Journal of Bioenergetics and Biomembranes, Vol. 24, No. 1, 1992

Original Article

Ionic Channels in the Plasma Membrane of *Schizosaccharomyces pombe*: Evidence from Patch-Clamp Measurements

Vladimír Vacata,[1,2] Milan Höfer,[1] H. Peter Larsson[2] and Harold Lecar[2]

Received October 1, 1992

Patch-clamp studies of the yeast *Schizosaccharomyces pombe* reveal that the plasma membrane contains a voltage-gated channel mildly selective for potassium over sodium, lithium, and chloride. The channel exhibits several conductances with a maximum of 153 pS. The channel gates in the region of physiologically relevant voltages, being closed at hyperpolarizing and open at depolarizing voltages. It is not inhibited by tetraethylammonium, quinine, or quinidine applied from the cytoplasmic side of the membrane; similarly, ATP and stretch have no effect. The frequency of its occurrence in patches implies that about 35 channels of this kind are present in the plasma membrane of a single cell.

KEY WORDS: Plasma membrane channels; channel gating; multilevel conductance; ionic selectivity; density of channels; *Schizosaccharomyces pombe*.

INTRODUCTION

The uptake of nutrients in yeast is driven by an electrochemical proton gradient, $\Delta\tilde{\mu}_{H^+}$, across the plasma membrane (Eddy, 1978, 1982), and the H^+ gradient, in turn, is generated by the plasma membrane-bound H^+-ATPase (Goffeau and Slayman, 1981; Bowman and Bowman, 1986; Goffeau and Boutry, 1986; Serrano, 1988; Goffeau *et al.*, 1989). Both processes are electrogenic and, on the scale of a whole cell, are balanced electrically by an opposing potassium current, presumably through ionic channels (Hauer and Höfer, 1982; Höfer, 1989). Using the patch-clamp technique (Hamill *et al.*, 1981) these channels can be identified and characterized.

Studies with yeast cells offer a model of higher plant cells with several advantages: rapid growth, homogeneity of cell population, and a suitability to genetic manipulation. The fission yeast *Schizosaccharomyces pombe*, strain 972h$^-$, was shown by Höfer and Nassar (1987) to take up D-glucose and non-metabolizable analogues, 2-DOG[3] and glucosamine, in symport with protons. Dufour and Goffeau (1980) isolated and purified the plasma membrane ATPase of this yeast and characterized its molecular and catalytic properties. Only little information, however, exists as to the molecular pathway of the counterbalancing potassium current.

The first direct observation of yeast plasma membrane channels came from patch clamp studies on the strain *Saccharomyces cerevisiae*. Gustin *et al.* (1986) reported a potassium channel with unit conductance of 20 pS susceptible to inhibition by TEA and exhibiting bursting kinetics. This potassium channel may be identical with the 17 pS potassium channel in the same

[1]Botanisches Institut der Universität Bonn, Kirschallee 1, 5300 Bonn 1, Germany.
[2]Division of Biophysics and Cell Physiology, University of California, Berkeley, California 94720.

[3]Abbreviations: BSA, bovine serum albumin; 2-DOG, 2-deoxy-D-glucose; DCCD, *N,N'*-dicyclohexylcarbodiimide; DTT, dithiothreitol; EGTA, ethyleneglycol-bis-(β-aminoethylether)-*N,N,N'N'*-tetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MES, 2(*N*-morpholino)ethanesulfonic acid; TEA, tetraethylammonium.

0145-479X/93/0100-0043$07.00/0

yeast reported by Ramirez *et al.* (1989). The latter exhibited similar gating behavior; however, in a plasma membrane H^+-ATPase mutant of the same yeast strain it displayed certain characteristics identified with plasma membrane H^+-ATPases: activation by ATP and inhibition by the H^+-ATPase-selective blocker DCCD. A later study by Gustin *et al.* (1988) showed that the plasma membrane of the wild-type harbors also a mechanosensitive channel permeable in various degrees to both cations and anions.

To study the ionic channels of the plasma membrane of the yeast *S. pombe*, we used the patch-clamp technique (Hamill *et al.*, 1981). A preliminary report on the results has appeared (Vacata *et al.*, 1990).

MATERIALS AND METHODS

Cultivation of Cells

Schizosaccharomyces pombe strain $972h^-$ (NCYC 1824) was kept on 2% agar slants containing 5.8% glucose and 2% yeast extract in a refrigerator, and reinoculated every 4 to 6 weeks. Yeast was cultivated according to a modified method described earlier (Höfer and Nassar, 1987). The cells were grown aerobically at 30°C on a gyratory shaker in a medium of the same composition. The pH was adjusted to 4.5 with HCl. A culture 1–4 days old was used to inoculate 50 ml fresh medium in a culture flask of 250 ml capacity. Cells were harvested after 19 h and washed three times in 30 ml distilled water by centrifugation in a J-2/C Beckman centrifuge. JA20 rotor, at 4000 *g* (6000 rpm) for 15 sec, and the pellet resuspended.

Preparation of Protoplasts

Protoplasts were prepared according to the modified method of Dickinson and Isenberg (1982). The fresh cell suspension was centrifuged at 4000 *g* for 15 sec., 250-mg portion of the cell pellet was transferred into 5 ml of freshly prepared cell pretreatment medium (CTM—100 mM Na_2EDTA, 100 mM MES and 40 mM DTT, pH 5.6) and incubated at 32°C for 30–40 min in a water bath. Cells were harvested by centrifugation at 4000 *g* (5500 rpm) for 15 s. They were then washed in 5 ml cell wash medium (CWM—300 mM KCl and 20 mM MES, pH 5.6), centrifuged again, and resuspended in 5 ml of freshly prepared protoplast forming medium (PFM—600 mM KCl, 20 mM K_2HPO_4/citric acid, pH 5.6, shortly before use, 20 mM DTT, 10 mM BSA, and 10 mg/ml NovoZym 234). The cells were then incubated for 70 min at 30°C in a water bath. Then 6 ml of ice-cold 1 M protoplast medium (1 M PM—1.0 M sorbitol, 10 mM KCl, 60 mM 2-DOG, 11 mM EGTA, 10 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES/KOH, pH 7.6) was added to the cell suspension, and the cultivation flask was placed on ice.

The treated cells were then observed under a microscope. If no protoplasts formed, cells were returned to the water bath and incubated another 30 min. When protoplasts formed, cells were washed three times by centrifugation at 1500 *g* (3000 rpm) for 1.5–2 min and resuspended in 3 or 9 ml (the last wash to improve protoplast separation from cell debris) or 0.75 M protoplast medium (0.75 M PM—0.75 M sorbitol, 140 mM KCl, 60 mM 2-DOG, 11 mM EGTA, 10 mM $MgCl_2$, 1 mM $CaCl_2$, and 10 mM HEPES/KOH, pH 7.6). After the third wash, the cells were resuspended in 3 ml of 0.75 M PM, placed on ice, and observed under a microscope. When protoplasts were abundant, we used 1.00 Osm patch-clamp medium; when protoplasts were scarce, we used 0.95 Osm patch-clamp medium (see below). The protoplast media contained 2-DOG to prevent cell wall regeneration.

Patch-Clamp Measurements

Patch micropipettes of resistances 4–8 MΩ were pulled in two stages from soft glass capillaries (Clay Adams) and backfilled. Protoplasts were transferred from 1 M PM medium into a patch-clamp medium (140 mM XY, XY being the salt of interest, 11 mM EGTA, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES/Tris, and 591–841 mM sorbitol, pH 7.2, 0.90–1.15 Osm) of appropriate osmolarity (see above). Protoplasts suitable for patch clamping had a diameter of 6–10 μm and were distinguished from spheroplasts by a low-contrast boundary. Seals with resistances of 1–15 GΩ formed within 0–5 min after touching the cells with the micropipette and applying suction of 0–50 mm Hg. After establishing a gigaohm seal, excised inside-out patches were obtained by shearing the cell away from the micropipette tip with a jet of liquid. All patch-clamp measurements were done in the inside-out mode, i.e., with the outer side of the membrane facing the inside of the micropipette.[4] The transmembrane currents were amplified by a List L/M

[4] A note on voltage polarity: In the inside-out mode used throughout the work, the voltage we refer to is that inside the electrode which, with respect to the orientation of the patch, represents that of the external medium of the cell. To translate the recording potentials reported throughout this paper into *in vivo* cell potentials, the sign must be changed.

EPC-7 amplifier, filtered usually at 800 Hz through an 8-pole Bessel filter (Frequency Devices, Model 902LPF), and fed through a TL-1 Interface (Axon Instruments) to an IBM-AT computer. The experiment was either controlled through a pCLAMP program (Axon Instruments), or the voltages were controlled manually while the signal was continuously recorded on a computer hard disk using an AxoTape program (Axon Instruments). Data were analyzed using AxoTape, pCLAMP, and StatGraphics (Statistical Graphics Corporation) software, and plotted on a Hewlett-Packard LaserJet III printer.

Chemicals

NovoZym 324 was purchased from NOVO INDUSTRI (Novo Biolabs, Bagsværd, Denmark), yeast extract from DIFCO, and 2-DOG and the rest of the chemicals of analytical grade from SIGMA.

RESULTS

Patch-Clamping of Yeasts

In the patch-clamp method, the tip of a glass micropipette is pressed against the cell's phospholipid membrane and suction applied until a tight mechanical and electrical seal is formed. Under these conditions currents through the membrane patch sealing the tip of the micropipette can be measured. The crucial prerequisite of this method is a direct contact between the glass and the phospholipid membrane of the cell; this condition is relatively easy to meet with animal cells, but plant cells and microorganisms are protected by a rigid cell wall, and sealing the micropipette to the membrane patch poses a difficult problem. The standard procedures for preparing protoplasts in other kinds of studies are not suitable to the patch-clamp technique because they do not render the cell membrane sufficiently "clean." We found that by resuspending the protoplasts in a mildly hypoosmotic patch-clamp medium, some of the slowly inflating protoplasts allow formation of a gigaohm seal. Electron-microscopic pictures of the protoplasts indicate that most of their surface is still covered with a certain amount of the cell wall or periplasmic material residue. The hypoosmotic inflation of the cells appears to "tear" this material and exposes the clean plasma membrane to the external medium. When viewed in a phase-contrast mode, these inflated protoplasts, some of which are patch-clampable, appear darker than the other cells. The patch-clampable protoplasts can also be found by a "rocking" test: of all the cells which rest on the glass bottom of the clamping chamber, only the ones with large areas of their membrane exposed to the outside will have affinity to this glass bottom; when the whole setup is gently rocked, these protoplasts, unlike the other cells, will appear to be attached to the bottom.

Two Types of Channels

The voltage-clamped, inside-out patches of the plasma membrane reveal a wide spectrum of conductances ranging from 8 to 160 pS. We divide these conductances into two categories.

Events associated with conductances in the range of 31–160 pS (Fig. 1) we ascribe to multilevel gating of a single species of channel; i.e., we conclude that they do not represent a superposition of current events in several different channels (see the next section). Events in this category were the most frequent and, since they appear to be functionally coupled, will be ascribed to multilevel gating of a single species of channel designated as "153-pS channel" according to the mean conductance implied by the largest occurring current events.

The second category comprises events associated with conductances in the range of 8–16 pS (Fig. 2). These events were rather rare, occurring in about one out of 20 patches. There is no suggestion of correlation among the observed events and therefore they might naturally be supposed to originate from several independent channels. However, one strong statistical argument suggests some kind of coupling of the observed current activities: in a majority of patches these current events cannot be seen; yet, when they occur, they always occur together. We mention these small current events just as a note of interest: the focus of this report is the 153-pS channel.

Multiple-Conductance Channel

An overview of gating of a single 153-pS channel at different voltages is shown in Fig. 3A. Figure 3B allows a detailed analysis of the multiplicity of the conductance levels at + 90 mV. It reveals the presence of five different conductance states termed *A* through *E* which, we believe, make up the total of the possible conductance states of a single multilevel channel protein. The figure serves to provide evidence of this conclusion by clearly illustrating the interconnection of the five conductance states. In Trace 1 of Fig. 3B,

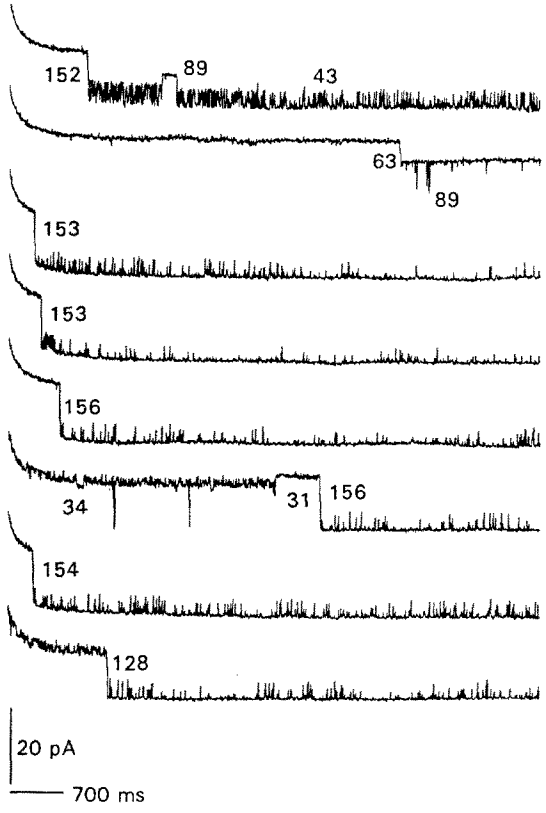

Fig. 1. Current events in the 31–160 pS region. The individual traces are all recorded from a single patch under identical conditions, and the beginning of each trace corresponds to the onset of an applied +90 mV voltage step. Between each step the patch was held for 20 s at voltage of −80 mV in order that all channels would be fully open. The patch was bathed in a standard clamp solution on both sides of the membrane: 140 mM KCl, 11 mM EGTA, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES/Tris, and 591 mM sorbitol, pH 7.2, 0.90 Osm. The numbers next to the current jumps indicate the corresponding change in conductance, in pS. The initial bending of traces is due to the capacitive current which follows the voltage step from −80 to +90 mV.

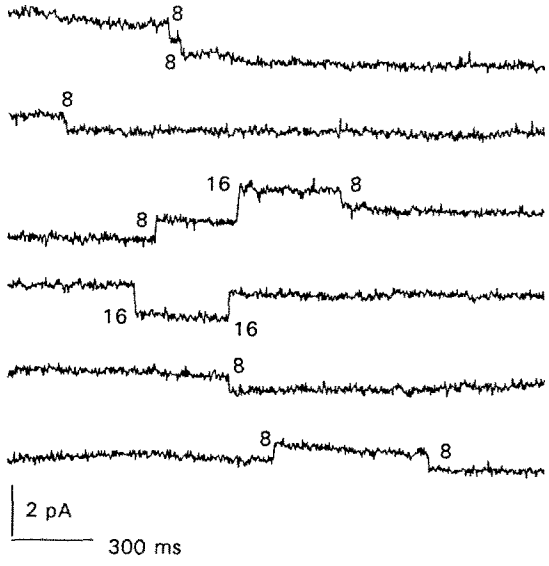

Fig. 2. Current events in the 8–16 pS region. All six traces represent one continuous record of the current through a membrane patch held at +80 mV for 30 s. The patch solution is the same as in Fig. 1. The numbers next to the current jumps indicate the change in conductance in pS. The trace does not suggest any correlation between the events. The first four events in the third trace might indicate the gating activity of two independent channels, in which case we see an 8-pS channel open, followed by a 16-pS channel, after which the 8-pS channel closes, again followed by the 16-pS channel.

as a first example, a jump in the current from a high conductance state, *E*, down to a lower conductance state, *A*, is accompanied by the onset of a rapid flickering between states *A* and *B*. Assuming these current events to be associated with the activity of membrane channels, the coincidence of the initial downward jump with the sudden onset of flickering seems too unlikely in any interpretation but one which ascribes the events to a single channel. Similarly, this argument applies to the subsequent step-like current event of ~200 ms duration occurring between levels *A* and *C*: the jump to *C* is coincident with an immediate cessation of the flicker, which then spontaneously resumes upon transition down to *A*. Therefore, conductance state *C* ought to be associated with *B*, and hence also *E*, and all three should be taken as states of a single channel. Additional records collected over the range of −90 to +90 mV show that states *A* and *E* represent the fully closed and the fully open conductance states of this channel.

Trace 2 of Fig. 3B shows a jump from a high conductance state to a lower one, followed by several rapid switches to a still lower state. The absolute magnitude of the current levels and the differences between them allows us to identify them with states *E*, *C*, and *A* of the one channel.

Traces 3 and 4 of Fig. 3B and reasoning similar to that above indicate yet another conductance state (or even a group of conductances), *D*. At the beginning of Trace 3 we see that the patch-current fluctuates between an amplitude of *D* and *E* and that this flicker is of a certain character. Following a transition between levels *E* and *A*, one familiar from Trace 1, a flickering of a visibly different character begins, which seems to be the already discussed transition *AB*.

A better resolved conductance switch between the fully open and the closed state is given in Fig. 3C. This and other records indicate that at a given resolution

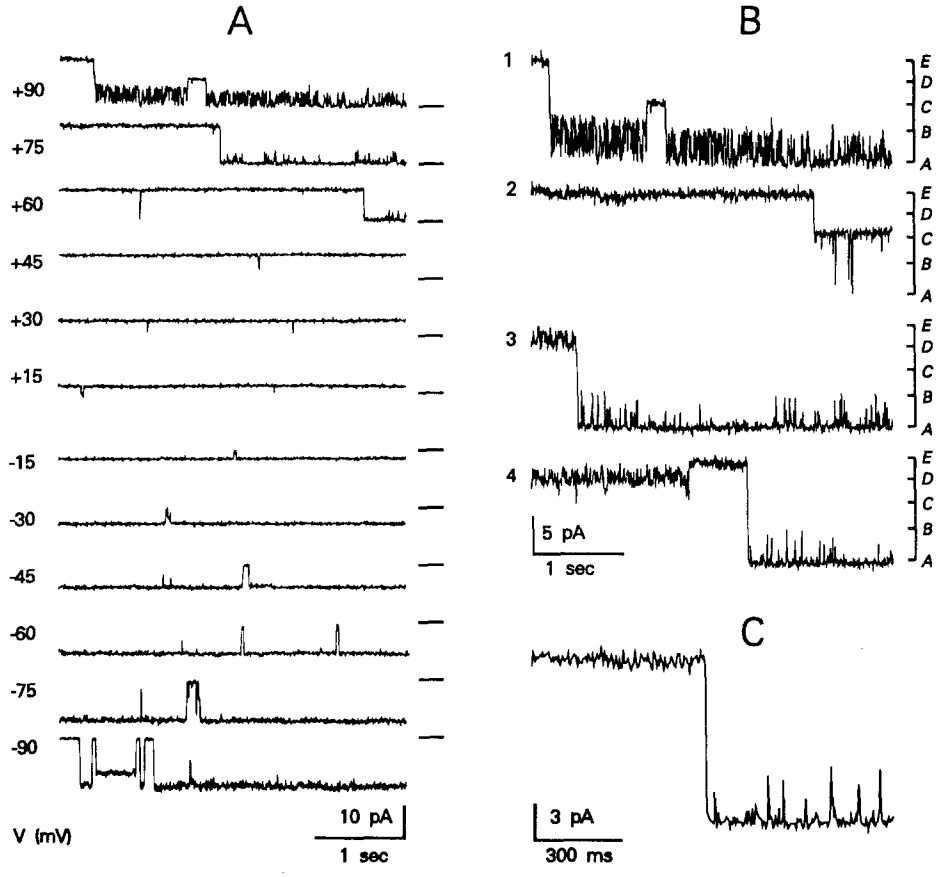

Fig. 3. (A) Gating activity of the potassium channel in the excised inside-out patches of the yeast *S. pombe*. Foe each current trace the membrane was held at a potential indicated to the left of the trace; between the episodes the patch was held at −80 mV for 20 s to open the channel for the next episode. The horizontal lines to the right of the current traces indicate the current level of the closed channel. The patch was bathed in a standard clamp solution on both sides of the membrane: 140 mM KCl, 11 mM EGTA, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES/Tris, and 591 mM sorbitol, pH 7.2, 0.90 Osm. The initial bending of traces is due to the capacitive current after switching the voltage from −80 mV to the clamp potential. (B) Subconductance states of the 153-pS channel. Excerpts from the current records of a single patch. Experimental setup as in (A). In all traces the membrane patch was clamped at +90 mV. The five conductance states are termed *A* through *E*, where *A* is the fully closed state and *E* is the fully open one. A detailed description of the conductance jumps is given in the text. (C) Highly resolved conductance jump between levels *E* and *A*. Clamp voltage +80 mV, filtering bandwidth 800 Hz.

the conductance switch between the two extreme conformational states of the channel can proceed directly, without any observable residency in the conductance substates. Such a current switch with a simultaneous opening or closing of four functionally unlinked smaller channels would have an infinitesimally small probability.

Together these observations strongly suggest that (1) the initial flickering in Trace 1 of Fig. 3B arises not from a small and distinct channel superimposed on the record over a larger one, (2) this flickering is connected with the channel responsible for the transition *EA*, and consequently that (3) level *D* is yet another conductance state of the same channel associated with states *A*, *B*, *C*, and *E*.

Interlevel Gating

Various transitions between the five conductance states were observed to occur. Figure 4 illustrates which interlevel transitions were observed at different voltages for a single patch. Nine of the ten possible

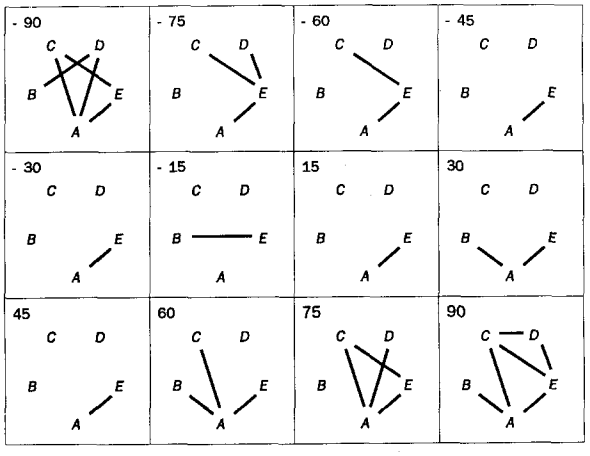

Fig. 4. Interlevel gating. The number at the upper left corner of each square indicates the voltage at which the transitions were observed. The probability of transitions between the lower-conductance states increases at voltages of absolute magnitude greater than 60 mV. For lower voltages the most frequent transition is that between the fully open and fully closed states. The only transition that has not been observed is that between states *B* and *C*.

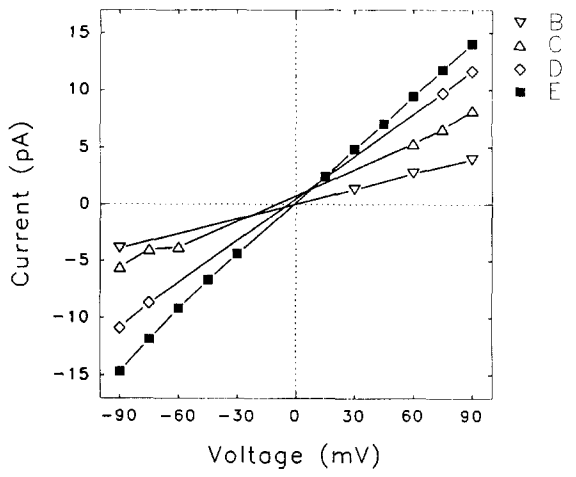

Fig. 5. *I–V* plots of all conductance states for transitions to and from the fully closed state. At positive voltages, the characteristics of all states are linear, while at negative voltages the characteristics deviate slightly from linearity. Conductance *C* either exhibits rectification at negative voltages, or owes its nonlinearity to another undiscerned sublevel of conductance. In the region of positive voltages the conductances are *B* 43 ± 5 pS, *C* 89 ± 7 pS, *D* 128 ±5 pS, and *E* 153 ± 6 pS (n = 12).

transitions between the five conductance states were recorded, only the transition between states *B* and *C* being absent. The probability of gating between one subconductance state and another, or between one substate and the fully open state, increases sharply at voltages of absolute magnitudes higher than 60 mV. For voltages between −60 and +60 mV the gating is more sporadic; the correlation of the five conductance states is much less readily apparent, and events might easily be taken as the activity of several channels.

Values of the Conductances of the Individual States

The current–voltage characteristics of the four open conductance states of the 153-pS channel are depicted in Fig. 5. At positive pipette voltages the current–voltage characteristics of all conductance states appear linear. At negative voltages, the characteristics deviate slightly from linearity, and the conductance transition denoted as *AC* shows some rectification, possibly caused by yet another conductance substate. From the figure, the conductances of the four open states for positive voltages are: *B* 43 ± 5 pS, *C* 89 ± 7 pS, *D* 128 ± 5 pS, and *E* 153 ± 6 pS (n = 12).

There is no conflict between these values and the values implied by certain other events, for example, the 31-pS transition shown in the sixth trace of Fig. 1: this event can be reasonably ascribed to an intersubstate transition *DE*, since according to the values reported above we would expect such a transition $DE = D - E = 25 \pm 11$ pS. Indeed, no less than nine categories of events were observed, all of which were ascribable to gating between the four different conductance states, and no other conductance sources need be postulated to account for the events recorded, excepting only the 8–16-pS events mentioned above.

Dwell Times of Conductance States and Time/Conductance-Averaged Currents

The dwell times of individual conductance states for a range of voltages are shown in Fig. 6. At the physiologically relevant voltages, +30 mV and above, the highest degree of substate activity is observed, whereas below +30 mV and at negative potentials the channel resides in the fully open state *E* the whole of the time (note that a dwell time of 8 s indicates the state was stable for the duration of the entire recording).

The time- and conductance-averaged current $\bar{I}(V)$ represents a general function which takes into consideration the fraction of time spent in, and conductance carried by, individual conductance states. It can be calculated as a function of voltage:

$$\bar{I}(V) = \frac{\sum_{i=1}^{n} G_i(V) \cdot P_i(V)}{n} \cdot V \qquad (1)$$

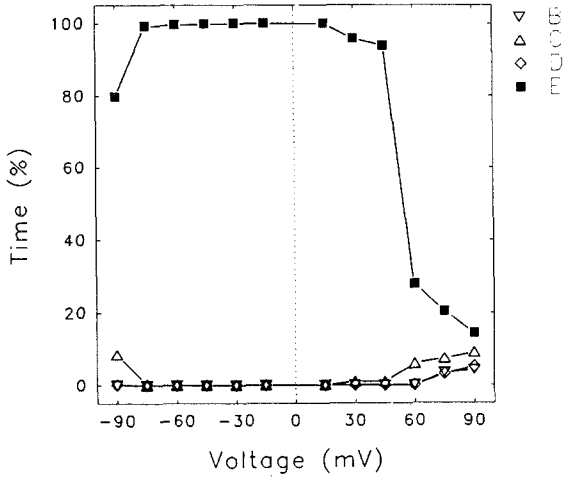

Fig. 6. Dwell times of conductance states. Points are derived from sixteen 8-s intervals following an applied voltage step from a holding potential of −80 mV to the various clamp potentials, and take into account events of greater than ~50 ms duration. *B* through *E* correspond to the conductance states discussed in the text. Notice that the main region of activity occurs at positive voltages, which are the physiologically relevant negative membrane potentials.

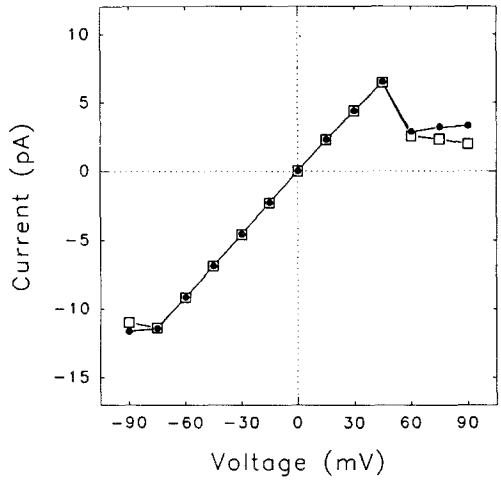

Fig. 7. Time/conductance-averaged currents. The averaged currents were calculated using Eq. (1). The data were compiled from 12 different experiments for the conductance values, and three experiments for the dwell-time values. Circles—averaged currents for all conductance sublevels; squares—averaged currents for only the fully open state *E* of the channel. The curves show strong rectification at the physiologically relevant voltages.

where $G_i(V)$ is the conductance of the i state at voltage V, $P_i(V)$ is the fraction of time the channel spends in that state, and n is the number of nonclosed conductance states. The resulting I–V curve, shown in Fig. 7, reveals strong rectification at the physiologically relevant voltages, leading to small positive currents. In this region the fully open state E accounts for roughly half of the channel's activity, as can be seen by comparing the two curves of Fig. 7. Hence, in intact yeast cells we would expect roughly half of the ionic current to flow through the fully open channels and the other half through channels in the other conformational substates.

Ion Selectivity

The ion selectivity of the channel was determined from its current–voltage characteristics, either with different KCl concentrations across the membrane, or under biionic conditions. The former measurement allows to determine the ratio of the potassium to chloride permeability using a rearranged Goldman–Hodgkin–Katz potential equation:

$$\frac{P_K}{P_{Cl}} = \frac{A - e^{(F/RT)V_{rev}}}{Ae^{(F/RT)V_{rev}} - 1} \qquad (2)$$

where V_{rev} is the reversal potential, P_K and P_{Cl} are the permeabilities of the channel with respect to potassium and chloride ions, $A = [K^+]_i/[K^+]_o = [Cl^-]_i/[Cl^-]_o$, F is the Faraday's constant, R is the gas constant, and T is the absolute temperature. The biionic measurement allows one to determine the selectivity of the channel to potassium relative to other cations according to the alternate expression of the Goldman–Hodgkin–Katz equation

$$\frac{P_K}{P_X} = \frac{B}{(B + 1)e^{(F/RT)V_{rev}} - 1} \qquad (3)$$

where $B = P_K/P_{Cl}$. The results of the measurements for a single patch are shown in Table I, in which we can see that the permeability of the channel to the ions tested goes $P_K > P_{Na} > P_{Li} > P_{Cl}$.

Table I. Ion Selectivities of the Channel[a]

	K^+	Na^+	Li^+	Cl^-
V_{rev} (mV)	0	−10.2	−15.1	−19.2
P_K/P_X	1	1.6	2.1	6.1

[a] Permeabilities were derived from the reversal potentials obtained at $T = 297$ K with 140 mM KCl inside the micropipette, and various bath solutions: 46.7 mM KCl for determination of P_K/P_{Cl} [Eq. (2), $A = 3$] and 140 mM LiCl and NaCl for determination of P_K/P_{Li} and P_K/P_{Na} [Eq. (3)], respectively. The tabulated values represent the average of two measurements each; therefore, no deviations are given.

Insensitivity to Common Potassium Channel Blockers, Stimulants, and Inhibitors of ATPases, and Mechanical Stretch

Since the channel appears to be potassium-selective, we checked the effect of the common potassium channel blockers TEA (10 mM), quinine, and quinidine (both 50 μM). The blockers were perfused through the bath solution external to the micropipette, and were therefore administered to the cytoplasmic side of the membrane patch. No effect was observed. Similarly, in light of the effects in *S. cerevisiae*, ATPase mutants reported by Ramirez *et al.* (1989) in which activation and blocking of that yeast's potassium channel were observed in response to perfusion of ATP and DCCD, respectively, we tested these substances, observing no effect.

To test for the sensitivity of the channel to mechanical stress, we applied negative pressures of up to 15 kN m^{-2} to all patches, regardless of the channel activity. This mechanical stretch of the membrane produced neither additional gating in the patches containing the channel, nor activation of any channel in patches which appeared to contain no channels.

Density of Channels in *S. pombe* Plasma Membrane

Based on the diameter of the average protoplast and micropipette tips, and statistics on the number of membrane patches which contained none, one, or more channels, we may estimate the density of expression of the 153-pS channels in the average yeast plasma membrane. The selection of small patches of membrane from whole yeast cells is a random process, and we can test the channel statistics by comparing the number of actually observed channels to the expected Poisson distribution:

$$P(k) = \frac{e^{-m}m^k}{k!} \tag{4}$$

where $P(k)$ is the probability of finding k channels in a patch, and m is the mean number of channels observed.

Out of a representative ensemble of 39 membrane patches, one patch contained two channels, eight patches contained one channel only, and 30 patches contained no channels at all. Therefore, for 10 channels per 39 patches, $m = 10/39 = 0.256$, and we can compare the theoretical distribution of channels in patches to the number actually counted on the basis of gating activity (Table II). The close agreement between the experimental and theoretical values demonstrates that the statistics follow Poisson distribution, that the number of channels observed corresponds to the number of channels present in the membrane patch, and that we may justifiably extrapolate from the observed density of channels in patches to the total population within the cell.

Table II. Probabilities of Finding Channel in a Membrane Patch

Probability	Experiment	Theory
$P(0)$	$30/39 = 0.769$	0.774
$P(1)$	$8/39 = 0.205$	0.200
$P(2)$	$1/39 = 0.0256$	0.0254

Since the average protoplast diameter is $\sim 8\,\mu$m, the total area of the whole cell plasma membrane is approximately 200 μm^2. The type and shape of micropipettes used imply (Ruknudin *et al.*, 1991) an average patch area of 1.5 μm^2. The representative ensemble of 39 membrane patches therefore represents a sampling of 58 μm^2 of the cell surrface, in which we found 10 channels. In the total surface of 200 μm^2 of the whole yeast protoplast one would thus expect about 35 channels.

A possible pitfall in the extrapolation of the channel population from the observation of the channel activity is that perhaps not all channels within the patch of membrane under observation are always active; i.e., perhaps there are significant periods of "dormancy" for the channel which is then not observed and counted.

DISCUSSION

The plasma membrane of *S. pombe* harbors at least three different types of ionic channels: one 153-pS potassium channel and two smaller ones, 8- and 16-pS channels. Our study concentrates on the former one.

The 153-pS channel is voltage-dependent, mildly selective for potassium over other ions. The channel activity fits well the presumed physiological role of the channel, which is to counterbalance the flow of protons across the membrane, be it in the outward direction through the H^+-ATPase or in the inward direction during the symport with various solutes. The observed channel activity meets the behavior of the expected potassium channel: for a depolarized membrane the channel is open, allowing potassium ions to

flow into the cell down the electrochemical gradient generated by the H^+-ATPase, while for a hyperpolarized membrane the channel shuts close, providing a mechanism to control the potassium gradient as well as the performance of the H^+-ATPase.

The channel activity is insensitive to the common potassium channel blockers TEA, quinine, and quinidine applied from the cytoplasmic side of the membrane. By its insensitivity to TEA, the channel differs from that reported in *S. cerevisiae* (Gustin *et al.*, 1986). The response of the *S. pombe* channel to TEA cannot, however, be considered as conclusive since TEA is fully effective when applied to the outside of the plasma membrane and at higher concentrations. On the other hand, in reconstituted plasma membrane vesicles from *Metschnikowia reukaufii* already 2 mM TEA inhibited the generation of ΔpH by 50% when applied from the cytoplasmic side of the inside-out vesicles (Gläser and Höfer, 1987). Van de Mortel *et al.* (1990) reported a massive, TEA-insensitive potassium efflux from *S. cerevisiae* through putative potassium channels induced by onset of glucose fermentation. Hence, the sensitivity of ionic channels of the yeast plasma membrane to TEA does not appear to be obligatory.

The occurrence of this 153-pS channel is reproducible and independent of any activating means, such as mechanical pressure or chemicals. The gating of this channel entity is complex, exhibiting several closely associated subconductance levels and thus raising the question of its nature.

Two models seem to be plausible in explaining the multiple conductance levels. In the first one, the channel entity can be seen as a single barrel which is capable of undergoing several conformational changes. The tight functional coupling, i.e., the coincidence of onset or cessation of flickering with the opening or closing of the channel, and the direct transitions between the closed and the fully open states, seems to be an intuitively acceptable feature of such a single-barrel channel. In the second model the channel consists of several identical and functionally tightly coupled barrels. This is suggested by the observation that the conductance transitions are, within experimental error, integral multiples of the smallest conductance step. The transition kinetics of this multi-barrel entity would be highly cooperative since most of the time the barrels stick together. At this moment there is no conclusive evidence for either of these two models. A blocker that would either close the whole channel (Model 1) or bring out individual sublevels (Model 2) may solve this problem. However, such a blocker has not yet been found.

The long-standing dilemma of the yeast plasma membrane physiology is the coupling of the proton to potassium currents across the membrane. Two views are being discussed at present. According to the first one the H^+-ATPase of fungal plasma membranes is a pure electrogenic proton pump (Goffeau and Slayman, 1981; Goffeau and Bountry, 1986; Serrano, 1988). The potassium channel is a protein entity independent of the H^+-ATPase, and the sole driving force for the uptake of potassium into the cell is the electrochemical gradient generated by the H^+-ATPase. The currents of the two ions are coupled indirectly by means of an obligatory charge compensation. The channel described in this paper might be a good candidate for this independent transporter: it is potassium-selective and exhibits the right gating characteristics.

According to the other view (Poole, 1978; Villalobo, 1982, 1984, 1988; Höfer and Gläser, 1989) the potassium channel is a part of the H^+-ATPase which operates as an electrogenic H^+/K^+ exchanger similar to the sodium pump of animal cells (Cantley, 1981). In this case the coupling of the two ionic currents would be direct via one protein complex energized by the hydrolysis of ATP. If this were the case with the plasma membrane of *S. pombe*, one would expect to see some effects on this channel of the H^+-ATPase activators or blockers. However, none was observed. Moreover, there is a discrepancy between the densities of the potassium channels and the H^+-ATPases in the plasma membrane of *S. pombe*: while the observed proton gradients have to be generated by 10^5–10^6 of slow-pumping H^+-ATPases (Slayman, 1987), there is only about 35 potassium channels in the plasma membrane of a single cell. Hence this second view does not seem to apply to the plasma membrane of *S. pombe*.

The main physiological role of the observed channel is, to our opinion, the control of the electrical potential difference across the yeast plasma membrane. The high potassium concentration gradient serves as a buffer of the membrane potential: by depolarizing the plasma membrane, the potassium channels open and the outflow of positive charges repolarizes the plasma membrane again. This happens under physiological conditions, e.g., by the onset of a symport. Indeed, a stoichiometric efflux of potassium coupled to H^+/sugar symport was observed in the yeast *Rhodotorula glutinis* (Hauer and Höfer, 1982).

The rather moderate specificity of the described

channel for potassium is also consistent with such a function of the channel. For an electrical charge compensation it is irrelevant which ionic species carries the compensating charges. In experiments with isolated plasma membrane from *S. pombe* incorporated in proteoliposomes the ionic preference for charge compensation during $\Delta\tilde{\mu}_{H+}$ generation was $K^+ > Na^+ \gg Li^+$ and $Cl^- = NO_3^- \gg SO_4^{2-}$ (Mair and Höfer, 1988). The activity of another yeast plasma membrane H^+-ATPase from *M. reukaufii* incorporated in proteoliposomes exhibited a remarkable stimulation by alkali ions, K^+ being the most effective one, followed by Na^+, NH_4^+, and with a considerably lower efficiency also by Li^+ (Gläser and Höfer, 1987). A similar low ion selectivity was reported for the mechanosensitive channel of the yeast *S. cerevisiae* (Gustin *et al.*, 1988). This may imply that the channel detected in *S. pombe* is of the same nature. However, the ionic channel of *S. pombe* plasma membrane exhibited no response to mechanical stretch. In mammalian membranes a low selectivity toward alkali ions was also reported, e.g., for the *c*GMP-dependent channel from bovine photoreceptor membranes (Hanke *et al.*, 1988; Lühring *et al.*, 1990).

In view of these results, we conclude that the identified ionic channel in the plasma membrane of *S. pombe* is a membrane protein entity different from the H^+-ATPase, serving to control the membrane potential. On the other hand, our results provide no evidence in favor of the H^+-ATPase being either a pure electrogenic H^+-pump or an electrogenic H^+/K^+-exchanger. Although the identified ionic channel and the H^+-ATPase are undoubtedly different from each other, the later may still catalyze the potassium uptake in exchange for H^+. This uptake would then be independent of the potassium flux through the channel.

The discovery of the multiconductance ionic channel in the yeast plasma membrane raises the question of the physiological significance of the various subconductances. In general, the main gating between the closed and the fully open states of the channel occurs at physiologically relevant voltages, allowing massive fluxes of electrical charge across the membrane. The physiological role of the low-conductance events may consist in a finer adjustment of the membrane potential by fine-tuning of the ionic flux through the channel. This role may be confirmed by further patch clamp examination of mutant cells with an altered ion transport.

One perplexing aspect of the plasma membrane physiology of *S. cerevisiae* is the rather large amount of different potassium channels reported to date (Gómez-Lagunas *et al.*, 1989; Gustin *et al.*, 1986, 1988; Ramirez *et al.*, 1989). The conductances of these potassium channels, as normalized to 140 mM KCl, which we used in our experiments, are approximately 20, 35, 50, 70, and 160 pS. It is not clear why a plasma membrane of one cell should harbor so many different types of potassium channels, and why different research groups would observe different potassium channels. In our case, with the yeast strain of *S. pombe*, we observed one 153-pS channel which, however, exhibits four open conductance levels, with conductances of 43 ± 5, 89 ± 7, 128 ± 5, and 153 ± 6 pS. Considering the fact that transitions between all conductance levels seem to be allowed, this channel makes possible a total of six distinctive conductance transitions that could be related to the channels reported earlier: 25 ± 11, 43 ± 5, 64 ± 13, 89 ± 7, 128 ± 5, 153 ± 6 pS. At low voltages some of these conductances occur sporadically, suggesting no link to a multiconductance channel (Fig. 3A, traces at -30, -45, -60 mV). Even though our observations were made with a different yeast strain, we do not exclude the possibility that some of the seemingly different potassium channels observed with the yeast *S. cerevisiae* are expressions of one multiconductance potassium channel.

Finally, the ability to record single-channel events in the plasma membrane of the yeast, a simple eukaryote which can easily be manipulated by genetic engineering, potentially allows an electrophysiological examination of foreign channel genes expressed in these cells. A successful expression of an algal glucose-symporter gene in *S. pombe* has already been reported (Sauer *et al.*, 1990).

ACKNOWLEDGMENTS

This work was supported by Deutsche Forschungsgemeinschaft Grant Ho555/13 to M.H., who was also a recipient of an academic fellowship by VW-Stiftung Hannover, and National Science Foundation Grant DCB-8904462 to H.L. We are grateful to Mr. Oliver Baker for his help in preparing the manuscript.

REFERENCES

Bowman, B. J., and Bowman, E. J. (1986). H^+ ATPases from mitochondria, plasma membranes, and vacuoles of fungal cells, *J. Membr. Biol.* **94**, 83–97.

Cantley, L. C. (1981). Structure and mechanisms of the (Na,K)-ATPase, in *Current Topics in Bioenergetics* (Sanadi, D. R., ed.), Vol. 11, Academic Press, New York, pp. 201–237.

Dickinson, D. P., and Isenberg, I. (1982). Preparation of spheroplasts of *Schizosaccharomyces pombe*, *J. Gen. Microbiol.* **128**, 651–654.

Dufour, J.-P., and Goffeau, A. (1980). Molecular and kinetic properties of the purified plasma membrane ATPase of the yeast *Schizosaccharomyces pombe*, *Eur. J. Biochem.* **105**, 145–154.

Eddy, A. A. (1978). Proton-dependent solute transport in microorganisms, *Curr. Top. Membr. Transp.* **10**, 278–360.

Eddy, A. A. (1982). Mechanisms of solute transport in selected eukaryotic microorganisms, *Adv. Microb. Physiol.* **23**, 1–78.

Gläser, H.-V., and Höfer, M. (1987). Ion-dependent generation of the electrochemical proton gradient $\Delta\tilde{\mu}_{H^+}$ in reconstituted plasma membrane vesicles from the yeast *Metschnikowia reukaufii*, *Biochim. Biophys. Acta.* **905**, 287–294.

Goffeau, A., and Bountry, M. (1986). Three proton-pumping ATPases in yeast, *Microbiol. Sci.* **3**, 164–168.

Goffeau, A., Coddington, A., and Schlesser, A. (1989). Plasma membrane H^+-ATPase: Metabolite transport in the yeast *Schizosaccaromyces pombe*. (1989). In *Molecular Biology of the Fission Yeast*. Academic Press, Inc., (Nasim, A., Young, P., and Johnson, B. F., eds.), Academic Press, San Diego, pp. 397–429.

Goffeau, A., and Slayman, C. W. (1981). The proton-translocating ATPase of the fungal plasma membrane, *Biochim. Biophys. Acta* **639**, 197–223.

Gómez-Lagunas, F., Peña, A., Liévano, A., and Darzson, A. (1989). Incorporation of ionic channels from yeast plasma membranes into black lipid membranes, *Biophys. J.* **56**, 115–119.

Gustin, M. C., Martinac, B., Saimi, Y., Culbertson, M. R., and Kung, C. (1986). Ion channels in yeast, *Science* **233**, 1195–1197.

Gustin, M. C., Zhou, X.-L., Martinac, B., and Kung, C. (1988). A mechanosensitive ion channel in the yeast plasma membrane, *Science* **242**, 762–765.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981). Improved patch clamp techniques for high-resolution current recording from cells and cell-free membrane patches, *Pflügers Arch.* **391**, 85–100.

Hanke, W., Cook, N. J., and Kaupp, U. B. (1988). *c*GMP-dependent channel protein from photoreceptor membranes: single-channel activity of the purified and reconstituted protein, *Proc. Natl. Acad. Sci. USA* **85**, 94–98.

Hauer, R., and Höfer, M. (1982). Variable H^+/substrate stoichiometries in *Rhodotorula gracilis* are caused by a pH-dependent protonation of the carrier(s), *Biochem. J.* **208**, 459–464.

Höfer, M. (1989). Accumulation of electroneutral and charged carbohydrates by proton cotransport in *Rhodotorula*, *Methods Enzymol.* **174**, 629–653.

Höfer, M., and Gläser, H.-V. (1989). K^+ ions stimulate both ATPase activity and ΔpH generation in reconstituted yeast plasma membrane vesicles, in *Highlights of Modern Biochemistry*, Vol. 1, VSP-International Science Publishers, Zeist, pp. 753–760.

Höfer, M., and Nassar, F. R. (1987). Aerobic and anaerobic uptake of sugars in *Schizosaccharomyces pombe*, *J. Gen. Microbiol.* **133**, 2163–2172.

Lühring, H., Hanke, W., Simmoteit, R., and Kaupp, U. B. (1990). Cation selectivity of the *c*GMP-gated channel of mammalian rod receptors, in *Sensory Transduction*, Plenum Press, New York, pp. 169–173.

Mair, T., and Höfer, M. (1988). ATP-transduced generation of pH gradient and/or membrane potential in reconstituted plasma membrane vesicles from *Schizosaccharomyces pombe*, *Biochem. Int.* **17**, 593–604.

Poole, R. J. (1978). Energy coupling for membrane transport, *Annu. Rev. Plant Physiol.* **29**, 437–460.

Ramirez, J. A., Vacata, V., McCusker, J. H., Haber, J. E., Mortimer, R. K., Owen, W. G., and Lecar, H. (1989). ATP-sensitive K^+ channels in a plasma membrane H^+-ATPase mutant of the yeast *Saccharomyces cerevisiae*, *Proc. Natl. Acad. Sci. USA* **86**, 7866–7870.

Ruknudin, A., Song, M. J., and Sachs, F. (1991). The ultrastructure of patch-clamped membranes: a study using high voltage electron microscopy, *J. Membr. Biol.* **112**, 125–134.

Sauer, N., Caspari, T., Klebl, F., and Tanner, W. (1990). Functional expression of the *Chlorella* hexose transporter in *Schizosaccharomyces pombe*, *Proc. Natl. Acad. Sci. USA* **87**, 7949–7952.

Serrano, R. (1988). Structure and function of proton translocating ATPase in plasma membranes of plants and fungi, *Biochim. Biophys. Acta* **947**, 1–28.

Slayman, C. L. (1987). The plasma membrane ATPase of *Neurospora*: a proton-pumping electroenzyme, *J. Bioenerg. Biomembr.* **19**, 1–20.

Vacata, V., Höfer, M., and Lecar, H. (1990). Ionic channels in the plasma membrane of *Schizosaccharomyces pombe*: Evidence from patch-clamp measurements, *Proceedings 8th Small Meeting on Yeast Transport and Energetics* (Kotyk, A., and Horák, J., eds.), Press Center Biol. Inst., Prague.

Van de Mortel, J. B. J., Theuvenet, A. P. R., and Borst-Pauwels, G. W. F. H. (1990). A putative K^+-selective channel in the plasma membrane of yeast that is blocked by micromolar concentrations of external divalent cations and is insensitive to tetraethylammonium, *Biochim. Biophys. Acta* **1026**, 220–224.

Villalobo, A. (1982). Potassium transport coupled to ATP hydrolysis in reconstituted proteoliposomes of yeast plasma membrane ATPase, *J. Biol. Chem.* **257**, 1824–1828.

Villalobo, A. (1984). Energy-dependent H^+ and K^+ translocation by reconstituted yeast plasma membrane ATPase, *Can. J. Biochem. Cell Biol.* **62**, 12826–12830.

Villalobo, A. (1988). Energetic efficiency of different mechanistic models for potassium ion uptake in lower eukaryotic cells, *Folia Microbiol.* **33**, 407–424.