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COMMENTARY

FtsN—Trigger for Septation[▽]

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The bacterial cell is encased in peptidoglycan, which maintains the shape of the cell and protects it from bursting due to the turgor pressure (10).(p. 7383–7401) Growth and cell division require enlargement of this layer, a delicate process, since incorporation of new material involves the hydrolysis of bonds that maintain the integrity of this layer. This is especially true during cell division, when a cross-wall is formed and eventually split in the production of progeny cells.

At one level, cell division in bacteria is rather simple—formation of a Z ring at midcell, recruitment of a full complement of the essential division proteins to form the divisome, and septation (9). Of course, this simple overview leaves many fascinating details to be worked out, especially in the last step. Among these are how the activities of cell wall synthetases and hydrolases are coordinated, what triggers septation, and what the biochemical activities of the 10 essential division proteins in *Escherichia coli* are. This is particularly true for FtsN, the last essential division protein that localizes to the divisome. First, the FtsN sequence is poorly conserved, so it is difficult to determine whether it is even present in bacteria other than enterics. Second, there is evidence that it interacts with many other components of the division machinery, including FtsA, FtsI, FtsQ, and FtsW, and with the peptidoglycan synthetases PBP1b and MgtA (6, 11, 14). Third, it is required for the recruitment of a host of nonessential proteins whose numbers continue to grow, proteins involved in splitting the septum and invaginating the outer membrane (2, 3, 8). How does it do this? What is its essential function? How does it localize to the division site? The work from de Boer's lab complemented by work from other labs answers some of these questions and raises the possibility that FtsN is the trigger to start septation (7). Furthermore, the work reveals a new mechanism for recruitment of proteins to the septum.

FtsN was isolated as a multicopy suppressor of a temperature-sensitive mutation in *ftsA* and subsequently shown to suppress a number of temperature-sensitive mutations in other cell division genes, although not one in *ftsZ* (4). FtsN is a bitopic protein with a short cytoplasmic tail, a transmembrane domain, and a periplasmic domain that can be subdivided into three regions: a membrane-proximal region containing three short helices, a C-terminal SPOR domain, and a glutamine-rich linker (5, 17). The only region that is conserved is the SPOR domain, which binds to peptidoglycan and has homology to domains present in some cell wall hydrolases (12). Deletion analysis showed that this domain is not essential (15), although de Boer's lab shows that in its absence septation is slowed down. Furthermore, earlier studies showed that the cytoplasmic tail and transmembrane domain are not essential (1). Thus, the essential region of FtsN is in the periplasm, but it is not the conserved SPOR domain.

de Boer's lab became interested in FtsN when it showed up in a screen for transposon insertions that were synthetically sick with deletion of the *min* locus (7). This screen had already yielded several genes encoding proteins involved in division or its regulation (EnvC, Tol-Pal, and SlmA) (2, 3, 8). The insertion occurred at codon 119, which, in combination with earlier results, indicated that the essential region of FtsN occurred somewhere between the end of the transmembrane domain (amino acid 54) and 119. The essential region was narrowed down to a small region of the periplasmic domain (corresponding to the second helix) which functions even when exported on its own to the periplasm. In this case, it had to be overexpressed, presumably to make up for a lack of a membrane tether that would concentrate it at the membrane. However, it did not localize to the septum—the essential activity and the ability to localize to the septum are separable.

How does FtsN localize to the septum? This was answered by exporting a green fluorescent protein-tagged version of the SPOR domain to the periplasm. It localized sharply to the site of septation but was not present at the newly formed poles, indicating that localization was transient. Localization of the SPOR domain depended upon coexpression of the essential domain of FtsN, the activity of FtsI (also called PBP3), and the presence of at least one of the three known amidases. This indicated that the SPOR domain does not localize until constriction is initiated and suggested that the SPOR domain binds to glycan chains that have their peptide side chains removed (i.e., that are denuded) by the activity of an amidase. This fits nicely with the earlier results from Vollmer's lab, who found that the SPOR domain of FtsN bound preferentially to peptidoglycan treated with an amidase (15). They also observed that the SPOR domain binds preferentially to longer glycan chains, which is consistent with cooperative binding. If so, then denuded glycan chains are transiently present during septation and are bound cooperatively by the SPOR domain.

This use of a SPOR domain to localize to the septum is not unique to FtsN. The SPOR domain of the *B. subtilis* CwlC protein also localizes nicely to the septum in *E. coli*, indicating

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that this mechanism is well conserved. CwlC also binds to denuded glycan chains (15). Three other proteins in *E. coli* also have a SPOR domain (DamX, DedD, and RlpA), and in each case it is sufficient to localize to the septum. At least two of these, although not essential, contribute to the process of septation and join the growing list of proteins that are recruited to the septum, probably in an FtsN-dependent fashion. There is some variation in the SPOR domains; for example, the one from CwlC appears to have a higher affinity for the septal target than that of FtsN. Why FtsN is needed brings us back to the essential function of FtsN.

The essential function of FtsN is required for the localization of FtsN′s SPOR domain. How it does this is unknown but likely involves stimulation of the constriction process possibly by acting on FtsI. Several residues in FtsI that are required for FtsN localization have been identified (16). They lie in the periplasmic, membrane-proximal region of FtsI, raising the possibility that they contact the essential region of FtsN, leading to FtsI activation. This in turn leads to the presence of denuded glycan chains, which recruit more FtsN through its SPOR domain, resulting in a self-enhancing process triggering septation. In this model, it is not clear how the initial denuded glycan chains appear (FtsN is required for recruitment of AmiC), but it may be due to delocalized amidase activity, which is further enhanced by the incoming FtsN. Finally, although FtsN is poorly conserved at the sequence level, a protein with similar properties was recently isolated from *Caulobacter crescentus*, suggesting that FtsN is likely to be present in at least most gram-negative bacteria (13).

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