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Corynebacterium glutamicum mechanosensing: From osmoregulation to L-glutamate secretion for the avian microbiota-gut-brain axis

Yoshitaka Nakayama [1], [2,*]

[1] Molecular Cardiology and Biophysics Division, Victor Chang Cardiac Research Institute, Darlinghurst, NSW, 2010, Australia 1; y.nakayama@victorchang.edu.au
[2] St Vincent's Clinical School, Faculty of Medicine, The University of New South Wales, Darlinghurst, NSW, 2010, Australia 2; y.nakayama@victorchang.edu.au
* Correspondence: y.nakayama@victorchang.edu.au; Tel.: +61 2 9295 8744

Abstract: After the discovery of *Corynebacterium glutamicum* from the avian feces contaminated soil, its enigmatic L-glutamate secretion by corynebacterial MscCG-type mechanosensitive channels has been utilized for the industrial monosodium glutamate production. Bacterial mechanosensitive channels are activated directly by increased membrane tension upon hypoosmotic downshock, thus the physiological significance of the corynebacterial L-glutamate secretion has been considered as adjusting turgor pressure by releasing cytoplasmic solutes. In this review, we present information that corynebacterial mechanosensitive channels have been evolutionally specialized as carriers to secrete L-glutamate into the surrounding environment in their habitats rather than osmotic safety valves. The lipid modulation activation of MscCG channels in L-glutamate production can be explained by the "Force-From-Lipids" and "Force-From-Tethers" mechanosensing paradigms and differs significantly from the mechanical activation upon hypoosmotic shock. The review also provides information on the search for possibilities that *Corynebacterium glutamicum* was originally a gut bacterium in the avian host in the aim of understanding physiological roles of corynebacterial mechanosensing. *Corynebacterium glutamicum* is able to secrete L-glutamate by mechanosensitive channels in the gut microbiota and help the host brain function via the microbiota-gut-brain axis.

Keywords: Mechanosensitive channel; *Corynebacterium glutamicum*; L-glutamate secretion

1. Introduction

Corynebacterial mechanosensitive channel model for the industrial L-glutamate production

Corynebacterium glutamicum was first isolated as a glutamate producer in the avian (most likely pigeon) feces-contaminated soil in 1957 (1), when Kinoshita and Udaka described the result of an elaborated screening method with a glutamate-auxotrophic bacterium *Leuconostoc mesenteroides* strain P-60 (2). This originated the microbial amino acid production and understanding *Corynebacterium glutamicum* physiology has made numerous paradigm shifts in biotechnological process. One of the most striking findings is the biotin-dependent L-glutamate secretion. At a growth-limiting concentration of biotin, *Corynebacterium glutamicum* releases massively L-glutamate into the culture media, but not in the normal culture (3). Later, several alternative ways to trigger L-glutamate secretion have been reported, and the conditions are surprisingly diversified as impacting the cell wall and membrane, such as adding of penicillin (4), ethambutol (5), fatty acid ester surfactants (6), local anesthetics (7), and temperature upshift (8,9). Correspondingly, a wide range of mechanistic models to explain the L-glutamate secretion was suggested, and a coherent model was

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expected in the puzzled situation. Since L-glutamate is a charged amino acid, passive efflux was rejected, and an active carrier was proposed as the "carrier model" (10). The leakage from damaged cell envelopes were considered as one of the main causes due to inhibited fatty acid biosynthesis and membrane alterations in L-glutamate production as the "leak model" (11–13). Metabolism in the biotin-limited condition was shifted from energy production via tricarboxylic acid cycle towards L-glutamate production. Thus, the "metabolic flow change model" was proposed to trigger the L-glutamate secretion (14–16).

This puzzled situation has ended by the serendipitous discovery of the major L-glutamate exporter, NCgl1221 (cg1434). In the process of the screening of the L-glutamate overproducing strains without any specific treatment, several mutations on the *NCgl1221* gene were identified in the resultants (17). NCgl1221 was predicted to be one of bacterial mechanosensitive channel of small conductance (MscS) homologs, however shows similarity only in the pore domain, and its entire structure differs from other MscS-type mechanosensitive channels (18). This finding has emphasized the importance to bacterial mechanosensing by mechanosensitive channels on the studies of corynebacterial amino acid exporters (19,20), and mechanosensitive channels are now emergent targets for transporter engineering to use *Corynebacterium glutamicum* as a microbial cell factory (21,22). Using patch-clamp technique with *E. coli* giant spheroplasts, NCgl1221 was demonstrated to function as mechanosensitive channel with a strong rectifying activity and a slight cation selectivity. Thus, NCgl1221 was renamed as "mechanosensitive channel of *Corynebacterium glutamicum* (MscCG)" (23). Further, using giant protoplasts of *Bacillus subtilis*, L-glutamate was shown to be passively exported by the open pore of MscCG channels (24). In addition to MscCG as the major L-glutamate exporter, a different mechanosensitive channel gene, *mscCG2*, as a minor L-glutamate exporter, was identified in several industrial *Corynebacterium glutamicum* strains (25). Conclusively, using a novel patch-clamp technique with *Corynebacterium glutamicum* giant spheroplasts, all endogenous mechanosensitive channels including MscCG and MscCG2 were recorded electrophysiologically in the native membrane environment (26). These studies strongly supported the "mechanosensitive channel model" (27) and explained the L-glutamate secretion mechanisms as follows: (1) Specific treatments alter membrane tension by inhibiting membrane lipids or cell wall synthesis, (2) MscCG and MscCG2 are activated by increased membrane tension, and (3) L-glutamate is exported through the open pore of the MscCG-type mechanosensitive channels (**Figure 1**). In this model, mechanosensing by MscCG-type mechanosensitive channels is the central physiological phenomenon of the L-glutamate secretion. To extend our understanding about corynebacterial mechanosensing, we will summarize the current progress on studies of corynebacterial mechanosensitive channels and discuss the potential physiological significance of the L-glutamate secretion in the corynebacterial habitats.

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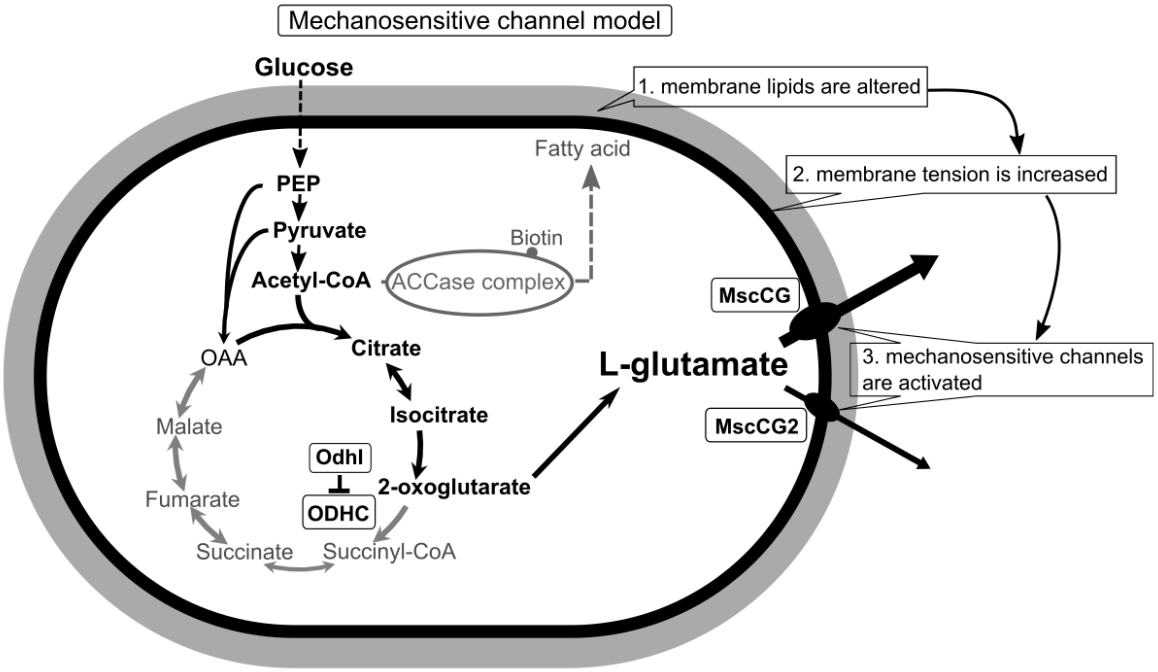

Figure 1 A scheme of the *Corynebacterium glutamicum* L-glutamate secretion triggered by biotin limitation as mechanosensitive channel model. Biotin limitation shifts metabolic flow to produce L-glutamate by inhibiting the 2-oxoglutarate dehydrogenase complex (ODHC) activity and inhibits the acetyl CoA carboxylase (ACCase) complex activity. As a result of fatty acid biosynthesis inhibition, the membrane lipids are altered to increase membrane tension. MscCG-type mechanosensitive channels, MscCG (major exporter) and MscCG2 (minor exporter), are activated by increased membrane tension to release L-glutamate.

2. Mechanosensitive solute efflux system functions for osmoregulation, but L-glutamate is not exported in *Corynebacterium glutamicum*.

Osmoregulation is an indispensable cellular function for corynebacteria to survive and adapt fluctuating osmotic environments in their various habitats in the soil, sewage, plants, food products, animal mucosa, skin flora (28–30). *Corynebacterium glutamicum* was discovered in avian feces-contaminated soil as a glutamate producer. Other heat-tolerant glutamate producers, *Corynebacterium efficiens* and *Corynebacterium suranareeae*, were identified from onion bulb (31) and soil contaminated with starling's feces (32), respectively. *Corynebacterium variabile* was isolated in the smear-ripened cheese in high salt environment (33,34). In general, the cytoplasmic concentration of osmotically active solutes is higher than the environment, thus causing water influx and cell swelling in the soil (35). Since the cytoplasmic membrane are freely permeable to water but forms a barrier for solutes between the environment and the cytoplasm, turgor pressure is exerted by the cytoplasmic membrane towards the cell wall. Bacterial turgor pressure has been estimated to be 3-5 atm for Gram-negative bacteria and 20 atm for Gram-positive bacteria, respectively (36). Obviously, corynebacteria were forced to develop adaptation mechanisms to cope with a huge turgor pressure that would easily break cells if they were not protected by the cell-wall.

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The main strategies of *Corynebacterium glutamicum* for osmoregulation are mechanosensitive solute efflux systems after an osmotic downshift and accumulation of compatible solutes, such as betaine, proline, glutamine, ectoine and trehalose, after an osmotic upshift (37–39) (**Figure 2**). An osmotic downshift leads to dramatically increased turgor pressure due to an excessive water influx. To avoid the osmotic cell lysis, *Corynebacterium glutamicum* activates within milliseconds the mechanosensitive solute efflux system that consists of two types of mechanosensitive channels, MscS-type (MscCG and MscCG2) and MscL-type (CgMscL), to release intracellular solutes swiftly into the environment and reduce the driving force for water entry. The mechanosensitive solute efflux system releases betaine or proline preferably to other amino acids of similar size and does not excrete ATP in *Corynebacterium glutamicum* (40). This is higher substance specificity than counterpart of the *E. coli* system that are non-selective and releases even small protein. After hypoosmotic shock, the cytoplasmic concentration of L-glutamate does not change even after the glutamate synthesis after K^+ accumulation in hyperosmotic condition (41,42). Thus, it is questionable whether L-glutamate plays important roles as an osmolyte in the osmoregulation in *Corynebacterium glutamicum*.

The bacterial mechanosensitive solute efflux system has been studied comprehensively by characterizing *E. coli* mechanosensitive channels of large conductance (MscL) and of small conductance (MscS) (43). *E. coli* has one *MscL* gene and six *MscS* genes (MscS, YnaI, YbdG, YbiO, YjeP(MscM), KefA(MscK)) (44,45). *E. coli* MscL and MscS mechanosensitive channels are required for survival upon hypoosmotic shock, and the double knockout among seven mechanosensitive channel genes leads to almost 90% decrease of survival rate in *E. coli* (46). In contrast, *Corynebacterium glutamicum* has one *MscL-like* gene and only one or two *MscS-like* genes (MscCG and MscCG2). Surprisingly, Nottebrock *et al* demonstrated that the deletion of all mechanosensitive channel genes did not change survival rate in *Corynebacterium glutamicum* (47). This evidences that the corynebacterial mechanosensitive solute efflux system is not required for survival upon hypoosmotic downshock. Instead of functioning as osmotic safety valves, corynebacterial mechanosensitive channels play roles in fine-tuning of cytoplasmic osmolarity in hyperosmotic environment. The mechanosensitive channel MscCG is coupled with the activity of betaine transporter BetP to regulate the cytoplasmic betaine concentration as the “pump and leak model” that MscCG exports and BetP imports betaine to balance the cytoplasmic osmolarity (23) (**Figure 2**). This

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indicates that the corynebacterial mechanosensitive efflux system exports mostly betaine as osmotically active solute rather than L-glutamate for osmoregulation.

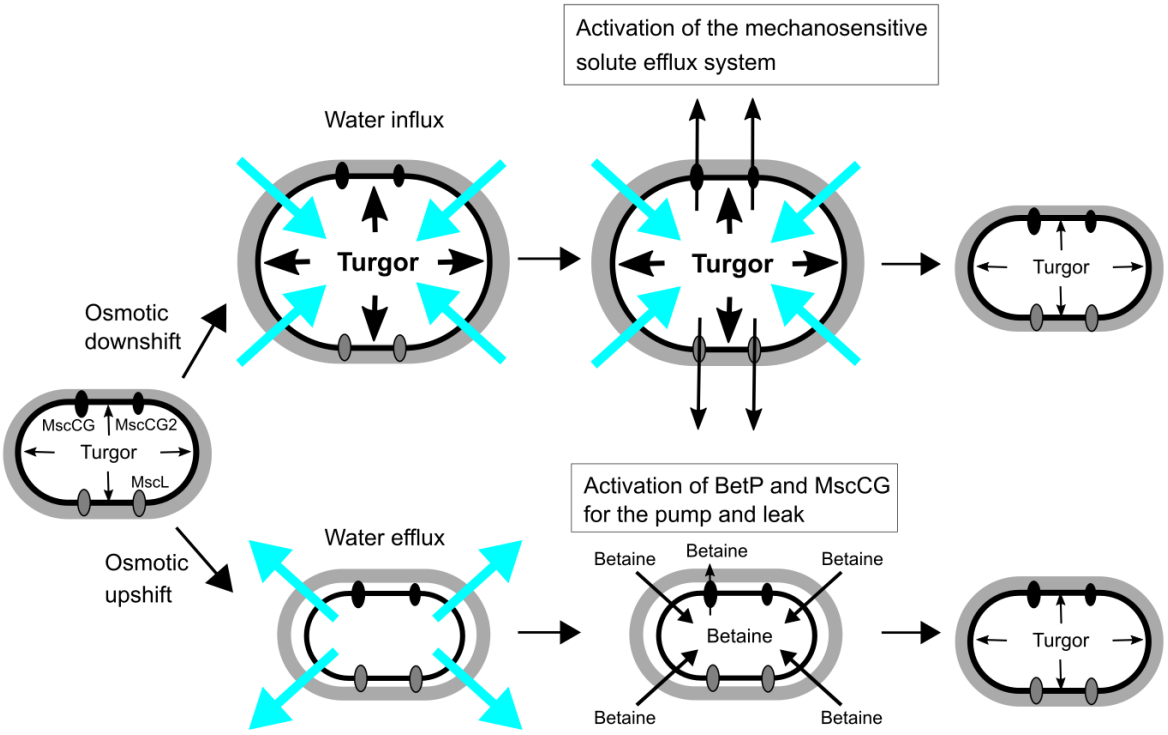

Figure 2 Osmoregulation of *Corynebacterium glutamicum* for osmotic down-/up-shift. Turgor pressure is increased by water influx upon osmotic downshift, and the mechanosensitive solute efflux system that consisted of the mechanosensitive channels, MscCG, MscCG2 and CgMscL, is activated to reduce osmotic gradient within milliseconds. Osmotic upshift causes water efflux, and thus cells intake betaine as major osmolytes from the environment by the activation of the betaine transporter BetP. The cytoplasmic betaine concentration is fine-tuned by the leakage through the mechanosensitive channel MscCG as the pump and leak mechanism.

3. Diversity of MscS mechanosensitive channel superfamily and the impact of the MscCG channel gating on L-glutamate secretion

The existence of MscS-type mechanosensitive channels in the cytoplasmic membranes of both prokaryotic organisms, *E. coli* and *Corynebacterium glutamicum*, indicates that these channels have developed throughout the evolution to cope with changes of mechanical environments, however, its functions as osmoregulator are significantly different (48). *E. coli* MscS has a nanometer scale large channel pore, and thus its conductance reaches 1 nS whereas *Corynebacterium glutamicum* MscCG has significantly smaller conductance of approximately 0.3 nS (23,26,49). As an osmoregulator, *E. coli* MscS has strong inactivation and adaptation mechanisms to mechanical stimuli that is relevant to avoid the over-efflux of cytoplasmic molecules upon osmotic downshock (50,51). However, *Corynebacterium glutamicum* MscCG does not have these features and tends to be open as metabolic valves rather than osmotic safety valves (52). Recent studies of MscS channel superfamily revealed that MscS-like channels are present among cell-walled organisms, bacteria, archaea, fungi, algae, and plants, and the physiological functions of these MscS-like channels are not simply osmotic safety

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valves upon hypoosmotic shock (18). In eukaryotes, algal and plant MscS-like channels (MSCs, MSLs) are much more complicated structures than *E. coli* MscS and found in the organellar membranes of chloroplasts (53–55) and mitochondria (56–58) for mechanosensing. Fungal MscS-like channels (Msy1 and Msy2) are localized in the endoplasmic reticulum membranes and involved in the osmotic Ca^{2+} signaling upon hypoosmotic shock (59,60).

Although the physiological functions of corynebacterial MscCG-type mechanosensitive channels are still controversial, the L-glutamate secretion is caused certainly by the conformational changes of the MscCG channels. *Corynebacterium glutamicum* MscCG is characterized to have the N-terminal pore domain (1-286 aa) corresponding to the entire *E.coli* MscS that has three transmembrane helices (TM1, TM2, and TM3) and a cytoplasmic cage domain in a monomer subunit **(Figure 3A).** Recently, Reddy *et al* refined the 3D structures of the full-length *E. coli* MscS by cryo-electron microscopy with nanodiscs (61) **(Figure 3B)**. MscCG shows the highest similarity in the pore-forming helix TM3 and adjacent regions. In contrast, the unique feature of MscCG type-mechanosensitive channels is the C-terminal domain (287-533 aa) including a cytoplasmic loop, the fourth transmembrane helix TM4, and a periplasmic loop (**Figure 3C**). This structure is highly conserved only in corynebacteria. Several gain- and loss-of-function mutations on MscCG were identified by the glutamate productivity assay and bacterial patch clamp technique (**Figure 3C**). Originally, Nakamura *et al* identified W15CSLW, A100T, A111T, A111V in the N-terminal pore domain and V419::IS1207, P424L in the periplasmic loop during the screening of L-glutamate overproducing strains. These mutations were reported to cause a spontaneous L-glutamate secretion in *Corynebacterium glutamicum* (17). Afterwards, Nakayama *et al* reported using *E. coli* patch-clamp that the spontaneous L-glutamate secretion is caused by the gain-of-function mutation on mechanosensitivity of MscCG channels (62). These findings proved that MscCG mechanosensitivity can be evaluated by L-glutamate productivity in *Corynebacterium glutamicum.* Using these assays, the functional domain of MscCG has been investigated thoroughly. Yamashita *et al* reported using L-glutamate productivity assay that the N-terminal pore domain (1-286 aa) is essential to export L-glutamate, and suggested using homology modelling that MscCG has an extra small loop structure (221–232 aa) and its deletion resulted in the loss of the channel functionality (63). Backer *et al* reported additional point mutations in the N-terminal pore domain and the impact of the C-terminal domain on the channel function (64,65). A106V was the gain-of-function mutation to cause the spontaneous L-glutamate secretion, and interestingly, Q112L and V115S double point mutation caused a loss-of-function mutation that *Corynebacterium glutamicum* cannot export L-glutamate even with penicillin treatment. In contrast, the deletion of the periplasmic loop (423-533aa) in the C-terminal domain caused the spontaneous L-glutamate secretion, but the further deletion of the periplasmic loop and the fourth transmembrane helix TM4, indicating that the fourth transmembrane helix TM4 is involved in the mechanosensitivity of MscCG channels. Moreover, Krumbach *et al.* identified further the gain-of-function mutations V422K, V422D, E423P, and E423S using the CRSPER/Cas12a genome editing technology (66).

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Based on the position of these mutations, the interaction with other components of the cell wall was suggested to be crucial for MscCG mechanosensitivity.

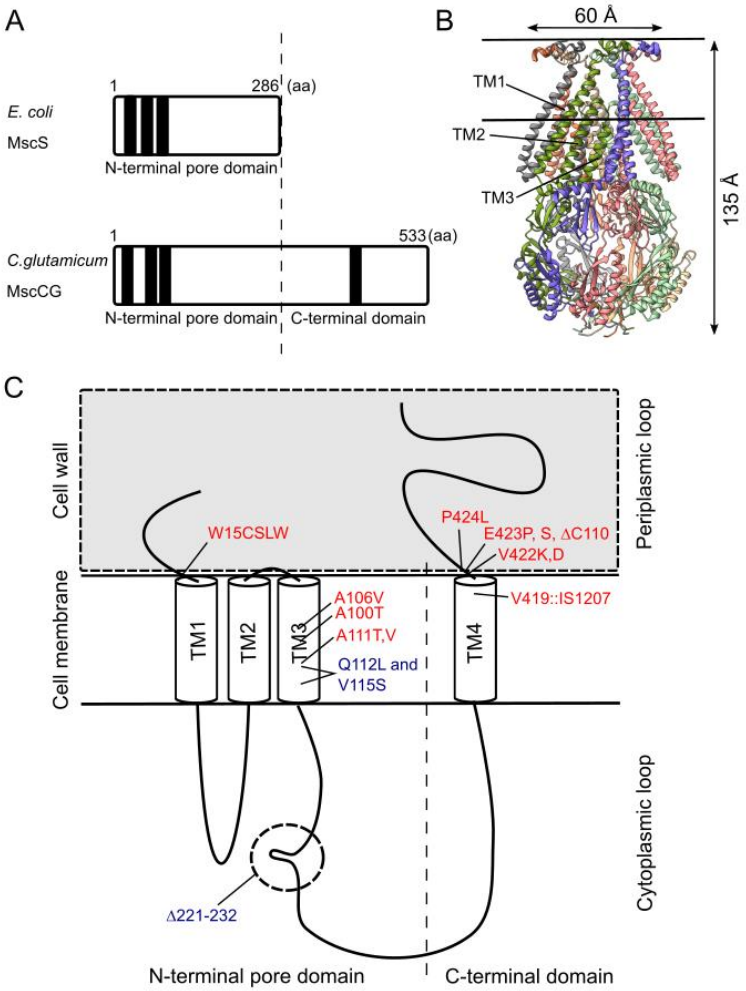

Figure 3 Structural features of the mechanosensitive channel MscCG. **A.** Secondary structure comparison between *E. coli* MscS and *C. glutamicum* MscCG. Black bars show predicted transmembrane (TM) helices by TOPCONS program (https://topcons.net/). **B.** The cryoEM 3D structure of *E. coli* MscS embedded in the nanodiscs with POPC:POPG=1:4 (The structure was cited from the Protein data bank 6PWP). **C.** The domain structure of the MscCG channel and the gain(red)- and loss(blue)-of-function mutations.

4. "Force-From-Lipids/Tethers" paradigms and bacterial cell/membrane mechanics for mechanosensing.

To understand mechanosensing by MscCG-type mechanosensitive channels, importantly hydrostatic pressure itself such as osmotic pressure does not activate directly force-sensing ion channels. Martinac *et al* demonstrated first the existence of MscS channels as "pressure-sensitive" channels in *E. coli* giant spheroplasts since suction was applied to activate the channels (67). Later, the responsible gene *yggB* was identified, and purified protein was successfully reconstituted into liposomes to confirm its mechanosensitivity in lipid bilayers (68). Using a high-speed camera, the membrane deformed by suction was visualized to calculate membrane tension (69), and the activation threshold of MscS channels was estimated approximately 6 mN/m (70). Moreover, Martinac *et al* elaborately proved that amphipaths, such as lysophosphatidylcholine and chlorpromazine, which generate local membrane curvature, can activate *E. coli* mechanosensitive channels MscS without applying suction (71). These findings led to establish the concept of the "Force-From-Lipids" paradigm for mechanosensing that membrane protein always senses the transbilayer force profile transmitted through membrane lipids (72–74) (**Figure 4**). In this paradigm, mechanical stimuli

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deforming cell shape, such as pressure, contact, indentation, increase membrane tension at the cellular level (micrometer scale), but more importantly, change the transbilayer force profile at the molecular level (nanometer scale) (75,76). For individual mechanosensitive channels in the cytoplasmic membrane, global membrane curvature in micrometer scale is too large to sense force like we live on the Earth but cannot feel its curvature on the ground. Thus, nanometer scale force-sensing is more critical for the activation of bacterial mechanosensitive channels than micrometer scale (71,77). In contrast to the "Force-From-Lipids" paradigm, most of eucaryotic mechanosensitive channels (TRPs and PIEZOs) are connected to cytoskeleton as molecular tethers, and mechanical force is transmitted directly or indirectly through the tethers to activate the channels (78–80). This idea is also widely accepted as the "Force-From-Tethers" paradigm (**Figure 4**). For corynebacterial mechanosensing by MscCG-type mechanosensitive channels, the cell wall may function as extracellular molecular tethers to transmit the mechanical force to the channels by connecting with the extracellular loop of MscCG channels since the gain-of-function mutations that cause the spontaneous L-glutamate secretion are localized at the extracellular boundary (66).

Mechanical properties of bacterial cells and membranes are distinct from animal counterparts since bacterial cells are protected by the cell wall and thus up to several hundred thousand times stiffer than animal cells (81,82). Young's elastic modulus, shear modulus, bending stiffness, and viscosity, are important mechanical properties of biological cell membranes. For the Gram-negative and Gram-positive bacterial cells, Young's elastic modulus were estimated between 50-150 and 100-200 MPa, respectively (81). Corynebacteria have a mycomembrane including mycolic acid layer, which is thicker than the cell wall of the Gram-positive bacteria, and thus this value is even larger. Based on these properties, the bacterial cell wall sustains approximately 90% of turgor pressure and leave 10% share to be sustained by the cytoplasmic membrane (83). This "force sharing" between the cell wall and membrane is large enough to activate mechanosensitive channels activated by membrane tension in the range of 6-12 mN/m in the cell membrane. In addition to bacterial cell mechanics, bacterial membrane mechanics is difficult to study due to the bacterial cell size and the cell wall. Using the micropipette aspiration technique, the mechanical membrane properties of *Corynebacterium glutamicum* giant spheroplasts were evaluated to be much softer mechanical properties compared to the *E. coli* giant spheroplasts membrane (26). *Corynebacterium glutamicum* has a significant amount of the negatively charged lipids cardiolipin (CL), phosphatidylglycerol (PG), and phosphatidylinositol (PI), but phosphatidylethanolamine (PE) in the cytoplasmic membrane. Consistently, liposomes made of the negatively charged lipids DOPG were shown to have softer mechanical properties than liposomes made of the neutral lipids DOPC by atomic force microscopy (84). These findings indicate that corynebacterial membranes are much more deformable by mechanical stimuli than other bacterial membranes, and therefore that membrane tension increased by largely expanded membranes upon osmotic downshock will activate *Corynebacterium glutamicum* mechanosensitive channels, MscCG, MscCG2 and CgMscL in the cytoplasmic membranes.

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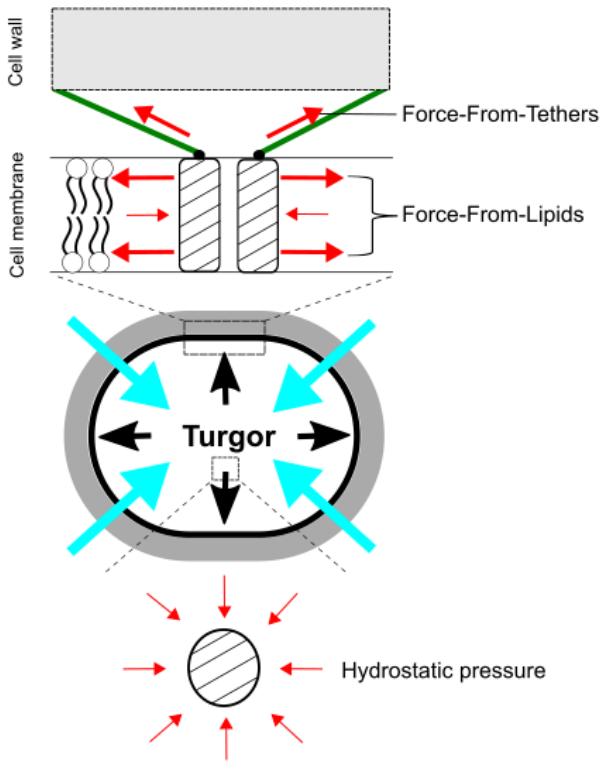

Figure 4 Force-From-Lipids and Force-From-Tethers paradigms for mechanosensing by mechanosensitive channels upon hypoosmotic downshift. Mechanosensitive channels sense transbilayer force profile in the lipid bilayers and are activated by increased membrane tension. Molecular tethers like cytoskeleton and peptidoglycan, also transmit mechanical force to activate mechanosensitive channels (top). In contrast, hydrostatic pressure does not activate directly mechanosensitive channels (bottom).

5. Mechanosensitive channel activation by altering the cell membrane and wall with specific triggers

In the mechanosensitive channel model, it has been proposed that membrane tension increase by specific triggers activates MscCG-type mechanosensitive channels for L-glutamate secretion (22,27). Truly, MscCG-type mechanosensitive channels can be activated mechanically by increased membrane tension with suction (26), however, the activation mechanisms in L-glutamate production are still mysterious. Firstly, the activation of MscCG-type mechanosensitive channels is exclusive in the presence of the other type of the mechanosensitive channel MscL. Secondly, the cell shape and size are not dramatically changed, thus it is unlikely that membrane tension is increased as during osmotic cell expansion. Thirdly, the conserved C-terminal domain of MscCG channels has impacts for the L-glutamate secretion although its molecular function is not understood. This indicates that MscCG-type mechanosensitive channels sense force from the lipids in the cell membrane in L-glutamate production differently from osmotic swelling.

The currently reported specific triggers to induce L-glutamate production can be grouped, such as inhibition of fatty acid biosynthesis, the alteration of mechanical properties of the cell membrane, and the degradation of the cell wall **(Figure 5)**. *Corynebacterium glutamicum* fatty acid biosynthesis system requires biotin since the α subunit of acetyl CoA carboxylase complex, AccBC, is biotinylated (85). Biotin limitation decreases the catalytic reaction to synthesize malonyl CoA from acetyl CoA and to synthesize α-carboxyl-acyl-CoA from acyl-CoA, thus the following fatty acid biosynthesis is inhibited as resulting in the alteration of both membranes, cell membrane and mycolic acid layer **(Figure 5)**. Other than biotin limitation, the fatty acid ester surfactants Tween 40 (Polyoxyethylene sorbitan

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monopalmitate) and Tween 60 (Polyethylene sorbitan monostearate) induce the L-glutamate production, but Tween 20 (Polyoxyethylene sorbitan monolaurate) and Tween 80 (Polyoxyethylene sorbitan monooleate) do not. To understand the molecular mechanisms, the *dtsR* (Detergent sensitivity rescuer) gene was isolated as a multicopy suppressor of a Tween 40-sensitive mutation of *Corynebacterium glutamicum* that requires fatty acid for the growth and secretes L-glutamate without biotin limitation (13). DtsR is the β subunits of the acetyl CoA carboxylase complex and renamed later AccD1 since *Corynebacterium glutamicum* has four acetyl CoA carboxylase β subunit genes (AccD1-4). AccD1 is involved in the catalytic reaction to synthesize malonyl CoA from acetyl CoA with AccBC (α-subunit) and AccE (ε-subunit) whereas AccD2 and AccD3 are involved in mycolic acid synthesis. Adding Tween 40 decreases the expression level of AccD1, and thus resulted in the inhibition of fatty acid biosynthesis to induce L-glutamate secretion like biotin limitation. From malonyl-CoA, *Corynebacterium glutamicum* synthesizes fatty acids with type-I fatty acid synthesis system that consists with two types of fatty acid synthase, FasA and FasB (86). Unlike other bacteria, *Corynebacterium glutamicum* does not have type-II fatty acid synthesis system. FasA is the main synthase for synthesizing oleoyl-CoA (C18:1) and palmitoyl-CoA(C16:0) and the deletion of FasA is lethal whereas FasB is the subordinate synthase for saturated stearoyl-CoA (C18:0) and palmitoyl-CoA (C16:0). The *fasA* mutant requires oleic acids (C18:1) for the growth and spontaneously secretes L-glutamate, implying that oleic acid auxotrophy is involved in the activation of MscCG mechanosensitive channels by changing membrane lipids. Further to fatty acid biosynthesis, *Corynebacterium glutamicum* synthesizes phospholipids from phosphatidic acid (PA) for making cell membranes consisted of negatively charged phospholipids: phosphatidylglycerol (PG), cardiolipin (CL), phosphatidylinositol (PI), and phosphatidylinositol mannosides (PIMs). The gene expression for each phospholipid synthesis alters the membrane lipid components. The overexpression of cardiolipin synthase *cls* causes the spontaneous glutamate secretion without any treatments (87), implying that increased amount of cardiolipin in the cell membrane may activate MscCG mechanosensitive channels for glutamate efflux. Cardiolipin is a four-tailed phospholipid, and its structure resembles two phosphatidylglycerols joined via the head groups. Due to its inversed conical shape, cardiolipin is a non-bilayer lipid and contributes to creating membrane curvature such as the curved poles of rod-shaped bacteria (88).

Mechanical properties of cell membranes affect mechanosensing because the force is transmitted to mechanosensitive channels through the viscoelastic cell membrane (89). Alterations of the membrane lipid composition, the ratio of saturated/unsaturated lipids, the membrane lipid chain length, have been reported to change mechanosensitivity of *E. coli* MscS and MscL as shown in modelling studies (70,90,91). In *Corynebacterium glutamicum*, temperature upshift from 30°C to 37-41°C induces the L-glutamate secretion, and this method has been considered as one of the cost-effective ways for the industrial production (8,92,93). The cell envelope fluidity of *Corynebacterium glutamicum* was analyzed with 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) during a temperature-triggered glutamate production and significant increase of the fluidity was

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reported (94). The membrane fluidity increase is not caused by high temperature itself, but mainly by changing membrane lipid composition after temperature upshift. This indicates the possibility of the temperature-dependent activation mechanisms of MscCG channels by changing membrane lipid composition. Related to the temperature-triggered glutamate production, Lambert *et al* reported that local anesthetics, such as chlorpromazine, tetracaine, butacaine, and benzocaine, also trigger the L-glutamate secretion by modulating the membrane state (7). In this method, the viscosity or fluidity of the membrane was proved not to be changed significantly using electron spin resonance spectroscopy with spin-labeled fatty acid probes. Thus, it was suggested that lipid bilayer elasticity, membrane shape, membrane bending energy, are critical rather than membrane viscosity and fluidity. Since local anesthetics can attribute to creating the local membrane curvature like amphipaths to change the transbilayer force profile (71), the activation of MscCG channels by local anesthetics is based on the "Force-From-Lipids" mechanosensing paradigm **(Figure 5**). Supportively to the above idea, the effects of local anesthetics and osmotic shifts were mutually interchangeable. A hyperosmotic shift inhibited tetracaine-triggered glutamate efflux whereas a hypoosmotic shift enhanced the action of tetracaine (7).

The corynebacterial cell wall has a unique cell surface structure only seen in the Corynebacteria-Mycobacteria-Nocardia group (95). The mycolic acid layer as the outer membrane mainly consists of trehalose corynomycolate (96). Underneath the mycolic acid layer, covalently linked arabinogalactan layer and peptidoglycan layer are present between the mycolic acid layer and cell membrane. Ethambutol and penicillin inhibit a series of arabinosyltransferases and enzymes for the cross-linking of peptidoglycans, respectively. Thus, these antibiotics cause the weakened cell wall due to less amount of arabinogalactan and fewer cross-links of peptidoglycans. As a specific trigger, adding ethambutol or penicillin induces the L-glutamate secretion that MscCG-type mechanosensitive channels are activated by a strongly disordered cell envelope due to the degradation of the cell-wall structure. To establish an efficient cellulosic glutamate production from lignocellulose feedstocks, the activation of MscCG by ethambutol and penicillin has been targeted (19). Without the mechanical support of the cell wall, cells are easily expanded by turgor pressure which may cause cells to burst; thus, they need to activate immediately the mechanosensitive solute efflux system. However, the L-glutamate secretion by adding ethambutol and penicillin is caused by the activation of MscCG-type channels exclusively similar to biotin limitation and adding fatty acid surfactants rather than osmotic swelling. The DNA microarray assay revealed that after adding ethambutol or penicillin, the expression of MscCG is significantly increased (5), indicating that the functions of MscCG channels are involved in the cell wall integrity. The structural alterations of the cell wall may change force transmitted to the channels tethered by peptidoglycan as "Force-From-Tethers" mechanosensing **(Figure 5)**.

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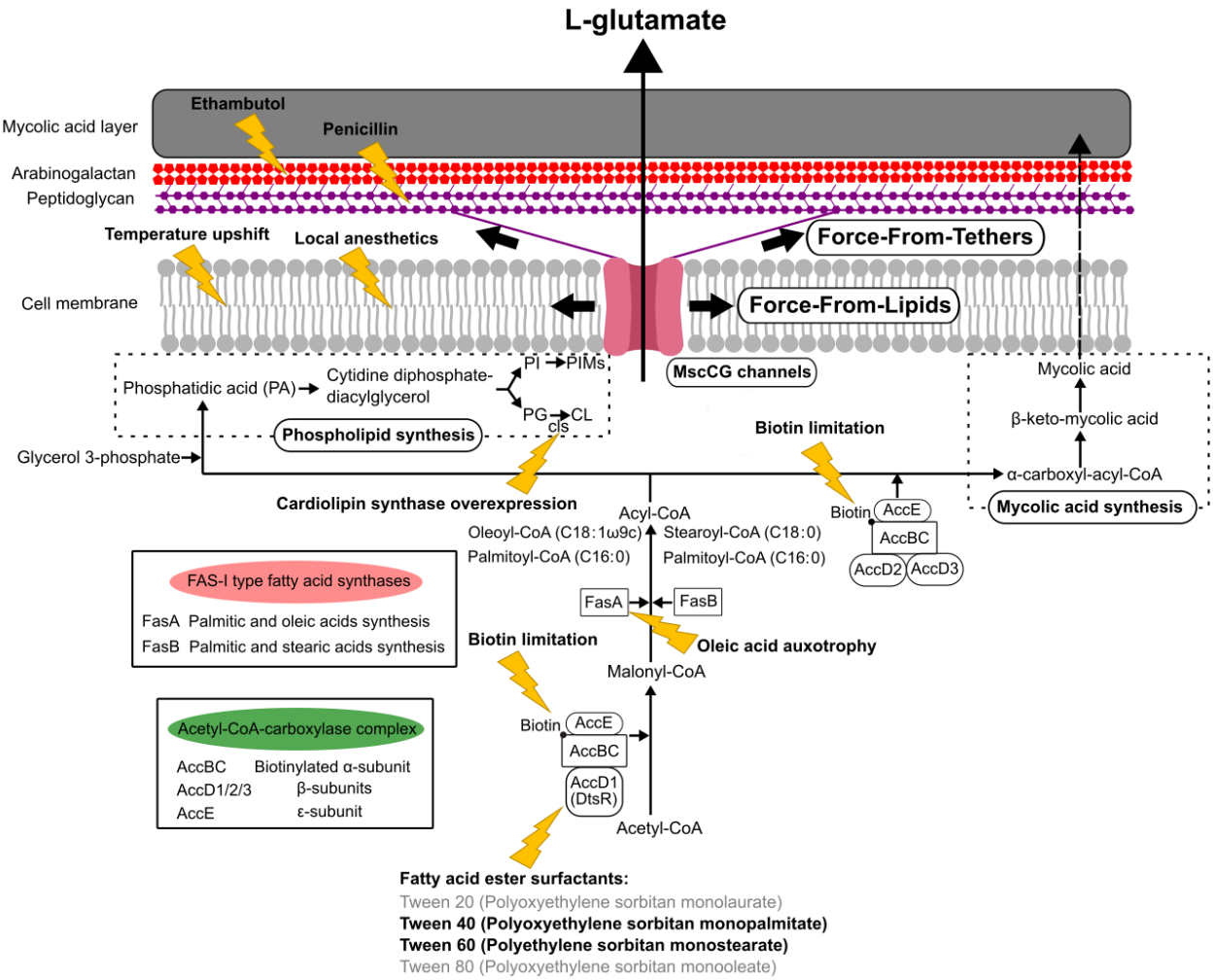

Figure 5 A scheme of "Force-From-Lipids/Tethers" activation mechanisms of the mechanosensitive channel MscCG in industrial L-glutamate production. Biotin limitation, adding fatty ester surfactants, oleic acid auxotrophy, overexpression of cardiolipin synthase, alter cell membranes and mycolic acid layers by changing fatty acid, phospholipid, and mycolic acid biosynthesis. Temperature upshift and local anesthetics change membrane mechanical properties. Adding ethambutol and penicillin degrade arabinogalactan and peptidoglycan layers in the cell wall, respectively, and activate MscCG channels.

6. Soil and gut bacterium scenario: L-glutamate secretion for the environmental signals in soil and the microbiota-gut-brain axis in the gut

Corynebacterium glutamicum has been considered as Gram-positive "soil" bacterium, and its L-glutamate export is for the "excretion" to reduce turgor pressure to protect cells from osmotic lysis. However, corynebacterial mechanobiology studies do not support this idea. The corynebacterial mechanosensitive solute efflux system is mainly adjusting the cytoplasmic concentration of betaine, and L-glutamate is not exported to reduce cytoplasmic osmolarity. Notably, mechanosensitive channels are not required for survival although turgor pressure upon osmotic downshock activates all mechanosensitive channels by increased membrane tension. In the soil environment, biotin-limiting, exposure to surfactants and penicillin, to trigger L-glutamate production can happen with other

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microbes, however it is unlikely that *Corynebacterium glutamicum* excretes L-glutamate for adapting to osmotic environments. Then, what is the physiological significance of the L-glutamate production for *Corynebacterium glutamicum*? Recent studies have revealed that glutamate is used for living organisms as signal rather than osmolytes. Since bacteria communicate through electrical signaling with ion channels (97). Bacterial biofilms expand outward until cells in the interior consumed the available reserves of the glutamate, which the bacteria use as a nitrogen source. Then the biofilms would stop expanding until the glutamate was replenished. Not only in microbial communications, L-glutamate is also used as environmental signals for plants. L-glutamate is commonly found in the phloem sap and performs a long-distance signaling role between the shoot and root (98). Thus, L-glutamate enables the shoot to communicate its changing Nitrogen/Carbon status to the root and thereby regulate the nitrate uptake system appropriately from the soil (99). Therefore, L-glutamate secreted by *Corynebacterium glutamicum* in soil can be used as an environmental signal for cell-cell communications among microbes and plants **(Figure 6)**.

In addition to roles in environments as a soil bacterium, it should be noted that *Corynebacterium glutamicum* was originally isolated in avian (most likely pigeon) feces contaminated soil in a Japanese park**.** Thus, *Corynebacterium glutamicum* can be a "gut" bacterium in avian. Indeed actinobacteria including Corynebacterium are the fourth most abundant phylum in the wild bird gastrointestinal tract (100). Gut microbiota are broadly involved in digestion of food products and help nutritional uptake for the host. For the host physiology, glutamate is absorbed by colonocytes and transferred from the lumen to portal circulation (**Figure 6**). In the brain, glutamate is the major excitatory neurotransmitter for N-methyl-d-aspartate receptor-mediated glutamatergic signaling, and thus the microbiota-gut-brain axis affects cognitive function of the host (101). Chang *et al* reported that plasma D-glutamate levels are associated with cognitive impairment in Alzheimer's disease and suggested that glutamate produced by bacteria like Corynebacteria in gut may modulate glutamatergic signaling in the brain (102). Moreover, bacterial glutamate is a substrate for gamma-aminobutyric acid (GABA) synthesis by decarboxylation with glutamate decarboxylase, thus it changes the host emotional behavior and moods by the microbiota-gut-brain axis on animal behaviors (103). Although the avian gut microbiota community is significantly different from the human gut microbiota due to it diets, it is possible that *Corynebacterium glutamicum* secretes glutamate for the brain function of the avian host. The MscCG mechanosensitive channel can be activated by the mammalian body temperature to secrete glutamate. Especially, body temperature in birds is the highest among mammals and reaches 41-43°C (104). The "soil" and "gut" bacterium scenario for the habitats of *Corynebacterium glutamicum* should be

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reconsidered to understand the *Corynebacterium glutamicum* physiology for the L-glutamate secretion.

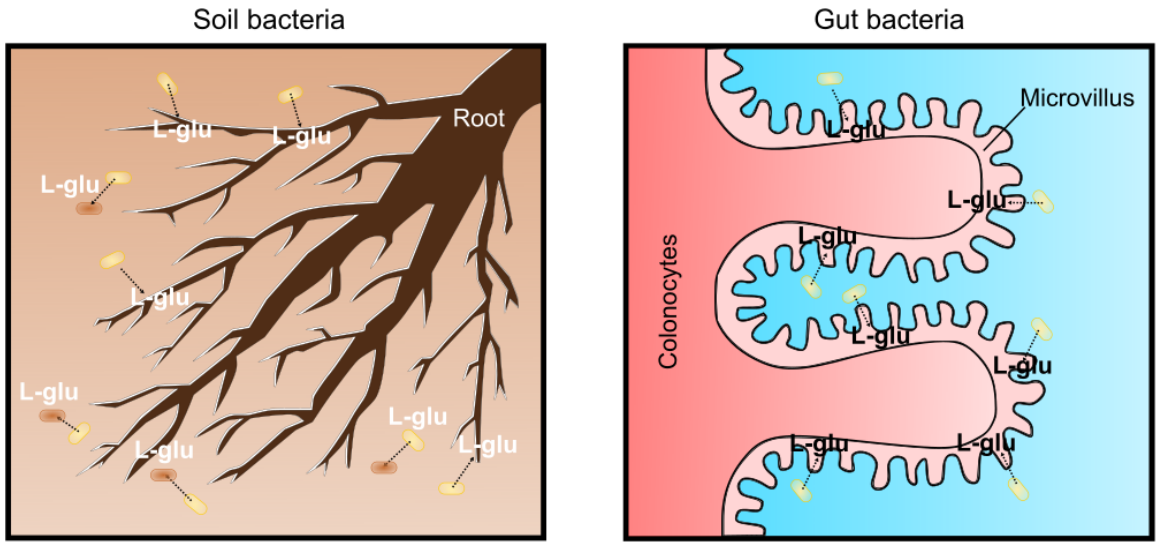

Figure 6 Physiological significance as soil and gut bacterium for *Corynebacterium glutamicum* L-glutamate secretion. In soil, L-glutamate is an environmental signal for microbes to communicate electrically and for plants to regulate the nitrate uptake system (left). In the avian gut, L-glutamate produced by bacteria is absorbed in colonocytes and circulate for the microbiota-gut-brain axis (right).

7. Conclusions & prospects

Mechanosensing is ubiquitously present for osmoregulation in bacteria. To manage huge turgor pressure, most of bacteria excrete L-glutamate to reduce osmotic gradient. However, the *Corynebacterium glutamicum* L-glutamate secretion is not simply for osmoregulation although MscCG-type mechanosensitive channels are activated to regulate cytoplasmic osmolarity. It is worth mentioning that corynebacterial mechanosensitive channels are not required for survival upon hypoosmotic shock at all due to its cell and membrane mechanics. The activation mechanism of MscCG-type mechanosensitive channels by altering cell membrane and wall in L-glutamate production differs significantly from the osmotic activation. However, the “Force-From-Lipids” and “Force-From-Tethers” paradigms for the gating mechanisms of mechanosensitive channels can explain the lipid modulation activation of MscCG channels in L-glutamate production. It is likely that *Corynebacterium glutamicum* uses L-glutamate as environmental signals for microbial and plant communications as a soil bacterium. Moreover, *Corynebacterium glutamicum* can be a gut bacterium to produce glutamate for the host brain function by the microbiota-gut-brain axis. Glutamate-producing corynebacteria have been found in the avian-feces contaminated soil in the world, and therefore, the physiological significance of *Corynebacterium glutamicum* L-glutamate secretion should be reconsidered in both soil and gut bacterium scenarios. In summary, understanding corynebacterial mechanosensing mechanisms by mechanosensitive channels promises to shed light on elucidating the physiological significance of *Corynebacterium glutamicum* L-glutamate secretion

and contribute to establish sustainable developments for the amino acid production by transporter engineering.

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References

1. Kinoshita S, Udaka S, Shimono M. Studies On the Amino Acid Fermentation Part I. Production of L-Glutamic Acid by Various Microorganisms∗. J Gen Appl Microbiol. 1957;
2. UDAKA S. Screening method for microorganisms accumulating metabolites and its use in the isolation of Micrococcus glutamicus. J Bacteriol. 1960;79:754–5.
3. Shiio I, Ôtsuka SIchir, Takahashi M. Effect of biotin on the bacterial formation of glutamic acid. J Biochem. 1962;
4. Nara T, Samejima H, Kinoshita S. Effect of Penicillin on Amino Acid Fermentation. Agric Biol Chem. 1964;
5. Radmacher E, Stansen KC, Besra GS, Alderwick LJ, Maughan WN, Hollweg G, et al. Ethambutol, a cell wall inhibitor of Mycobacterium tuberculosis, elicits L-glutamate efflux of Corynebacterium glutamicum. Microbiology. 2005;
6. Takinami K, Yoshii H, Tsuri H, Okada H. Biochemical Effects of Fatty Acid and its Derivatives on L-Glutamic Acid Fermentation: Part III Biotin-Tween 60 Relationship in the Accumulation of L-Glutamic Acid and the Growth of Brevibacterium. Agric Biol Chem. 1965;
7. Lambert C, Erdmann A, Eikmanns M, Kramer R. Triggering glutamate excretion in Corynebacterium glutamicum by modulating the membrane state with local anesthetics and osmotic gradients. Appl Environ Microbiol. 1995;
8. Delaunay S, Lapujade P, Engasser JM, Goergen JL. Flexibility of the metabolism of Corynebacterium glutamicum 2262, a glutamic acid-producing bacterium, in response to temperature upshocks. J Ind

 doi:10.20944/preprints202012.0807.v1

Microbiol Biotechnol. 2002;

9. Delaunay S, Gourdon P, Lapujade P, Mailly E, Oriol E, Engasser JM, et al. An improved temperature-triggered process for glutamate production with Corynebacterium glutamicum. Enzyme Microb Technol. 1999;

10. Gutmann M, Hoischen C, Krämer R. Carrier-mediated glutamate secretion by Corynebacterium glutamicum under biotin limitation. BBA - Biomembr. 1992;

11. Demain AL, Birnbaum J. Alteration of permeability for the release of metabolites from the microbial cell. Current topics in microbiology and immunology. 1968.

12. Hoischen C, Kramer R. Membrane alteration is necessary but not sufficient for effective glutamate secretion in Corynebacterium glutamicum. J Bacteriol. 1990;

13. Kimura E, Yagoshi C, Kawahara Y, Ohsumi T, Nakamatsu T, Tokuda H. Glutamate overproduction in corynebacterium glutamicum triggered by a decrease in the level of a complex comprising dtsR and a biotin-containing subunit. Biosci Biotechnol Biochem. 1999;

14. Asakura Y, Kimura E, Usuda Y, Kawahara Y, Matsui K, Osumi T, et al. Altered metabolic flux due to deletion of odhA causes L-glutamate overproduction in Corynebacterium glutamicum. Appl Environ Microbiol. 2007;

15. Kim J, Hirasawa T, Saito M, Furusawa C, Shimizu H. Investigation of phosphorylation status of OdhI protein during penicillin- and Tween 40-triggered glutamate overproduction by Corynebacterium glutamicum. Appl Microbiol Biotechnol. 2011;

16. Kimura E. L-glutamate production. In: Handbook of Corynebacterium Glutamicum. 2005.

17. Nakamura J, Seiko Hirano S, Ito H and Wachi M. Mutations of the Corynebacterium glutamicum NCgl1221 Gene, Encoding a Mechanosensitive Channel Homolog, Induce L-Glutamic Acid Production. APPLIED AND ENVIRONMENTAL MICROBIOLOGY; 2007.

18. Malcolm HR, Maurer JA. The Mechanosensitive Channel of Small Conductance (MscS) Superfamily: Not Just Mechanosensitive Channels Anymore. ChemBioChem. 2012.

19. Wen J, Bao J. Engineering Corynebacterium glutamicum triggers glutamic acid accumulation in biotin-rich corn stover hydrolysate. Biotechnol Biofuels. 2019;

20. Lanéelle MA, Tropis M, Daffé M. Current knowledge on mycolic acids in Corynebacterium glutamicum and their relevance for biotechnological processes. Applied Microbiology and Biotechnology. 2013.

21. Kawasaki H, Martinac B. Mechanosensitive channels of Corynebacterium glutamicum functioning as exporters of L-glutamate and other valuable metabolites. Curr Opin Chem Biol [Internet]. 2020;59:77–83. Available from: https://doi.org/10.1016/j.cbpa.2020.05.005

22. Nakayama Y, Hashimoto K ichi, Sawada Y, Sokabe M, Kawasaki H, Martinac B. Corynebacterium glutamicum mechanosensitive channels: towards unpuzzling “glutamate efflux” for amino acid production. Biophysical Reviews. 2018.

23. Börngen K, Battle A, Möker N, Morbach S, Marin K, Martinac B, and Krämer R. The properties and contribution of the Corynebacterium glutamicum MscS variant to fine-tuning of osmotic adaptation. Biochimica et Biophysica Acta; 2010.

24. Hashimoto KI, Murata J, Konishi T, Yabe I, Nakamatsu T, Kawasaki H. Glutamate is excreted across the cytoplasmic membrane through the NCgl1221 channel of Corynebacterium glutamicum by passive diffusion. Biosci Biotechnol Biochem. 2012;76(7):1422–4.

25. Wang Y, Cao G, Xu D, Fan L, Wu X, Ni X, et al. A novel Corynebacterium glutamicum L-glutamate exporter. Appl Environ Microbiol. 2018;84(6).

26. Nakayama Y, Komazawa K, Bavi N, Hashimoto K ichi, Kawasaki H, Martinac B. Evolutionary specialization of MscCG, an MscS-like mechanosensitive channel, in amino acid transport in Corynebacterium glutamicum. Sci Rep. 2018 Dec 1;8(1).

27. Hirasawa T, Wachi M. Glutamate fermentation-2: Mechanism of L-Glutamate overproduction in corynebacterium glutamicum. In: Advances in Biochemical Engineering/Biotechnology. 2017. p. 57–72.

28. Yu QL, Yan ZF, He X, Tian FH, Jia CW, Li CT. Corynebacterium defluvii sp. nov., isolated from Sewage. J Microbiol. 2017;

29. Cavana P, Zanatta R, Nebbia P, Miniscalco B, Vittone V, Zanoni MG, et al. Corynebacterium urealyticum urinary tract infection in a cat with urethral obstruction. J Feline Med Surg. 2008;

30. Cimprich RE, Rooney JR. Corynebacterium equi Enteritis in Foals. Vet Pathol. 1977;

31. Fudou R, Jojima Y, Seto A, Yamada K, Kimura E, Nakamatsu T, et al. Corynebacterium efficiens sp. nov., a glutamic-acid-producing species from soil and vegetables. Int J Syst Evol Microbiol. 2002;

32. Nantapong N, Matsutani M, Kanchanasin P, Kataoka N, Paisrisan P, Matsushita K, et al. Corynebacterium suranareeae sp. Nov., a glutamate producing bacterium isolated from soil and its complete genome-based analysis. Int J Syst Evol Microbiol. 2020;

33. Hahne J, Kloster T, Rathmann S, Weber M, Lipski A. Isolation and characterization of Corynebacterium spp. from bulk tank raw cow’s milk of different dairy farms in Germany. PLoS One. 2018;

34. Mounier J, Goerges S, Gelsomino R, Vancanneyt M, Vandemeulebroecke K, Hoste B, et al. Sources of the adventitious microflora of a smear-ripened cheese. J Appl Microbiol. 2006;

35. Morbach S, Krämer R. Osmoregulation. In: Handbook of Corynebacterium Glutamicum. 2005.

36. Whatmore AM, Reed RH. Determination of turgor pressure in Bacillus subtilis: A possible role for K+ in turgor regulation. J Gen Microbiol. 1990;

37. Wood JM. Bacterial osmoregulation: A paradigm for the study of cellular homeostasis. Annu Rev Microbiol. 2011;

38. Sleator RD, Hill C. Bacterial osmoadaptation: The role of osmolytes in bacterial stress and virulence. FEMS Microbiology Reviews. 2002.

39. Krämer R. Osmosensing and osmosignaling in corynebacterium glutamicum. Amino Acids. 2009;37(3):487–97.

40. Ruffert S, Lambert C, Peter H, Wendisch VF, Krämer R. Efflux of compatible solutes in Corynebacterium glutamicum mediated by osmoregulated channel activity. Eur J Biochem. 1997;

41. Wolf A, Krämer R, Morbach S. Three pathways for trehalose metabolism in Corynebacterium glutamicum ATCC13032 and their significance in response to osmotic stress. Mol Microbiol. 2003;

42. Guillouet S, Engasser JM. Sodium and proline accumulation in Corynebacterium glutamicum as a response to an osmotic saline upshock. Appl Microbiol Biotechnol. 1995;

43. Cox CD, Bavi N, Martinac B. Bacterial Mechanosensors. Vol. 80, Annual Review of Physiology. Annual Reviews Inc.; 2018. p. 71–93.

44. Edwards MD, Black S, Rasmussen T, Rasmussen A, Stokes NR, Stephen TL, et al. Characterization of three novel mechanosensitive channel activities in Escherichia coli. Channels. 2012;

45. Naismith JH, Booth IR. Bacterial mechanosensitive channels-MscS: Evolution's solution to creating sensitivity in function. Annual Review of Biophysics. 2012.

46. Levina N, Tötemeyer S, Stokes NR, Louis P, Jones MA, Booth IR. Protection of Escherichia coli cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: Identification of genes required for MscS activity. EMBO J. 1999;

47. Nottebrock D, Meyer U, Krämer R, Morbach S. Molecular and biochemical characterization of mechanosensitive channels in Corynebacterium glutamicum. FEMS Microbiol Lett. 2003;

48. Cox CD, Nakayama Y, Nomura T, Martinac B. The evolutionary 'tinkering' of MscS-like channels: generation of structural and functional diversity. Pflugers Archiv European Journal of Physiology. 2014.

49. Nakayama Y, Becker M, Ebrahimian H, Konishi T, Kawasaki H, Krämer R, et al. The impact of the C-terminal domain on the gating properties of MscCG from Corynebacterium glutamicum. Biochim Biophys Acta - Biomembr. 2016;

50. Kamaraju K, Belyy V, Rowe I, Anishkin A, Sukharev S. The pathway and spatial scale for MscS inactivation. J Gen Physiol. 2011;

51. Boer M, Anishkin A, Sukharev S. Adaptive MscS gating in the osmotic permeability response in E. coli: The question of time. Biochemistry. 2011;

52. Nakayama Y, Yoshimura K, Iida H. Electrophysiological characterization of the mechanosensitive channel mscCG in corynebacterium glutamicum. Biophys J. 2013;

53. Nakayama Y, Fujiu K, Sokabe M, Yoshimura K. Molecular and electrophysiological characterization of a mechanosensitive channel expressed in the chloroplasts of Chlamydomonas. Proc Natl Acad Sci U S A. 2007;

54. Haswell ES, Meyerowitz EM. MscS-like proteins control plastid size and shape in Arabidopsis thaliana. Curr Biol. 2006;

55. Veley KM, Marshburn S, Clure CE, Haswell ES. Mechanosensitive channels protect plastids from hypoosmotic stress during normal plant growth. Curr Biol. 2012;

56. Li Y, Hu Y, Wang J, Liu X, Zhang W, Sun L. Structural Insights into a Plant Mechanosensitive Ion Channel MSL1. Cell Rep [Internet]. 2020;30(13):4518-4527.e3. Available from: https://doi.org/10.1016/j.celrep.2020.03.026

57. Lee JS, Wilson ME, Richardson RA, Haswell ES. Genetic and physical interactions between the organellar mechanosensitive ion channel homologs MSL1, MSL2, and MSL3 reveal a role for inter-organellar communication in plant development. Plant Direct. 2019;

58. Lee CP, Maksaev G, Jensen GS, Murcha MW, Wilson ME, Fricker M, et al. MSL1 is a mechanosensitive ion channel that dissipates mitochondrial membrane potential and maintains redox homeostasis in mitochondria during abiotic stress. Plant J. 2016;

59. Nakayama Y, Yoshimura K, Iida H. Organellar mechanosensitive channels in fission yeast regulate the hypo-osmotic shock response. Nat Commun. 2012;

60. Nakayama Y, Hirata A, Iida H. Mechanosensitive channels Msy1 and Msy2 are required for maintaining organelle integrity upon hypoosmotic shock in Schizosaccharomyces pombe. FEMS Yeast Res. 2014;

61. Reddy B, Bavi N, Lu A, Park Y, Perozo E. Molecular basis of force-from-lipids gating in the mechanosensitive channel MscS. Elife [Internet]. 2019 Dec 27;8. Available from: https://elifesciences.org/articles/50486

62. Nakayama Y, Yoshimura K, Iida H. A Gain-of-function mutation in gating of Corynebacterium glutamicum NCgl1221 causes constitutive glutamate secretion. Applied and Environmental Microbiology. 2012.

63. Yamashita C, Hashimoto KI, Kumagai K, Maeda T, Takada A, Yabe I, et al. L-glutamate secretion by the N-terminal domain of the corynebacterium glutamicum NCgl1221 mechanosensitive channel. Biosci Biotechnol Biochem. 2013;

64. Becker M, Börngen K, Nomura T, Battle AR, Marin K, Martinac B, et al. Glutamate efflux mediated by Corynebacterium glutamicum MscCG, Escherichia coli MscS, and their derivatives. Biochim Biophys Acta - Biomembr. 2013;

65. Becker M, Krämer R. MscCG from Corynebacterium glutamicum: functional significance of the C-terminal domain. Eur Biophys J. 2015;

66. Krumbach K, Sonntag CK, Eggeling L, Marienhagen J. CRISPR/Cas12a Mediated Genome Editing to Introduce Amino Acid Substitutions into the Mechanosensitive Channel MscCG of Corynebacterium glutamicum. ACS Synth Biol. 2019;8(12):2726–34.

67. Martinac B, Buechner M, Delcour AH, Adler J, Kung C. Pressure-sensitive ion channel in Escherichia coli. Proc Natl Acad Sci U S A. 1987;84(8):2297–301.

68. Sukharev S. Purification of the small mechanosensitive channel of Escherichia coli (MscS): The subunit structure, conduction, and gating characteristics in liposomes. Biophys J. 2002;

69. Sokabe M, Sachs F. The structure and dynamics of patch-clamped membranes: A study using differential interference contrast light microscopy. J Cell Biol. 1990;

70. Nomura T, Cranfield CG, Deplazes E, Owen DM, Macmillan A, Battle AR, et al. Differential effects of lipids and lyso-lipids on the mechanosensitivity of the mechanosensitive channels MscL and MscS. Proc Natl Acad Sci U S A. 2012;109(22):8770–5.

71. Martinac B, Adler J, Kung C. Mechanosensitive ion channels of E. coli activated by amphipaths. Nature. 1990;

72. Teng J, Loukin S, Anishkin A, Kung C. The force-from-lipid (FFL) principle of mechanosensitivity, at large and in elements. Pflugers Archiv European Journal of Physiology. 2014.

73. Nakayama Y, Hashimoto K ichi, Kawasaki H, Martinac B. “Force-From-Lipids” mechanosensation in Corynebacterium glutamicum. Biophysical Reviews. 2019.

74. Cox CD, Bavi N, Martinac B. Origin of the Force: The Force-From-Lipids Principle Applied to Piezo Channels. Curr Top Membr. 2017;

75. Gullingsrud J, Schulten K. Lipid Bilayer pressure profiles and mechanosensitive channel gating. Biophys J. 2004;

76. Bavi O, Cox C, Vossoughi M, Naghdabadi R, Jamali Y, Martinac B. Influence of Global and Local Membrane Curvature on Mechanosensitive Ion Channels: A Finite Element Approach. Membranes (Basel). 2016;

77. Martinac B, Bavi N, Ridone P, Nikolaev YA, Martinac AD, Nakayama Y, et al. Tuning ion channel mechanosensitivity by asymmetry of the transbilayer pressure profile. Biophysical Reviews. 2018.

78. Heureaux J, Chen D, Murray VL, Deng CX, Liu AP. Activation of a bacterial mechanosensitive channel

 doi:10.20944/preprints202012.0807.v1

in mammalian cells by cytoskeletal stress. Cell Mol Bioeng. 2014;

79. Zanini D, Göpfert MC. Mechanosensation: Tethered ion channels. Current Biology. 2013.

80. Cox CD, Bae C, Ziegler L, Hartley S, Nikolova-Krstevski V, Rohde PR, et al. Removal of the mechanoprotective influence of the cytoskeleton reveals PIEZO1 is gated by bilayer tension. Nat Commun. 2016;

81. Tuson HH, Auer GK, Renner LD, Hasebe M, Tropini C, Salick M, et al. Measuring the stiffness of bacterial cells from growth rates in hydrogels of tunable elasticity. Mol Microbiol. 2012;

82. Steltenkamp S, Rommel C, Wegener J, Janshoff A. Membrane stiffness of animal cells challenged by osmotic stress. Small. 2006;

83. Martinac B, Nikolaev YA, Silvani G, Bavi N, Romanov V, Nakayama Y, et al. Cell membrane mechanics and mechanosensory transduction. Vol. 86, Current Topics in Membranes. 2020.

84. Yuki Takechi-Haraya,† Yukihiro Goda and KS-K. Atomic Force Microscopy Study on the Stiffness of Nanosized Liposomes Containing Charged Lipids.

85. Gande R, Dover LG, Krumbach K, Besra GS, Sahm H, Oikawa T, et al. The two carboxylases of corynebacterium glutamicum essential for fatty acid and mycolic acid synthesis. J Bacteriol. 2007;

86. Radmacher E, Alderwick LJ, Besra GS, Brown AK, Gibson KJC, Sahm H, et al. Two functional FAS-I type fatty acid synthases in Corynebacterium glutamicum. Microbiology. 2005;

87. Nampoothiri K, Hoischen C, Bathe B, Möckel B, Pfefferle W, Krumbach K, et al. Expression of genes of lipid synthesis and altered lipid composition modulates L-glutamate efflux of Corynebacterium glutamicum. Appl Microbiol Biotechnol. 2002;

88. Oliver PM, Crooks JA, Leidl M, Yoon EJ, Saghatelian A, Weibel DB. Localization of anionic phospholipids in Escherichia coli cells. J Bacteriol. 2014;

89. Bavi N, Nakayama Y, Bavi O, Cox CD, Qin QH, Martinac B. Biophysical implications of lipid bilayer rheometry for mechanosensitive channels. Proc Natl Acad Sci U S A. 2014;

90. Ridone P, Nakayama Y, Martinac B, Battle AR. Patch clamp characterization of the effect of cardiolipin on MscS of E. coli. Eur Biophys J. 2015;

91. Ridone P, Grage SL, Patkunarajah A, Battle AR, Ulrich AS, Martinac B. “Force-from-lipids” gating of mechanosensitive channels modulated by PUFAs. J Mech Behav Biomed Mater. 2018;

92. Lapujade P, Goergen JL, Engasser JM. Glutamate Excretion as a Major Kinetic Bottleneck for the Thermally Triggered Production of Glutamic Acid by Corynebacterium glutamicum. Metab Eng. 1999;

93. Shi T, Fan X, Wu Y, Ma Q, Xu Q, Xie X, et al. Mutation of genes for cell membrane synthesis in Corynebacterium glutamicum causes temperature-sensitive trait and promotes L-glutamate excretion.

 doi:10.20944/preprints202012.0807.v1

Biotechnol Biotechnol Equip [Internet]. 2020;34(1):38–47. Available from: https://doi.org/10.1080/13102818.2019.1711186

94. Bokas D, Uy D, Grattepanche F, Duportail G, Guedon E, Delaunay S, et al. Cell envelope fluidity modification for an effective glutamate excretion in Corynebacterium glutamicum 2262. Appl Microbiol Biotechnol. 2007;76(4):773–81.

95. Chiaradia L, Lefebvre C, Parra J, Marcoux J, Burlet-Schiltz O, Etienne G, et al. Dissecting the mycobacterial cell envelope and defining the composition of the native mycomembrane. Sci Rep. 2017;

96. Puech V, Chami M, Lemassu A, Lanéelle MA, Schiffler B, Gounon P, et al. Structure of the cell envelope of corynebacteria: Importance of the non-covalently bound lipids in the formation of the cell wall permeability barrier and fracture plane. Microbiology. 2001;147(5):1365–82.

97. Prindle A, Liu J, Asally M, Ly S, Garcia-Ojalvo J, Süel GM. Ion channels enable electrical communication in bacterial communities. Nature. 2015;

98. Toyota M, Spencer D, Sawai-Toyota S, Jiaqi W, Zhang T, Koo AJ, et al. Glutamate triggers long-distance, calcium-based plant defense signaling. Science (80-). 2018;

99. Forde BG, Lea PJ. Glutamate in plants: Metabolism, regulation, and signalling. In: Journal of Experimental Botany. 2007.

100. Grond K, Sandercock BK, Jumpponen A, Zeglin LH. The avian gut microbiota: community, physiology and function in wild birds. J Avian Biol. 2018;49(11):1–19.

101. Baj A, Moro E, Bistoletti M, Orlandi V, Crema F, Giaroni C. Glutamatergic signaling along the microbiota-gut-brain axis. International Journal of Molecular Sciefile:///C:/Users/yosnak/Downloads/ijms-20-01482.pdfnces. 2019.

102. Chang C, Lin C, Lane H. d -glutamate and Gut Microbiota in Alzheimer ' s Disease. :1–17.

103. Mazzoli R, Pessione E. The neuro-endocrinological role of microbial glutamate and GABA signaling. Frontiers in Microbiology. 2016.

104. Prinzinger R, Preßmar A, Schleucher E. Body temperature in birds. Comparative Biochemistry and Physiology -- Part A: Physiology. 1991.