# Chloride Channels in the Nuclear Membrane

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**Summary.** Chloride-selective ion channels were measured from isolated rat liver nuclei. Single ion channel currents were recorded in both "nuclear-attached" and in excised patches in the inside-out configuration of the patch-clamp technique. Two types of chloride conductance were defined, a large conductance (150 pS; $i_{Cl,N}$) channel with complex kinetics and multiple substates, and a second smaller conductance (58 pS; $I_{Cl,n}$) channel sensitive to block by ATP. The channels were inhibited by pharmacological agents known to block chloride channels and were insensitive to internal and external changes in calcium and magnesium. Presumably the channels reside in the external membrane of the nuclear double membrane and may mediate charge balance in the release and uptake of calcium from the perinuclear space.

**Key Words** nuclear membrane · ion channels · chloride channels · patch clamp · nucleus

## Introduction

The nuclear envelope consists of an inner and an outer nuclear membrane, nuclear pore complex, and nuclear lamina. In most cells, the outer nuclear membrane is continuous with the rough and smooth endoplasmic reticulum (ER) and the space between the two nuclear membranes, known as the perinuclear space, is continuous with the lumen of the ER. Besides the nucleo-cytoplasmic flow of substances through the nuclear pores, little is known about the function of the rest of the nuclear membrane. However, the nuclear envelope contains ion channels (Mazzanti et al., 1990) and specific binding sites for insulin, thyroxine, and steroid hormones, as well as an ATP-dependent Ca uptake system. Here we report ion channels measured from the outer membrane of the rat hepatocyte nuclear envelope. Our results show that the ionic permeability of this mem-

brane is clearly dominated by a high conductance to chloride (Cl) ions determined by at least two types of highly-selective $Cl^-$ channels. The functional significance of the chloride channels as a counterion mechanism is discussed.

## Materials and Methods

### ISOLATION OF NUCLEI

Nuclei were obtained using a modified method of Blobel and Potter (1966) for nuclear isolation. Livers were removed from female Sprague Dawley rats, placed in ice-cold 0.25 M sucrose solution containing (in mM); 40 KCl, 5 MgCl₂, 2 EGTA, 10 HEPES, pH 7.84 at 4°C, minced, and homogenized using a motor-driven Teflon pestle. The homogenate was filtered and spun at low speed (2500 rpm) for 5 min. The supernatant was discarded and 2 ml of the 0.25 M sucrose solution mixed with the homogenate. The sucrose concentration of the homogenate was raised to 1.6 M by mixing it with 2.3 M sucrose solution containing (in mM); 65 KCl, 5 MgCl₂, 2 EGTA, 10 HEPES, pH 7.84 at 4°C. The suspension was layered over 2.3 M sucrose solution and spun for 20 min at 76,000 × g. The nuclear pellet was suspended in standard bath solution containing (in mM): 140 KCl, 2 MgCl₂, 1.1 EGTA, 0.1 CaCl₂, 10 HEPES, pH 7.3, pelleted and resuspended in standard bath solution.

### ELECTRICAL RECORDING

All channels were measured using the tight-seal patch-clamp technique (Hamill et al., 1981). Nuclei were kept in ice-cold standard bath solution until experiments were performed 1–36 hr after isolation. Nuclei were allowed to adhere to the glass at least one-half hour before recording. Patch electrodes were fabricated from Corning #7052 glass. Data were stored and analyzed using an Instrutech system (Mineloa, New York). Raw recordings of channels were printed on a Gould TA 2000 thermal array recorder ($f_c$ = 2 kHz). Positive charges flowing out of the pipette are shown as outward deflections. All recordings were made at room temperature (20–22°C). Solutions were adjusted to 270–290 mOsm with the pipette solution made 5 mOsm hypoosmotic in relation to the bath.

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## Results

The ion channels in the outer nuclear membrane of isolated nuclei from hepatocytes (Blobel & Potter, 1966) were studied using the patch-clamp technique. Single ionic currents were recorded in the "nuclear-attached" configuration and then, after excision, in the "inside-out" configuration. In both cases the cytoplasmic side of the outer nuclear membrane was exposed to the solution in the recording pipette. Although the nuclear pore is a highly permeable molecular sieve with an effective radius of about 5 nm (Paine, Moore & Horowitz, 1975) surprisingly, high resistance seals were frequently formed (> 15 GΩ). Thus, in our preparation, most of the pores must have been occluded, as suggested by electron micrographs of isolated nuclei. Of more than 300 patches measured, approximately 90% displayed chloride-selective ion channels at estimated densities of 1 to 5/patch. Typically, two types of $Cl^-$ channels were distinguished on the basis of their conductance and gating kinetics. Figure 1 shows these channels recorded in the nuclear-attached configuration. Besides the main conductance state, at least two substates for the large conductance channel ($I_{Cl,N}$) were found. A third substate was seen in the flickering baseline with occasional sojourns to the basal state. The activity of the large conductance channel usually disappeared after a time ranging from seconds to minutes. In contrast, the activity of the intermediate channel ($I_{Cl,n}$) was longlasting and stable. A third very low conductance $Cl^-$ channel was recorded in some patches, but its characteristics could not be studied in detail. We concentrated our efforts on the large ($I_{Cl,N}$) and the intermediate ($I_{Cl,n}$) size channels. Figure 1c shows the I-V relation for both large and intermediate channels in two different asymmetrical $Cl^-$ solutions. The large channel had a slope conductance of 150 pS (from +30 to −50 mV, $V_p$) in symmetrical 140 mM KCl (not shown). Under the same conditions, the intermediate channels' slope conductance was 58 pS.

The gating behavior of the channels was complex with at least four substates of conductance and three closed states for $I_{Cl,N}$ and two substates of conductance and three closed states for $I_{Cl,n}$. At 0 mV the activity of both channels occurred in bursts of openings separated by long closed periods. As the cytoplasmic side of the outer membrane was made more positive, the steady-state activity of the channel greatly increased. Both channels displayed voltage dependence, but plots of net channel activity ($Np_o$, where $N$ = number of channels and $p_o$ = probability of opening) vs. voltage varied quantitatively from patch to patch.

To determine the ionic selectivity of the nuclear envelope ions channels, solutions were used in which chloride was partially replaced by glutamate. The reversal potential of the single channel current was measured using either the nuclear-attached or excised patch configurations. When the recordings were made from a nuclear-attached patch, the channels' reversal potentials closely approximated $E_{Cl}$ corresponding to the Cl gradient between the pipette and the bath. After excision, the same (now "inside-out") patch showed an identical I-V relation and reversal potential as in the nuclear-attached mode. This suggests the [Cl] is equivalent on both sides of the membrane under our conditions. We do not know if the chloride channels span the two membranes of the envelope or only the outer membrane. Figure 2a shows the measured single channel reversal potentials as a function of chloride concentration gradient. As expected for chloride-selective conductances, the plot of reversal potentials versus the ratio of internal to external chloride concentrations followed the predicted Nernst relation with a slope of −58 mV/decade at 22°C. Under these asymmetrical Cl conditions, the reversal potential was consistently found to be close to $E_{Cl}$ by symmetrical substitution of K, Na, Cs, or choline as cations. These results show that both channel types are highly Cl-selective over cations.

The nuclear chloride channels were inhibited by anion transport inhibitors. In the presence of DIDS (4,4' diisothiocyanotostilbene, 2,2' disulfonic acid) both $I_{Cl,N}$ and $I_{Cl,n}$ were blocked (Fig. 2b) in a dose-dependent manner. Niflumic acid, another agent known to suppress chloride channels (White & Alwyn, 1990), also blocked the large and intermediate conductances. Interestingly, ATP blocked $I_{Cl,n}$ from either side of the membrane. The ATP block was dose dependent and induced a rapid flicker with a complete suppression of channel activity at 5 mM (free ATP 1.7 mM; Fig. 3). ATP has previously been shown to block chloride channels from platelet membranes reconstituted into planar phospholipid bilayers (Manning & Williams, 1989). In contrast, $I_{Cl,N}$ was not blocked by concentrations up to 13 mM ATP. Substitution of the buffer BES for HEPES did not alter the gating behavior of the channels, as has been reported for some chloride channels (Tabcharani & Hanrahan, 1989). Varying $Ca^{2+}$ on either side of the membrane had no effect on either channel in the range from 10 nM to 3.5 μM. Tetraethylammonium Cl (TEA; up to 5 mM) and varying $[Mg^{2+}]$ on either side of the membrane from 0 to 2 mM did not alter the channels' gating.

## Discussion

This report demonstrates the presence of chloride channels in the nuclear membrane of rat hepato-
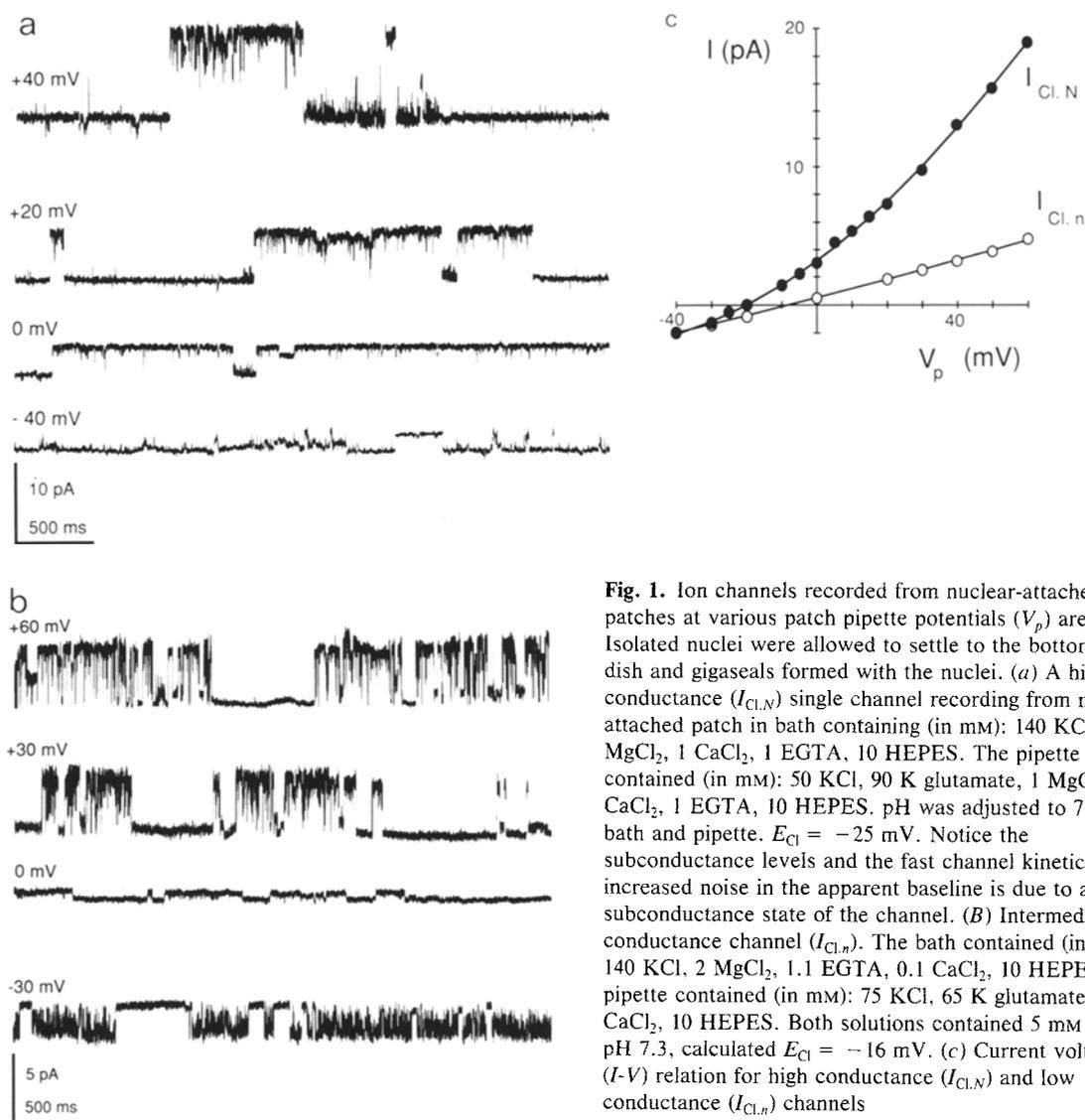
**Fig. 1.** Ion channels recorded from nuclear-attached patches at various patch pipette potentials $(V_p)$ are shown. Isolated nuclei were allowed to settle to the bottom of a dish and gigaseals formed with the nuclei. (a) A high conductance $(I_{Cl.N})$ single channel recording from nuclear-attached patch in bath containing (in mM): 140 KCl, 1 MgCl₂, 1 CaCl₂, 1 EGTA, 10 HEPES. The pipette contained (in mM): 50 KCl, 90 K glutamate, 1 MgCl₂, 1 CaCl₂, 1 EGTA, 10 HEPES. pH was adjusted to 7.3 for bath and pipette. $E_{Cl} = -25$ mV. Notice the subconductance levels and the fast channel kinetics. The increased noise in the apparent baseline is due to a small subconductance state of the channel. (B) Intermediate conductance channel $(I_{Cl.n})$. The bath contained (in mM): 140 KCl, 2 MgCl₂, 1.1 EGTA, 0.1 CaCl₂, 10 HEPES. The pipette contained (in mM): 75 KCl, 65 K glutamate, 0.15 CaCl₂, 10 HEPES. Both solutions contained 5 mM TEA, pH 7.3, calculated $E_{Cl} = -16$ mV. (c) Current voltage $(I$-$V)$ relation for high conductance $(I_{Cl.N})$ and low conductance $(I_{Cl.n})$ channels

cytes. Patches from these nuclei were dominated by chloride conductances, and we observed no measurable cationic conductances. A previous report revealed potassium channels in the mouse pronucleus membrane (Mazzanti et al., 1990). The properties of the Cl⁻ channels described here differ from previously reported Cl⁻ channels of other intracellular membranes. The mitochondrial outer membrane Cl⁻ channel (Roos, Benz & Brdiczka, 1982) has a much larger conductance (480 pS in 100 mM KCl), and the inner mitochondrial membrane is only slightly anion selective (Sorgato, Keller & Stühmer, 1987). Chloroplast Cl⁻ channels have a conductance similar to that of $I_{Cl.N}$ but differs in

its voltage-dependence (Schonknecht et al., 1988). A Cl⁻ channel of similar conductance to $I_{Cl.n}$ has been reported from heart sarcolemma (Coronado & Latorre, 1982).

Studies of mouse nuclear membranes suggested that nuclear pores are present at densities of approximately 3.3 pores/$\mu$m² (Mazzanti et al., 1990). The tip area of our pipette was $\sim 1$ $\mu$m² $(R_{pip} \geq 10$ M$\Omega)$, thus the number of patent nuclear pores may range from 0–4 per patch. The conductance of the nuclear pore is unknown, but given the large diameter ($\sim 9$ nm) of the pores, it is often thought that the nuclear pores cannot provide significant resistance to ion flow. However, calculations of pore resistances
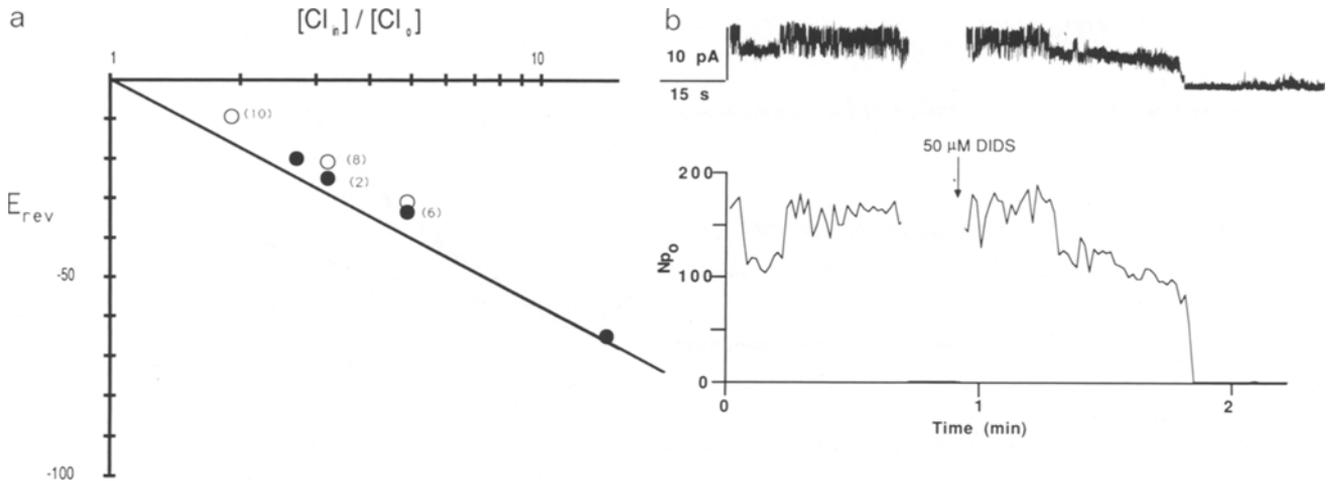
**Fig. 2.** Nuclear channels are chloride selective. (*a*) Reversal potentials of nuclear channels were averaged and plotted against the ratio of chloride in the bath to the pipette ($Cl_{in}/Cl_{out}$). The number of reversal potential measured are given in parentheses. The expected Nernst relation is shown as a solid line (slope $-58$ mV/decade). Closed circles, $I_{Cl.N}$; open circles, $I_{Cl.n}$. Best fits for the large and intermediate single conductance channel gave slopes of $-62$ and $-53$ mV/decade, respectively. (*b*) Large and intermediate conductance channels were blocked by 50 $\mu$M DIDS. DIDS was reversible only when washed out shortly after addition. Electrical artifact due to the application of DIDS to the bath has been removed from the record. Channel activity is quantified as open probability ($Np_o$) $\times$ 100. $Np_o$ is calculated as the integral of net current ($I$) divided by single channel amplitude, $i$ ($I = Np_o i$)
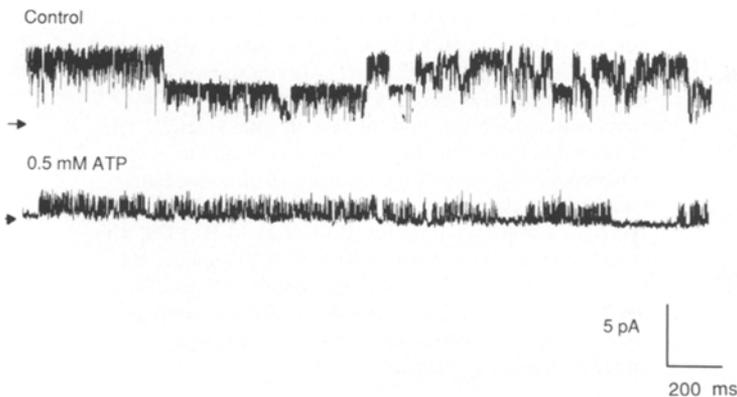


**Fig. 3.** $I_{Cl.n}$ is blocked by ATP. An excised nuclear patch was exposed to 500 $\mu$M ATP (free ATP, 22 $\mu$M) during a 5 sec period between the top and bottom trace. The channel is rapidly blocked, producing a flicker and apparent reduction in amplitude. Arrows indicate baseline. Solution for pipette contained (in mM): 115 K glutamate, 25 KCl, 2 MgCl$_2$, 0.345 CaCl$_2$, 10 HEPES, pH 7.3; solution for the bath contained (in mM): 140 KCl, 2 MgCl$_2$, 1.1 EGTA, 0.1 CaCl$_2$, 10 HEPES, pH 7.3, $V_p$ +30 mV

based on diameters are not inconsistent with pores as ion channels (Paine & Horowitz, 1980). Using microelectrodes to measure nuclear envelope electrical resistances in *Chironomus* salivary gland cells, Loewenstein and coworkers (Kanno & Loewenstein, 1963; Ito & Loewenstein, 1965) observed a significant barrier to ion movement (0.5–2 $\Omega$/cm$^2$). We do not know if the chloride channels are due to nuclear pores or other membrane proteins. It is also possible that the patch recording distorts the normal function of the nuclear pores and eliminates them from our recording or that many of the pores

are normally occluded by molecules in transport across the nuclear membrane. Whatever the case, chloride-selective conductances are present in the outer nuclear membrane.

The method of nuclear isolation used (Blobel & Potter, 1966) reliably gave relatively pure nuclear preparations. By electron microscopy most of the nuclei appeared intact and clean, although detached small fragments of ER were occasionally found in some areas of the nuclear envelope. The small contamination by ER membranes could not be the origin of the recorded membrane conductances given the

high rate of sealing and recording. However, the outer nuclear membrane is continous with that of the ER, making it likely there are structural similarities between these two membranes; e.g., the phospholipid distribution of both membranes is similar (Khandwala & Kasper, 1971) and anion channels exist in the ER (Schmid et al., 1988). The detection of Cl conductances in the nuclear membranes of rat hepatocytes could well be related to the presence of similar conductances in the ER.

A more detailed understanding of the possible physiological function and regulation of the nuclear $Cl^-$ channels is clearly important. In other intracellular membranes (i.e., in SR, mitochondria, and chloroplasts) the flow of $Cl^-$ and $K^+$ counteract the electrogenic movement of other ions such as $H^+$ or $Ca^{2+}$ (Garcia & Miller, 1984; Sorgato et al., 1987; Schonknecht et al., 1988). Although it has been well established that the endoplasmic reticulum plays an important role in $Ca^{2+}$ storage in liver cells, a similar role of the perinuclear space has not yet been demonstrated. Nevertheless, the hypothesis that the perinuclear space can also be a $Ca^{2+}$ reservoir now is supported in cells such as hepatocytes where an ATP-dependent $Ca^{2+}$ uptake stream in the nuclear envelope modulates the transport of $Ca^{2+}$ (Kulikova et al., 1982; Nicotera et al., 1989). This system seems to be related to cytoplasmic-independent transient $Ca^{2+}$ accumulations in the nucleus and associated endoplasmic reticulum (Williams et al., 1985; Williams, et al., 1988; Nicotera et al., 1989). The presence of $IP_3$ receptors has not yet been reported for the nuclear envelope of liver cells but is present in brain cerebellar Purkinje cell (Ross et al., 1989; Satoh et al., 1990). The chloride-selective channels reported here could provide a mechanism for charge balance during the electrogenic movement of $Ca^{2+}$ through the nuclear membrane. In this way, chloride-selective channels, together with the $Ca^{2+}$ transport mechanism, may participate in nuclear processes during the cell cycle. An alternative possibility to be considered is that these $Cl^-$ channels may participate in the traffic of negatively charged macromolecules, such as RNA, from the nucleus to the cytoplasm, as has been suggested for the $Cl^-$ channels on the inner membrane of mitochondria (Hurt & van Loon, 1986; Sorgato et al., 1987).

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