The conserved DNA-binding protein WhiA is involved in cell division in Bacillus subtilis

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Bacterial cell division is a highly coordinated process that begins with the polymerization of the tubulin-like protein FtsZ at midcell. FtsZ polymerization is regulated by a set of conserved cell division proteins, including ZapA. However, a zapA mutation does not result in a clear phenotype in *Bacillus subtilis*. In this study, we used a synthetic-lethal screen to find genes that become essential when ZapA is mutated. Three transposon insertions were found in *yvcL*. The deletion of *yvcL* in a wild-type background had only a mild effect on growth, but a *yvcL zapA* double mutant is very filamentous and sick. This filamentation is caused by a strong reduction in FtsZ-ring assembly, suggesting that YvcL is involved in an early stage of cell division. YvcL is 25% identical and 50% similar to the *Streptomyces coelicolor* transcription factor WhiA, which induces *ftsZ* and is required for septation of aerial hyphae during sporulation. Using green fluorescent protein fusions, we show that YvcL localizes at the nucleoid. Surprisingly, transcriptome analyses in combination with a ChIP-on-chip assay gave no indication that YvcL functions as a transcription factor. To gain more insight into the function of YvcL, we searched for suppressors of the filamentous phenotype of a *yvcL zapA* double mutant. Transposon insertions in *gtaB* and *pgcA* restored normal cell division of the double mutant. The corresponding proteins have been implicated in the metabolic sensing of cell division. We conclude that YvcL (WhiA) is involved in cell division in *B. subtilis* through an as-yet-unknown mechanism.

In most bacteria, cell division begins with the polymerization of the tubulin-like protein FtsZ into a ring-like structure at midcell. This Z-ring serves as a scaffold for other proteins that are required for septum biosynthesis. Several proteins support the assembly of the Z-ring. The protein FtsA contains a membrane anchor and anchors the Z-ring to the cell membrane (1, 2). The protein ZapA cross-links FtsZ-polymers and promotes polymer bundling (3, 4). In Gram-positive bacteria and cyanobacteria the protein SepF supports the bundling of FtsZ polymers, as well (5–8). The absence of SepF results in irregular division septa (9). EzrA is another conserved protein that interacts directly with FtsZ. This protein contains a transmembrane anchor at its N terminus. It can inhibit bundling of FtsZ polymers (10), but it is also involved in shuttling of the penicillin-binding protein PBP1, which is involved in cell wall synthesis, between the lateral wall and the division site (11). Assembly of the Z-ring at midcell is regulated in part by the Min and Noc systems (12). MinCD prevent polymerization of FtsZ close to cell poles, and mutations in minC or minD lead to minicell formation (13). MinC interacts with FtsZ and inhibits FtsZ polymerization (14). MinC is activated by MinD, which is anchored to the membrane through its amphipathic C terminus (15, 16). The polar localization of MinCD in *Bacillus subtilis* is determined by the proteins MinJ and DivIVA (17–19). The Z-ring does not mature in the area of the cell that is occupied by the nucleoid. In *B. subtilis*, this nucleoid occlusion mechanism is regulated by Noc that binds DNA and prevents FtsZ polymerization (20, 21). Finally, the frequency of cell division is related to cell mass and the glycosyltransferase UgtP has been shown to inhibit FtsZ assembly and to function as a metabolic regulator of Z-ring assembly (22). The activity of UgtP is determined by the concentration of UDP-glucose, which is abundantly produced in cells grown in rich media.

ZapA is conserved and present in most bacterial species (4). A zapA mutant is very sensitive to reduced FtsZ levels. High levels of ZapA counteract the division inhibition caused by overexpression of MinCD (4). The crystal structure of ZapA from *Pseudomonas aeruginosa* revealed a tetramer formed by two antiparallel dimers (23), and several biochemical studies have shown that ZapA is capable of promoting the lateral bundling of FtsZ protofilaments (4, 23, 24). A deletion of zapA does not result in a clear phenotype in *B. subtilis*, and it was postulated that ZapA is only required under circumstances when cells have difficulties forming a Z-ring (25). To find new cell division proteins that become essential when ZapA is absent, we used a synthetic-lethal screening and identified the protein YvcL.

Deletion of *yvcL* in a zapA mutant background results in very filamentous cells that are blocked in proper Z-ring formation. YvcL is a DNA-binding protein and shows strong homology with the protein WhiA from *Streptomyces coelicolor*. WhiA regulates the expression of several genes during sporulation, including *ftsZ* (26, 27). Surprisingly, transcriptome analyses of a *yvcL* mutant did not show a transcriptional effect on known cell division genes in *B. subtilis*, and the localization of YvcL binding sites on the genome gave no clear indication that YvcL functions as a transcription factor.

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factor. Finally, a transposon screen revealed that the cell division defect of a yvcL zapA double mutant can be suppressed by inacti-
vating the genes gltB, pgcA, or ugtP. These genes are part of a
metabolic sensor pathway that couples nutritional availability to
cell division (28), providing further evidence that YvcL is involved in
cell division in B. subtilis.

MATERIALS AND METHODS

Bacterial strains, plasmids, and oligonucleotides. The strains and plas-
mids used in the present study are listed in Table S1 in the supplement-
ary material. The oligonucleotide sequences are listed in Table S2 in the sup-
plemental material. Bacterial cultures were grown at 30°C or 37°C in liq-
uid Luria-Bertani (LB) medium, in competence medium, or on solid nu-
trient agar (Oxoid). DNA manipulations were carried out by standard
techniques, and chromosomal DNA was purified by using phenol-chlo-
roform extraction. Transformations of cells were carried out as described
previously (29), and transformants were plated on nutrient agar sup-
plemented with ampicillin (100 µg/mL), chloramphenicol (5 µg/mL),
erthyroycin (1 µg/mL), glucose (0.4 to 0.8%), kanamycin (5 µg/mL),
spectinomycin (50 µg/mL), tetracycline (12 µg/mL), X-Gal (5-bromo-
4-chloro-3-indolyl-β-D-galactopyranoside; 160 µg/mL), IPTG (isopro-
pyl-β-D-thiogalactopyranoside; 0.5 to 1 mM), or xylose (0.0025 to 1%).

Construction of yvcL mutant strains. To construct strain Ks207, which
contains an insertion of pMutin4 in yvcL, thereby disrupting yvcL
expression, a 581-bp DNA fragment (primers yvcL-F1 and yvcL-R1) was
digested with HindIII and BamHI, and cloned into pMutin4 digested with
the same enzymes. The resulting plasmid pMutin4yvcL was integrated into
B. subtilis by Campbell integration.

In strain Ks400, yvcL is substituted by a kanamycin resistance cassette.
DNA fragments outside of yvcL were amplified by using the primer pairs
Ks80/Ks95 and Ks84/Ks83, resulting in 940- and 984-bp DNA frag-
ments, respectively. The kanamycin resistance cassette was amplified
from pBEST501 (30) with the primers km3 and km4. After the fragments
were digested with BamHI and EcoRI and ligated, the mixture was trans-
formed into B. subtilis.

Strain Ks696 is a markerless yvcL mutant strain. To generate this strain,
plasmid pMutin4yvcLKO was constructed. A 1,094-bp fragment,
comprising the 3' end of yvcL and the 5' end of yvcL, was amplified by
using the primers Ks94 and yvcL-R1 and subsequently cloned into pMutin4
after digestion with HindIII and BamHI. Site-directed mutagenesis on the
plasmid was performed using the primers Ks120 and Ks121 that intro-
duced an EcoRI site and a stop codon in the beginning of yvcL (32 bp from
start). The resulting plasmid pMut4yko was transformed into B. subtilis
cells and selected for erythromycin resistance and blue colonies on X-Gal
plates. In order to excise the plasmid, one of the transformants was grown
in competence medium for ~10 generations without any antibiotic pres-
sure and screened for the loss of the plasmid on plates with X-Gal. White
and erythromycin-sensitive colonies were cultured, and the chromosomal
DNA checked for the EcoRI site in yvcL. The yvcL region was also analyzed
by sequencing.

The conditional mutant in yvcL-chr-yycN was constructed by using
plasmid pMutIyvcL. An ~400-bp region upstream of yvcL was amplified
by using the primers Ks94 and Ks95. The fragments and plasmids were cut
with HindIII and BamHI and cloned into pMutin4 plasmid digested with
the corresponding enzymes. Plasmid pMutIyvcL was transformed into B.
subtilis and selected for single-crossover events that led to insertion of the
IPTG-inducible Pspac promoter upstream of yvcL (strain Ks438). To allow
tight regulation of yvcL expression, an extra copy of lacI was introduced by
transforming plasmid pAPNC213 (31), which integrates into aprE locus,
resulting in strain Ks891.

To construct a xylose-inducible YvcL overexpression strain, the gfp
gene was removed from plasmid pSG1729 by digestion with Kpn1 and
XhoI, followed by insertion of the yvcL gene, which was amplified using
the primers Ks98 and yvcL-5', and then digested with the same enzymes.

The ensuing plasmid pSG1729YvcL-GFP was transformed into B. subtilis,
resulting in strain Ks596.

Construction of the GFP-yvcL fusion protein. To construct a xylose-
inducible GFP-YvcL fusion, plasmid pSG1729YvcL was generated by li-
gating the amyE-integration vector pSG1729 and a 1,020-bp PCR frag-
ment (primers yvcL-N5 and yvcL-N3), both digested with HindIII and
EcoRI. Plasmid pSG1729YvcL was used for a QuikChange mutagenesis
reaction with oligonucleotides HS410 and HS411 in order to introduce the
A206K mutation in the green fluorescent protein (GFP) coding se-
quence to reduce protein dimerization (32). The resulting plasmid was
verified by sequencing and pSG1729YvcL(mGFP). Plasmid pSG1729YvcL(mGFP)
was transformed into B. subtilis, resulting in strain PG732. The amyE::mGFP-yvcL::yvcL-spc allele of PG732 was combined with a
yvcL mutation (strain Ks400) so that mGFP-yvcL is the only copy of
yvcL in the cell (strain Ks736).

YvcL antibody. To raise antibodies against YvcL, an expression vector
pQE60EYvcL was created, which allows the expression of a C-terminally
tagged YvcL-His6 fusion protein. To construct pQE60EYvcL, a 967-bp
PCR product (primers Ks89 and Ks99) was cloned into pQE60E using
BamHI and BglII. Escherichia coli XL1-Blue was used as a host for cloning
and protein expression. The resultant E. coli XL1-Blue strain containing
pQE60EYvcL (Ks432) was used for the expression of the fusion protein as
follows. Strain Ks542 was inoculated into 300 mL of LB medium supple-
mented with ampicillin and 0.8% glucose (to allow tight repression).
When the cell density reached an optical density at 600 nm (OD600) of
~0.5, expression was induced with 1 mM IPTG for 3.5 h. The pellets were
resuspended in 1.2 mL of buffer (100 mM NaCl, 50 mM Tris·Cl [pH
8.0]) and sonicated. The inclusion bodies containing YvcL-His6 were se-
parated by centrifugation and isolated on a 12% SDS-PAA gel. The protein
band corresponding to YvcL-His6 was cut from the gel and used to raise
rabbit polyclonal antiserum (Eurogentec, Ltd). For Western blotting, a
10,000×-diluted anti-YvcL serum was used.

Synthetic lethal screen. To perform a synthetic lethal screen with
Δzapa strain, we used the method described by Claessen et al. (11).
In brief, the zapa-yshB genes were amplified using yshA-F and yshB-R prim-
ers and cloned into the unstable plasmid pLSSO*. pLSSO* contains the
lacZ reporter gene, which enables blue-white screening as an indicator for
plasmid stability. To prevent possible induction of the endogenous β-
galactosidase, the lacZ gene was also deleted (33). The resulting plasmid
pLSSO-zapa was transformed into cells with a zapa-yshB deletion (strain
Ks6) and subsequently a lacZ::cat deletion was introduced to prevent
transposon insertions that would activate the native B. subtilis β-galacto-
sidase (strain Ks50). This strain was transformed by pMarB, which carries
the transposon TnYLB-1 (34), and the transposon mutant library was
screened on nutrient agar plates supplemented with X-Gal and 1 mM
MgSO4. Magnesium was added to the media, since it enhanced blue col-
ony formation. Loss of pLSSO-zapa was further stimulated by incubating
the plates at 50°C. After selection for blue colonies, chromosomal DNA
of positive clones was backcrossed into strain Ks50 to check whether the
transposon stabilized pLSSO-zapa in cells. To map the transposon in-
sertions, DNA fragments were TaqI digested, ligated, and amplified by
inverse PCR using the primers OFPCR1 and OFPCR2. The chromosomal
position of the transposon was mapped by sequencing using primer
OFPCR3 (34).

Suppressor screen for yvcL zapa double mutant. To select trans-
poson mutants that suppress the lethal phenotype of a yvcL zapa double
deletion, the following procedure was developed. First, the unstable plas-
mid pLSSO-YvcL was constructed by cloning yvcL (amplified by Ks128
and Ks97) into pLSSO*. Leaky transcription from the Pspac promoter gave
sufficient levels of YvcL to prevent cell death in the yvcL zapa double-
mutant background (strain Ks742). Nevertheless, 0.1 mM IPTG and
spectinomycin was used during construction of the strains. This strain
also contained a lacZ deletion.

Strain Ks742 was used for transposon mutagenesis using pMarB (34),
and the transposon mutant library was screened on nutrient agar plates
supplemented with X-Gal, 0.1 mM IPTG, and 1 mM MgSO4, followed by incubation at 37°C. After screening and selection for white colonies, the chromosomal DNA of 82 positive clones was purified and transformed into the conditional yvcL zapA mutant strain (KS859). Suppressor mutations that were able to recover growth of KS859 in the absence of IPTG were mapped as described for the synthetic lethal screen.

Microscopic imaging. Before the cells were mounted onto microscopic slides, the slides were covered with a thin layer of 1.5% agarose solution. For fluorescence microscopy, also 1 mM MgSO4 and 0.5% glucose were added to the agarose. Zeiss Axiolab 200M microscope was used to capture images. For analyses of all microscopic pictures, ImageJ software (http://rsb.info.nih.gov/ij/) was used.

Microarray analysis. To identify differences in gene expression between wild-type B. subtilis (strain 168), the KS400 strain, and the KS696 strain, microarray analyses were performed using a 135K tiling array that was designed using the National Center for Biotechnology Information Bacillus subtilis 168 uido57675 Fasta sequence (26 January 2011, Refseq NC_000964.3, gi/255767013). Probes (60 nucleotides and a Blatbitscore threshold of 80) were designed with a tile step of 32 bases with an overlap of 28 bases between probes on opposite strands.

To isolate RNA, cell pellets were flash frozen in liquid nitrogen immediately after harvesting and stored at −80°C prior to RNA extraction. Frozen pellets were ground by using a mortar and pestle before immersion in 300 μl of Qiazol reagent (Qiagen). RNA was isolated using the Qiazol protocol and further purified using an E.Z.N.A. MicroElute RNA cleanup kit (Omega Biotek), including an on-column treatment with the RNase-free DNase set (Life Technologies). RNA was quantified on a NanoDrop ND-1000 (Thermo Scientific), and RNA integrity was measured with a BioAnalyzer (Agilent Technologies) using an RNA Nano6000 kit (Agilent Technologies), yielding RIN values of ≥9.7. Labeling was performed by reverse transcription using random octamers, incorporating Cy3 for the test samples and Cy5 for the common reference, as described previously (35). Hybridization, washing, and scanning were performed as described elsewhere (36).

All arrays were subjected to a set of quality control checks, such as visual inspection of the scans, checking for spatial effects through pseudocolor plots, and inspection of pre- and post-normalized data with box plots, density plots, ratio-intensity plots, and principal component analysis. Expression values were calculated by using the robust multi-array average (RMA) algorithm (37), where probe-sets were defined based on the coding sequences with a BSU locus tag. Differences in gene expression between wild-type B. subtilis, the KS400 strain, and the KS696 strain, were statistically analyzed using the Limma package in R 2.14.1 (http://cran.r-project.org/). Empirical Bayes test statistics were used for hypothesis testing (38), and all P values were corrected for false discoveries (39). Gene expression data and array design have been deposited at the public Gene Expression Omnibus, accession number GSE45824. The processed array data are listed in Table S4 in the supplemental material.

ChIP-on chip. Chromatin immunoprecipitation (ChIP) and ChIP-on-chip analysis of YvlK was performed as described previously (40). ChIP was performed with YvlK antibody bound to protein A-coated magnetic Dynabeads (Invitrogen). The input (whole DNA) and immunoprecipitated DNA was amplified, labeled, and applied onto Nimblegen custom-made chips (~54,000 50-bp oligonucleotides in array) containing genome-wide probes. The oligonucleotides used for quantitative PCRs (qPCRs) are listed in Table S2 in the supplemental material.

RESULTS

Synthetic lethal screen. The cell division protein ZapA is conserved in Gram-positive and Gram-negative bacteria, and yet a deletion of zapA does not result in a clear cell division defect (4, 41). To find new cell division proteins that become essential when ZapA is absent, we applied a synthetic lethal screen using the unstable plasmid pLOSS* (11), zapA was cloned into pLOSS*, resulting in pLOSS-zapA, and the plasmid was introduced into a zapA mutant. pLOSS* contains the lacZ reporter gene which enables blue-white screening as an indicator for plasmid stability. Transposon mutagenesis was performed using the mariner transposon (34). We isolated three mutants that formed blue colonies, indicating that they maintained pLOSS-zapA and required ZapA for growth (Fig. 1A and B). When chromosomal DNA from these mutants was backcrossed into a zapA mutant, only very small colonies appeared (Fig. 1C). Cells in these colonies were very filamentous and lysed easily (Fig. 1E). Mapping of the transposons by reversed PCR revealed three independent transposon insertions into the gene yvcL (Fig. 1F).

Analysis of the yvcL operon. The yvcL gene is part of the yvcL-yvcN operon (Fig. 1F) (42). YvlK and YvcN encode proteins of unknown function. YvcJ is a GTPase required for full induction of genetic competence, although the mechanism of this regulation is unclear (43). YvcK is involved in cell wall synthesis under gluco-
neogenic growth conditions and is required for the correct localization of penicillin-binding protein PBP1 (44, 45). crh, located downstream of yvcL, is involved in the control of carbon flux (42, 46). The inactivation of neither crh nor yvcN resulted in a clear growth defect, but to exclude any polar effects of the transposon insertions, an IPTG-inducible Pspac promoter was integrated either up- or downstream of yvcL and introduced into a zapA mutant. To obtain full repression of the Pspac promoter, an extra copy of lacI was introduced, as well. IPTG was only required for normal growth in the construct that contained Pspac upstream of yvcL (data not shown), thus confirming that the synthetic lethal phenotype is indeed linked to yvcL.

YvcL shows a high homology with WhiA from Streptomyces coelicolor with 25% identity and 50% similarity (see Fig. S1 in the supplemental material). The name was derived from the fact that a whiA mutant forms white colonies as a result of its inability to form gray-pigmented spores (47). The sporulation defect appears to be a consequence of the inability of whiA mutants to form division septa in aerial hyphae. Despite its homology with WhiA, a yvcL mutation has a relatively mild effect on the sporulation in B. subtilis and reduces the sporulation efficiency by only 30 to 40% (see Table S3 in the supplemental material).

**Growth defect of the yvcL mutant.** We noticed that in a wild-type background the transposon insertions resulted in smaller colonies. This reduction in growth may be caused by a polar effect, and therefore a markerless mutation was constructed by introducing a stop codon at the beginning of yvcL (strain KS696). However, even this mutant grows slower compared to the wild-type strain (Fig. 2A). Microscopic analyses revealed that the mutant cells are longer (Fig. 2B and C). Deletion of the upstream yvcK gene is known to affect growth and cell shape, and this can be compensated by the addition of an excess of Mg2+ (44). However, addition of Mg2+ did not abrogate the lower growth rate or increased cell length of a yvcL mutant (not shown).

*S. coelicolor* WhiA is upregulated when sporulation is initiated (26). To examine whether synthesis of YvcL may be growth phase dependent, we purified the protein and raised antibodies. Western blot analysis with YvcL-specific antibodies indicated that the protein is constitutively expressed throughout the growth phase (Fig. 2D). This is in agreement with a recent comprehensive transcriptome study that demonstrated the constitutive transcription of yvcL (48). Apparently, the function of YvcL is not restricted to a certain developmental stage in *B. subtilis*.

**Effect with other cell division mutants.** The synthetic sick phenotype when a yvcL mutation is combined with a zapA deletion suggests that YvcL might affect the activity of FtsZ. If this is the case, then it is likely that the introduction of a yvcL mutation in other cell division mutants will also result in a cell division phenotype. Like ZapA, the cell division protein SepF stimulates bundling of FtsZ protofilaments. However, when a yvcL deletion was introduced into a sepF mutant no effect on cell division was observed, and the double knockout grew fine (Fig. 3). Interestingly, when a yvcL deletion was combined with a mutation in either ezrA, minCD, or noc, the resulting transformants grew poorly and formed very filamentous cells (Fig. 3). These findings further support the suggestion that YvcL influences the activity of FtsZ.

It has been shown that a zapA ezrA double mutation is sick and grows filamentous (4, 9). However, deleting zapA in either a minCD or noc mutant does not result in impaired growth or excessive filamentation (Fig. 3). Thus, YvcL and ZapA are not functionally redundant.
The \( yvcL \) mutant is sensitive to reduced FtsZ concentrations. If \( yvcL \) supports the assembly of the Z-ring, it is likely that a \( yvcL \) mutant is sensitive for a reduction of intracellular FtsZ levels. To test this, an IPTG-inducible \( ftsZ \) allele was introduced into the \( H9004 \ yvcL \) mutant as the only copy of \( ftsZ \) (strain KS748). Serial dilutions were spotted onto plates containing low (30 \( \mu \)M) or high (500 \( \mu \)M) IPTG concentrations. As shown in Fig. 4A, the \( yvcL \) mutant shows reduced growth at low FtsZ concentrations (low IPTG), although the effect is much less compared to a \( zapA \) mutant (middle panel). This raises the question whether overexpression of FtsZ might rescue the synthetic lethal phenotype of a \( yvcL \ zapA \) double mutant. Both the \( yvcL \) and the \( zapA \) mutations...

**FIG 3** Transformation of a \( yvcL \) deletion into either \( ezrA \), \( minCD \) or \( noc \) mutant strains results in filamentation. (A) Colony formation of the different mutants: \( \Delta yvcL \) (KS267), \( \Delta yvcL \Delta sepF \) (KS341), \( \Delta yvcL \Delta ezrA \) (KS344), \( \Delta yvcL \Delta minCD \) (KS356), \( \Delta yvcL \Delta noc \) (KS354), \( \Delta zapA \) (KS6), \( \Delta zapA \Delta minCD \) (PG740), and \( \Delta zapA \Delta noc \) (PG739) strains. (B) Phase-contrast image of \( \Delta yvcL \Delta ezrA \) (left panel) and \( \Delta yvcL \Delta minCD \) (right panel) cells. The \( \Delta yvcL \Delta noc \) double mutant shows comparable filamentous cells (not shown). Insets show cells from \( \Delta ezrA \) and \( \Delta minCD \) single mutants (strains KS44 and KS338, respectively). Scale bar, 5 \( \mu \)m.

**FIG 4** Sensitivity of \( yvcL \) mutants for altered FtsZ levels. (A) \( yvcL \) mutant is sensitive to reduced cellular FtsZ levels. Serial dilutions of \( \Delta yvcL \) and \( \Delta zapA \) mutant strains containing an IPTG-inducible \( ftsZ \) gene (KS268, \( P_{spac}\)-\( ftsZ \); KS162, \( \Delta zapA \ P_{spac}\)-\( ftsZ \); and KS748, \( \Delta yvcL \ P_{spac}\)-\( ftsZ \)). Dilutions were spotted onto plates with 30 or 500 \( \mu \)M IPTG. (B) FtsZ overexpression restores growth and cell division of a \( yvcL \ zapA \) double mutant. FtsZ overexpression was accomplished by the introduction of an ectopic \( P_{xylose}\)-driven \( ftsZ \) copy. Serial dilutions of strains PG8 (\( P_{xylose}\)-\( ftsZ \)), PG735 (\( \Delta zapA \ P_{xylose}\)-\( ftsZ \)), KS737 (\( \Delta yvcL \ P_{xylose}\)-\( ftsZ \)), and PG738 (\( \Delta zapA \Delta yvcL \ P_{xylose}\)-\( ftsZ \)) were spotted onto plates with 0, 0.025, 0.05, and 0.075% xylose. (C) Phase-contrast images of strain PG738 (\( \Delta zapA \Delta yvcL \ P_{xylose}\)-\( ftsZ \)) grown in the absence or presence of 0.025% xylose, indicating that overexpression of FtsZ restores cell division. Scale bar, 5 \( \mu \)m.

The \( \Delta yvcL \) mutant is sensitive to reduced FtsZ concentrations. If \( yvcL \) supports the assembly of the Z-ring, it is likely that a \( yvcL \) mutant is sensitive for a reduction of intracellular FtsZ levels. To test this, an IPTG-inducible \( ftsZ \) allele was introduced into the \( yvcL \) mutant as the only copy of \( ftsZ \) (strain KS748). Serial dilutions were spotted onto plates containing low (30 \( \mu \)M) or high (500 \( \mu \)M) IPTG concentrations. As shown in Fig. 4A, the \( yvcL \) mutant shows reduced growth at low FtsZ concentrations (low IPTG), although the effect is much less compared to a \( zapA \) mutant (middle panel). This raises the question whether overexpression of FtsZ might rescue the synthetic lethal phenotype of a \( yvcL \ zapA \) double mutant. Both the \( yvcL \) and the \( zapA \) mutations...
were introduced into a strain carrying an extra copy of \textit{ftsZ} driven by the strong xylose-inducible \textit{Pxyl} promoter (strain PG738). In the absence of xylose, this strain forms very small colonies and filamentous cells (Fig. 4B and C). The induction of \textit{ftsZ} (0.025 to 0.05\% xylose induction) clearly stimulated colony formation of the double mutant (Fig. 4B), and microscopic analyses indicated that cell division was restored (Fig. 4C).

Although increased \textit{ftsZ} levels do stimulate growth of the \textit{yvcL zapA} double mutant, the colonies are still slightly smaller compared to the single mutants (Fig. 4B). In fact, detailed growth analyses in microtiter plates revealed that \textit{ftsZ} overexpression neither restores the growth rate reduction of the \textit{yvcL} single mutant nor that of the \textit{yvcL zapA} double mutant (see Fig. S2 in the supplemental material). In addition, Fig. 4B shows that high levels of \textit{ftsZ} (0.075\% xylose) causes lyses of the \textit{yvcL} mutants, which is not observed with wild-type cells or the \textit{zapA} mutant. These data suggest that YvcL is not simply a regulator of \textit{ftsZ} activity.

\textbf{Reduced Z-ring formation in a \textit{yvcL zapA} double mutant.} The sensitivity of a \textit{yvcL} mutant for reduced \textit{ftsZ} concentrations suggests that the filamentous phenotype of a \textit{yvcL zapA} double mutant is caused by a defect in Z-ring assembly. To examine this, a GFP-\textit{ftsZ} reporter fusion was introduced into a strain containing an IPTG-inducible \textit{yvcL} and a \textit{zapA} deletion (strain 754). When grown in the presence of IPTG, normal cells were formed with clear fluorescent bands indicative of Z-rings (Fig. 5, left panel). However, in the filamentous cells that are formed when IPTG is omitted, no clear Z-rings could be observed (Fig. 5, right panel). Thus, a \textit{yvcL zapA} double mutant has difficulties to complete the first step in cell division; the assembly of a Z-ring.

\textbf{YvcL localizes at the nucleoid.} The crystal structure of a WhiA homolog from \textit{Thermatoga maritima} indicated that the conserved C-terminal region comprises a typical DNA-binding helix-turn-helix domain (49). To examine whether YvcL binds to DNA in \textit{B. subtilis}, an N-terminal mGFP fusion (monomeric GFP) was constructed under the control of the xylose-inducible \textit{Pxyl} promoter (strain PG736). The mGFP-YvcL fusion appears to be active since it restored growth of a \textit{yvcL zapA} double mutant (data not shown). Western blot analyses indicated that induction with 0.01\% xylose resulted in mGFP-YvcL levels that are comparable to wild-type YvcL (see Fig. S3 in the supplemental material). Figure 6 shows the localization of induced mGFP-YvcL in exponentially growing cells. The GFP signal clearly localizes at the nucleoid. This is even more apparent at higher xylose concentrations (see Fig. S3 in the supplemental material) and supports the assumption that YvcL binds to DNA.

\textit{Transcriptome analysis of \textit{yvcL} mutants.} \textit{S. coelicolor} WhiA binds to its own promoter and is required for its own expression (26, 50), the sporulation-dependent expression of \textit{ftsZ}, and the expression of other sporulation genes (27, 51, 52). The nucleoid-binding activity of YvcL and its homology to WhiA suggest that
YvcL functions as a transcription factor, possibly regulating ftsZ expression. To identify genes that are regulated by YvcL, a genome-wide transcriptome analysis was performed by using tiling arrays. Wild-type _B. subtilis_ and the _yvcL::kan_ deletion strain (KS400) were grown in LB medium to an OD_{600} of ~0.5, followed by the isolation of RNA. The microarray results indicated that the downstream located _yvcN_ and _crh_ genes were significantly upregulated (Table 1). This is likely a consequence of the kanamycin marker that deletes _yvcL_ and reads into _crh_ and _yvcN_. Therefore, a new transcriptome analysis was performed, this time using the markerless _yvcL_ mutant (KS696). In this case, there was no difference in _yvcN_ and _crh_ expression between _A. vvcL_ cells and wild-type cells (Table 1). The data sets of both transcriptome analyses were combined. A total of 49 genes showed 2-fold downregulation and 92 genes showed 2-fold upregulation in both _yvcL_ mutants. The significance cutoff was set at an adjusted P value of <1.0E-5.

**Table 1** lists 46 genes that show a >4-fold difference in expression in both data sets. Surprisingly, we found no significant difference in the expression of known cell division genes, including _ftsZ_. Western blot analyses confirmed that FtsZ levels are not markedly affected in a _yvcL_ mutant (see Fig. S4 in the supplemental material). The overexpression of YvcL has also no effect on cellular FtsZ levels (see Fig. S4 in the supplemental material) and does not suppress the synthetic cell division defect of a _zapA_ _ezrA_ double mutant (4). Finally, the presence of many up- and downregulated genes in the transcriptome profiles might be related to the fact that a _yvcL_ mutant grows slower (Fig. 2).

**Identification of YvcL binding sites on the genome.** The GFP-YvcL fusion indicated that the protein accumulates at the nucleoid but the transcriptome analysis did not identify any YvcL-regulated gene that could explain why this protein becomes important when ZapA, EzrA, MinCD, or Noc are absent. To determine whether the transcriptome profile can be linked to specific YvcL operator sites on the genome, the chromosomal YvcL-binding sites were determined using ChIP combined with microarrays (ChIP-on-chip assay). After cross-linking, chromosomal DNA was isolated, and YvcL-DNA fragments were immunoprecipitated with YvcL antibodies. The DNA fragments were amplified, fluorescently labeled, and hybridized to Nimblegen tiling arrays, as previously described (40). The intensity plot of YvcL-enriched genomic regions is shown in Fig. 7A. We noticed that several of the peaks are also present in the published ChIP-on-chip profiles with Noc and Smc (21, 40). These peaks might therefore indicate unspecific amplification. The peaks unique for YvcL are marked in red (Fig. 7A). The strongest peaks were verified with a ChIP experiment, followed by qPCR, whereby DNA from a _yvcL_ mutant served as a negative control (Fig. 7B). The YvcL peaks do not seem to reveal a special binding pattern and could not be assigned to genes that showed up in the transcriptome analysis. The protein is enriched at an actively transcribed region that encompasses the ribosomal genes _rpsL_, _rplB_, and _rplN_, but in fact a closer inspection of peaks indicated that YvcL binds within coding regions instead of promoter regions (Fig. 7C). Thus, the ChIP-on-chip data do not support the assumption that YvcL functions as a transcription factor.

**Isolation of suppressor mutants.** Possibly, the identification of mutants that suppress the filamentous phenotype of a _yvcL_ _zapA_ double mutant might shed light on the function of YvcL. To find such suppressors, we again made use of the instable pLOSS* plasmid, but this time _yvcL_ was cloned into the plasmid. When the plasmid was introduced into a _yvcL_ _zapA_ double mutant, the resulting transformants formed blue colonies of normal size on X-Gal-containing plates. After transposon mutagenesis, a few white colonies of normal size appeared. These colonies had lost the plasmid and contained a transposon insertion that suppressed the sick phenotype caused by the combined deletion of _yvcL_ and _zapA_. Eventually, three suppressors were identified. Two clones had a transposon insertion in _gtaB_, and one clone contained a transposon in _pgcA_. Interestingly, mutations in _gtaB_ and _pgcA_ have been shown to increase the frequency of cell division (28). GtaB and PgcA provide the UDP-glucose substrate for the glucosyltransferase UgtP, and this protein binds directly to FtsZ and inhibits the assembly of FtsZ (28). Indeed, when a _ugtP_ deletion was introduced into a conditional _yvcL_ _zapA_ double mutant, the transformants formed normally sized colonies without IPTG (Fig. 8). Microscopic analyses also showed that the disruption of _gtaB_, _pgcA_, or _ugtP_ suppresses the cell division defect of the _yvcL_ _zapA_ double mutant (Fig. 8).

**DISCUSSION**

Using a synthetic lethal screen, we have identified a new protein involved in cell division in _B. subtilis_. This protein, YvcL, is required for normal growth and cell division when the FtsZ regulators ZapA, EzrA, MinCD, or Noc are absent. In the filamentous _yvcL_ _zapA_ double mutant, no clear Z-rings are observed. This phenotype can be suppressed by blocking the activity of UgtP, the metabolic regulator that inhibits Z-ring formation. Together, these data suggest that YvcL acts at the level of Z-ring formation, which is supported by the finding that a _yvcL_ mutant is sensitive for reduced FtsZ concentrations, and that increased FtsZ levels suppress the cell division defect in the _yvcL_ _zapA_ double mutant.

YvcL belongs to the conserved protein family DUF199 whose members are assumed to act as transcriptional regulators (53). The crystal structure of _Thermatoga maritima_ WhiA revealed a typical helix-turn-helix fold related to bacterial sigma-70 factors (49). This domain is required for binding of _S. coelicolor_ WhiA to its own promoter (50). WhiA is essential for the induction of _ftsZ_ during sporulation in _S. coelicolor_ (37, 54), and constitutive _ftsZ_ expression rescues sporulation in a _whiA_ mutant (55). Because of these structural and functional homologies, we propose to use the name WhiA instead of YvcL.

Considering the homologies between _B. subtilis_ and _S. coelicolor_ WhiA, it was surprising to find that _B. subtilis_ WhiA does not regulate _ftsZ_ or other known cell division genes. _S. coelicolor_ WhiA is also required for the expression of _parAB_, _whiB_, and _hupS_ during sporulation (26, 51, 52). We could not detect transcriptional changes in _soj/spo0J_, which are the _B. subtilis_ equivalents of _parA/B_, in strains lacking WhiA. _B. subtilis_ does not encode a _whiB_ homologue, and _hupS_ encodes a histone-like protein typical for Actinomycetes. The expression of _B. subtilis_ chromosome architectural proteins Hbs, ScpA/B, and Smc was also not altered in a _whiA_ mutant. Thus far, any possible transcriptional activity of WhiA fails to explain why this protein is required for cell division in _B. subtilis_.

The exact function of _B. subtilis_ WhiA remains elusive. Because the protein binds DNA, it might play a role in nucleoid occlusion. However, it is unlikely that WhiA regulates Noc directly since a _whiA noc_ double mutant shows a severe cell division phenotype that is not observed with the single mutants. We have examined whether the nucleoid localization of Noc is affected in a _whiA_ mutant, but that is not the case (see Fig. S5 in the supplemental
### TABLE 1 Transcriptome analysis of \textit{yvcL} mutants

<table>
<thead>
<tr>
<th>Gene</th>
<th>KS400/wt</th>
<th>KS696/wt</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{yvcL}</td>
<td>0.01</td>
<td>1.07</td>
<td>Putative morphogen</td>
</tr>
<tr>
<td>\textit{mtnA}</td>
<td>0.02</td>
<td>0.02</td>
<td>Methylthioribose-1-phosphate isomerase (methionine salvage pathway)</td>
</tr>
<tr>
<td>\textit{mtnK}</td>
<td>0.02</td>
<td>0.02</td>
<td>Methylthioribose kinase (methionine salvage pathway)</td>
</tr>
<tr>
<td>\textit{clid}</td>
<td>0.09</td>
<td>0.15</td>
<td>Holin regulator of murein hydrolases</td>
</tr>
<tr>
<td>\textit{lrgA}</td>
<td>0.10</td>
<td>0.11</td>
<td>Anti-holin factor controlling activity of murein hydrolases</td>
</tr>
<tr>
<td>\textit{yxiM}</td>
<td>0.16</td>
<td>0.14</td>
<td>Putative esterase (lipoprotein)</td>
</tr>
<tr>
<td>\textit{yxiK}</td>
<td>0.14</td>
<td>0.10</td>
<td>Putative phage head maturation protein</td>
</tr>
<tr>
<td>\textit{yxiJ}</td>
<td>0.11</td>
<td>0.07</td>
<td>Conserved hypothetical protein</td>
</tr>
<tr>
<td>\textit{yxiI}</td>
<td>0.09</td>
<td>0.07</td>
<td>Conserved hypothetical protein</td>
</tr>
<tr>
<td>\textit{yxxG}</td>
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<td>0.07</td>
<td>Putative nucleic acid binding protein</td>
</tr>
<tr>
<td>\textit{yxiG}</td>
<td>0.10</td>
<td>0.06</td>
<td>Conserved hypothetical protein</td>
</tr>
<tr>
<td>\textit{yxxC}</td>
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<td>0.06</td>
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<td>\textit{yxiF}</td>
<td>0.09</td>
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<td>Putative phage reverse transcriptase or polymerase</td>
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<td>0.07</td>
<td>Hypothetical protein</td>
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<td>Putative membrane protein with diguanylate cyclase domain</td>
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<tr>
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<tr>
<td>\textit{ydaM}</td>
<td>0.19</td>
<td>0.13</td>
<td>Putative glycosyl transferase associated to biofilm formation</td>
</tr>
<tr>
<td>\textit{ydaN}</td>
<td>0.20</td>
<td>0.11</td>
<td>Putative regulator</td>
</tr>
<tr>
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<td>0.21</td>
<td>Proline transporter</td>
</tr>
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<td>0.19</td>
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<td>16.6</td>
<td>Putative lipoprotein</td>
</tr>
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<td>7.4</td>
<td>ABC transporter involved in the signaling pathway that activates KinA</td>
</tr>
<tr>
<td>\textit{bmrD}</td>
<td>7.9</td>
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<td>ABC transporter involved in the signaling pathway that activates KinA</td>
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<td>\textit{yvcN}</td>
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<td>1.0</td>
<td>Putative acetyltransferase</td>
</tr>
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<td>\textit{crh}</td>
<td>6.2</td>
<td>1.0</td>
<td>Catabolite repression HPr-like protein</td>
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<td>\textit{yvcA}</td>
<td>5.4</td>
<td>8.7</td>
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<td>5.4</td>
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<td>Antiholin factor</td>
</tr>
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<td>Hypothetical protein</td>
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<td>7.8</td>
<td>Sucrase-6-phosphate hydrolase</td>
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<tr>
<td>\textit{sacP}</td>
<td>5.4</td>
<td>7.8</td>
<td>Phosphotransferase system (PTS) sucrose-specific enzyme</td>
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<tr>
<td>\textit{yfjA}</td>
<td>5.3</td>
<td>7.1</td>
<td>Putative lipoprotein</td>
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<tr>
<td>\textit{yfjB}</td>
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<td>5.9</td>
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<td>5.8</td>
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<td>\textit{yfjB}</td>
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<td>11.8</td>
<td>Siderophore bacillibactin synthetase</td>
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<td>\textit{dhbB}</td>
<td>4.4</td>
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<td>Isochorismatase</td>
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<td>2,3-Dihydroxybenzoate-AMP ligase (enterobactin synthetase component E)</td>
</tr>
<tr>
<td>\textit{dhbA}</td>
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<td>2,3-Dihydro-2,3-dihydroxybenzoate dehydrogenase</td>
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<td>Protein involved in biofilm formation</td>
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<td>Major biofilm matrix component</td>
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<td>\textit{sacB}</td>
<td>4.0</td>
<td>6.3</td>
<td>Levansucrase</td>
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</table>

*Genes with a 4-fold expression difference and an adjusted \( P \) value of \(<1.0E^{-5}\) present in both transcriptome experiments are listed (except for \textit{yvcL}, \textit{yvcN}, and \textit{crh}). KS400 contains a kanamycin marker in \textit{yvcL}, and KS696 is the markerless \textit{yvcL} mutation. Fold differences are shown, and genes shifted to the right in column 1 indicate operons. wt, wild type.*
Moreover, we failed to notice the classical FtsZ rings and spirals that overlap the nucleoid or chromosome bisection, which are typical for noc mutants (21).

Depletion of FtsZ, although causing filamentation that makes cells prone to lysis, does not reduce cell growth (elongation) (56). However, a whiA mutant clearly grows slower, and the additional expression of FtsZ does not suppress this growth defect. It is unclear how the activity of WhiA links cell growth with cell division. Interestingly, inactivation of UgtP, the metabolic sensor of cell division, suppresses the severe cell growth defect of the whiA zapA double mutant. UgtP interacts with FtsZ and inhibits assembly of FtsZ when cells grow in rich medium (28). Possibly, WhiA represses directly or indirectly the activity of UgtP. The transcriptome data indicate that the expression levels of ugtP or other genes involved in the activity of UgtP (pgcA, gtaB) are unaffected in a whiA knockout strain. We also could not detect increased septal localization of UgtP in such backgrounds (data not shown). Further research will be necessary to determine whether WhiA controls UgtP activity.

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