Activation of $K^+$ channels and increased migration of differentiated intestinal epithelial cells after wounding

JALADANKI N. RAO,1,2 OLEKSANDR PLATOSHYN,3 LI LI,1,2 XIN GUO,1,2 VERA A. GOLOVINA,4 JASON X.-J. YUAN,3 AND JIAN-YING WANG1,2,5

Departments of 1Surgery, 4Physiology, and 5Pathology, University of Maryland School of Medicine and 2Baltimore Veterans Affairs Medical Center, Baltimore, Maryland 21201; and 3Department of Medicine, School of Medicine, University of California, San Diego, California 92103

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Rao, Jaladanki N., Oleksandr Platoshyn, Li Li, Xin Guo, Vera A. Golovina, Jason X.-J. Yuan, and Jian-Ying Wang. Activation of $K^+$ channels and increased migration of differentiated intestinal epithelial cells after wounding. Am J Physiol Cell Physiol 282: C885–C898, 2002. First published November 21, 2001; 10.1152/ajpcell.00361.2001.—Early mucosal restitution occurs by epithelial cell migration to reseal superficial wounds after injury. Differentiated intestinal epithelial cells exhibit increased migration by altering voltage-gated $K^+$ (Kv) channel expression. Stable Cdx2-transfected IEC-6 cells (IEC-Cdx2L1) with highly differentiated phenotype expressed higher basal levels of Kv1.1 and Kv1.5 mRNAs and proteins than parental IEC-6 cells. Neither IEC-Cdx2L1 cells nor parental IEC-6 cells expressed voltage-dependent $Ca^{2+}$ channels. The increased expression of Kvl channels in differentiated IEC-Cdx2L1 cells was associated with an increase in whole cell $K^+$ currents, membrane hyperpolarization, and a rise in $[Ca^{2+}]_{cyt}$. The migration rates in differentiated IEC-Cdx2L1 cells were about four times those of parental IEC-6 cells. Inhibition of Kv channel expression by polyamine depletion decreased $[Ca^{2+}]_{cyt}$, reduced myosin stress fibers, and inhibited cell migration. Elevation of $[Ca^{2+}]_{cyt}$ by ionomycin promoted myosin II stress fiber formation and increased cell migration. These results suggest that increased migration of differentiated intestinal epithelial cells is mediated, at least partially, by increasing Kv channel activity and $Ca^{2+}$ influx during restitution.

Address for reprint requests and other correspondence: J.-Y. Wang, Dept. of Surgery, Baltimore Veterans Affairs Medical Center, 10 North Greene St., Baltimore, MD 21201 (E-mail: jwang@smail.umd.edu).

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entry is a major source for \([Ca^{2+}]_{cyt}\), membrane depolarization would decrease \([Ca^{2+}]_{cyt}\) in cells lacking L-type voltage-dependent Ca\(^{2+}\) channels (VDCC) (13, 30, 48). In contrast, membrane hyperpolarization would increase the Ca\(^{2+}\) driving force, enhance Ca\(^{2+}\) influx, and increase \([Ca^{2+}]_{cyt}\) in the nonexcitable cells.

Our (37, 48) previous studies have demonstrated that intestinal epithelial cells do not express VDCC and that induced activation of Kv channels causes membrane hyperpolarization, enhances Ca\(^{2+}\) entry by increasing the driving force for Ca\(^{2+}\) influx, raises \([Ca^{2+}]_{cyt}\), and promotes cell migration after wounding in undifferentiated parental IEC-6 cells. Expression of the Kv channel genes in IEC-6 cells requires cellular polyamines, including spermidine, spermine, and their precursor putrescine. Depletion of cellular polyamines by inhibition of ornithine decarboxylase (ODC), a key enzyme for polyamine synthesis, with \(N\)-difluoromethylornithine (DFMO) decreases Kv channel expression, causes membrane depolarization, reduces \([Ca^{2+}]_{cyt}\), and decreases cell migration (48). We (37) have further demonstrated that RhoA of small GTPases is a downstream target of elevated \([Ca^{2+}]_{cyt}\) after activation of K\(^+\) channels by increased cellular polyamines and that Ca\(^{2+}\)-activated RhoA activity increases the formation of actomyosin stress fibers in migrating cells during restitution.

The current study tests the hypothesis that differentiated intestinal epithelial cells exhibit increased migration after wounding by altering Kv channel expression and \([Ca^{2+}]_{cyt}\). First, we compared the Kv channel expression, \(E_{m}\) and resting \([Ca^{2+}]_{cyt}\) in differentiated intestinal epithelial cells (stable Cdx2-transfected IEC-6 line) with those in undifferentiated parental cells (IEC-6 line). Second, we determined whether inhibition of Kv channel expression by polyamine depletion decreased \([Ca^{2+}]_{cyt}\) in differentiated intestinal epithelial cells and further investigated whether manipulating \([Ca^{2+}]_{cyt}\), either by increase or decrease, altered cell migration. Third, we determined whether observed changes in \([Ca^{2+}]_{cyt}\) affected the cellular distribution of nonmuscle myosin II.

**MATERIALS AND METHODS**

**Materials.** Disposable culture ware was purchased from Corning Glass Works (Corning, NY). Tissue culture media and dialyzed fetal bovine serum (dFBS) were obtained from GIBCO-BRL (Gaithersburg, MD), and biochemicals were from Sigma (St. Louis, MO). The primary antibody, an affinity-purified rabbit polyclonal antibody against Kv1.1 or Kv1.5, was purchased from Alomone Labs. The specific rabbit polyclonal antibody against nonmuscle myosin II was obtained from Biomedical Technologies (Stoughton, MA). Anti-rabbit IgG, FITC isomer conjugate, and ionomycin were purchased from Sigma. DFMO was purchased from Ilex Oncology (San Antonio, TX).

**Cell culture and general experimental protocol.** The IEC-6 cell line was purchased from American Type Culture Collection at passage 13. The cell line was derived from normal rat intestine and was developed and characterized by Quaroni et al. (35). IEC-6 cells originated from intestinal crypt cells, as judged by morphological and immunologic criteria. They are nontumorigenic and retain the undifferentiated character of intestinal epithelial crypt cells. Stock cells were maintained in T-150 flasks in DMEM supplemented with 5% heat-inactivated FBS, 10 μg insulin, and 50 μg gentamicin sulfate/ml. Flasks were incubated at 37°C in a humidified atmosphere of 90% air-10% CO\(_2\). Stock cells were subcultured once a week at 1:20, and the medium was changed three times per week. The cells were restarted from original frozen stock every seven passages. Tests for mycoplasma were routinely negative, and passages 15–20 were used in the experiments. There were no significant changes of biological function and characterization from passages 15 to 20.

The stable Cdx2-transfected IEC-6 cells (IEC-Cdx2L1 cells; kind gift of Dr. P. G. Traber, University of Pennsylvania, Philadelphia, PA) were developed and characterized by Suh and Traber (42). The expression vector, the LacSwitch system (Strategene, La Jolla, CA), was used for directing the conditional expression of Cdx2, and isopropyl \(\beta\)-d-thiogalactopyranoside (IPTG) served as the inducer for the gene expression. IEC-6 cells were transfected with pOPRSVCdx2 by electroporation technique, and clones resistant to selection medium containing 0.6 mg G418/ml and 0.3 mg hygromycin B/ml were isolated and screened for Cdx2 expression by Northern blot, RNase protection assays, and electrophoretic mobility shift assay. Stock stable Cdx2-transfected IEC-6 cells were grown in DMEM used in parental nontransfected IEC-6 cells. Before experiments, cells were grown in DMEM containing 4 mM IPTG for 16 days to induce cell differentiation.

The general protocol of the experiments and the methods used were similar to those described previously (36, 47). Briefly, IEC-6 and Cdx2-transfected cells were plated at 6.25 \(\times\) 10\(^4\) cells/cm\(^2\) in DMEM plus 5% dFBS, 10 μg/ml insulin, 50 μg/ml gentamicin sulfate, and 4 mM IPTG. The cells were incubated in a humidified atmosphere at 37°C in 90% air-10% CO\(_2\) (vol/vol) for 4 days, which was followed by a period of different experimental treatments.

In the first series of studies, we examined changes in the Kv channel expression, voltage-gated K\(^+\) currents \([I_{Kv}]\), \(E_{m}\), and \([Ca^{2+}]_{cyt}\) in differentiated Cdx2-transfected IEC-6 cells and then compared the differences between Cdx2-transfected cells and nontransfected parental cells. Cells were grown in standard DMEM for 4 days after initial plating, and the cell layers were washed three times with ice-cold Dulbecco’s PBS. Different solutions were then added according the assays to be conducted.

In the second series of studies, we examined whether inhibition of Kv channel expression by polyamine depletion decreased \([Ca^{2+}]_{cyt}\) in Cdx2-transfected IEC-6 cells and further determined the role of \([Ca^{2+}]_{cyt}\) in the process of increased cell migration after wounding. The cells were grown in the control cultures and cultures containing either DFMO (5 mM) alone or DFMO plus 5 μM spermidine for 4 days, and then the levels of Kv channel mRNAs and proteins were measured. Cell migration was assayed 4 and 6 h after removal of part of the cell layers. The Ca\(^{2+}\) ionophore ionomycin was used to increase \([Ca^{2+}]_{cyt}\), whereas the Ca\(^{2+}\)-free medium was employed to decrease \([Ca^{2+}]_{cyt}\). The measurements of \([Ca^{2+}]_{cyt}\) and cell migration were carried out at various times after treatment with ionomycin or the Ca\(^{2+}\)-free medium.

In the third series of studies, we investigated whether observed changes in \([Ca^{2+}]_{cyt}\) affected the distribution of nonmuscle myosin II in migrating cells after wounding. After cells were grown in the presence or absence of DFMO or DFMO plus spermidine for 4 days, they were exposed to ionomycin or the Ca\(^{2+}\)-free medium immediately after...
wounding, and cellular distribution of nonmuscle myosin II was assayed 6 h after treatment.

Electron microscopy. Cells were fixed at room temperature in 2.5% glutaraldehyde-3.2% paraformaldehyde buffered with 0.1 M sodium cacodylate (pH 7.4). Cells were then postfixed in 2% osmium tetroxide in the same buffer, dehydrated, and embedded in Epon as described previously (36). Ultrathin sections were examined in an electron microscope.

RT-PCR. Total RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform extraction method (6). Specific primers for Kv channel α- (pore forming subunit) and β-subunits (cytoplasmic regulatory subunit), L-type VDCC α1- and β1-subunits, and transient receptor potential channel genes (TRPC) were designed from the cDNA sequences of the coding regions corresponding to the channel genes (Table 1). These particular sequences were chosen on the basis of previously established specificity (48–50, 53). RT-PCR was performed as we (47) described previously. To quantify the PCR products (the amounts of mRNA) of Kv, VDCC, and TRPC, an invariant mRNA of β-actin was used as an internal control.

The optical density values in the gel were measured by a gel documentation system (UVP, Upland, CA), and the channel signals were normalized to the optical density values in the β-actin signals (48).

Western blot analysis. Cell samples, dissolved in SDS sample buffer (250 mM Tris·HCl, pH 6.8, 2% SDS, 20% glycerol, and 5% mercaptoethanol), were sonicated and centrifuged at 2,000 rpm for 15 min. The protein concentration of the supernatant was boiled for 5 min and then subjected to electrophoresis on 10% acrylamide gels according to the method of Laemmli (23). Briefly, after the transfer of protein onto nitrocellulose filters, the filters were incubated overnight at 4°C in 5% nonfat dry milk in 1× PBS-Tween 20. Immunologic evaluation was then performed for 90 min in 1% BSA-PBS-Tween 20 buffer containing affinity-purified antibody against Kv1.1 or Kv1.5 channel protein. The filters were subsequently washed with 1× PBS-Tween 20 and incubated with an IgG second antibody conjugated to peroxidase by protein cross-linking with 0.2% glutaraldehyde. The immunocomplexes on the filters were reacted for 1 min with chemiluminescence reagent (NEL-100, DuPont NEN).

Electrophysiological measurements. Whole cell K+ currents (IK) were recorded with an Axopatch-1D amplifier and a DigiData 1200 interface (Axon Instruments, Foster City, CA) by the patch-clamp technique (50). Patch pipettes (2–4 MΩ) were made on a Sutter electrode puller using borosilicate glass tubes and fire polished on a Narishige microforge. Step-pulse protocols and data acquisition were performed with pCLAMP software. Currents were filtered at 1–2 kHz (~3 dB) and digitized at 2–4 kHz with the Axopatch-1D amplifier. To record optimal IK(Kv), we replaced CaCl2 with equimolar MgCl2 in the bath solution. Series resistance and capacitance were routinely compensated (for 60–80%) by adjusting the internal circuitry of the patch-clamp amplifier. Leakage currents were subtracted with the P/V–4 protocol in pCLAMP software. Em in single IEC-6 or IEC-Cdx2L1 cells was measured in current-clamp mode (I = 0) using whole cell

Table 1. Oligonucleotide sequences of primers used for RT-PCR

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<tr>
<th>Names</th>
<th>Size, bp</th>
<th>Sense/Antisense</th>
<th>Location, nt</th>
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<tr>
<td>α-Subunit</td>
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<td></td>
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<tr>
<td>Kv1.1 (X12589)</td>
<td>594</td>
<td>5'-ATCTTGAAAATCTCCCGCGACTCGAGGG-3'</td>
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<td></td>
<td>5'-CTGCTTTTTAAAAGATGCT-3'</td>
<td>1724–1744</td>
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<td>267</td>
<td>5'-ACGTGCGGACGGACGAACTCA-3'</td>
<td>2085–2106</td>
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<td></td>
<td>5'-GTTGGCCTTTCTGTCTCCAG-3'</td>
<td>2330–2351</td>
</tr>
<tr>
<td>Kv2.1 (X16476)</td>
<td>269</td>
<td>5'-ACGCAACTACACCTCTCCAGG-3'</td>
<td>2868–2889</td>
</tr>
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<td></td>
<td></td>
<td>5'-CTAAATGTCAGCTGACCGCG-3'</td>
<td>3115–3136</td>
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<tr>
<td>Kv4.3 (U42975)</td>
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<td></td>
<td></td>
<td>5'-CTGCTGGGTTGGCGGAGAAAGTC-3'</td>
<td>789–810</td>
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<td>5'-CTGAGAGTTACTGGAGGAC-3'</td>
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<td>5'-AGGTTTGTGTGGAGAAATCTAATCC-3'</td>
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<td>5'-CAGACAGCATGGAAGGAAATGGT-3'</td>
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<td>mTRPC6 (U49069)</td>
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<td></td>
<td>5'-ACGCCGCTCAGCTCTCAGTT-3'</td>
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<td>Voltage-gated Ca2+ channels</td>
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<td>VDCC-α1 (M67515)</td>
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<td>5'-ATCCCGAAGAAGACGGACGAGG-3'</td>
<td>3876–3896</td>
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<td></td>
<td></td>
<td>5'-GGTATGAGATGCGGGAGGG-3'</td>
<td>4227–4247</td>
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<tr>
<td>VDCC-β1 (M80545)</td>
<td>549</td>
<td>5'-TGCTGGATAGAAGAGGCTGTT-3'</td>
<td>682–702</td>
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<tr>
<td></td>
<td></td>
<td>5'-GCCGTCGTGAAATAATCGGTCC-3'</td>
<td>1210–1230</td>
</tr>
</tbody>
</table>

Kv, voltage-gated K+; TRPC, transient receptor potential channels; m, mouse; h, human; VDCC, voltage-dependent Ca2+ channels. The GenBank accession numbers (which are given in parentheses) for the sequences were used in designing the primers.
Measurement of \([Ca^{2+}]_{cyt}\). The digital imaging methods used for measuring \([Ca^{2+}]_{cyt}\) were as previously described (51). Briefly, IEC-6 or IEC-Cdx2L1 cells were plated on 25-mm coverslips and incubated in culture medium containing 3.3 \(\mu M\) fura 2-AM for 30–40 min at room temperature (22–24°C) under an atmosphere of 10% CO\(_2\) in air. The fura 2-loaded cells were then superfused with standard bath solution for 20–30 min at 22–24°C to wash away extracellular dye and permit intracellular esterases to cleave cytosolic fura 2-AM into active fura 2. Fura 2 fluorescence (510 nm emission; 380 and 360 nm excitation) from the cells and background fluorescence were imaged using a Nikon Diaphot microscope equipped for epifluorescence. Fluorescent images were obtained using a microchannel plate image intensifier (Amperex XX1381; Opelco, Washington, DC) coupled by fiber optics to a Pulnix charge-coupled device video camera (Stanford Photonics, Stanford, CA).

Image acquisition and analysis were performed with a MetaMorph imaging system (Universal Imaging). Video frames containing images of fura 2 fluorescence from cells and the corresponding background images (fluorescence from fields devoid of cells) were digitized at a resolution of 512 horizontal \(\times\) 480 vertical pixels and 8 bits using a Matrix LC imaging board operating in an IBM-compatible computer. Images were acquired at a rate of one averaged image every 3 s when \([Ca^{2+}]_{cyt}\) was changing and every 60 s when \([Ca^{2+}]_{cyt}\) was relatively constant. \([Ca^{2+}]_{cyt}\) was calculated from fura 2 fluorescence emission excited at 380 and 360 nm using the ratio method (32). In most experiments, multiple cells (usually 10–15 cells) were imaged in a single field, and one arbitrarily chosen peripheral cytosolic area (4–6 \(\times\) 4–6 pixels) from each cell was spatially averaged.

Measurement of cell migration. The migration assays were carried out as we (47, 48) described previously. Cells were plated at 6.25 \(\times\) 10\(^4\) cells/cm\(^2\) in DMEM plus dFBS on 60-mm dishes thinly coated with Matrigel according to the manufacturer’s instructions and incubated as described for stock cultures. The cells were fed on day 2 and migration tested on day 4. To initiate migration, we scratched the cell layer with a single-edge razor blade cut to \(\sim\)27 mm in length. The scratch began at the diameter of the dish and extended over an area 7–10 mm wide. The migrating cells in six contiguous 0.1-mm squares were counted at \(\times\)100 magnification beginning at the scratch line and extending as far out as the cells had migrated. All experiments were performed in triplicate, and the results were reported as the number of migrating cells per millimeter of scratch.

Nonmuscle myosin II staining. The immunofluorescence procedure was carried out according to the method of Vieldkind and Swierenga (45) with minor changes (37). The primary antibody recognizes the 200-kDa nonmuscle myosin II in immunoblots of IEC-Cdx2L1 cell extracts and does not cross-react with other cytoskeletal proteins (36). Nonspecific slides were incubated without antibody to nonmuscle myosin II. Slides were viewed through a Zeiss confocal microscope (model LSM410).

HPLC analysis of cellular polyamines. The cellular polyamine content was determined as previously described (46). Briefly, after the cells were washed three times with ice-cold Dulbecco’s PBS, we added 0.5 M perchloric acid. The cells were then frozen at \(-80°C\) until ready for extraction, dianalysis, and HPLC. The standard curve encompassed 0.31–10 \(\mu\)M. Values that fell >25% below the curve were considered not detectable. Protein was determined by the Bradford method (4). The results are expressed as nanomoles of polyamines per milligram of protein.

Statistical analysis. All data are expressed as means \(\pm\) SE from six dishes. Autoradiographic and immunofluorescence labeling results were repeated three times. The significance of the difference between means was determined by ANOVA. The level of significance was determined using Dunnett’s multiple-range test (14).

RESULTS

Changes in cell migration in differentiated intestinal epithelial cells. Forced expression of the Cdx2 gene in the stable Cdx2-transfected IEC-6 cells (IEC-Cdx2L1) induced a significant development of differentiated phenotype as indicated by electron microscopic features (Fig. 1A) and molecular evidence (Fig. 1B). Non-transfected parental IEC-6 cells showed a simple...
monolayer of flat epithelial cells with no evidence of cellular differentiation (Fig. 1). However, the IEC-Cdx2L1 cells treated with 4 mM IPTG for 16 days exhibited multiple morphological and molecular characteristics of intestinal epithelial differentiation (Fig. 1). These enterocyte-like cells were polarized, showed lateral membrane interdigitations, a well-demarcated basal lamina, and microvilli at the apical pole and also expressed brush-border enzymes such as sucrase-isomaltase.

These differentiated intestinal epithelial cells migrated over the wounded edge much faster than undifferentiated parental IEC-6 cells after wounding in a model that mimics the early stage of epithelial restitution in vitro. The numbers of cells migrating over the wounded edge in differentiated IEC-Cdx2L1 cells were almost four times that of undifferentiated parental IEC-6 cells at 6 h after wounding (122 ± 5 cells/mm in IEC-6 cells to 410 ± 12 cells/mm in IEC-Cdx2L1 cells, n = 12, P < 0.05). Increased migration in Cdx2-transfected cells does not result simply from clonal variation, because identical results were observed when another independently transfected clone, IEC-Cdx2L2, was analyzed (data not shown). In addition, increased migration in differentiated Cdx2-transfected cells is not due to the effects of G418, hygromycin B, and IPTG. There were no significant differences in the rates of cell migration between nontransfected IEC-6 cells (122 ± 5 cells/mm, n = 6) and cells transfected with the empty vector containing no Cdx2 cDNA but maintained in G418 and hygromycin B and exposed to 4 mM IPTG for 16 days (127 ± 7 cells/mm, n = 6). Treatment with 4 mM IPTG for 16 days also did not affect the migration rates in nontransfected parental IEC-6 cells (122 ± 5 vs. 119 ± 6 cells/mm, n = 12, P > 0.5). Furthermore, the rate of migration in Cdx2-transfected IEC-6 cells before treatment with IPTG to induce differentiation was identical to that of nontransfected parental IEC-6 cells (128 ± 8 vs. 122 ± 5 cells/mm, n = 12, P > 0.5).

*Kv* channel expression in differentiated IEC-Cdx2L1 cells. The mRNA expression of Kv1.1 and Kv1.5 channels increased significantly in differentiated IEC-Cdx2L1 cells that exhibited increased migration after wounding. As shown in Fig. 2, the mRNA levels of Kv1.1 and Kv1.5 in differentiated IEC-Cdx2L1 cells were ~2.2- and ~1.9-fold greater, respectively, than those of undifferentiated parental IEC-6 cells. On the other hand, there were no significant differences in mRNA expression of Kv2.1, Kv4.3, Kv9.3, and Kvβ1.1 between differentiated IEC-Cdx2L1 cells and parental IEC-6 cells. The mRNA levels of Kv1.1 and Kv1.5 channels in differentiated IEC-Cdx2L2 cells were similar to those observed in IEC-Cdx2L1 cells (data not shown). We also examined the effects of G418, hygromycin B, and IPTG on Kv channel expression and demonstrated that mRNA levels of Kv1.1, Kv1.5, Kv2.1, Kv4.3, Kv9.3, and Kvβ1.1 in the empty vector-transfected IEC-6 cells were indistinguishable from those in nontransfected parental IEC-6 cells. Exposure of nontransfected parental IEC-6 cells to IPTG or

![Fig. 2. Changes in the mRNA expression of voltage-gated K⁺ (Kv) channel α- and β-subunits in parental IEC-6 and stable Cdx2-transfected IEC-6 cells (IEC-Cdx2L1). Before experiments, IEC-Cdx2L1 cells were grown in DMEM containing 5% FBS in the presence of 4 mM IPTG (the inducer for gene expression) for 16 days to induce cell differentiation. Both parental IEC-6 and differentiated IEC-Cdx2L1 cells were then cultured in DMEM containing 5% FBS for 4 days, and total cellular RNA was harvested for RT-PCR analysis. A: RT-PCR-amplified products displayed in agarose gels. Ae: Kv1.1 (594 bp). Ab: Kv1.5 (267 bp). Ac: Kv2.1 (269 bp). Ad: Kv4.3 (270 bp). Ae: Kv9.3 (570 bp). Af: Kvβ1.1 (150 bp). Ag: β-actin (244 bp). The first-strand cDNAs, synthesized from total cellular RNA, were amplified with the specific sense and antisense primers (see Table 1). B: data normalized to the amount of α5-actin (optical density of channel mRNA/optical density of β-actin mRNA) are expressed as means ± SE from 3 separate experiments. *P < 0.05 compared with parental IEC-6 cells.](http://ajpcell.physiology.org/)
Cdx2-transfected IEC-6 cells before treatment with IPTG to induce differentiation was not associated with increased expression of Kv1.1 and Kv1.5 channels (data not shown).

Increased Kv1.1 and Kv1.5 mRNAs in differentiated IEC-Cdx2L1 cells were paralleled by increases in the Kv channel proteins as measured by Western blot analysis (Fig. 3). The concentrations of Kv1.1 and Kv1.5 in differentiated IEC-Cdx2L1 cells were more than two times greater than in parental IEC-6 cells.

The high levels of Kv channel mRNA and protein expression in IEC-Cdx2L1 cells were associated with an increase in $I_{K(v)}$ and membrane hyperpolarization (Fig. 4). In this study, whole cell $I_K$ were elicited by depolarizing the cells to a series of test potentials ranging from $-60$ to $+80$ mV in 20-mV increments from a holding potential of $-70$ mV.

**Fig. 3.** Protein levels of Kv1.1 and Kv1.5 channels in parental IEC-6 and IEC-Cdx2L1 cells described in the Fig. 2 legend. A: representative autoradiograms of Western blots. Whole cell lysates from parental IEC-6 and differentiated IEC-Cdx2L1 cells were harvested, applied to each lane (20 µg), and subjected to electrophoresis on a 10% acrylamide gel. Kv1.1 (~86 kDa) and Kv1.5 (~75 kDa) channel proteins were identified by probing nitrocellulose with the specific antibodies. After the blot was stripped, actin (~45 kDa) immunoblotting was performed as an internal control for equal loading. B: quantitative analysis of Western immunoblots by densitometry from cells described in A. Values are means from 3 separate experiments; relative Kv1.1 and Kv1.5 channel protein levels were corrected for loading as measured by densitometry of actin. *P < 0.05 compared with parental IEC-6 cells.

**Fig. 4.** Changes in voltage-gated K$^+$ currents [$I_{K(v)}$] and resting membrane potential ($E_m$) in parental IEC-6 and IEC-Cdx2L1 cells described in the Fig. 2 legend. A: representative families of currents elicited by depolarizing the cells from a holding potential of $-70$ mV to a series of test potentials ranging from $-60$ to $+80$ mV at 20-mV increments in IEC-6 and IEC-Cdx2L1 cells. Ba: composite current-voltage relationships ($I$-$V$ curves) from IEC-6 and IEC-Cdx2L1 cells. The $I$-$V$ curve in differentiated IEC-Cdx2L1 cells is significantly different from the curve of undifferentiated parental IEC-6 cells. Bb: average currents at $+80$ mV obtained from both IEC-6 and IEC-Cdx2L1 cells tested; the currents were normalized to the maximal amplitude of each current record and are expressed as % of the maximal currents ($I/I_{max}$). C: time constants of current activation ($\tau_{act}$) and inactivation ($\tau_{inact}$) of the averaged currents. D: summarized data showing $E_m$ from IEC-6 and IEC-Cdx2L1 cells described in A. Data are expressed as means ± SE ($n$ = 25). *P < 0.05 compared with parental IEC-6 cells.
to 5 mM 4-aminopyridine (4-AP), a K⁺ channel blocker, therefore the currents were 4-AP-sensitive Iₖ(V) (data not shown). Consistent with the activation of Kv channel mRNA expression, the amplitudes of Iₖ(V) in differentiated IEC-Cdx2L1 cells were higher than in parental IEC-6 cells (Fig. 4A). The current-voltage relationships indicate that the increase in Iₖ(V) in IEC-Cdx2L1 cells appears to be voltage dependent (Fig. 4B). The K⁺ currents at +80 mV were 365 ± 12 pA in IEC-Cdx2L1 cells (n = 32) and 238 ± 8 pA in parental IEC-6 cells (n = 31, P < 0.05). The normalized whole cell currents at +80 mV, which were averaged from all IEC-Cdx2L1 and IEC-6 cells, showed that the currents were rapidly activated and slowly inactivated (Fig. 4Bb). The time constants for current activation were 3.2 ± 0.2 ms in IEC-Cdx2L1 cells and 2.2 ± 0.1 ms in IEC-6 cells (Fig. 4Ca), whereas the time constants for current inactivation were 135 ± 13 ms in IEC-Cdx2L1 cells and 150 ± 18 ms in IEC-6 cells, respectively (Fig. 4Cb).

Because the membrane input resistance in intestinal epithelial cells was very high under resting conditions (~8 to 9 MΩ), a small change in Iₖ(V) would cause a large change in Eₘ. Indeed, the augmentation of Iₖ(V) in differentiated IEC-Cdx2L1 cells resulted in a significant membrane hyperpolarization (Fig. 4D). The resting Eₘ was −43 ± 2.5 mV in IEC-Cdx2L1 cells and −28 ± 1.1 mV in parental IEC-6 cells (n = 25, P < 0.05). These results suggest that activation of Kv channel expression and the resultant increase in Kv channel activity would cause a significant membrane hyperpolarization.

**Effect of hyperpolarized Eₘ on [Ca²⁺]ᵢ in IEC-Cdx2L1 cells.** In excitable cells, such as smooth muscle cells and neurons, VDCC are major pathways for Ca²⁺ influx. Therefore, membrane depolarization would open VDCC and promote Ca²⁺ influx (28, 32, 44). In contrast, nonexcitable cells, including intestinal epithelial cells, do not express VDCC and membrane depolarization would decrease the Ca²⁺ driving force and attenuate Ca²⁺ influx through store-operated Ca²⁺ channels that may be formed by TRPC (49, 52, 53). As shown in Fig. 5, both differentiated IEC-Cdx2L1 cells and parental IEC-6 cells expressed TRPC1 and TRPC5, which encode the Ca²⁺-permeable channels involved in capacitative Ca²⁺ entry in mammalian cells (52, 53). The level of TRPC1 mRNA in IEC-Cdx2L1 cells increased significantly and was approximately twofold greater than in parental IEC-6 cells (Fig. 5A). Although TRPC4 and TRPC6 were highly expressed in rat pulmonary artery smooth muscle cells, they were not detectable in IEC-Cdx2L1 and IEC-6 cells by RT-PCR analysis (Fig. 5, B and D). Neither differentiated IEC-Cdx2L1 cells nor parental IEC-6 cells expressed VDCC (Fig. 5, E and F). The pore-forming (α₁-subunit) and regulatory subunits (β₁-subunit) of L-type VDCC were not detectable in both IEC-Cdx2L1 and IEC-6 cells but were highly expressed in rat pulmonary artery smooth muscle cells. These results indicate that differentiated IEC-Cdx2L1 cells do not express VDCC but express TRPC1 and TRPC5 channels that may be responsible for the capacitative Ca²⁺ entry in intestinal epithelial cells.

As described above, membrane hyperpolarization in cells that do not express VDCC would increase the Ca²⁺ driving force for Ca²⁺ influx and raise [Ca²⁺]ᵢ. Indeed, resting [Ca²⁺]ᵢ in differentiated IEC-Cdx2L1 cells, in which Kv1.1 and Kv1.5 channels were highly expressed and the membrane was hyperpolarized, increased significantly compared with that of parental IEC-6 cells (Fig. 6). [Ca²⁺]ᵢ was 143 ± 3.6 nM in IEC-Cdx2L1 cells and 116 ± 3 nM in parental IEC-6 cells (n = 25, P < 0.05). These results suggest that increased Kv channel activity and the resultant membrane hyperpolarization in differentiated IEC-Cdx2L1 cells increase the driving force for Ca²⁺ influx and thus...
raise \([\text{Ca}^{2+}]_{\text{cyt}}\), which may play a critical role in the increase in cell migration after wounding.

**Effect of inhibition of Kv channel expression by polyamine depletion on \([\text{Ca}^{2+}]_{\text{cyt}}\) and cell migration.** It has been shown (48) that Kv channel expression in intestinal epithelial cells requires polyamines and that depletion of cellular polyamines decreases expression of the Kv channel genes and reduces \(I_{K(v)}\). Exposure of IEC-Cdx2L1 cells to 5 mM DFMO (a specific inhibitor for ODC) for 4 days almost completely depleted cellular polyamines. Putrescine and spermidine were undetectable, whereas spermine was decreased by \(>65\%\) on day 4 in the DFMO-treated IEC-Cdx2L1 cells (data not shown).

Polyamine depletion by DFMO significantly inhibited expression of Kv1.1 and Kv1.5 channels (Fig. 7) but had no effect on expression of Kv2.1, Kv4.3, Kv9.3, and Kv\(\beta\)1.1 channels (data not shown) in differentiated IEC-Cdx2L1 cells. The mRNA levels of Kv1.1 and Kv1.5 in cells exposed to DFMO for 4 days were only \(~20\%\) and \(~30\%\) of the normal values (without DFMO), respectively (Fig. 7A). The decreased mRNA levels of Kv1.1 and Kv1.5 channels were completely prevented by exogenous spermidine (5 \(\mu\)M) given together with DFMO. The decreased mRNA levels of Kv1.1 and Kv1.5 channels were paralleled by decreases in the channel proteins (Fig. 7B). The level of Kv1.1 protein in cells treated with DFMO for 4 days was \(~35\%\) of normal value, whereas the Kv1.5 channel protein was \(~40\%\) of control. The protein levels of Kv1.1 and Kv1.5 channels returned to normal when DFMO was given together with exogenous spermidine.

Because IEC-Cdx2L1 cells did not express VDCC, inhibition of Kv channel activity by polyamine depletion would decrease the \(\text{Ca}^{2+}\) driving force through membrane depolarization, inhibit \(\text{Ca}^{2+}\) influx, and reduce \([\text{Ca}^{2+}]_{\text{cyt}}\). The results presented in Fig. 8 clearly show that depletion of cellular polyamines by DFMO significantly decreased the resting \([\text{Ca}^{2+}]_{\text{cyt}}\), which was associated with an inhibition of cell migration. \([\text{Ca}^{2+}]_{\text{cyt}}\) was decreased by \(~35\%\) (from 138 \pm 6 \(\text{nM}\) in control cells to 90 \pm 3 \(\text{nM}\) in DFMO-treated cells; \(n = 25\), \(P < 0.05\); Fig. 8A), whereas the rate of cell migration was decreased by \(~80\%\) in DFMO-treated cells (Fig. 8B and C). Addition of spermidine to the cultures containing DFMO not only reversed the inhibitory effects of polyamine depletion on \([\text{Ca}^{2+}]_{\text{cyt}}\) but also restored cell migration to normal levels. Differentiated intestinal epithelial cells seemed to migrate as a sheet into the wounded area (Fig. 8, Ba and Bc), although the mechanism involved is unknown. Furthermore, removal of extracellular \(\text{Ca}^{2+}\) from the culture medium immediately after wounding completely prevented the restoration of cell migration by exogenous spermidine in polyamine-deficient cells. There was no apparent loss of cell viability in cells treated with DFMO alone, DFMO plus spermidine, or spermidine plus the \(\text{Ca}^{2+}\)-free medium containing DFMO as assayed by the trypan blue staining method (data not shown).

**Effect of increasing \([\text{Ca}^{2+}]_{\text{cyt}}\) on cell migration.** The relationship between \([\text{Ca}^{2+}]_{\text{cyt}}\) and cell migration in differentiated IEC-Cdx2L1 cells was further examined by using the \(\text{Ca}^{2+}\)-ionophore ionomycin. Exposure to 1 \(\mu\)M ionomycin reversibly increased \([\text{Ca}^{2+}]_{\text{cyt}}\) by promoting \(\text{Ca}^{2+}\) influx regardless of the presence or absence of polyamines (Fig. 9A). In normal cells, \([\text{Ca}^{2+}]_{\text{cyt}}\) was dramatically increased after the addition of ionomycin for 5 min (from 141 \pm 7 to 485 \pm 23 \(\text{nM}\), \(n = 10\), \(P < 0.05\)). When ionomycin was washed out, \([\text{Ca}^{2+}]_{\text{cyt}}\) rapidly returned to basal levels (Fig. 9Aa). Exposure of polyamine-deficient cells to ionomycin also remarkably increased \([\text{Ca}^{2+}]_{\text{cyt}}\) (from 88 \pm 4 to 340 \pm 15 \(\text{nM}\), \(n = 10\), \(P < 0.05\)), but the peak of ionomycin-induced \(\text{Ca}^{2+}\)
influx was significantly reduced compared with that of controls (Fig. 9, Aa vs. Ab). This reduced response of DFMO-treated cells to ionomycin was apparently due to a decrease in the Ca$^{2+}$ driving force as a result of inhibition of Kv channel expression by polyamine depletion (Fig. 7).

Consistent with the augmenting effect on [Ca$^{2+}$]$_{cyt}$, treatment with ionomycin also increased cell migration in control and polyamine-deficient IEC-Cdx2L1 cells. Ionomycin given immediately after wounding increased the rate of cell migration by $\sim 20\%$ in control cells (without DFMO) (Fig. 9Ba). Cell migration in polyamine-deficient cells was also increased by ionomycin (Fig. 9Bb). At all time points studied (4 and 6 h after wounding), the rates of cell migration in polyamine-deficient cells exposed to ionomycin were significantly increased compared with those observed in cells treated with DFMO alone (from $70 \pm 4$ to $123 \pm 5$ cells/mm at 4 h; $83 \pm 4$ to $136 \pm 6$ cells/mm at 6 h, $n = 6$, $P < 0.05$) (Fig. 9Bb). These results indicate that a rise in [Ca$^{2+}$]$_{cyt}$ induced by the activation of Kv channel expression and membrane hyperpolarization in differentiated IEC-Cdx2L1 cells plays a critical role in the increased rate of cell migration after wounding.

**Effect of [Ca$^{2+}$]$_{cyt}$ on distribution of nonmuscle myosin II.** To determine the possible mechanism by which [Ca$^{2+}$]$_{cyt}$ mediates cell migration in differentiated intestinal epithelial cells, the effects of changes in [Ca$^{2+}$]$_{cyt}$, either decreased or increased, on cellular distribution of nonmuscle myosin II were examined in control and polyamine-deficient IEC-Cdx2L1 cells. As shown in Fig. 10A, a network of long stress fibers that traversed the cytoplasm was observed in the control group. This thick network of cortical myosin II fibers was just beneath the plasma membrane. Exposure of control cells to the Ca$^{2+}$-free medium during migration significantly decreased the formation of myosin II stress fibers (Fig. 10, A vs. B). The distribution of

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**Fig. 7. Effect of depletion of cellular polyamines by treatment with N-$\alpha$-difluoromethylornithine (DFMO) on Kv1.1 and Kv1.5 expression in IEC-Cdx2L1 cells.** Cells were cultured in DMEM containing 5% dFBS and 5 mM DFMO in the presence or absence of 5 $\mu$M exogenous spermidine (SPD) for 4 days. A: Kv channel mRNA expression as measured by RT-PCR analysis. Aa: PCR-amplified products displayed in agarose gels for Kv1.1 (594 bp), Kv1.5 (267 bp), and $\beta$-actin (244 bp). Ab: quantitative analysis of RT-PCR results by densitometry from cells described in Aa. Data were normalized to the amount of $\beta$-actin (optical density of the channel mRNA/optical density of the $\beta$-actin mRNA) and are expressed as means $\pm$ SE from 3 separate experiments. B: Kv channel protein expression as measured by Western blot analysis. Ba: immunoblots of IEC-Cdx2L1 cell proteins (25 $\mu$g/lane) were incubated with affinity-purified anti-Kv1.1, anti-Kv1.5, or anti-actin antibodies. Whole cell lysates from each group were subjected to electrophoresis on a 10% acrylamide gel. Kv1.1 ($\sim 86$ kDa) and Kv1.5 ($\sim 75$ kDa) channel proteins were identified by probing nitrocellulose with the specific antibodies. After the blot was stripped, actin ($\sim 45$ kDa) immunoblotting was performed as an internal control for equal loading. Bb: quantitative analysis of Western immunoblots by densitometry from cells described in Ba. Values are means $\pm$ SE from 3 separate experiments; relative Kv1.1 and Kv1.5 channel protein levels were corrected for loading as measured by densitometry of actin. *$P < 0.05$ compared with control and DFMO + SPD.
myosin II stress fibers was sparse and devoid of long stress fiber formation. Polyamine depletion by DFMO also affected cellular organization of nonmuscle myosin II in differentiated IEC-Cdx2L1 cells (Fig. 10, A vs. C). Long stress fibers disappeared in polyamine-deficient cells, and there were no distinct myosin II stress fibers in the cytoplasm. On the other hand, either elevation of [Ca^{2+}]_{cyt} by treatment with ionomycin in polyamine-deficient cells (Fig. 10D) or spermidine given together
stress fibers in migrating IEC-Cdx2L1 cells are significantly regulated by elevation of \([\text{Ca}^{2+}]_{\text{cyt}}\) during restitution after wounding.

**DISCUSSION**

Early rapid intestinal mucosal reepithelialization following superficial wounding is a complex process that includes the flattening, spreading, migrating, and repolarizing of differentiated columnar epithelial cells, but the exact mechanisms involved in this primary repair modality are still unclear. We (36) have recently demonstrated that differentiated intestinal epithelial cells induced by forced expression of the Cdx2 gene (IEC-Cdx2L1 cells) migrate over the wounded edge much faster than undifferentiated parental crypt cells (IEC-6 line) in an in vitro model mimicking the early cell division-independent stage of epithelial restitution. These findings (36) are of important biological significance because the rapid mucosal restitution of superficial wounds in vivo is the function of differentiated intestinal epithelial cells from the surface of the mucosa rather than from the undifferentiated epithelial cells within the crypts. In this study, we provide direct evidence to support the contention that activation of Kv channel expression and the resultant elevation of \([\text{Ca}^{2+}]_{\text{cyt}}\) due to enhanced Ca\(^{2+}\) driving force play a critical role in the process by which the rate of differentiated intestinal epithelial cell migration is increased after wounding.

Differentiated IEC-Cdx2L1 cells highly expressed Kv1.1 and Kv1.5 channels, associated with an increase in \(I_{K(v)}\) and membrane hyperpolarization. At the molecular level, the Kv channels in mammalian cells are composed of the pore-forming α-subunits and the regulatory β-subunits (16). It has been shown (11–13) that the function and number of Kv channels are major determinants of \(E_m\) in many types of cells. In resting cells, \(E_m\) is a function of the Na\(^+\), K\(^+\), and Cl\(^-\) concentration gradients across the plasma membrane and the relative ion permeability. Because transmembrane \(P_K > P_{Na} > P_{Cl}\) (\(P_{Na}/P_{K}/P_{Cl} = 1.00:4.00:4.45\)) is predominant under physiological conditions, \(E_m\) is controlled primarily by \(P_K\) and \(K^+\) concentration gradients. \(P_K\) (and thus \(E_m\)) is directly related to \(I_{K(v)}\), which is dependent on the total number of functional K\(^+\) channels and single-channel (unitary) current (18, 29).

When K\(^+\) channel opens or K\(^+\) channel expression rises, \(P_K\) is increased, leading to membrane hyperpolarization (13, 30). As shown in Figs. 2 and 3, the levels of Kv1.1 and Kv1.5 channel mRNAs and proteins in differentiated IEC-Cdx2L1 cells are approximately twofold higher than those of undifferentiated parental IEC-6 cells, indicating that membrane hyperpolarization in differentiated epithelial cells results, at least partially, from the increased expression of Kv channels.

\(E_m\) regulates \([\text{Ca}^{2+}]_{\text{cyt}}\) through controlling the Ca\(^{2+}\) driving force for Ca\(^{2+}\) influx in nonexcitable cells that do not express VDCC (13, 48). \([\text{Ca}^{2+}]_{\text{cyt}}\), which regulates a large number of biological functions, is con-
trolled by Ca$^{2+}$ influx through Ca$^{2+}$-permeable channels in the plasma membrane and Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores (32, 44). Under physiological conditions, extracellular Ca$^{2+}$ concentration is 1.6–1.8 mM, −10,000–20,000-fold higher than the resting [Ca$^{2+}$]$_{cyt}$ (50–150 nM), which provides a seemingly inexhaustible supply of Ca$^{2+}$ for its diverse intracellular function. The transmembrane Ca$^{2+}$ influx depends on the Ca$^{2+}$ driving force (i.e., the electrochemical gradient across the plasma membrane), which is predominantly regulated by $E_m$ while the Ca$^{2+}$ concentration gradient is constant (11–13, 18). In nonexcitable cells, including epithelial cells and lymphocytes, membrane hyperpolarization raises [Ca$^{2+}$]$_{cyt}$ by increasing the Ca$^{2+}$ driving force, whereas membrane depolarization reduces [Ca$^{2+}$]$_{cyt}$ by decreasing the Ca$^{2+}$ driving force (13, 30, 48). However, in excitable cells such as neurons and muscle cells that highly express VDCC, membrane depolarization increases [Ca$^{2+}$]$_{cyt}$ by opening VDCC (28, 44). Although differentiated IEC-Cdx2L1 cells did not express VDCC, they highly expressed TRPCs (Fig. 5), which are Ca$^{2+}$-permeable channels responsible for capacitative Ca$^{2+}$ entry (49, 52, 53). It is possible that the elevation of [Ca$^{2+}$]$_{cyt}$ in differentiated IEC-Cdx2L1 cells (Fig. 6) is partially due to the increase in capacitative Ca$^{2+}$ entry via TRPCs following membrane hyperpolarization induced by activation of Kv channel expression. Because passive Ca$^{2+}$ leakage, receptor-operated Ca$^{2+}$ channels, and nonselective cation channels all contribute to Ca$^{2+}$ influx (15, 30, 34, 44, 54), other Ca$^{2+}$ channels also may be involved in the process leading to the induction of [Ca$^{2+}$]$_{cyt}$ in differentiated IEC-Cdx2L1 cells.

Elevation of [Ca$^{2+}$]$_{cyt}$ in differentiated IEC-Cdx2L1 cells is a major mediator for the increased migration after wounding. Our (37, 48) previous studies have shown that Kv channels play a critical role in the regulation of cell migration by controlling $E_m$ and [Ca$^{2+}$]$_{cyt}$ in undifferentiated parental IEC-6 cells. Expression of Kv channels requires cellular polyamines, and depletion of cellular polyamines by treatment with DFMO decreases Kv channel gene expression, results in membrane depolarization, reduces [Ca$^{2+}$]$_{cyt}$, and inhibits cell migration. To determine the role of increased expression of Kv channels and the resultant elevation of [Ca$^{2+}$]$_{cyt}$ in the process of epithelial migration in differentiated IEC-Cdx2L1 cells after wounding, we examined the effects of inhibition of Kv channel expression by polyamine depletion on [Ca$^{2+}$]$_{cyt}$ and cell motility in the IEC-Cdx2L1 cells. As shown in Fig. 7, depletion of cellular polyamines by treatment with DFMO resulted in a remarkable decrease in levels of Kv1.1 and Kv1.5 channel mRNAs and proteins, although it negligibly affected expression of Kv2.1, Kv4.3, Kv9.3, and Kv$\beta$1.1 channels (data not shown). Reduced expression of Kv1.1 and Kv1.5 channels was associated with significant decreases in both [Ca$^{2+}$]$_{cyt}$ and cell migration in differentiated IEC-Cdx2L1 cells (Fig. 8). Exogenous spermidine given together with DFMO not only completely prevented the inhibition of Kv channel expression but also restored cell migration to normal levels. These findings are consistent with those observed (37, 48) in undifferentiated parental IEC-6 cells and strengthen the evidence that the activation of Kv channels plays an important role in the regulation of cell migration during early restitution by controlling $E_m$ and [Ca$^{2+}$]$_{cyt}$, which is regulated by cellular polyamines.

The decrease in the migration rate in the polyamine-deficient cells is primarily due to the decrease in [Ca$^{2+}$]$_{cyt}$ rather than to the alteration of differentiation, because polyamine depletion by DFMO fails to affect characteristics of differentiated phenotype in IEC-Cdx2L1 cells (36). In support of this possibility, removal of extracellular Ca$^{2+}$ from the culture medium immediately after wounding almost completely prevented the restoration of cell migration by exogenous spermidine in polyamine-deficient IEC-Cdx2L1 cells (Fig. 8, B and C). This contention is further supported by the results presented in Fig. 9 showing that increasing [Ca$^{2+}$]$_{cyt}$ by treatment with the Ca$^{2+}$-ionophore ionomycin significantly increased cell migration in polyamine-deficient IEC-Cdx2L1 cells.

To investigate how elevated [Ca$^{2+}$]$_{cyt}$ modulates cell migration during restitution in differentiated intestinal epithelial cells, we examined the distribution of cytoskeletal protein nonmuscle myosin II in IEC-Cdx2L1 cells in the presence or absence of Ca$^{2+}$. Nonmuscle myosin II is a major cellular motor molecule of the intestinal epithelial cells (9, 17) and is implicated in the formation of stress fibers that regulate cell adhesion, spreading, and motility (1, 17, 20, 31, 41). Dysfunction of myosin II, by either microinjection of myosin II antibody, antisense RNA, or recombination dominant negative mutants, significantly decreases cell migration in nonmuscle cells (22, 40). Figure 10 shows that [Ca$^{2+}$]$_{cyt}$ regulates the migration of differentiated IEC-Cdx2L1 cells at least partially through alteration of the formation of actomyosin stress fibers. When [Ca$^{2+}$]$_{cyt}$ was decreased by exposure to the Ca$^{2+}$-free medium or inhibition of Kv channel expression via polyamine depletion, the number of long stress fibers of myosin decreased significantly, and in some cells they disappeared completely from the cytoplasm. In contrast, increased [Ca$^{2+}$]$_{cyt}$ via treatment with the Ca$^{2+}$-ionophore ionomycin in polyamine-deficient cells promoted the formation of myosin II stress fibers. These results are consistent with data from other studies (19–21) finding that the formation and function of stress fibers are regulated by Ca$^{2+}$-dependent signaling pathways. Although the exact mechanisms involved in downstream targeting in this process are still unknown, we (37) have recently demonstrated that the stimulation of myosin II stress fiber formation by polyamines is a result of Ca$^{2+}$-induced activation of RhoA protein in undifferentiated parental IEC-6 cells.

In summary, these results indicate that differentiated IEC-Cdx2L1 cells highly express Kv1.1 and Kv1.5 channel mRNAs and proteins, which are associated with a significant increase in $I_{Kv}$ and membrane hyperpolarization. Because IEC-Cdx2L1 cells do not express VDCC, the membrane hyperpolarization in dif-
Differented intestinal epithelial cells increases the Ca$^{2+}$ driving force for Ca$^{2+}$ influx and raises [Ca$^{2+}$]$_{cyt}$. Inhibition of Kv channel expression by depletion of cellular polyamines with DFMO decreases [Ca$^{2+}$]$_{cyt}$ and inhibits cell migration during restitution. Increasing [Ca$^{2+}$]$_{cyt}$ by exposure to the Ca$^{2+}$ ionophore ionomycin after wounding promotes cell migration in normal and polyamine-deficient IEC-Cdx2L1 cells. Elevation of [Ca$^{2+}$]$_{cyt}$ also increases the formation of myosin II stress fibers in differented IEC-Cdx2L1 cells, while decreased [Ca$^{2+}$]$_{cyt}$ after inhibition of Kv channel expression or removal of extracellular Ca$^{2+}$ results in the reorganization of myosin II, along with a marked reduction of stress fibers. These findings suggest that differented intestinal epithelial cells exhibit increased migration after wounding, at least partially, by the activation of Kv channel expression, leading to the increase in the Ca$^{2+}$ driving force for Ca$^{2+}$ influx during restitution.

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