YKC1 encodes the depolarization-activated K^+ channel in the plasma membrane of yeast

Xin-Liang Zhou[a,*], Brian Vaillant[a,*], Stephen H. Loukin[a], Ching Kung[a,b,*], Yoshiro Saimi[a]

[a]*Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706, USA*
[b]*Department of Genetics, University of Wisconsin, Madison, WI 53706, USA*

Received 29 August 1995

Abstract Our previous patch-clamp studies showed that depolarization activates a K^+-specific current in the plasma membrane of the budding yeast, *Saccharomyces cerevisiae* [Gustin et al. (1986) Science 233, 1195–1197]. The Yeast Genome Sequencing Project has now uncovered on the left arm of chromosome X an open reading frame (ORF) that predicts a 77-kDa protein reminiscent of a *shaker*-like α subunit with 6 membrane spans followed by a subunit with 2 spans. We found that deleting this ORF removes the yeast K^+ current. Furnishing the ORF from plasmids restores or even greatly amplifies this current. These manipulations have no effects on the 40-pS mechanosensitive conductance also native to this membrane. Thus, this ORF, named *YKC1* here, likely encodes a structure for the K^+-specific channel of the yeast plasma membrane. This and other K^+ channel subunits are compared and the possible uses of this gene in research are discussed. *YKC1* has recently been shown by others to induce in frog oocytes a K^+ current. Its activation is coupled to E_{K^+} and its outward rectification depends on external divalent cations. We found the *YKC1* channel in its native membrane activates at low voltages largely independent of E_{K^+} and it remains so despite removal of divalents by chelation.

Key words: YKC1; Yeast; K^+ channels; Plasma membrane; Outward rectification

1. Introduction

Depolarization activated K^+ channels govern K^+ efflux and membrane repolarization. They underlie delayed rectification and spike spacing in excitable tissues. Isolation of the *Shaker* gene, which encodes an a subunit of K^+ channels in *Drosophila* [1], initiated the molecular dissections that have led to a deeper understanding in the pore lining, voltage sensor, and inactivation particle [2].

Although research concentrates on channels of animal tissues, microbes also have channels that may be important to their physiologies and such channels are of comparative and evolutionary interest. Besides, they and their genes may provide new opportunities to further study channel structures and functions using modern microbial genetic techniques. Voltage-gated K^+ conductances in several microbes have been documented using classical voltage clamp and patch clamp [3]. Up to now, however, the proteins or genes corresponding to these conductances remained mostly undefined. On the other hand, nucleotide sequences partly resembling animal K^+ channel α subunit genes have been recognized in *Paramecium* [4] and in *E. coli* [5], but their corresponding conductances, if any, have not been identified.

The budding yeast, *Saccharomyces cerevisiae*, is commonly used as a model eukaryotic cell because of the ease of its genetic manipulation. Our patch-clamp analyses of its plasma membrane have revealed the activities of a mechanically gated channel [6] and a voltage gated K^+ channel [7]. The latter shows a strong selection of K^+ over Na^+ and is activated by membrane depolarization but not by hyperpolarization. It has a unit conductance of ca. 20 pS (at 120 mM K^+, 5°C) and is blocked by external Ba^{2+} or TEA [7] and by internal Ca^{2+} [8,9] or H^+ [9].

Two types of animal K^+ channel pore-forming subunits are recognized [12]: the *Shaker* (*Sh*) family K^+ channels, usually activated by depolarization, and the IRK1-type channels, showing inward rectification. The *Sh*-type channel is composed of four α subunits, each of which can be modeled to have 6 membrane-spanning domains (M1–M6) with a loop (P) between M5 and M6 that is believed to line part of the channel pore. An IRK1-type channel, on the other hand, is made of subunits each modeled to have only two membrane-spanning domains flanking a P loop. The P region, being conserved, is often diagnostic of these K^+ channels.

Investigators from the European Yeast Genome Sequencing Project have uncovered an open reading frame (J0911) in a cosmid bearing sequences from the left arm of chromosome X [10]. We and others [11] have recognized that the J0911 ORF predicts a 691 residue protein having eight putative membrane-spanning domains (M1–M8) with a P loop between M5 and M6 and an additional P loop between M7 and M8, as if it is a fusion of a subunit with the *Sh* motif (and some sequence homology) and one with the IRK1 motif (but little sequence homology). Intrigued by the similarity and peculiarity, we tested and report here that this ORF indeed corresponds to the yeast plasma membrane depolarization activated K^+ conductance we reported in 1986 [7].

2. Materials and methods

2.1. Yeast stocks and media

Saccharomyces cerevisiae haploid strains αW303 and αW303ΔJO911 were gifts of T. Miosga. The genotype of αW303 is *MATa, ade2-1, his3-11/15, leu2-3/112, trp1-1, ura3-1, can1-100*. αW303ΔJO911 (αW303,*ykc1Δ*::URA3) was derived from αW303 by the replacement of a 1.02-kb genomic fragment, extending from 190 bp 5′ of the start codon of the *YKC1 ORF* through bp 830 of the ORF, with a 1.23-kb fragment containing the *URA3* gene [10]. αW303 and αW303ΔJO911 strains were maintained on YEPD plates [13]. αW303ΔJO911U$^-$ was a non-reverting *ura3*$^-$ derivative of αW303ΔJO911 isolated by selection on 5-fluroorotic acid containing plates [14].

*Corresponding author. Fax: (1) (608) 262-4570.
E-mail: ckung@facstaff.wisc.edu

**The first two authors contributed equally in this work. B.V. matched J0911 to animal K^+ channel genes, X.-L.Z. performed the patch-clamp analyses.

0014-5793/95/$9.50 © 1995 Federation of European Biochemical Societies. All rights reserved.
SSDI 0014-5793(95)01035-1

αW303ΔJO911U⁻(SUFYKC) was αW303ΔJO911U⁻ transformed with plasmid pSUFYKC bearing *URA3*, the selectable marker. αW303Δ-JO911U⁻(SUFYKC) stock was maintained on synthetic SD plates lacking uracil.

2.2. Plasmid constructions

pSUFYKC was constructed for the expression of the *YKC1* ORF from the promoter of the *SUF14* gene, which encodes a constitutively expressed ribosomal subunit protein [15]. A 2.1-kb fragment was PCR amplified with Pfu DNA polymerase (Stratagene) from yeast genomic DNA, using an oligonucleotide primer containing the predicted start of the *YKC1* ORF preceded by an additional *Bam*HI site (CGGGA-TCCCGATGACAAGGTTCATGAACAG) and a 2nd primer containing the antisense of the predicted end of the *YKC1* ORF followed by an additional *Hin*dIII site (CCCAAGCTTGGGTCAAAGTGTC-TTTCTATGCT). This PCR product was digested with *Bam*HI and *Hin*dIII and inserted into the same sites of YEPSUF14B-3A.3B, a plasmid derived from YEP352 [15], containing the *SUF14* promoter immediately followed by a synthetic *Bam*HI site [16]. These *URA3*-bearing plasmids have the origin of replication from the 2 μm plasmid and, under URA selection, exist in multiple copies per cell.

2.3. Cell cultures

In preparation for patch clamp, yeast cells were inoculated from fresh plates into YEPD at a density of ca. 10^6 cells/ml, grown for 16 h to early stationary phase, and then reinoculated at a 1/20 dilution into fresh YEPD and grown for 2 h. We found this growth regimen to be optimal in preparing spheroplasts for patch clamp purposes. Because of this optimum and because we wished to compare all strains under the same conditions, all cells were cultured in the rich YEPD. αW303ΔJO911U⁻(SUFYKC) thrived in YEPD but tended to segregate its plasmids since the medium did not require the uracil prototrophy. Immediately before spheroplasting, aliquots of αW303ΔJO911U⁻(SUFYKC) cells were spread on SD plates with or without uracil to assess plasmid loss. As expected, many cells reverted back to uracil auxotrophy, indicating plasmid loss. The fraction of *ura*⁻ colonies varied among cultures. This loss is consistent with the finding that many spheroplasts made from these cells had no K^+ current (see section 3). Because only a few freshly prepared spheroplasts could be sampled per culture, the loss of the current has not been statistically correlated with the loss of uracil prototrophy, however.

2.4. Patch clamp techniques

These techniques are similar to those we have described [6,7,17]. Briefly, cells were collected by centrifugation and the cell pellet was resuspended and incubated at 30°C for 20 min with 0.13 mg/ml Zymolyase (ICN) in 0.4 M sorbitol to partially digest the cell wall while supporting the emerging spheroplasts osmotically. The preparation was examined under a microscope to monitor the progress of spheroplast formation. Spheroplasts were pelleted, washed, and resuspended in

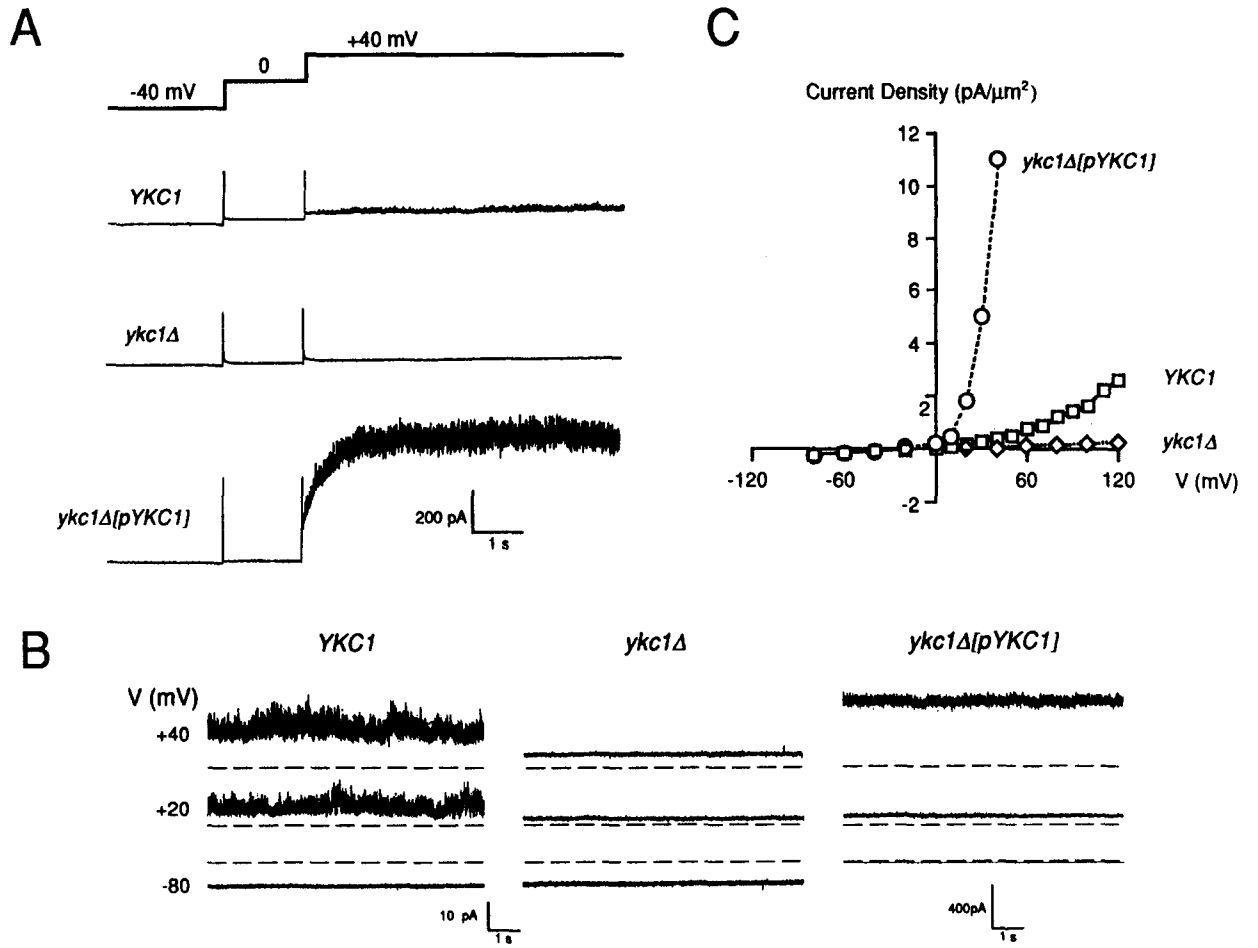

Fig. 1. The ensemble K^+ current correlates with the *YKC1* gene. (A) Whole-cell current elicited by command voltages from −40 to 0 to 40 mV from a spheroplast of the *YKC1* wild-type parental strain (*YKC1*, top current trace), the *YKC1*-deleted strain (*ykc1Δ*, middle) and the *YKC1*-replenished plasmid transformant strain (*ykc1Δ*[YKC1], bottom). (B) Sample current traces at −80, 20 and 40 mV from cells of the parental (left), the deleted (center) and the plasmid transformed strain (right). Broken lines mark the zero current levels. Note scale change to accommodate the large currents of the transformant. (C) Plots the whole cell currents against the clamped voltage for cells of the parent (□), the deleted (◇) and the plasmid transformed strain (○). Whole-cell mode recording with a pipet solution of 180 mM KCl, 0.5 mM EDTA, 5 mM HEPES, pH 7.2 and a bath of 20 mM KCl, 100 mM NaCl, 50 mM $MgCl_2$, 5 mM HEPES, pH 7.2. In this and the following figures '*YKC1*' stands for αW303, the parental strain with the wild-type *YKC1* gene; '*ykc1Δ*' stands for αW303ΔJO911 with its chromosomal *YKC1* deleted; and *ykc1Δ*[YKC1] stands for αW303ΔJO911U⁻[pSUFYKC], which is the deletion strain transformed with *YKC1*-bearing plasmids.

120 mM KCl, 50 mM $MgCl_2$, 5 mM HEPES, pH 7.2. An aliquot of spheroplast suspension was diluted into the experimental chamber containing a perfusable bath of 20 mM KCl, 100 mM NaCl, 50 mM $MgCl_2$, 5 mM HEPES, pH 7.2. A Boralex glass pipet was micromanipulated in the chamber on an inverted microscope to approach the clean surface of a free-floating spheroplast. Only spheroplasts of diameters of 4–6 mm were used. Sustained suction on the surface usually yielded gigaseals. Only preparations with seal resistance over 10 GΩ were used. The electrode was attached to the head stage of a patch clamp amplifier (List-Medical System EPC7). Pressure could be applied through a syringe connected to the electrode pipet to activate mechanosensitive channels [6]. Membrane currents were recorded at room temperature (19–23°C) on chart (Gould) or tape (Vetter). Signals were digitized at 10 kHz, filtered at 1 kHz (8-pole Bessel filter) before analyzed with pCLAMP Program (Axon Instrument). All experiments were performed in whole-cell mode [18].

3. Results

3.1. Ensemble K^+ current correlates with the YKC1 gene

Under patch clamp, spheroplasts of the parental yeast strain (αW303), from which the *YKC1*-deletion strain is derived, have an electric activity indistinguishable from which we have previously identified as the voltage-dependent K^+ current of another wild-type strain (D41) [7]. The whole-cell current of αW303 spheroplasts clearly increases upon depolarization and not hyperpolarization (Fig. 1A, top current trace). As has been established, the depolarization activated current is carried by K^+ and can be blocked by tetramethylammonium (TEA) [7]. Such a current has been observed in every one of over 1,000 wild-type spheroplasts in the last 10 years in our laboratory. This current is an ensemble of several tens of unit events in rapid state transitions [7] (Fig. 1B, left). In contrast, it is entirely missing from W303ΔJO911, the *YKC1*-deleted spheroplasts (Fig. 1A, second current trace; Fig. 1B, center). *YKC1* deletion does not cause a general perturbation of the plasma membrane. αW303ΔJO911 spheroplasts have membrane resistances and leakage currents similar to those of αW303. Whole-cell currents plotted against voltage (Fig. 1C) show the *YKC1*-encoded current (Fig. 1C, squares) activates upon depolarization, while there is no current above leakage background in the *YKC1*-deleted spheroplasts (diamonds).

We have transformed the deleted strain with a 2μ-based plasmid containing a constitutively promoted *YKC1* gene. Spheroplasts from this transformed strain, αW303ΔJO911U$^-$(SUFYKC), not only can have the K^+ current restored, but it can sometimes be greatly enhanced presumably because of the presence of multiple copies of the *YKC1*-bearing plasmid (Fig. 1A, bottom; Fig. 1B, right). We have confirmed that this current, like the wild-type current [7], can also be reversibly blocked by externally applied TEA (data not shown). The whole-cell current of the transformant shown in Fig. 1 is more than 30 times that of the wild-type parent. There appears no rigid control on channel density imposed by cell structures or by biophysical limitations here. Not all restored cells have amplified current and not all spheroplasts prepared from these YEPD cultures inoculated with transformant colonies from selection plates show the K^+ current. This is expected since the cells are not under selection in the rich YEPD medium to retain their plasmids (see section 2).

3.2. Unit K^+ conductance of the YKC1 product

With little or no internal Ca^{2+}, the ensemble K^+ currents are evident (Fig. 1). 1 mM internal Ca^{2+} was included in the pipet solution to block most openings so as to better examine the occasional unit conductance [8,9]. Unit currents through the plasma membrane of αW303 are observed upon depolarization (Fig. 2, left, upper 3 traces) but not hyperpolarization (bottom trace). This unit conductance is nearly identical to that observed in D41 [7] with a conductance of ca. 30 pS in the conditions used here (180 mM K^+, room temperature). Similar examinations with more than 30 spheroplasts of the *YKC1*-deleted αW303ΔJO911 strain never revealed such a channel activity (Fig. 2, center). Spheroplasts of αW303ΔJO911U$^-$[SUFYKC] have a unit conductance indistinguishable from that of the founding parental strain αW303 (Fig 2, right).

3.3. The yeast mechanosensitive channel is not related to YKC1

The yeast plasma membrane is equipped with a set of 40-pS mechanosensitive channels [6]. Pressure applied to spheroplasts activates these channels in the wild type (Fig. 3, top current

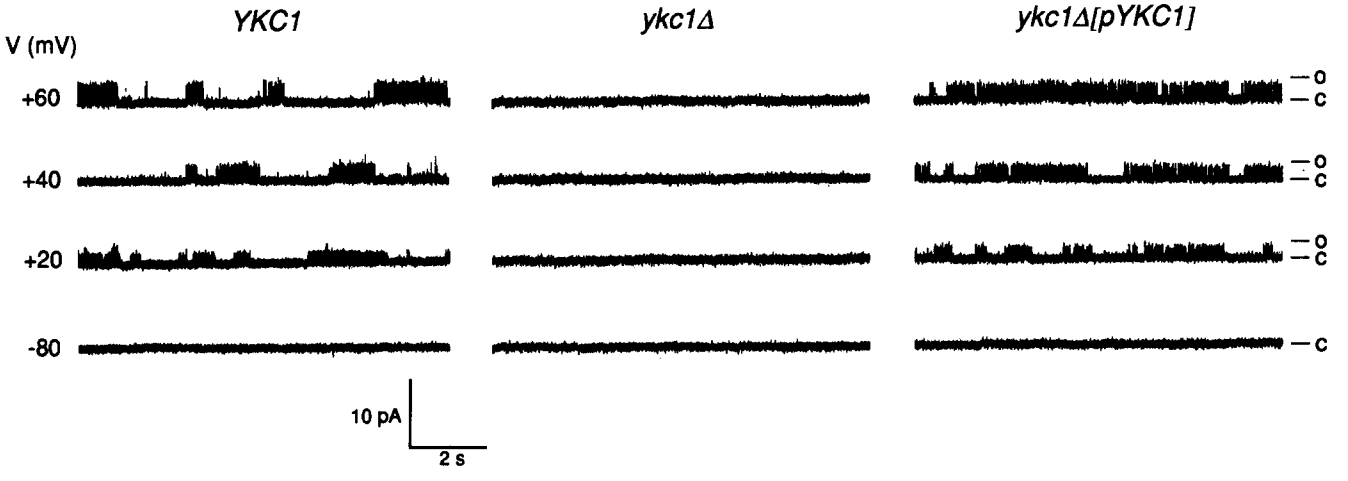

Fig. 2. Unit K^+ currents correlate with the *YKC1* gene. Shown are membrane currents at −80, 20, 40 and 60 mV recorded from whole cells with 1 mM internal Ca^{2+} to suppress most opening, so as to examine the occasional unit events. Left: *YKC1* (the *YKC1* wild-type parent). Center: *ykc1Δ* (the *YKC1*-deleted strain). Right: *ykc1Δ*[YKC1] (the *YKC1*-restored transformant strain; this spheroplast has restored but not greatly enhanced ensemble K^+ current). C = closed; O = open level. Pipets: 180 mM KCl, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.2. Bath: 20 mM KCl, 100 mM NaCl, 50 mM $MgCl_2$, 5 mM HEPES, pH 7.2.

trace), in *YKC1* deletion strain (second current trace) and in *YKC1*-plasmid transformed deletion strain (third current trace). Thus the mechanosensitive channel is not encoded by *YKC1*, and its deletion has no general effects on other conductances in the plasma membrane. The mechanosensitive channels are usually examined upon hyperpolarizations so that their activities can be separated from those of the K^+ channels. In the case of αW303ΔJO911, however, the K^+ channels have been deleted from the membrane. Here then, we have genetically created a situation in which to clearly observe the activities of the yeast mechanosensitive channels upon membrane depolarizations (Fig. 3, bottom two traces) as well as hyperpolarizations.

3.4. The native K^+ current differs from the one induced by *YKC1* in oocytes

A recent report [11] showed that a K^+ current appears when the J0911 ORF is expressed in frog oocytes. The activation of this heterologously expressed current is coupled to E_{K^+}. That membrane currents become symmetric at either polarizations when such oocytes are bathed with 2 mM EDTA and 2 mM EGTA led to the conclusion that the outward rectification of the expressed K^+ current depends on external divalent cation(s) [11].

We have reexamined the native K^+ current and found no coupling to E_{K^+} and no dependence on divalents. For example, changing the E_{K^+} by 55 mV changes the current amplitudes as expected, but does not noticeably affect the threshold of wild-type channel activation (Fig. 4A). Also, the K^+ current of αW303 spheroplasts remains strongly outwardly rectifying regardless of the external concentration of divalent cations (Fig. 4B). No current is seen to activate upon hyperpolarization, even upon strong chelation (Fig. 4B, bottom right trace).

4. Discussion

We report here that an ORF uncovered by the Yeast Genome Sequencing Project corresponds to the depolarization-activated K^+ conductance we described previously [7]. This ORF is apparently a functional gene in yeast, which we now call *YKC1*. The product of *YKC1* is apparently located in the plasma membrane. The deletion and the restoration of *YKC1* correlate with the loss and the reappearance of the K^+ current (Figs. 1,2). This, together with YKC1's structural similarities to other K channel genes (Fig. 5), makes it likely that it is indeed the pore-forming subunit of the yeast channel. Heterologous expression of this gene by another group [11] further supports this conclusion.

The K^+ current expressed in *Xenopus* oocytes [11] and the K^+ current native to the yeast plasma membrane have some similarities: Both are activated upon depolarization, highly selective for K^+, and blockable by external Ba^{2+} or TEA. On the other hand, the activation of the heterologous current is coupled to E_{K^+}, and the current loses its rectification when chelators are added to the bath. These properties could not be demonstrated with the native K^+ current (Fig. 4). The origin of these differences is obscure. There may be additional subunits besides the pore former in the native yeast channel not found in oocytes. The *YKC1* product may also have acquired from or conferred to the oocyte membrane unusual properties.

The structural peculiarity of the protein predicted by *YKC1*

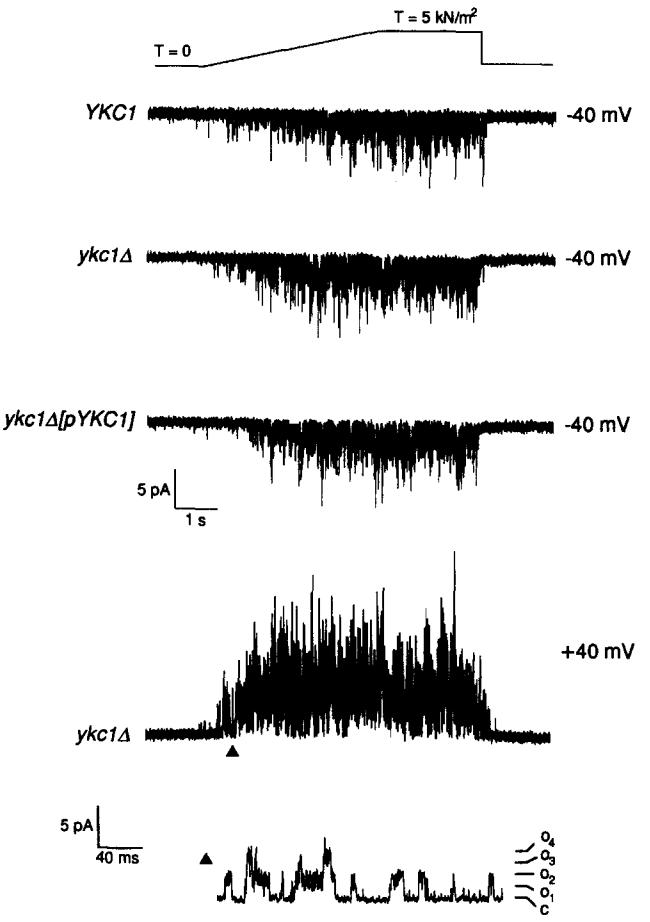

Fig. 3. Activities of the 40 pS mechanosensitive channels. Whole-cell membrane currents induced by pressure exerted on spheroplasts are shown. Top diagram: approximates the membrane tension as calculated from the applied pressure and cell diameter. Top current trace: *YKC1* (the *YKC1* wild-type parent). Second trace: *ykc1Δ* (the *YKC1*-deleted strain). Third trace: *ykc1Δ*[YKC1] (the *YKC1*-restored transformant strain). Top three traces were recorded at −40 mV. At +40 mV, pressure-induced currents convole with the K^+ current except when the latter is genetically deleted (Fourth trace, same spheroplast as the second trace). The Bottom trace shows at higher sweep speed the events marked with 'D' on the fourth trace, to show clearly the unit events; C = closed level, O_1 to O_4 = one to four-open levels. Pipets: 180 mM KCl, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.2. Bath: 20 mM KCl, 100 mM NaCl, 50 mM $MgCl_2$, 5 mM HEPES, pH 7.2.

[10,11] is obvious: it resembles a fusion of a *Sh*-like subunit at the N-terminal and a IRK1-type subunit at the C-terminal side (Fig. 5A). The region from residue 70 to 325 predicts 6 transmembrane domains (M1 through M6) with a pore-forming region (P1) between M5 and M6, as in *Sh*-type α subunits. There is considerable sequence homology between the P1–M6 portion of YKC1 and those of other *Sh*-like α subunits (Fig. 5B). The best match of this portion is with *elk*, the *eag*-like putative K^+ channel in *Drosophila*, with 28% identity and 77% similarity [20]. No significant matches of other regions can be detected between *YKC1* and other *Sh*-like α subunits, including the M4 domain, which appears to be a part but not the entirety of the voltage sensor in *Sh*-like channels [24]. The M4 of *YKC1* has only one basic residue, in contrast to the M4's of *Sh*-like a subunits which have several evenly spaced basic residues. The region between residue 383 and 455 of YKC1 predicts two

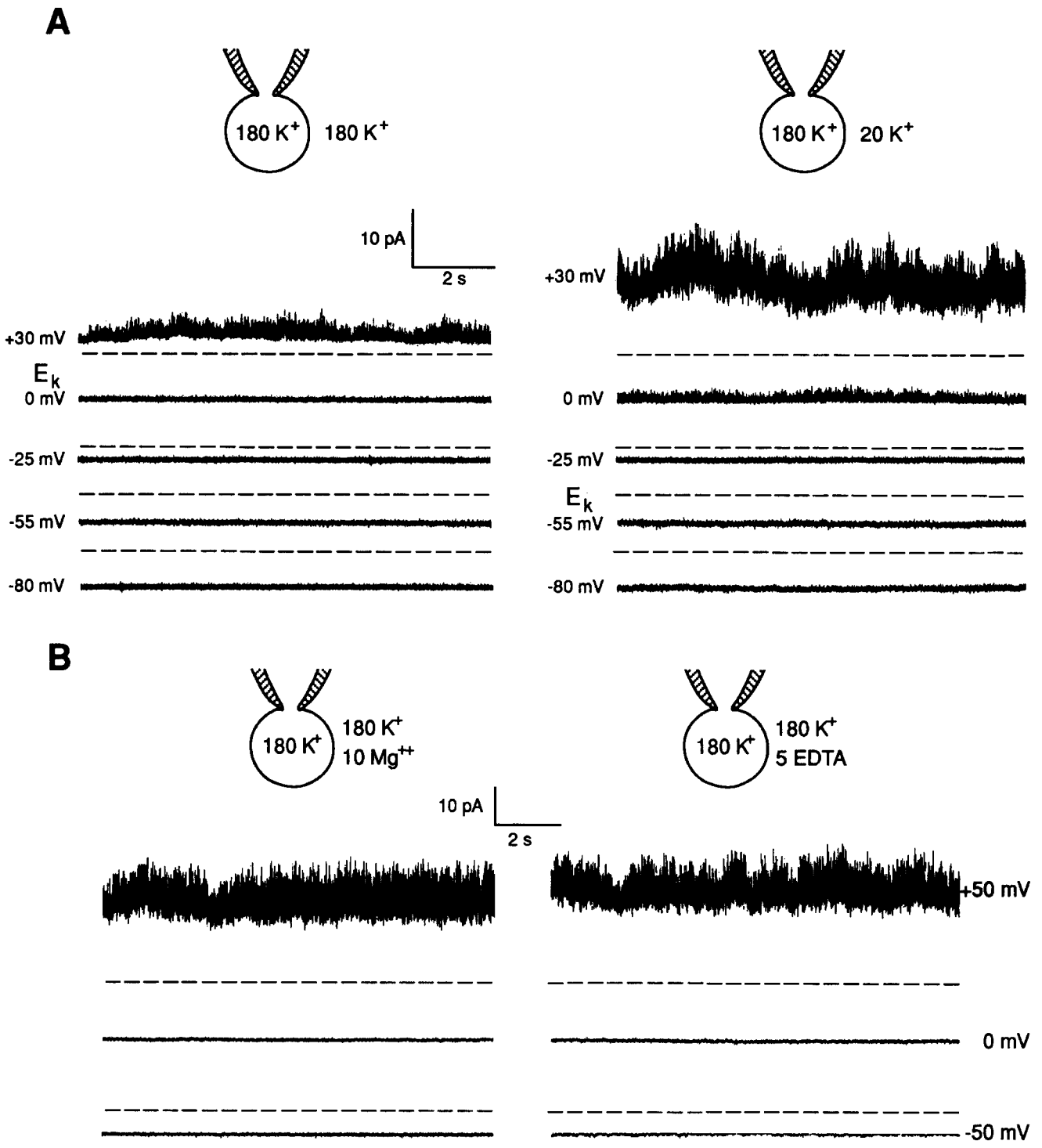

Fig. 4. The activation of the native yeast K^+ channel is largely independent on E_K and remains outwardly rectifying regardless of external concentrations of divalent cations. (A) Whole cell currents at the marked voltages from a spheroplast bathed in symmetric 180 mM K^+ (left) and after reducing external K^+ to 20 mM (right). Broken lines mark the zero-current levels. A depolarization from 0 to 30 mV clearly activates the currents in both cases, although the current is larger for the asymmetric condition because of the larger driving force. In this asymmetric condition, a 30 mV depolarization from −55 (=E_K) to −25 mV does not activate channel current. The same αW303 (wild type) spheroplast was recorded in whole-cell mode with pipet solution of 180 mM KCl, 0.5 mM EDTA, 5 mM HEPES, pH 7.2, and bath solution of the same composition, which was then changed to 20 mM KCl, 320 mM sorbitol, 0.5 mM EDTA, 5 mM HEPES, pH 7.2, by perfusion. (B) Whole-cell currents at the marked voltages from a different spheroplast bathed in the presence (left) and after depletion of external divalent cations (right). An αW303 spheroplast was recorded with the above pipet solution and a bath of 180 mM KCl, 10 $MgCl_2$, 5 mM HEPES, pH 7.2. The bath was then perfused thoroughly with 180 mM KCl, 5 mM EDTA, pH 7.2. Removal of divalent cations made only minimal differences. Note that no inward currents are activated upon hyperpolarizations.

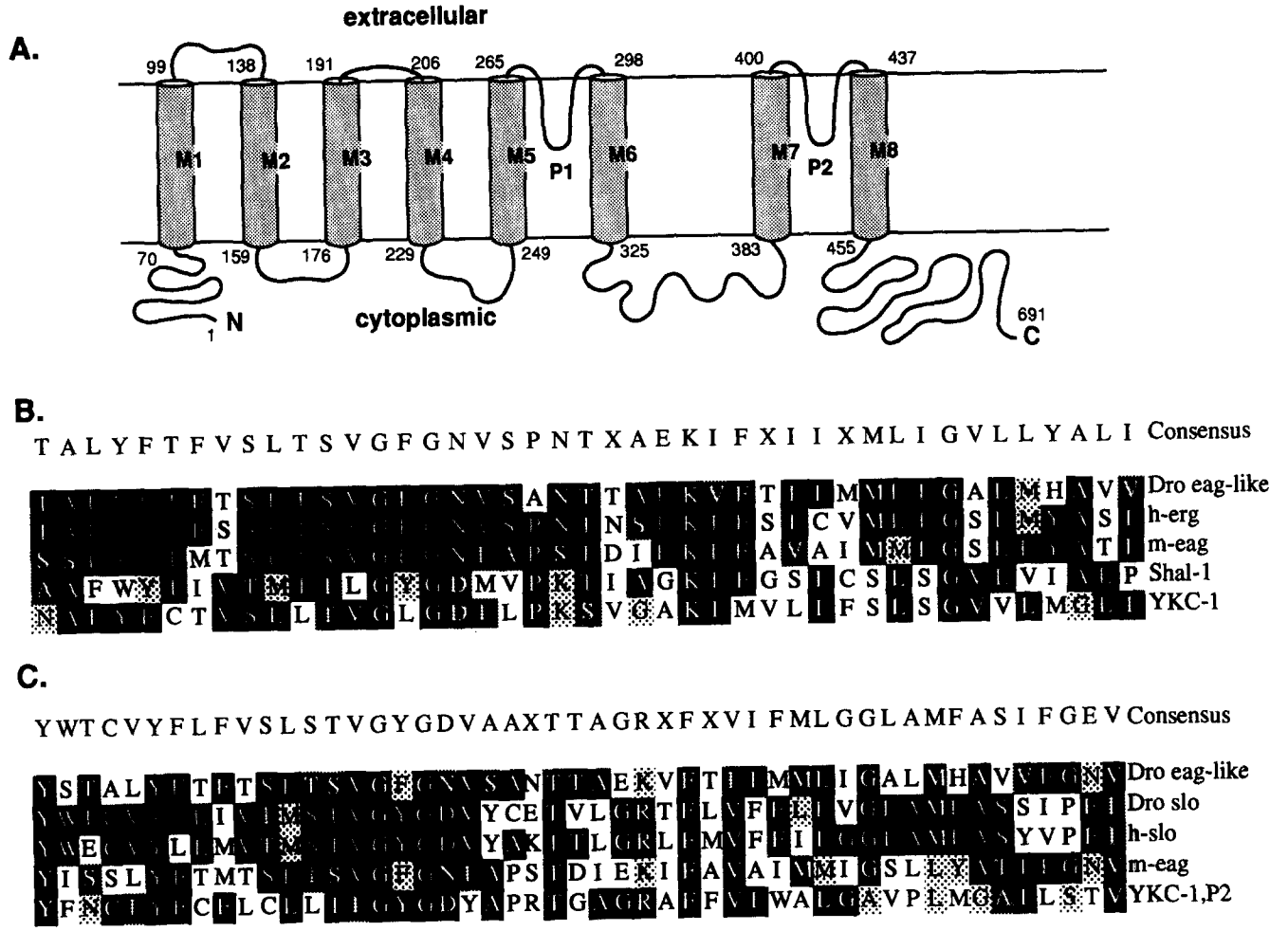

Fig. 5. Sequence analysis of the YKC1 protein. (A) A diagram showing the putative arrangement of the different domains of the YKC1 protein with respect to the plasma membrane. Numbered residues delimit the putative membrane spanning domains. These domains contain residues judged to be within the membrane by the Predict Protein Program [19]. (B) Comparison of amino acid sequence of the P1 and the adjacent part of M6 of YKC1 and the best fitting P and M6 sequences of other presumed K^+ channel proteins. The consensus sequence is shown on top. The degree of shading indicates similarity to this consensus; darkest indicates identity. Sequences are from *Drosophila elk* putative K^+ channel (Dro eag-like) [20], human *erg*K^+ channel (h-erg) [20], mouse *eag* K^+ channel (m-*eag*) [20], rat *Shal-1* K^+ channel (Shal-1) [21]. (C) Comparison of amino acid sequence of the P2 and part of the M8 domains of YKC1 with the P and M6 domains of the best fitting sequences of other presumed K^+ channel proteins in the database. Sequences are from *Drosophila elk* putative K^+ channel (Dro eag-like) [20], *Drosophila slowpoke* Ca^{2+}-activated K^+ channel (Dro slo) [22], human *slowpoke* homolog (h-slo) [23] and mouse *eag* K^+ channel (m-eag) [20].

transmembrane domains flanking a pore forming region (P2) as in the IRK-type subunits [25]. However, the sequence does not match significantly with IRK-type channels, but rather with the *eag*-like or *slowpoke*-type channels of *Drosophila* [20,22,23]. The homology is restricted to part of M7, P2, and M8, best matching the *Drosophila* Ca^{2+}-dependent K^+ channel (*slowpoke*) [22], with 31% identity and 75% similarity (Fig. 5C). Thus, neither the sequence analysis or the mechanism of activation of YKC1 in its native membrane (Fig. 4) indicate features akin to inward rectifiers [11].

In summary, we present strong evidence that the *YKC1* gene encodes the K^+ channel native to yeast plasma membrane. This discovery provides new opportunities for a deeper understanding of voltage-gated ion channels. For example, it might be possible to examine channel properties after randomly mutagenizing *YKC1* so as to gain insights into structure–function relations unencumbered by preconceived hypotheses that drive site-directed mutageneses. It might even be possible to collect milligrams of natively expressed K^+ channel protein for direct structural studies.

Acknowledgements: We thank T. Miosga for yeast strains, and J. Hendrick for the SUF14 expression plasmid, S.A.N. Goldstein for sharing results before publication. Supported by NIH GM 22714, and 36386.

References

[1] Jan L.Y. and Jan Y.-N. (1992) Annu. Rev. Physiol. 54, 537–555.
[2] Catterall, W.A. (1995) Annu. Rev. Biochem. 64, 493–531.
[3] Saimi, Y., Martinac, B., Preston, R.R., Zhou, X.-L. Sukharev, S., Blount, P. and Kung, C. (1994) in: Molecular Evolution of Physiological Processes (Fambrough, D. ed.) pp. 179–195, Rockefeller University Press, New York.
[4] Jegla, T. and Salkoff, L. (1995) Receptors and Channels 3, 51–60.
[5] Milkman, R. (1994) Proc. Natl. Acad. Sci. USA 91, 3510–3514.
[6] Gustin, M.C., Zhou, X.-L., Martinac, B. and Kung, C. (1988) Science 242, 762–765.
[7] Gustin, M.C., Martinac, B., Saimi, Y., Culbertson, R. and Kung, C. (1986) Science 233, 1195–1197.

[8] Bertl, A., Slayman, C.L. and Gradmann, D. (1993) J. Membrane Biol. 132, 183–199.
[9] Zhou, X.-L. (1995) unpublished observation.
[10] Miosga, T., Witzel, A. and Zimmermann, F.K. (1994) Yeast 10, 965–973.
[11] Ketchum, K.A., Joiner, W.J., Sellers, A.J., Kaczmarek, L.K. and Goldstein, S.A.N. (1995) Nature 376, 690–695.
[12] Jan, L.Y. and Jan. Y.N. (1994) Nature 371, 119–122.
[13] Sherman, F. (1991) in: Methods in Enzymology, vol. 194, Guide to Yeast Genetics and Molecular Biology (Guthrie, C. and Fink, G.R. eds.) pp. 3–21, Academic Press, San Diego, CA.
[14] Boeke, J.D., LaCroute, F. and Fink, G.R. (1984) Mol. Gen. Genet. 197, 345–346.
[15] Hill, J.E., Myers, A.M., Koerner, T.J. and Tzagoloff, A. (1993) Yeast 2, 163–167.
[16] Hendricks, J. (1995) The Characterization of SUF-13 and SUF-14: Two Frameshift Mutation Suppressors in *S. cerevisiae*, Ph.D. Dissertation, University of Wisconsin, Madison, WI.
[17] Saimi, Y., Martinac, B., Delcour, A.H., Minorsky, P.V., Gustin, M.C., Culbertson, M.R., Adler, J. and Kung, C. (1992) in: Methods in Enzymology, vol. 207, Ion Channels (Rudy, B. and Iverson, L.E. eds.) pp. 681–691, Academic Press, New York.
[18] Hamill, O.P., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.J. (1981) Pluegers Arch. 391, 95–100.
[19] Rost, R., Casadio, R., Fariselli, P. and Sander, C. (1995) Protein Sci. 4, 521–533.
[20] Warmke, J.W. and Ganetzky, B. (1994) Proc. Natl. Acad. Sci. USA 91, 3438–3442.
[21] Baldwin, T.J., Tsaur, M.L., Lopez, G.A., Jan, Y.N. and Jan, L.Y. (1991) Neuron 7, 471–483.
[22] Atkinson, N., Robertson, G. and Ganetzky, B. (1991) Science 253, 551–555.
[23] Tseng-Crank, J., Foster, C.D., Krause, J.D., Mertz, R., Godinot, N., DiChiara, T.J. and Reinhart, P.H. (1994) Neuron 13, 1315–1330.
[24] Papazian, D.M., Timpe, L.C., Jan Y.-N. and Jan L.Y. (1991) Nature 349, 305–310.
[25] Kubo, Y., Baldwin, T.J., Jan Y.-N. and Jan, L.Y. (1993) Nature 362, 127–133.