

Washington University in St. Louis Washington University Open Scholarship

Biology Faculty Publications & Presentations

Biology

2012

A role for mechanosensitive channels in chloroplast and bacterial fission.

Margaret E. Wilson

Washington University in St Louis

Elizabeth S. Haswell

Washington University in St Louis, ehaswell@wustl.edu

Follow this and additional works at: https://openscholarship.wustl.edu/bio_facpubs

 Part of the [Biology Commons](#)

Recommended Citation

Wilson, Margaret E. and Haswell, Elizabeth S., "A role for mechanosensitive channels in chloroplast and bacterial fission." (2012).

Biology Faculty Publications & Presentations. 10.

https://openscholarship.wustl.edu/bio_facpubs/10

This Article is brought to you for free and open access by the Biology at Washington University Open Scholarship. It has been accepted for inclusion in Biology Faculty Publications & Presentations by an authorized administrator of Washington University Open Scholarship. For more information, please contact digital@wumail.wustl.edu.

A role for mechanosensitive channels in chloroplast and bacterial fission

Margaret E. Wilson and Elizabeth S. Haswell*

Department of Biology; Washington University in Saint Louis; Saint Louis, MO USA

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-1¹² caused extreme cellular filamentation (Fig. 1A and B). The severity of filamentation varied greatly, with cells from the same sample ranging from 5 μ m to over 50 μ m 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, immunofluorescence microscopy using an anti-FtsZ antibody¹³ 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 cephalixin (compare Fig. 1F and I). The average distance between Z-rings in cells expressing MSL2 was 1.28 \pm 0.4 μ m, while in cephalixin-treated cells it was 6.03 \pm 1.6 μ m (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

*Correspondence to: Elizabeth S. Haswell; Email: ehaswell@wustl.edu
Submitted: 10/27/11; Accepted: 11/10/11
<http://dx.doi.org/10.4161/psb.18735>

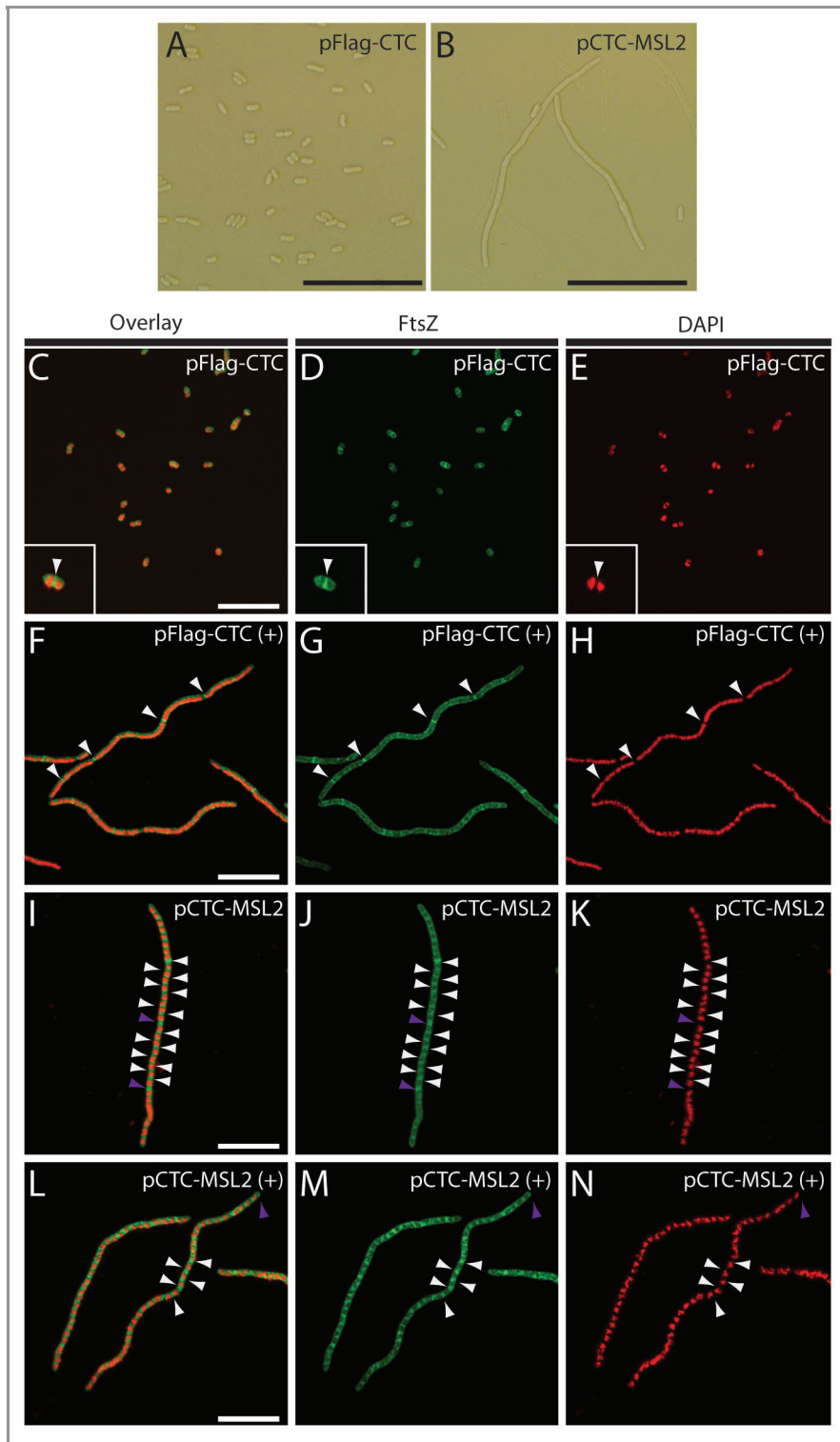


Figure 1. Heterologous expression of MSL2 in *E. coli* causes filamentation and disrupts normal Z-ring placement. (A and B). Light micrographs of Frag-1 cells harboring the indicated constructs after 1 h of induction in 0.1 mM IPTG. Cells were immobilized on agarose pads as previously described.²⁶ (C–N). 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 cephalixin for 1 h prior to fixing. White and purple arrowheads indicate single and double or polar FtsZ rings, respectively. Size bar = 10 μ m.

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* homologs¹⁵ 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 over-expression 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

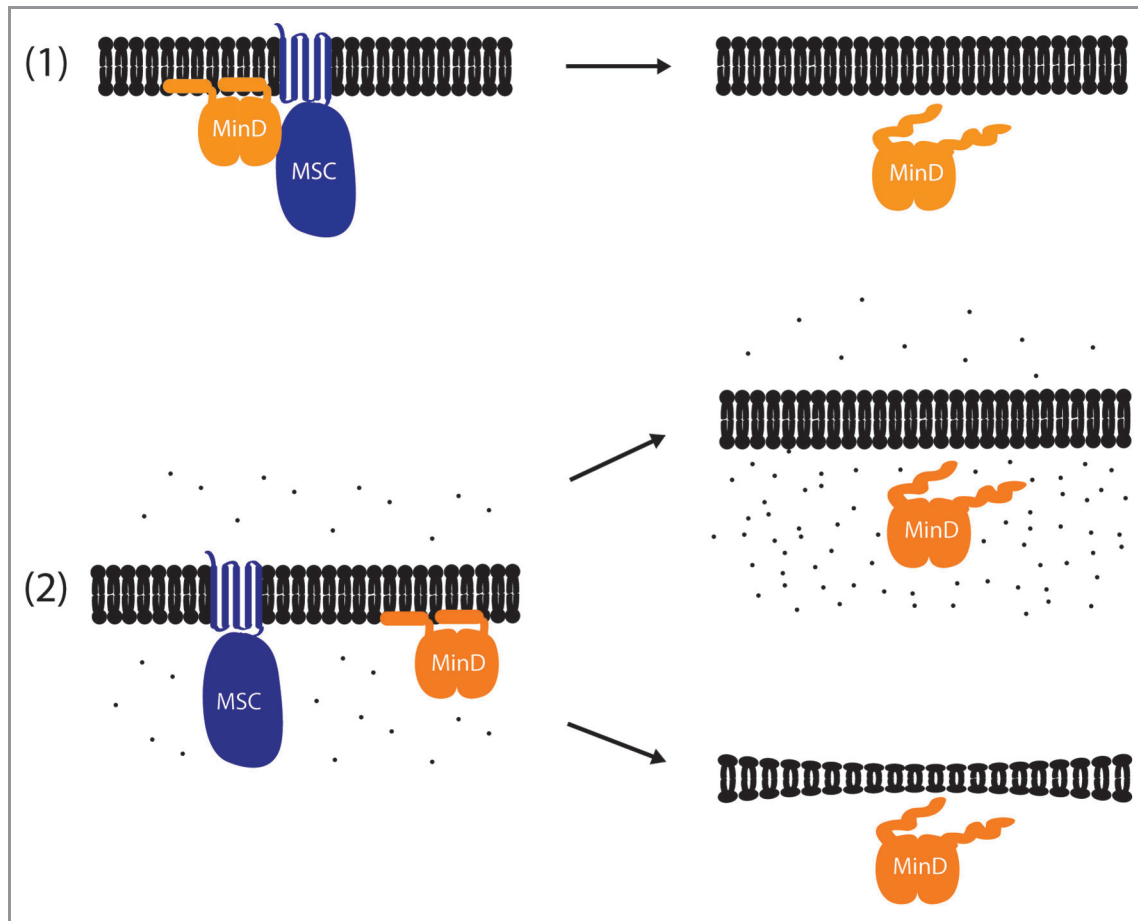


Figure 2. Mechanisms by which MS channels may influence chloroplast or *E. coli* cell division. The possible effects of mechanosensitive channel (MSC) activity (represented in blue, using the predicted topology of Arabidopsis MSL2) on the membrane association and/or function of components of the division 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 helices of MinD²¹ to interact with membrane phospholipids. These indirect models are difficult to test, but measuring chloroplastidic ion concentrations in *msl2 msl3* mutants via ICP-MS²⁵ 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.

Acknowledgments

We thank Gregory Jensen for his initial experiments on the expression of MSL2 in *E. coli* and Bill Margolin for insightful comments on these results. We also are grateful to David Weiss for the anti-FtsZ antibodies and Ian Booth for the MFJ465 and Frag-1 strains. This research was funded by NSF Award 0816627.

References

- Lopez-Juez E, Pyke KA. Plastids unleashed: their development and their integration in plant development. *Int J Dev Biol* 2005; 49:557-77; PMID:16096965; <http://dx.doi.org/10.1387/ijdb.051997el>
- Glynn JM, Miyagishima SY, Yoder DW, Osteryoung KW, Vitha S. Chloroplast division. *Traffic* 2007; 8:451-61; PMID:17451550; <http://dx.doi.org/10.1111/j.1600-0854.2007.00545.x>
- de Boer PA. Advances in understanding *E. coli* cell fission. *Curr Opin Microbiol* 2010; 13:730-7; PMID:20943430; <http://dx.doi.org/10.1016/j.mib.2010.09.015>
- Miyagishima SY, Kabeya Y. Chloroplast division: squeezing the photosynthetic captive. *Curr Opin Microbiol* 2010; 13:738-46; PMID:21041111; <http://dx.doi.org/10.1016/j.mib.2010.10.004>
- Lutkenhaus J. Assembly dynamics of the bacterial MinCDE system and spatial regulation of the Z ring. *Annu Rev Biochem* 2007; 76:539-62; PMID:17328675; <http://dx.doi.org/10.1146/annurev.biochem.75.103004.142652>
- Yang Y, Glynn JM, Olson BJ, Schmitz AJ, Osteryoung KW. Plastid division: across time and space. *Curr Opin Plant Biol* 2008; 11:577-84; PMID:18990608; <http://dx.doi.org/10.1016/j.pbi.2008.10.001>
- Maple J, Møller SG. The complexity and evolution of the plastid-division machinery. *Biochem Soc Trans* 2010; 38:783-8; PMID:20491665; <http://dx.doi.org/10.1042/BST0380783>
- Wilson ME, Jensen GS, Haswell ES. Two mechanosensitive channel homologs influence division ring placement in *Arabidopsis* chloroplasts. *Plant Cell* 2011; 23:2939-49; PMID:21810996; <http://dx.doi.org/10.1105/tpc.111.088112>
- Pivetti CD, Yen MR, Miller S, Busch W, Tseng YH, Booth IR, et al. Two families of mechanosensitive channel proteins. *Microbiol Mol Biol Rev* 2003; 67:66-85; PMID:12626684; <http://dx.doi.org/10.1128/MMBR.67.1.66-85.2003>
- Haswell ES, Meyerowitz EM. MscS-like proteins control plastid size and shape in *Arabidopsis thaliana*. *Curr Biol* 2006; 16:1-11; PMID:16401419; <http://dx.doi.org/10.1016/j.cub.2005.11.044>
- Levina N, Töttemeyer 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; 18:1730-7; PMID:10202137; <http://dx.doi.org/10.1093/emboj/18.7.1730>
- Epstein W, Kim BS. Potassium transport loci in *Escherichia coli* K-12. *J Bacteriol* 1971; 108:639-44; PMID:4942756
- Mercer KL, Weiss DS. The *Escherichia coli* cell division protein FtsW is required to recruit its cognate transpeptidase, FtsI (PBP3), to the division site. *J Bacteriol* 2002; 184:904-12; PMID:11807049; <http://dx.doi.org/10.1128/jb.184.4.904-912.2002>
- Yu XC, Margolin W. FtsZ ring clusters in min and partition mutants: role of both the Min system and the nucleoid in regulating FtsZ ring localization. *Mol Microbiol* 1999; 32:315-26; PMID:10231488; <http://dx.doi.org/10.1046/j.1365-2958.1999.01351.x>
- Booth IR, Edwards MD, Miller S, Li C, Black S, Bartlett W, et al. Structure-function relations of MscS. In: OP H, ed. *Curr Top Membr*, 2007:269-94.
- Justice SS, Hunstad DA, Cegelski L, Hultgren SJ. Morphological plasticity as a bacterial survival strategy. *Nat Rev Microbiol* 2008; 6:162-8; PMID:18157153; <http://dx.doi.org/10.1038/nrmicro1820>
- Mattick KL, Rowbury RJ, Humphrey TJ. Morphological changes to *Escherichia coli* O157:H7, commensal *E. coli* and *Salmonella* spp in response to marginal growth conditions, with special reference to mildly stressing temperatures. *Sci Prog* 2003; 86:103-13; PMID:12838606; <http://dx.doi.org/10.3184/003685003783238725>
- Pyke K. Plastid division: the squeezing gets tense. *Curr Biol* 2006; 16:R60-2; PMID:16431362; <http://dx.doi.org/10.1016/j.cub.2006.01.003>
- Schwacke R, Schneider A, van der Graaff E, Fischer K, Catoni E, Desimone M, et al. ARAMEMNON, a novel database for *Arabidopsis* integral membrane proteins. *Plant Physiol* 2003; 131:16-26; PMID:12529511; <http://dx.doi.org/10.1104/pp.011577>
- de Boer PA, Crossley RE, Hand AR, Rothfield LI. The MinD protein is a membrane ATPase required for the correct placement of the *Escherichia coli* division site. *EMBO J* 1991; 10:4371-80; PMID:1836760
- Szeto TH, Rowland SL, Rothfield LI, King GF. Membrane localization of MinD is mediated by a C-terminal motif that is conserved across eubacteria, archaea, and chloroplasts. *Proc Natl Acad Sci U S A* 2002; 99:15693-8; PMID:12424340; <http://dx.doi.org/10.1073/pnas.232590599>
- Aldridge C, Møller SG. The plastid division protein AtMinD1 is a Ca²⁺-ATPase stimulated by AtMinE1. *J Biol Chem* 2005; 280:31673-8; PMID:16014621; <http://dx.doi.org/10.1074/jbc.M505126200>
- Strahl H, Hamoen LW. Membrane potential is important for bacterial cell division. *Proc Natl Acad Sci U S A* 2010; 107:12281-6; PMID:20566861; <http://dx.doi.org/10.1073/pnas.1005485107>
- Phillips R, Ursell T, Wiggins P, Sens P. Emerging roles for lipids in shaping membrane-protein function. *Nature* 2009; 459:379-85; PMID:19458714; <http://dx.doi.org/10.1038/nature08147>
- Jeong J, Cohu C, Kerke L, Pilon M, Connolly EL, Guerinot ML. Chloroplast Fe(III) chelate reductase activity is essential for seedling viability under iron limiting conditions. *Proc Natl Acad Sci U S A* 2008; 105:10619-24; PMID:18647837; <http://dx.doi.org/10.1073/pnas.0708367105>
- Levin PA. Light microscopy techniques for bacterial cell biology. *Method Microbiol* 2002; 31:115-32; [http://dx.doi.org/10.1016/S0580-9517\(02\)31007-9](http://dx.doi.org/10.1016/S0580-9517(02)31007-9)