Involvement of an essential gene, mviN, in murein synthesis in Escherichia coli. †

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Running title: E.coli essential mviN
We have isolated a temperature-sensitive mutant of mviN, an essential gene in Escherichia coli. At the non-permissive temperature, mviN mutant cells swelled and burst. An intermediate in murein synthesis, polyprenyl diphosphate-N-acetylmuramic acid-(pentapeptide)–N-acetyl-glucosamine, accumulated in mutant cells. These results indicated that MviN is involved in murein synthesis.

**Keywords**: mviN / murein / Escherichia coli

The mviN gene was first identified in Salmonella typhimurium as a gene in a chromosomal region required for virulence in a mouse model of typhoid-like disease (1, 3). One of the putative virulence genes in this region was termed mviS for mouse virulence Salmonella. Subsequently, mviS was identified as fltA, a gene that encodes a sigma factor necessary for the expression of the fla operon, which includes 14 genes that are essential for flagella synthesis (2, 3, 5, 14). Thus, although the original terminology has been maintained, there is no direct evidence that mviN is involved in virulence, and its function has not been fully elucidated.

In our lab, we are carrying out a systematic construction of long-range chromosomal deletion mutants of E. coli (7, 12). Previously, we reported that the chromosomal region containing mviN is essential for cell growth. Viable mutants carrying a deletion of this chromosomal region were isolated only in the presence of a mini-F plasmid carrying the rimJ-yceH-mviM-mviN locus (12). To determine whether mviM and mviN are essential genes, we constructed mviM::Cm and mviN::Cm disruptants in the presence or absence of the complementing rimJ-yceH-mviM-mviN mini-F plasmid (see supplemental material for details). The deletion mutants were used as donors in P1 phage-mediated transduction, and the gene disruptions were introduced into strains that contained either the complementing mini-F plasmid, or vector control. Cm-resistant mviM deletion mutants were obtained in the presence or absence of the complementing mini-F plasmid. However, Cm-resistant mviN deletion mutants could only grow in the presence of the complementing plasmid, not the control empty vector. These results indicated that mviN, but not mviM, is essential for growth.
To further characterize the function of mviN, we isolated a temperature-sensitive (ts) mutant of mviN, termed mviN<sup>ts555</sup>, by plasmid shuffling, which is a kind of localized mutagenesis using a mini-F plasmid (9, 10, 11). The growth of the ts mutant at the non-permissive temperature was restored by pBAD-mviN, which confirmed that mviN<sup>ts555</sup> is an mviN mutant (see supplemental material for details). Sequence analysis of mviN<sup>ts555</sup> revealed a deletion in the mviN upstream region (basepairs 1,125,808 - 1,127,003 of the E. coli genome), which included the 3' region of yceH, mviM, and a predicted promoter of the mviN gene (the profiling of E. coli chromosome (PEC) database (http://shigen.lab.nig.ac.jp/ecoli/pecplus/index.jsp)). We did not find any mutations in the coding region of mviN, which suggests that the ts phenotype is due to decreased mviN expression (Fig. 1).

When we observed the mutant cells by microscopy after incubation at the non-permissive temperature, the cells appeared to be swollen, and many cells had burst into ghosts (Fig. 2 B). These results suggest a defect in the rigidity of the cell wall. To determine whether the defect involved impaired peptidoglycan synthesis, we introduced a multi-copy plasmid carrying the ispU (<i>rth</i>) gene, which encodes an undecaprenyl diphosphate synthase involved in peptidoglycan synthesis, into mviN ts mutants (10). Expression of <i>ispU</i>, but not in the presence of vector control, suppressed the growth defect of mviN<sup>ts555</sup> cells at 42°C, indicating that mviN is involved in peptidoglycan synthesis.

We next examined the level of intermediates in cell wall biosynthesis in mviN mutants. Recently, Guan et al. have reported that lipid intermediates in cell wall biosynthesis, polypropenyl diphosphate- <i>N</i>-acetylmuramic acid -(pentapeptide) –<i>N</i>-acetyl-glucosamine’s (mixture of decaprenyl and undecaprenyl derivatives), were extracted specifically into chloroform from an acidified mixture (6). We labeled mutant and wild type cells with [<sup>3</sup>H] diaminopimelic acid (DAP), which is incorporated predominantly into the cell wall (see supplemental material for details), and then measured the levels of radioactivity in various fractions. The incorporation of radioactive DAP into the lipid intermediates of mutant cells was markedly higher than in wild type cells (Table 1). The ratio of lipid intermediates to total incorporation was 4 times higher in mutant cells than in wild-type cells, while the ratio of radioactivity into the cell wall fraction was lower in mutant cells than in wild-type cells. We
next cultivated cells in the presence of $[^{14}\text{C}]$ isopentenyl diphosphate (IPP) to label the polyprenyl moiety of the lipid intermediates (see supplemental material for details). Because IPP is not incorporated into untreated growing cells, $[^{14}\text{C}]$IPP was added to lyophilized cells, and then the cells were re-cultivated. Radiolabeled polyprenyl phosphate derivatives were extracted with chloroform from neutral and acidified mixtures, and polyprenyl phosphates and polyprenyl diphosphates that were extracted from neutral mixtures were separated by ion-exchange chromatography (Table 2). The ratio of lipid intermediates to total radioactivity for all the polyprenyl phosphate derivatives was higher in mutant than in wild-type cells. Thus, based on the results of two separate labeling experiments, in which we labeled pentapeptide moieties by $[^{3}\text{H}]$DAP and polyprenyl moieties by $[^{14}\text{C}]$IPP, the lipid intermediates accumulated to higher levels in mutant cells than in wild-type cells. These results indicated that the mviN gene is involved in the metabolism of the lipid intermediates of the peptidoglycan synthesis.

What is the function of MviN in cells? We analyzed the putative amino acid sequence of MviN with Pfam and SOSUI, and found that the protein has 14 trans-membrane domains, which indicates that MviN is an integral membrane protein. The protein also appeared to be a member of the "MviN, MATE (multi Antimicrobial Extrusion) -like superfamily", which is comprised of integral membrane proteins. Members of the MATE family have been shown to function as drug/sodium antiporters (15). These proteins have also been shown to mediate resistance to a wide range of cationic dyes, fluoroquinolones, aminoglycosides and other structurally diverse antibodies and drugs (15). MATE proteins have also been implicated in the production of polysaccharides, such as RfbX (Wzx) (4) and WzxE (17), which have been implicated in E. coli O antigen biosynthesis, Bacillus subtilis SpoVB, which is involved in spore cortex biosynthesis (16, 19), and eukaryotic RFT1, which is required for the translocation of the dolichyl diphosphate-Man$_5$GlcNAc$_2$ intermediate, an oligosaccharide complex used in protein glycosylation, from the cytosolic side of the ER membrane to the lumen during the biosynthesis of dolichyl diphosphate-Glc$_3$Man$_9$GlcNAc$_2$ (8). These results suggest that MviN may be involved in the transmembrane transport of peptidoglycan precursors across the inner membrane. This transport process was recently reconstituted in
vitro (18), which will enable further characterization of the function of MviN. Alternatively, MviN may be indirectly involved in peptidoglycan synthesis. For example, it may play a role in folding or localization of other proteins involved in peptidoglycan synthesis because the precursors have been shown to accumulate by a wide variety of treatments that inhibit peptidoglycan synthesis or that block cell division (13). Further analyses are necessary to clarify its specific role.

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References


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FIG. 1 Inoue et al.
TABLE 1. Incorporation of $[^3]H$DAP into the cell wall and cell wall synthesis intermediates<sup>a</sup>

<table>
<thead>
<tr>
<th>mviN gene</th>
<th>Radioactivity (dpm) x 10&lt;sup&gt;-3&lt;/sup&gt; b</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WSI</td>
<td>LI</td>
</tr>
<tr>
<td>mviN&lt;sup&gt;f&lt;/sup&gt;</td>
<td>131 (13)</td>
<td>1.6 (0.1)</td>
</tr>
<tr>
<td>mviN&lt;sup&gt;ts555&lt;/sup&gt;</td>
<td>282 (15)</td>
<td>12 (4)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data represents the averages and standard deviations (in parentheses) of three independent assays.  
<sup>b</sup>WSI, water soluble intermediates; LI, lipid intermediates; CW, cell wall fraction.
TABLE 2. Incorporation of [14C]IPP into polyprenyl phosphate derivatives

<table>
<thead>
<tr>
<th>mviN gene</th>
<th>Radioactivity (dpm) x 10^{-3} b</th>
<th>Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP</td>
<td>PDP</td>
</tr>
<tr>
<td>mviN+</td>
<td>16.1 (1.5)</td>
<td>0.64 (0.02)</td>
</tr>
<tr>
<td>mviNts555</td>
<td>15.3 (2.4)</td>
<td>0.61 (0.17)</td>
</tr>
</tbody>
</table>

aData represents the averages and standard deviations (in parentheses) of three independent assays.  bPP, polyprenyl phosphates; PDP, polyprenyl diphosphates; LI, lipid intermediates.
**Figure legends**

**FIG. 1.** Sequence of the \( mviN^{ts555} \) ts mutation. \( mviN^{ts555} \) mutants carried a deletion that extended from the \( NruI \) site of \( yceH \) (nucleotide 429 of the open reading frame) to the upstream region of \( mviN \) (nucleotide –57 relative to the initiation codon at +1). Small letters indicate open reading frame sequences. The termination codon of \( yceH \) and the initiation codon of \( mviN \) are underlined. The underlined capital letters represent the promoter of \( mviN \).

**FIG. 2.** Cell morphology of \( mviN^{ts555} \). (A) wild-type \( mviN^+ \), strain MG1655 \( \Delta mviN::Sm^{recA::Tn10 / mini-F (ApR)} - mviN^+ \); (B) ts mutant \( mviN^{ts555} \), strain MG1655 \( \Delta mviN::Sm^{recA::Tn10 / mini-F (ApR)} - mviN^{ts555} \). Cells were incubated at 42°C for 2 hours and observed without fixation by phase contrast microscopy. Magnification, 100X objective. Arrows indicate ghosts.