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Mechanosensitive behavior of bacterial cyclic nucleotide gated (bCNG) ion channels: Insights into the mechanism of channel gating in the mechanosensitive channel of small conductance superfamily

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ABSTRACT

We have recently identified and characterized the bacterial cyclic nucleotide gated (bCNG) subfamily of the larger mechanosensitive channel of small conductance (MscS) superfamily of ion channels. The channel domain of bCNG channels exhibits significant sequence homology to the mechanosensitive subfamily of MscS in the regions that have previously been used as a hallmark for channels that gate in response to mechanical stress. However, we have previously demonstrated that three of these channels are unable to rescue *Escherichia coli* from osmotic downshock. Here, we examine an additional nine bCNG homologues and further demonstrate that the full-length bCNG channels are unable to rescue *E. coli* from hypoosmotic stress. However, limited mechanosensation is restored upon removal of the cyclic nucleotide binding domain. This indicates that the C-terminal domain of the MscS superfamily can drive channel gating and further highlight the ability of a superfamily of ion channels to be gated by multiple stimuli.

1. Introduction

We previously identified a novel class of ligand-gated bacterial ion channels, the bacterial cyclic nucleotide gated (bCNG) ion channel family [1]. These channels are composed of three domains: a channel domain, a linker domain, and a cyclic nucleotide binding domain (Fig. 1). The pore lining helix of the channel domain has significant homology to the mechanosensitive channel of small conductance from *Escherichia coli* (*E. coli*) (Ec-MscS) and the cyclic nucleotide monophosphate (cNMP) binding domain has high homology to known cNMP binding domains, including the cNMP binding domain of MloK1, a bacterial cyclic nucleotide regulated potassium channel [2–4]. The linker domain is not highly conserved within the family and does not exhibit significant homology to known protein sequences. In previous studies, we demonstrated that three members of this channel family gate in response to cyclic adenosine monophosphate (cAMP) [1].

The strong homology of bCNG channels to Ec-MscS indicates that these channels are members of the MscS superfamily of ion channels, which is primarily composed of mechanosensitive channels from all phylogenetic kingdoms [5–7]. However, we have previously demonstrated that three members of the family do not gate in response to mechanical stress [1]. The inability of bCNG channels to gate in response to mechanical stress is surprising, due to their significant similarity to the pore lining helix and upper vestibule domain of Ec-MscS (Fig. 2). Previously, sequence homology in these regions has been used as a benchmark for the identification of ion channels that gate in response to membrane tension [5,8]. Using this approach a broad range of mechanosensitive ion channels, across all phylogenetic kingdoms, have been identified that include mechanosensitive ion channels in plants, bacteria, and archea [5–7].

Here we examine the ability of a larger subset of the bCNG ion channel family to rescue *E. coli* from osmotic downshock. Strikingly, we find that none of the channels examined are capable of rescuing *E. coli*, suggesting that the bCNG ion channel family has evolved to be non-mechanosensitive. To further examine the structural source of this decreased mechanosensitivity, we removed the cyclic nucleotide binding domain and linker region (Fig. 1) from four of these channels to determine if these domains interfered with the channels' ability to respond to mechanical stress. The removal of the C-terminal cyclic nucleotide binding domain produced bCNG channels that were slightly mechanosensitive. The increased mechanosensation of these truncated channels provides insight into the gating mechanism of bCNG ion channels.

Abbreviations: bCNG, bacterial cyclic nucleotide gated; MscS, mechanosensitive channel of small conductance; *E. coli* and Ec, *Escherichia coli*; cAMP, cyclic adenosine monophosphate; cNMP, cyclic nucleotide monophosphate.

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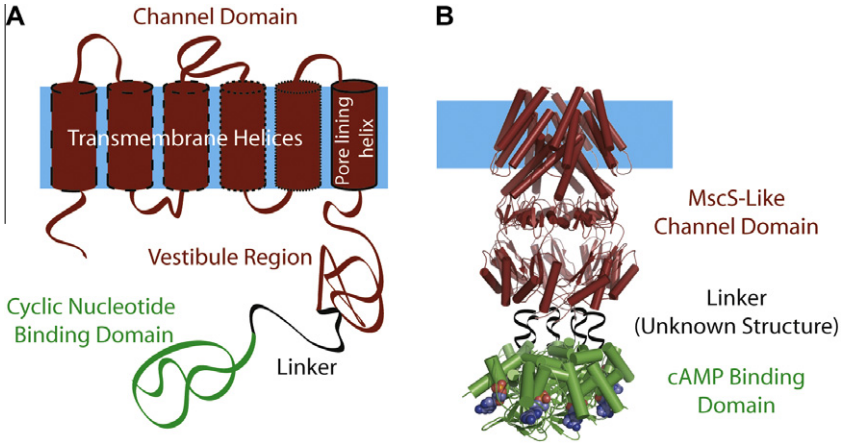

Fig. 1. (A) Graphical representation of bCNG channels labeling the various channel domains and regions. Relative helix conservation across the bCNG family is indicated by dashed lines around each helix with the totally conserved pore lining helix shown as a solid line and the poorly conserved sixth helix shown with the largest spacing between dashes. (B) Schematic representation of a three transmembrane domain bCNG channel based on the MscS structure (2OAU) [21,22] and the binding domain of MloK1 (IVP6) [23].

2. Materials and methods

2.1. Strains and plasmids

The *E. coli* strain MJF465 (*MscS, MscL, MscK* null) was used for osmotic downshock assays [9,10]. Cloning was conducted using the Top10F′ *E. coli* strain (Invitrogen). Channels were initially cloned into pET46 vector (Novagen), before being subcloned into the pB10b vector for osmotic downshock assays [11,12].

2.2. Cloning and subcloning into pB10b

Ac-bCNGa, Ac-bCNGb, Bg-bCNGa, Bx-bCNGa, Bx-bCNGb, Bx-bCNGc, Rr-bCNG, Se-bCNG, and Ss-bCNGb were cloned from genomic DNA into pET46 as previously described for Cv-bCNG, Ml-bCNG, and Te-bCNGb by Caldwell et al. [1]. Primers used for cloning are given in Supplementary Table 1. Bacterial genomic DNA was obtained from the following sources: *Azorhizobium caulinodas* ORS 571 (Ac) from T. Aono at U. Tokyo, *Burkholderia graminis* C4D1M (Bg) from S. Brady at Rockefeller University, *Burkholderia xenovorans* LB400 (Bx) from J. Tiedje at Michigan State University, *Agrobacterium tumefaciens str.* C58 (Rr) was purchased from ATCC, *Synechococcus elongatus* PCC 7942 (Se) from S.S. Golden at UCSD, *Synechocystis* sp. PCC6803 (Ss) from KAZUZA. Channels were subsequently subcloned into pB10b for osmotic downshock assays, as previously described [1]. Primers used for sub-cloning are given in Supplementary Table 1. Constructs were verified by enzymatic

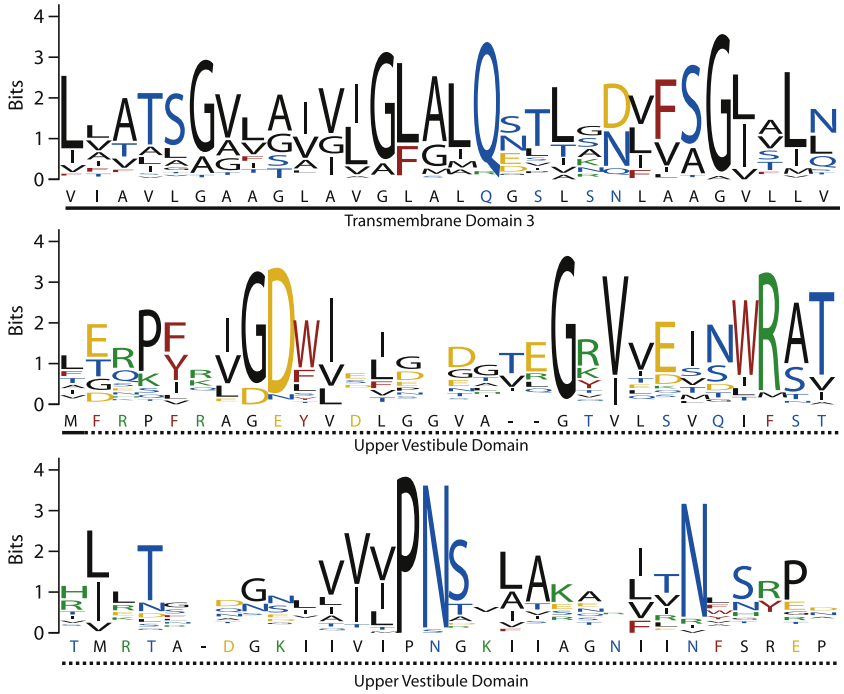

Fig. 2. Conservation of amino acids in the pore lining helix and the upper vestibule domain for the entire bCNG channels is shown using WebLogos [24,25]. The corresponding residues in MscS are indicated below each logo. Hydrophobic residues (I, P, L, M, V, A, G), are colored black; aromatic residues (F, W, Y) are colored red; polar residues (S, T, Q, N, C) are colored blue; basic residues (K, R, H) are colored green; and acidic residues (D, E) are colored yellow. The *Y*-axis, in bits, gives the maximum sequence conservation, $\log_2(20) = 4.13$ [24,25], larger letters indicate higher conservation of that residue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)