

Corynebacterium Glutamicum Mechanosensing

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Corynebacterium glutamicum is a workhorse for industrial amino acid production, and the most striking feature of this bacterium is L-glutamate secretion. L-glutamate secretion is triggered by altering cell surface structures by biotin limitation, adding surfactants, penicillin, etc. Mechanosensing by MscCG-type mechanosensitive channels is the central physiological phenomenon for the L-glutamate secretion, and understanding the structural and functional diversity of MscS bacterial mechanosensitive channel superfamily will solve long-puzzling questions in *Corynebacterium glutamicum*.

bacterial mechanosensitive channel

MscS-like channel

glutamate exporter

1. Introduction

Corynebacterial Mechanosensitive Channel Model for the Industrial L-Glutamate Production

The *Corynebacterium glutamicum* ATCC13032 strain was first isolated as a glutamate producer in the avian (most likely pigeon) feces-contaminated soil in 1957 by Kinoshita and Udaka ^[1], who described the result of an elaborated screening method with a glutamate-auxotrophic bacterium *Leuconostoc mesenteroides* strain, P-60 ^[2]. This sparked microbial amino acid production and our understanding *C. glutamicum* physiology has made numerous paradigm shifts in the biotechnological process. Since then, several *Corynebacterium glutamicum* subspecies, other than the ATCC13032 strain, have been isolated from different materials for industrial production. One of the most striking findings of *C. glutamicum* studies is biotin-dependent L-glutamate secretion. In nature, glutamate has two optical isomers, d(-) and l(+), and the l form (L-glutamate) is the most widely occurring, while the d form (D-glutamate) occurs rarely in the bacterial cell walls as a component of peptidoglycan layers. At a growth-limiting concentration of biotin, *C. glutamicum* massively releases L-glutamate into the culture media; however, this bacterium does not produce it at all under normal culture conditions ^[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 the adding of penicillin ^[4], ethambutol ^[5], ciprofloxacin ^[6], fatty acid ester surfactants ^[7], local anesthetics ^[8], and temperature upshift ^{[9][10]}. Correspondingly, a wide range of mechanistic models to explain L-glutamate secretion was suggested, and a coherent model was expected to elucidate the situation. Since L-glutamate is a charged amino acid, passive efflux was rejected, and an active carrier was proposed as the “carrier model” ^[11]. The leakage from damaged cell envelopes was considered as one of the main causes due to inhibited fatty acid biosynthesis and membrane alterations in L-glutamate production, proposed as the “leak model” ^{[12][13][14]}. Metabolism in the biotin-limited condition was shifted from energy

production via tricarboxylic acid (TCA) cycle towards L-glutamate production. Thus, the “metabolic flow change model” was proposed to trigger L-glutamate secretion [15][16][17]. This puzzling situation has ended with 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 as the result [18]. NCgl1221 was predicted to be a homologue of bacterial mechanosensitive channels of small conductance (MscS); however, it shows similarity only in the pore domain, and its entire structure differs from other MscS-type mechanosensitive channels [19]. This finding has emphasized the importance of bacterial mechanosensing by mechanosensitive channels on the studies of corynebacterial amino acid exporters [20][21], and mechanosensitive channels are now emergent targets for transporter engineering to use *C. glutamicum* as a microbial cell factory [22][23]. Conclusively, using a novel patch-clamp technique with *C. glutamicum* giant spheroplasts of the industrial strain ATCC13869, all endogenous mechanosensitive channels including MscCG and MscCG2 were recorded electrophysiologically in the native membrane environment [24]. These studies strongly supported the “mechanosensitive channel model” [25] 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; (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.

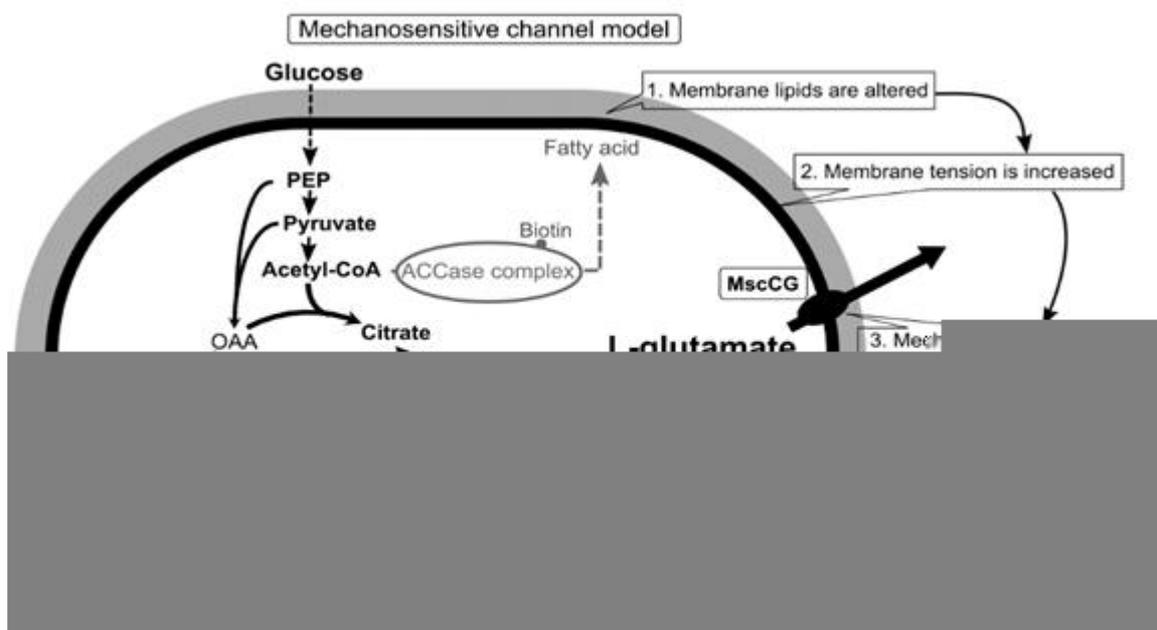


Figure 1. A scheme of the *C. glutamicum* L-glutamate secretion triggered by biotin limitation as mechanosensitive channel model. Biotin limitation shifts metabolic flow to produce L-glutamate by inhibiting the 2-oxoglutarate dehydrogenase complex (ODHC) activity and inhibits the acetyl CoA carboxylase (ACCase) complex activity. As a result of fatty acid biosynthesis inhibition, membrane lipids are altered to increase membrane tension. In the ATCC13032 strain, the MscS-like mechanosensitive channel MscCG is activated exclusively as major exporter by increased membrane tension to release L-glutamate. In several industrial strains, another MscS-like

mechanosensitive channel, MscCG2, is also activated as a minor exporter. However, the MscL-type mechanosensitive channel CgMscL is not activated in L-glutamate secretion.

2. 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 *C. glutamicum*, indicates that these channels have developed through evolution to cope with changes of mechanical environments; however, its functions as an osmoregulator are significantly different [26]. *E. coli* MscS has a large open channel pore (~16 Å), and thus its conductance reaches 1 nS, whereas *C. glutamicum* MscCG has a significantly smaller conductance of approximately 0.3 nS [27][24][28]. As an osmoregulator, *E. coli* MscS has strong inactivation and adaptation mechanisms to mechanical stimuli that are needed to avoid the over-efflux of cytoplasmic molecules upon osmotic downshock [29][30]. However, *C. glutamicum* MscCG does not have these features and tends to be open as a metabolic valve rather than an osmotic safety valve [31]. Recent studies of the 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 those of osmotic safety valves upon hypoosmotic shock [19]. In eukaryotes, algal and plant MscS-like channels (MSCs, MSLs) are much more complicated structures than *E. coli* MscS and are found in the organellar membranes of chloroplasts [32][33][34] and mitochondria [35][36][37] for mechanosensing. Fungal MscS-like channels (Msy1 and Msy2) are localized in the endoplasmic reticulum membranes and are involved in osmotic Ca²⁺ signalling upon hypoosmotic shock [38][39].

Although the physiological functions of corynebacterial MscCG-type mechanosensitive channels are still controversial, L-glutamate secretion is certainly caused by the conformational changes of the MscCG and MscCG2 channels. *C. glutamicum* MscCG and MscCG2 are 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 (Figure 2A). Recently, Reddy et al. refined the 3D structures of the full-length *E. coli* MscS embedded in lipid bilayers by cryo-electron microscopy with nanodiscs [40] (Figure 2B). MscCG and MscCG2 show the highest similarity in the pore-forming helix TM3 and adjacent regions. The unique feature of the mechanosensitive channel MscCG is the C-terminal domain (287–533 aa), including a cytoplasmic loop, the fourth transmembrane helix, TM4, and a periplasmic loop. In contrast, MscCG2 does not have these features (Figure 2C). 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 2C). Originally, Nakamura et al. identified W15CSLW, A100T, A111T, A111V in the N-terminal pore domain and V419::I51207, 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 *C. glutamicum* [18]. Afterwards, Nakayama et al. reported using *E. coli* patch-clamp that the spontaneous L-glutamate secretion was caused by the gain-of-function mutation on mechanosensitivity of MscCG channels [41]. These findings proved that MscCG mechanosensitivity can be

evaluated by L-glutamate productivity in *C. glutamicum*. Using these assays, the functional domain of MscCG has been thoroughly investigated. 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 channel functionality [42]. Becker et al. reported additional point mutations in the N-terminal pore domain and the impact of the C-terminal domain on the channel function [43][44]. 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, such that *C. glutamicum* cannot export L-glutamate, even with penicillin treatment. In contrast, the deletion of the periplasmic loop (423–533 aa) in the C-terminal domain caused spontaneous L-glutamate secretion, but not 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. further identified the gain-of-function mutations V422K, V422D, E423P, and E423S using CRSPER/Cas12a genome-editing technology [45]. Based on the position of these mutations, the interaction with other components of the cell wall was suggested to be crucial for MscCG mechanosensitivity.

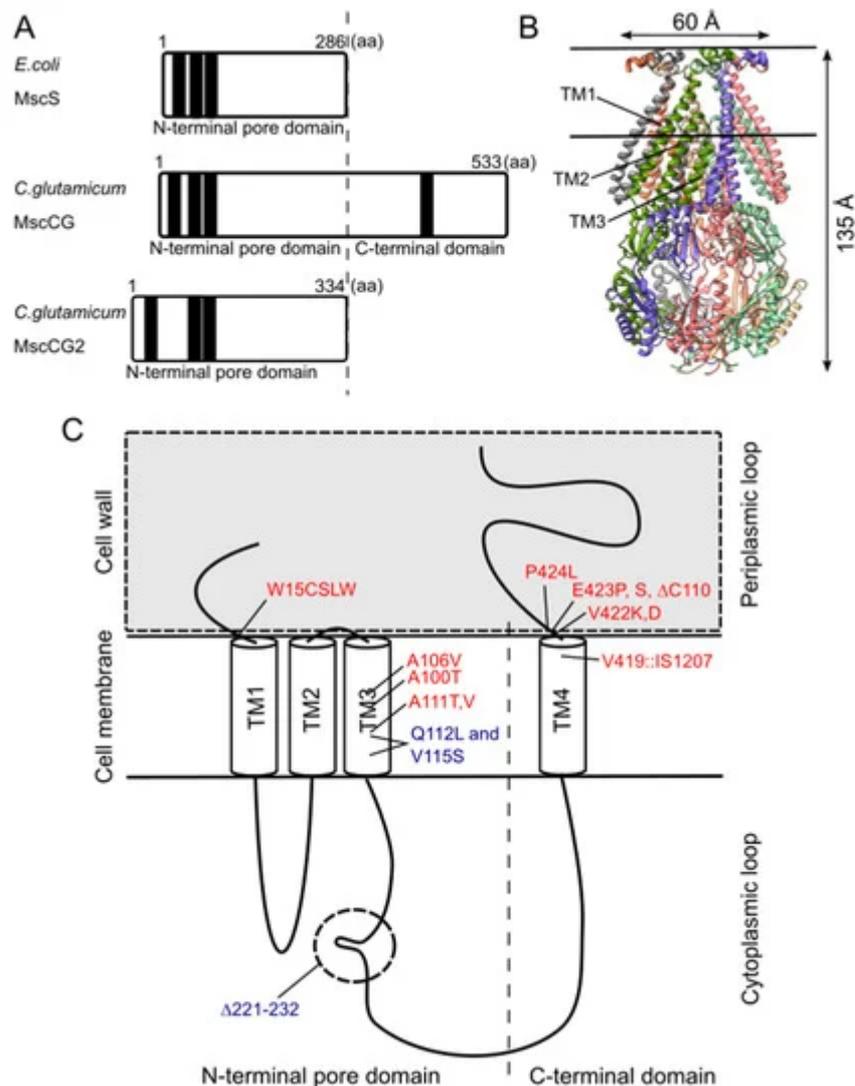


Figure 2. Structural features of the mechanosensitive channel MscCG. **(A).** Secondary structure comparison among *E. coli* MscS, *C. glutamicum* MscCG, and MscCG2. Black bars show predicted transmembrane (TM) helices by TOPCONS program (<https://topcons.net/>). **(B).** The cryoEM 3D structure of *E. coli* MscS embedded in nanodiscs with POPC:POPG = 1:4 (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.

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, 3, 193–205.
2. Udaka, S. Screening method for microorganisms accumulating metabolites and its use in the isolation of *Micrococcus glutamicus*. *J. Bacteriol.* 1960, 79, 754–755.
3. Shiio, I.; Ôtsuka, S.I.; Takahashi, M. Effect of biotin on the bacterial formation of glutamic acid. *J. Biochem.* 1962, 51, 56–62.
4. Nara, T.; Samejima, H.; Kinoshita, S. Effect of penicillin on amino acid fermentation. *Agric. Biol. Chem.* 1964.
5. Radmacher, E.; Stansen, K.C.; Besra, G.S.; Alderwick, L.J.; Maughan, W.N.; Hollweg, G.; Sahm, H.; Wendisch, V.F.; Eggeling, L. Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits L-glutamate efflux of *Corynebacterium glutamicum*. *Microbiology* 2005, 151, 1359–1368.
6. Lubitz, D.; Wendisch, V.F. Ciprofloxacin triggered glutamate production by *Corynebacterium glutamicum*. *BMC Microbiol.* 2016, 16, 235.
7. Takinami, K.; Yoshii, H.; Tsuru, 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, 29, 351–359.
8. 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, 61, 4334–4342.
9. Delaunay, S.; Lapujade, P.; Engasser, J.M.; Goergen, J.L. Flexibility of the metabolism of *Corynebacterium glutamicum* 2262, a glutamic acid-producing bacterium, in response to temperature upshocks. *J. Ind. Microbiol. Biotechnol.* 2002, 28, 333–337.
10. Delaunay, S.; Gourdon, P.; Lapujade, P.; Mailly, E.; Oriol, E.; Engasser, J.M.; Lindley, N.D.; Goergen, J.L. An improved temperature-triggered process for glutamate production with *Corynebacterium glutamicum*. *Enzym. Microb. Technol.* 1999, 25, 762–768.
11. Gutmann, M.; Hoischen, C.; Krämer, R. Carrier-mediated glutamate secretion by *Corynebacterium glutamicum* under biotin limitation. *BBA Biomembr.* 1992, 1112, 115–123.

12. Demain, A.L.; Birnbaum, J. Alteration of permeability for the release of metabolites from the microbial cell. *Curr. Top. Microbiol. Immunol.* 1968, 46, 1–25.
13. Hoischen, C.; Kramer, R. Membrane alteration is necessary but not sufficient for effective glutamate secretion in *Corynebacterium glutamicum*. *J. Bacteriol.* 1990, 172, 3409–3416.
14. 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, 63, 1274–1278.
15. Asakura, Y.; Kimura, E.; Usuda, Y.; Kawahara, Y.; Matsui, K.; Osumi, T.; Nakamatsu, T. Altered metabolic flux due to deletion of *odhA* causes l-glutamate overproduction in *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 2007, 73, 1308–1319.
16. 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, 91, 143–151.
17. Kimura, E. *Handbook of Corynebacterium glutamicum: Chapter 19 l-Glutamate Production*; CRC Press: Boca Raton, FL, USA, 2005; pp. 439–463. ISBN 9780429129056.
18. Nakamura, J.; Hirano, S.; Ito, H.; Wachi, M. Mutations of the *Corynebacterium glutamicum* NCgl1221 gene, encoding a mechanosensitive channel homolog, induce l-Glutamic acid production. *Appl. Environ. Microbiol.* 2007, 73, 4491–4498.
19. Malcolm, H.R.; Maurer, J.A. The Mechanosensitive Channel of Small Conductance (MscS) Superfamily: Not Just Mechanosensitive Channels Anymore. *ChemBioChem* 2012, 13, 2037–2043.
20. Wen, J.; Bao, J. Engineering *Corynebacterium glutamicum* triggers glutamic acid accumulation in biotin-rich corn stover hydrolysate. *Biotechnol. Biofuels* 2019, 12.
21. Lanéelle, M.A.; Tropis, M.; Daffé, M. Current knowledge on mycolic acids in *Corynebacterium glutamicum* and their relevance for biotechnological processes. *Appl. Microbiol. Biotechnol.* 2013, 97, 9923–9930.
22. Kawasaki, H.; Martinac, B. Mechanosensitive channels of *Corynebacterium glutamicum* functioning as exporters of l-glutamate and other valuable metabolites. *Curr. Opin. Chem. Biol.* 2020, 59, 77–83.
23. Nakayama, Y.; Hashimoto, K.I.; Sawada, Y.; Sokabe, M.; Kawasaki, H.; Martinac, B. *Corynebacterium glutamicum* mechanosensitive channels: Towards unpuzzling “glutamate efflux” for amino acid production. *Biophys. Rev.* 2018, 10, 1359–1369.

24. Nakayama, Y.; Komazawa, K.; Bavi, N.; Hashimoto, K.I.; Kawasaki, H.; Martinac, B. Evolutionary specialization of MscCG, an MscS-like mechanosensitive channel, in amino acid transport in *Corynebacterium glutamicum*. *Sci. Rep.* 2018, 8.
25. Hirasawa, T.; Wachi, M. Glutamate fermentation-2: Mechanism of l-glutamate overproduction in *Corynebacterium glutamicum*. In *Advances in Biochemical Engineering/Biotechnology*; Springer: Tokyo, Japan, 2017; Volume 159, pp. 57–72.
26. Cox, C.D.; Nakayama, Y.; Nomura, T.; Martinac, B. The evolutionary ‘tinkering’ of MscS-like channels: Generation of structural and functional diversity. *Pflugers Arch. Eur. J. Physiol.* 2014, 467, 3–13.
27. Börngen, K.; Battle, A.R.; Möker, N.; Morbach, S.; Marin, K.; Martinac, B.; Krämer, R. The properties and contribution of the *Corynebacterium glutamicum* MscS variant to fine-tuning of osmotic adaptation. *Biochim. Biophys. Acta Biomembr.* 2010, 1798, 2141–2149.
28. Nakayama, Y.; Becker, M.; Ebrahimian, H.; Konishi, T.; Kawasaki, H.; Krämer, R.; Martinac, B. The impact of the C-terminal domain on the gating properties of MscCG from *Corynebacterium glutamicum*. *Biochim. Biophys. Acta Biomembr.* 2016, 1858, 130–138.
29. Kamaraju, K.; Belyy, V.; Rowe, I.; Anishkin, A.; Sukharev, S. The pathway and spatial scale for MscS inactivation. *J. Gen. Physiol.* 2011, 138, 49–57.
30. Boer, M.; Anishkin, A.; Sukharev, S. Adaptive MscS gating in the osmotic permeability response in *E. coli*: The question of time. *Biochemistry* 2011, 50, 4087–4096.
31. Nakayama, Y.; Yoshimura, K.; Iida, H. Electrophysiological characterization of the mechanosensitive channel MscCG in *Corynebacterium glutamicum*. *Biophys. J.* 2013, 105, 1366–1375.
32. 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. USA* 2007, 104, 5883–5888.
33. Haswell, E.S.; Meyerowitz, E.M. MscS-like proteins control plastid size and shape in *Arabidopsis thaliana*. *Curr. Biol.* 2006, 16, 1–11.
34. Veley, K.M.; Marshburn, S.; Clure, C.E.; Haswell, E.S. Mechanosensitive channels protect plastids from hypoosmotic stress during normal plant growth. *Curr. Biol.* 2012, 22, 408–413.
35. Li, Y.; Hu, Y.; Wang, J.; Liu, X.; Zhang, W.; Sun, L. Structural Insights into a Plant Mechanosensitive Ion Channel MSL1. *Cell Rep.* 2020, 30, 4518–4527.e3.
36. Lee, J.S.; Wilson, M.E.; Richardson, R.A.; Haswell, E.S. 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, 3.

37. Lee, C.P.; Makshev, G.; Jensen, G.S.; Murcha, M.W.; Wilson, M.E.; Fricker, M.; Hell, R.; Haswell, E.S.; Millar, A.H.; Sweetlove, L.J. MSL1 is a mechanosensitive ion channel that dissipates mitochondrial membrane potential and maintains redox homeostasis in mitochondria during abiotic stress. *Plant J.* 2016, 88, 809–825.
38. Nakayama, Y.; Yoshimura, K.; Iida, H. Organellar mechanosensitive channels in fission yeast regulate the hypo-osmotic shock response. *Nat. Commun.* 2012, 3.
39. 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, 14, 992–994.
40. Reddy, B.; Bavi, N.; Lu, A.; Park, Y.; Perozo, E. Molecular basis of force-from-lipids gating in the mechanosensitive channel MscS. *Elife* 2019, 8.
41. Nakayama, Y.; Yoshimura, K.; Iida, H. A Gain-of-function mutation in gating of *Corynebacterium glutamicum* NCgl1221 causes constitutive glutamate secretion. *Appl. Environ. Microbiol.* 2012, 78, 5432–5434.
42. Yamashita, C.; Hashimoto, K.I.; Kumagai, K.; Maeda, T.; Takada, A.; Yabe, I.; Kawasaki, H.; Wachi, M. I-glutamate secretion by the N-terminal domain of the *Corynebacterium glutamicum* NCgl1221 mechanosensitive channel. *Biosci. Biotechnol. Biochem.* 2013, 77, 1008–1013.
43. Becker, M.; Börngen, K.; Nomura, T.; Battle, A.R.; Marin, K.; Martinac, B.; Krämer, R. Glutamate efflux mediated by *Corynebacterium glutamicum* MscCG, *Escherichia coli* MscS, and their derivatives. *Biochim. Biophys. Acta Biomembr.* 2013, 1828, 1230–1240.
44. Becker, M.; Krämer, R. MscCG from *Corynebacterium glutamicum*: Functional significance of the C-terminal domain. *Eur. Biophys. J.* 2015, 44, 577–588.
45. Krumbach, K.; Sonntag, C.K.; 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, 2726–2734.

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