Detection of a large cation-selective channel in nuclear envelopes of avian erythrocytes

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To determine whether the nuclear envelope of eukaryotic cells has the capability to regulate ion fluxes, we have used the patch-clamp technique to detect ion channels in this membrane system. Since possible sites for ion channels in the nuclear envelope include not only the nuclear pores, but also both the inner and outer nuclear membranes, we have patched giant liposomes composed of phosphatidylcholine and nuclear envelope fragments isolated from mature avian erythrocytes. A large, cation-selective channel with a maximum conductance of approximately 800 pS in symmetrical 100 mM KCl was detected. This channel is a possible candidate for a nuclear pore.

Cation channel; Liposome; Nuclear envelope; Nuclear membrane; Nuclear pore; Patch clamp

1. INTRODUCTION

The nuclear envelope (NE) of eukaryotic cells is a complex structure comprising two lipid bilayers (the inner and outer nuclear membranes; INM and ONM, respectively), which are fused at the nuclear pores, through which nucleo-cytoplasmic transport of macromolecules is believed to occur [1,2] (Fig. 1). Although the nuclear pores are thought to be large aqueous channels which offer no resistance to the movement of inorganic ions and small molecules [1,2], experiments with Ca-sensitive [3] and potential-sensitive fluorescent dyes [4] suggest that this is not always the case. Consistent with these results, an electrical potential difference between the cytoplasm and nucleoplasm (nucleoplasm negative) has been measured in some cell types [5–8]. Therefore, even though the nuclear pores in certain cells (most notably oocytes [9,10]) appear to be freely permeable to ions, it cannot be assumed that they are permanently open in all cell types, or that their conductance properties are unaffected by different physiological conditions. In addition, it is possible – irrespective of ion flow through the nuclear pores – that the INM and ONM contain channels which control ion fluxes into and out of the perinuclear space [4], a compartment enclosed by these two membranes (Fig. 1A).

We report here the use of the patch-clamp technique to identify a large cation-selective channel in NEs of avian erythrocytes. This channel, which has a maximum conductance of approx. 800 pS, is possibly a nuclear pore.

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2. MATERIALS AND METHODS

The procedure of Jackson [11] was used to prepare nuclear envelopes (NEs) from mature erythrocytes obtained from fresh chicken or turkey blood. Giant liposomes were prepared from NEs and L-α-phosphatidylcholine (from soybean, Type II-S, Sigma, Munich) according to published procedures [12,13], using a phosphatidylcholine-to-NE protein ratio in the range of approx. 100:1. The liposomes were rehydrated in solution of 100 mM KCl, 0.1 mM $CaCl_2$ and 5 mM Hepes, pH 7.4.

Patch-clamp experiments were carried out according to standard techniques [14]. Unless otherwise noted in the figure legends, both the bath and pipet solutions contained: 100 mM KCl, 5 mM Tris-HCl, pH 7.4, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$. In some experiments, 100 mM KCl was substituted by 100 mM CsCl or 100 mM K-gluconate, respectively. Filled patch pipets had a resistance of approx. 5 MΩ. The electrical recordings were performed at room temperature with an EPC-7 amplifier (List-Electronic, Darmstadt). The stated potentials are those read from the amplifier, and refer to the pipet potential. The EPC-7 output signal was filtered at 2 kHz (8-pole Bessel filter, Frequency Devices Inc., Haverhill, MA, USA), and monitored on a storage oscilloscope (Telonik, Cologne). Data were stored first in digital form (Instrutech Corp., Elmont, NY, USA) on a video tape recorder. After filtering at 2000 kHz (8-pole Bessel filter) and digitizing at a sample rate of 20 kHz, data were analyzed using the pClamp program from AXON Instruments on a personal computer.

3. RESULTS

Because NE ion channels could conceivably be present at three different locations, and those in the INM might be inaccessible to the patch pipet in the nucleus-attached mode, we have used giant liposomes composed of phosphatidylcholine and NE fragments (NE-liposomes; Fig. 1). To obtain as homogeneous an

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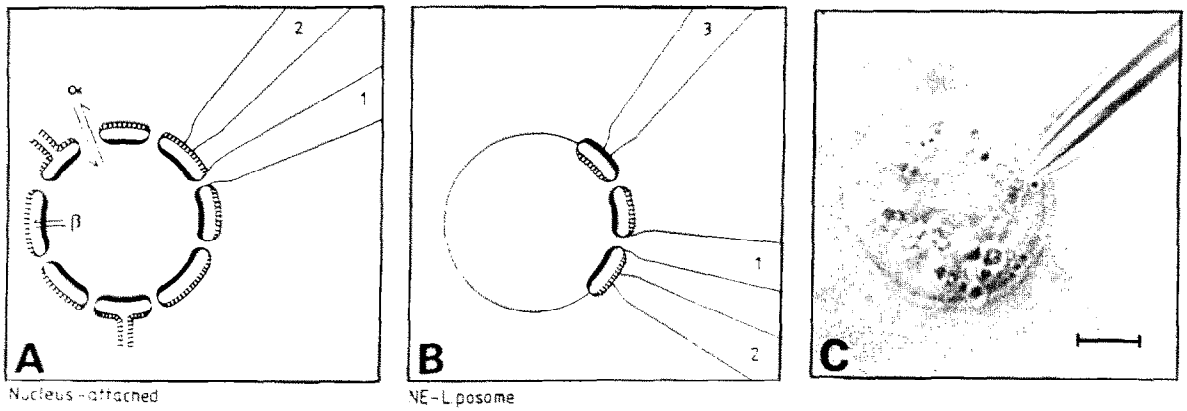

Fig. 1. Patch clamping of nuclear envelopes (NEs). In NEs, the inner nuclear membrane (INM; heavy black line) and outer nuclear membrane (ONM; hatched line) are joined at the nuclear pores, thus forming a compartment, the perinuclear space. As shown in this drawing, the ONM is often continuous with the endoplasmic reticulum (ER) in many cell types; this is not the case, however, in avian erythrocytes, which contain little or no ER. Two transport pathways at the nuclear periphery are (α) between cytoplasm and nucleoplasm, through the nuclear pores; and (β) between perinuclear space and nucleoplasm, across the INM. Two modes of patch clamping are possible: (A) nucleus-attached or (B) giant liposomes containing NE fragment. In (A), ONM (pipet 2) or nuclear pores (pipet 1) would be accessible to the patch pipet. Assuming that NE fragments can be incorporated into liposomes in either orientation (B), the INM (pipet 3) would also be accessible to the pipet. It is not possible at present to separate the INM from the ONM. (C) Light micrograph of a giant liposome with attached pipet. Scale bar represents 10 μM. The 800 pS channel was most frequently observed in such transparent liposomes with granular structures.

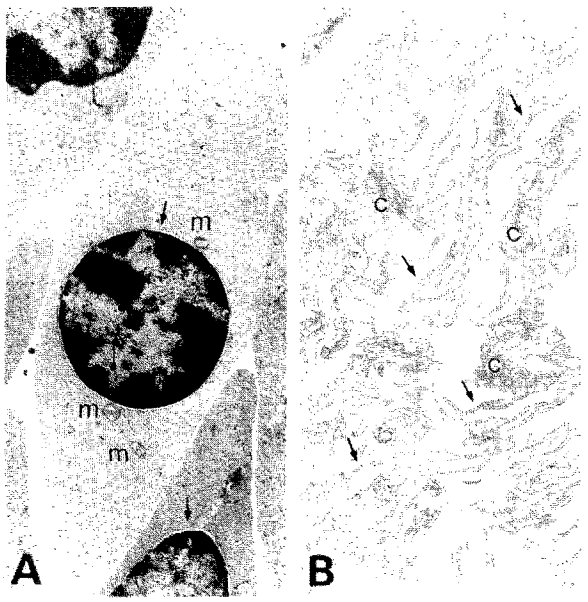

Fig. 2. Electron micrographs of mature chicken erythrocytes (A) and NEs isolated from these cells (B). (A) Note the absence of endomembranes, such as ER and Golgi vesicles, and the paucity of mitochondria (m). The ONM and perinuclear space (arrows) are clearly visible. The ONM is unbroken, again demonstrating the lack of ER membranes, with which the ONM is continuous in most other cell types. The nuclei in these cells are too small to patch directly. In (B), a double membrane system (arrows) and residual chromatin (c), which are characteristic of NEs [11,19] are apparent. Although there is no unique enzyme marker for NEs, the lack of both ouabain-sensitive ATPase activity and monoamine oxidase activity (diagnostic for plasma membrane and mitochondria, respectively) demonstrated the absence of these two possible sources of contamination (data not shown). The nuclear pore density in these NEs is approximately 3/μm^2 [18]. Magnification in (A), 7500 ×; in (B) 11000 ×.

envelope preparation as possible, NEs were isolated from avian erythrocytes, since these cells contain very few mitochondria and are deficient in other endomembranes, such as endoplasmic reticulum and Golgi vesicles (Fig. 2).

Fig. 3 shows current traces obtained during patch-clamp experiments using symmetrical 100 mM KCl. A large channel with a conductance of approx. 800 pS was observed at pipet potentials between −30 and +30 mV. This channel was mostly in the open configuration at these lower potentials, with only infrequent, brief closures. In contrast, at potentials ranging from +40 to +70 mV and −40 to −70 mV, increased activity and smaller openings predominated. These smaller openings were generally in multiples of approx. 50 pS (Fig. 4), with conductances of 400–600 pS frequently observed. The maximum conductance of 800 pS was rarely seen at these higher potentials, although it reappeared as the primary event when the potential was returned to lower values.

The NE channel conducted primarily cations, as indicated by a reversal potential of −15.7 mV (pipet potential) with a bath solution of 100 mM KCl and a pipet solution of 200 mM KCl (Fig. 5). Since the behavior of the channel was similar using either CsCl or K-gluconate (data not shown), the channel was not specific for K^+, but was probably a general cation channel.

4. DISCUSSION

We report in this paper the use of the patch clamp technique to identify a large, cation-selective channel in

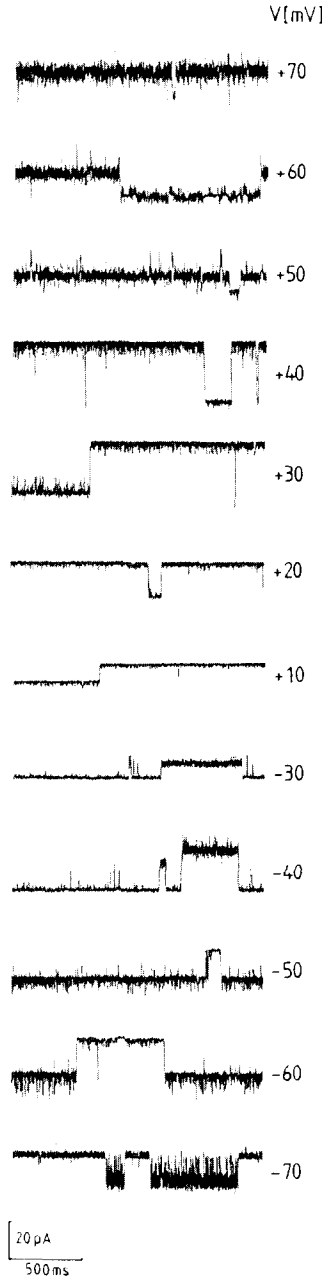

Fig. 3. Current traces of the NE channel. Measurements on NE-liposomes were made in symmetrical 100 mM KCl. These traces are examples obtained from a single liposome. The large channel (with a maximum conductance of approx. 800 pS, shown here at pipet potentials of 10–30 mV) was observed in approx. 25% of liposomes for which a gigaohm seal was successfully obtained. At potentials higher than ± 40 mV, increased activity and intermediate stages of conductance were observed.

liposomes containing NEs isolated from avian erythrocytes. At potentials between −30 and +30 mV, this channel was mostly open in the maximum conducting state of approx. 800 pS; at higher potentials, intermediate states of conductance, in multiples of approx. 50 pS, were observed. These results can be interpreted as reflecting the existence of a large channel

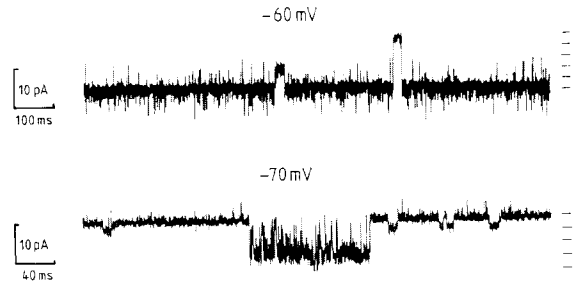

Fig. 4. Intermediate conductance stages of the NE channel. These stages were in multiples of approx. 50 pS (distance between bars at the right), and were observed at higher potentials (examples shown at −60 and −70 mV).

composed of a number of subunits which can open and close either individually or cooperatively. At low potentials, cooperative opening of the subunits predominates, resulting in openings with the maximum conductance. At higher potentials, the subunits are more active, but do not open cooperatively: intermediate states, with openings of 50–100 pS within a maximum range of 800 pS, are most frequently observed. A channel with these characteristics has not been described previously from any other type of biological membrane, including the only two possible sources of contamination in our NE preparation: plasma membrane [15] and mitochondria [16].

Only one other study so far has used the patch clamp technique to address the possibility that ion channels exist in the NE. Mazzanti et al. [8] have identified, using the nucleus-attached mode (Fig. 1A), a K^+-selective channel in mouse pronuclei. The maximum conductance of this channel was 200 pS, and thus smaller than

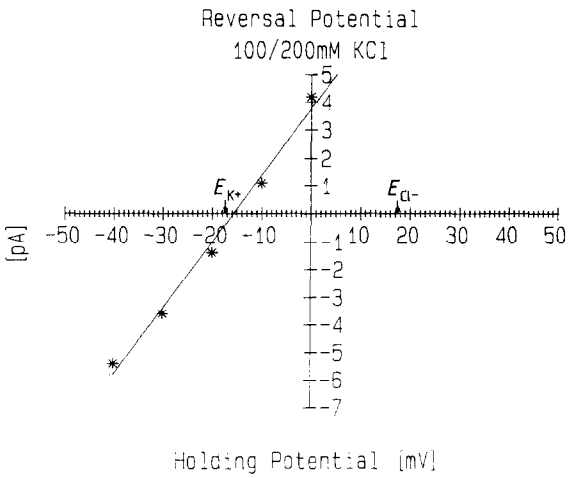

Fig. 5. Ion selectivity of the NE channel as determined by reversal potential. The bath and pipet solutions contained 100 mM KCl and 200 mM KCl, respectively. As shown, the reversal potential was −15.7 mV, which is close to the expected reversal potential of −17.45 mV, calculated from the Nernst equation for K^+ under these conditions.

the one we have observed. However, they also detected smaller conductance states, the most common being 55 pS; this is close to the value of approx. 50 pS which was the smallest opening we detected. Because of differences in the sources of NEs and the patch clamp modes used, though, we cannot conclude that the channel observed in mouse pronuclei by Mazzanti and coworkers is the same as the one we have detected in avian erythrocyte NEs. However, since the NE could contain different channels in various sites (Fig. 1), and species or cell-type differences in NE channel properties might be expected, any results showing ion channel activity in this membrane system merit further consideration.

We cannot distinguish at this time whether the channel we have observed is a nuclear pore or another channel in either the INM or ONM. However, the conductance we observed (800 pS) is similar to that expected (1000 pS) for a channel having the estimated dimensions of a nuclear pore (diameter of 9 nm, length of 80 nm, containing a medium of 100 $\Omega \cdot$cm) [8]. Furthermore, nuclear pores are believed to be composed of 8 subunits in an octagonal array [17], a structure which would be consistent with our data suggesting the presence of 8–16 substates (50–100 pS each) of the 800 pS opening. Each of the 8 subunits of the nuclear pore could operate independently or cooperatively, depending on the voltage, thus resulting in a number of substates of activity such as we have observed.

Such versatility in conductance properties could be essential for a complex structure such as the NE, which must regulate the nucleo-cytoplasmic traffic of not only macromolecules (RNA and proteins), but also smaller molecules which may be required as substrates or regulators for nuclear functions. In addition, if the NE controls ion fluxes at the nuclear periphery, this in turn could affect the structure, interactions and activities of macromolecules in this region of the nucleus. Since specific DNA sequences have been found associated with nuclear membranes [18,19], the functional consequences of this relationship must be considered in view of the polyionic nature of nucleic acids and proteins [20–22], and the probable dynamic electrical properties of the nuclear membranes.

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