Barttin modulates trafficking and function of ClC-K channels

Ute Scholl†*, Simon Hebeisen††, Audrey G. H. Janssen†‡, Gerhard Müller-Neven§, Alexi Alekov*, and Christoph Fahlke†¶**

*Abteilung Neurophysiologie, Medizinische Hochschule Hannover, 30625 Hannover, Germany; †Abteilung Physiologie and Institut für Biochemie, Rheinisch-Westfälische Technische Hochschule (RWTH) Aachen, 52074 Aachen, Germany; §Centro de Estudios Científicos (CECS), Valdivia 509000, Chile; and ¶Zentrum für Systemische Neurowissenschaften Hannover (ZSN), 30559 Hannover, Germany

Barttin is an accessory subunit of a subgroup of ClC-type chloride channels expressed in renal and inner ear epithelia. In this study, we examined the effects of barttin on two ClC-K channel isoforms, rat ClC-K1 and human ClC-Kb, using heterologous expression, patch clamping, confocal imaging, and flow cytometry. In the absence of barttin, only a small percentage of rClC-K1 and hClC-Kb channels are inserted into the plasma membrane. Coexpression of barttin enhances surface membrane insertion and furthermore modifies permeation and gating of ClC-K channels. hClC-Kb channels are nonfunctional without barttin and require the coexpressed accessory subunit to become anion conducting. In contrast, rClC-K1 channels are active without barttin, but at the cost of reduced unitary conductance as well as altered voltage dependence of activation. We mapped the separate functions of barttin to structural domains by a deletion analysis. Whereas the transmembrane core is necessary and sufficient to promote ClC-K channel exit from the endoplasmic reticulum, a short cytoplasmic segment following the second transmembrane helix modifies the unitary conductance. The entire cytoplasmic carboxyl terminus affects the open probability of ClC-K channels. The multiple functions of barttin might be necessary for a tight adjustment of epithelial Cl− conductances to ensure a precise regulation of body salt content and endocochlear potential.

Results

Barttin Promotes Surface Membrane Insertion of rClC-K1 and hClC-Kb.

We expressed rClC-K1 and hClC-Kb channels alone or together with human barttin in mammalian cells and measured anion currents with the patch clamp technique. Consistent with previous reports, we observed anion currents in the absence of barttin only in cells expressing rClC-K1 (10), but not in those expressing hClC-Kb (11). Coexpression of barttin with rClC-K1 and hClC-Kb, respectively, results in large whole-cell anion current amplitudes in tsA201 cells (Fig. 1A and B). To study the effect of barttin on the subcellular ClC-K channel distribution, we generated fluorescent protein-tagged constructs (Fig. 1C and D) and examined transfected cells by confocal imaging and flow cytometry. Barttin inserts itself into the plasma membrane (Fig. 1E), whereas ClC-K1 and ClC-Kb channels are predominantly present in the endoplasmic reticulum (Fig. 1F and H). Cotransfection of barttin results in a redistribution of the chloride channels to the surface membrane (Fig. 1G and J). Barttin exerts similar effects on ClC-K channel trafficking in MDCKII cells, a cell line forming confluent monolayers with characteristics of in vivo renal epithelia (12) (Fig. 1), and in tsA201 cells (Fig. 6, which is published as supporting information on the PNAS website), the cell line we routinely use for electrophysiological experiments.

We next quantified the effect of barttin on the subcellular distribution of ClC-K channels by means of flow cytometry. We expressed mutant ClC-K channels with an amino-terminal GFP protein and a FLAG epitope between transmembrane domains L and M (Fig. 1D) in tsA201 cells, applied anti-FLAG antibodies to nonpermeabilized cells, and individually measured GFP and antibody fluorescence levels. Because the engineered FLAG epitope is located extracellularly (13, 14), a plot of the antibody fluorescence levels versus the GFP fluorescence (Fig. 1J and K) provides information about the fraction of channels inserted into the plasma membrane. Antibody fluorescence levels were negligible in cells expressing channel constructs lacking the FLAG-epitope (Fig. 1J and K), demonstrating the specificity of antibody binding. In the absence of barttin, antibody fluorescence levels of cells with FLAG-tagged ClC-K constructs are significantly larger than background levels (P < 0.05; Fig. 1J and K). Therefore, hClC-Kb and rClC-K1 insert into the plasma membrane even without barttin. Barttin increases the ratio of antibody fluorescence to GFP fluorescence and thus the fraction of channels in the surface membrane severalfold (Fig. 1J and K). Both in the presence and in the absence of barttin, a larger percentage of rClC-K1 channels could traffic to the surface membrane than hClC-Kb.

ClC-Kb Channels Are Nonfunctional in the Absence of Barttin. Because hClC-Kb channels can insert into the cell surface even in the absence of barttin (Fig. 1J), the complete lack of anion currents

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

†U.S. and S.H. contributed equally to this work.

**To whom correspondence should be addressed. E-mail: fahlke.christoph@mh-hannover.de.

© 2006 by The National Academy of Sciences of the USA

www.pnas.org/cgi/doi/10.1073/pnas.0601631103

PNAS | July 25, 2006 | vol. 103 | no. 30 | 11411–11416
Fig. 1. Barttin promotes surface membrane insertion of CIC-K channels. (A and B) Representative currents from cells coexpressing hClC-Kb (A) or rClC-K1 (B) with barttin and mean isochronal current amplitudes determined 2 ms after a voltage step to −155 mV (n > 8) from cells expressing channels with and without barttin. (C and D) Topology model of barttin (C) and CIC-K channels (D). The position of the FLAG epitope used for FACS analysis is marked by an arrow. (E) Confocal images of live MDCK cells coexpressing a barttin-YFP fusion protein and a fluorescent marker for the membrane surface (CFP-Mem; Clontech). CFP is shown in green, and YFP is shown in red. This color code results in an orange coloring of regions where both proteins overlap. (F and H) Confocal images of live MDCK cells coexpressing a fluorescent marker for the endoplasmic reticulum (CFP-ER; Clontech) together with YFP-hClC-Kb (F) and YFP-rClC-K1 (H). (G and I) Confocal images of live MDCK cells coexpressing a barttin-CFP fusion protein and YFP-hClC-Kb (G) or YFP-rClC-K1 (I). (Scale bars: 5 μm.) (J and K) Surface expression of hClC-Kb (J) and rClC-K1 (K) with and without (*) barttin in transiently transfected tsA201 cells determined by flow cytometry. Shown are plots of fluorescence levels of an anti-Flag antibody versus the GFP fluorescence. Data were binned in four groups where means and SEMs were obtained. GFP-hClC-Kb without FLAG (●) was used as a control. For both channels, antibody fluorescence is significantly different from control level (P < 0.05), and barttin significantly increases the fluorescence levels (P < 0.05).

Fig. 2. hClC-Kb protopores are functional only in the presence of barttin. (A) Surface expression of hClC-Kb in transiently transfected tsA201 cells determined by flow cytometry, when expressed alone (●), together with hClC-1 (■), or with barttin (▲). GFP-hClC-Kb without FLAG (●) was used as a control. (B and C) Representative current recordings from a cell expressing the hClC-1-hClC-Kb concatamer alone (B) and from another cell expressing it together with barttin (C).

(distinguish between these possibilities, we studied heterodimeric channels consisting of hClC-Kb and another CIC isoform that inserts into the plasma membrane independently of barttin, hClC-1 (15). We reasoned that hClC-Kb might reach the surface membrane at higher density as a component of a heterodimeric channel together with such related protein. By means of flow cytometry, we first compared surface membrane expression in cells expressing hClC-Kb alone and in cells coexpressing hClC-Kb and hClC-1. Cotransfection of hClC-1 increases surface insertion of hClC-Kb (P < 0.05; Fig. 2A), demonstrating that the two different CIC isoforms can assemble to heterodimeric channels and that these heterodimeric channels effectively travel to the plasma membrane. If one assumes that hClC-Kb does not display anion currents in the absence of barttin because of the low membrane insertion, the cotransfection with hClC-1 should result in the appearance of an hClC-Kb current component. In contrast to this prediction, coexpression of hClC-1 and hClC-Kb without barttin resulted in anion currents that were indistinguishable from pure hClC-1 currents (data not shown).

In coexpression experiments, a quantitative analysis of the effect of barttin is difficult, because the contribution of homodimeric channels to the observed current amplitudes is not known. To solely express heterodimeric channels (16), we engineered a concatameric construct that links one hClC-1 and one hClC-Kb coding region into a single ORF. In the absence of barttin, expression of this construct alone results in anion currents that were undistinguishable from pure hClC-1 currents (data not shown).
mV were applied. WT rClC-K1 channels are open at the holding potential, and current amplitudes change instantaneously upon voltage steps in both directions. For membrane hyperpolarization, this instantaneous rise is followed by a time-dependent current increase. The time-dependent changes of rClC-K1 currents are comparable for rClC-K1 channels with and without barttin (Fig. 3A and B). However, the voltage dependence of the relative open probability is shifted to more positive potentials by barttin (Fig. 3C). Therefore, barttin increases the channel open probability in the physiological voltage range.

We then used nonstationary noise analysis to study the effects of barttin on unitary channel properties. This analysis was not feasible for WT rClC-K1 channels because of the limited time-dependent changes of the macroscopic current amplitude upon voltage steps (Fig. 3A and B). We therefore used a mutant rClC-K1 bearing a single point mutation, V166E. Because single channel experiments on ClC-0 channels demonstrated that substitutions of this acidic side chain did not affect the single channel amplitude (17), we expect unitary current amplitudes of V166E rClC-K1 to be identical to those of WT channel. V166E alters gating of rClC-K1 (Fig. 3D–F). Without barttin, V166E rClC-K1 channels are activated by membrane hyperpolarization (Fig. 3D and F), whereas cotransfection of barttin inverts the voltage dependence of activation (Fig. 3E), resulting in a depolarization-induced activation (Fig. 3F).

Nonstationary noise analyses (Supporting Text and Fig. 7, which are published as supporting information on the PNAS web site) demonstrated that the unitary conductance of V166E rClC-K1 was 3-fold increased by barttin (without barttin, 6.5 ± 0.3 pS; with barttin, 19.4 ± 0.9 pS; n > 6). These results were corroborated by direct measurements of unitary current amplitudes (Supporting Text and Fig. 8, which is published as supporting information on the PNAS web site). Single channel recordings demonstrated the occurrence of channels with a unitary conductance of 20.5 pS in cells cotransfected with V166E rClC-K1 and barttin and of 8.6 pS in cells expressing V166E rClC-K1 alone.

Barttin thus changes gating and ion permeation of rClC-K1 channels. Assuming that ClC-K channels exhibit a three-dimensional structure comparable with the recently solved structure of an Escherichia coli ClC transporter (14), barttin most likely does not contribute to the formation of the ion conduction pathway, but rather allosterically modifies the ion conduction pathway of V166E rClC-K1.

Separate Domains of Barttin Exhibit Distinct Functions. The predicted transmembrane topology of barttin contains two transmembrane helices encompassing the amino acids between 9 and 54 and a cytoplasmic carboxyl terminus of 266 aa. We generated various truncation mutants and studied the effect of these truncated barttins on the subcellular distribution and on the functional properties of ClC-K channels (Fig. 4). An isolated carboxyl-terminal fragment Cterm(T61-G320) and three barttin truncation mutants (C54X, Y57X, and T61X) do not result in macroscopic chloride currents in cells when expressed with hClC-Kb (Fig. 4A). In contrast, all other tested mutants with longer carboxyl termini (I72X, L85X, S115X, and L243X) result in current amplitudes comparable with WT barttin. For rClC-K1, even C54X and T61X cause significant increases of macroscopic current amplitudes (Fig. 4B). Confocal microscopy revealed that all truncated barttin mutants longer than C54X cause hClC-Kb channels to be present predominantly in the surface membrane (Fig. 4D–F). Our results demonstrate that trafficking and function of ClC-K channels are conferred by separate regions of barttin. Whereas the transmembrane core of barttin is sufficient for ClC-K channel exit from the endoplasmic reticulum and incorporation into the surface membrane, an additional stretch of 15–17 aa (I72X barttin) is necessary to turn hClC-Kb into a conductive anion channel.

The isolated carboxyl-terminal fragment Cterm(T61-G320)-
CFP did not change ClC-K currents (Fig. 4B). It is soluble and small enough to diffuse through nuclear pores into the cell nucleus, resulting in a fluorescent staining of the whole cell (Fig. 4K). There is no redistribution of CFP fluorescence in experiments involving coexpression with hClC-Kb. Our data suggest that the carboxyl-terminal fusion protein of barttin does not stably bind to the CIC channel protein.

To define barttin domains involved in modifying unitary current amplitudes and in gating of V166E rClC-K1 channels, we next studied the effect of truncated barttins on these channels. Fig. 5A shows normalized representative current recordings from cells expressing V166E rClC-K1 alone, with three truncated barttin constructs (T61X, S115X, and L243X) and with WT barttin. Whereas channels without barttin are activated by hyperpolarization, all other tested barttin constructs induce channel activation by membrane depolarization. Fig. 5B and C shows the effects of various barttin truncations on the voltage dependences of the absolute open probability and unitary current amplitudes determined by nonstationary noise analysis. V166E rClC-K1 channels with truncated barttins exhibit a smaller absolute open probability than those with WT barttin at positive potentials. However, at negative potentials, all tested barttins reduce the absolute open probability compared with barttin-less V166E rClC-K1. The alteration of unitary current amplitude by mutant barttin requires domains differing from those involved in the setting of the absolute open probability. All barttins longer than I72X result in the same unitary current amplitude as WT barttin, whereas Y57X and T61X barttin increase the unitary current amplitude, but not to the WT level (Fig. 5C and Table 1). The transmembrane core together with a short stretch of 15–17 aa is sufficient to increase the unitary conductance of V166E rClC-K1 channels to the level of their WT barttin-associated counterparts.
at physiological voltages (Fig. 3), it slightly decreases this value
causes a shift of the relative open probability of WT rClC-K1 to
a glutamate residue in the signature sequence GKE
ClC-K channels by barttin. This is not a surprising result because
hClC-Ka by barttin is therefore more similar to hClC-Kb than to
absence of this accessory subunit (3, 4, 11). The regulation of
nor rClC-K2 and hClC-Kb can be functionally expressed in the
hClC-Ka, the functional homologue to rClC-K1 in humans (8),
treatment of hypertension or renal failure.
channel modifiers to stimulate water and salt excretion in the
by barttin, such components could be used as selective ClC-K
of small molecules that selectively modify the various functions
core. The modular architecture of barttin might allow the design
results define barttin as a protein with separate functional

15–17 adjacent amino acids of the carboxyl terminus switch
hClC-Kb channels from closed to open state (Fig. 4A) and
increase the unitary current amplitudes of rClC-K1 ~3-fold (Fig.
5C). The remainder of the carboxyl-terminal tail is necessary for
setting the absolute channel open probability (Fig. 5B). These
results define barttin as a protein with separate functional
domains: the transmembrane core associates with the pore-
forming subunit of the channel and acts as a chaperone for
intracellular trafficking. The cytoplasmic carboxyl terminus modifies the functional properties of the CIC-K/barttin channel
when associated with CIC-K channels via the transmembrane
core. The modular architecture of barttin might allow the design
of small molecules that selectively modify the various functions
of the CIC-K

Thus far, rClC-K1 is the only mammalian CIC-K isoform that
is functional without barttin in a heterologous system. Neither
hCIC-Ka, the functional homologue to rClC-K1 in humans (8),
nor rCIC-K2 and hCIC-Kb can be functionally expressed in the
absence of this accessory subunit (3, 4, 11). The regulation of
hCIC-Ka by barttin is therefore more similar to hCIC-Kb than to
rCIC-K1, indicating a species specificity in the regulation of CIC-K
channels by barttin. This is not a surprising result because
hCIC-Ka and hCIC-Kb exhibit a higher sequence identity to each
other than to rCIC-K1 or to rCIC-K2 (11).

CIC-K channels are the only mammalian CIC channels lacking
a glutamate residue in the signature sequence GKE/VGPPXH
(18) close to the selectivity filter of this channel family (14). The
functional modification of rCIC-K1 by barttin assigns a likely
physiological significance to this uniqueness. Whereas barttin
causes a shift of the relative open probability of WT rCIC-K1 to
more positive potentials and thus increases the open probability
at physiological voltages (Fig. 3), it slightly decreases this value
for V166E rCIC-K1 (P_{open} at −75 mV: 0.27 ± 0.02 (n = 6)
without barttin and 0.24 ± 0.02 (n = 6) with barttin). CIC-K
channels might have lost the conserved negative side chain as an
evolutionary adjustment to enable an increase of the channels’
open probability by barttin.

Barttin affects the subcellular trafficking as well as the ion
conduction and gating of renal and inner ear chloride channels.
The number of functions of barttin is unprecedented among
accessory subunits, demonstrating that CIC-type chloride
channels are unique not only in molecular architecture and gating
mechanisms, but also in their regulation by non pore-forming
subunits.

Methods

Mutagenesis and Channel Expression. tsA201 and MDCKII cells
were transfected with 0.1–10 μg of plasmid DNA encoding WT
and mutant rCIC-K1, hCIC-Kb, and human barttin by using a
calcium phosphate precipitation method (16). Cells were typically
examined 2 days after transient transfection. For electrophysiological experiments, cells were cotransfected with a plasmid
encoding the CDS antigen and incubated 5 min before use
with polystyrene microbeads precoated with anti-CD8 antibodies
(Dynabeads M-450 CD 8; Dynal, Great Neck, NY) (19). Only
cells decorated with microbeads were used for electrophysiological
recordings. Methods for generation of the transfected plasmids are provided in Supporting Text.

Electrophysiology. Standard whole-cell or excised outside-out patch clamp recordings were performed by using an EPC9 or 10 (HEKA
Electronics, Lambrecht, Germany) amplifier (20, 21). Pipettes
were pulled from borosilicate glass and had resistances between 1.0 MΩ
and 2.2 MΩ for whole-cell recordings and >10 MΩ for single-
channel recordings. In whole-cell experiments, 60–80% of the
series resistance was compensated by an analog procedure, so that
the calculated voltage error due to access resistance was always <2
mV. The standard extracellular solution contained 140 mM NaCl,
4 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, and 5 mM Hepes; the
standard intracellular solution contained 120 mM NaCl, 2 mM
MgCl₂, 5 mM EGTA, and 10 mM Hepes. All solutions were
adjusted to pH 7.4 with NaOH. Cells/patches were clamped to 0
mV between test sweeps. For single-channel recordings, currents
were filtered with a 0.5 or 1 kHz low-pass Bessel filter and sampled
at 10 kHz. Capacitive and leak currents were compensated by
subtracting scaled control traces recorded at the calculated anion
reversal potential (~−5 mV).

Data were analyzed by a combination of PulseTools (HEKA
Electronics, Lambrecht, Germany) and Sigmaplot (Jandel
Scientific, San Rafael, CA) programs. Isochronal current amplitudes
were measured 2 ms after the voltage step. To determine
the unitary current amplitudes as well as absolute open proba-
bilities of rCIC-K1 channels, nonstationary noise analysis was
performed as described (20). Amplitude histograms were gen-
erated from 10-s voltage steps to various potentials. The single-
channel amplitude and the absolute open probability were
determined by fitting Gaussian distributions to these histograms.
In patches with more than one channel, the open probability of
a single channel was calculated assuming a binomial distribution
of channel openings. Unitary conductances were calculated by
fitting linear relationships to the voltage dependences of the
unitary current amplitudes (Figs. 5 and 8). To obtain the voltage
dependence of activation, isochronal current amplitudes were
determined at a voltage step to −125 mV after 200-ms prepulses
to various voltages, normalized by their maximum value and
plotted versus the preceding potential. The absolute voltage
dependence was obtained by adjusting the relative activation
curve to the open probability at +75 mV calculated by nonsta-
tionary noise analyses. Activation curves were fit with a sum of
a voltage-independent minimum open probability (P_{open min}) and a

Table 1. Permeation and gating properties of V166E rClC-K1 channels coexpressed with WT and mutant barttin
or without barttin, respectively

<table>
<thead>
<tr>
<th>pore-forming and accessory subunit</th>
<th>unitary conductance, pS</th>
<th>V_0.5, mV</th>
<th>apparent gating charge, e_o</th>
<th>P_{open min}</th>
<th>P_{open max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>V166E rCIC-K1</td>
<td>6.5 ± 0.3, n = 11</td>
<td>−110.2 ± 2.8, n = 8</td>
<td>−1.0 ± 0.0, n = 8</td>
<td>1.0</td>
<td>0.14</td>
</tr>
<tr>
<td>+ Y57X barttin</td>
<td>9.1 ± 0.3, n = 6</td>
<td>31.2 ± 1.4, n = 16</td>
<td>1.1 ± 0.1, n = 16</td>
<td>0.32</td>
<td>0.19</td>
</tr>
<tr>
<td>+ T61X barttin</td>
<td>13.1 ± 0.5, n = 7</td>
<td>38.0 ± 2.3, n = 12</td>
<td>1.3 ± 0.1, n = 12</td>
<td>0.55</td>
<td>0.27</td>
</tr>
<tr>
<td>+ I72X barttin</td>
<td>18.1 ± 0.4, n = 12</td>
<td>17.7 ± 1.9, n = 19</td>
<td>1.1 ± 0.0, n = 19</td>
<td>0.88</td>
<td>0.21</td>
</tr>
<tr>
<td>+ L85X barttin</td>
<td>16.6 ± 0.6, n = 9</td>
<td>15.3 ± 2.3, n = 14</td>
<td>1.1 ± 0.0, n = 14</td>
<td>0.71</td>
<td>0.19</td>
</tr>
<tr>
<td>+ S115X barttin</td>
<td>18.7 ± 0.6, n = 7</td>
<td>11.4 ± 1.3, n = 17</td>
<td>1.2 ± 0.0, n = 17</td>
<td>0.84</td>
<td>0.33</td>
</tr>
<tr>
<td>+ L243X barttin</td>
<td>18.5 ± 0.7, n = 10</td>
<td>14.7 ± 2.8, n = 6</td>
<td>1.0 ± 0.1, n = 6</td>
<td>0.87</td>
<td>0.30</td>
</tr>
<tr>
<td>+ WT barttin</td>
<td>19.4 ± 0.9, n = 6</td>
<td>11.6 ± 3.5, n = 15</td>
<td>0.9 ± 0.0, n = 15</td>
<td>1.00</td>
<td>0.20</td>
</tr>
</tbody>
</table>
voltage-dependent term: \( I(V) = \text{Amp}/(1 + e^{zeo(V - V_0)/kT}) \) + \( P_{\text{min}} \), with \( zeo \), being the apparent gating charge and \( \text{Amp} + P_{\text{min}} \) being the maximum open probability. For the determination of mean peak current amplitudes (Fig. 4), whole cell and excised outside-out patch currents were averaged. To account for the distinct membrane area in patches compared with a whole cell, the patch current amplitudes were multiplied by the ratios of resting conductance before excision by the corresponding value after excision (21).

Confocal Microscopy and Flow Cytometry. Live cell confocal imaging was carried out with transiently transfected MDCKII or tsA201 cells on a glass coverslip 48–72 h after transfection as described (21). For flow cytometry, \( 5 \times 10^5 \) to \( 1 \times 10^6 \) transfected tsA201 cells were resuspended in cold PBS supplemented with 5% FCS and 0.1% sodium azide (PBS/azide) and incubated with 2 \( \mu \)g/ml mouse anti-flag antibody (Sigma) for 30 min at 4°C. Cells were washed with cold PBS/azide and subsequently incubated in darkness with a 1:50 dilution of an R-phycoerythrin-conjugated α-mouse antibody for 30 min at 4°C. Cells were washed again and resuspended in PBS/azide. Then \( 10^4 \) cells per sample were analyzed by flow cytometry using a FACScanlibr (Becton Dickinson) equipped with a 488-nm argon laser. Data were analyzed by a combination of CellQuest Pro (BD Biosciences), WinMDI version 2.8, and SigmaPlot (Jandel Scientific). GFP fluorescence of individual cells was sorted into four bins, and average antibody fluorescence in each of these bins was plotted against average GFP fluorescence. For statistical evaluation, ratios of average GFP and antibody fluorescence of the region with the largest GFP fluorescence were compared by using Student’s \( t \) test.

We thank Drs. A. L. George (Vanderbilt University, Nashville, TN) and S. Uchida (Tokyo Medical and Dental University) for providing the expression constructs for hCIC-Kb, rCIC-K1, and barttin. We thank Drs. Tania Alekova, Patricia Hidalgo, Heider Linder, Günther Schmalzing, and Saba Sile for helpful discussions; Dr. S. Radtke for help with the flow cytometry; Drs. A. Luckhoff and P. Heinrich for support; Barbara Poser for excellent technical assistance; and Dr. Stefan Wullner (RWTH, Aachen, Germany) for providing the MDCK cells. These studies were supported by Deutsche Forschungsgemeinschaft Grants FOR450, TP10 (to C.F.), and SFB 542, Zentralprojekt Z1 (to G.M.-N.) and by the Studienstiftung des deutschen Volkes (U.S.).