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# A role for mechanosensitive channels in chloroplast and bacterial fission.

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# A role for mechanosensitive channels in chloroplast and bacterial fission

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**Keywords:** *Arabidopsis thaliana*, *Escherichia coli*, mechanosensitive ion channel, chloroplast division, bacterial fission

**Abbreviations:** MscS, mechanosensitive channel of small conductance; DAPI, 4',6-diamidino-2-phenylindole; FtsZ, filamentous temperature sensitive Z; Z-ring, FtsZ ring; IPTG, isopropyl-β-D-1-thiogalactopyranoside; MS, mechanosensitive; MSL, MscS-Like

The division site in both chloroplasts and bacteria is established by the medial placement of the FtsZ ring, a process that is in part regulated by the evolutionarily conserved components of the Min system. We recently showed that mechanosensitive ion channels influence FtsZ ring assembly in both *Arabidopsis thaliana* chloroplasts and in *Escherichia coli*; in chloroplasts they do so through the same genetic pathway as the Min system. Here we describe the effect of heterologous expression of the Arabidopsis MS channel homolog MSL2 on FtsZ ring placement in *E. coli*. We also discuss possible molecular mechanisms by which MS channels might influence chloroplast or bacterial division.

Chloroplasts are plant-specific organelles responsible for several vital metabolic reactions, including photosynthesis,[1] and they undergo many rounds of division during the life of a plant in order to maintain their population.[2] Many of the proteins and regulatory mechanisms involved in chloroplast division have evolutionary counterparts in bacterial fission.[3,4] For example, in both plants and bacteria, division site selection is determined by the placement of the polymer-forming GTPase filamentous temperature sensitive Z (FtsZ), which associates with the inner membrane to form a ring-like structure known as the Z-ring. Medial placement of the Z-ring is regulated in part by the Min system, which in *E. coli* is composed of the MinD/MinC complex and the topological specificity factor MinE.[3,5] Normal Z-ring assembly in *Arabidopsis thaliana* chloroplasts requires homologs of MinD and MinE, as well as several proteins of eukaryotic origin, ARC3, MCD1 and PARC6.[4,6,7]

Recently we showed that two mechanosensitive (MS) channel homologs, MscS-Like2 (MSL2) and MSL3, influence Z-ring assembly in Arabidopsis chloroplasts and that they do so in the same pathway as the land plant Min system.[8] MSL2 and MSL3 are related to a well-studied MS channel from *E. coli*, the mechanosensitive channel of Small conductance (MscS) and are required for normal plastid size and shape.[9,10] Furthermore, we observed that the *E. coli* strain MJF465, in which the genes encoding MscS and two other MS channels of the *E. coli* inner membrane, MscK and MscL, are disrupted,[11] showed inappropriate Z-ring placement similar to that observed in *msl2 msl3* mutant chloroplasts. Our results thus link membrane tension and the process of Z-ring assembly in both chloroplasts and bacteria.[8] Here we present the surprising effects of heterologous expression of MSL2 in *E. coli*, and discuss several possible mechanisms by which MS channels might influence Z-ring assembly in an evolutionarily conserved manner.

## Expression of MSL2 in *E. coli* Causes Defects in Septation and Z-ring Placement

During the course of unrelated experiments, we fortuitously observed that isopropyl-β-D-1-thiogalactopyranoside (IPTG)-induced expression of MSL2 in the wild type *E. coli* strain Frag-1[12] caused extreme cellular filamentation (**Fig. 1A and B**). The severity of filamentation varied greatly, with cells from the same sample ranging from 5 μm to over 50 μm in length. This phenotype, and our previous observation that MS channels are required for normal Z-ring placement in *E. coli*, lead us to investigate Z-ring placement in *E. coli* cells expressing MSL2. As shown in **Figure 1 C–N**, immunoflorescence microscopy using an anti-FtsZ antibody[13] revealed that the filaments generated by expressing MSL2 contained a high number of Z-rings when compared with the filaments generated by treatment with the septation inhibitor cephalexin (compare **Fig. 1F and I**). The average distance between Z-rings in cells expressing MSL2 was 1.28 +/– 0.4 μm, while in cephalexin-treated cells it was 6.03 +/– 1.6 μm (n = 55 cells for each condition). In addition to closely spaced Z-rings (white arrows), polar and double Z-rings were often observed in cells expressing MSL2 (purple arrows).

Multiple, closely-spaced, and polar-localized Z-rings have been described in *E. coli* mutants lacking a functional Min system,[14] and, as summarized above, we previously observed a similar phenotype in the *E. coli* MJF465 strain.[8] Perhaps the most

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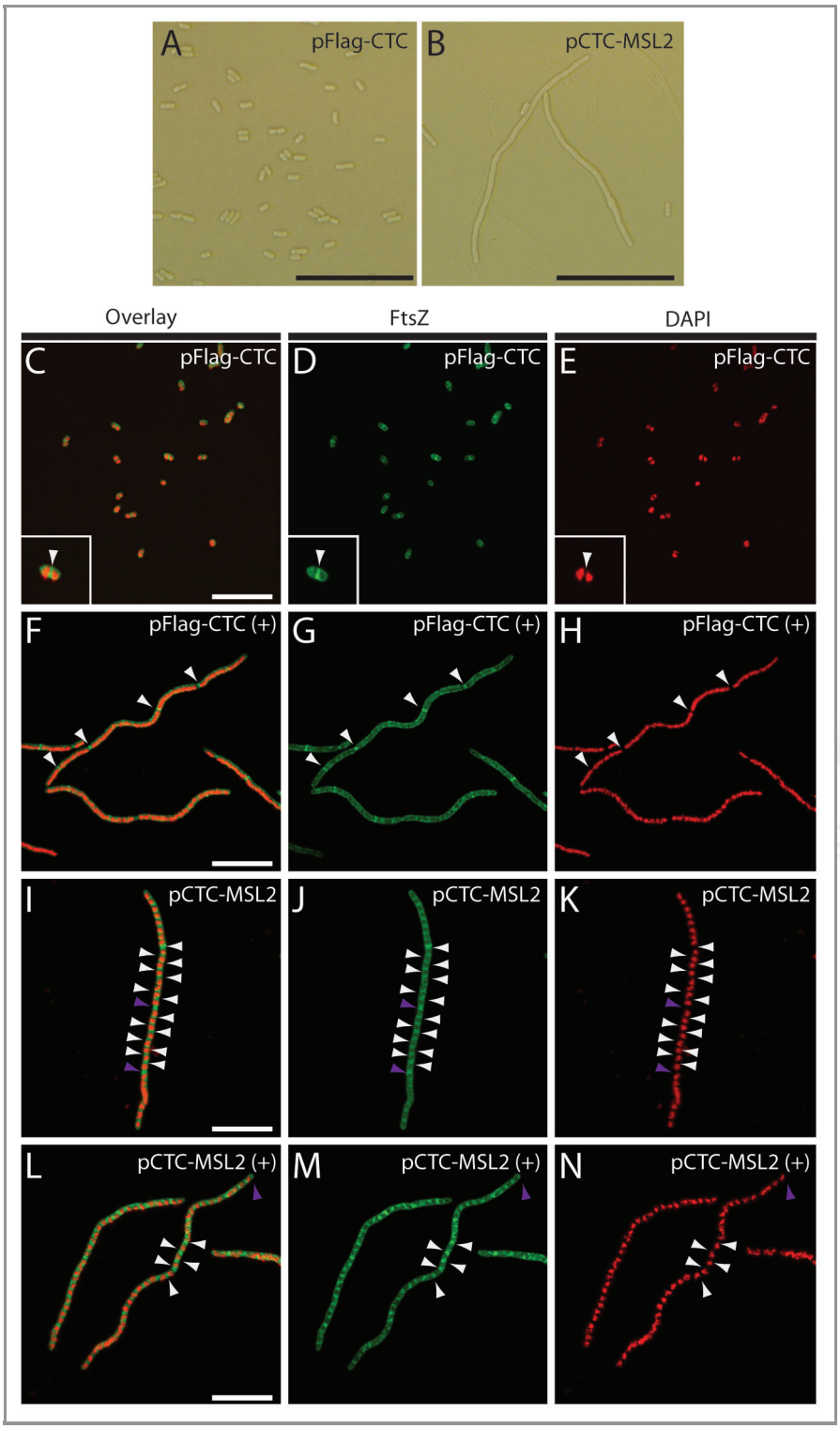


**Figure 1.** Heterologous expression of MSL2 in *E. coli* causes filamentation and disrupts normal Z-ring placement. (A and B). Light micrographs of Frag-1 cells harboring the indicated constructs after 1 h of induction in 0.1 mM IPTG. Cells were immobilized on agarose pads as previously described.[26] (C–N). Immunofluorescence micrographs of FtsZ (pseudocolored green) and 4′,6-diamidino-2-phenylindole (DAPI) staining of nucleoids (pseudocolored red) in Frag-1 cells harboring an empty vector (C–H) or pCTC-MSL2 (I–N). Immunofluorescence and DAPI staining were performed as described previously.[8] All cells were treated with 0.1 mM IPTG for 20 min; + signs indicate cells that were further treated with 10 mM cephalexin for 1 h prior to fixing. White and purple arrowheads indicate single and double or polar FtsZ rings, respectively. Size bar = 10 μm.

parsimonious explanation for the observed proliferation of FtsZ rings in response to heterologous expression of MSL2 is that MSL2 prevents the function of endogenous MS channels or specific components of the *E. coli* Min system. An attractive hypothesis is that MSL2 inhibits one or more of its six *E. coli* homologs[15] through the formation of defective heteromeric channels in the membrane or by sequestration away from the membrane. However, this model does not explain the observed defect in septation, and we cannot rule out the possibility that the entire effect of MSL2 expression is nonspecific, as filamentation and alterations in FtsZ levels are frequently observed in bacteria in response to stress.[16,17] Future potentially informative experiments include examining cell morphology and Z-ring production in response to MSL2 expression in an *E. coli* strain lacking all seven endogenous MS channels, and in response to overexpression of these endogenous channels.

## How do MS Channels Influence Z-ring Placement in Chloroplasts and Bacteria?

MS channels are excellent candidates to convey information about osmotic stress, plastid size and shape, or plastid crowding to division machinery,[18] but the molecular mechanism by which they might do so has yet to be determined. Below we discuss two general models for the interaction between MS channels and known regulators of Z-ring placement in *E. coli* cells and Arabidopsis chloroplasts. These models are also diagrammed in **Figure 2**, using MSC and MinD to representative MS channels and plastid division proteins, respectively.

**(1) MS channels interact directly with FtsZ or its regulators.** MSL2 and MSL3 co-localize with MinE to distinct foci on the inner membrane of chloroplasts and their soluble C-terminal domains are predicted to localize to the stroma,[10,19] where they could stabilize or localize membrane-bound components of the division machinery through direct protein-protein interactions (**Fig. 2**, **Model 1**). FtsZ, MinD, MinE, ARC3, MCD1, ARC6 and PARC6 all show peripheral or integral membrane association and could be influenced by MSL2 or MSL3.[4,6,7] However, both yeast two-hybrid and co-immunoprecipitation assays have

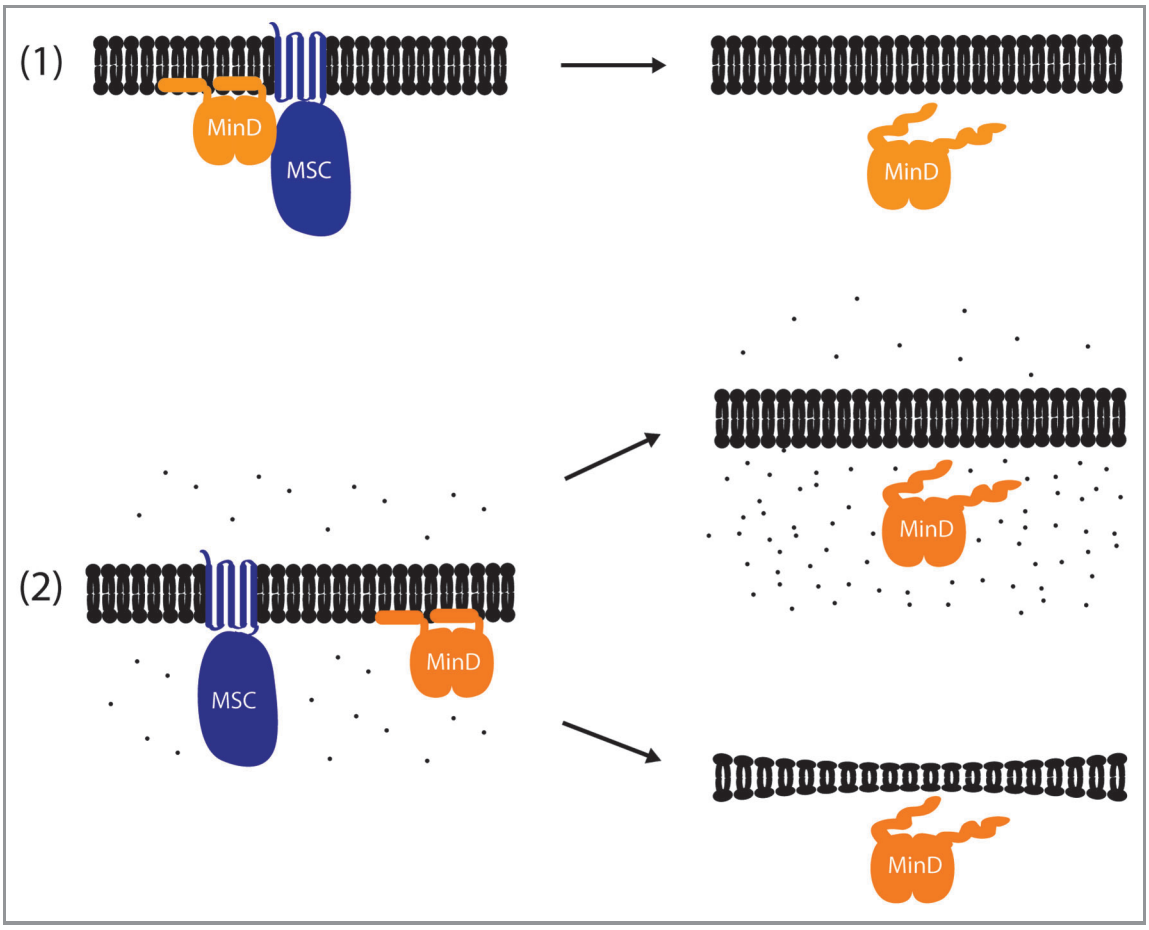


**Figure 2.** Mechanisms by which MS channels may influence chloroplast or *E. coli* cell division. The possible effects of mechanosensitive channel (MSC) activity (represented in blue, using the predicted topology of Arabidopsis MSL2) on the membrane association and/or function of components of the division machinery (MinD is used as an example and is represented in orange). In Model 1, MSCs directly interact with MinD, stabilizing its association with the membrane and allowing it to function in the inhibition of FtsZ ring assembly. In the absence of the MSC, MinD is unable to properly associate with the membrane and FtsZ assembly is no longer appropriately inhibited. In Model 2, MSCs are required to maintain chloroplast ion homeostasis or to relieve membrane tension in the inner plastid envelope for normal MinD function. Top, in the absence of the MSC, $Ca^{2+}$ or $Mg^{2+}$ ions accumulate in the plastid stroma, enhancing the ATPase activity of MinD and leading to its continual membrane disassociation. Bottom, increased membrane tension alters the biophysical properties of the membrane, preventing MinD from inserting its amphipathic helix into the lipid bilayer.

failed to support a direct interaction between MSLs and any of these proteins (unpublished data).

(2) **MS channels indirectly influence Z-ring assembly by altering ion homeostasis or membrane tension.** As we have been unable to obtain evidence for direct interactions between MSL2, MSL3 and the Arabidopsis Min system, we instead favor an alternate model (**Fig. 2**, **Model 2**). Both membrane tension and ion homeostasis are likely altered in a bacterium or plastid lacking MS ion channels, potentially disrupting the membrane localization and therefore the function of division machinery components indirectly.

One component that might be altered by ion homeostasis is MinD. *E. coli* MinD is a membrane $Mg^{2+}$-dependent ATPase; MinD dimers bind the membrane via their C-terminal amphipathic helices.[20,21] ATP-bound MinD recruits MinC to the membrane where the MinC/D complex acts to inhibit FtsZ ring assembly. Upon ATP hydrolysis, MinD loses its membrane association.[3,5] How Arabidopsis MinD affects Z-ring assembly is not clear, though it has been established that its ATPase activity is stimulated by $Ca^{2+}$ and MinE.[22] Thus, the accumulation of high levels of cations in the absence of MS channels could favor the dissociation of MinD from bacterial or chloroplast membranes. Altered membrane potential has also been recently shown to disrupt the localization of a number of *E. coli* proteins including MinD.[23]

Alternatively, rather than releasing ions, MS channels might be required to release membrane tension in the chloroplast or bacterial envelope for proper Z-ring placement. Membrane tension affects both the physical and electrical properties of the membrane and of the proteins embedded within it.[24] For example, tension-induced changes in lipid spacing and bilayer thickness could interfere with the ability of the amphipathic helixes of MinD[21] to interact with membrane phospholipids. These indirect models are difficult to test, but measuring chloroplastidic ion concentrations in *msl2 msl3* mutants via ICP-MS[25] may provide some additional insight.

In conclusion, many intriguing mechanisms can be envisioned by which MS channels may impact Z-ring assembly during chloroplast and bacterial division, and of course, the mechanism used does not need to be the same for both chloroplasts and bacteria. We anticipate that future research into the specific effects of MS channels on ion homeostasis and membrane tension and the impact of these parameters on components of the Min system will identify the precise mechanism by which MS channel influence Z-ring assembly in both chloroplasts and bacteria.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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