Involvement of an Essential Gene, \textit{mviN}, in Murein Synthesis in \textit{Escherichia coli}†

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We isolated a temperature-sensitive mutant with a mutation in \textit{mviN}, an essential gene in \textit{Escherichia coli}. At the nonpermissive temperature, \textit{mviN} mutant cells swelled and burst. An intermediate in murein synthesis, polypropyl diphosphate–N-acetylmuramic acid–(pentapeptide)–N-acetyl-glucosamine, accumulated in mutant cells. These results indicated that MviN is involved in murein synthesis.

The \textit{mviN} gene was first identified in \textit{Salmonella enterica} serovar Typhimurium as a gene in a chromosomal region required for virulence in a mouse model of typhoidlike disease (1, 3). One of the putative virulence genes in this region was designated \textit{mviS} (mouse virulence \textit{Salmonella}). Subsequently, \textit{mviS} was identified as \textit{fliA}, a gene that encodes a sigma factor necessary for expression of the \textit{flg} operon, which includes 14 genes that are essential for flagellum synthesis (2, 3, 5, 14). Thus, although the original terminology has been maintained, there is no direct evidence that \textit{mviN} is involved in virulence, and its function has not been fully elucidated.

In our lab, we are carrying out systematic construction of \textit{Escherichia coli} long-range chromosomal deletion mutants (7, 12). Previously, we reported that the chromosomal region containing \textit{mviN} is essential for cell growth. Viable mutants having a deletion of this chromosomal region were isolated only in the presence of a mini-F plasmid carrying the \textit{rntl-yeCH-mviM-mviN} locus (12). To determine whether \textit{mviM} and \textit{mviN} are

![Diagram](http://jb.asm.org/)
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 the 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 a vector control. Chloramphenicol-resistant mviM deletion mutants were obtained in the presence or absence of the complementing mini-F plasmid. However, chloramphenicol-resistant mviN deletion mutants could grow only in the presence of the complementing plasmid and not in the presence of the empty control 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) mviN mutant, designated mviN\textsuperscript{ts555}, by plasmid shuffling, which is a kind of localized mutagenesis performed using a mini-F plasmid (9, 10, 11). The growth of the TS mutant at the nonpermissive temperature was restored by pBAD-mviN, which confirmed that mviN\textsuperscript{ts555} is an mviN mutant (see the supplemental material for details). Sequence analysis of mviN\textsuperscript{ts555} revealed a deletion in the mviN upstream region (base pairs 1,125,808 to 1,127,003 of the E. coli genome), which included the 3’ region of yceH, mviM, and a predicted promoter of the mviN gene (based on the E. coli chromosome database PEC [http://www.shigen.nig.ac.jp/ecoli/pec/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 examined the mutant cells using microscopy after incubation at the nonpermissive temperature, the cells appeared to be swollen, and many cells had burst to form ghosts (Fig. 2B). These results suggest that there was a defect in the rigidity of the cell wall. To determine whether the defect involved impaired peptidoglycan synthesis, we introduced a multicopy plasmid carrying the ispU\textsuperscript{rth} gene, which encodes an undecaprenyl diphosphate synthase involved in peptidoglycan synthesis, into mviN TS mutants (10). Expression of ispU suppressed the growth defect of mviN\textsuperscript{ts555} cells at 42°C, indicating that mviN is involved in peptidoglycan synthesis, but it did not do this in the presence of the vector control.

We next examined the levels of cell wall biosynthesis intermediates in mviN mutants. Recently, Guan et al. reported that cell wall biosynthesis lipid intermediates, including polypropenyl diphosphate-N-acetylmuramic acid—(pentapeptide)-N-acetylglucosamines (a mixture of decaprenyl and undecaprenyl derivatives), were extracted specifically from an acidified mixture into chloroform (6). We labeled mutant and wild-type cells with [3H]diaminopimelic acid (DAP), which is incorporated predominantly into the cell wall (see the 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 greater than the incorporation of radioactive DAP into the lipid intermediates of wild-type cells (Table 1). The ratio of lipid intermediates to the total compounds was four times higher in mutant cells than in wild-type cells, while the ratio of radioactivity in the cell wall fraction was lower in mutant cells than in wild-type cells. We next cultivated cells in the presence of [14C]isopentenyl diphosphate (IPP) to label the polypropenyl
mviN gene | Water-soluble intermediates | Lipid intermediates | Cell wall fraction | Lipid intermediates/ (water-soluble intermediates + lipid intermediates + cell wall fraction) | Cell wall fraction/ (water-soluble intermediates + lipid intermediates + cell wall fraction) |
--- | --- | --- | --- | --- | --- |
$mviN^+$ | 131 (13) | 1.6 (0.1) | 245 (20) | 0.44 (0.06) | 65 (2) |
$mviN^{++}$ | 282 (15) | 12 (4) | 367 (33) | 1.76 (0.55) | 56 (1.0) |

*The data are the averages and standard deviations (in parentheses) of three independent assays.

moiety of the lipid intermediates (see the supplemental material for details). Because IPP is not incorporated into untreated growing cells, $[^{14}C]IPP$ was added to lyophilized cells, and then the cells were recultivated. Radiolabeled polyisoprenyl phosphate derivatives were extracted from neutral and acidified mixtures with chloroform, and polyisoprenyl phosphates and polyisoprenyl diphosphates that were extracted from neutral mixtures were separated by ion-exchange chromatography (Table 2). The ratio of the radioactivity of lipid intermediates to the total radioactivity for all the polyisoprenyl phosphate derivatives was higher in mutant cells than in wild-type cells. Thus, based on the results of two separate labeling experiments, in which we labeled pentapeptide moieties by using $[^3H]$DAP and polyisoprenyl moieties by using $[^14C]IPP$, the lipid intermediates accumulated at higher levels in mutant cells than in wild-type cells. These results indicated that the $mviN$ gene is involved in metabolism of the lipid intermediates of 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 transmembrane 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 antibiotics 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 translocation of the dolichyl diphosphate-Man$_5$GlcNAc$_2$ intermediate, an oligosaccharide complex used in protein glycosylation, from the cytosolic side of the endoplasmic reticulum membrane to the lumen during the biosynthesis of dolichyl diphosphate-Glc$_3$Man$_5$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 should 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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TABLE 1. Incorporation of $[^3H]$DAP into the cell wall and cell wall synthesis intermediates

<table>
<thead>
<tr>
<th>mviN gene</th>
<th>Water-soluble intermediates</th>
<th>Lipid intermediates</th>
<th>Cell wall fraction</th>
<th>Lipid intermediates/ (water-soluble intermediates + lipid intermediates + cell wall fraction)</th>
<th>Cell wall fraction/ (water-soluble intermediates + lipid intermediates + cell wall fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$mviN^+$</td>
<td>131 (13)</td>
<td>1.6 (0.1)</td>
<td>245 (20)</td>
<td>0.44 (0.06)</td>
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<td>282 (15)</td>
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<td>367 (33)</td>
<td>1.76 (0.55)</td>
<td>56 (1.0)</td>
</tr>
</tbody>
</table>

*The data are the averages and standard deviations (in parentheses) of three independent assays.

TABLE 2. Incorporation of $[^{14}C]IPP$ into polyisoprenyl phosphate derivatives

<table>
<thead>
<tr>
<th>mviN gene</th>
<th>Polyprenyl phosphates</th>
<th>Polyprenyl diphosphates</th>
<th>Lipid intermediates</th>
<th>Lipid diphosphates + lipid intermediates</th>
<th>Ratio (%) (lipid intermediates/ [polyprenyl phosphates + polyprenyl diphosphates + lipid intermediates])</th>
</tr>
</thead>
<tbody>
<tr>
<td>$mviN^+$</td>
<td>16.1 (1.5)</td>
<td>0.64 (0.02)</td>
<td>0.59 (0.14)</td>
<td>3.4 (0.8)</td>
<td></td>
</tr>
<tr>
<td>$mviN^{++}$</td>
<td>15.3 (2.4)</td>
<td>0.61 (0.17)</td>
<td>1.16 (0.62)</td>
<td>6.5 (2.7)</td>
<td></td>
</tr>
</tbody>
</table>

*The data are the averages and standard deviations (in parentheses) of three independent assays.

REFERENCES


