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Interaction of Streptococcus mutans YidC1 and YidC2 with Translating and Nontranslating Ribosomes

Zht Cheng Wu, Jeanine de Keyzer, Greetje A. Berrelkamp-Lahpor, Arnold J. M. Driessen

The YidC/OxaI/Alb3 family of membrane proteins is involved in the biogenesis of integral membrane proteins in bacteria, mitochondria, and chloroplasts. Gram-positive bacteria often contain multiple YidC paralogs that can be subdivided into two major classes, namely, YidC1 and YidC2. The Streptococcus mutans YidC1 and YidC2 proteins possess C-terminal tails that differ in charges (+9 and +14) and lengths (33 and 61 amino acids). The longer YidC2 C terminus bears a resemblance to the C-terminal ribosome-binding domain of the mitochondrial OxaI protein and, in contrast to the shorter YidC1 C terminus, can mediate the interaction with mitochondrial ribosomes. These observations have led to the suggestion that YidC1 and YidC2 differ in their abilities to interact with ribosomes. However, the interaction with bacterial translating ribosomes has never been addressed. Here we demonstrate that Escherichia coli ribosomes are able to interact with both YidC1 and YidC2. The interaction is stimulated by the presence of a nascent membrane protein substrate and abolished upon deletion of the C-terminal tail, which also abrogates the YidC-dependent membrane insertion of subunit c of the F$_{1}$F$_{0}$-ATPase into the membrane. It is concluded that both YidC1 and YidC2 interact with ribosomes, suggesting that the modes of membrane insertion by these membrane insertases are similar.

The bacterial membrane protein YidC is a member of YidC/OxaI/Alb3 protein family that is universally conserved in all three domains of life (1). YidC/OxaI/Alb3 proteins are known to contribute to the insertion, folding, and multisubunit complex assembly of membrane proteins (2, 3). Several studies have shown that the function of YidC family members is conserved and that these membrane protein insertases are interchangeable. For instance, Escherichia coli YidC and its mitochondrial inner membrane homologue OxaI can be functionally replaced (4, 5). The numbers of YidC/OxaI/Alb3-like proteins differ between species and range from one single YidC protein in Gram-negative bacteria up to six copies in the plant Arabidopsis thaliana (2, 6, 7). Gram-positive bacteria, such as bacilli, lactobacilli, streptococci, and actinobacteria, often harbor two YidC homologs (1). For instance, Bacillus subtilis YigG and SpoIIIJ are homologous to E. coli YidC; and either protein suffices to support vegetative growth (8, 9). On the other hand, YigG and SpoIIIJ fulfill specific functions in natural competence development and sporulation, respectively (10, 11). Likewise, Streptococcus mutans contains two YidC homologs, termed YidC1 and YidC2 (Fig. 1A). Either of these is sufficient for viability, but the proteins also perform specific roles as single gene deletions affect the cell function differently (12). Deletion of the yidC1 gene results in impaired biofilm formation (13), while deletion of the yidC2 gene causes a stress-sensitive phenotype comparable to that of mutants lacking the SRP pathway (12). The latter concerns impaired biofilm formation, decreased ATPase activity, and growth defects under acid, osmotic, and oxidative stress conditions (12). Interestingly, the stress tolerance and ATPase activity defects of the ΔyidC2 mutant are complemented by a chimeric YidC1 protein that bears the C terminus of YidC2 (14). Therefore, the C terminus appears to play a key role in the specific YidC function.

The C termini of YidC1 and YidC2 are both highly positively charged, but the C terminus of YidC2 is much longer (61 residues) and carries a higher net charge (+14) than the C terminus of YidC1 (33 residues, +9) (Fig. 1A). In this respect, the YidC2 C terminus more closely matches the C terminus of OxaI (86 residues, +14), which acts as a binding site for mitochondrial ribosomes (15). In the yeast Saccharomyces cerevisiae, YidC2 and chimeras of OxaI or Escherichia coli YidC carrying YidC2 C terminus are able to partially complement the OxaI deletion phenotype for growth on nonfermentable carbon sources (16). In contrast, chimeras of YidC2 with the YidC1 C terminus were unable to complement the OxaI function (16), while complementation by YidC1 could not be determined as this protein did not reach the mitochondrial inner membrane. Furthermore, YidC2 and the chimeras of OxaI and the E. coli YidC carrying the C terminus of YidC2 were shown to coprecipitate with mitochondrial ribosomes (16). These observations point at an important structural and functional role of the C-terminal tail in mitochondrial ribosome binding and led to the suggestion that YidC1 and YidC2 may differ in their modes of action, suggesting that only the latter is able to support cotranslational membrane insertion through a direct interaction with ribosomes (16). Importantly, mitochondrial and bacterial ribosomes are very different in terms of composition and structure. While the RNA/protein ratio for some mitochondrial ribosomes is 1/3, bacterial ribosomes usually have a ratio of 2/1, which corresponds to a much greater RNA content of the bacterial ribosomes (17). Although detailed structural information on mitochondrial ribosomes is limited, cryo-electron microscopy
(cryo-EM) and biochemical studies indicate substantial differences in the exit tunnel area of bacterial and mitochondrial ribosomes (18–20). This part of the ribosome is considered to constitute the YidC binding site, yet which specific characteristics determine the interaction is largely unresolved. The features that contribute to the binding of mitochondrial ribosomes to OxaI may differ from those of the bacterial ribosome-YidC interaction. Moreover, with the mitochondrial ribosomes, the impact of the presence of a nascent chain on the binding characteristics has never been assessed, whereas this may be a critical feature of protein targeting to YidC. Therefore, we have investigated the interaction of S. mutans YidC1 and YidC2 with translating and nontranslating ribosomes derived from E. coli. Using surface plasmon resonance (SPR), we demonstrate that both membrane insertases are capable of E. coli ribosome binding involving their positively charged C termini. This interaction appears to be essential for membrane insertion of subunit c of the F₁Fₒ-ATPase (F₝c), suggesting that the modes of membrane insertion by these membrane insertases are similar.

**MATERIALS AND METHODS**

**Strains and plasmids.** yidC1 (SMU.337) and yidC2 (SMU.1727) were PCR amplified using Phusion polymerase (Finnzymes) with genomic DNA of S. mutans UA159 (21) as the template and the forward and reverse primers listed in Table 1. PCR products were cleaved with NcoI and BamHI and ligated into the corresponding sites of pTRC99a (22), yielding pZW337 and pZW1727, respectively. The same yidC2 (SMU.1727) PCR product was also ligated between the NcoI and BamHI sites of pET15b, yielding pJK821. To delete the C terminus of YidC, yidC1 was amplified using SMU.337fw and SMU.337(1–240)rev (Table 1) and ligated into pTRC99a, yielding pZW337(1–240). To delete the C-terminal 36 residues of YidC2, yidC2(1–274) was amplified using SMU.1727(1–274)rev, cleaved with NcoI and BamHI, and cloned into pET15b, yielding pJK823. To obtain stable ribosomes charged with F₝c nascent chains, the PstI-EcoRV fragment of the plasmid pUC19Strep3FtsQSecM (23) was exchanged with a fragment coding for the first 44 residues of E. coli F₝c, which includes first transmembrane domain and part of the cytoplasmic loop. The resulting plasmid (pJK763) encodes the F₝c fragment preceded by an N-terminal triple Strep-tag and a C-terminal SecM stalling sequence yielding a nascent chain length of 123 amino acids. All plasmids were
verified by sequence analysis. The *E. coli* strain Lemo21(DE3) was from New England BioLabs.

**Overexpression of YidC1 and YidC2 in *E. coli*.** *E. coli* BL21(ADE3) (Table 2) was transformed with pZW337, pZW1727, or pZW337(1–240). Cells were cultured in LB supplemented with 0.5% glucose and 100 μg/ml ampicillin at 30°C to an optical density at 600 nm (OD$_{600}$) of 0.7. Overexpression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and cells were grown for an additional 30 min. Cells were harvested using the Beckmann JLA8.1000 rotor (6,900 rpm, 15 min, 4°C), subjected to ultracentrifugation using the Beckmann JLA8.1000 rotor (6,900 rpm, 15 min, 4°C), and inner membrane vesicles (IMVs) were isolated as described previously (24). For the overexpression of YidC2 and YidC2(1–274) in *E. coli* Lemo21(DE3), cells were transformed with pJK821 or pJK823 and cultered in LB supplemented with 0.25 mM thiamine at 30°C to an OD$_{600}$ of 0.5. Overexpression was induced with 0.4 mM IPTG, and cells were grown for an additional 3 h before harvesting.

**Ribosomes and RNC complex purification.** Ribosomes and F$_c$-ribosome nascent chain (RNC) complexes were isolated from *E. coli* BL21(DE3)Δtig (Table 2) or BL21(DE3)Δtig transformed with pJK763 as described previously (25). The trigger factor deletion strain was used to prevent possible copurification of this abundant chaperone with the nascent chains. However, since identical results were obtained with ribosomes isolated from the wild-type *E. coli* strain MRE600 (data not shown), there is no evidence that trigger factor would influence the binding of ribosomes to YidC. The ribosome concentration was determined spectrophotometrically at a wavelength of 260 nm using an extinction coefficient of 4.2 × 10$^4$ liters mol$^{-1}$ cm$^{-1}$. Stalled nascent chains were detected by SDS-PAGE followed by Western blotting using an antibody against the Strept-tag (IBA).

**SPR.** Surface plasmon resonance (SPR) analysis of the binding of ribosomes or RNCs to YidC1- or YidC2-containing membranes was performed on Biacore 2000 and 3000 systems (GE Healthcare) as described previously (25, 26). IMVs isolated from BL21(DE3) or LEMO(DE3) cells or IMVs containing overexpressed levels of YidC1 or YidC2 were immobilized on a 1.1 sensor chip (GE Healthcare) in separate channels with similar loading levels (∼4,000 response units [RU]) in each channel. Ribosomes or RNCs in buffer B (50 mM Tris-HCl [pH 7.5], 150 mM KCl, 5 mM MgCl$_2$, 1 mM dithiothreitol [DTT]), 0.5 mg/ml bovine serum albumin (BSA) were injected into the channel at a flow rate of 20 μl/min. The binding surface was regenerated by a brief injection of 100 mM Na$_2$CO$_3$ (pH 10). Data were corrected for background binding to wild-type IMVs to obtain specific binding signals and analyzed using BiaEvaluation software version 4.1 (Biacore). This background signal consists of the contributions of the bulk flow and ribosome binding to the endogenous levels of the *E. coli* YidC and SecYEG proteins.

**Miscellaneous methods.** The *in vitro* insertion of F$_c$ into IMVs was performed as described previously (27). SDS-PAGE was performed according to standard protocols. IMV concentrations were determined using the Bio-Rad DC protein assay kit using BSA as the standard.

**RESULTS**

**Functional overexpression of *S. mutans* YidC1 and YidC2 in *E. coli*.** The *S. mutans* yidC1 and yidC2 genes were cloned into pTRC99A and overexpressed in *E. coli* BL21(DE3). SDS-PAGE analysis of isolated inner membrane vesicles (IMVs) showed additional bands of 23 and 27 kDa corresponding to full-length YidC1 and YidC2, respectively (28) (Fig. 1B). Previously it was shown that *S. mutans* YidC1 and YidC2 can be functionally expressed in *E. coli*, as they partially complement the defects in growth and membrane insertion of the c subunit of the F$_c$-F$_o$ ATPase (F$_c$ of an *E. coli* YidC deletion strain (28)). F$_c$ is a small double-spanning membrane protein that inserts in a YidC-dependent manner (29). To confirm that the overexpressed YidC1 and YidC2 were active, their ability to mediate F$_c$ insertion was examined in an *in vitro* membrane insertion assay. *E. coli* F$_c$ was synthesized *in vitro* in the presence of wild-type IMVs containing endogenous levels of *E. coli* YidC or IMVs containing overexpressed YidC1 or YidC2, and F$_c$ membrane insertion was assayed by its resistance to proteinase K (29). Overexpression of

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tr>
<td>SMU.337 fw</td>
<td>CGAGATCGCCATGGTAAAGAAAATATAGAATTATG</td>
</tr>
<tr>
<td>SMU.1727 fw</td>
<td>CGGTCTCGCATTGGTAAAGAAAATATAGACGTCTTTATTTT</td>
</tr>
<tr>
<td>SMU.337 rv</td>
<td>CGGCCCGGATCCTTATTGCTTATGGTGACG</td>
</tr>
<tr>
<td>SMU.1727 rv</td>
<td>CGGCCCGGATCCTTATTGCTTATGGTGACG</td>
</tr>
<tr>
<td>SMU.337(1–240) rv</td>
<td>CGACAGCGGATCTTAAAGGGATATTTCAAACAGAATCTG</td>
</tr>
<tr>
<td>SMU.1727(1–274) rv</td>
<td>CCCCGCGGGATCCTTATTGCTTATGGTGACG</td>
</tr>
</tbody>
</table>

**Table 1** PCR primers used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain or plasmid</td>
<td>Characteristic(s)</td>
</tr>
<tr>
<td>Bl21(ADE3)</td>
<td>F$^-$ dcm ompT hsdS$_E$(r$_E$− m$_E^−$) gal (ADE3)</td>
</tr>
<tr>
<td>Bl21(DE3)Δtig</td>
<td>F$^-$ dcm ompT hsdS$_E$(r$_E$− m$_E^−$) gal (DE3)Δtig</td>
</tr>
<tr>
<td>Lemo21(DE3)</td>
<td>fluA2 [lon] ompT gal (ADE3) dcm ΔhsdS/pLemo(Cam)$'$</td>
</tr>
<tr>
<td>pTRC99A</td>
<td>Expression vector for <em>E. coli</em> based on pKK233-2, carrying hybrid trp/lac promoter and multiple-cloning site of pUC18; Amp$'$</td>
</tr>
<tr>
<td>pET15b</td>
<td>Expression vector with T7 promoter</td>
</tr>
<tr>
<td>pZW337</td>
<td><em>S. mutans</em> yidC1 in pTRC99A</td>
</tr>
<tr>
<td>pZW1727</td>
<td><em>S. mutans</em> yidC2 in pTRC99A</td>
</tr>
<tr>
<td>pZW337(1–240)</td>
<td><em>S. mutans</em> YidC1(1–240) in pTRC99A</td>
</tr>
<tr>
<td>pJK821</td>
<td><em>S. mutans</em> yidC2 in pET15</td>
</tr>
<tr>
<td>pJK823</td>
<td><em>S. mutans</em> YidC2(1–274) in pET15b</td>
</tr>
<tr>
<td>pJK763</td>
<td>pUC19-based plasmid for expression of first 44 residues of <em>E. coli</em> F$_c$; preceded by triple Strept-tag, followed by SecM stalling sequence</td>
</tr>
</tbody>
</table>
YidC1 or YidC2 stimulated the membrane insertion of FocC above the levels observed with only the endogenous YidC (Fig. 2A). These data show that YidC1 and YidC2 of S. mutans can be functionally overexpressed in E. coli and that both proteins support the membrane insertion of FocC.

**YidC1 and YidC2 interact with translating and nontranslating E. coli ribosomes.** Surface plasmon resonance (SPR) was used to analyze qualitatively the binding of E. coli ribosomes to the membrane-embedded S. mutans YidC1 or YidC2 proteins. SPR was previously shown to accurately detect the high-affinity interaction between ribosomes and the SecYEG complex (25). Here we used E. coli ribosomes as so far there is no method described for the production of RNCs from S. mutans. Our in vitro work described in the previous section demonstrates that E. coli ribosomes functionally cooperate with YidC1/YidC2 and, previous work has demonstrated that S. mutans YidC1/YidC2 can functionally complement the growth defect of an E. coli YidC deletion strain (28). IMVs containing overexpressed YidC1 or YidC2 were immobilized at comparable amounts on an L1 chip (25, 26), and E. coli ribosomes were injected in the flow. To compensate for “bulk” contributions to the SPR signal and background binding of ribosomes to membranes, all measurements were corrected for binding to similar amounts of wild-type IMVs that only contain the native levels of YidC and SecYEG present in a reference channel (26) (see Fig. S1 in the supplemental material). Ribosome binding to YidC2 resulted in a specific binding response of 88 RU (Fig. 3C). Surprisingly, the E. coli ribosomes also interacted with YidC1, resulting in an even higher binding response of 132 RU (Fig. 3C). Although the difference in binding responses may be due to a difference in overexpression levels (Fig. 1B), these data clearly demonstrate that both YidC1 and YidC2 are able to interact with E. coli ribosomes.

We have recently shown that the presence of a nascent chain at the ribosome highly stimulates the interaction between ribosomes and the SecYEG complex (25, 26). To determine whether this is also the case with YidC, ribosomes carrying a chimeric nascent chain of E. coli FocC were isolated. To halt translation, a construct was made in which the stalling motif of the E. coli secretion monitor protein SecM (30) was fused behind the first 44 amino acids of FocC, resulting in nascent chains 83 amino acids long (23). At this nascent chain length, the first transmembrane segment (TMS) of FocC will be exposed from the ribosomal exit tunnel (Fig. 3A). Nascent FocC was preceded by a triple Strep-tag to allow for the specific isolation of the RNCs (31) (Fig. 3B), extending the nascent chain by 40 amino acids. The presence of the FocC nascent chain resulted in a dramatic increase in ribosome binding to YidC1 and YidC2, yielding responses of 380 and 220 RU, respectively (Fig. 3D).

Previously, we observed that the interaction between ribosomes or RNCs and the SecYEG complex cannot be described by simple bimolecular interaction models (25). Similarly, simple data-fitting models did not accurately describe the interaction between RNCs and YidC1 or YidC2 (data not shown). Best fits were obtained using a “bivalent analyte” interaction model yielding K_D (equilibrium dissociation constant) values in the low nanomolar range (~0.2 nM) that did not significantly differ for YidC1 and YidC2. Such high affinity is also evident from sensorgrams displaying a very slow dissociation of the RNCs and the need to perform high-pH carbonate treatment to regenerate the binding sites. Although the exact mechanism of the interaction cannot be explained by fitting algorithms alone, these data clearly indicate that both YidC1 and YidC2 interact very strongly with the RNCs. Taken together, our data show that both S. mutans YidC proteins bind ribosomes and this interaction is enhanced by the presence of nascent FocC.

**Deletion of the C-terminal tail of YidC1 and YidC2 abrogates binding of ribosomes and RNCs.** Previous studies indicate that the long (61 residues) positively charged C-terminal tail of YidC mediates the interaction with mitochondrial ribosomes (16). To determine whether this region is also involved in E. coli ribosome binding, a C-terminal deletion mutant was created. Complete deletion of the C-terminal tail of YidC2 ([i.e., YidC2(1–254)], did not result in significant overexpression in E. coli (data not shown). However, shortening the C terminus only by 36 residues, yielding YidC2(1–274) and thereby reducing the net charge from +14 to +5, allowed overexpression in E. coli Lemo21(DE3) to a similar level as full-length YidC2 (Fig. 1C). This partial deletion of the C terminus completely abolished the ability of YidC2 to interact with ribosomes (Fig. 4C) and FocC-RNCs (Fig. 4D). Although the C-terminal tail of YidC1 is significantly shorter (33 residues) than the C terminus of YidC2, it also carries a high positive charge (+9). To test whether the YidC1-ribosome interaction also involves the C-terminal tail, a YidC1 mutant was constructed in which the C-terminal 31 residues were deleted. YidC1(1–240) was overexpressed in E. coli BL21(DE3) (Fig. 1B). Also, the YidC1(1–240) mutant was unable to interact with ribosomes (Fig. 4A) or FocC-RNCs (Fig. 4B). This implies that with both YidC1 and YidC2, an intact C-terminal tail is essential for ribosome binding.

**YidC1 and YidC2 proteins with a deleted C-terminal tail are defective in membrane protein insertion.** To determine whether the ribosome binding defect also results in a functional defect in membrane protein insertion, YidC1(1–240) and YidC2(1–274) were tested for their ability to mediate the in vitro membrane insertion of FocC.
insertion of F$_c$. Overexpression of either YidC1(1–240) or YidC2(1–274) failed to stimulate membrane insertion of F$_c$ (Fig. 2A, lane 8, versus 10, and Fig. 2B lane 7 versus 8). Therefore, it is concluded that the binding of ribosomes to the C-terminal tail of YidC1 and YidC2 is crucial for membrane insertion of F$_c$.

**DISCUSSION**

We have investigated the ability of *S. mutans* YidC1 and YidC2 to interact with ribosomes in order to examine the hypothesis that these paralogous YidC proteins differ in their modes of interaction with ribosomes. Our data demonstrate that both YidC1 and YidC2 can interact with translating and nontranslating ribosomes isolated from *E. coli* and that the highly positively charged C-terminal tail of these YidC proteins is essential for this interaction. Moreover, deletion of the C-terminal tail blocked the YidC-dependent membrane insertion of subunit c of the F$_1$F$_c$-ATPase, suggesting that ribosome binding is essential for the insertase activities of YidC1 and YidC2. It is concluded that YidC1 and YidC2 function by similar mechanisms and that both support the cotranslational mode of membrane insertion.

**FIG 3** Specific binding of ribosomes and F$_c$-RNCs to YidC1 and YidC2. (A) Schematic illustration of the SecM-stalled F$_c$-RNCs. (B) Coomassie brilliant blue (CBB) staining (left panel) and Western blotting (right panel) using a Strep-tag antibody of the purified F$_c$-RNCs. The lower band is the tRNA dissociated form of the F$_c$ fragment, whereas the upper band is the tRNA associated form that could be completely dissociated using 100 mM NAOH (not shown). (C) SPR sensorgram of the binding of nontranslating ribosomes to YidC1 (dotted line) or YidC2 (solid line) IMVs. (D) Binding of F$_c$-RNCs to YidC1 (dotted line) or YidC2 (solid line) IMVs. All of the binding measurements were done at 25°C with a flow rate of 20 μl/min and a ribosome/RNC concentration of 27 nM. Each binding experiment was performed at least 3 times using independently isolated IMVs.
The finding that YidC2 binds *E. coli* ribosomes confirms earlier studies with mitochondrial ribosomes (16). However, while mitochondrial ribosomes are unable to interact with the C terminus of YidC1 (16), YidC1 readily binds *E. coli* ribosomes (this study). Clearly, the reduced net positive charge and/or length of the YidC1 C terminus is affecting the interaction with mitochondrial and *E. coli* ribosomes differently, suggesting that *E. coli* and mitochondrial ribosomes have different requirements for YidC binding.

Even though structural information on fungal mitochondrial ribosomes is limited, cryo-electron microscopy (cryo-EM) analysis of mitochondrial ribosomes of animals and protists indicates major structural differences with bacterial ribosomes (19, 20). Importantly, the regions around the polypeptide exit tunnel show significant structural variation (20). This is supported by analysis of the composition of the yeast ribosomal exit tunnel that identified proteins that were homologous to the bacterial exit tunnel proteins but contained mitochondrion-specific domains and the N and C termini (18). In addition, three mitochondrion-specific proteins were found to be close to the exit tunnel. YidC binding likely involves the rRNA and protein interface near or at this ribosomal exit tunnel, and structural constraints may play a critical role in this process. As the C terminus of YidC1 is significantly shorter than the C terminus of YidC2 or Oxa1, the YidC1 C-terminal tail may extend insufficiently from the membrane surface to support a functional interaction with mitochondrial ribosomes. Bacterial ribosomes may thus be less critical toward the length and net charge of the YidC C terminus than mitochondrial ribosomes.

Importantly, our study also addressed the binding of translating ribosomes to YidC1/C2, showing that the presence of the nascent chain enhances the binding. This aspect could not be addressed with mitochondrial ribosomes as these are inactive for translation in vitro. Therefore, we conclude that the C-terminal region of YidC fulfills an important but more subtle role in ribosome binding than previously anticipated.

The observation that both YidC1 and YidC2 can actively insert the small membrane protein Foc and bind ribosomes is in agree-
ment with their (partial) overlapping function in the native host (14, 16, 28). Such overlapping function has also been observed with the YidC homologs of *Bacillus subtilis* (8, 10). In *S. mutans*, it has been proposed that the overlapping function of YidC1 serves as a “backup mechanism” for YidC2 because only a double deletion is lethal for *S. mutans* (28). Nevertheless, YidC1 cannot fully replace YidC2 unless its C terminus is replaced for the one of YidC2 (14). It is still unclear why the C terminus has such a major impact on specificity and which substrates this concerns. Since both YidC1 and YidC2 can interact with *E. coli* ribosomes (this study), the YidC2 C terminus must have an additional role in conferring substrate specificity. To elucidate this question, further insight into the exact substrate specificity of these YidC proteins is required.

In summary, our results demonstrate that both *S. mutans* YidC1 and YidC2 functionally interact with (translating) ribosomes and that this interaction depends on the C-terminal tails of both proteins. Future studies should be directed to determine the molecular basis of the specificity of the YidC1 and YidC2 proteins.

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REFERENCES


Supplemental FIG. 1. Specific binding of ribosomes (panels A and B) and F0c-RNCs (panels C and D) to YidC1 and YidC2 showing the sensograms of the channels loaded with the IMVs bearing overexpressed YidC1/2 (dashed lines) and the control channels loaded with IMVs bearing only the endogenous levels of the E. coli YidC (dotted lines). Also shown is the specific binding after subtraction of the control channel (solid lines). Further details are as in the legend of Figure 3 and the Experimental Procedures section.