MjK1, a K^+ channel from *M. jannaschii*, mediates K^+ uptake and K^+ sensitivity in *E. coli*

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Abstract The methanogenic and hyperthermophilic deep-sea archaeon *Methanococcus jannaschii* has three putative K^+ channels, MVP (Mj0139), MjK1 (Mj0138.1) and MjK2 (Mj1357). The physiological function of these K^+ channels was examined in a viability assay, using the *Escherichia coli* mutant LB2003 (kup1, ΔkdpABC5, ΔtrkA). While MjK2 expression had no effects on the potassium-dependent phenotype of LB2003, MVP and MjK1 complemented the deficiency at a concentration of 1 mM KCl. In contrast to KcsA, MthK and MVP, MjK1 strongly affected host cell viability at 10 and 100 mM KCl. The toxic effects were less pronounced when growth media were supplemented with the K^+ channel blocker $BaCl_2$.

Key words: Complementation; Archaebacterial potassium channel; Affinity purification

1. Introduction

Potassium is an essential nutrient which plays an important role in many aspects of life. Thus most bacteria have developed K^+ transporters and channels, which enable molecular adaptation to different environmental conditions. K^+ channels are ion-selective pores, composed of two or four subunits, which conduct potassium with high selectivity along the electrochemical gradient. Most of the knowledge about their tetrameric architecture and function was gained from studies on the bacterial K^+ channels, KcsA (*Streptomyces lividans*), MthK (*Methanobacterium thermoautotrophicum*) and K*v*AP (*Aeropyrum pernix*) [1–3].

Recently two K^+ channels (MjK1, MjK2) with two putative transmembrane helices per subunit and one with six (MVP, methanococcal voltage-gated potassium channel) were identified in the genome of the hyperthermophilic deep-sea archaeon *Methanococcus jannaschii* [4]. MVP mediated potassium uptake in *Escherichia coli* and yeast; electrophysiological measurements exhibited activation of the channel upon hyperpolarization [5]. In contrast, little is known about the biological function of bacterial K^+ channels with two transmembrane helices per subunit, although the structure of the related channel MthK has been solved [6].

In order to examine the physiological function of the K^+ channels of *M. jannaschii*, we cloned their genes for heterologous expression in *E. coli*. A subsequent phenotypic analysis using an *E. coli* mutant strain that is deficient in the three major potassium uptake systems Trk, Kup and Kdp, identified characteristic physiological properties for each channel.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Genomic DNA for cloning was isolated from *M. jannaschii* DSM 2661 cells (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). The *E. coli* strain TOP10 (Invitrogen) was used as a host for recombinant plasmids and grown at 37°C in 1.5 l Luria Bertani medium (LB) using an ampicillin concentration of 100 μg ml^{-1}. Once the cell culture reached the mid-log phase (OD_{600} = 0.7) the protein expression was induced with isopropyl-β-D-1-thiogalactoside (IPTG) (1 mM) and incubation was continued for 4 h until the cells were harvested.

2.2. Cloning

The open reading frames Mj0138.1, Mj0139 and Mj1357 were amplified with SAWADY-PWO polymerase from chromosomal DNA of *M. jannaschii* as described recently [7]. Mj0138.1 and Mj0139 genes were cloned into the one step cloning vector pTcHis2TOPO (Invitrogen), whereas the Mj1357 gene was cloned into pTrcHis2B using *Bam*H1 and *Hin*dIII restriction sites generated by the primers. The following primers were used for amplification: Mj0138.1 forward: 5′-ATG GAA ACT TAT GAG AAG ATA GAG CTT G-3′, reverse: 5′-TTC TAC TCC CTC AAC ATA TCT TTT TAA C-3′; Mj0139 forward: 5′-ATG AAC TTA AAA GAT AGG CGG TTA AAG A-3′, reverse: 5′-ATC TCC CTT TAA TGC CTT TTG TAA AGA-3′; Mj1357 forward: 5′-ATG GAT CCG ATG GAG ACG TCA A-3′, reverse: 5′-TTT GTT CTA GAA AGC TTT GTA ACT-3′ (for pTrcHis2B). The resulting plasmids were named pMjK1 (Mj0138.1), pMjK2 (Mj0137) and pMVP (Mj0139), respectively.

2.3. Complementation

LB2003 cells [8] carrying either pMVP, pMjK1, pMjK2 or pTrcHis2C (Invitrogen) as negative control were grown at 37°C in KML medium (46 mM Na_2HPO_4, 23 mM NaH_2PO_4, 8 mM $(NH_4)_2SO_4$, 1 mM sodium citrate, 0.4 mM $MgSO_4$, 6 μM $FeSO_4$, 1 mg l^{-1} thiamine, 10 mM glucose, 100 μg ml^{-1} ampicillin (the number after the name of the medium indicates the potassium concentration in mM). The control and cells carrying pMjK2 were grown in KML100 and cells carrying pMVP and pMjK1 were grown in KML1. After the cultures had reached an OD_{600} of 0.6, the cells were sedimented at 3500×*g* for 10 min. The pellet was resuspended in 20 ml KML0 and sedimented again. After three cycles of washing the OD_{600} of each sample was adjusted to 0.5 with KML0. For growth experiments on solid media 50 μl aliquots of this suspension were plated on

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Abbreviations: RCK, regulators of K^+ conductance; MVP, methanococcal voltage-gated potassium channel; MjK1 and MjK2, *Methanococcus jannaschii* K^+ channels 1 and 2; MthK, *Methanobacterium thermoautotrophicum* K^+ channel; KcsA, K^+ channel A *Streptomyces lividans*; TrkA-N and TrkA-C, domains of potassium uptake systems

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KML media containing the indicated potassium concentration and 1.3% agar. The growth experiments in liquid media were performed by diluting 10 μl aliquots of the aforementioned suspension in 20 ml of KML (KCl added as indicated).

2.4. Immunological detection of fusion protein

Membrane vesicles (50 μg protein) or purified proteins were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and blotted to nitrocellulose (Biometra). C-terminal fusion peptides were detected using anti-myc antibody (Invitrogen) coupled to alkaline phosphatase, according to the manufacturers instructions.

3. Results and discussion

3.1. Structural features of M. jannaschii K^+ channels

MjK1 and MjK2 subunits consist of two putative transmembrane helices that surround the pore sequence TXGYGD. In both channels a cytoplasmic RCK domain (regulators of K^+ conductance) forms the C-terminus and makes up two thirds of the protein (Fig. 1) [6,9,10]. The RCK domains contain two conserved regions, domain of potassium uptake system TrkA-N (domain of potassium uptake

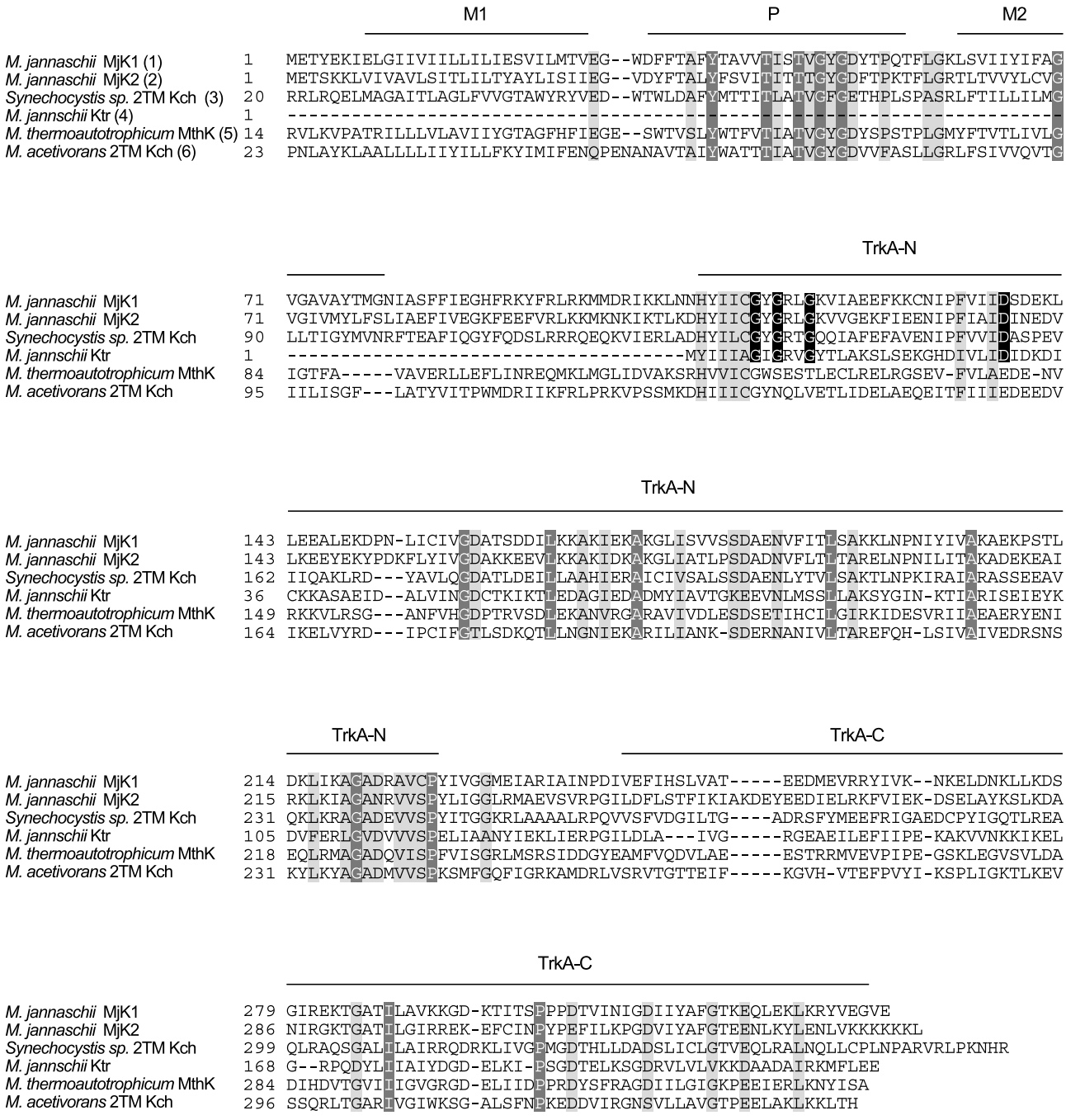

Fig. 1. Alignment of MjK1 and MjK2 with the deduced amino acid sequences of other K^+ channels with two transmembrane domains. The sequences were aligned with DNASTAR software using the Clustal W algorithm. Identical and conserved amino acids are highlighted in dark and light gray, respectively. The Rossmann fold is highlighted in black. Transmembrane domains (M1–M2), the pore region (P) as well as the conserved domains TrkA-N and TrkA-C are marked above the alignment. The NCBI accession numbers refer to the arbitrary numeration in the alignment: (1) Q57604, (2) Q58752, (3) NP_440478, (4) Q58505, (5) O27564, (6) NP_617354.

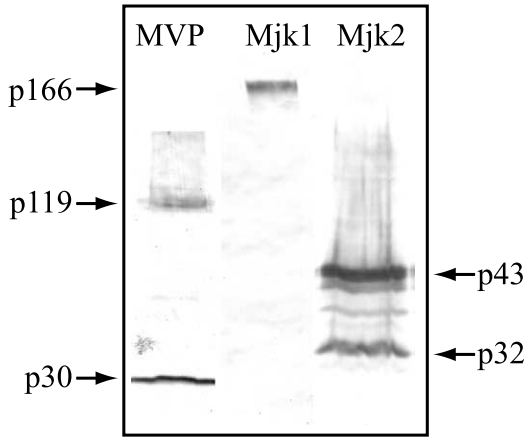

Fig. 2. Identification of MVP, MjK1 and MjK2. Membrane vesicles (50 μg total protein) were subjected to SDS–PAGE and separated proteins were blotted to nitrocellulose. Recombinant fusion proteins were identified by anti-*myc* antibody coupled to alkaline phosphatase.

systems, residues 108–228) and TrkA-C (residues 269–330), which occur in many transporters and potassium channel [6,10]. The former includes a Rossmann motif, which may bind to nicotinamide adenine dinucleotide (NAD) or reduced NAD (NADH), thereby mediating conformational switches [11]. Since TrkA-N and TrkA-C are also present in the MthK channel, a similar domain organization for MjK1 and MjK2 is probable.

MVP, the hyperpolarization activated K^+ channel of *M. jannaschii*, has six transmembrane helices per subunit and shares homology with other voltage-gated channels in two regions, the pore segment P and the putative voltage sensor region in S4 [5]. The cytoplasmic regions of MVP are short and comprise of only six and 11 amino acids, respectively. Therefore, regulatory domains that modulate function of the homologous channels Kat1 (*Arabidopsis thaliana*) and Shaker (*Drosophila melanogaster*) are not present.

3.2. Identification of MjK channels in E. coli membranes

In order to examine the activities of MjK1, MjK2, and MVP the corresponding open reading frames were cloned into vectors of the ptrcHis series, where gene expression is under control of a *trc* promoter [7,12]. The plasmids provide a C-terminal *myc*-epitope tag for immune detection, followed by a histidine hexamer for affinity purification. The tagged protein subunits have predicted molecular masses of 30 kDa (MVP), 42 kDa (MjK1) and 43 kDa (MjK2), respectively. Fig. 2 shows the immune analyses of expressed K^+ channels in *E. coli* membranes. Two main protein bands, with the predicted molecular masses of the mono- and the tetramer of MVP occur at approximately 30 and 119 kDa, respectively (Fig. 2, first lane). MjK1 was identified at 166 kDa, which corresponds to the predicted molecular mass of the channel tetramer (Fig. 2, second lane). In contrast, two proteins of 43 and 32 kDa were identified for MjK2 (Fig. 2, third lane). Amino acid sequencing confirmed that p43 corresponds to the complete open reading frame of MjK2, whereas the p34 band represents the RCK domain of MjK2, beginning at the internal methionine residue M99. Since M99 is homologous to M107 (MthK), the N-terminal amino acid of the soluble RCK domains of MthK, it is likely that p34 expression is initiated at an internal start codon. However, generation by proteolysis is also possible. Since the formation of soluble RCK domains has been reported for the K^+ channels MthK and EcKch as well, it seems to be a species-independent mechanism [6,10].

3.3. Complementation of LB2003 cells

Functional expression of ion channels has profound consequences for the viability of the host cells, as shown by numerous complementation studies with bacterial and plant ion channels [5,13–15].

In order to examine whether all *M. jannaschii* K^+ channels restore the capability for potassium uptake, the plasmid constructs were introduced into the *E. coli* strain LB2003. This mutant lacks the three major potassium uptake systems Kdp, Kup, and Trk and requires 25 mM potassium for half-maximal growth [8,16]. The phenotypic analysis was performed on solid minimal growth media with defined potassium concentrations (Fig. 3a, upper row). The corresponding growth characteristics in liquid cultures are shown in Fig. 3a (lower row).

On solid media the control and cells expressing MjK2 could grow in the presence of 100 mM KCl, but not on lower concentrations (Fig. 3a, upper row). MVP mediated growth between 1 and 100 mM KCl. In contrast, the expression of MjK1 allowed growth between 0.5 and 5 mM KCl.

Similar growth characteristics were observed in liquid media, but generally toxic effects of potassium were more pronounced (Fig. 3a, lower row). MjK2 and the control could grow at 100 mM KCl after 16 h. Incubation over 64 h revealed that cells expressing MjK2 could grow slightly faster than the control in the presence of 10 mM KCl.

The susceptibility of MVP and MjK1 for K^+ channel blockers was analyzed by placing a paper disk, impregnated with the appropriate blocker solution, on a growing mat of bacteria (Fig. 3c). After overnight incubation a clear halo around the paper disk indicated growth inhibition. Cs^+ and Ba^{2+} inhibited growth mediated by MjK1. MVP displayed only a weak susceptibility for Cs^+, even when the potassium concentration was growth limiting. Both channels were not sensitive for tetraethylammonium (TEA) (Table 1).

A major difference between MjK2 and MjK1 is their expression level in *E. coli*. Whereas up to 1 mg MjK2 per l cell culture could be expressed without deleterious effects for the host (data not shown), the expression rate of MjK1 was in the range of ng l^{-1}. KcsA could also be purified in high amounts from *E. coli* with only minor toxic effects for the host and electrophysiological data and complementation suggest that

Table 1
Diameter of halo upon growth inhibition of LB2003 (pMjK1) and LB2003 (pMVP) cells by K^+ channel blockers

Inhibitor	1 mM KCl			0.5 mM KCl			0.25 mM KCl		
	$BaCl_2$	TEA	CsCl	$BaCl_2$	TEA	CsCl	$BaCl_2$	TEA	CsCl
LB2003 (pMjK1)	2.7 cm	–	3.0 cm	3.3 cm	–	5.6 cm	NG	NG	NG
LB2003 (pMVP)	–	–	–	–	–	–	–	–	1.6 cm

NG, no growth supported at this potassium concentration; –, no inhibition.

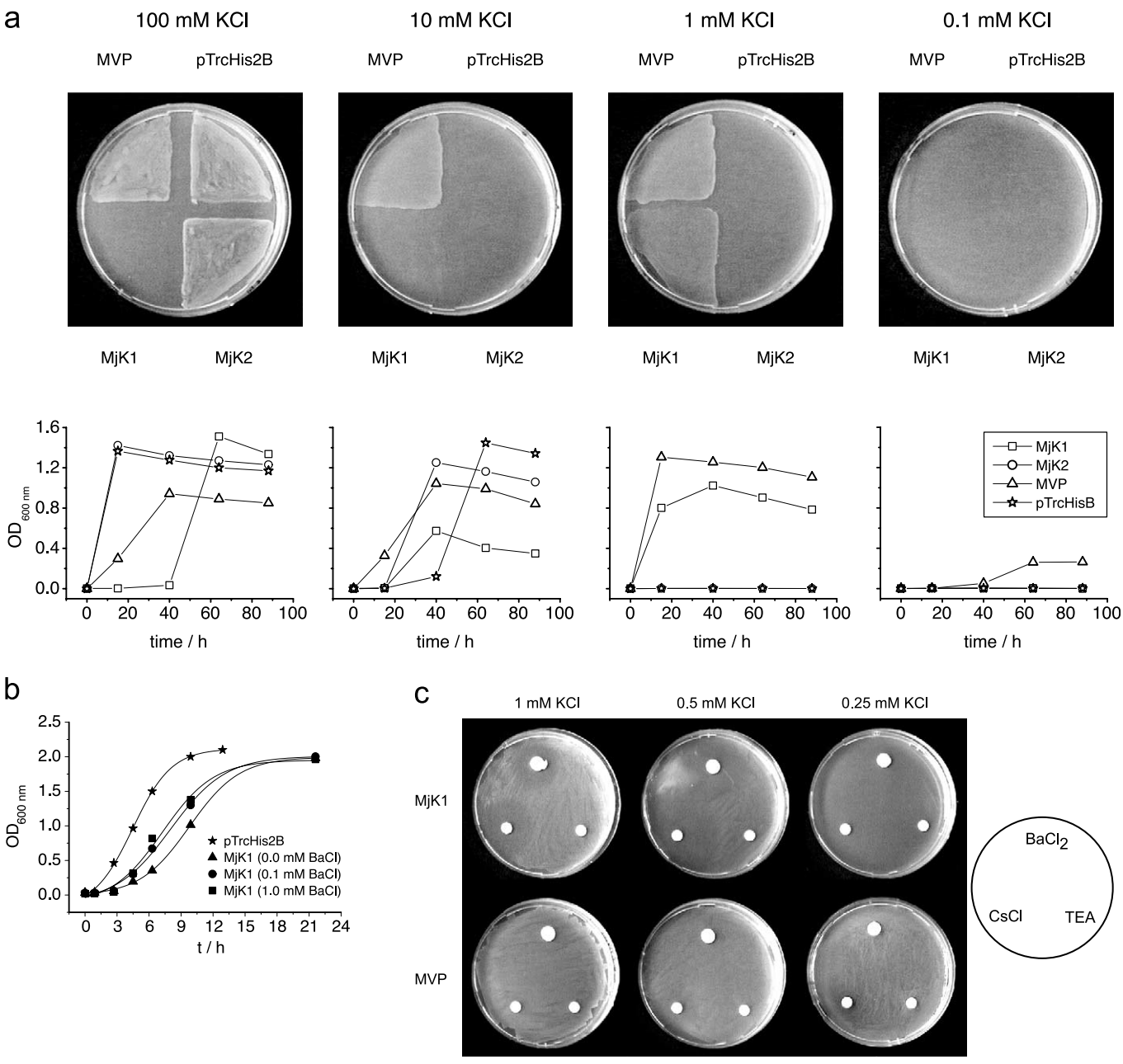

Fig. 3. Complementation of the potassium-dependent phenotype of LB2003 cells. The effect of different K^+ concentrations on growth of the *E. coli* strain LB2003 carrying the plasmids pMVP, pMjK1, pMjK2 and pTrcHis2C was analyzed. a: Upper row: growth after 16 h on solid KML media. Lower row: growth kinetics over 88 h in liquid KML media. b: Effect of Ba^{2+} on growth kinetics of cells expressing MjK1 in the presence of 5 mM KCl. c: Effect of K^+ channel blockers on growth mediated by MVP and MjK1. Whatman filter papers (diameter 1 cm) soaked with 10 μl of 1 M $BaCl_2$, CsCl or TEA were placed on a growing mat of LB2003 cells expressing either MjK1 or MVP. Incubation was performed overnight on KML media at 37°C. Growth inhibition produced a clear halo around the filter disk.

the channel is mainly in a closed state [15]. This may be also true for MjK2 and could explain failed complementation.

Biochemical data revealed that the channels KcsA, MthK and MjK1 form SDS stable tetramers [6,17], whereas mainly monomers of MjK2 were obtained under these conditions (loading buffer without mercaptoethanol, loading without previous heating) [18]. Size exclusion chromatography under non-denaturing conditions (solubilization with dodecylmaltoside) also confirmed that MjK2 subunits are not assembled in *E. coli* (Zeilinger et al., in preparation). This may point to inactivity of MjK2 due to failed formation of channels in *E. coli*. Recently it was shown that the presence of lipids is a crucial parameter for K^+ channel assembly [19]. The lipid composition of the *E. coli* membrane may not meet the requirements for MjK2 folding.

Interestingly, a potassium concentration of 100 mM is not toxic for the *E. coli* mutant carrying the control vector pTrcHis2B, pMVP or pMjK2, whereas expression of MjK1 was toxic for the host above a concentration of 5 mM. This toxic effect of KCl was reduced in a concentration-dependent manner, when the media were supplemented with $BaCl_2$. However, the growth characteristics of the wild-type could not be achieved, suggesting that the channels were not blocked quantitatively (Fig. 3b).

The toxic effects on host cells and the results of the complementation strongly suggest that MjK1 is open under physiological conditions of *E. coli*. This is in marked contrast to the results obtained for KcsA, which is mainly closed. However, it should be noted that KcsA mutants with altered pH response allowed the mutant TK2420 to grow after 3 days [15].

Since the free intracellular potassium concentration in *E. coli* is in the range of 0.1–0.2 M [20], a membrane potential of ~ -85–-120 mV in *E. coli* would cause a non-physiological elevation of intracellular potassium at external potassium concentration at >2 mM. MVP is opened by high negative potentials $V_{1/2} = -175$ mV. Therefore, excessive potassium influx may be limited due to depolarization below the threshold potential of MVP, which could explain why the channel is not toxic for *E. coli* at high potassium concentrations [5]. In contrast, MjK1 likely behaves like the structural homolog MthK and conducts potassium at low membrane potentials. This in turn would uncouple the membrane potential, thereby perturbing potential-driven transport processes and potassium homeostasis [21].

To our knowledge, the internal potassium concentration of *M. jannaschii* has not been determined, yet. However, even non-halophilic archaeabacteria have higher internal potassium concentrations than bacteria (0.6–1.1 M) [22]. Assuming comparable membrane potentials in *E. coli* and *M. jannaschii*, the archaeon may tolerate an external potassium of 6–11 mM, which is close to the potassium content of seawater (9.1–10.7 mM), the environment of *M. jannaschii*.

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