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NOTES

Role for the Nonessential N Terminus of FtsN in Divisome Assembly▽

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FtsN, the last essential protein in the cell division localization hierarchy in *Escherichia coli*, has several peculiar characteristics, suggesting that it has a unique role in the division process despite the fact that it is conserved in only a subset of bacteria. In addition to suppressing temperature-sensitive mutations in *ftsA*, *ftsK*, *ftsQ*, and *ftsI*, overexpression of FtsN can compensate for a complete lack of FtsK in the cell. We examined the requirements for this phenomenon. We found that the N-terminal terminal region (cytoplasmic and transmembrane domains) is critical for suppression, while the C-terminal murein-binding domain is dispensable. Our results further suggest that FtsN and FtsK act cooperatively to stabilize the divisome.

Cell division in *Escherichia coli* requires the concerted action of at least 10 essential proteins at midcell. FtsZ and the FtsZ-binding proteins FtsA and ZipA assemble at midcell to form the Z-ring. The Z-ring then serves as a scaffold for assembly of the remaining late proteins, which occurs according to a linear hierarchy (FtsK → FtsQ → FtsL/B → FtsW → FtsI → FtsN). One gene, *ftsN*, was found to be a multicopy suppressor of a temperature-sensitive mutation in *ftsA*. Surprisingly, it could also act as a multicopy suppressor of a variety of other cell division defects, including temperature-sensitive alleles of *ftsQ* and *ftsI* (3). Overexpression of FtsN was even able to rescue cells in which *ftsK* was deleted (6).

FtsN requires multiple contacts with multiple upstream proteins in order to localize to midcell, whereas the other late division proteins associate in a simple fashion with proteins immediately upstream of them in the hierarchy (8, 9). For example, FtsI, the protein immediately upstream of FtsN, localizes normally in the absence of FtsA or FtsQ provided that FtsW is targeted to midcell using a technique known as premature targeting. In contrast, FtsN fails to localize in cells missing FtsA or FtsQ, even if all other upstream proteins are present at midcell.

We revisited the ability of FtsN overexpression to rescue the growth of cells that have lost the essential cell division protein FtsK. We reasoned that an understanding of the molecular mechanism behind this phenotype could shed light on the function of FtsN in cell division. In particular, we wanted to know (i) to what degree the functions of FtsK and FtsN are redundant, (ii) whether suppression restores relatively normal divisome assembly or allows cells to divide via a novel alternate pathway that does not require assembly of late divisomal proteins, and (iii) to what degree the nonessential domains of FtsN play a role in this process.

In the course of analyzing the localization of division proteins in cells in which FtsK is depleted, we noticed that cells expressing a green fluorescent protein (GFP)-FtsN fusion exhibited frequent midcell fluorescence and were significantly shorter than the cells of isogenic strains expressing other GFP fusions. The FtsK depletion strain JOE563 carries an *ftsK*::*cat*-Δ*5* null allele at the endogenous locus and is complemented from a spectinomycin-resistant (Spc^r), low-copy-number, arabinose-inducible pBAD42 plasmid (pJC85). Upon repression of the plasmid-borne copy of *ftsK* by growth on glucose, the FtsK protein is depleted to such an extent that the levels are undetectable by Western blotting (1). Under these conditions, GFP fusions to most downstream proteins that normally depend on FtsK (FtsK-dependent proteins), including FtsQ, FtsL, and FtsI, fail to localize and cells lose the ability to divide (2). However, we found that when a single copy of the GFP-FtsN fusion was integrated into the chromosome in an FtsK depletion strain (JOE702) and induced with 10 μM isopropyl-β-D-thiogalactopyranoside (IPTG), growth was rescued and GFP-FtsN localized efficiently to the midcell of suppressed cells (Table 1) (9).

We wondered whether under suppressing conditions division occurred independent of the localization of FtsK-dependent division proteins that are normally required for FtsN′s localization. We examined the localization of two such proteins, FtsL and FtsQ, both of which fail to localize in cells in which FtsK is depleted (2). Upon overexpression of FtsN from pDSW204 in such strains (15), localization of FtsL and FtsQ to potential division sites was largely restored (Fig. 1C and D). Although suppression was not complete (the cells were somewhat longer and occasionally branched), the majority of cells (>50%) contained a fluorescent band at midcell.

This finding is consistent with prior work on FtsK suppression by pZAQ, a multicopy plasmid that increases the levels of FtsZ, FtsA, and FtsQ in the cell. Interestingly, suppression by pZAQ resulted in the localization of FtsI, indicating that division in this case was also not independent of the localization of FtsK-dependent division proteins (7). Thus, regardless of whether suppression of Δ*ftsK* cells is provided by overexpres-

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TABLE 1. GFP-FtsN suppression

Construct[a]	Mutant background	Temp (°C)	Growth on:		
			Ara	Glc	Glc + IPTG
GFP-FtsN	pBAD-*ftsK*[b]	37	+++[e]	++	+++
GFP-FtsN	Δ*ftsK*[c]	37	−	−	+
GFP-FtsL	pBAD-*ftsK*	37	+++	−	−
GFP-FtsN	pBAD-*ftsK*	42	+++	−	−
GFP-FtsN	pBAD-*ftsN*[d]	42	+++	+++	+++

[a] All GFP constructs were expressed from a single-copy pDSW204 promoter.
[b] The FtsK depletion strain was Δ*ftsK*::*cat*-Δ*5* complemented by pJC85 (pBAD42-*ftsK*).
[c] Δ*ftsK*::*cat*-Δ*5* with no complementing plasmid.
[d] FtsN depletion strain.
[e] +++, wild-type growth; ++, slightly wrinkled colonies; +, poor growth and wrinkled colonies; −, no growth.

sion of proteins upstream (FtsZ, FtsA) or downstream (FtsQ, FtsN) of FtsK in the localization hierarchy, localization of FtsK-dependent proteins is restored.

Given the multiple mechanisms by which FtsK can be suppressed and the fact that none of the proteins shares a similar sequence or membrane topology with FtsK, it is unlikely that overexpression suppression is due to complementation of a specific activity that FtsK$^-$ cells lack. It has been speculated that the primary defect could be destabilization of the divisome, resulting in a failure to recruit downstream division proteins (7). FtsN may counteract this effect, stabilizing assembly via weak affinities to multiple division proteins. Such a model is consistent with recent bacterial two-hybrid studies which revealed potential interactions between FtsN and FtsA, FtsQ, FtsW, and FtsI (5, 12). As one measure of this stabilization activity, we tested the ability of FtsN to suppress at high temperatures, as higher temperatures might additionally destabilize the divisome. Strikingly, despite the fact that GFP-FtsN complemented a strain in which FtsN was depleted and localized normally at 42°C, overexpression of GFP-FtsN was unable to restore viability to a strain in which FtsK was depleted at the same temperature (Table 1). Consistent with a loss of divisome stability, both FtsL and FtsQ did not localize under these conditions, and cells were unable to divide (Fig. 1E and F).

Previous studies have reported rescue of cells lacking FtsK by FtsN overexpression. However, the resulting cells grow poorly, form filaments, and show a loss of viability (6, 7). We considered the possibility that the growth defects seen in these studies compared to the relatively efficient suppression in our analysis might have been due to residual FtsK expression from the repressed pBAD42 (Spc^r) plasmid pJC85. We examined whether an FtsK depletion strain expressing GFP-FtsN (JOE702) could lose the FtsK complementing plasmid when it was transformed with incompatible empty plasmid pBAD42 (Kan^r) and plated under GFP-FtsN overexpression conditions (with IPTG). When we plated JOE702 cells transformed with pBAD42(Kan^r) on NZ arabinose plus IPTG, we found two colony types. Ninety percent of the colonies had wild-type morphology, and all of the colonies tested had not lost the complementing plasmid, pJC85 (all 24 colonies were Spc^r). However, 10% of the colonies were extensively wrinkled (Fig. 2A). Cells in these colonies had lost pJC85 (none of 23 colonies were Spc^r) and required IPTG (GFP-FtsN expression) for growth, regardless of the sugar present (Table 1). In contrast, when we used a control strain expressing GFP-FtsL (JOE701), none of the >200 colonies examined were wrinkled, pJC85 was maintained in all isolates tested (all 24 isolates were Spc^r), and arabinose was required for growth (Table 1). PCR analysis confirmed that *ftsK* was not present in JOE702-derived cells that had lost the complementing plasmid and that the locus containing the *ftsK*::*cat*-Δ*5* allele was intact (Fig. 2B). Inspection of cells in the colonies revealed that they were highly filamentous, and the cells grew very poorly in liquid media, consistent with previous studies of FtsN multicopy suppression (data not shown). Thus, FtsN overexpression can rescue the viability of a strain completely lacking FtsK. However, this suppression is not sufficient to provide for normal division.

Comparison of the suppression phenotype in strains in which FtsK was depleted to the phenotype of strains in which *ftsK* was deleted also indicated that minute quantities of FtsK in the cell (due to incomplete repression of the pBAD promoter) potentiated the ability of FtsN to rescue growth. Such synergy suggests that these proteins do not act redundantly at midcell. Rather, it seems likely that the two proteins contribute to divisome stability via distinct and potentially cooperative mechanisms.

Neither the N-terminal domain comprising the cytoplasmic tail and transmembrane (N_{cytoTM}) domain nor the C-terminal murein-binding domain of FtsN (N_{MB}) is essential for growth (4, 14). We asked whether either domain is required for rescue of *ftsK* cells. We were particularly interested in the idea that the murein-binding element allows FtsN to bind to septal murein. Once at midcell, FtsN would be in a position to back-recruit division proteins.

In addition to full-length FtsN, we cloned two constructs:

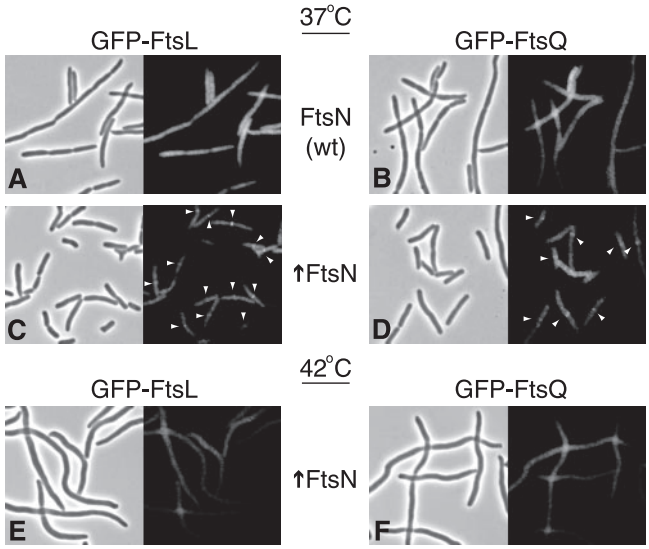

FIG. 1. FtsN overexpression restores localization of FtsL and FtsQ in cells in which FtsK is depleted at 37°C but not at 42°C. FtsK was depleted in isogenic FtsN-overexpressing cells (↑FtsN) and control cells (wt) at 37°C or 42°C for 3 to 5 h until control samples showed filamentation. Localization of GFP fusions to FtsQ and FtsL (expressed from λ*att*) is shown along with corresponding phase-contrast images. Expression of FtsN was from pDSW204.

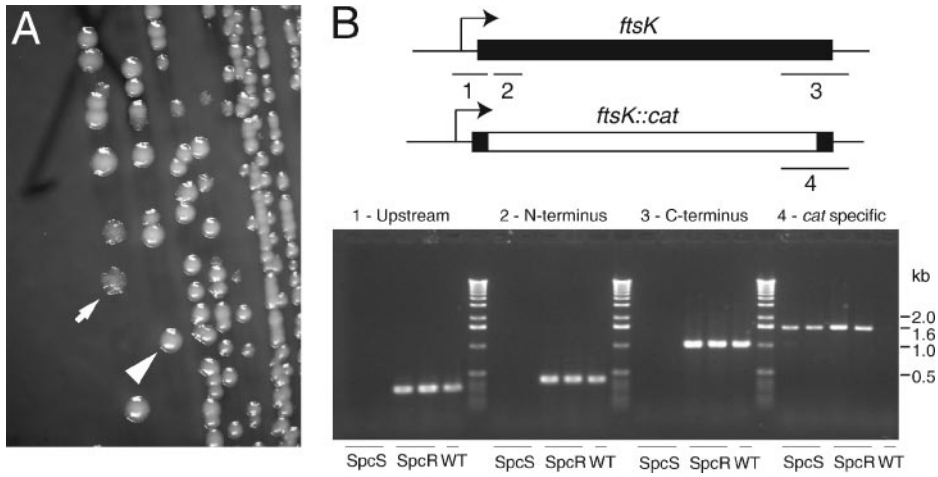

FIG. 2. Complete loss of FtsK under FtsN overexpression conditions results in a cell division defect. (A) When JOE702 (Δ*ftsK* Δλ*att*::P_{207}-*ftsN*/pBAD42-*ftsK*) was transformed with an incompatible Kan^r plasmid, grown in the presence of 0.2% arabinose, 10 μM IPTG, and 40 μg/ml kanamycin, and plated on the same medium, a mixture of wrinkled and smooth colonies was obtained. The arrow indicates a wrinkled colony. The arrowhead indicates a smooth colony. (B) PCR analysis of wrinkled (Spc^s) colonies, which lost the pBAD42-*ftsK*(Spc^r) complementing plasmid, failed to show products with primer pairs specific for *ftsK* (products 1, 2, and 3), consistent with plasmid loss, while yielding the expected ~1.6-kb fragment for the *ftsK*::*cat*-Δ5 allele (product 4). Smooth (Spc^r) colonies which maintained the complementing plasmid contained both the *ftsK*::*cat*-Δ*5* allele and the plasmid-encoded *ftsK* gene. PCR analysis of wild-type cells yielded products consistent with the presence of *ftsK* but not with the presence of the *ftsK*::*cat*-Δ5 allele. The results for two independent colonies of each colony type are shown along with the results for a single wild-type (WT) control. PCR products are mapped on the corresponding alleles (*ftsK* or *ftsK*::*cat*-Δ*5*).

$FtsN_{241}$, in which N_{MB} was removed, and FFN, in which N_{cytoTM} was swapped with the first 39 amino acids of MalF (which comprise the first cytoplasmic and transmembrane domains). When expressed from pDSW204 (15), all three constructs readily complemented an FtsN depletion strain at uninduced levels, consistent with prior reports of the dispensability of both the N- and C-terminal domains (Table 2).

TABLE 2. FtsN swap suppression

Construct[a]	Mutant background[b]	Temp (°C)	Complementation with IPTG at a concn of [c]:				Notes[d]
			1 μM	10 μM	100 μM	1,000 μM	
FtsN	pBAD-*ftsN*	37	+++	+++	+++	+/−	
FFN	pBAD-*ftsN*	37	+++	+++	+++	ND[e]	
$FtsN_{241}$	pBAD-*ftsN*	37	+++	+++	+++	ND	
FtsN	Δ*ftsK*	37	+	+	−	−	P1
FFN	Δ*ftsK*	37	−	−	−	−	P1
$FtsN_{241}$	Δ*ftsK*	37	+	+	−	−	P1
FtsN	pBAD-*ftsK*	37	++	++	−	−	
FFN	pBAD-*ftsK*	37	−	−	−	−	
$FtsN_{241}$	pBAD-*ftsK*	37	++	++	−	−	
FtsN	*ftsQ1*(Ts)	42	+	+/−	−	−	
FFN	*ftsQ1*(Ts)	42	−	−	−	−	
$FtsN_{241}$	*ftsQ1*(Ts)	42	+/−	−	−	−	

[a] Constructs were expressed with a C-terminal 3×Myc epitope from the multicopy pDSW204 plasmid.
[b] Same mutant backgrounds as those in Table 1.
[c] +++, wild-type growth; ++, slightly wrinkled colonies; + and +/−, very poor growth and small wrinkled colonies (+ indicates that there was efficient recovery of transductants); −, no growth.
[d] P1, tested by P1 transduction.
[e] ND, not done.

Surprisingly, while $FtsN_{241}$ suppressed an FtsK depletion strain and allowed us to obtain uncomplemented *ftsK*::*cat*-Δ*5* transductants, FFN was unable to suppress (Table 2). We worried that the failure to suppress could have been due to reduced levels of FFN. Indeed, Western blot analysis revealed that at equivalent IPTG levels, the FFN construct produced

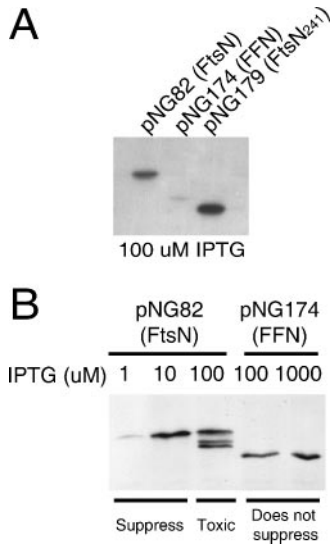

FIG. 3. Western blot of FtsN swap constructs. In panel A, FtsN, FFN, and $FtsN_{241}$ were induced with 100 μM IPTG. In panel B, FtsN and FFN were induced with various levels of IPTG, as indicated above the lanes. In all cases, equal cell volumes were loaded and proteins were detected by their C-terminal 3×Myc tags using polyclonal anti C-myc antibody (Sigma). "Suppress" and "Does not suppress" refer to the abilities of the constructs to suppress a FtsK depletion strain at the levels of IPTG indicated above the lanes.

significantly lower levels of protein than both the wild-type FtsN construct and $FtsN_{241}$ produced (Fig. 3A). However, FFN was unable to suppress even at higher IPTG levels (100 and 1,000 μM) despite the fact that it was produced at levels equivalent to or greater than the levels of the wild-type FtsN construct required for suppression (1 to 10 μM IPTG) (Table 2 and Fig. 3B). Thus, we concluded that N_{cytoTM}, which does not show obvious conservation, is essential for suppression of an FtsK depletion strain, while the relatively conserved C-terminal murein-binding domain (N_{MB}) is dispensable.

We also asked whether N_{cytoTM} is required for FtsN overexpression rescue of other division defects as well. In fact, we found that N_{cytoTM} is also necessary to suppress the *ftsQ1*(Ts) allele as FFN failed to rescue at all levels of induction. Similar to the case with the FtsK depletion strain and in contrast to FFN, $FtsN_{241}$ was also able to suppress *ftsQ1*(Ts). However, in this case, the suppression was somewhat less than that seen with full-length FtsN (Table 2). Thus, N_{cytoTM}, previously postulated to be important only for promoting translocation and anchoring of the periplasmic domain in the appropriate cellular compartment, turns out to play a general role in facilitating divisome assembly. In contrast, although we obtained some evidence that N_{MB} allows FtsN to suppress *ftsQ1*(Ts) more efficiently, if N_{MB} does in fact play a role in cell division, its contribution is likely to be minor or redundant.

Previous work on mutant cell division proteins has focused primarily on testing for the abilities of these proteins to complement null alleles. Such an approach has yielded significant insights into the essential domains of the division proteins, providing a framework for understanding protein function. This work, however, highlights the limitations of relying on complementation for growth and division as the only measure of *fts* protein function, particularly because our understanding of the ultimate functions of many of the division proteins is incomplete. Here, by assaying the role of FtsN with a nonstandard assay (suppression of the Δ*ftsK*::*cat*-Δ*5* allele) we were able to discover a novel role for a nonessential domain of FtsN.

This finding adds to the increasing number of observations of a specific role for the cytoplasmic and/or transmembrane segments of cell division proteins in divisome assembly. This role appears to be established even though, at least in the case of the bitopic membrane proteins (FtsQ, FtsL, FtsB, FtsI, and FtsN), there is no obvious conservation in these regions and in several cases (FtsQ and FtsN) these N-terminal regions have been shown to be "nonessential" for function in standard complementation assays (7, 10, 11, 13, 16). Together, these observations point to a model in which the N-terminal cytoplasmic and/or transmembrane domains of (most likely all) division proteins contribute to the interactions among divisomal components.

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