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Antisense RNA Targeting the First Periplasmic Domain of YidC Appears to Reduce Cell Growth at 30°C but Does Not Induce a Filamentous Phenotype

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YidC is an essential protein in *Escherichia coli* that is evolutionarily conserved. It is involved in the proper folding and insertion of many membrane proteins. YidC is a transmembrane protein that consists of six transmembrane domains and a major 30 kDa periplasmic domain between transmembrane domains one and two. In this study, we sought to develop a method to study the role of the YidC major periplasmic domain by knocking down expression of full length YidC using antisense RNA designed to bind to the sequence of messenger RNA corresponding to this region of YidC. Mutant forms of YidC (lacking the major periplasmic domain) could then be studied using this system. In our study, we investigated whether a knockdown of YidC would result in a growth defect or defined phenotype. We used an IPTG-inducible antisense RNA targeting the first periplasmic domain of YidC. The antisense RNA product was designed to suppress YidC protein expression by binding to a 248 bp and 50 bp region of mRNA coding for the periplasmic domain of YidC. Uninduced and IPTG induced *E. coli* DH5α cells harbouring these constructs were compared at 4 hours and 24 hours post-induction. When grown on solid media, cells harbouring the pNEARH2481 antisense RNA construct showed reduced growth when compared to cells harbouring the parental vector. This is consistent with the crucial role of YidC in the insertion of essential proteins in the inner membrane, as decreased expression of YidC lead to reduced cell growth. Microscopic observations of these cells did not show a filamentous morphology relative to their uninduced control suggesting that the expression of the antisense RNA did not impact cell division under the conditions tested.

Membrane proteins often play a crucial role in cell systems, as they are involved in the regulation of many biological processes essential for cell survival (1). YidC is an evolutionarily conserved chaperone protein located in the inner membrane of many Gram-negative pathogenic bacteria (1, 2). It aids in the proper folding and insertion of other membrane proteins (3). YidC is made up of 548 amino acid residues and contains six transmembrane domains (1). The periplasmic domain following the first transmembrane domain consists of 319 residues (1). It has been shown that this large periplasmic domain is not required for YidC function and does not affect cell growth, however its function is still unknown (1).

It has been proposed that YidC interacts with Sec-dependent proteins found in the inner membrane, such as FtsQ. (4, 5). FtsQ, a type 2 inner membrane protein, stabilizes the Z-ring formed during cell division (4). Research has suggested that YidC releases proteins from the Sec system into the inner membrane (6). Although the interaction between the two proteins is not required, it appears YidC increases the efficiency of FtsQ insertion into the inner membrane (1). The YidC pathway has been implicated as the sole pathway responsible for insertion of the Sec-independent protein F_1F_0 ATP synthase subunit (1). As the F_1F_0 ATP synthase subunit is essential to cell survival, due to its role in producing ATP molecules for various cellular processes, it has been suggested that this interaction is the main reason why YidC is essential for *E. coli* survival (1).

Studies using *yidC* knockout mutants have revealed a role for YidC in cell growth and survival. Samuelson *et al.* demonstrated that depletion of YidC by complementing a *yidC* knockout mutant with an arabinose inducible plasmid rendered cells non-viable (5). Employing the same knockout mutant, Wang *et al.* examined the effect of YidC depletion in the expression of other genes in the cell. They utilized a gene chip method to examine the transcriptome of the *yidC* knockout mutant as compared to a control strain (7). The study revealed that genes involved in DNA/RNA repair, energy metabolism, and transcription/translation functions were altered when YidC was depleted (7). In particular, genes involved in the stress response, such as PspA, were upregulated, as were certain proteases and chaperones (7). The results suggest that the cells are under stress when YidC is depleted (7). Wang *et al.* also observed a reduced growth rate in the *yidC* knockout mutants when compared to the control (7). The cells were also nonmotile and longer than the control cells (7). Wang *et al.* suggests that the cells were not dividing properly due to the disruption of cell membrane structure when YidC is depleted (7).

Similar studies were consequently conducted to investigate the role of YidC in cell growth; however, these studies were performed through a different approach. asRNA was used to suppress translation of YidC (3, 8). In these studies, DNA segments encoding asRNA were designed such that the resulting asRNA would complement to regions of mRNA encoding YidC (3, 8). This would result in double stranded regions of mRNA that block ribosomes from translating past these sites and thus expression of YidC is reduced (10). However, as the gene was not deleted, and expression of some mRNA encoding YidC is still possible, these studies yielded different phenotypes from those observed by Samuelson *et al* (5).

In the study conducted by Patil *et al.*, it was demonstrated that asRNA targeting the RBS of YidC reduced cell growth (3). Drawing from this conclusion from Patil *et al.*, Lalani *et al.* used asRNA to target the region of mRNA encoding the first periplasmic domain of YidC rather than the RBS (6). It is unusual to target the first periplasmic domain, since targeting the RBS yields better knockdown results; however, Lalani *et al.* did so in order to study the role of the first periplasmic domain by complementation with a plasmid encoding YidC (6, 8). It was observed that expression of the asRNA did not affect growth rate of the cells (6). However, similar to the observations made by Wang *et al.*, cells with a YidC knock down appeared to exhibit a partial filamentous phenotype (7).

Based on these previous studies, we hypothesize that expression of asRNA targeting the region of mRNA encoding the first periplasmic domain of YidC would lead to a filamentous phenotype and reduced growth at 25°C and 30°C due to the possible enhanced annealing of the asRNA to the mRNA at lower temperatures. Our data has shown that induction of asRNA targeting the YidC major periplasmic domain results in reduced growth at 30°C but did not appear to result in a filamentous phenotype.

MATERIALS AND METHODS

Growth of *E. coli* DH5α harboring pHN678, pZ, pNEARH50 and pNEARH2481. *E. coli* DH5α cells harboring pHN678, pZ, pNEARH50, and pNEARH2481 (Fig. S1) were obtained from Lalani *et al*. (2015). Cells were grown at 37°C on Luria plates (1% w/v tryptone, 0.5% w/v yeast extract, 0.5% w/v NaCl, 1.5% w/v agarose) with 30 µg/ml chloramphenicol to select for cells containing these plasmids. Overnight cultures were grown in Luria broth (1% w/v tryptone, 0.5% w/v yeast extract, 0.5% w/v NaCl) with 30 µg/ml chloramphenicol at 37°C and shaken at 200 rpm for 18 hours.

Microscopic observation of *E. coli* DH5α expressing pHN678, pZ, pNEARH50 and pNEARH2481. Overnight cultures of *E. coli* DH5α harboring the pHN678, pZ, pNEARH50, and pNEARH2481 were used to inoculate 30 ml of Luria broth (with 30 µg/ml chloramphenicol), shaken at 37°C, and grown to an OD_{600} of approximately 0.200. Three 5 ml aliquots of each culture were transferred to sterile test tubes and were uninduced or induced with 0.32 or 1 mM of isopropyl β-D-1-thiogalactopyranoside (IPTG) and grown at 37°C. Wet mounts were prepared and observed at 4 hours and 24 hours post-induction using the Unilux-12 Microscope (Kyowa) at 1000X magnification with oil immersion. Images of the cells were obtained using the imaging software AxioVision.

Growth of *E. coli* DH5α harboring pHN678, pZ, pNEARH50 and pNEARH2481 at varying concentrations of IPTG and temperatures. Isolated colonies of *E. coli* DH5α harboring pHN678, pZ, pNEARH50, and pNEARH2481 were used to inoculate 1 ml of Luria broth and incubated for 1 hour at 37°C. Luria agar plates with 30 µg/ml chloramphenicol were spread plated with 50 µl of 1 mM, 10 mM or 20 mM of IPTG. 5 µl of the cultures were spotted onto the plates with or without IPTG, and grown at 30°C and 37°C for 24 hours, and 25°C for 48 hours. Growths of cells on plates with or without IPTG were compared by using the unaided eye.

RESULTS

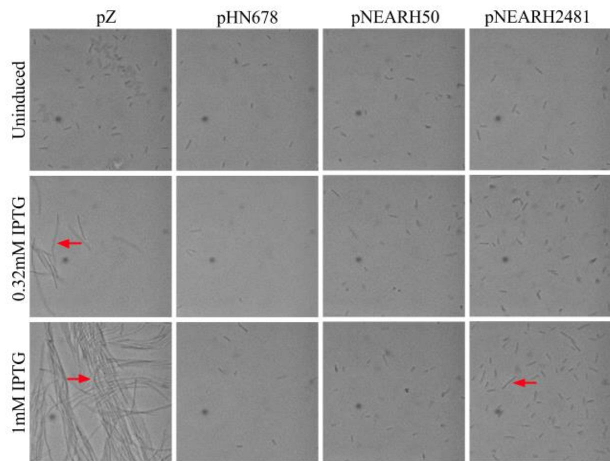

FIG 1 Expression of pNEARH50 and pNEARH2481 in *Escherichia coli* DH5α for 4 hours does not induce a distinct filamentous phenotype. Cells harboring pZ (*ftsZ* asRNA), pHN678 (vector only), pNEARH50 (*yidC* asRNA) and pNEARH2481 (*yidC* asRNA) were induced with 0 mM (uninduced), 0.32 mM or 1 mM IPTG for 4 hours at 37°C. Wet mounts of the cells were prepared and observed using an upright light microscope at 1000X magnification. Images were obtained using AxioVision. Red arrows point to filamentous or elongated cells.

Antisense RNA targeting the first periplasmic domain of YidC did not result in filamentous phenotype. We attempted to replicate the partial filamentous phenotype observed by Lalani *et al. E. coli* DH5α containing pHN678, pZ, pNEARH50, and pNEARH2481 were grown in LB culture supplemented with chloramphenicol at 37°C. Each culture was split and grown in the presence or absence of 1 mM IPTG to induce expression of asRNA. pNEARH2481 carries asRNA that targets a 248 bp region of the first periplasmic domain of YidC on the mRNA while pNEARH50 targets a 50 bp region. At both 4 hours and 24 hours post-induction, 4 µl of each culture was observed microscopically. From fig. 1, uninduced cells containing 4 different constructs exhibited identical rod shaped morphologies. Cells harboring the empty vector pHN678 and pZ, which regulates the expression FtsZ (6), were induced with IPTG to act as negative and positive controls, respectively. The disruption of FtsZ, an essential protein for cell division, has been shown to induce highly filamentous phenotype in cell morphology (8). Negative control cells containing pHN678 (vector only) exhibited no difference in morphology 4 hours post-induction with up to 1 mM IPTG (Fig. 1). Induction of the cells with 0.32 mM was added to our experiment as it was previously demonstrated that 0.32 mM induced high levels of expression of the asRNA (3). Cells containing pZ express asRNA that targets FtsZ. The positive control cells containing pZ demonstrated a change to a highly filamentous phenotype in cell morphology at 4 hours post-induction (Fig. 1), thereby confirming IPTG induction. In addition, the filamentous phenotype was more pronounced at 1 mM IPTG versus 0.32 mM IPTG, demonstrating a

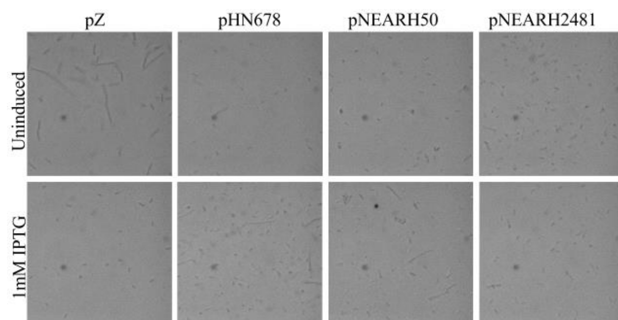

FIG 2 Expression of pZ, pHN678, pNEARH50, or pNEARH2481 does not induce filamentous phenotype in *Escherichia coli* DH5α at 24 hours. *E. coli* DH5α harboring pZ (*ftsZ* asRNA), pHN678 (vector only), pNEARH50 (*yidC* asRNA) and pNEARH2481 (*yidC* asRNA) were uninduced or induced with 1 mM IPTG for 24 hours at 37°C. Wet mounts of the cells were prepared and observed using a light microscope at 1000X magnification. Images were obtained using AxioVision. IPTG induction of of cells with pZ, pHN678, pNEARH2481 or pNEARH50 did not appear to lead to a filamentous phenotype. Red arrows point to filamentous or elongated cells.

dose response effect with increasing IPTG concentration (Fig. 1). At 4 hours post-induction, both pNEARH2481 and pNEARH50 containing cells exhibited no difference in cell morphology at 1 mM or 0.32 mM IPTG compared to the uninduced samples (Fig. 1).

At 24 hours post-induction, cells harbouring pNEARH2481 and pNEARH50 also did not appear to show significant differences in cell morphology at 1 mM IPTG when compared to uninduced cells (Fig 3). Negative control cells containing pHN678 showed consistent results with the 4 hour induction, as no changes were observed in cell morphology (Fig. 2). Positive control cells harbouring pZ at 24 hours post 1 mM IPTG induction demonstrated a reduction in filamentous phenotype when compared to the cells imaged at 4 hours post-induction (Fig. 2). In both induced and uninduced samples, several cells were observed to be elongated in their cell morphology. However, over half of the cells per field appear to be largely unchanged and the presence of the elongated cells were also observed in the negative control cells. As the induced and uninduced samples of pNEARH2481 and pNEARH50 at both time points appeared to have similar morphology, it appears that the asRNA targeting the first periplasmic domain region of YidC on the mRNA did not result in the development of filamentous phenotype in *E. coli* cells.

No significant differences were observed in the growth of *E. coli* DH5α cells containing pNEARH2481 upon induction of YidC antisense RNA. *E. coli* DH5α containing pHN678, pZ, and pNEARH2481 were spot plated on LB agar plates with chloramphenicol and incubated overnight at 25°C, 30°C, and 37°C for a spot growth assay. The cells were also induced with 0 mM, 1 mM, 10 mM, or 20 mM IPTG overnight. A range of IPTG concentration was tested, as the optimal concentration for overnight stimulation was undetermined. It was proposed that difference in growth rates may not be distinct if colonies are overgrown, thus a variety of temperature were tested to determine the optimal temperature to observe growth differences between conditions. As observed in Fig 4, cells containing pNEARH2481 demonstrated no significant difference in growth when grown under the different concentrations of IPTG at both 30°C and 37°C. Cells harboring pNEARH2481 did not grow at 25°C (Fig. 3). Negative control cells containing pHN678 also did not exhibit a difference in growth rate at the three temperatures. Cells containing pZ, which showed filamentous morphology upon induction, was expected to exhibit reduced growth rate due to disruption of cell division. Positive control cells containing pZ showed reduced growth as the concentration of IPTG increased to 20 mM when grown at 37°C. The results indicate that temperature, rather than the concentration of IPTG, appears to have a more pronounced effect on the growth rate of cells harboring pNEARH2481, while both conditions contribute to the reduced growth rate observed for pZ; neither conditions appear to have an effect on the growth rate of cells harboring pHN678.

***E. coli* DH5α cells containing pNEARH2481 exhibit a slower growth rate relative to cells containing pHN678 and pZ.** *E. coli* DH5α containing pHN678, pZ, and pNEARH2481 were spot plated on LB agar plates with chloramphenicol and incubated overnight at 25°C, 30°C, and 37°C. The cells were also induced with 0 mM, 1 mM, 10 mM, or 20 mM IPTG overnight. Cells containing pNEARH2481 showed reduced cell growth relative to cells containing pHN678 at all three temperature conditions (Fig. 3). It was also observed that cells harboring pNEARH2481 exhibited reduced cell growth relative to cells containing pZ except when induced with 20 mM IPTG (Fig. 3). Cells harboring pNEARH2481 also appeared to grow slower at 30°C than at 37°C, while cells harboring pZ and pHN678 grew similarly at the two temperatures (Fig. 3). Both pZ and pHN678 grew slower at 25°C, and pNEARH2481 did not grow at 25°C (Fig. 3). The growth rate of these cells was based on the density and size of the colonies grown on the plate. It was speculated that the slower growth rate of cells containing the pNEARH2481 and pNEARH50 could be a result of leaky expression of the construct. LB was used as the culture medium and contains lactose that may lead to activation and expression of these constructs. This indicates that the asRNA encoded in pNEARH2481 has detrimental effects on the growth rate of *E. coli* DH5α cells, and the effects may be more apparent at lower temperatures, however the lack of growth at 25°C may not be completely attributed to the asRNA, as pZ and pHN678 also had reduced cell growth at 25°C.

DISCUSSION

The aim of the study was to examine the effect on morphology and growth of cells with YidC knockdown at different temperatures. Lalani *et al.* constructed plasmids pNEARH2481 and pNEARH50, which are inducible by IPTG and contain asRNA targeting the first periplasmic domain of YidC. The asRNA binds the

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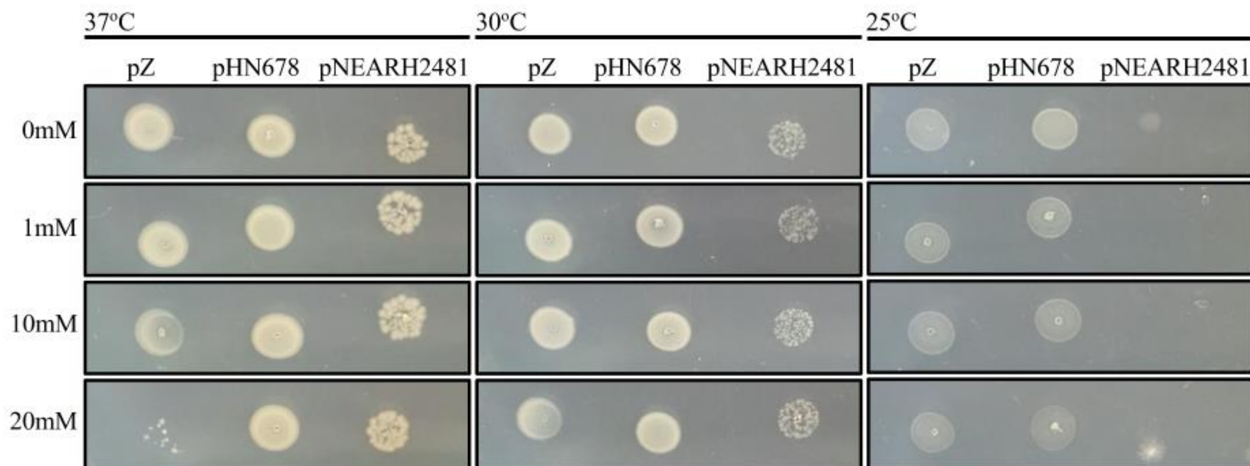

FIG 3 Growth of *E. coli* DH5α harboring pNEARH2481 appear to decrease at lower temperatures but not at increasing IPTG concentration relative to pHN678. 5 µl of *E. coli* DH5α harboring pZ (*ftsZ* asRNA), pHN678 (vector only) and pNEARH2481 (*yidC* asRNA) were spotted onto Luria agar plates with 30 µg/ml chloramphenicol containing 0, 1, 10, or 20 mM IPTG and grown at 37°C, 30°C for 24 hours and at 25°C for 48 hours.

complementary sequences of the YidC mRNA and prevents translation and synthesis of the protein (Fig. 4). It was demonstrated that *E. coli* DH5α expressing the asRNA did not exhibit a defect in growth (6) as observed in cells depleted of YidC (9). However, the cells appeared to display a filamentous phenotype compared to the uninduced cells, although the phenotype observed was not rigorously characterized due to project time constraints (6).

The cells were also examined at a later time point of 24 hours post-induction as it was possible that the cells were not fully expressing the asRNA at 4 hours. However, at 24 hours the filamentous phenotype observed in cells containing pZ was no longer present, and cells containing pNEARH2481 and pNEARH50 were also not filamentous (Fig. 2). From these results, it appears knockdown of YidC with the asRNA constructs did not lead to a change to its normal rod-shaped phenotype. The study from Wang *et al.* observed reduced growth in cells depleted of YidC (9). A result from their study suggests that these cells stopped growing at approximately 7 hours post-induction (9). Therefore, it is suggested that cells expressing the asRNA constructs may exhibit reduced growth rate and thus were outcompeted by cells that were not affected by the knockdown. This would result in a population mainly consisting of cells that appear to be not affected by the asRNA construct. Therefore, cells expressing asRNA targeting the periplasmic domain of YidC may experience reduced growth and only survivors, displaying wild-type phenotype, would have been observed under the microscope.

As a result, we examined the growth of cells containing pNEARH2481 at different temperatures to better understand the effect of the knockdown on the cells. This was achieved through a spot growth assay where the same volume of each cell condition was spotted onto the same LB plate instead of examining cell morphology. If cells were indeed dying due to the actions of the asRNA, a growth assay would be better able to detect the effect of temperature than examining individual cells. Cells harboring pHN678, pZ, pNEARH2481 and pNEARH50 were spotted onto plates containing different IPTG concentrations and grown at 25°C, 30°C and 37°C. It was expected that at lower temperature the stability of RNA-RNA interaction between the asRNA and the target mRNA would improve, thus we hypothesized that the growth of our cells at lower temperatures and higher concentrations of IPTG will increase the efficiency of the knockdown of YidC.

It was observed that the concentration of IPTG did not affect the growth of cells harboring pNEARH2481 (Fig. 3). It appears low level basal expression of the construct without IPTG has the same effect on growth as high concentration of IPTG such as 20 mM. In contrast, cells containing pZ appeared to exhibit reduced growth with increasing IPTG concentration indicative of a dose response to IPTG (Fig. 3). Cells containing pHN678 did not exhibit a reduced growth rate at higher IPTG concentrations. This indicated that the IPTG induction was successful in inducing the expression of the asRNA in the construct. Cells harbouring the asRNA construct exhibit an IPTG-independent reduction in growth rate.

Lastly, it was observed that at all temperature conditions, cells containing pNEARH2481 exhibited a decrease in growth rate compared to cells containing pHN678. Although all cells grew slower at 25°C, pNEARH2481 exhibited minimal growth at 25°C (Fig. 3). However, the cultures used to spot the plates were

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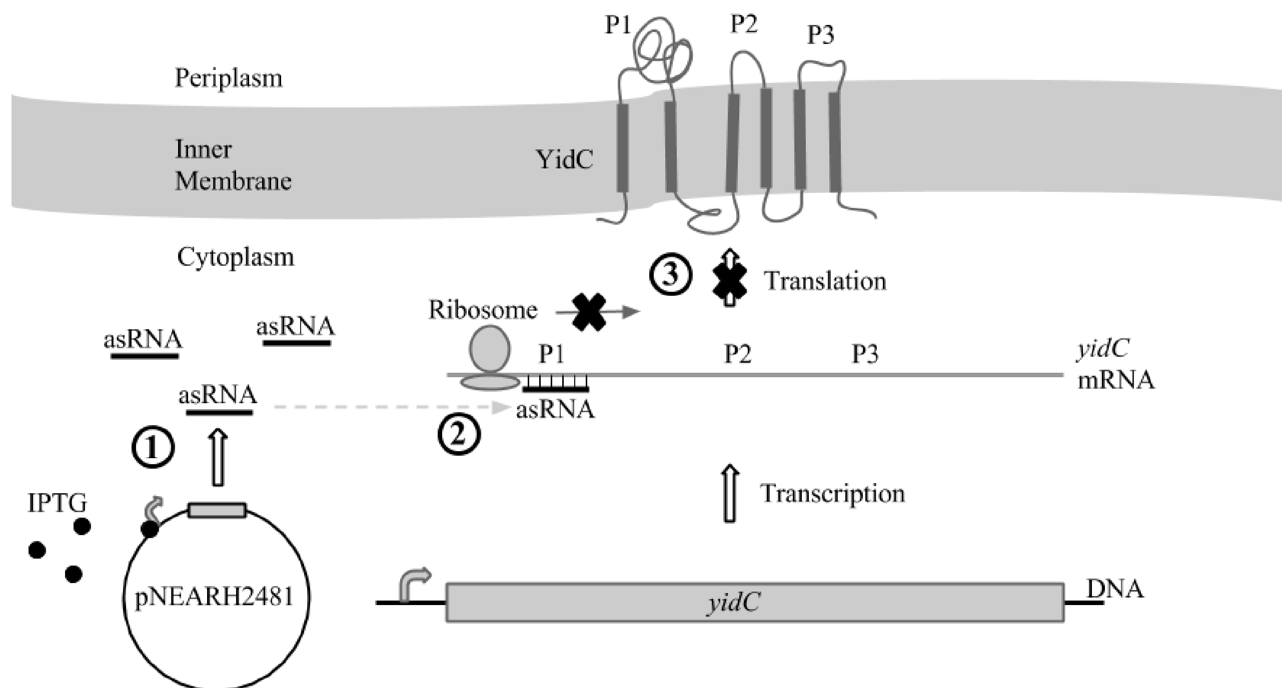

FIG 4 Model of the action of antisense RNA targeting the first periplasmic domain of *yidC*. YidC is a transmembrane protein with three periplasmic domains (P1-3). (1) pNEARH2481 expresses antisense RNA (asRNA) targeting P1 in the presence of IPTG induction. (2) asRNA anneals to P1 region of the *yidC* mRNA and (3) prevents translation of the *yidC* mRNA by the ribosome.

made from an isolated colony and were not normalized to the same concentration. It is possible a higher concentration of pZ and pHN678 were spotted onto the plates, which would contribute to the greater growth observed on the plates. It is also possible that the increased size of the pNEARH2481 could have resulted in the slower growth rate due to longer replication time as it has been shown that cells with larger plasmids may experience longer lag phases relative to cells with smaller plasmids. (11). However, it appears cells containing pNEARH2481 grew slower at 30°C than at 37°C, which was not observed in the controls (Fig. 3). This may suggest that at 30°C, a stronger interaction between the asRNA and the mRNA of *yidC* is occurring. As a result, the cells would exhibit a greater growth defect at 30°C than at 37°C. The growth observed at 37°C may be due to only a partial knockdown of YidC. A reduced growth rate in cell knockdown of YidC at lower temperatures is consistent with studies that have observed a reduced growth rate in cells depleted of YidC or knockdown of YidC with asRNA targeting the RBS of yidC (3, 8). The knockdown of YidC in these studies may have been more pronounced, and as a result a reduced growth rate was observed even at 37°C. However, when we lowered the temperature, where RNA-RNA interaction are predicted to be more stable and may be expressed at higher levels resulting in more effective knockdown of YidC would occur, a similar defect growth rate is observed (12). This provides support for the importance of YidC and its role as one of the proteins that is involved in the insertion of essential proteins such as the F_1F_0 ATP synthase subunit. Therefore, the knockdown of YidC results in the reduction of protein insertion and could lead to cell death several hours post-induction (7).

In conclusion, a distinct filamentous phenotype was not observed in cells expressing asRNA that targets the first periplasmic domain of YidC when induced with IPTG for 4 hours or 24 hours. asRNA targeting of the first periplasmic domain of YidC does not induce a change in cell morphology upon IPTG induction. However, it is possible that the cells expressing the asRNA were killed off during induction, and only mutants were observed under the microscope during the time of imaging. This alternative was not explored due to time constraints of this project. Cells containing pNEARH2481 exhibit a reduced growth rate at 30°C compared to at 37°C relative to the positive and negative controls. The concentration of IPTG did not affect growth.

FUTURE DIRECTIONS

Patil *et al.* successfully knocked down YidC using asRNA targeting the RBS of the YidC transcript (3). However, the cells still exhibited growth, suggesting the knockdown was also not complete, as cells exhibiting a loss of YidC are not expected to survive due to disruption of essential protein insertion. The microscopic examination and

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characterization of the phenotype of these cells may provide further insight on the role of YidC in cell morphology and division of these normal rod-shaped cells. As we have found that cells expressing asRNA to the periplasmic domain did not induce filamentous morphology found in cells containing pZ with FtsZ disruption. Construction of plasmids targeting the RBS may lead to a more efficient knockdown of YidC relative to pNEARH2481 and pNEARH50, however, Patil *et al.* did not study microscopic phenotype and morphology of the YidC knockdown cells. It is speculated that these cells will exhibit a pronounced filamentous morphology similar to YidC depleted cells observed in Wang *et al.* In addition, the growth rates of these cells should be examined through a spot growth assay on LB plates at varying temperatures to determine the optimal conditions for the knockdown. This will help determine the most optimal time and condition to observe a morphology change in these cells as it was found in Wang *et al.* that cells with depleted YidC might not survive for extended period time, thus allowing normal cells to overtake the culture or plate.

Lastly, if the cells display a filamentous phenotype, complementation of the cells with wild type YidC can be performed. If the cells no longer display a filamentous phenotype, this suggests that the effects observed were due to the effects of the knockdown of YidC and not due to other factors.

ACKNOWLEDGEMENTS

We would like to thank Dr. David Oliver, Jia Wang, and Celine Michiels for their continued assistance and support throughout the project. We would like to thank Lalani *et al*. for providing the antisense RNA constructs pNEARH2481 and pNEARH50. We would also like to thank the University of British Columbia and the Department of Microbiology and Immunology (Vancouver, BC) for their laboratory equipment and financial support over the course of this project.

REFERENCES

1. **Kiefer, D, Kuhn, A.** 2007. YidC as an Essential and Multifunctional Component in Membrane Protein Assembly. International Review of Cytology. **259:**113-138.
2. **Kuhn, A, Stuart, R, Henry, R, Dalbey, RE.** 2003. The Alb3/Oxa1/YidC protein family: membrane-localized chaperones facilitating membrane protein insertion? Trends Cell Biol. **13:**510-516.
3. **Patil, SD, Sharma, R, Srivastava, S, Navani, NK, Pathania, R.** 2013. Downregulation of yidC in *Escherichia coli* by antisense RNA expression results in sensitization to antibacterial essential oils eugenol and carvacrol. PloS One. **8:**e57370.
4. **Urbanus, ML, Scotti, PA, Fröderberg, L, Sääf, A, de Gier, JL, Brunner, J, Samuelson, JC, Dalbey, RE, Oudega, B, Luirink, J.** 2001. Sec-dependent membrane protein insertion: sequential interaction of nascent FtsQ with SecY and YidC. EMBO reports. **2:**524-529.
5. **Samuelson, JC, Jiang, F, Dalbey, RE, Möller, I, Chen, M, Wiedmann, M, Phillips, GJ, Kuhn, A.** 2000. YidC mediates membrane protein insertion in bacteria. Nature **406:**637-641.
6. **Lalani R, Susilo N, Xiao E, Xu A.** 2015. Antisense RNA Targeting the First Periplasmic Domain of YidC in Escherichia coli Appears to Induce Filamentation but Does Not Affect Cell Viability. Journal of Experimental Microbiology and Immunology (JEMI).
7. **Wang, P, Kuhn, A, & Dalbey, RE.** 2010. Global change of gene expression and cell physiology in YidC-depleted *Escherichia coli*. Journal of bacteriology: **192**:2193-2209.
8. **Feucht, A, Errington, J.** 2005. ftsZ mutations affecting cell division frequency, placement and morphology in *Bacillus subtilis*. Microbiology. **151:**2053-2064.
9. **Waters LS, Storz G.** 2009. Regulatory RNAs in bacteria. Cell **136**:615-628.
10. **Beck, K., Eisner, G., Trescher, D., Dalbey, R. E., Brunner, J., and Müller, M**. (2001). YidC, an assembly site for polytopic *Escherichia coli* membrane proteins located in immediate proximity to the SecYE translocon and lipids. EMBO Rep. **2**:709–714.
11. **Smith, CA, Robertson, D, Yates, B, Nielsen, DM, Brown, D, Dean, RA, Payne, GA**. 2008. The effect of temperature on Natural Antisense Transcript (NAT) expression in Aspergillus flavus. Curr. Genet. **54**:241-269.
12. **Smith, MA, Bidochka, MJ.** 1998. Bacterial fitness and plasmid loss: the importance of culture conditions and plasmid size. Can. J. Microbiol. **44:**351-355.