JCI The Journal of Clinical Investigation

# Spontaneously oscillating K+ channel activity in transformed Madin-Darby canine kidney cells.

A Schwab, … , S Wünsch, H Oberleithner

*J Clin Invest.* 1993;92(1):218-223. https://doi.org/10.1172/JCI116553.

**Research Article**

Intracellular alkalinization is known to be associated with tumorigenic transformation. Besides phenotypical alterations alkali-transformed Madin-Darby canine kidney (MDCK) cells exhibit a spontaneously oscillating cell membrane potential (PD). Using single-channel patch clamp techniques, it was the aim of this study to identify the ion channel underlying the rhythmic hyperpolarizations of the PD. In the cell-attached patch configuration, we found that channel activity was oscillating. The frequency of channel oscillations is 1.1 +/- 0.1 min-1. At the peak of oscillatory channel activity, single-channel current was -2.7 +/- 0.05 pA, and in the resting state it was -1.95 +/- 0.05 pA. Given the single-channel conductance of 53 +/- 3 pS for inward (and of 27 +/- 5 pS for outward) current the difference of single-channel current amplitude corresponded to a hyperpolarization of approximately 14 mV. The channel is selective for K+ over Na+. Channel kinetics are characterized by one open and by three closed time constants. The channel is Ca2+ sensitive. Half maximal activation in the inside-out patch mode is achieved at a Ca2+ concentration of 10 mumol/liter. In addition, we also found a 13-pS K+ channel that shows no oscillatory activity in the cell-attached patch configuration and that was not Ca2+ sensitive. We conclude that the Ca(2+)-sensitive 53-pS K+ channel is underlying spontaneous oscillations of the PD. It has virtually identical biophysical […]



# Spontaneously Oscillating $K^+$ Channel Activity in Transformed Madin-Darby Canine Kidney Cells

**Albrecht Schwab, Hans-Jürgen Westphale,* Leszek Wojnowski, Stefan Wünsch, and Hans Oberleithner**
*Department of Physiology, University of Würzburg, D-W-8700 Würzburg, Germany; and *Medizinische Hochschule Hannover, Zentrum für Innere Medizin, Abteilung für Nephrologie, D-W-3000 Hannover 61, Germany*

## Abstract

**Intracellular alkalinization is known to be associated with tumorigenic transformation. Besides phenotypical alterations alkali-transformed Madin-Darby canine kidney (MDCK) cells exhibit a spontaneously oscillating cell membrane potential (PD). Using single-channel patch clamp techniques, it was the aim of this study to identify the ion channel underlying the rhythmic hyperpolarizations of the PD. In the cell-attached patch configuration, we found that channel activity was oscillating. The frequency of channel oscillations is 1.1±0.1 $min^{-1}$. At the peak of oscillatory channel activity, single-channel current was −2.7±0.05 pA, and in the resting state it was −1.95±0.05 pA. Given the single-channel conductance of 53±3 pS for inward (and of 27±5 pS for outward) current the difference of single-channel current amplitude corresponded to a hyperpolarization of ~ 14 mV. The channel is selective for $K^+$ over $Na^+$. Channel kinetics are characterized by one open and by three closed time constants. The channel is $Ca^{2+}$ sensitive. Half maximal activation in the inside-out patch mode is achieved at a $Ca^{2+}$ concentration of 10 μmol/liter. In addition, we also found a 13-pS $K^+$ channel that shows no oscillatory activity in the cell-attached patch configuration and that was not $Ca^{2+}$ sensitive. We conclude that the $Ca^{2+}$-sensitive 53-pS $K^+$ channel is underlying spontaneous oscillations of the PD. It has virtually identical biophysical properties as a $Ca^{2+}$-sensitive $K^+$ channel in nontransformed parent MDCK cells. Hence, alkali-induced transformation of MDCK cells did not affect the channel protein itself but its regulators thereby causing spontaneous fluctuations of the PD. (*J. Clin. Invest.* 1993. 91:218–223.) Key words: Madin-Darby canine kidney cell • transformation • $K^+$ channel • $Ca^{2+}$ • oscillation**

## Introduction

It is well known that cells alkalinize during proliferation (see reference 1 for a review). Moreover, intracellular alkalinization is also recognized as a possible pathogenetic mechanism of carcinogenesis (2). Madin-Darby canine kidney (MDCK)[1] cells, a renal epithelial cell line derived from collecting duct of dog kidney, could be transformed by prolonged exposure to an alkaline growth medium (3). Alkali-induced transformation of MDCK cells resulted in dramatic changes of cell morphology with loss of contact inhibition and pronounced pleomorphism. Some of these acquired morphological characteristics have also been described for MDCK cells transformed with viral ras-oncogene (4) or with viral tyrosine kinase $pp60^{v\text{-}src}$ (5). Functionally, alkali-transformed MDCK cells, hereafter referred to as MDCK-F cells according to their ability to form foci, are distinguished from their parental cells by a spontaneously oscillating cell membrane potential (6) and by constant migratory activity (7). In contrast, parent MDCK cells have a constant cell membrane potential (8, 9), and they do not migrate.

Transformation also induces oscillations of the cell membrane potential in fibroblasts. Whereas nontransformed fibroblasts only transiently hyperpolarize after the addition of bradykinin, ras-transformed fibroblasts exhibit sustained oscillations of the cell membrane potential and of intracellular $Ca^{2+}$ concentration (10, 11).

Using single-channel patch clamp techniques, we wanted to identify the putative $K^+$ channel underlying the rhythmic hyperpolarizations of the cell membrane potential in alkali-transformed MDCK cells. Microelectrode studies suggested a $Ca^{2+}$-activated $K^+$ channel (6). Such a channel has been described in nontransformed MDCK cells, too, and its regulation has been studied extensively (12, 13). Furthermore, it is known that ras-transformation of fibroblasts leads to the expression of a novel $Ca^{2+}$-activated $K^+$ current (14). Therefore, we were particularly interested in the properties of the putative $Ca^{2+}$-activated $K^+$ channel in alkali-transformed MDCK cells. We raised the following questions: Is it a novel channel? Is it a modified form, or is it the "original" channel as it is found in nontransformed parent MDCK cells?

Our results show that alkali-transformed MDCK cells express the "original" $Ca^{2+}$-activated $K^+$ channel, thereby implying modifications of its regulators.

## Methods

*Cell culture.* Experiments were carried out on MDCK-F cells. As described in detail (3), transformation of MDCK cells was achieved by prolonged exposure of the cells to an alkaline growth medium (pH 7.7). Both cell types, nontransformed parent MDCK cells and transformed MDCK-F cells, are shown in Fig. 1. Like the parent cells, MDCK-F cells were kept under standard cell culture conditions at 37°C in humidified air containing 5% $CO_2$. Cells were grown in bicarbonate-buffered MEM with Earle's salts (Biochrom KG, Berlin, Germany) supplemented with 10% FCS (Biochrom). When MDCK-F cells had reached subconfluency they were in part passaged and in part seeded in low density on small coverslips loaded with poly-L-lysine (0.1 g/liter) (Serva, Heidelberg, Germany). Cells on these small coverslips could be transferred to the stage of an inverted microscope (ID 02 MT; Zeiss, Oberkochen, Germany) for patch clamp experiments. Experiments were carried out on day 1 and 2 after seeding of the cells.

Address correspondence to Albrecht Schwab, Department of Physiology, Röntgenring 9, D-W-8700 Würzburg, Germany.

*Received for publication 17 August 1992 and in revised form 5 February 1993.*

1. *Abbreviations used in this paper:* MDCK, Madin-Darby canine kidney (cells); MDCK-F, alkali-transformed MDCK cells.

J. Clin. Invest.

0021-9738/93/07/218/06 $2.00
Volume 92, July 1993, 218–223

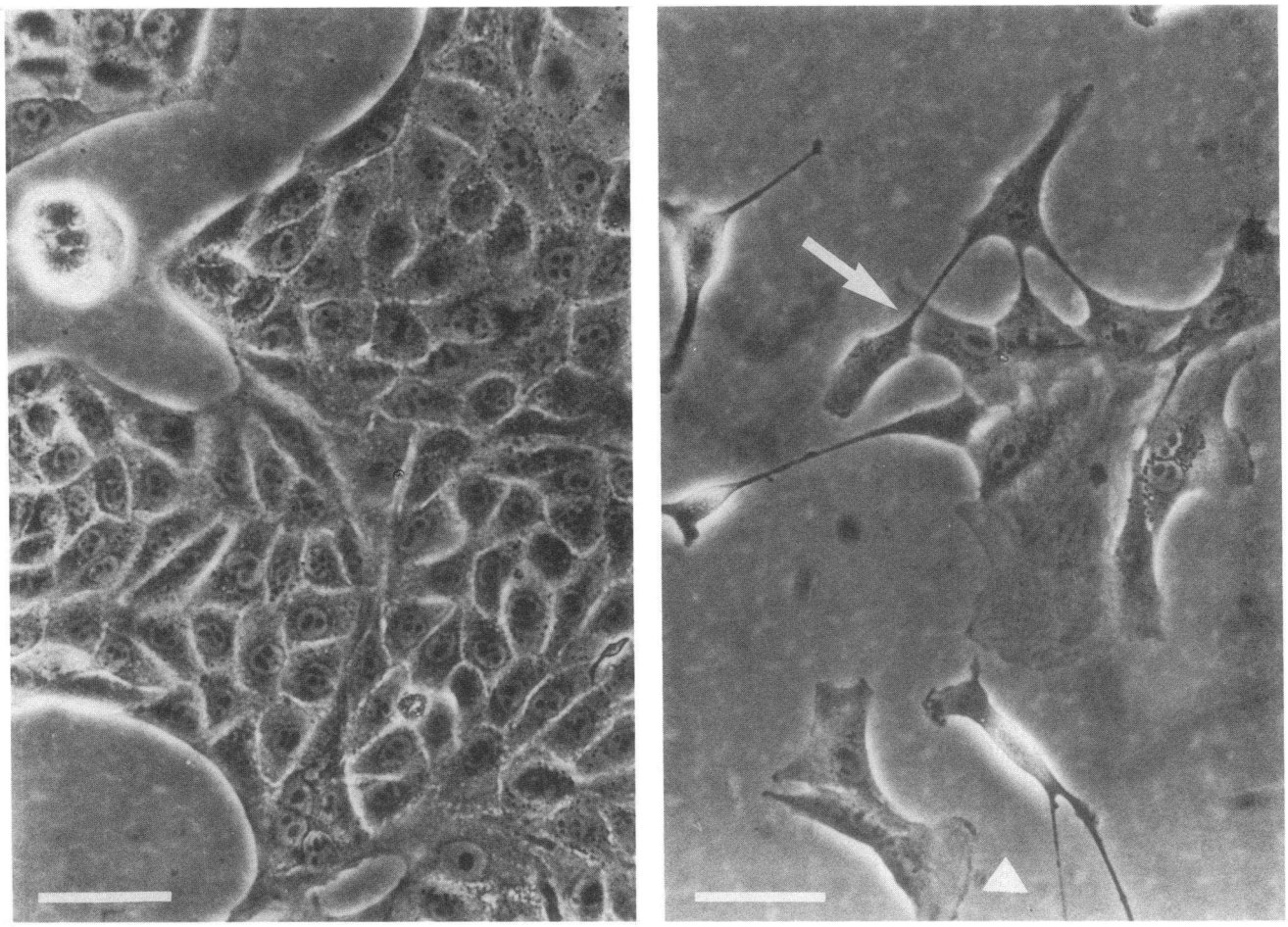

*Figure 1.* Comparison of nontransformed parent MDCK cells (*A*) and alkali-transformed MDCK-F cells (*B*). Note the dramatically altered morphology of alkali-transformed MDCK cells. In contrast to "normal" MDCK cells, MDCK-F cells no longer form epithelial layers, they lack contact inhibition, and grow over each other (*arrow*). Moreover, they are moving over the bottom of the culture flask indicated by the ruffled membrane areas (*arrowhead*).

*Experimental solutions.* Cells were bathed during all experiments in Ringer's solution of the following composition (mmol/liter): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 5 glucose, 10 Hepes, titrated to pH 7.4 with 1 mol/liter NaOH (control Ringer's). For low $Ca^{2+}$ Ringer's solutions (100 nmol/liter–50 μmol/liter), we used 1.3 mmol/liter EGTA as $Ca^{2+}$ buffer and calculated the amount of $Ca^{2+}$ necessary to yield the desired free $Ca^{2+}$ activities. The pipette solution contained (mmol/liter): 140 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes, titrated with 1 mol/liter KOH to pH 7.4. All experiments were carried out at 37°C.

*Patch clamp technique.* Patch clamp experiments were performed according to the methods described by Hamill et al. (15). Pipettes, pulled from micropipettes (Hirschmann Laborgeräte, Eberstadt, Germany), had resistances of 6–10 MΩ when filled with 140 mmol/liter KCl. Recordings were made using a patch clamp amplifier (L/M-EPC7; List Medical Electronic, Darmstadt, Germany). Single-channel currents were filtered at 1.5 kHz by an eight-pole Bessel filter (902 LPF; Frequency Devices Inc., Haverhill, MA). Data were stored on a video cassette recorder (JVC HR-D430 EG; Victor Company, Japan) via a digital pulse code modulator (PCM-501 ES; Sony). For data analysis, digitized current records were read into an IBM-compatible computer via an interface (TL-1 DMA; Axon Instruments Inc., Foster City, CA) and analyzed with software (pclamp; Axon Instruments Inc.).

Open probability $P_o$ was determined according to the equation:

$$P_o = \sum_{n=1}^{N}(t_n \times n)/(N \times T)$$

where $t_n$ is the dwell time spent at current levels corresponding to $n = 1, 2, \cdots N$ channels in the open state, $n$ is the number of open channels, $N$ the total number of active channels in the patch, and $T$ is the total time of the analyzed record. Current amplitudes were determined by fitting Gaussian distribution curves to amplitude histograms.

For kinetic analysis, histograms for open and closed dwell times were created, and time constants were determined by performing maximal likelihood fitting with one or more exponentials for the open and closed time histograms, respectively (16).

*Statistics.* All values are given as mean±SEM. Paired or unpaired Student's $t$ test was performed where applicable. Significance was assumed when $P < 0.05$.

## Results

In a first series of experiments, we looked for spontaneous channel activity in the cell-attached patch configuration. Using identical experimental solutions (bath, control Ringer's solution; pipette, 140 mmol/liter KCl) as in our previous study performed in parent MDCK cells (13), we found, in contrast to the former ones, spontaneous rhythmic channel activity. Fig. 2 provides an example of a representative experiment. Periods with high channel activity can be clearly separated from periods in which the channels remain closed. In this example, at least four channels are intermittently activated.

To correlate the observed rhythmic channel activity with

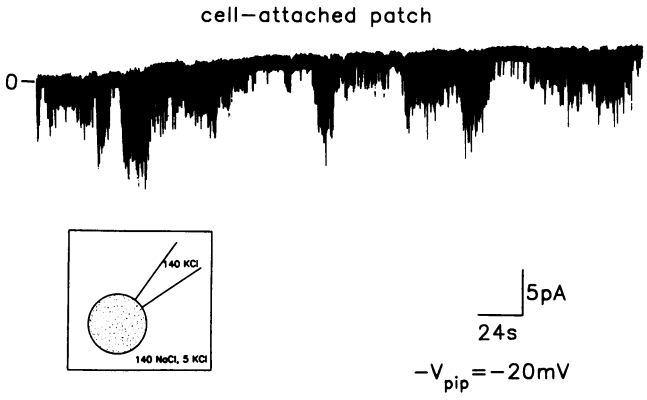


*Figure 2.* Oscillating activity of at least four channels recorded in the cell-attached patch configuration. Periods with high channel activity are alternating with periods in which channels remain closed. A second channel of lower current amplitude can be seen as small deflection of the baseline current. There is no clear evidence for oscillating activity of this channel. Channel openings are shown as downward deflections from closed state (*0*).

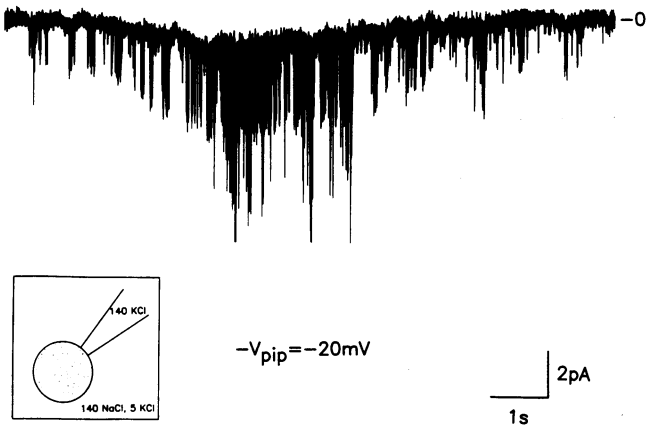


*Figure 4.* Period of high channel activity displayed at high time resolution in the cell-attached patch configuration. In parallel to an increase of channel activity, the single channel current amplitude rises from −2 to ~ −3 pA thereby reflecting the simultaneous hyperpolarization of the cell membrane potential. The cell membrane under the patch was hyperpolarized by 20 mV to better visualize the current flow. Channel openings are shown as downward deflections from closed state (*0*).

oscillations of the cell membrane potential (6), we next analyzed whether both (i.e., cell membrane potential and $K^+$ channels) are oscillating with a similar frequency. These results are summarized in Fig. 3. We measured a frequency of 1.1±0.1 $min^{-1}$ ($n$ = 16) for oscillations of channel activity. This is virtually the same frequency as previously measured for oscillations of the cell membrane potential (6), thereby indicating a close correlation between channel events and hyperpolarizations of the cell membrane potential.

When the cell membrane potential hyperpolarizes, the driving force for $K^+$ ions to move from the pipette into the cell will increase, and the single channel amplitude should rise. In our experiments, we determined the following values for single-channel current amplitudes during periods of high channel activity and during periods with low channel activity. During high activity that is at the peak of an oscillation, single-channel current amounted to −2.7±0.05 pA, whereas in the resting state, single-channel current was −1.95±0.05 pA. Given a single-channel conductance of 53 pS (see below), this difference of current amplitude approximately corresponded to a hyperpolarization of the cell membrane potential of 14 mV. This is in the same order of magnitude as the cell membrane potential fluctuations measured with microelectrodes (6). Moreover, values for single channel current amplitudes also correlate well with the absolute value of cell membrane potential.

Fig. 4 provides a recording of a period of high channel activity in an expanded time scale. It is clearly visible that the single-channel current amplitude dramatically increases when the channels become active.

In Fig. 5, this phenomenon is shown in a quantitative way. It demonstrates the analysis of an experiment in which oscillatory channel activity was observed in the cell-attached patch configuration. The upper panel shows the channel open probability as a function of time and the lower panel displays the single-channel current amplitude at the same time. Each point

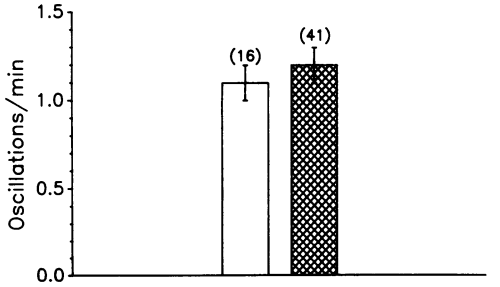


*Figure 3.* Frequency of oscillations of channel activity (left bar) and of oscillations of the cell membrane potential (right bar). The mean value for the cell membrane potential is taken from Westphale et al. (6). Briefly summarized, MDCK-F cells were fused to giant cells according to the protocol previously described (8) and plated on coverslips. 24–72 h after fusion cells were impaled with microelectrodes filled with 1 mol/liter KCl and the cell membrane potential was recorded continuously. During an experiment cells were superfused with Ringer's solution. Both techniques yield virtually identical values indicating that $K^+$ channel oscillations displayed in Fig. 2 correspond to oscillations of the cell membrane potential. □, Patch clamp; ■, microelectrode.

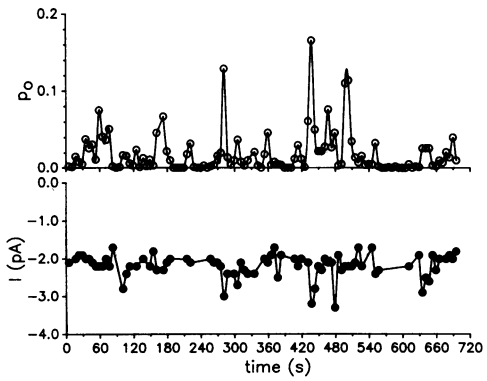


*Figure 5.* Simultaneous analysis of channel open probability and of single channel current amplitude for a cell-attached patch recording with oscillating channel activity. Each data point represents the mean value for 5 s. Correlation between open probability and single channel current amplitude is statistically significant.

represents the mean value for a time interval of 5 s. As expected, open probability and single-channel current amplitude are positively correlated ($P < 0.05$). We tested for correlation between open probability and single-channel amplitude in all experiments. In all but one, we found a positive correlation ($P < 0.05$). This further underlines that it is the observed intermittently active channel that is responsible for the oscillations of the cell membrane potential.

After demonstrating the presence of oscillating channel activity, we wanted to characterize this channel and compare its properties with a channel from parent MDCK cells. First, we determined the conductance of the channel. In the cell-attached patch configuration, we measured a slope conductance of 53±3 pS for inward current (pipette potential ranged from $-V_{pip} = -20$ to $-60$ mV) and of 27±5 pS for outward current (pipette potential ranged from $-V_{pip} = 40$ to 60 mV). Fig. 6 represents the corresponding current-voltage relationship of seven experiments. These are virtually identical conductances as described for the $K^+$ channel from normal MDCK cells. Inward rectification had also been confirmed in exised patches using symmetrical solutions with 140 mmol/liter KCl (13).

Given the resting cell membrane potential of −20–−25 mV (6) and the composition of the pipette solution (140 mmol/liter KCl), the reversal potential of 20 mV is indicative of a $K^+$ channel. This is confirmed in experiments using the inside-out patch configuration. The channel is selective for $K^+$ over $Na^+$. The relative channel selectivity was determined from the shift of the reversal potential (~ 40 mV) after a change of the bath solution from control Ringer's (140 mmol/liter NaCl, 5 mmol/liter KCl) to one containing 140 mmol/liter KCl. The selectivity ratio $K^+/Na^+$ is ≥ 5:1 ($n = 5$), which is the same value as in parent MDCK cells.

We then examined the kinetic behavior of the channel in the inside-out patch configuration with control Ringer's solution containing 1 mmol/liter $Ca^{2+}$ in the bath. Again, we found great similarities between the $K^+$ channels from parent and from transformed MDCK-F cells. Under these conditions, channel kinetics are characterized by brief channel openings and closures interrupted by intermediate and rarely by long-lasting channel closures. We determined one open time constant ($t_{open}$) and three closed time constants ($t_{c1-3}$) ($n = 4$): $t_{open}$ = 11.3±4 ms; $t_{c1}$ = 1.5±0.2 ms, $t_{c2}$ = 9.8±1.8 ms, $t_{c3}$ = 106.6±27.7 ms.

So far, our measurements indicate that transformed MDCK cells express the same $K^+$ channel as parent MDCK cells. In parent MDCK cells, $K^+$ channel activity shows a strong $Ca^{2+}$ dependence (13). Microelectrode studies suggested a role of intracellular $Ca^{2+}$ in triggering the rhythmic activation of $K^+$ channels in MDCK-F cells. Therefore, it was of great importance to examine the $Ca^{2+}$ sensitivity of the $K^+$ channel in transformed MDCK-F cells. Using the inside-out patch configuration, we exposed the intracellular surface of the patch membrane to varying $Ca^{2+}$ concentrations ranging from ~ 10 pmol/liter (nominally $Ca^{2+}$ free plus 1.3 mmol/liter EGTA) to 1 mmol/liter. The results of these experiments are summarized in Fig. 7. The channel is virtually closed at a free $Ca^{2+}$ activity of 100 nmol/liter and reaches its half maximal activity at a $Ca^{2+}$ concentration of 10 μmol/liter. Hence, $K^+$ channels in MDCK-F cells exhibit a 10 times higher $Ca^{2+}$ sensitivity than $K^+$ channels from parent MDCK cells (13).

In addition to the 53-pS $Ca^{2+}$-sensitive $K^+$ channel, we found a second $K^+$ channel with a slope conductance of 13±2 pS. This channel can be seen in Fig. 2 as small deflections of the baseline current and in Fig. 8 recorded in the inside-out patch configuration. We could find no evidence for oscillating activity of this channel in the cell-attached patch mode. Moreover, as evident from Fig. 8, in inside-out patches it showed no $Ca^{2+}$ sensitivity. Hence, it is unlikely that the small $K^+$ channel is underlying the oscillating cell membrane potential.

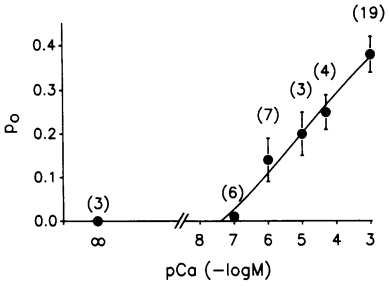


*Figure 7.* Dose response curve of open probability for $Ca^{2+}$ for the 53-pS $K^+$ channel recorded in the inside-out patch configuration. The channel is virtually closed at a $Ca^{2+}$ concentration of 100 nmol/liter and it is half maximally activated at 10 μmol/liter. The number of experiments given in parentheses. ∞ is nominally $Ca^{2+}$ free with 1.3 mmol/liter EGTA.

## Discussion

MDCK cells transformed by sustained alkaline stress have acquired new characteristic properties which clearly distinguish them from "normal," nontransformed MDCK cells (3).

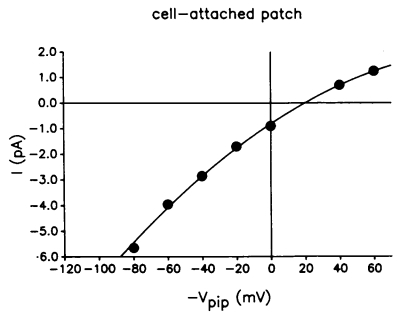


*Figure 6.* Current-voltage relation for a $K^+$ channel recorded in seven experiments in the cell-attached patch configuration. Current is plotted as function of varying pipette potential. Curve fitting done according to least square method (Sigmaplot 3.1; Jandel Scientific, Corte Madera, CA). Error bars are within the symbols.

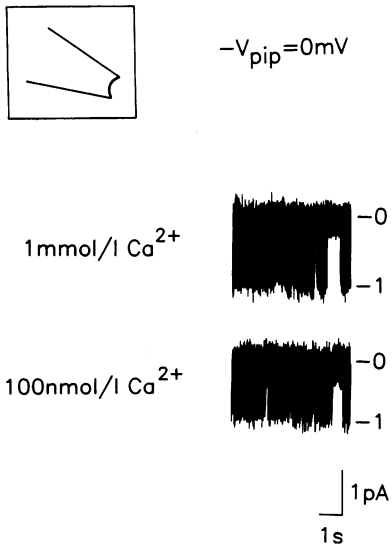


*Figure 8.* Recording of the small $K^+$ channel in the inside-out patch configuration. In this experiment, the channel was exposed to 1 mmol/liter and 100 nmol/liter $Ca^{2+}$ to demonstrate its $Ca^{2+}$ insensitivity. Channel openings shown as downward deflections from the closed state 0. Bath: Ringer's solution; pipette: 140 mmol/liter KCl.

MDCK-F cells lack contact inhibition, they migrate over the bottom of the culture flasks (7) and, most importantly in the context of this study, they exhibit an unstable cell membrane potential with spontaneous hyperpolarizations (6). In this study we characterized the $K^+$ channel underlying the instability of the cell membrane potential. We identified a $Ca^{2+}$-sensitive $K^+$ channel with almost identical biophysical properties as the $K^+$ channel from nontransformed MDCK cells. This also includes the voltage insensitivity of the channel in the physiological voltage range (results not shown). One could have anticipated a model in which the channel was activated at depolarized (i.e., −20 mV) and inactivated at more hyperpolarized cell membrane potentials (i.e., −40 mV) thereby causing periodic fluctuations of the cell membrane potential. However, the rhythmic changes of channel open probability cannot be accounted for by a voltage dependence of the channel. Therefore, the oscillating $K^+$ channel activity cannot be explained on the basis of a modification of the biophysical properties of the channel protein itself.

Our experiments rather point to modified regulators of the $K^+$ channel in transformed MDCK cells. In parent MDCK cells, the $K^+$ channel is regulated by protein kinase C, which dramatically increases its $Ca^{2+}$ sensitivity in the inside-out patch configuration (13). The notion of a modified regulation of the $K^+$ channel in MDCK-F cells is supported by the following observations: In parent MDCK cells no spontaneous channel activity is present in the cell-attached patch configuration. The $Ca^{2+}$-sensitive $K^+$ channel has to be activated by agonists such as serotonin or minoxidil sulfate which exert their effect via the PKC signalling pathway (12, 13). In contrast, in transformed MDCK-F cells, the $Ca^{2+}$-sensitive $K^+$ channel shows spontaneous activity in an oscillatory pattern. However, rhythmically changing activity of the $K^+$ channel is not observed in the cell-free inside-out patch configuration (results not shown). Therefore, we have to postulate an intracellular trigger that intermittently activates the $K^+$ channel in MDCK-F cells.

The trigger that causes activation of the $K^+$ channel is most likely intracellular $Ca^{2+}$. This hypothesis is supported by the finding that oscillations of the cell membrane potential cease when $Ca^{2+}$ is removed from the bathing medium (6). Moreover, the $Ca^{2+}$ dependence of the $K^+$ channel also points to this possibility. Direct evidence for the involvement of intracellular $Ca^{2+}$ comes from experiments in which the fluorescent $Ca^{2+}$-sensitive dye fluo-3 was used for monitoring intracellular $Ca^{2+}$. It could be demonstrated that the intensity of the fluorescence signal is not stable, but also exhibits spontaneous oscillations of similar frequency as the $K^+$ channel activity (17). These $Ca^{2+}$ measurements also provide strong support for the contention that the small, $Ca^{2+}$-insensitive $K^+$ channel is not responsible for the oscillations of the cell membrane potential. Simultaneous oscillations of $K^+$ channel activity and of intracellular $Ca^{2+}$ have also been found in mammary epithelial cells (18).

Our experiments do not allow to identify the primary "motor" for the oscillations. However, our observations of oscillating $K^+$ channels in MDCK-F cells and of biophysically identical $K^+$ channels in parent MDCK cells that are active only upon application of agonists, are quite similar as results from ras-transformed fibroblasts (19). In $ras^+$ fibroblasts, bradykinin causes oscillations of the cell membrane potential by periodic activation of $Ca^{2+}$-sensitive $K^+$ channels whereas $ras^-$ fibroblasts only respond with a transient hyperpolarization. In $ras^+$ fibroblasts elevated levels of 1,4,5-inositol-triphosphate were found. Moreover, in $ras^-$ fibroblasts pretreated with lithium, elevated levels of 1,4,5-inositol-triphosphate were found and bradykinin also caused oscillations of the cell membrane potential. These experiments provide strong evidence for the involvement of the protein kinase C signaling pathway in generating oscillations of the cell membrane potential in ras-transformed NIH 3T3 fibroblasts. They are in good agreement with experiments in ras-transformed rat fibroblasts in which activation of protein kinase C and enhancement of phosphoinositol metabolism was observed (20).

Interestingly, in normal MDCK cells oleoylacetylglycerol, a synthetic activator of protein kinase C, elicits oscillatory activity of the $Ca^{2+}$-sensitive $K^+$ channel, too (13). It is therefore tempting to speculate that protein kinase C also plays a crucial role in modulating spontaneously oscillating $K^+$ channel activity in alkali-transformed MDCK cells. Further experiments are needed to determine whether the increased $Ca^{2+}$ sensitivity of the 53 pS $K^+$ channel can be accounted for by a permanent activation of protein kinase C in alkali-transformed MDCK cells.

As already pointed out, transformation of MDCK cells does not affect the biophysical properties of the channel protein. Similarly, normal and carcinomatous murine mammary carcinoma cells also express the same $Ca^{2+}$-activated $K^+$ channel. In normal mammary epithelial cells, oscillatory $K^+$ channel activity can be induced by epidermal growth factor, whereas $K^+$ channel activity is spontaneously oscillating in carcinomatous mammary cells (21, 22). These authors discussed the possibility that oscillating $K^+$ channels might be related to the proliferation of mammary cells. This would be consistent with observations made in fibroblasts in which mitogens such as FCS induce oscillations of intracellular $Ca^{2+}$- and of a $Ca^{2+}$-sensitive $K^+$ current (23). It is also well known that cell divisions of oocytes are accompanied by oscillations of the cell membrane potential and of intracellular $Ca^{2+}$ (24).

We do not believe that oscillating $K^+$ channel activity is related to altered proliferation of MDCK-F cells, since growth of nontransformed MDCK cells and of MDCK-F cells does not differ when observed for 8 d (unpublished observation from our laboratory). However, we have evidence that oscillatory $K^+$ channel activity is related to migration of MDCK-F cells. MDCK-F cells are spontaneously migrating over the bottom of the culture flask at a rate of 0.9 $\mu$m/min (7). In contrast, nontransformed MDCK cells whose cell membrane potential is not oscillating do not change their position when observed over extended time periods. Moreover, recent experiments demonstrate that $K^+$ channel blockers such as barium or tetraethylammonium (7) also interfere with cell motility, thereby pointing to the functional link between $K^+$ channel oscillations and migration. In human neutrophils, a correlation between oscillations and migration was postulated, too. Here, transient increases of intracellular $Ca^{2+}$ were found to be required for the cells to migrate (25).

In summary, we identified a 53-pS $Ca^{2+}$-sensitive $K^+$ channel as underlying spontaneous oscillations of the cell membrane potential of alkali-transformed MDCK-F cells. Nontransformed parent MDCK cells express a virtually identical $K^+$ channel. We conclude that oscillations of the cell membrane potential of alkali-transformed MDCK cells cannot be explained on the basis of a modification of the biophysical properties of the $Ca^{2+}$-sensitive $K^+$ channel, but rather by a modification of its regulators.

## Acknowledgments

We thank Drs. S. Silbernagl and M. Hunter for fruitful discussions during the course of the experiments, and Dr. G. Giebisch for introducing A. Schwab to patch clamp technology. We are grateful to Mrs. B. Schuricht and B. Gassner for their skillful technical assistance, and to Mrs. M. Schulze for preparing the figures.

This work was supported by Deutsche Forschungsgemeinschaft, SFB 176, A6.

## References

1. Moolenaar, W. H., A. J. Biermann, and S. W. de Laat. 1987. Effects of growth factors on $Na^+/H^+$ exchange. *In* $Na^+/H^+$ Exchange. S. Grinstein, editor. CRC Press, Inc., Boca Raton, FL 227–234.

2. Perona, R., F. Portillo, F. Giraldez, and R. Serrano. 1990. Transformation and pH homeostasis of fibroblasts expressing yeast $H^+$-ATPase containing site-directed mutations. *Mol. Cell. Biol.* 10:4110–4115.

3. Oberleithner, H., H.-J. Westphale, and B. Gassner. 1991. Alkaline stress transforms Madin-Darby canine kidney cells. *Pfluegers Arch.* 419:418–420.

4. Schoenenberger, C.-A., A. Zuk, D. Kendall, and K. S. Matlin. 1991. Multilayering and loss of apical polarity in MDCK cells transformed with viral K-*ras*. *J. Cell Biol.* 112:873–889.

5. Warren, S. L., and W. J. Nelson. 1987. Nonmitogenic morphoregulatory action of $pp60^{v\text{-}src}$ on multicellular epithelial structures. *Mol. Cell. Biol.* 7:1326–1337.

6. Westphale, H.-J., L. Wojnowski, A. Schwab, and H. Oberleithner. 1992. Spontaneous membrane potential oscillations in Madin-Darby canine kidney cells transformed by alkaline stress. *Pfluegers Arch.* 421:218–223.

7. Schwab, A., H.-J. Westphale, L. Wojnowski, S. Wünsch, and H. Oberleithner. 1992. Oscillating $K^+$ channels in spontaneously migrating alkali-transformed MDCK cells. *J. Am. Soc. Nephrol.* 3:819 (Abstr.)

8. Kersting, U., L. Wojnowski, W. Steigner, and H. Oberleithner. 1991. Hypotonic stress-induced release of $KHCO_3$ in fused renal epitheloid (MDCK) cells. *Kidney Int.* 39:891–900.

9. Stefani, E., and M. Cereijido. 1983. Electrical properties of cultured epitheloid cells (MDCK). *J. Membr. Biol.* 73:177–184.

10. Lang, F., F. Friedrich, E. Kahn, E. Wöll, M. Hammerer, S. Waldegger, K. Maly, and H. Grunicke. 1991. Bradykinin-induced oscillations of the cell membrane potential in cells expressing the Ha-ras oncogene. *J. Biol. Chem.* 266:4938–4942.

11. Wöll, E., S. Waldegger, F. Lang, K. Maly, and H. Grunicke. 1992. Mechanism of intracellular calcium oscillations in fibroblasts expressing the ras oncogene. *Pfluegers Arch.* 420:208–212.

12. Friedrich, F., M. Paulmichl, H. A. Kolb, and F. Lang. 1988. Inward rectifyer K channels in renal epitheloid cells (MDCK) activated by serotonin. *J. Membr. Biol.* 106:149–155.

13. Schwab, A., J. Geibel, W. Wang, H. Oberleithner, and G. Giebisch. 1993. Mechanism of activation of $K^+$ channels by Minoxidil sulfate in Madin-Darby canine kidney cells. *J. Membr. Biol.* In press.

14. Rane, S. G. 1991. A $Ca^{2+}$ activated $K^+$ current in ras-transformed fibroblasts is absent from nontransformed cells. *Am. J. Physiol.* 260(Cell Physiol. 29):C104–C112.

15. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved techniques for high-resolution current recording from cell-free membrane patches. *Pfluegers Arch.* 391:85–100.

16. Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single channel records. *In* Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 191–263.

17. Wojnowski, L., H.-J. Westphale, A. Schwab, and H. Oberleithner. 1992. $Ca^{2+}$ dependent oscillations of the plasma membrane potential in MDCK cells transformed by alkaline stress. 8th International Symposium on $Ca^{2+}$ binding proteins and $Ca^{2+}$ function in health and disease, 23–27 August 1992, Davos, Switzerland.

18. Furuya, K., and K. Enomoto. 1990. Real-time imaging of intracellular calcium changes with simultaneous single channel recording in mammary epithelial cells. *Brain Res. Bull.* 25:779–781.

19. Waldegger, S., G. Pinggera, K. Kloiber, M. Ritter, E. Wöll, E. Humpeler, K. Maly, H. Grunicke, and F. Lang. 1993. Further studies on the nature of cell membrane potential oscillations in NIH 3T3 fibroblasts expressing the ras oncogene. *Cell. Physiol. Biochem.* 3:89–96.

20. Huang, M., K. Chida, N. Kamata, K. Nose, M. Kato, Y. Homma, T. Takenawa, and T. Kuorki. 1988. Enhancement of inositol phospholipid metabolism and activation of protein kinase C in ras-transformed rat fibroblasts. *J. Biol. Chem.* 263:17975–17980.

21. Enomoto, K., K. Furuya, T. Maeno, C. Edwards, and T. Oka. 1991. Oscillating activity of a calcium-activated $K^+$ channel in normal and cancerous mammary cells in culture. *J. Membr. Biol.* 119:133–139.

22. Furuya, K., K. Enomoto, S. Furuya, S. Yamagishi, C. Edwards, and T. Oka. 1989. Single calcium-activated potassium channel in cultured mammary epithelial cells. *Pfluegers Arch.* 414:118–124.

23. Peres, A., and S. Giovannardi. 1990. Mitogen-induced oscillations of membrane potential and $Ca^{2+}$ in human fibroblasts. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 261:35–38.

24. Grandin, N., and M. Charbonneau. 1991. Intracellular free calcium oscillates during cell division of *Xenopus* embryos. *J. Cell Biol.* 112:711–718.

25. Marks, P. W., and F. R. Maxfield. 1990. Transient increases in cytosolic free $Ca^{2+}$ appear to be required for the migration of adherent human neutrophils. *J. Cell Biol.* 110:43–52.