PBP1a glycosyltransferase and transpeptidase activities are both required for maintaining cell morphology and envelope integrity in *Shewanella oneidensis*.

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**One sentence summary**

The combined enzymatic activities of glycosyltransferase and transpeptidase are essential for cell wall homeostasis.

**ABSTRACT**

In rod-shaped Gram-negative bacteria, penicillin binding protein 1a (PBP1a) and 1b (PBP1b) form peptidoglycan-synthesizing complexes with the outer membrane lipoprotein LpoA and LpoB, respectively. *Escherichia coli* mutants lacking PBP1b/LpoB are sicker than those lacking PBP1a/LpoA. However, we previously found that mutants lacking PBP1a/LpoA but not PBP1b/LpoB are deleterious in
**Shewanella oneidensis.** Here, we show that *S. oneidensis* PBP1a (SoPBP1a) contains conserved signature motifs with its *E. coli* counterpart, EcPBP1a. Although EcPBP1a play a less prominent role in *E. coli*, it is capable of substituting for the SoPBP1a in a manner dependent on SoLpoA. In *S. oneidensis*, expression of PBP1b is lower than PBP1a, and therefore the additional expression of SoPBP1b at low levels can functionally compensate for the absence of SoPBP1a. Importantly, *S. oneidensis* PBP1a variants lacking either glycosyltransferase (GTase) or transpeptidase (TPase) activity fail to maintain normal morphology and cell envelope integrity. Similarly, SoPBP1b variants also fail to compensate for the loss of SoPBP1a. Furthermore, overproduction of variants of SoPBP1a, but not SoPBP1b, has detrimental effects on cell morphology in *S. oneidensis* wild type cells. Overall, our results indicate that the combined enzymatic activities of SoPBP1a are essential for cell wall homeostasis.

**Keywords**

Peptidoglycan; Penicillin binding protein; Cell morphology; Envelope integrity;

*Shewanella oneidensis*

**INTRODUCTION**

Peptidoglycan of the bacterial cell wall is essential for maintaining cell shape and providing mechanical strength to prevent osmotic rupture. This structure is composed of glycan chains with the repeating disaccharide N-acetylmuramic acid-β-1,4-N-acetylglucosamine (MurNAc-GlcNAc). The neighboring glycan chains are cross-linked by pentapeptide attached to the lactyl moiety of MurNAc sugar (Vollmer *et al.*, 2008; Typas *et al.*, 2012). Peptidoglycan synthesis requires glycosyltransferase (GTase) and transpeptidase (TPase) activities to polymerize and cross-link the glycan chains, respectively. The most important peptidoglycan synthases are high molecular weight penicillin-binding-proteins (HMW-PBPs), the
targets of β-lactam antibiotics. The HMW-PBPs are divided into two varieties: the class A PBPs (aPBPs) are bifunctional and have both GTase and TPase activities, while class B PBPs (bPBPs) are monofunctional and possess only TPase activity (Sauvage et al. 2008). Except for HMW-PBPs, recent studies demonstrated that SEDS (shape, elongation, division, and sporulation) proteins are a widespread family of peptidoglycan polymerases (Cho et al. 2016; Meeske et al. 2016).

To maintain peptidoglycan homeostasis, the activities of peptidoglycan synthases must be spatially and temporally controlled. In Escherichia coli, PBP1a and PBP1b are the major aPBPs and their activities are posttranslational regulated by the outer membrane lipoproteins LpoA and LpoB, respectively (Paradis-Bleau et al. 2010; Typas et al. 2010; Egan et al. 2014; Lupoli et al. 2014). It was found that each lipoprotein specifically interacts with its cognate PBP, and then stimulates the GTase and TPase activities. Therefore, two peptidoglycan-synthesizing complexes are formed, PBP1a/LpoA and PBP1b/LpoB. Many other factors including bPBPs, cytoskeletal-like elements, and peptidoglycan hydrolases may also be incorporated into the two PBP/Lpo complexes (Typas et al. 2012; Cho et al. 2016; Lai et al. 2017). Subsequently, the two complexes were identified in Vibrio cholerae, Shewanella oneidensis and Pseudomonas aeruginosa (Dörr et al. 2014; Greene et al. 2018), suggesting that lipoprotein-mediated activation of PBPs is conserved in many Gram-negative species.

In E. coli, the absence of either PBP1a or PBP1b does not affect cell viability whereas loss of both results in cell lysis, suggesting that PBP1a and PBP1b have redundant roles in peptidoglycan synthesis (Yousif et al. 1985; Paradis-Bleau et al. 2010; Typas et al. 2010). Nevertheless, phenotypes of strains lacking PBP1a or PBP1b are not identical. Strain lacking PBP1b but not PBP1a is more sensitive to β-lactam antibiotics and various stresses (Pepper et al. 2006; Paradis-Bleau et al. 2010; Sarkar et al. 2012; Mueller et al. 2019), and exhibits decreased biofilm formation (Kumar et al. 2012), suggesting that PBP1b is more prominent than PBP1a in E. coli.
Shewanella species are renowned for their respiratory versatility and regarded as reservoirs for antibiotic resistance genes (Poirel et al. 2004; 2005; Fredrickson et al. 2008; Canteón 2009; Allen et al. 2010; Potron et al. 2011). Our previous studies found that inactivation of PBP1a/LpoA but not PBP1b/LpoB in S. oneidensis, the representative of the Shewanella genus, drastically enhances β-lactamase expression, thus conferring the bacterium with hyperresistance to many β-lactams (Yin et al. 2013; 2014; 2015; 2018a; 2018b). Unlike strains lacking PBP1b/LpoB, strains lacking PBP1a/LpoA are more sensitive to SDS, vancomycin, and grow poorly in the absence of NaCl, suggesting that PBP1a/LpoA is required to maintain cell envelope integrity in S. oneidensis (Yin et al. 2018b). Intriguingly, loss of PBP1a/LpoA results in aberrant cell morphology, including branches and bulges (Yin et al. 2018b). These results indicate that PBP1a rather than PBP1b plays prominent roles in S. oneidensis.

Here we present evidence that E. coli PBP1a and S. oneidensis PBP1b, the less prominent aPBP in each strain, can functionally compensate for the loss of PBP1a in S. oneidensis. We further demonstrate that GTase and TPase activities of PBP1a are both essential for maintaining cell morphology and envelope integrity. Likewise, the combined enzymatic activities of PBP1b are required to compensate for the absence of PBP1a. Finally, we show that overexpression of inactive PBP1a variants, but not PBP1b variants, leads to changes in cell morphology.

MATERIALS AND METHODS

Bacterial strains, plasmids, PCR primers and culture conditions

The bacterial strains and plasmids used in this study are listed in Table 1. The primers used in this study are listed in Table S1. S. oneidensis and E. coli were grown in Luria-Bertani (LB) medium (Difco, Detroit, MI) at 30°C and 37°C, respectively. When appropriate, the growth medium was supplemented with chemicals at the following concentrations: 2,6-diaminopimelic acid (DAP), 0.3 mM; kanamycin (Km), 10 μg/mL; and gentamicin (Gm), 15 μg/mL.
Controlled expression of relevant genes

To assess the effects of the genes of interest expressed at various levels on cell morphology and envelope integrity, we amplified and placed each of them under the IPTG-inducible promoter $Ptac$ within pHGE-$Ptac$ (Luo et al. 2013) or under the arabinose-inducible promoter pBAD within pHGC02 (Yin et al. 2015). PCR amplification was carried out with genomic DNAs from the $S. oneidensis$ and $E. coli$ strains as the templates with the primers listed in Table S1. The resulting PCR products were digested by restriction enzymes corresponding to the restriction enzyme sites included in the primers, and then ligated to vectors with T4 DNA ligase and transformed into $E. coli$ WM3064. After verification by sequencing, the vectors were transferred into the relevant strains via conjugation. Cells carrying the vectors of interest were grown in the LB media in the presence of IPTG or arabinose at various levels.

Site-directed mutagenesis

pHGC02-$mrcA$ and pHGE-$mrcB$ were used as the template for site-directed mutagenesis with a QuikChange II XL kit (Stratagene) according to the method described before (Sun et al. 2013). Mutated PCR products were generated with primers listed in Table S1 in the supplemental material, subsequently digested by DpnI, and transformed into $E. coli$ WM3064. After sequencing verification, the resulting plasmid was transferred into the $S. oneidensis$ strains by conjugation.

Growth of $S. oneidensis$

For measuring growth of different $S. oneidensis$ strains, overnight cultures were diluted 1:100 in 3 mL fresh LB broth and incubated at 30°C with 200 rpm shaking. The optical density at 600 nm (OD$_{600}$) was recorded every hour after initial inoculation.
Spotting assay

The spotting assay was used to assess the susceptibility of *S. oneidensis* strains to hypo-osmotic stress and different antibiotics (Yin *et al.* 2018b). Overnight bacterial cultures were subcultured 1:100 in 3 mL fresh LB broth and grown to an OD$_{600}$ of 0.6 at 30°C with 200 rpm shaking. Samples were serially diluted, and then 3 μL of each dilution was spotted onto LB plates (containing 85 mM NaCl), LB-no salt plates, LB plates supplemented with 50 μg/mL vancomycin or 25 μg/mL ampicillin. When needed, different concentrations of isopropyl-β-D-thiogalactopyranoside (IPTG) or arabinose were added to the plates. The plates were incubated for 18 h at 30°C and then photographed.

Promoter activity assay

The promoter activity was determined using a markerless integrative lacZ reporter system as described previously (Yin *et al.* 2014). A segment containing the target promoter was amplified from genomic DNA and inserted into pHGEI01 by restriction enzyme digestion and ligation (Fu *et al.* 2014). After being verified by sequencing, the resultant vector was transferred to relevant *S. oneidensis* strains by conjugation for integration into the degenerated nrfCD locus. Cells grown to the mid-logarithmic (OD$_{600}$ of 0.4) were collected, and β-galactosidase activity assays were determined by monitoring color development at 420 nm using a Sunrise Microplate Reader (Tecan) as described previously (Yin *et al.* 2014).

Phase-contrast microscopy

*S. oneidensis* strains were cultivated to the logarithmic phase (OD$_{600}$ of 0.4) or stationary phase (OD$_{600}$ of 1.2). The morphology of cells was observed with a Motic BA410E phase-contrast microscope. Micrographs were captured with a Moticam Pro 285A digital camera and Motic images advanced 3.2 software (Motic Incorporation, China).
RESULTS

*S. oneidensis* PBP1a activity can be functionally substituted by *E. coli* PBP1a

The prominent aPBP in *S. oneidensis* (%PBP1a) is different from that in *E. coli* (%PBP1b). To determine why mutants lacking PBP1a are deleterious in *S. oneidensis*, we first compared the amino acid sequence of %PBP1a and %PBP1b with *E. coli* aPBPs. Indeed, %PBP1a shares a higher identity with %PBP1a than %PBP1b (50% vs. 21%), while %PBP1b shows more identity with %PBP1b than %PBP1a (40% vs. 27%). Similar to *E. coli* aPBPs (Typas et al. 2010), %PBP1a and %PBP1b are both anchored to the inner membrane by an N-terminal transmembrane region (TM) and composed of three domains, GTase, TPase and PBP-docking domains (ODD in %PBP1a and UB2H in %PBP1b) (Fig. 1A). %PBP1a and %PBP1b contain five signature motifs in the GTase domain and three signature motifs in the TPase domain, which are highly conserved with their *E. coli* counterparts (Born et al. 2006) (Fig. 1A and 1B).

We next asked whether %PBP1a or %PBP1b can functionally substitute for %PBP1a activity. To test this, the coding region of %mrcA or %mrcB was ligated into an IPTG-inducible plasmid pHGE-Ptax (Luo et al. 2013), and introduced into *S. oneidensis* mutants lacking %PBP1a (ΔmrcA). This mutant harboring the empty vector failed to grow on LB-no salt medium, a phenotype indicative of defects in the cell envelope as we described previously (Yin et al. 2018b). As expected, ΔmrcA cells expressing %mrcA in trans restored growth on LB-no salt medium (Fig. 1C). Meanwhile, expression of %mrcA restored growth of the ΔmrcA strain on LB-no salt medium. By contrast, expression of %mrcB was unable to restore growth of the strain under this hyp-osmotic condition (Fig. 1C). Consistent with our previous results (Yin et al. 2018b), ΔmrcA cells carrying the control vector exhibited aberrant cell morphology (including branches and bulges), especially in stationary phase. These cells expressing %mrcA, %mrcA but not %mrcB returned to wild type rod shapes (Fig. 1D). Collectively, these results suggest that %PBP1a but not %PBP1b is capable of
substituting for SoPBP1a activity, even though EcPBP1a plays a less prominent role in E. coli.

Additionally, the plasmid harboring Ec mrcA was introduced into a strain lacking SoPBP1a and its activator SoLpoA simultaneously (ΔmrcAΔlpoA). The ΔmrcAΔlpoA strain displayed phenotypes resembling the ΔmrcA strain (Yin et al. 2018b). Intriguingly, expression of Ec mrcA did not fully rescue the growth defect of the ΔmrcAΔlpoA strain on LB-no salt medium (Fig. 1C), suggesting that SoLpoA is required for EcPBP1a activity in S. oneidensis.

**Additional PBP1b can functionally compensate for PBP1a mutation**

Although S. oneidensis possesses another bifunctional aPBP, PBP1b, our preceding results indicated that PBP1b cannot support cell morphology and envelope integrity in the absence of PBP1a (Yin et al. 2018b). One possible explanation is that the abundance of PBP1b is lower than PBP1a in S. oneidensis. To test this, we employed a markerless integrative lacZ-reporter system (pHGEI01) to determine the promoter activities of mrcA (PmrcA) and mrcB (PmrcB) (Fu et al. 2014). In the wild type strain, the expression level of β-galactosidase driven by PmrcA was ~5-fold higher than that induced by PmrcB (Fig. 2A). Notably, the activity of PmrcB in the ΔmrcA strain equated approximately to that in the wild type strain (Fig. 2A). Therefore, the expression of PBP1b is lower than that of PBP1a in S. oneidensis.

Given that the normal amount of PBP1b is insufficient to compensate for the loss of PBP1a, we proposed that additional PBP1b would rescue the phenotypes of PBP1a mutation. To confirm, the coding region of mrcB was ligated into plasmid pHGE-Ptac, and then introduced into the ΔmrcAΔlpoA strain. While the introduction of the empty vector or IPTG did not affect any phenotypes, the ΔmrcAΔlpoA strain harboring the recombinant plasmid restored the ability to grow on the LB-no salt medium and vancomycin-supplemented medium following induction with 0.01 mM or even without IPTG (Fig. 2B and Fig. S1). It has been reported that the tac promoter (Ptac) within pHGE-Ptac is leaky (Shi et al. 2014; Yin et al. 2016), implying that the
leaky or low-level expression of mrcB driven by the Ptac is adequate for maintaining cell envelope integrity of the ΔmrcAΔlpoA strain. However, the strain failed to grow on the LB-no salt medium and vancomycin-supplemented medium when treated with high levels of IPTG (>0.05 mM) (Fig. 2B).

Consistently, the ΔmrcAΔlpoA strain carrying the recombinant plasmid displayed a normal rod shape in the presence of low levels of IPTG (<0.01 mM), whereas all cells displayed aberrant cell shapes after induction with high levels of IPTG (>0.05 mM), which reproduces the morphology of the ΔmrcAΔlpoA strain (Fig. 2C). Moreover, β-lactam resistance of the ΔmrcAΔlpoA strain was partially restored by the induction of mrcB when compared to the wild type strain (Fig. S1). These data, collectively, suggest that the upregulation of mrcB can functionally compensate for the absence of PBP1a/LpoA complex.

**PBP1a GTase and TPase activities are both required for its function**

PBP1a is bifunctional and possesses both GTase and TPase activities. To determine which catalytic function is responsible for cell morphology and envelope integrity, we expressed SoPBP1a variants lacking GTase or TPase activity in the ΔmrcA strain. In EcPBP1a, the E86Q and S465A mutation inactivate the GTase and TPase activity, respectively (Born et al. 2006). Based on the aforementioned sequence identity between EcPBP1a and SoPBP1a, it is reasonable that the E86Q and S464A mutation inactivate the GTase and TPase activity of SoPBP1a, respectively (Fig. 1B). The SoPBP1a variants were constructed by site-directed mutagenesis of the plasmid pHGC02-mrcA, which contains the mrcA gene under the control of the arabinose-inducible araBAD promoter (pBAD) (Yin et al., 2015). However, the PBP1a variants lacking GTase (mrcA^{E86Q}) or TPase (mrcA^{S464A}) failed to rescue the growth defect and the morphological defect of the ΔmrcA strain (Fig. 3A and 3B). Additionally, the ampicillin resistance of the ΔmrcA strain was not affected when complemented by PBP1a variants lacking either GTase or TPase activity (Fig. 3A).
These results suggest that the GTase and TPase activities of PBP1a are both required for its normal physiological function.

**PBP1b GTase and TPase are both required for functional compensation**

To determine which activity of PBP1b is essential for functional compensation, the ΔmrcAΔlpoA strain was provided with PBP1b variants lacking GTase or TPase activity. It was shown that the E233Q mutation inactivates the GTase activity and that the S510A mutation inactivates the TPase activity of PBP1b in *E. coli* (Terrak *et al.* 1999; Meisel *et al.* 2003). Correspondingly, the E193Q and S469A mutation should inactivate the GTase and TPase activity of *S. oneidensis* PBP1b, respectively (Fig. 1B). In contrast to the wild type PBP1b, the PBP1b variants lacking GTase (mrcB<sup>E193Q</sup>) or TPase (mrcB<sup>S469A</sup>) failed to restore any phenotypes of the ΔmrcAΔlpoA strain in the absence or presence of IPTG (Fig. 4A and 4B), suggesting that both GTase and TPase activities of PBP1b are indispensable for functional compensation.

**Overexpression of PBP1a variants affects cell morphology**

It has been reported that the overproduction of inactive PBP1b variants leads to lysis of the wild type cells in *E. coli*, which is similar to the case of penicillin-induced lysis (Meisel *et al.* 2003). To investigate the effects of aPBPs overexpression on wild type cells morphology in *S. oneidensis*, we expressed native aPBPs or aPBPs variants in the wild type strain. The wild type strain harboring native aPBPs or aPBPs variants grew normally in the absence of inducer. However, overexpression of PBP1a variants led to a slight reduction in stationary phase OD, while overexpression of PBP1b variants negatively affected growth of the wild type strain (Fig. S2).

We next examined the morphology of stationary-phase cells by phase-contrast microscopy. Wild type cells harboring the empty vector or native aPBPs displayed typical rod-shaped morphology under all test conditions (Fig. 5A and 5B). Interestingly, overexpression of inactive PBP1a or PBP1b variants had different effects on cell morphology. Regardless of IPTG concentration, wild type cells...
expressing PBP1b variants lacking either GTase \((mrcB^{E193Q})\) or TPase \((mrcB^{S469A})\) activity retained normal rod shapes (Fig. 5B). However, wild type cells producing PBP1a variant lacking GTase \((mrcA^{E86Q})\) retained its normal rod shape in the presence of arabinose at low level (0.002%) but became spherical when supplemented with arabinose at high level (0.2%), whereas cells carrying PBP1a variant lacking TPase \((mrcA^{S464A})\) became spherical even in the presence of arabinose at low level (0.002%) (Fig. 5A). Overall, these data demonstrate that overexpression of inactive variants of PBP1a but not PBP1b has detrimental effects on cell morphology in \(S.\ oneidensis\).

**DISCUSSION**

Two multienzyme complexes, PBP1a/LpoA and PBP1b/LpoB, are employed by rod-shaped Gram-negative bacteria for peptidoglycan synthesis (Typas et al. 2012; Greene et al. 2018). Although they appear to be largely redundant \textit{in vivo}, strains lacking individual complexes display different phenotypes. In \(E.\ coli\), the biological roles of PBP1b/LpoB are more important when compared to PBP1a/LpoA (Ranjit et al. 2017). However, our previous studies demonstrated that PBP1a/LpoA play more prominent roles than PBP1b/LpoB in \(S.\ oneidensis\) (Yin et al. 2015; 2018b), which is in agreement with that in \(V.\ cholerae\) (Dörr et al. 2014). \(S.\ oneidensis\) is relatively distant from \(E.\ coli\) among the Gammaproteobacteria, it is therefore likely that PBP1a/LpoA and PBP1b/LpoB evolve different functions after the divergence of the orders \textit{Alteromonadales} and \textit{Enterobacteriales}.

In \(E.\ coli\), \(Ec\)PBP1b and \(Ec\)LpoB (1000 and 2300 molecules per cell respectively) have higher copy numbers than \(Ec\)PBP1a and \(Ec\)LpoA (both 500 molecules per cell) (den Blaauwen and Nanninga, 1990; Paradis-Bleau et al. 2010). Meanwhile, the \textit{in vitro} activities of \(Ec\)PBP1b are more active than \(Ec\)PBP1a (Kraus and Höl tj e 1987; Egan et al. 2015). These results suggest that \(Ec\)PBP1a and \(Ec\)PBP1b have different protein levels and enzymatic activities, which may account for the different biological
roles of EcPBP1a and EcPBP1b. In this study, we found that the expression of SoPBP1b is lower than that of SoPBP1a in S. oneidensis, and additional expression of SoPBP1b at low levels rescues almost all phenotypes of SoPBP1a mutation. Therefore, it is reasonable that the low abundance of SoPBP1b may explain why endogenous SoPBP1b cannot compensate for the loss of SoPBP1a fully in S. oneidensis. Another possibility that cannot be ruled out is that SoPBP1b may be a less efficient enzyme.

In contrast to SoPBP1b and EcPBP1a, expression of EcPBP1b fails to restore the phenotypes of SoPBP1a mutation. In E. coli, EcLpoB is required to bind to the UB2H domain of EcPBP1b and then stimulate EcPBP1b activity (Paradis-Bleau et al. 2010; Typas et al. 2010). It is notable that the UB2H domain of SoPBP1b is 33% identical and 54% similar to that of EcPBP1b. More importantly, our previous study showed that SoLpoB shares only 17% identity and 29% similarity with EcLpoB (Yin et al. 2015). Based on these, we can speculate that SoLpoB may not interact specifically with EcPBP1b, which explain why EcPBP1b cannot compensate for the loss of SoPBP1a. Consistent with this conclusion, P. aeruginosa PBP1b also cannot functionally substitute for E. coli aPBP Activity (Greene et al. 2018).

Our results demonstrate that the GTase and TPase activities of SoPBP1a are both essential for maintaining cell wall homeostasis in S. oneidensis. On the one hand, inactive SoPBP1a variants lacking GTase or TPase fail to maintain cell morphology and envelope integrity. Inactive SoPBP1b variants lacking GTase or TPase also fail to compensate for the absence of SoPBP1a. Consistently, EcPBP1b GTase and TPase activities are both required to create normally shaped cells de novo in the absence of a preexisting cell wall template (Ranjit et al. 2017). Additionally, the combined enzymatic activities of EcPBP1b are essential to prevent lysis in E. coli strain lacking EcPBP1b (Jorgenson et al. 2019). On the other hand, overexpression of inactive SoPBP1a variants results in aberrant cell morphology in the wild type strain. Similar results were observed in E. coli, in which overproduction of the inactive variants of EcPBP1b causes a lysis effect similar to the case of penicillin-induced lysis (Meisel et al. 2003). The most probable explanation is that the inactive variants of SoPBP1a or
EcPBP1b replace the native enzyme in the multienzyme peptidoglycan-synthesizing complex. The resulting complexes still contain active peptidoglycan hydrolases, thus disturbing the balance between peptidoglycan synthesis and hydrolysis. Consistent with this explanation, overexpression of *E. coli* D,D-carboxypeptidases, which remove the terminal D-Alanine from the pentapeptide side-chains, results in cell morphology irregularities and increased cell envelope permeability (Ghosh *et al.* 2008; Yang *et al.* 2019).

Interestingly, SoPBP1a variant lacking TPase (*mrcA*<sup>S464A</sup>) has more detrimental effects than SoPBP1a variant lacking GTase (*mrcA*<sup>E86Q</sup>) on cell morphology. It is notable that the TPase activity requires ongoing GTase activity in *E. coli* (Egan *et al.* 2015), suggesting that SoPBP1a variant lacking GTase in *S. oneidensis* may also lack TPase activity. However, SoPBP1a variant lacking TPase still retains GTase activity and is capable of catalyzing glycan strand polymerization but not peptide cross-linking. It is reminiscent of the lethal activity of mecillinam and other β-lactams. The inhibition of TPase by β-lactams in *E. coli* leads to the formation of uncrosslinked glycans that are rapidly degraded into fragments, eventually inducing a deleterious futile cycle of cell wall synthesis (Cho *et al.* 2014). Therefore, the overproduction of SoPBP1a variant lacking TPase may stimulate the futile cycle of peptidoglycan synthesis. Overall, the expression and activities of peptidoglycan synthases have to be tightly regulated to ensure shape-maintaining growth and cell division (Typas *et al.* 2012; Egan *et al.* 2015; 2017).

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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Figure 1. *E. coli* PBP1a is capable of substituting for the *S. oneidensis* PBP1a. (A) Domain organization and signature motifs in *S. oneidensis* PBP1a (SoPBP1a) and PBP1b (SoPBP1b). Black bars, signature motifs of the GTase and TPase domains. (B) Sequence alignment of the signature motifs between *E. coli*=
Figure 2. Phenotypes of PBP1a/LpoA mutation are rescued by low levels of PBP1b but not high levels. (A) Expression of β-galactosidase controlled by the promoter of mrcA (P\textsubscript{mrcA}) and mrcB (P\textsubscript{mrcB}) in the wild type (WT) and ΔmrcA strains. The data represented the mean of three biological replicates with standard deviation. (B) The sensitivity of S. oneidensis cells to hypo-osmotic stress (LB-no salt) and vancomycin (VAN). Experiments were performed the same as described in Fig. 1. 3 μl of each dilution was spotted onto the indicated plates in the presence of IPTG at various concentrations. (C) Phase-contrast microscopy of S. oneidensis cells at the stationary phase (OD\textsubscript{600nm} of 1.2). In both panels B and C, vec represents the empty vector pHGE-P\textsubscript{tac}. Also see data in Fig. S1.
Figure 3. Contribution of PBP1a GTase and TPase activities to cell morphology and envelope integrity. (A) Growth of *S. oneidensis* strains on LB, LB-no salt, and LB plates supplemented with 50 μg/ml vancomycin (VAN) or 25 μg/ml ampicillin (AMP). Experiments were performed the same as described in Fig. 1. (B) Phase contrast microscopy of *S. oneidensis* cells at the stationary phase (OD$_{600nm}$ of 1.2). In both panels, vec, *mrcA*$_{E86Q}$ and *mrcA*$_{S464A}$ represent the empty vector pHGC02, a plasmid expressing *S. oneidensis* PBP1a variant with the E86Q mutation and a plasmid expressing *S. oneidensis* PBP1a variant with the S464A mutation, respectively. Scale bar, 2.5 μm.
**Figure 4.** Inactive PBP1b variants cannot functionally compensate for PBP1a/LpoA mutation. (A) The sensitivity of *S. oneidensis* cells to hypo-osmotic stress (LB-no salt) and vancomycin (VAN) in the presence of 0.01 mM IPTG. (B) Phase-contrast microscopy of *S. oneidensis* cells at the stationary phase (OD_{600nm} of 1.2) with or without IPTG. Experiments were performed the same as described in Fig. 1. In both panels, vec, *mrcB_{E193Q}* and *mrcB^{S469A}* represent the empty vector pHGE-P**tac**, a plasmid expressing *S. oneidensis* PBP1b variant with the E193Q mutation and a plasmid expressing *S. oneidensis* PBP1a variant with the S469A mutation, respectively. Scale bar, 2.5 μm.
Figure 5. Phase-contrast microscopy of *S. oneidensis* wild type cells expressing native or inactive variants of PBP1a (A) and PBP1b (B). In panel A, native PBP1a (*mrcA*), PBP1a variants with E86Q mutation (*mrcA*<sup>E86Q</sup>) or S464A mutation (*mrcA*<sup>S464A</sup>) were under the control of the pBAD promoter. In panel B, native PBP1b (*mrcB*), PBP1b variants with E193Q mutation (*mrcB*<sup>E193Q</sup>) or S469A mutation (*mrcB*<sup>S469A</sup>) were under the control of the Ptac promoter. Also see data in Fig. S2.
Table 1 Bacterial strains and plasmids used in this study.

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<th>Strain or plasmid</th>
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<td><strong>S. oneidensis strains</strong></td>
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<td>MR-1</td>
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<td>HG0280</td>
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<td>Yin et al. 2015</td>
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<td><strong>Plasmids</strong></td>
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<td>pHGEI01</td>
<td>Integrative E. coli lacZ reporter vector</td>
<td>Fu et al. 2014</td>
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<td>pHGC02</td>
<td>Arabinose-inducible expression vector containing the pBAD promoter</td>
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<td>pHGE-Ppac</td>
<td>IPTG-inducible expression vector containing the Ppac promoter</td>
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<sup>a</sup> UIUC, University of Illinois at Urbana-Champaign.