Volume 302, number 1, 81–85 FEBS 10997 May 1992

A large conductance ion channel in the nuclear envelope of a higher plant cell

A.J.M. Matzke, C. Behensky, T. Weiger and M.A. Matzke

Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, A-5020 Salzburg, Austria

Received 10 March 1992

To detect and characterize ion channel activity in the nuclear envelope of a higher plant cell, we performed patch clamp experiments on nuclei isolated from coconut endosperm cells and on giant liposomes containing nuclear envelope fragments prepared from the same cells. An ion channel exhibiting a number of conductance substates, with a maximum of ca. 1,000 pS, was observed. Above an applied potential of ± 100 mV, the behavior of the channel was similar in isolated nuclei and liposomes, indicating that both patch clamp modes were detecting the same channel. That such a channel has now been identified in members of both the animal and plant kingdoms reinforces the notion that the nuclear pores are not always open to ions.

Coconut endosperm; Ion channel; Nuclear envelope; Nuclear membrane; Nuclear pore; Patch clamp

1. INTRODUCTION

Despite several early studies with microelectrodes demonstrating the presence of an electrical potential across the nuclear envelope (NE) of several cell types [1–4], a variety of non-electrophysiological investigations have since led to wide acceptance of the view that the nuclear pores are large aqueous channels (open diameter ca. 9 nm) which present no barrier to the passive diffusion of inorganic ions and small molecules with a molecular mass less than ca. 5 kDa [5,6]. Consequently, attention has been focussed on determining the mechanisms by which the nuclear pores mediate the efficient transport of macromolecules into and out of the nucleus [7,8]. However, recent electrophysiological studies using the patch clamp technique on nuclei and NEs of animal cells have revealed large conductance ion channels, which might be the same as the nuclear pores [9–11]. These results, and others obtained using Ca^{2+}- sensitive [12] or potential-sensitive [13] fluorescent dyes, have reopened the discussion on the ion permeability of the NE [14]. We show in this report that the NE of coconut endosperm cells also contains a large ion-conducting channel, thus demonstrating the general occurrence of such a channel in the NE of both higher plants and animals.

2. MATERIALS AND METHODS

For experiments using the nucleus-attached mode, nuclei were isolated rapidly without homogenization from the gelatinous endosperm of immature coconuts (18 × 15 cm) (supplied by Lever Solomons Ltd., Solomon Islands) by simply pouring a mixture of endosperm from 1 coconut and buffer (usually coconut water) through a 120 μm Nybolt sieve, followed by centrifugation at 250 × *g* to sediment the nuclei. The nuclear pellet was resuspended in the same buffer and kept in a tube on ice until patch clamp experiments were performed, which was always within 4 h after isolation. To isolate nuclear envelopes (NEs), the procedure was scaled up by grinding gently with a Dounce homogenizer up to 30 g of endosperm from 3–5 coconuts. Nuclei were collected on Percoll gradients as described previously [15]. Isolated nuclei were quick frozen in a 1:1 mixture of glycerol and TKMS buffer (0.1 M sucrose, 50 mM Tris–HCl, 25 mM KCl, 5 mM $MgCl_2$, pH 7.5) and stored at −80°C until isolation of NEs. This was done essentially according to Jackson [16], except that the NaCl treatment was eliminated. Isolated NEs were resuspended in 10 mM Tris–HCl, 0.1 mM $MgCl_2$, pH 7.5, then quick-frozen in liquid N_2 and stored at −80°C until use. Giant liposomes containing NE fragments were prepared according to the procedure of Criado and Keller [17]. Electron microscopy and freeze fracture were done using standard techniques [18, 19].

Patch clamp experiments were carried out at room temperature as described previously [10]. The compositions of bath and pipet solutions are indicated in the figure legends. Filled patch pipets had resistances of ca. 5 MΩ. The output signal from the EPC-7 amplifier (List-Electronic, Darmstadt) was filtered at 2 kHz with an 8-pole Bessel filter (Frequency Devices Inc., Haverhill, MA, USA). The digital sampling frequency was 20 kHz. Data were analyzed using the pClamp program from Axon Instruments on a personal computer. Current amplitude histograms were derived from all points amplitude histograms.

3. RESULTS AND DISCUSSION

To investigate the presence and properties of ion channels in the endosperm nuclei, several patch clamp configurations were used (Fig. 1C,F). When a steady voltage of ca. ± 100 mV or higher was applied in the nucleus-attached mode (Fig. 1C), it was possible to observe ion channel activity, which was characterized by large, infrequent openings encompassing a range of

Correspondence address: A.J.M. Matzke, Institute of Molecular Biology, Austrian Academy of Sciences, Billrothstrasse 11, A-5020 Salzburg, Austria. Fax: (43) (662) 24961 29.

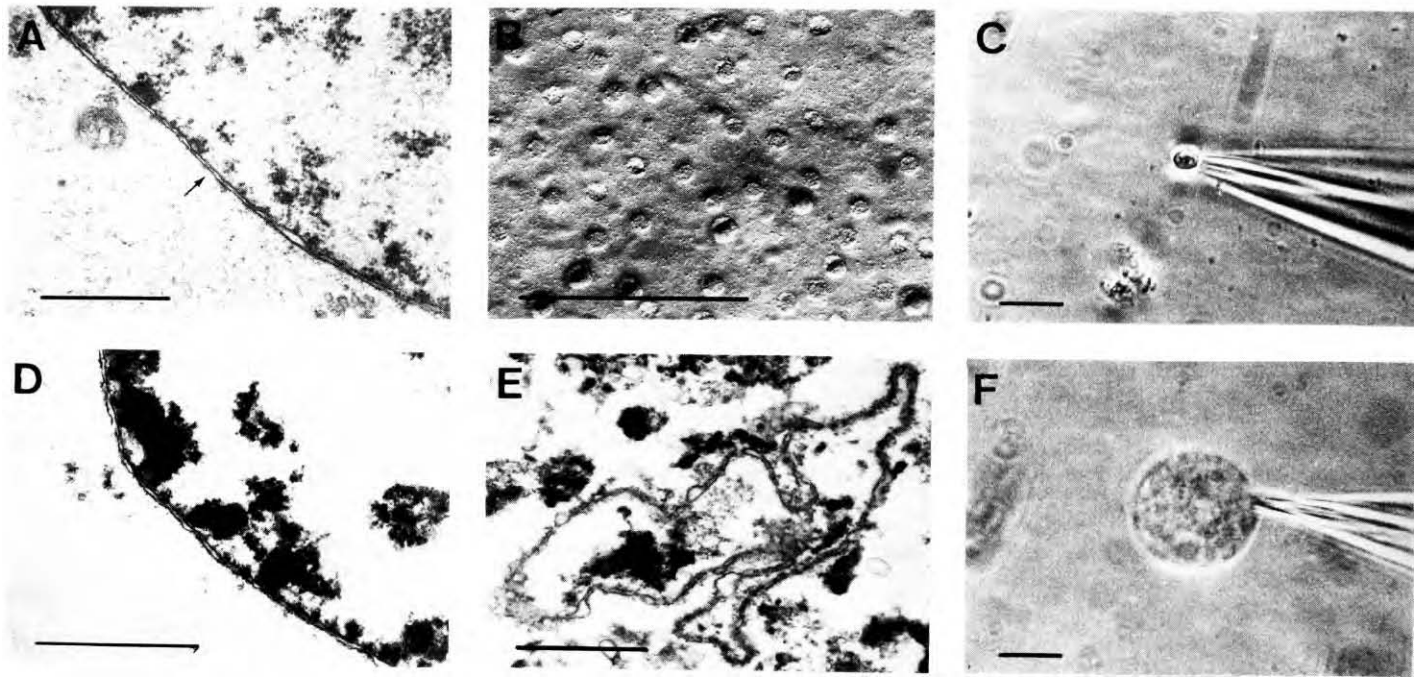

Fig. 1. Description of nuclei and NEs used in this study. As a source of plant nuclei, we chose a relatively soft, non-photosynthetic tissue: the gelatinous endosperm of immature coconuts [25,26], since the use of harsh grinding methods and nonionic detergents (which must be used with other plant tissues to break cell walls and to solubilize chloroplasts, respectively) could be avoided. The endosperm cells also contain few endomembranes which could contaminate the NE. (A) Electron microscopic appearance of the double membrane system, which is characteristic of the NE, in an unbroken coconut endosperm cell. Note that the outer nuclear membrane is intact (arrow), because of the paucity of endoplasmic reticulum, which is often continuous with the outer nuclear membrane in other cell types. Bar=1 μm. (B) Freeze-fracture of the NE of an endosperm cell showing that the density of nuclear pores is 8–10 pores/μm^2. The external tip diameter of a fire-polished patch pipet was ca. 1 μm, which should cover an approximate surface area of 0.79 μm^2 containing ca. 7 pores. This is a minimum estimate; from capacitance measurements of plasma membranes [27], it has been determined that a patch pipet 1–2 μm in diameter will enclose a membrane surface of 5–6 μm^2. However, it is not clear whether this value applies to nuclear membranes. Bar=1 μm. (C) Patch clamping of an isolated endosperm nucleus (nucleus-attached mode). The patch pipet was placed directly on the surface of isolated nuclei. The endosperm nuclei varied in size, ranging from 3 to 10 μm in diameter. In our experiments, smaller nuclei (3–5 μm in diameter) provided the most reliable results. That GΩ seals could be obtained at all with these nuclei indicated that the nuclear pores were not freely permeable to ions. The patched regions could also be detached and, if the seal remained stable, measurements continued. Unlike the nucleus-attached mode, using detached patches allowed the calculation of conductances, since the concentrations of conductiong ions in both the pipet and bathing solutions were known. Bar=10 μm. (D) Electron microscopic appearance of the double membrane system of an isolated endosperm nucleus. Note the relatively intact membranes, and the lack of contaminating cytoplasmic material and plasma membrane. Bar=1 μm. (E) Example of an isolated nuclear envelope. Such intact double membranes, along with membrane fragments and residual chromatin remained following DNase treatment of isolated endosperm nuclei. Bar=1 μm. (F) Patch-clamping of a giant liposome composed of phosphatidyl choline and isolated nuclear envelope fragments (NE-liposome) (part E). Bar=10 μm.

conductance states (Fig. 2A–C). These probably represent the activity of one or only a few channels, since at least 500 ms of inactivity often preceded and followed the events, and it is unlikely that many single channels would open and close so synchronously at such infrequent intervals [20,21]. The conductance limits of the various substates or channels were not strictly defined, a feature which has been found for other large channels, such as the giant channel in the inner mitochondrial membrane [21], and in the cell membrane of a Gram-positive bacterium [22]. Since the ion concentrations in the nucleoplasm of the endosperm nuclei were not known, only approximate conductances could be calculated. Assuming, however, that the K^+ concentration in the nucleoplasm was the same as in the patch pipet and bath (100–200 mM), the estimated maximal conductance of a single pore was ca. 1,000 pS (Fig. 2A,C). This value was confirmed with detached nuclear patches, in which maximum conductances of ca. 1,000 p*S* were also detected (Fig. 2B).

In contrast to isolated nuclei, giant liposomes prepared with NE fragments (Fig. 1E) contained an activity which could be detected at lower potentials ranging between ca. −30 and +60 mV. The maximum conductance was again ca. 1,000 pS in symmetrical 100 mM KCl. Substates were visible, the most common was about half the maximum (ca. 450 p*S*) but ones as small as 50 and 100 pS were also seen (Fig. 3D,E). In general, at low potentials (± 10 mV) the channel was mostly open at higher conductance levels (Fig. 3A,B). Increasing the potential to ± 20–30 mV resulted in more frequent closures, and smaller or intermediate conduct-

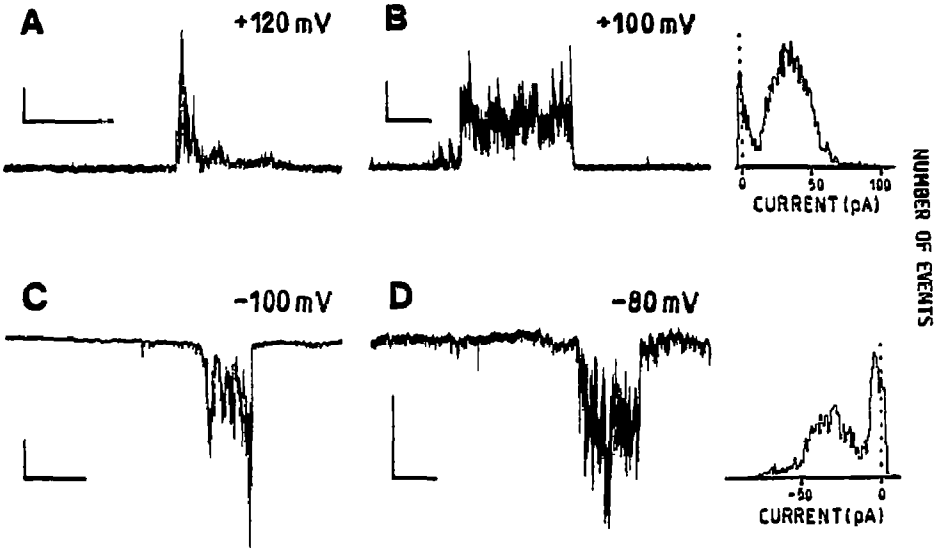

Fig. 2. Current traces from endosperm nuclei. For measurements in the nucleus-attached mode a total of 548 nuclei were tested. For 25%, a GΩ seal was obtained, and in 15%, this seal was sufficiently stable to obtain data at a range of potentials. In this mode, and also with detached nuclear patches, activity was routinely seen only at higher potentials (usually at least ± 100 mV). In general, this activity was infrequent and had a spiky appearance. V_p=pipet voltage. Bars: 30 pA, 100 ms. In the current amplitude histograms shown at the right of trace (B) and (D), zero current is designated by the dotted vertical line. (A) Nucleus-attached: V_p=+120 mV; maximum opening ca. 850 pS. (B) Detached nuclear patch: V_p=+100 mV; maximum openings of ca. 975 pS. The current amplitude histogram was plotted from points taken during 300 ms comprising the major event. Conductance substates in intervals of 50–100 pS are apparent; the predominant conductance was ca. 400 pS. Bin width 1.11; 12 events/division. (C) Nucleus-attached: V_p=−100 mV; maximum openings of 950 pS and 1680 pS. This patch probably contains at least 2 pores which have opened simultaneously, producing the larger opening, which approaches twice the estimated maximum single channel conductance (1,000 pS). (D) NE-liposome: V_p=−80 mV. 980 pS opening. The current amplitude histogram was plotted from points taken during the 200 ms which included the major event. The predominant conductances were ca. 50 pS and 400 pS. Bin width 0.52; 10 events/division. The pipet and bath solutions in part (A) were 200 mM KCl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Tris–HCl, pH 7.4, and in parts (B) and (C), 100 mM KCl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM MES, pH 4.9. Similar results were obtained with the two different buffer systems used (MES, pH 4.9 and Tris, pH 7.4). The MES buffer was tested because it had the same pH as the 'water' which bathed the endosperm tissue in unbroken coconuts. In part (D), the internal solution of the liposomes was 100 mM KCl, 0.1 mM $CaCl_2$, 5 mM HEPES buffer, pH 7.4. The bath and pipet solutions contained 100 mM KCl, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Tris–HCl, pH 7.4.

ance substates were observed more often (Fig. 3C–E). The channel often became inactive at negative holding potentials ranging from −30 to −50 mV (Fig. 3E). If the voltage was then raised above ± 60 mV, a restoration of activity, which again resembled that seen in the nucleus-attached configuration (Fig. 2D), was obtained. This suggested that the same channel was measured in both modes. The activity seen at lower potentials in liposomes, but not nuclei, may reflect the removal constraints which influence the channel in an intact nucleus.

We attempted to study the ion selectivity of the NE channel by determining the reversal potential in asymmetrical KCl, although the abundance of current magnitudes complicated the analysis of current/voltage relationships for single conductances [21]. The reversal potential was ca. −5 mV, suggesting that at lower conductance states, the channel was slightly cation selective. Accordingly, the activity was undiminished in CsCl, NaCl and K-gluconate (data not shown). In the fully open state (ca. 1,000 pS), the channel probably behaves as a general diffusion pore, which is consistent with previous results [5,6].

Other patch clamp investigations on NEs of animal cells have shown that in several types of murine nuclei, K^+-selective channels were present. Those channels were smaller than the ones we have observed, being only 200 pS or approximate multiples thereof [9, 11]. The NE channel described in this paper resembles most closely that previously detected in giant liposomes containing NE fragments of chicken erythrocytes, which had a maximal conductance of ca. 800 pS, comprising substates of 50–100 pS [10]. These maximum conductance values approach that expected (1000 pS) for a channel with the dimensions of a nuclear pore (diameter 9 nm, length 80 nm, containing a medium of 100 Ω·cm) [9].

The behavioral similarities of the NE channel and another eukaryotic channel, the megachannel (maximum conductance ca. 1.3 nS in symmetrical 150 mM KCl) found in the inner mitochondrial membrane [22, 23] are also striking. Interestingly, the latter is also possibly involved in the transport of macromolecules (into and out of mitochondria), and to be situated at the sites of contact between the inner and outer mitochondrial membranes [22].

In summary, an increasing number of electrophysiological studies indicate that the nuclear pores can adopt

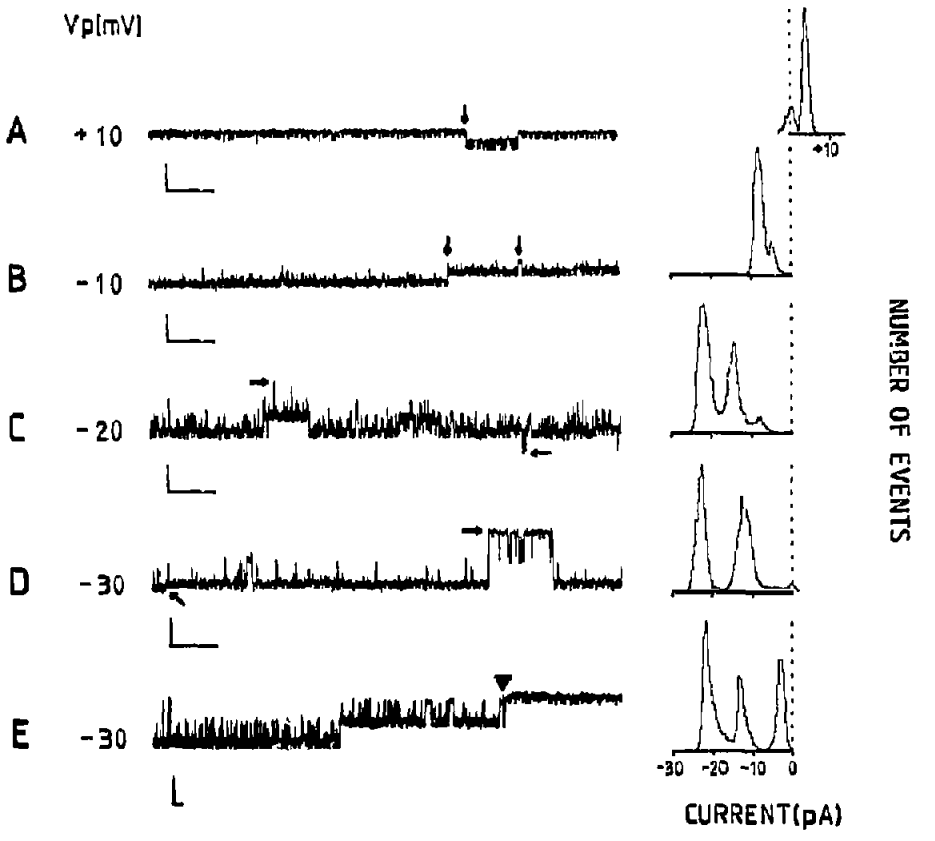

Fig. 3. Typical current records from giant liposomes containing NE fragments (NE-liposomes). A total of 118 liposomes were tested, 44% of which formed stable GΩ seals. Activity similar to that displayed was observed in 22 liposomes (ca. 18%) at potentials between −30 and +60 mV. At potentials above ± 60 mV, 17 showed in addition the spiky activity characteristic of the nucleus-attached mode (Fig. 2D). This indicated that the same channel could exhibit both types of behavior. Traces in A–D and in Fig. 2D, are from the same liposome. Bars: 10 pA, 70 ms. Current amplitude histograms are shown to the right of each trace. These histograms are derived from current records (which contained the representative traces shown) lasting ca. 5.5 s, except for the trace in (A), which was obtained from 350 mS of current trace including the event shown. The traces show examples of maximum conductances; from the current amplitude histograms, we have calculated the most frequent conductances (corresponding to different substates) obtained over the period sampled. Zero current is indicated by the dotted vertical line. The bin width was 0.4 in all cases. The pipet, bath and internal liposome solutions were the same as in Fig. 2D. (A) V_p=+10 mV. ca. 900 pS maximum closure (arrow) and subsequent opening. Major conductance: 460 pS. 73 events/division. (B) V_p=−10 mV. Arrows: two closures, ca. 480 pS each (960 pS total). Major conductances: 520 pS, 810 pS. 900 events/division. (C) V_p=−20 mV. 1,180 pS maximum conductance (current measured between arrows). In the trace shown, the channel is mostly in an intermediate state of conductance. Major conductances: 1,180 pS, 800 pS, 460 pS. 440 events/division. (D) V_p=−30 mV. Arrows: ca. 50 pS closure; ca. 900 pS closure with subsequent ca. 450 pS flickerings. Major conductances: 800 pS, 450 pS. 490 events/division. (E) Inactivation of the NE channel in liposomes frequently occurred after the applied voltage was adjusted to between −30 and −50 mV. This trace (V_p=−30 mV) illustrates this behavior. Following ca. 1 min of relatively continuous activity, including closures of ca. 870 pS and ca. 460 pS, the channel became progressively less active, then closed (arrowhead). Reactivation could take place if the potential was increased up to at least ± 60 mV, but usually ± 100 mV was required. The activity then resembled that seen in the nucleus-attached mode (see Fig. 2D; compare with Fig. 2A–C). Major conductances: 100 pS, 450 pS, 750 pS. 500 events/division.

a variety of conductance states, and can be closed to ions. Therefore, it will be important to determine factors which control nuclear transport of both large and small molecules in vivo, as well as the physiological consequences of ion gradients across the nuclear envelope. Possibilities include the balance of charges during nucleo-cytoplasmic transport of macromolecules [9,11], and the regulation of ion concentrations in the nucleoplasm, which could be a factor influencing the conformations and interactions of macromolecules in the nucleus [3,4,24].

Acknowledgements: We thank the Austrian Fonds zur Förderung der wissenschaftlichen Forschung for financial support, R. Hedrich for advice and help with preliminary experiments, and A. Trewavas and A. Slabas for helpful discussions.

REFERENCES

[1] Loewenstein, W.R. and Kanno, Y.J. (1963) J. Gen. Physiol. 46, 1125–1140.
[2] Wiener, J., Spiro, D. and Loewenstein, W.R. (1965) J. Cell Biol. 27, 107–117.
[3] Kroeger, H. and Lezzi, M. (1965) Annu. Rev. Entomol. 11, 1–22.

[4] Guilian, D. and Diacumakos, E.G. (1977) J. Cell Biol. 72, 86–103.
[5] Paine, P.L. and Horowitz, S.B. (1980) in: Cell Biology: A Comprehensive Treatise (Prescott, D.M. and Goldstein, L., eds.) Vol. 4, Academic Press, New York.
[6] Gerace, L. and Burke, B. (1988) Annu. Rev. Cell Biol. 4, 335–374.
[7] Dingwall, C. and Laskey, R. (1986) Annu. Rev. Cell Biol. 2, 367–390.
[8] Silver, P.A. (1991) Cell 64, 489–497.
[9] Mazzanti, M., DeFelice, L.J., Cohen, J. and Malter, H. (1990) Nature 343, 764–767.
[10] Matzke, A.J.M., Weiger, T.M. and Matzke, M.A. (1990) FEBS Lett. 271, 161–164.
[11] Mazzanti, M., DeFelice, L.J. and Smith, E.F. (1991) J. Memb. Biol. 121, 189–198.
[12] Williams, D.A., Fogarty, K.E., Tsien, R.Y. and Fay, F.S. (1985) Nature 318, 558–561.
[13] Matzke, M.A., Matzke, A.J.M. and Neuhaus, G. (1988) Plant, Cell Environ. 11, 157–163.
[14] Dingwall, C. (1991) BioEssays 13, 213–218.
[15] Willmitzer, L. and Wagner, K.G. (1981) Exp. Cell Res. 135, 69–77.
[16] Jackson, R.C. (1976) Biochemistry 15, 5641–5651.
[17] Criado, M. and Keller, B.U. (1987) FEBS Lett. 224, 172–176.
[18] Sabatini, D.D., Bensch, K. and Barnett, R.J. (1963) J. Cell Biol. 17, 19–58.
[19] Plattner, H. and Zingsheim, H.P. (1987) Elektronmikroskopische Methodik in der Zell- und Molekularbiologie, Gustav Fischer, Stuttgart.
[20] Fox, J.A. (1987) J. Memb. Biol. 97, 1–8.
[21] Petronilli, V., Szabò, I. and Zoratti, M. (1989) FEBS Lett. 259, 137–143.
[22] Zoratti, M. and Petronilli, V. (1988) FEBS Lett. 240, 105–109.
[23] Szabò, I. and Zoratti, M. (1991) J. Biol. Chem. 266, 1–4.
[24] Matzke, A.J.M. and Matzke, M.A. (1991) Bioelectrochemistry and Bioenergetics 25, 357–370.
[25] Cutter, V.M. and Freeman, B. (1954) Nature 173, 827–828.
[26] Cutter, V.M., Wilson, K.S. and Freeman, B. (1955) Am. J. Botany 42, 109–115.
[27] Mazzanti, M. and DeFelice, L.J. (1987) Biophys. J. 52, 95–100.