Oscillating activity of a Ca(2+)-sensitive K+ channel. A prerequisite for migration of transformed Madin-Darby canine kidney focus cells.

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Research Article

Migration plays an important role in the formation of tumor metastases. Nonetheless, little is known about electrophysiological phenomena accompanying or underlying migration. Previously, we had shown that in migrating alkali-transformed Madin-Darby canine kidney focus (MDCK-F) cells a Ca(2+)-sensitive 53-pS K+ channel underlies oscillations of the cell membrane potential. The present study defines the role this channel plays in migration of MDCK-F cells. We monitored migration of individual MDCK-F cells by video imaging techniques. Under control conditions, MDCK-F cells migrated at a rate of 0.90 +/- 0.03 microns/min (n = 201). Application of K+ channel blockers (1 and 5 mmol/liter Ba2+, 5 mmol/liter tetraethylammonium, 100 mumol/liter 4-aminopyridine, 5 nmol/liter charybdotoxin) caused marked inhibition of migration, pointing to the importance of K+ channels in migration. Using patch-clamp techniques, we demonstrated the sensitivity of the Ca(2+)-sensitive 53-pS K+ channel to these blockers. Blockade of this K+ channel and inhibition of migration were closely correlated, indicating the necessity of oscillating K+ channel activity for migration. Migration of MDCK-F cells was also inhibited by furosemide or bumetanide, blockers of the Na+/K+/2Cl- cotransporter. We present a model for migration in which oscillations of cell volume play a central role. Whenever they are impaired, migration is inhibited.

Oscillating Activity of a Ca^{2+}-sensitive K^+ Channel

A Prerequisite for Migration of Transformed Madin-Darby Canine Kidney Focus Cells

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Abstract

Migration plays an important role in the formation of tumor metastases. Nonetheless, little is known about electrophysiological phenomena accompanying or underlying migration. Previously, we had shown that in migrating alkali-transformed Madin-Darby canine kidney focus (MDCK-F) cells a Ca^{2+}-sensitive 53-pS K^+ channel underlies oscillations of the cell membrane potential. The present study defines the role this channel plays in migration of MDCK-F cells. We monitored migration of individual MDCK-F cells by video imaging techniques. Under control conditions, MDCK-F cells migrated at a rate of 0.90±0.03 μm/min (n = 201). Application of K^+ channel blockers (1 and 5 mmol/liter Ba^{2+}, 5 mmol/liter tetraethylammonium, 100 μmol/liter 4-aminopyridine, 5 nmol/liter charybdotoxin) caused marked inhibition of migration, pointing to the importance of K^+ channels in migration. Using patch-clamp techniques, we demonstrated the sensitivity of the Ca^{2+}-sensitive 53-pS K^+ channel to these blockers. Blockade of this K^+ channel and inhibition of migration were closely correlated, indicating the necessity of oscillating K^+ channel activity for migration. Migration of MDCK-F cells was also inhibited by furosemide or bumetanide, blockers of the $Na^+/K^+/2Cl^-$ cotransporter. We present a model for migration in which oscillations of cell volume play a central role. Whenever they are impaired, migration is inhibited. (*J. Clin. Invest.* 1994. 93:1631–1636.) Key words: alkali-transformed Madin-Darby canine kidney focus cell • migration • K^+ channel • oscillation • cell volume

Introduction

Cell migration plays a pivotal role in such diverse processes as immune defense, embryogenesis, wound healing, and tumor metastasis (1). The clinical relevance of migration is manifested among others by its positive correlation with metastatic potential of tumor cells (2, 3). Accordingly, modern chemotherapeutic agents are aiming at mechanisms involved in the generation of cell motility (4).

Despite its great physiological significance, there is still controversy concerning the mechanisms responsible for migration (5). The lipid flow model predicts that migration is based on endo-/exocytotic recycling of plasma membrane with exocytosis of membrane vesicles taking place at the leading edge of the lamellipodium (6, 7). Other models are based on intricate interactions of cytoskeletal components which exert force on cytoskeleton-associated adhesion molecules (8–10).

Whereas elaborate experiments were performed to demonstrate lipid flow (7, 11, 12) or to visualize components of the cytoskeleton during migration (13, 14), little is known about electrophysiological phenomena or ion fluxes accompanying or underlying cell migration. However, ion movement across the plasma membrane must occur during migration since the surface to volume ratio may change when lamellipodia are formed or when the "tail" of a migrating cell is retracted. Changes of the surface to volume ratio are a signal for cells to regulate their volume. Many cell types accomplish volume regulation by the movement of K^+, Cl^-, or HCO_3^- across the plasma membrane followed by respective changes of the cell membrane potential (15–17). Hence, one can assume that the cell membrane potential of migrating cells is not always constant. Macrophages, for example, may exhibit rhythmic hyperpolarizations of the cell membrane potential or oscillating activity of Ca^{2+}-sensitive K^+ channels (18, 19). In our previous study we described oscillating activity of a Ca^{2+}-sensitive 53-pS K^+ channel in migrating, alkali-transformed Madin-Darby canine kidney focus (MDCK-F)[1] cells (20). Neurite outgrowth in neuroblastoma cells, a process functionally related to migration, also relies on the activation of K^+ channels (21).

In the present study, we investigated whether migration of MDCK-F cells depends on oscillating K^+ channel activity. Using differential interference contrast (DIC) video microscopy we monitored migration of individual MDCK-F cells. The role of the oscillating Ca^{2+}-sensitive 53-pS K^+ channel in migration was assessed with various K^+ channel blockers. In patch-clamp experiments, we studied the sensitivity of the formerly described K^+ channel to the blockers applied in migration experiments.

Our results show that oscillating activity of the Ca^{2+}-sensitive 53-pS K^+ channel is a prerequisite for migration of alkali-transformed MDCK-F cells. We propose a tentative model in which cell volume is the link between migration and K^+ channel activity.

Methods

Cell culture. Experiments were carried out on "normal" MDCK cells (catalogue number CCL 34; American Type Culture Collection, Rockville, MD) and on alkali-transformed MDCK-F cells (American Type

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1. *Abbreviations used in this paper:* 4-AP, 4-aminopyridine; CTX, charybdotoxin; DIC, differential interference contrast; MDCK-F, Madin-Darby canine kidney focus cell; TEA, tetraethylammonium; VRD, volume regulatory decrease.

Culture Collection). MDCK cells were transformed by prolonged exposure to an alkaline growth medium (pH 7.7) (22). Parent MDCK cells and transformed MDCK-F cells were kept under identical 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 KG). For experiments carried out between 4 h and 2 d after seeding, cells were plated on poly-L-lysine (0.1 g/liter; Serva, Heidelberg, Germany) coated coverslips.

Migration experiments. For screening migration of parent MDCK cells and of alkali-transformed MDCK-F cells, cells in culture flasks were placed on the heated table (37°C) of an inverted microscope (ID 02 MT; Zeiss, Oberkochen, Germany). Using phase contrast optics, time lapse photography was performed for up to 12 h, and migration was quantitated from calibrated prints of these photographs.

The effect of K^+ channel blockers on migration of MDCK-F cells was studied with a system allowing paired experiments on a single cell level. Coverslips with MDCK-F cells were mounted on the stage of an inverted microscope equipped with DIC optics (IM 35; Zeiss). A polyethylene ring was glued onto the coverslips so that they served as perfusion chambers for continuous superfusion with preheated solutions (37°C).

MDCK-F cells were monitored with a video camera (Panasonic TV camera; Matsushita Communication Industrial Co. Ltd., Osaka, Japan) lined up to the microscope. The video signal was transferred to an image processing board (Java; Jandel Scientific, Corte Madera, CA). Depending on the experimental conditions, videoprints (Video Graphic Printer UP 850; Sony, Köln, Germany) were made in 1–5-min intervals. Videoprints were calibrated and taken for the analysis of migration. As shown in Fig. 1, we measured the distance the leading edge of a lamellipodium advanced with time. To distinguish migration from cell spreading, only those cells were analyzed whose cell body moved in the same direction as the lamellipodium.

The composition of the superfusion solution was as follows (in mmol/liter): 122 NaCl, 5.4 KCl, 1.2 $CaCl_2$, 0.8 $MgCl_2$, 1 NaH_2PO_4, 5.5 glucose, 10 Hepes, titrated to pH 7.4 with 1 mol/liter NaOH. $BaCl_2$, tetraethylammonium (TEA), 4-aminopyridine (4-AP; Sigma Chemical Co., Deisenhofen, Germany), charybdotoxin (CTX; Alomone Laboratories, Jerusalem, Israel), furosemide, and bumetanide were added to the superfusion solution. In 50 mmol/liter KCl Ringer's solution, NaCl was isosmotically replaced by KCl.

Patch-clamp technique. Patch-clamp experiments were performed according to the methods described by Hamill et al. (23). Recordings were made in the inside-out patch configuration using a patch-clamp amplifier (L/M-EPC7; List Medical Electronic, Darmstadt, Germany). Single channel currents were filtered at 1.5 kHz by an 8-pole Bessel filter (902 LPF; Frequency Devices Inc., Haverhill, MA). Data were stored on a video cassette recorder (HR-D430 EG; JVC, Eschborn, Germany) via a digital pulse code modulator (PCM-501 ES; Sony). Current records were read into a computer via a TL-1 DMA Interface (Axon Instruments, Inc., Foster City, CA), and open probability and single channel current amplitudes were analyzed with pclamp software (Axon Instruments, Inc.).

Patch-clamp experiments were performed on parent MDCK cells. Before the experiments, cells were slightly trypsinized so that they were spreading while under investigation.

During patch-clamp experiments, cells were bathed in Ringer's solution of the following composition (in mmol/liter): 140 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 10 Hepes, titrated to pH 7.4 with 1 mol/liter NaOH. The pipette solution contained (in mmol/liter): 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 Hepes, titrated to pH 7.4 with 1 mol/liter KOH. K^+ channel blockers were applied to the pipette solution in identical concentrations as for migration experiments.

Statistics. Data are given as mean±SEM. Paired or unpaired Student's t tests were performed where applicable. Significance was assumed when $P < 0.05$.

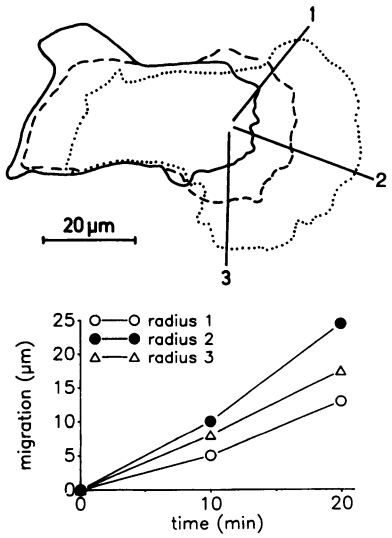

Figure 1. Analysis of migration of MDCK-F cells. (*Top*) Outlines of a migrating MDCK-F cell redrawn at three different times (0, 10, and 20 min) during the course of an experiment. *Solid line,* 0 min; *broken line,* 10 min; *dotted line,* 20 min. (*Bottom*) Distance the leading edge of the lamellipodium advanced in three different directions (radius 1–3) plotted as a function of time. This cell migrated at a rate of 1.2 µm/min into the direction of radius 2.

Results

Migration of parent MDCK cells and of alkali-transformed MDCK-F cells. To link migration to alkali transformation, we compared migration of parent (normal) MDCK cells and of alkali-transformed MDCK-F cells. Parent MDCK cells migrate only when they have no contact with neighboring cells. Under cell culture conditions, their rate of migration is 0.2±0.02 µm/min (n = 16). After forming cell–cell contacts, parent MDCK cells stop migrating. This was confirmed in experiments with continuous superfusion using DIC video microscopy. Only 2 out of 18 cells migrated.

In contrast, MDCK-F cells migrate over each other since contact inhibition is lacking. In addition, MDCK-F cells migrate much faster than normal MDCK cells: 0.9±0.03 µm/min (n = 201). Fig. 2 shows a migrating MDCK-F cell monitored with DIC video microscopy. The cell extends a flat lamellipodium in the direction of migration, and the cell body is "dragged behind."

After cell cleavages, daughter cells of normal MDCK cells remained in close contact, whereas MDCK-F daughter cells migrated immediately into opposite directions.

These observations demonstrate that migration of MDCK-F cells is causally linked to their transformation.

Migration of MDCK-F cells is linked to K^+ channel activity. To demonstrate the functional link between oscillating K^+ channel activity and migration, we monitored migration of MDCK-F cells with DIC video microscopy under control conditions and in the presence of K^+ channel blockers. Such a paired experiment is displayed in Fig. 3. After a control period of 16 min, we applied 5 nmol/liter CTX, a highly specific blocker of Ca^{2+}-sensitive K^+ channels. The cell immediately stopped. After 5 min, CTX was washed out, and the cell resumed migration. In 13 similar experiments, migration rate was reduced to −0.08±0.2 µm/min or to 2±14% of the respective control values.

In this way we also tested the effect of the following K^+ channel blockers on migration: Ba^{2+} (1 and 5 mmol/liter), TEA (5 mmol/liter), and 4-AP (100 µmol/liter). The results of these experiments are summarized in Fig. 4 *A*. All K^+ channel blockers dramatically reduced the rate of migration: 5 mmol/liter Ba^{2+}, −0.49±0.36 µm/min (−43±31% of con-

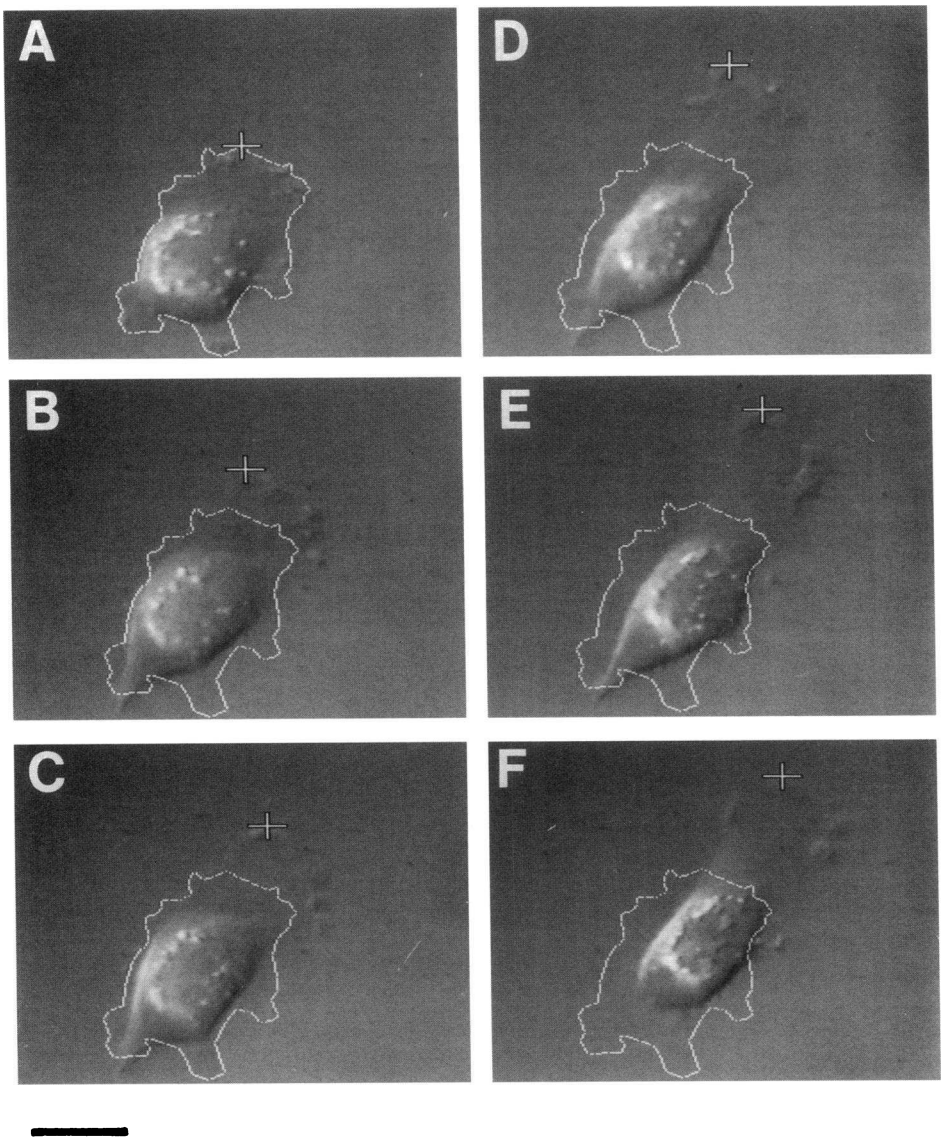

Figure 2. Migrating MDCK-F cell. *A–F* were taken in 5-min intervals. The white line represents the outline of the cell at the beginning of the experiment (corresponding to solid line in Fig. 1). The cross points to the farthest advanced part of the cell. The flat lamellipodium and the prominent cell body are clearly distinguishable. Bar = 20 μm.

trol); 1 mmol/liter Ba^{2+}, 0.35±0.1 μm/min (37±12% of control); 5 mmol/liter TEA, 0.26±0.15 μm/min (38±16% of control); 100 μmol/liter 4-AP: 0.03±0.09 μm/min (−1±13% of control). A negative rate of migration after the application of 5 mmol/liter Ba^{2+} or of CTX signifies a retraction of the lamellipodium.

A similar inhibition of migration was seen in experiments in which the rhythmic hyperpolarizations of the cell membrane potential were erased with 50 mmol/liter KCl Ringer's solution. 50 mmol/liter KCl abolishes the electrochemical driving force for K^+ ions across the cell membrane. Although K^+ channels are still triggered by oscillations of intracellular Ca^{2+} (24) there is no K^+ net movement. Migration is reduced to 0.15±0.1 μm/min (22±13% of control; n = 11). These results are also summarized in Fig. 4 *A*.

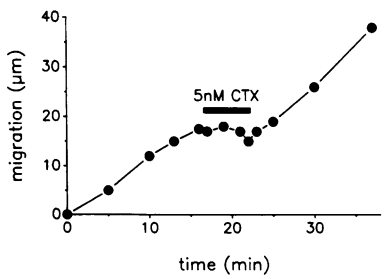

Figure 3. Migration experiment using the K^+ channel blocker CTX. Migration is plotted as a function of time. The K^+ channel blocker CTX (5 nmol/liter) reversibly inhibits migration of a single MDCK-F cell.

Sensitivity of the 53-pS K^+ channel to the blockers of migration. To correlate the inhibitory effect of the K^+ channel blockers on migration with the Ca^{2+}-sensitive 53-pS K^+ channel, we studied its sensitivity to these blockers in the inside-out patch configuration. This channel underlies oscillations of the cell membrane potential and is expressed with identical biophysical properties in alkali-transformed MDCK-F cells and in normal parent MDCK cells (20). For two reasons we studied this channel in excised patches of parent MDCK cells. Most importantly, the channel in MDCK-F cells regularly "runs down" within a few minutes, i.e., its activity spontaneously decays. Hence, it would be difficult to distinguish run down from channel blockade. Secondly, patches from MDCK-F cells are mechanically very fragile so that most of them break upon excision to the inside-out patch configuration.

We do not know the basis for channel run down, but it could reflect altered channel regulation in MDCK-F cells. In addition, the ultrastructure of patches may be different in

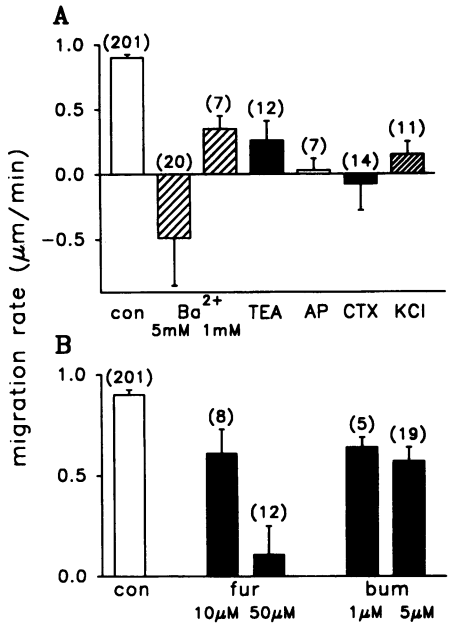

Figure 4. Summary of migration experiments. (*A*) The effect of the K^+ channel blockers Ba^{2+}, TEA, 4-AP, and CTX and of a 50 mmol/liter KCl containing Ringer's solution (*KCl*) on migration. (*B*) The effect of furosemide (*fur*) and of bumetanide (*bum*) on migration.

MDCK-F cells, explaining mechanical instability and accelerated diffusion of factors necessary for maintaining channel activity without run down. Nonetheless, we are dealing with identical channels in both cell types (20).

Fig. 5 shows original recordings of the Ca^{2+}-sensitive 53-pS K^+ channel in the inside-out patch configuration. Trace *A* depicts the K^+ channel under control conditions. In eight experiments, open probability and current amplitude were 0.52±0.14 and −2.5±0.1 pA, respectively, which is in good agreement with previous findings (25). The following traces demonstrate the effects of 5 mmol/liter Ba^{2+} (*B; n* = 7), 1 mmol/liter Ba^{2+} (*C; n* = 7), 5 mmol/liter TEA (*D; n* = 9), 100 μmol/liter 4-AP (*E; n* = 11), and 5 nmol/liter CTX (*F; n* = 7)

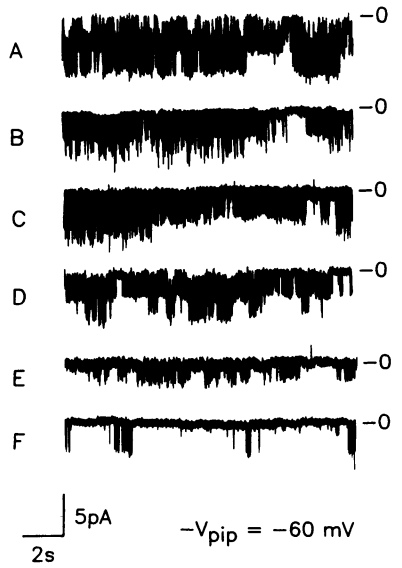

Figure 5. Original recordings of the Ca^{2+}-sensitive 53-pS K^+ channel in the inside-out patch configuration. *A*, control; *B*, 5 mmol/liter Ba^{2+}; *C*, 1 mmol/liter Ba^{2+}; *D*, 5 mmol/liter TEA; *E*, 100 μmol/liter 4-AP; *F*, 5 nmol/liter CTX. 0 marks the closed level of channels.

on K^+ channel activity. The blockers were always applied to the pipette solution. Ba^{2+} caused a dose- and voltage-dependent block with hyperpolarization enhancing the inhibition. TEA only moderately reduced open probability and current amplitude. Whereas Ba^{2+}, TEA, and 4-AP are rather unspecific K^+ channel blockers, CTX is highly specific. At a concentration of 5 nmol/liter, open probability is reduced to 8% of control.

In Fig. 6 we compare K^+ channel blockade with the blocker-induced inhibition of migration. We have normalized migration and channel activity to the respective control values which are taken as 100%. Blockade of the Ca^{2+}-sensitive K^+ channel and inhibition of migration are closely correlated. Almost complete blockade of the K^+ channel by CTX is followed by complete inhibition of migration. Reduction of channel activity to 38% by the application of 1 mmol/liter Ba^{2+} reduces migration to 40% of control. These results confirm our hypothesis that oscillating activity of the 53-pS K^+ channel is a prerequisite for migration of MDCK-F cells.

K^+ channels may modulate migration via controlling cell volume. The following experiments were designed to test whether cell volume played an important role in migration. Intermittent activation of the 53-pS K^+ channel in MDCK-F cells causes periodic K^+ efflux and thereby periodic reduction of cell volume. However, at the beginning of each spike of K^+ channel activity the cell must have regained K^+ (and cell volume) since K^+ channel oscillations do not decay (26). Except for the Na^+ K^+-ATPase, the $Na^+/K^+/2Cl^-$ cotransporter is one of the most important transporters mediating K^+ influx into MDCK cells (27).

According to this hypothesis, application of K^+ channel blockers impaired migration by inhibiting "cell shrinkage." We therefore tested whether migration is also inhibited if "cell swelling" is impaired after the application of furosemide or bumetanide, potent blockers of the $Na^+/K^+/2Cl^-$ cotransporter. The results of these experiments are summarized in Fig. 4 *B*. Both drugs reversibly inhibited migration of MDCK-F cells. 10 and 50 μmol/liter furosemide reduced migration rate to 0.61±0.12 and 0.11±0.14 μm/min (49±12 and 10±17% of control). 1 and 5 μmol/liter bumetanide lowered migration to 0.64±0.05 and 0.57±0.07 μm/min (74±8 and 62±9% of control).

For reasons of electroneutrality, K^+ flux has to be accompanied by anion movement. The involvement of anion channels

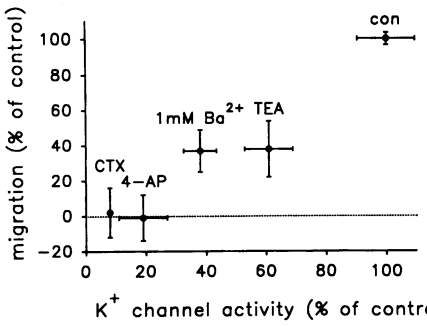

Figure 6. Migration plotted as a function K^+ channel activity. Both K^+ channel activity and migration are normalized. 100% corresponds to control values. It is clearly evident that migration is closely correlated to K^+ channel activity.

was indirectly demonstrated by the use of 5-nitro-2-(3-phenylpropylamino)-benzoic acid (1 μmol/liter). It reduced migration to 0.15±0.15 μm/min (n = 5), further underlining the significance of volume regulatory processes for migration (data not shown).

Discussion

In this study we demonstrated that K^+ channel activity is closely related to migration of MDCK-F cells. Inhibition of migration was achieved with the same blockers that inhibited a Ca^{2+}-sensitive 53-pS K^+ channel. Previously, we had shown that this K^+ channel underlies spontaneous oscillations of the cell membrane potential and is expressed in identical form in parent MDCK cells and in MDCK-F cells (20). Our observations point to an aspect of cell migration which has often been neglected. It is the contribution of ion channels and of the cell membrane potential for this very complex process. We are considering two major mechanisms by which the cell membrane potential regulates migration: (*a*) via reflecting cell volume regulation and, (*b*) via control of intracellular Ca^{2+}.

Cell membrane potential controls migration via cell volume regulation. Based on measurements of the membrane conductance, we predict that oscillating K^+ channel activity causes rhythmic changes of cell volume of MDCK-F cells. During the hyperpolarizing spikes, the driving force for K^+ ions is such that they exit the cell. Due to the high anion conductance of MDCK cells Cl^- or HCO_3^- ions will follow (28), i.e., the content of intracellular solutes is reduced and the cells will "shrink."

During volume regulatory decrease (VRD) after a hypotonic shock, parent MDCK cells initially lose K^+. This is reflected by a transient hyperpolarization (15) caused by the activation of the same Ca^{2+}-sensitive K^+ channel which oscillates in MDCK-F cells (29). In pancreatic secretory cells, ion channel activity, triggered by elevations of intracellular Ca^{2+}, and cell volume are closely correlated. In these cells intracellular Ca^{2+} rises upon stimulation with agonists. Subsequently, cell volume is reduced due to the activation of K^+ and Cl^- channels. When oscillations of intracellular Ca^{2+} are induced, cell volume also oscillates (30, 31).

Hence, each period of high K^+ channel activity in MDCK-F cells resembles the initial phase of a VRD. However, at the beginning of each spike, MDCK-F cells must have recovered from the volume and K^+ loss since K^+ channel oscillations are not decaying (26). Besides the Na^+ K^+-ATPase, the $Na^+/K^+/2Cl^-$ cotransporter, which is present in MDCK cells (27) and which plays an important role in volume regulation (32), is the most powerful mechanism for K^+ uptake and hence for volume recovery. Our results suggest that "K^+ refilling" of MDCK-F cells is at least partially mediated by this transporter.

The $Na^+/K^+/2Cl^-$ cotransporter is activated in other transformed cells. In NIH 3T3 fibroblasts expression of the Ha-ras oncogene predominantly stimulated furosemide-sensitive, ouabain-resistant rubidium-86 uptake. Accordingly, ras oncogene–expressing cells have an increased cell volume (33), and only in ras^+ fibroblasts does regulatory volume increase after hypertonic shock depend on the function of the $Na^+/K^+/2Cl^-$ cotransporter (34).

We do not know yet whether the $Na^+/K^+/2Cl^-$ cotransporter is upregulated in alkali-transformed MDCK-F cells too. We are aware that our results provide only indirect evidence for its participation and for the involvement of cell volume changes in the events leading to migration of MDCK-F cells. Direct measurements of rubidium-86 flux and of cell volume in transformed MDCK-F cells are yet to be performed.

Recent experiments performed on human melanoma cells point to the correlation between migration and the ability to regulate cell volume. Cells from melanoma cell lines deficient for actin-binding protein are unable to migrate, nor can they regulate their cell volume (35, 36). It is of interest that completely different mechanisms, a defect in the cytoskeleton or inhibition of ion transport with external blockers, have the same effects on migration. In contrast to actin-binding protein–deficient melanoma cells, normal MDCK cells which do not migrate can regulate their volume (15). This further underlines that the ability to regulate volume or oscillations of the cell volume per se is not sufficient for migration. Further experiments are needed for elucidating the molecular basis of the different migratory behaviors of parent and of transformed MDCK cells. Presently, it appears premature to generalize our model for all migrating cells since the correlation of oscillations with migration has not yet been studied systematically.

Cell membrane potential controls migration via intracellular Ca^{2+}. Previously, we had shown that oscillations of intracellular Ca^{2+} are the "motor" for oscillating K^+ channel activity in MDCK-F cells (37). In the present study we looked as selectively as possible at the effect of K^+ channels on migration. Therefore, a key experiment was to superfuse the cells with 50 mmol/liter KCl Ringer's solution (24). Ca^{2+} oscillations are not affected, but migration of MDCK-F cells is inhibited nonetheless. These findings confirmed the significance of K^+ movement across the cell membrane for locomotion.

Migration experiments using 5 mmol/liter Ba^{2+} are examples for the simultaneous block of K^+ channels and Ca^{2+} oscillations (unpublished observation from our laboratory). Possibly, the dramatic effect of 5 mmol/liter Ba^{2+} on the lamellipodium is caused by this dual effect, i.e., the retraction of lamellipodia probably involves more than impaired volume regulation.

Ca^{2+} oscillations of MDCK-F cells depend on Ca^{2+} influx from the extracellular space (24). Ca^{2+} influx is of great importance for migration too (38). Migration of developing postmitotic cerebellar cells, for example, is coupled to the expression of N-type Ca^{2+} channels in the plasma membrane (39). Similarly, *N*-methyl-D-aspartate receptor stimulation or inhibition affected migration of cerebellar cells via altered Ca^{2+} influx (40). We do not know to what extent Ca^{2+} influx into MDCK-F cells is changed by reducing the electrochemical driving force for Ca^{2+} entry after the application of K^+ channel blockers or 50 mmol/liter KCl. However, Ca^{2+} oscillations per se cannot maintain migration.

The dependence of Ca^{2+} oscillations on Ca^{2+} influx raises the question of whether Ca^{2+} influx into MDCK-F cells also depends on changes of cell volume. VRD of MDCK cells is preceded by a La^{3+} inhibitable increase of intracellular Ca^{2+} (41). It is remarkable that La^{3+} also blocks the Ca^{2+} influx necessary for maintaining oscillations of intracellular Ca^{2+} in MDCK-F cells (24). Future studies will be needed to determine whether the La^{3+}-inhibitable Ca^{2+} influx in MDCK-F cells is mediated by a volume-sensitive pathway.

Conclusion. We suggest the following sequence of events causing migration of MDCK-F cells. Oscillations of intracellular Ca^{2+} are the primary motor. They trigger the Ca^{2+}-sensitive

53-pS K^+ channel underlying oscillations of the cell membrane potential (20). Because of the ensuing loss of K^+ (and probably Cl^- or HCO_3^-) ions during each burst of K^+ channel activity the cell shrinks periodically. At the beginning of each spike of K^+ channel activity the cell must have replenished K^+ ions and regained its volume. K^+ refilling is at least partially accomplished via the $Na^+/K^+/2Cl^-$ cotransporter. The distribution of K^+ channels and transporters mediating K^+ uptake has to be polarized. Our working hypothesis is that K^+ channels are confined to the cell body ("rear end") of a migrating MDCK-F cell and that transporters like the $Na^+/K^+/2Cl^-$ cotransporter are predominantly located in the lamellipodium ("front"). In such a scenario, shrinkage (rear end) and swelling (front) would be able to assist the cytoskeleton to advance the cell. Currently, experiments are undertaken to verify the polarized distribution of the relevant K^+ channels and transporters.

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