1993). Nuclear factor kappa B, NF-κB, was chosen because it is a ubiquitous transcription factor which seems most extensively exploited in the immune system (e.g., Lenardo & Baltimore, 1989; Fujita et al., 1992; Schreck et al., 1992; Ueberla et al., 1993; Zabel et al., 1993; *reviewed in* Baeuerle & Henkel, 1994). NF-κB participates directly in nucleocytoplasmic signaling and it is capable of activating transcription of a great variety of genes encoding immunologically relevant proteins. Recently, NF-κB was shown to activate the tumor suppressor p53 and thus be part of an important mechanism for suppressing cell growth in response to stress (Wu & Lozano, 1994). NF-κB was also shown to be inhibited by aspirin (Kopp & Ghosh, 1994) and, therefore, to form part of the mechanisms underlying the actions of this universal drug. SP1 was tested because this TF regulates transcription through the SV40 promoter and because the SP1 transcriptional control sequence is found in a large number of viral and cellular promoters including the HIV long terminal repeat (Briggs et al., 1986; Kadonaga et al., 1987, 1988; *reviewed in* Pugh & Tjian, 1990). The present studies demonstrate for the first time that patch clamp, along with the macromolecule-conducting channel paradigm (Simon & Blobel, 1991, 1992; Bezkurov et al., 1994; *see* Bustamante et al., 1995*a*) can be used to detect, measure and understand translocation of transcription factors along the NPC channel and to determine novel actions of TFs on NPCs.

Materials and Methods

Nuclei, Chemical Reagents and Other Substances

Cardiac myocyte nuclei and salt solutions were prepared as detailed in the preceding paper (Bustamante et al., 1995*a*). The test solutions, containing the transcription factors, were prepared in high-K saline (mM: 150 KCl, 5 $MgCl_2$, 10 HEPES, and 4 KOH; pH 7.2–7.3). c-Jun (40 kD) and NF-κB (49 and 50 kD subunits) were expressed in *Escherichia coli* from full-length human cDNA clones (Promega). SP1 (≈100 kD) was expressed from a full-length human cDNA clone in HeLa cells (Promega). Neutral dextrans (Molecular Probes) were selected as negative controls to avoid possible voltage-dependent effects. All solutions and teflon labware were sterile and thoroughly cleaned with Type I reagent grade water. Liquid handling and storage was performed with sterile DNAse-, RNase-, pyrogen-free materials (Biopur, Eppendorf). Teflon needles (Hamilton) were mounted on 1% accuracy pipettors (Eppendorf) and used to fill the patch clamp pipettes with 10 μl of the test solution (Bustamante, 1992, 1993)[1].

Patch Clamp

Patch clamp procedures were carried out as described in the preceding paper (Bustamante et al., 1995*a*). Due to the long duration of the experiments (2–72 hr), electrode polarization was monitored and minimized to <2 mV by overnight equilibration of the pipette and bath electrodes (Bustamante, 1992, 1993) with the additional measure of leaving all electronic and computer equipment turned on. These long-lasting experiments also forced us to verify the nucleus-attached status of the patch. This could only be accomplished by lifting the pipette at the end of the experiment. If the pipette did not pull the nucleus it was concluded that the patch had been excised during the experiment. Two experiments demonstrated that the patch had been excised at an undetermined moment during the experiment. Therefore, these two experiments demonstrated that the TF effects were not mediated by intranuclear mechanisms. Compared to control experiments (without TF), where tight-seals were lost within 2 hr, all experiments with these natural nucleophilic proteins were terminated at will. To minimize TF action during pipette approach to the nuclear envelope (observed during screening trials), the pipette was double-loaded with 5 μl of control solution and then 5 μl of test solution. This procedure also allowed control activity during the first few moments of recording (*see* Bustamante et al., 1995*a*). The experiments were carried at 22–24°C.

[1] TFs are sold on the basis of their footprinting activity, expressed in terms of footprint units (fpu). Conversion of fpu into moles per liter was carried out by taking into account the molecular weight of each TF and the weight per vial provided by the manufacturer. Due to manufacturing and marketing practices, the values given here are likely underestimates.

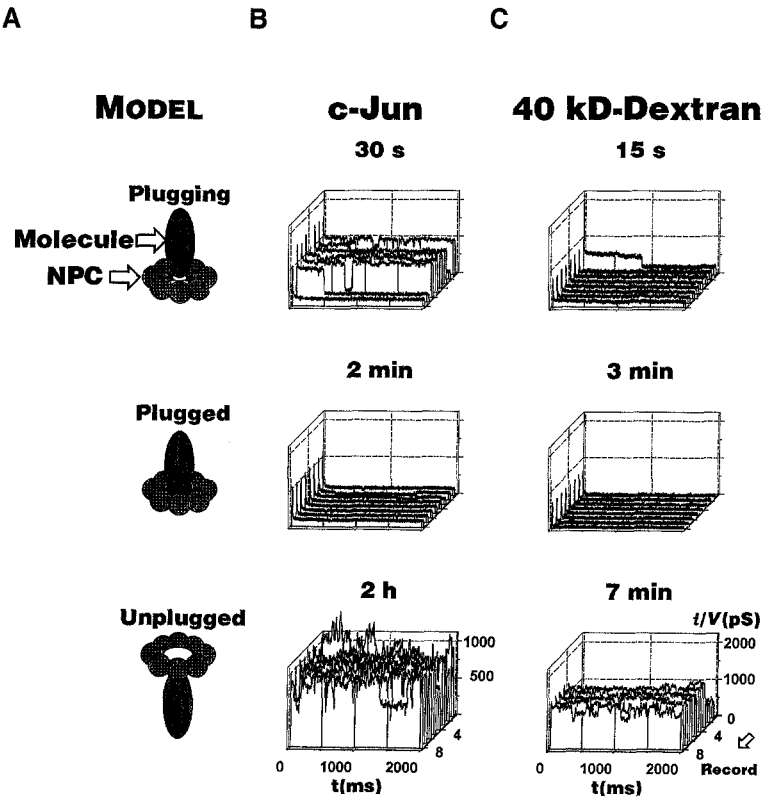

Fig. 2. Translocation of c-Jun and 40 kD-dextran transiently reduces ion conductance of the NPC channel. (*A*) Macromolecule-conducting model used to explain the experimental observations corresponding to the experiments shown in the two panels on the right. The NPC is symbolized by a single ring of eight macromolecular complexes. (*B*) Effects of 100 fM c-Jun (40 kD) at 30 sec (start of plugging as shown by last record), 2 min (maximal plugging), and 2 hr (clearance of NPC channel). The records of patch ion conductance, *i/V*, were obtained with 0 to +20 mV pulses applied to the patch clamp pipette in nucleus-attached mode. (*C*) Effects of neutral dextran (40 kD) at 15 sec (start of plugging as shown by first record), 3 min (maximal plugging) and 7 min (channel clearance). Pulses from 0 to −10 mV.

Results

c-Jun, NF-κB and SP1 Upregulate Ion Conductance and Stabilize NPCs

Figure 2*B* illustrates the effects of 100 fM cytosolic c-Jun in saline alone. Six experiments with 5–500 fM of the protein showed similar phenomenology. As shown in Fig. 2*B*, ion flow rapidly disappeared (<2 min), resulting in a minimal patch ion conductance, $\Gamma = i/V$ (where i and V represent, respectively, the patch current and voltage). After 2 hr, ion flow, and thus patch ion conductance, resumed but with higher amplitude. A comparison of the maximal current amplitudes at the beginning and at the end of the experiment indicates that the number of functional channels increased from 1 to 2. In 8 of 12 initial screening experiments carried out with 1–100 pM of c-Jun, the channels did not close at all. A major observation with c-Jun was the stability that it conferred to the preparation. Recordings could be carried out for more than 72 hr and all experiments were terminated at will. Negative controls were carried out with 10 and 40 kD neutral dextran molecules because these molecules are thought not to interact chemically with NPCs. Translocation of 10 kD-dextran molecules reduced γ by ≈10% (50 fM, $n = 3$; *not shown*) in agreement with a reduction of the conductivity of the medium (e.g., Bezkurov et al., 1994). Translocation of the 40-kD dextran molecules, however, transiently plugged the NPCs (50 fM, $n = 3$), as shown in Fig. 2*C*.

Figure 3 shows that the transient reduction of γ observed with c-Jun could be reproduced with NF-κB (40 kD subunit: $n = 6$; 50 kD subunit, $n = 12$) and SP1 ($n = 12$). Figure 3*B* illustrates that channel plugging took about 2 min to develop when 25 fM of NF-κB-50 kD was used. NPC ion channel activity (and thus ion flow) resumed after 45 min, an indication that the NPC channel had been cleared (unplugged) by a successful macromolecular translocation. NPC ion channel conductance, γ, was similarly modified with 25–250 fM SP1 ($n = 12$). However, as shown in Fig. 3*C*, SP1 (25 fM, $n = 3$) translocation lasted several hours due to the larger size of the protein molecule. As expected from concepts of macromolecular transport, translocation could be assisted by addition of 2 mM ATP (25 fM, $n = 3$). Figure 3*D* illustrates that translocation was accelerated to <5 min in the presence of ATP (compare to panel *C*). These effects could not be duplicated when MgATP was substituted with 2 mM of its slowly hydrolyzable analogue MgATPγS. ATP was not tested for dextrans since NPC plugging by 40 kD-dextran was short-lived (Fig. 2*C*).

Effects of Transcription Factors on Channel Stochastic Properties

Figure 4 illustrates our approach to characterizing the statistical behavior of the NPC channels for a test with

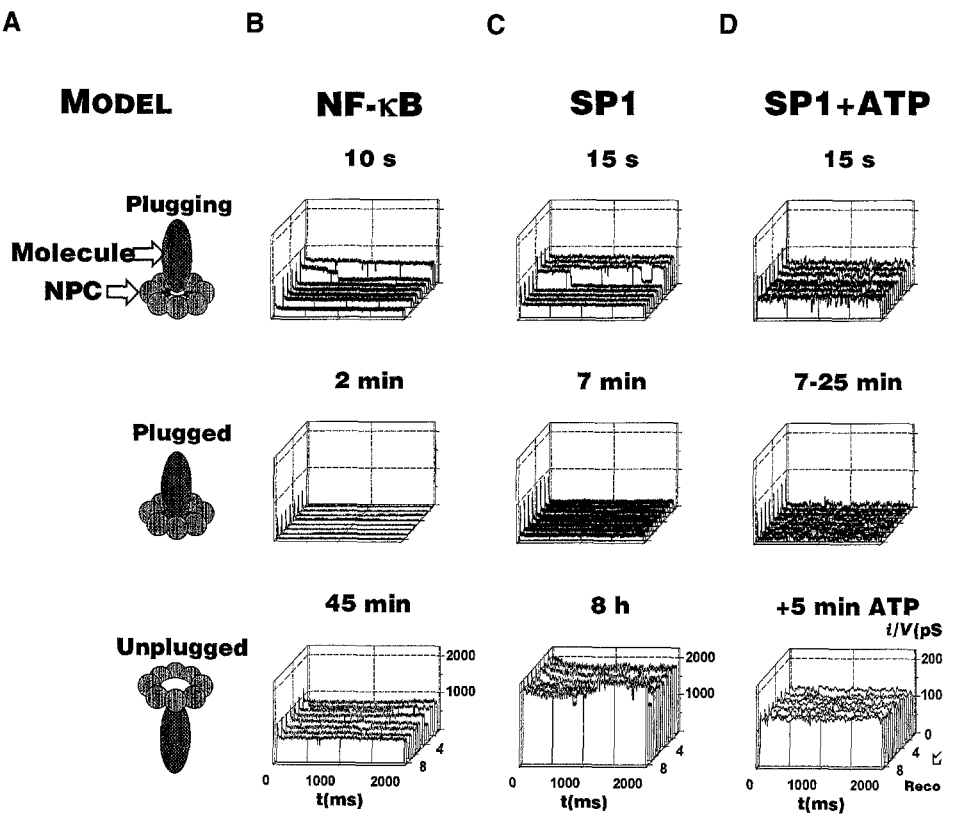

Fig. 3. Effects of NF-κB and SP1 on NPC channel conductance. (*A*) Model explaining the experimental observations on panels *B–D*. (*B–D*) Record ensembles of patch ion conductance, *i/V*, obtained with 0 to −10 mV pulses. Ensemble times (on top of the plots) were measured from the moment of attaining the pipette-nuclear envelope seal. (*B*) Effects of 25 fM NF-κB (50 kD subunit) at 15 sec (start of translocation), 2 min (complete plugging of NPC channel), and 45 min (clearance of channel). (*C*) Effects of 25 fM (100 kD) at 15 sec (start of translocation), 7 min (plugging of NPC channel), and 8 hr (clearance of channel).(*D*) SP1 translocation could be accelerated with addition of 2 mM ATP. Control recordings are shown at 15 sec. Records representative of the plugging effect are shown for 7–25 min. Traces demonstrating clearance of the NPC channel are shown after 5 min treatment with ATP (i.e., 30 min).

2.5 pM c-Jun. As explained in the previous paper (Bustamante et al., 1995*a*), averages were taken from current ensembles, <*i*>. The averages were then divided by the applied voltage, *V*, to obtain the average conductance of the patch as shown in panel *A* of Fig. 4 (the top two graphs). The open probability of the patch channel population, P_o, ($P_o = Np_o$; the number of channels in the patch, *N*, times the open probability of a single NPC channel, p_o) was obtained by dividing the average conductance values in Fig. 4*A* by the single channel conductance, γ, obtained from direct measurements of open ↔ close jumps in the records (i.e., $P_o = <i>/V/\gamma$). A total of 31 jumps were measured, giving a $\gamma = 329 \pm 47$ pS (mean ± SD, $P < 0.0001$). The two graphs in Fig. 4*B* give the plot of P_o derived from the averages in Fig. 4*A*. The P_o values were normalized to the maximum for comparison to the results in other experiments (two bottom plots, Fig. 4*C*). It is obvious that the initial high P_o of the patch was immediately reduced to zero within 2.5 min, only to recover after 1 hr. At 25 fM, the time to channel plugging was 1.9 ± 0.8 min ($n = 12$). Plug time was 8.4 ± 3.3 hr (n = 5). MgATP, 5 mM, reduced plugging time to 5.1 ± 2.3 min ($n = 4$).

Figure 5 illustrates an experiment with 10 fM NF-κB, 49 kD subunit. Fig 5*A* shows the time course of the average conductance at selected periods of observation during the experiment. The data in Fig. 5 were obtained with −10 mV pulses. The single channel conductance, γ, did not change with voltage polarity. A value of 382 ± 64 pS was obtained for γ, from 44 mean single conductance values corresponding to 44 record ensembles. Single channel conductance did not change significantly throughout the experiment. Figure 5*B* shows the time course of open probability, $P_o = Np_o$, calculated from the values in panel *A* and the calculated γ. The time course for the relative P_o is given in the plots of Fig. 5*C*. The central plots in Fig. 5*A–C* show that after roughly 3 hr the ion-conducting channels became plugged. At that time, we added 5 mM MgATP to test the nucleocytoplasmic transport paradigm that macromolecular transport is

mediated by ATP hydrolysis. As Fig. 5 shows, the channels became unplugged and, since γ did not change, the maneuver demonstrated that other channels had been plugged at the beginning of the experiment. Note that, at difference from the effects of c-Jun, increased Np_o occurred only when ATP was added to the bath, indicating that the increase was associated with ATP and not NF-κB. At 10 fM, the time to channel plugging was about 3 hr ($n = 2$). At ten times that concentration, the transcription factor plugged the channels within 2 min 1.5 ± 3.2 min ($n = 3$). When 5 mM MgATP was added to the bath solution, unplugging followed within 4.7 ± 3.2 min ($n = 3$).

The effects of 50 fM SP1 on the statistical properties of single NPC channel gating are illustrated in Fig. 6. Panels *A* to *C* give plots similar to those of Fig. 5. After a prolonged period of negligible activity (here 13 hr), opening of the channels ensued. The conductance before and after plugging was about the same, 310 ± 23 pS ($n = 40$), but was rapidly reduced to zero during plugging. P_o at the beginning of the experiments was 0.28 ± 0.14 ($n = 12$) in relation to its final value. At 50 fM, the time to channel plugging was 2.3 ± 0.9 min ($n = 12$). Plugging of the channels lasted several hours and working conditions during this experimental series did not allow for long-term follow up to recovery in all the patches. Plug time was 12.7 ± 2.8 hr ($n = 2$). In the presence of 5 mM MgATP unplugging was always achieved, reducing plug time to 6.3 ± 1.5 min ($n = 4$).

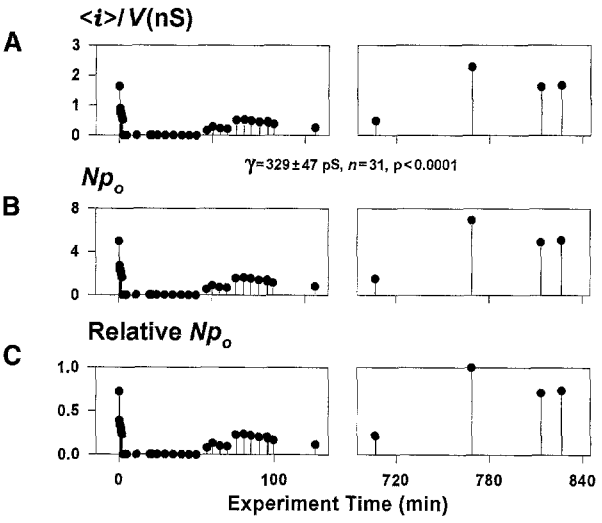

Fig. 4. Effect of 2.5 pM c-Jun on open probability of the NPC channel population. (*A*) Plots of average patch ion conductance *vs.* time of acquisition during the experiment. Ensemble averages of conductance were obtained by dividing the current ensemble averages, <*i*>, by the applied voltage, *V*. The conductance of a single NPC channel, γ, was measured directly from the current jumps between conducting and nonconducting states (open ↔ close). Thirty-one jumps were detected in this experiment. Error bars superimposing the data points give the corresponding standard deviations. (*B*) The open probability for the population of channels in the patch, P_o, was equal to the single channel open probability, p_o, times the number of channels in the patch N (i.e., $P_o = Np_o$, and all channels assumed equal and independent). (*C*) Relative P_o was computed in relation to its maximum value.

Discussion

Transcription Factor Translocation Along The NPC Channel Causes Transient Interruption of Ion Flow

As demonstrated in the preceding companion paper (Bustamante et al., 1995*a*), the γ values for these experiments did not differ from those recorded in control experiments (without protein added to the pipette): 421 ± 46 pS (mean ± SD). These control values were similar to those reported for this preparation (Bustamante, 1992, 1993, 1994*a*). The present patch clamp investigations show that during their translocation along the NPC channels, all the three TFs tested interrupt ion flow along the channel. This observation is predicted by the model of macromolecule-conducting channels (Simon & Blobel, 1991, 1992; Bezkurov et al., 1994; Bustamante et al., 1995*a*). Occasionally, we observed slow (10–100 msec) transitions between the ion conducting and nonconducting (on-off) states of the pore. We previously reported the rare occurrence of channel state transitions with similar characteristic times but failed to find an adequate explanation for our observations (Bustamante, 1994*a*). The slow transitions between the open and closed states suggest a sluggish movement of the channel gate(s) but it appears more likely that these slow transitions result from smaller macromolecules squeezing through the NPC channel (*see* Bezkurov et al., 1994). These observations are in agreement with the macromolecule-conducting channel model (*see* Bustamante et al., 1995*a*). Since the length of the NPC channel is about 10 times longer than the diameter of the channel we think that at a given time several molecules occupied the channel until diffusional equilibrium was reached. As the 40 kD-dextran molecules translocated readily, c-Jun translocation seems to be determined, in great measure, by nominal simple diffusion. ATP was not tested for dextrans since NPC plugging by 50 kD-dextran was short-lived (Fig. 2*C*). This observation suggests that the prolonged NPC plugging by the transcription factors was due to stronger interactions of the molecule with the lining of the NPC channel or due to other mechanisms yet to be identified such as friction (*see* Bezkurov et al., 1994).

Transcription Factor Stabilization of NPCs

The stabilizing effect of the transcription factors may be related to the recently recognized architectural role of

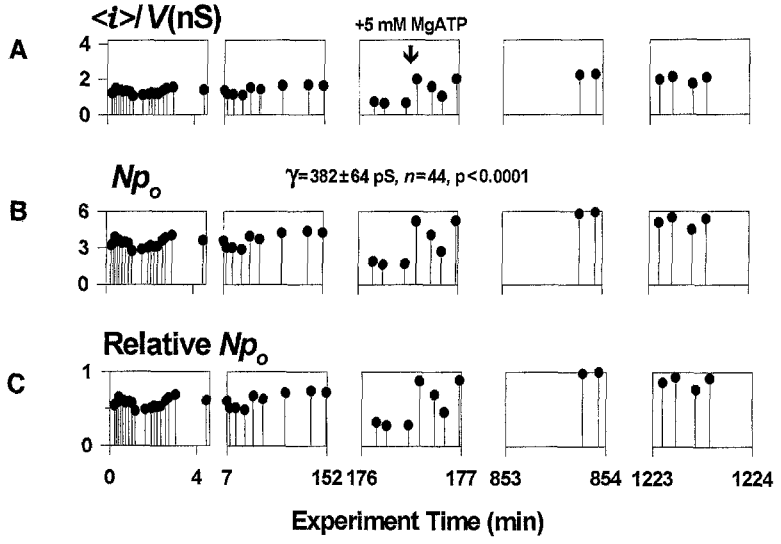

Fig. 5. Effect of 10 fM NF-κB-49 kD on open probability of NPC channels. (*A*) Plots of average patch ion conductance *vs.* time of acquisition at selected times highlighting this experiment. Single NPC channel conductance, γ, was obtained from 44 mean values of open ↔ close transitions. Error bars superimposing the data points give the corresponding standard deviations. (*B*) Plots of open probability, $P_o = Np_o$, of the patch channel population calculated from the values in (*A*). (*C*) plots of relative P_o computed from (*B*).

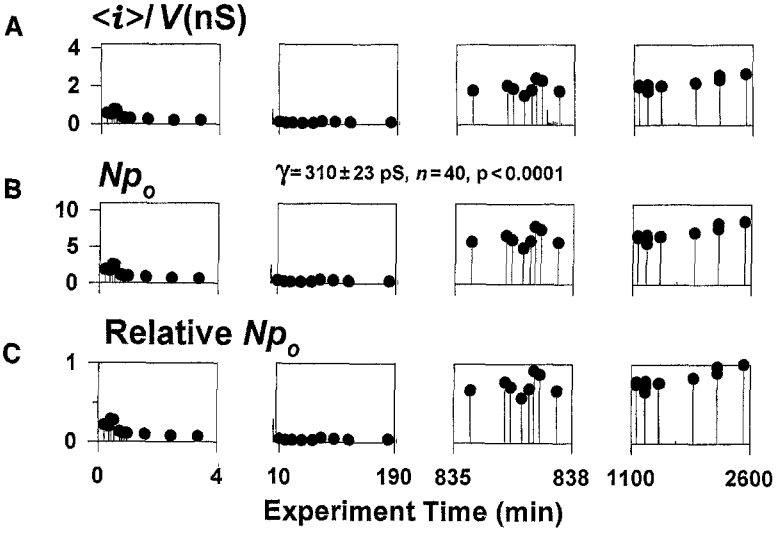

Fig. 6. Effect of 50 fM SP1 on open probability of NPC channels. (*A*) Plots of average patch ion conductance *vs.* time of acquisition at selected times during the experiment. Single NPC channel conductance, γ, was obtained from 40 mean values of open ↔ close transitions. (*B*) Plots of open probability, $P_o = Np_o$, calculated from the values in (*A*). (*C*) Plots of relative P_o computed from (*B*).

transcription factors (Wolffe, 1994). This effect may be cause for optimism since it is the first time that a substance is identified as conferring such a robust life to a patch clamp preparation, a length of time far beyond those reported for any patch clamp study (a few hours at best). Therefore, transcription factors, or their mediating mechanism(s) (e.g., structural stabilization through multimerization—*see* Bustamante et al., 1995*c*) may prove crucial in preventing uncontrollable rundown of patch clamp and of other preparations. The low concentration levels at which the transcription factors had noticeable effects qualify as homeopathic and, therefore, a discussion of this subject is highly desirable and necessary. First, at the lowest concentration level of 1 fM (a level we did not use but that helps our discussion by setting the most stringent limit), the number of TF molecules contained in the 10 μl-pipette was in the order of 10^4 (10^{-15} moles.l^{-1} × 10^{-5} l × 6 × 10^{23} molecules.mole^{-1}). The pipette length filled with solution was roughly 10 mm. A rough approximation suggests that there is one molecule per μm of pipette length. The average time, $\langle t \rangle$, required for a neutral particle to move an average distance $\langle l \rangle$ can be obtained from the diffusion coefficient, *D*, according to the following relationship for Brownian, random motion (*see* Jingersons & Straumanis, 1954; Hille, 1992):

$$\langle t \rangle = \langle l \rangle^2 / D$$

Since values of diffusion coefficients are not available for transcription factors, we used the worst case for dextran diffusion within the cytoplasm obtained in a nucleocytoplasmic transport study (Paine et al., 1975; *see* Table II in Paine & Horowitz, 1980). A value of ~10^{-7}

cm^2/s (~10 $\mu m^2/s$) was found for 24 kD dextran (Paine et al., 1975). That is, the random movement of a single molecule will cover an area of 1 μm^2 (equivalent to a distance of 1 μm) in 0.1 sec. This estimate agrees with that calculated elsewhere (e.g., Paine & Horowitz, 1980). Since at 1 fM there is roughly one TF molecule per μm of pipette length, it takes less than 1 sec (or a few seconds at worst) for a molecule to reach the pipette tip. That is, TF molecules were readily available to the NPCs within a few seconds after gigaseal formation at all levels of concentration used. Consequently, the apparently low number of NPCs displaying ion channel behavior (i.e., flip-flopping between open and closed states) at the beginning of the experiment, reflected by a low relative Np_o, appears to have resulted from several NPC channels being plugged by the TF molecules at the start of the recording as a result of the absence of ATP (required for macromolecular transport; *see* Miller et al., 1991). It is interesting to note that at high TF concentration (e.g., >25 pM), replugging of the channels was not observed. This suggests an additional effect of the TFs on NPCs because the source of TF molecules in the patch-pipette was far from exhausted at these concentrations levels. That is, the absence of replugging at high TF concentrations indicates that TF molecules induced the permanent opening of the NPC channels but failed to gain access to them after their initial action.

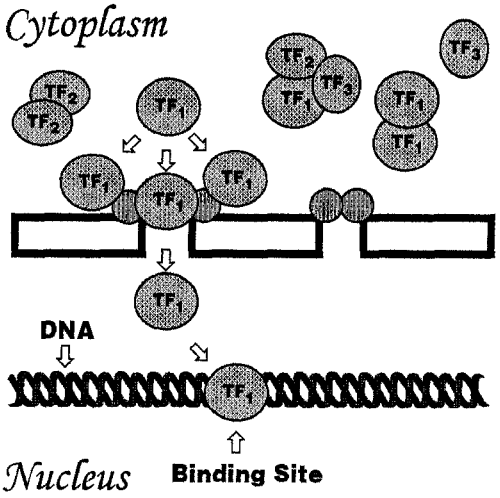

Fig. 7. Paradigm explaining transcription factor, TF, action on NPC ion conductance. Transcription factor molecules (TF_1, TF_2, TF_3, etc.) open and stabilize NPCs. The proteins may form homo- and/or heteromultimers and use this combinatorial mechanism to modulate TF action. Upon translocation, TFs bind to the corresponding DNA binding site. During translocation, the lumen of the NPC channel is plugged by the TF, resulting in interruption of ion flow and a negligible NPC ion conductance, γ. Transduction and other cytosolic signals (e.g., Zn^{2+}, G-proteins, protein kinases/phosphatases) also determine the conductance and open probability of the NPC channel gating.

Transcription Factor Upregulation of NPC Ion Channel Conductance

Both c-Jun and SP1, but not NF-κB, increased patch ion conductance. In most of the experiments, the single channel conductance, γ, was practically unchanged. Therefore, the increased P_o (=Np_o) of the patches suggest an increase in the number of ion-conducting channels. We think that this may be the result of having several NPCs plugged at the beginning of the experiment and then, as time passed, the NPCs became unplugged due to the successful translocation of the TF molecules. The lack of effect by NF-κB may be due to a less important role of this transcription factor in regulating NPCs in general or it may be specifically related to our preparation: cardiac myocytes. Upregulation of patch ion conductance by TFs supports classical microelectrode experiments with *in situ* nuclei demonstrating a reduction of nuclear envelope ion resistance (i.e., increased ion conductance) in cells with increased transcriptional capacity (e.g., *Xenopus laevis* oocytes in Loewenstein et al., 1966; *see* Bustamante, 1994*b;* Bustamante et al., 1994).

NPCs Mediate Transcription Factor Translocation to the Nucleus

Since expression of the *c-jun* gene is an early response to cellular deformation (e.g., stretch) which, in turn, leads to muscle growth and hypertrophy (Parker & Schneider, 1991; Sadoshima et al., 1992), our results with cardiac myocytes may serve as the basis for future experiments aimed at understanding cardiac hypertrophy, in particular, and abnormal cell growth and function, in general. Our results suggest that blockade of the NPC channel should prevent c-Jun translocation and, consequently, mechanically induced alterations in cellular structure. The significance of our observations with NF-κB is not clear as this transcription factor has not been studied in cardiac myocytes. However, one may speculate that NPC mediation of NF-κB translocation to the nucleus is similar to that proposed for c-Jun. It seems that a better understanding of NPC significance to NF-κB translocation may be obtained from cells where this TF is known to play an important role (i.e., cell from the immune system, such as lymphocytes—*see* Bauerle & Henkel, 1994). NPCs mediation of SP1 translocation should be of relevance to studies where the SP1 promoter plays a role in transcriptional activity of other DNA-binding proteins and, therefore, gene expression.

New Model of Transcription Factor Access to DNA-binding Sites

The results, summarized in Fig. 7, indicate that NPCs may serve as an integration point for transcription and for signal transduction to the nucleus. Since NPCs me-

diate mRNA export, they also play an important role in gene expression. These investigations provide additional support to the potential of patch clamp in assessing the role of NPCs in the control and integration of signal transduction to the nucleus, and, therefore, in mediating the regulation gene expression (Hoffman, 1993; Marx, 1993). Since *tempo* is of critical importance to transcription (O'Farrell, 1992), and since up- and downregulation of NPC channel gating is significant to macromolecular transport, NPCs clearly play a pivotal mediator role in transcription and, therefore, in gene expression. New strategies in drug therapy that target transcription factors and nuclear localization signals may benefit from our observations (e.g., Kopp & Gosh, 1994; Nebert, 1994; Pennypacker et al., 1994; Peterson & Tupy, 1994; Prabhakar & Kayastha, 1994). The NPC channel itself may prove to be a convenient pharmacological target.

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