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Novel Mechanism of *Escherichia coli* Porin Regulation

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A novel mechanism of *Escherichia coli* porin regulation was discovered from multicopy suppressors that permitted growth of cells expressing a mutant OmpC protein in the absence of DegP. Analyses of two suppressors showed that both substantially lowered OmpC expression. Suppression activities were confined to a short DNA sequence, which we designated *ipeX* for *i*nhibition of *p*orin *e*xpression, and to DNA containing a 3′-truncated *ompR* gene. The major effect of *ipeX* on *ompC* expression was exerted posttranscriptionally, whereas the truncated OmpR protein reduced *ompC* transcription. *ipeX* was localized within an untranslated region of 247 base pairs between the stop codon of *nmpC*—a remnant porin gene from the cryptic phage *qsr′* (DLP12) genome—and its predicted Rho-independent transcriptional terminator. Interestingly, another prophage, PA-2, which encodes a porin similar to NmpC, known as Lc, has sequences downstream from *lc* identical to that of *ipeX*. PA-2 lysogenization leads to Lc expression and OmpC inhibition. Our data show that the synthesis of the *lc* transcript, whose 3′ end contains the corresponding *ipeX* sequence, inhibits OmpC expression. Overexpression of *ipeX* RNA inhibited both OmpC and OmpF expression but not that of OmpA. *ompC-phoA* chimeric gene constructs revealed a 248-bp untranslated region of *ompC* required for *ipeX*-mediated inhibition. However, no sequence complementarity was found between *ipeX* and this region of *ompC*, indicating that inhibition may not involve simple base pairing between the two RNA molecules. The effect of *ipeX* on *ompC*, but not on *ompF*, was independent of the RNA chaperone Hfq.

A special class of *Escherichia coli* outer membrane proteins (OMPs) called porins forms water-filled channels through which hydrophilic solutes gain access into the bacterial cell (27). Porins have led the way in providing insights on the structure, regulation, and assembly of membrane proteins. The two classical porins OmpC and OmpF consist of three 16-stranded β barrels, each of which forms a channel that is restricted in the middle due to the inward folding of a loop (8). By demanding bacterial growth on sugars too large to normally diffuse through porin channels, alterations in the channel loop resulting in functionally large channels were obtained (19). One such alteration in the OmpC porin was an R74C substitution ($OmpC_{R74C}$, or $OmpC_{1Cys}$). This mutant porin and its derivative, $OmpC_{R74C,G154C}$, or $OmpC_{2Cys}$ (17), are the subjects of this work and are further discussed below.

The OmpC and OmpF porin genes are transcriptionally regulated by a classical two-component signal transduction regulatory system consisting of the OmpR and EnvZ proteins (12, 13). OmpC and OmpF are also subject to posttranscriptional regulation including the small regulatory RNA molecules *micC* (7) and *micF* (23), respectively. A characteristic of these regulatory RNA molecules is that they prevent translation by base pairing with their target mRNAs in the region encompassing the translation start site (31).

There also have been reports of some unusual and less well-understood mechanisms of porin regulation. For instances, it has been shown that the absence of an OMP, TolC, leads to lower OmpF levels (25). Although it is known that this effect on OmpF in *tolC*-null mutants involves *micF* up-regulation (22), the molecular mechanism behind this up-regulation is unknown. Another fascinating but even less understood example of porin regulation involves the lysogenization of *E. coli* K-12 cells by the PA-2 phage (28). Here, the lysogenization event leads to the expression of a phage-encoded porin, Lc, and the inhibition of the host's OmpC porin. The work conducted in this study will shed light on how phage porin expression regulates that of *ompC*.

Porins have also served as excellent models to study OMP assembly because they are abundant and a great deal is known about their genetics, biochemistry, and structures. A generally accepted view of the porin assembly pathway is that after the removal of the signal peptide from precursors, mature porin molecules transiently exist in the periplasm as soluble or peripherally membrane-associated, thermolabile intermediates. These intermediates interact with various soluble folding factors to gain assembly competence (9). The insertion of these intermediates into the outer membrane may be facilitated by YaeT (Omp85), an essential OMP (34, 35). Misfolded porins are degraded by DegP, a periplasmic protease (5, 20, 21).

The subject of this study is a mutant porin protein, $OmpC_{2Cys}$, which contains two nonnative cysteine residues at positions 74 and 154 of the mature sequence (17). In the oxidizing environment of the periplasm, the two cysteine residues form disulfide bonds, causing $OmpC_{2Cys}$ misfolding and a partial loss of its cellular activities, including porin and phage receptor functions (17, 33, 34). These functions and normal folding of $OmpC_{2Cys}$ are restored in a background deficient in DsbA's periplasmic disulfide isomerase activity (17, 36). Expression of $OmpC_{2Cys}$ in a *degP*-null background is lethal at all growth temperatures unless *dsbA* is disrupted (5). Expressing a variant of DegP, $DegP_{S210A}$ (30), which lacks the proteolytic activity but maintains the normal polypeptide binding capacity (15), can reverse the $OmpC_{2Cys}$-mediated lethality (5). This reversal is shown to be due to the capture of $OmpC_{2Cys}$ by

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TABLE 1. Bacterial strains and plasmids used in this study

Strain/plasmid	Relevant characteristics	Reference/source
Strains		
MC4100	F$^-$ *araD139* Δ(*argF-lac*)*U139 rpsL150 flbB5301 ptsF25 deoC1 thi-1 rbsR relA*	4
PLB3260	MC4100 Δ*lamB106* Φ*ompF′*::*lacZ*$^+$	S. Benson
PLB3261	MC4100 Δ*lamB106* Φ*ompC′*::*lacZ*$^+$	S. Benson
RAM411	PLB3261 *ompC*$^+$ *zei*::Tn*10* (50% linkage to *ompC* by P1 transduction)	17
RAM412	PLB3261 *ompC234* ($OmpC_{1Cys}$) *zei*::Tn*10*	17
RAM415	PLB3261 *ompC402* ($OmpC_{2Cys}$) *zei*::Tn*10*	17
RAM1126	RAM415 *degP*::Km^r pCS10	5
RAM1304	RAM412 *hfq1*::ΩKm^r (*hfq* null) pTrc99A	This study; 32
RAM1305	RAM412 *hfq2*-ΩKm^r (*hfq*$^+$) pTrc99A	This study; 32
RAM1306	RAM412 *hfq1*::ΩKm^r (*hfq* null) pTrc99-*ipeX*	This study; 32
RAM1307	RAM412 *hfq2*-ΩKm^r (*hfq*$^+$) pTrc-*ipeX*	This study; 32
CS109	W1485 F$^-$	C. Schnaitman
CS137	W1485 F$^-$ PA-2 lysogen	C. Schnaitman
Plasmids		
pACYC184	Cm^r Tc^r; p15A replicon	6
pC510	pACYC-$degP_{S210A}$	30
pBAD33	Cm^r; expression vector; p15A/M13 replicons	11
pBR322	Ap^r Tc^r; pMB1 replicon	3
pTrc99A	Ap^r; expression vector; pMB1 replicon	Pharmacia
pTrc-*ybcQ*	The *ybcQ* clone in pTrc99A encompassing *ybcQ, ipeX* and the 3′ end of *nmpC*	This study
p*ybcQ* plus SL-1 through SL-5	The *ybcQ* clone in pTrc99A encompassing *ybcQ* and all five stem-loops of *ipeX*	This study
p*ybcQ* plus SL-2 through SL-5	The *ybcQ* clone in pTrc99A encompassing *ybcQ* and stem-loops 2 to 5 of *ipeX*	This study
p*ybcQ* plus SL-3 through SL-5	The *ybcQ* clone in pTrc99A encompassing *ybcQ* and stem-loops 3 to 5 of *ipeX*	This study
pTrc-*ipeX*	The *ipeX* clone in pTrc99A containing all five stem loops of *ipeX* and the 3′ ends of *ybcQ* and *nmpC*	This study
pTrc-*ipeX* (SL-2*)	The *ipeX* clone in pTrc99A with a 3-base alteration in the stem-loop 2 of *ipeX*	This study
pRAM1006	A 3.0-kb HindIII chromosomal fragment containing *ompC* was cloned into a pSC101-derived plasmid (Ap^r Km^r)	R. Misra
pTrc-*ompC*	A clone carrying the *ompC* coding region under the control of the pTrc99A promoter	This study
p*ompC*-UTR[ss]-*phoA*	An *ompC-phoA* chimeric clone in pACYC184 from which PhoA, with OmpC's signal sequence, was synthesized under *ompC*'s transcriptional and translational controls	This study
p*ompC*-UTR-*phoA*	An *ompC-phoA* chimeric clone in pACYC184; PhoA, with its native signal sequence, was synthesized under *ompC*'s transcriptional and translational controls	This study

$DegP_{S210A}$, sequestering it away from the normal assembly pathway (5).

In this study we screened a plasmid library to identify cellular factors which, when overexpressed, can rescue $OmpC_{2Cys}$-mediated lethality in a *degP*-null *dsbA*$^+$ background. Two plasmid clones carrying different regions of the chromosome were characterized in detail. Although both plasmid clones inhibited $OmpC_{2Cys}$ expression, they achieved this feat by different mechanisms: one involved a well-known porin transcription factor, OmpR (12, 13), while the other involved a poorly understood strategy of porin regulation employed by certain temperate phages (28).

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth media. Bacterial strains and plasmids used in this study are listed in Table 1. Luria broth (LB) and Luria agar (1.5% [wt/vol]) media were prepared according to the method of Silhavy et al. (29). When required, ampicillin (50 μg/ml), kanamycin (25 μg/ml), chloramphenicol (25 μg/ml), tetracycline (25 μg/ml), IPTG (isopropyl-β-D-thiogalactopyranoside) (0.4 mM), and L-arabinose (0.2%) were added to the growth medium.

DNA methods. A previously constructed plasmid library was used to identify multicopy suppressor clones. The plasmid library was constructed by ligating Sau3A-digested chromosomal DNA fragments to BamHI-digested pBR322. Using this gene library, we have been able to isolate plasmids carrying a number of different genes, indicating that the library is highly representative. Smaller plasmid clones were constructed by cloning DNA fragments amplified by PCR using the original 12- and 76-minute suppressor plasmids as templates (see Table 1 for further description). Table 2 lists primers used for DNA amplifications. Some of these primers also introduced restrictions sites that were used for subsequent cloning into plasmid vectors. Site-directed mutagenesis was performed by using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) per the manufacturer's instructions. Sequences of only forward primers used for mutagenesis are shown in Table 2. Clones and site-directed mutagenesis changes were confirmed by DNA sequence analysis.

RNA methods. Total cellular RNA was purified using the TRIzol Max bacterial RNA isolation kit (Invitrogen). LB (4 ml) containing ampicillin, with and without IPTG, was inoculated with 1:50 dilution of overnight cultures. Cultures were grown at 37°C in a shaking water bath to an optical density at 600 nm (OD_{600}) of 0.5. Cells from 3-ml cultures were pelleted in a microcentrifuge for

TABLE 2. Primers used in this study.

Primer	Sequence (5′ to 3′)
Primers used for *ybcQ* and *ipeX* cloning and *ipeX* mutagenesis	
ybcQ	
Forward	TCTGGCTGGATccTGCTCAGGTCG (BamHI)
Reverse	GCACTGATGAatTCGTTGCTGTAGG (EcoRI)
ipeX	
Reverse	CCGGTTAGAGATGGATccCGTTG (BamHI)
Forward	GCACTGATGAatTCGTTGCTGTAGG (EcoRI)
SL-1	CTAATCgaATTcCGAAAAAGATATGTTGCGGGAGGCG (EcoRI)
SL-2	gGaattcGCCTCCCCAACATATAAGTGGC (EcoRI)
SL-3	CCCTCAAGCgAaTTCCTTTAGAAGC (EcoRI)
ipeX SL-2 mutant	CCCCAACATATAAGTGGCTCaaaCAAGCCACTTCCTTTAGAAGC
Primers used to construct *ompC* and *ompC-phoA* clones	
ompC	
Forward	CTTGcATgcTTATTGCTTGATGTTAGGTGC (SphI)
Reverse	CTTtCATGATATTAACCCTCTGTTATATGCC (BspHI)
SS	AACTTcaTGAGCGTTTGCTGCGC (BspHI)
Whole	ATCCtCATGAGAACGGTCGCAAGAG (BspHI)
phoA	
Forward	
SS	AAATAtcaTGAAACAAAGCACTATTGCACTGGC (BspHI)
BspHI	acatcAtGaaCGGACACCAGAAATGCCTG (BspHI)
Reverse	CGAAgcTTCACTGCCGGG (HindIII)
Primers used in RT-PCR analysis	
ipeX	
Forward (RT)	CCCTCAAGCgAaTTCCTTTAGAAGC (EcoRI)
Reverse	CCGGTTAGAGATGGATccCGTTG (BamHI)
ipeX-SL-4 reverse	GGGTAATATATAACAGAAGGTTTATATAG
lc	
Forward	GGTCTGAACTTTGCTGCTCAGTACCAAGGC
Reverse	CTGGTAAACCAGACCTACAGCAAC
ompA	
Forward	GCTATCGCGATTGCAGTGGCAC
Reverse	CTGGAGCCGGAGCAACTACTG
ompC	
Forward	CGGTAAAGTAGACGGCCTGCAC
Reverse	CTGGTTGTCGTCCAGCAGGTTG

[a] Restriction sites are underlined and mutational sites are in lowercase letters.

15 s at 15,000 × *g*. RNA was isolated from cell pellets after they had been resuspended in 200 μl of Max bacterial enhancement reagent preheated to 95°C.

RNA decay studies were conducted essentially as described by Gudapaty et al. (10). LB was inoculated with 1:50 dilution of an overnight culture, and cells were grown to OD_{600} of 0.5. At this point, rifampin (200 μg/ml final concentration) was added, and 2-ml samples were collected 0, 2, 8, and 16 min after the addition of rifampin. Withdrawn cells were centrifuged immediately at 15,000 × *g* for 15 s, and pellets were frozen in a dry-ice ethanol bath. RNA was then isolated, quantified, and stored at −80°C.

RT-PCR. cDNA was obtained from total RNA by using the ProtoScript first-strand cDNA synthesis kit (New England BioLabs). PCR was performed using DynaZyme EXT DNA polymerase (Finnzymes) on serial dilutions of the cDNA template. Prior to the reverse transcription (RT) reaction, 1 μg RNA was treated with amplification grade DNase I (Invitrogen) to remove any traces of genomic DNA. RT reactions were performed using each of the 120 ng/μl *ompC* and *ompA* reverse primers (Table 2), after which RNA templates were removed by incubation with RNase H (2 U/μl). Undiluted and diluted cDNA (1:5, 1:25, and 1:125) from RT reactions were used as templates for PCR. Amplification reactions rendered *ompC* and *ompA* DNA fragments of approximately 900 and 600 nucleotides, respectively. For the *ompC* RNA decay study, 1:10 dilution cDNA template was used in the PCR. RT-PCR was performed to detect *ipeX* RNA in cells. All DNA products were separated in 0.8% agarose gels except for the small *ipeX* PCR product, which was separated in a 2% agarose gel. DNA bands were stained with ethidium bromide and visualized in a Bio-Rad Fluor-S imager.

β-Galactosidase assay. *ompC* and *ompF* transcriptional activities were determined by measuring β-galactosidase activities of *ompC′*::*lacZ*$^+$ and *ompF′*::*lacZ*$^+$ operon fusions. Cells were grown to an OD_{600} of 0.5 in LB supplemented with or without IPTG to induce *ipeX* expression from a plasmid clone. Assays were carried out in duplicate as described by Miller (16).

Protein analysis. Whole cell envelopes were extracted by the French press cell lysis method as described previously (18). Membrane pellets were resuspended in 20 mM Tris-HCl, pH 7.5, and stored frozen at −20°C. Membrane proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide (11%) gel electrophoresis. To better resolve OmpC and OmpF, 4 M urea was added to the separation gel solution. For Western blot analysis, proteins from SDS-polyacrylamide gel electrophoresis gels were transferred onto Immobilon-P polyvinylidene difluoride transfer membranes (Millipore) by using a Mini Transblot electrophoretic transfer cell (Bio-Rad). Membrane blots were blocked overnight in 5% (wt/vol) nondairy cream. The next day, they were incubated for 1.5 h with primary rabbit antibodies against OmpC (1:10,000 dilution), followed by 1-h incubation with goat anti-rabbit immunoglobulin G secondary antibodies (Sigma). Detection was carried out using ECF substrate (Pierce) as per manufacturer's instructions. Bands were visualized using a Molecular Dynamics Storm imager.

RESULTS

Multicopy suppressors of $OmpC_{2Cys}$. We sought plasmid clones that could overcome the lethal effect of $OmpC_{2Cys}$ in a *degP*-null background. The viability of the *ompC*$_{2Cys}$ *degP*::Km^r strain depends on the expression of protease-deficient $DegP_{S210A}$ by an IPTG-inducible plasmid promoter. That is, the *ompC*$_{2Cys}$ *degP*::Km^r/p*degP*$_{S210A}$ strain (RAM1126) grows on media supplemented with IPTG but not on media lacking IPTG. A random chromosomal gene library, generated on a

plasmid with a replicon (ColE1) and an antibiotic resistance gene (Ap^r) compatible with the resident p$degP_{S210A}$ plasmid (p15A; Cm^r), was transformed into RAM1126, and transformants were selected at 30°C on antibiotic plates lacking IPTG. Several hundred transformants were obtained, and due to this large number, they were pooled and the plasmids extracted from this pool were retransformed into RAM1126 to ensure that plasmid-encoded functions rescued the $OmpC_{2Cys}$ lethality. When MC4100-competent cells were transformed with the same amount of the same gene library DNA, approximately 100,000 transformants were typically obtained. Thus, about 0.1% of transformants contained plasmid genes that could reverse the conditional lethal phenotype of RAM1126.

EcoRI restriction analysis of plasmids from fifty random colonies obtained through the second round of transformation revealed five distinct groups of plasmid clones. DNA sequence analysis from one member of each group revealed three subgroups that encompassed DNA from the 12-, 56-, and 76-minute region of the chromosome. Plasmids from these subgroups were tested to see whether they could support the viability of the $ompC_{2Cys}$ *degP*::Km^r strain in the absence of p$degP_{S210A}$. Since the $ompC_{2Cys}$ *degP*::Km^r strain could not be constructed in the absence of p$degP_{S210A}$, the three suppressor plasmids and the vector plasmid were first introduced into the $ompC_{2Cys}$ $degP^+$ strain, followed by transduction of the *degP*::Km^r-null allele by the P1 phage. The fact that stable Km^r (*degP*::Km^r) transductants were obtained only when the recipient $ompC_{2Cys}$ $degP^+$ cells carrying the suppressor plasmids (but not the vector plasmid) were used showed that the three suppressor plasmids are capable of supporting growth without the p$degP_{S210A}$ plasmid. We have been unable to resolve the mechanism of suppression by the plasmid encompassing the 56-minute chromosomal region. Results for the other two suppressor plasmids are presented here.

Suppression of $OmpC_{2Cys}$-mediated lethality by a plasmid clone encompassing the 12-minute chromosomal region. A suppressor plasmid that reversed the lethal phenotype of $OmpC_{2Cys}$ in a $DegP^-$ background carried a chromosomal insert of 4,212 bp and contained six complete genes (*ybcN*, *ninE*, *ybcO*, *rus*, *ybcQ*, and *trs5-2*) flanked by two truncated genes (*ybcM′* and *nmpC′*) at both ends (Fig. 1A). All of these genes are from the genome of a cryptic lambdoid phage *qsr′* (DLP12) integrated at the 12-minute region of several *E. coli* strains, including MC4100. Of the six complete genes, the functions of only *rus* and *trs5-2* are known, and they are involved in recombination events. The *trs5-2* gene is a part of the IS*5* element that disrupts the phage-encoded *nmpC* porin gene in most laboratory *E. coli* K-12 strains (2, 28). In the absence of any obvious candidate for the suppression, subcloning experiments were carried out to narrow down the responsible gene. Initially, two subclones were constructed, one carrying *ybcN* to *rus* and the other carrying *ybcQ* to the truncated *nmpC* gene. The subclone carrying *ybcN* to *rus* could not suppress $OmpC_{2Cys}$-mediated lethality but the *ybcQ* to *nmpC′* clone could. Moreover, an even smaller subclone (referred to as the *ybcQ* clone in Fig. 1A) containing the *ybcQ* gene, and 318 bp upstream and 230 bp downstream from the *ybcQ* open reading frame (ORF) was found to suppress $OmpC_{2Cys}$-mediated lethality. Western blot analysis of envelopes revealed that the presence of the *ybcQ* clone substantially reduced $OmpC_{2Cys}$ levels (Fig. 1B, lanes 1 and 3), suggesting that the plasmid clone most likely achieved suppression by lowering the mutant OmpC protein level.

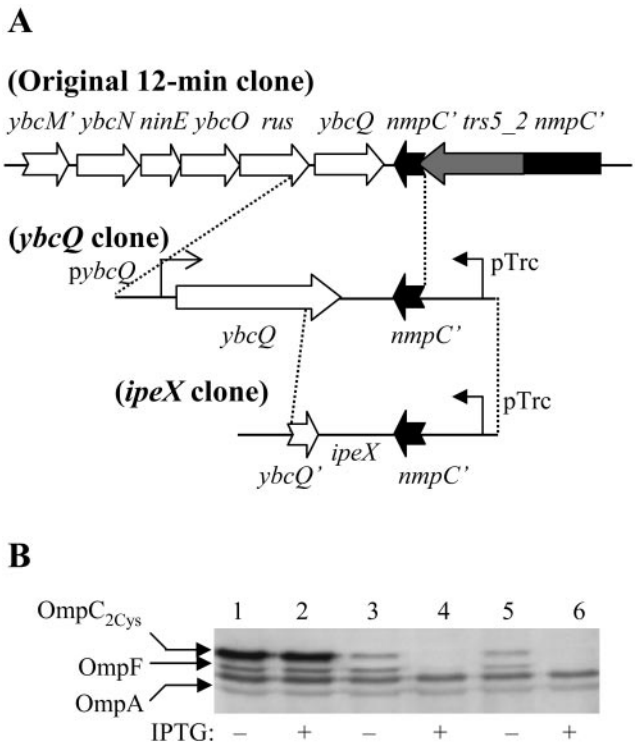

FIG. 1. (A) Schematic diagram of the plasmid clones that suppress $OmpC_{2Cys}$-mediated lethality. As described in the text, the *ybcQ* and the *ipeX* clones, both of which lower $OmpC_{2Cys}$ and OmpF expression, were subcloned from the original 12-minute clone. (B) OmpC, OmpF, and OmpA from RAM415 were detected in whole cell envelopes by Western blot analyses using OmpC polyclonal antibodies that also recognize OmpF and OmpA. Envelope samples were obtained from cells containing pTrc99A vector plasmid (lanes 1 and 2), the *ybcQ* clone (lanes 3 and 4) and the *ipeX* clone (lanes 5 and 6). The presence (+) or absence (−) of IPTG in bacterial cultures is indicated.

The *ybcQ* ORF within the 922-nucleotide-long *ybcQ* clone is oriented in the opposite direction of the IPTG-inducible promoter of pTrc99A (Fig. 1A). We noted that this clone produced a dramatically greater inhibitory effect on *ompC* expression when IPTG was present in the growth medium than when it was absent (Fig. 1B, lanes 3 and 4). This indicated that expression of a product in the direction of the pTrc99A promoter is possibly involved in porin inhibition (see below). The effect of the *ybcQ* clone was not specific to $OmpC_{2Cys}$, as the levels of $OmpC_{1Cys}$ and wild-type OmpC were also significantly reduced (data not shown). Surprisingly, when IPTG was present, the *ybcQ* clone also exerted a strong inhibitory effect on OmpF but not on OmpA (Fig. 1B). Lastly, the inhibitory effect of the *ybcQ* clone on OmpC and OmpF was not influenced by the presence or absence of DegP, even though the original plasmid clone was isolated in a genetic background lacking DegP (data not shown).

Identification of the porin inhibitor sequence. The negative effects on OmpC and OmpF described above most likely resulted from the expression of some insert DNA under the

control of the IPTG-regulated plasmid promoter of the *ybcQ* clone. The *ybcQ* ORF is oriented opposite to the IPTG-regulated plasmid promoter, which suggested that *ybcQ* might not be involved in inhibiting porin expression. We proceeded to test this by both disrupting the *ybcQ* reading frame and constructing subclones that lacked the majority of the *ybcQ* gene. The *ybcQ* gene encodes a 127-residue-long protein that is homologous to the lambda antitermination protein Q. We introduced a premature stop codon by altering the 40th codon of the *ybcQ* ORF. The plasmid clone capable of expressing only the truncated YbcQ protein suppressed lethality and reduced OmpC and OmpF levels as effectively as the parental plasmid containing the intact *ybcQ* ORF (data not shown). Thus, it is unlikely that the *ybcQ* ORF mediates suppression.

Next, we built several smaller subclones to further pinpoint the region responsible for *ompC* and *ompF* inhibition. The porin inhibition activity was narrowed down to a 247-bp sequence that excluded all but 17 nucleotides from the 3′ end of the *ybcQ* ORF (Fig. 1A). We call this 247-bp sequence *ipeX*, for *i*nhibitor of *p*orin *ex*pression. The *ipeX* clone was as effective in reducing OmpC and OmpF levels (Fig. 1B, lanes 5 and 6) as the *ybcQ* clone (Fig. 1B, lanes 3 and 4). A hypothetical ORF of 78 nucleotides can be identified within the 247-bp DNA insert. But clones lacking the potential Shine-Dalgarno (SD) sequence and start codon of this hypothetical ORF retained the inhibitory effect on porins (data not shown), thus eliminating the possibility that a small peptide from the *ipeX* sequence mediated the inhibition of porin expression. Taken together, these results suggested that an RNA molecule made from the ORF-free DNA sequence is the inhibitor.

Characteristics of *ipeX*. MFOLD software (http://bioweb.pasteur.fr/seqanal/interfaces/mfold-simple.html), which predicts RNA secondary structures, suggested multiple secondary structures within the *ipeX* sequence. Most notably, it predicted five adjacent stem-loop structures of 34, 22, 24, 22, and 18 nucleotides (SL-1 to SL-5) (Fig. 2A and 3A). SL-5, which is followed by a string of five consecutive U's, resembles a conventional Rho-independent transcription terminator (Fig. 2A). Synthesis of a small RNA corresponding to the *ipeX* DNA on a plasmid clone was confirmed by RT-PCR analysis (Fig. 2B and C). A greater amount of *ipeX* RNA was present when IPTG was added in the culture, showing both leaky (without IPTG) and induced expression of *ipeX* from the pTrc promoter of the *ipeX* clone (Fig. 2C, lanes 2 and 3). These results are consistent with the notion that the elevated *ipeX* RNA level in the presence of IPTG produces a greater inhibitory effect on porin expression. *ipeX* RNA could not be detected in bacterial cells devoid of the *ipeX* plasmid clone (Fig. 2C, lane 1), suggesting that *ipeX* is not normally expressed from the MC4100 chromosome.

The involvement of *ipeX* stem-loops on porin expression was investigated by constructing various plasmids lacking one or more predicted stem-loop structures (Fig. 3A). The data showed that the presence of all five stem-loops imposed the strongest inhibitory effect on *ompC* expression, a modest effect in the presence of stem-loops 2 to 5, and no inhibition when only stem-loops 3 to 5 were present (Fig. 3B). Thus, the region encompassing stem-loops 1 and 2 is mandatory for porin inhibition. However, we cannot completely rule out the possibility that a lack of the inhibitory effect of smaller clones is due to the

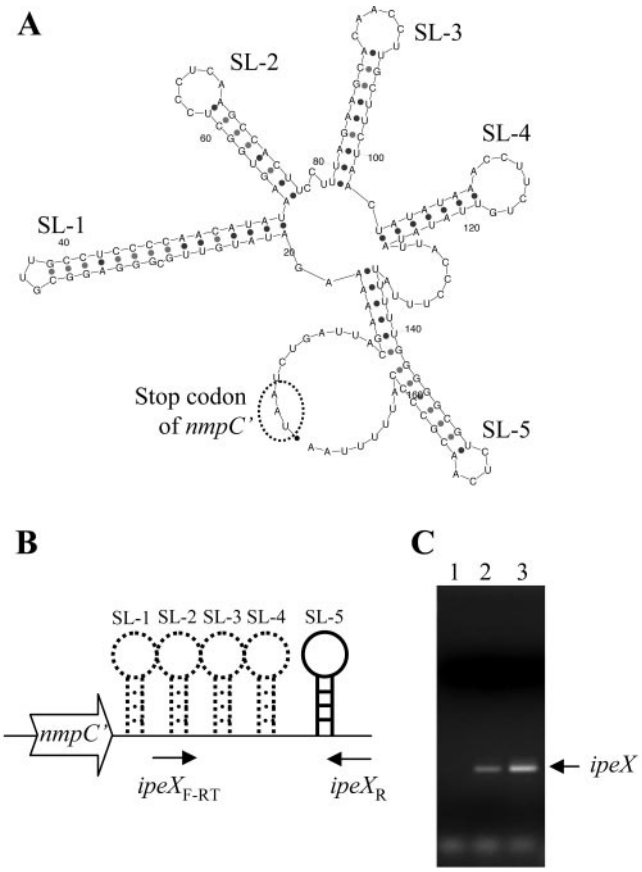

FIG. 2. (A) The 167-bp-long portion of the *ipeX* RNA sequence was analyzed using MFOLD software, which predicted the RNA secondary structure. There are four stem-loop structures (SL-1 to SL-4) and a presumed Rho-independent transcription terminator (SL-5). The stop codon of the truncated *nmpC′* gene is circled. (B) A cartoon showing the *ipeX* region and locations of *ipeX* primers used in the RT-PCR analysis. (C) *ipeX* RNA was analyzed by RT-PCR from cells containing just the vector plasmid (lane 1) and the *ipeX* clone (lanes 2 and 3). RNA was isolated from cells grown in the absence (lane 2) and presence (lane 3) of IPTG to induce *ipeX* expression from the pTrc99A promoter.

instability of the truncated *ipeX* transcript. It is unclear why the *ybcQ* clone with all five stem-loops but lacking 38-bp from the 3′ end of *nmpC′* imposes a greater inhibitory effect on porin expression than a similar clone containing the 38-bp 3′ *nmpC* DNA (Fig. 3B, lanes 4 and 5).

Previously two regulatory RNA molecules that negatively regulate *ompC* and *ompF* expression have been identified, *micC* (7) and *micF* (23), respectively. Both of these RNA molecules have extended sequence complementarities to the 5′ end of their target mRNAs and exert their inhibitory effects on porin expression by preventing translation (7, 23). No such extended complementarity between *ipeX* and *ompC* or *ompF* was found. Interestingly, however, bases present in the loop region of stem-loop 2 LSL-2 of *ipeX* are complementary to the SD region of *ompF* (6 of 6 nucleotides), *ompC* (5 of 6 nucleotides), and *ompA* (4 of 6 nucleotides). Although all 6 bases of LSL-2 are complementary to the *ompF* SD region, the effect of *ipeX* on *ompC* is more severe than on *ompF*, suggesting that a

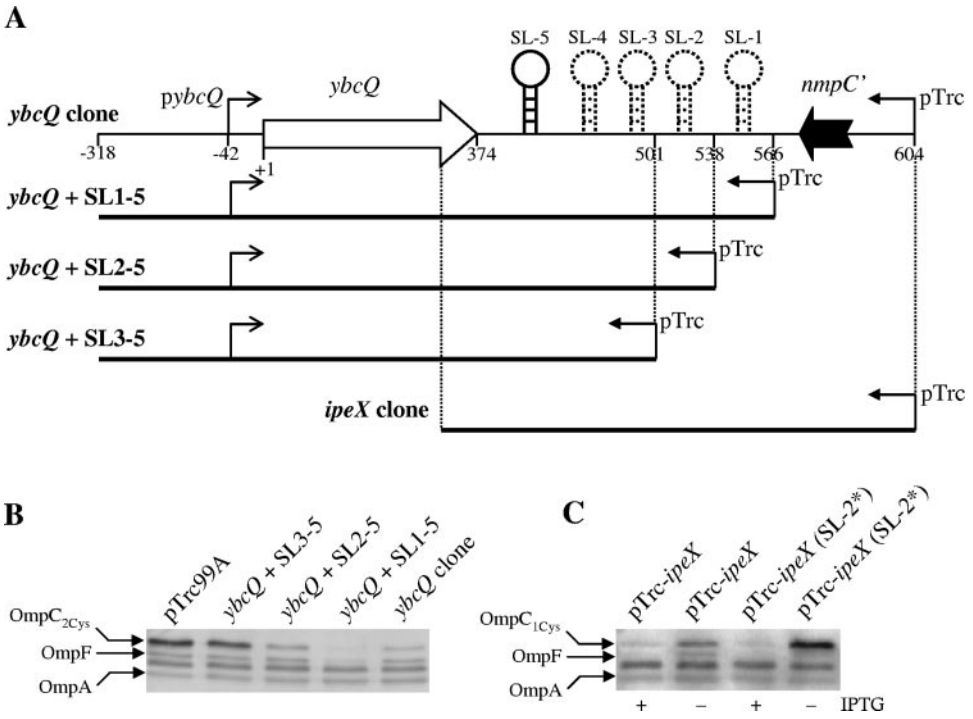

FIG. 3. (A) Schematic diagrams of the *ybcQ* clone and its derivatives lacking one or two predicted stem-loops (SL) or almost the entire *ybcQ* gene (*ipeX* clone). Small leftward arrows indicate the location of IPTG-inducible pTrc99A promoter while small rightward arrows show the location of the predicted *ybcQ* transcription start site. (B) OmpC, OmpF, and OmpA were detected in whole cell envelopes by Western blot analyses with OmpC polyclonal antibodies that also recognize OmpF and OmpA. Shown are the names of the plasmids that were transformed into RAM415 ($OmpC_{2Cys}$). *ipeX* expression was not induced by IPTG; instead, it occurred from the leaky pTrc99A promoter. (C) OMPs were detected from whole cell envelopes. Shown are the names of plasmids transformed into RAM412 ($OmpC_{1Cys}$). pTrc-*ipeX* (SL-2*) refers to a mutant plasmid derived from pTrc-*ipeX* in which three residues of *ipeX* SL-2, which are complementary to the *ompC* and *ompF* SD sequences, have been altered by site-directed mutagenesis. *ipeX* expression was induced by IPTG.

sequence other than that in LSL-2 plays a greater role in porin regulation. To assess the involvement of the LSL-2 sequence in *ompC* regulation, we altered 3 of its 6 nucleotides (CCCTCA to CAAACA) by site-directed mutagenesis and found only a modest reduction (without overexpression) in the ability of the mutant *ipeX* clone to reduce OmpC levels (Fig. 3C). These results affirmed that although the LSL-2 sequence plays a role in regulation, additional *ipeX* sequences must also participate in porin regulation and inhibition specificity.

In PA-2 lysogens, *ipeX* is transcribed in *cis* with the phage porin gene. The *ipeX* sequence located downstream of the *nmpC* porin gene of the cryptic phage *qsr′* is identical to the sequence present downstream of the *lc* gene of the PA-2 phage. We asked whether *ipeX* RNA is made in PA-2 lysogens and, if so, whether it is made as a part of the *lc* transcript. These questions were addressed by conducting RT-PCR analysis of RNA obtained from the PA-2 lysogen (CS137) and its parental strain (CS109) (Fig. 4). In the PCR step, we carried out two separate amplifications using the same forward primer, which was specific to the *lc* gene but different reverse primers, one specific to the *lc* gene while the other specific to the *ipeX* sequence (Fig. 4A). Transcription of the *lc* gene from PA-2 lysogens should give a RT-PCR product using *lc*-specific forward (lc_F) and reverse (lc_R) primers. If *ipeX* is transcribed as part of *lc*, we should detect a RT-PCR product by using lc_F and $ipeX_R$ primers. The data presented in Fig. 4B showed a RT-PCR product of the expected size from PA-2 lysogens, using lc_F and $ipeX_R$ primers, thus showing that *ipeX* RNA is synthesized attached to the 3′ end of the *lc* RNA.

***ipeX* exerts its negative effect by destabilizing the target mRNA.** The IPTG-induced expression of *ipeX* from the plasmid clone only moderately reduced *ompC* (50%) and *ompF* (20%) transcription from the *ompC′*::*lacZ*$^+$ and *ompF′*::*lacZ*$^+$ operon fusion constructs, respectively (Fig. 5). This effect of *ipeX* on *ompC* and *ompF* transcription was significantly less than that on OmpC and OmpF protein levels (Fig. 1B), suggesting that the bulk of the inhibitory effect must be exerted posttranscriptionally. Since pulse-chase experiments failed to show any OmpC protein when cells expressing high levels of *ipeX* were labeled (data not shown), indicating an inhibition on OmpC protein synthesis, we focused on RNA analysis.

Direct evidence for the posttranscriptional effect of *ipeX* on *ompC* and *ompF* expression came from RT-PCR analysis of RNA isolated from cells expressing *ipeX*. *ompA* mRNA was analyzed as a control, since OmpA levels remained unaffected when *ipeX* was overexpressed (Fig. 1B). When cells were grown in the absence of IPTG so that only leaky expression of *ipeX* could occur from the plasmid promoter, a modest reduction in *ompC* mRNA level was observed (Fig. 6A). In contrast, a dramatic reduction in *ompC* mRNA resulted when *ipeX* expression was fully induced by the addition of IPTG to the growth medium, while *ompA* mRNA levels remained unaf-

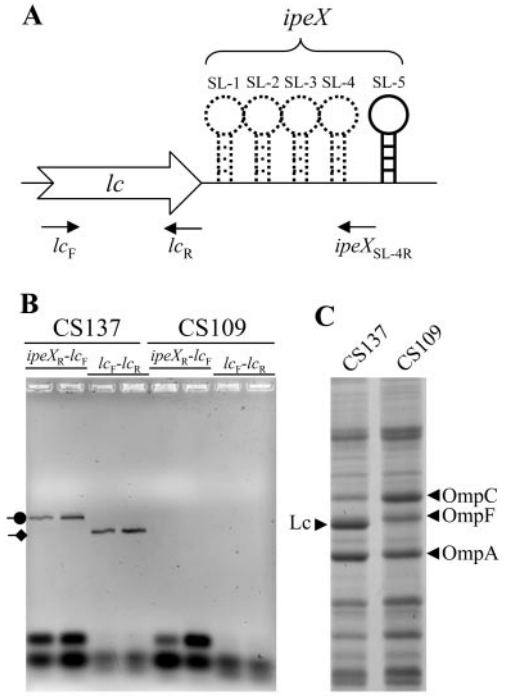

FIG. 4. RT-PCR and envelope protein analyses from the parental (CS109) and PA-2 lysogen (CS137) strains. (A) A cartoon showing the *lc-ipeX* region of the PA-2 phage genome. Locations of various stem-loops (SL-1 to SL-5) and primers used in the RT-PCR analysis are shown. (B) After RT reactions using the *ipeX* reverse primer ($ipeX_R$), a subsequent PCR step was carried out using primers to amplify the *lc* region (lc_F and lc_R) and the *lc-ipeX* region (lc_F and $ipeX_R$). PCR-amplified products were analyzed on an agarose gel, and bands were visualized after ethidium bromide staining. PCR-amplified products corresponding to *lc* (diamond arrowhead) and *lc-ipeX* (circle arrowhead) RNA were obtained only from the PA-2 lysogen strain. (C) Envelopes isolated from CS109 and CS137 were analyzed by SDS-polyacrylamide gel electrophoresis. Proteins bands were visualized after Coomassie brilliant blue staining. Positions of Lc, OmpC, OmpF, and OmpA are shown.

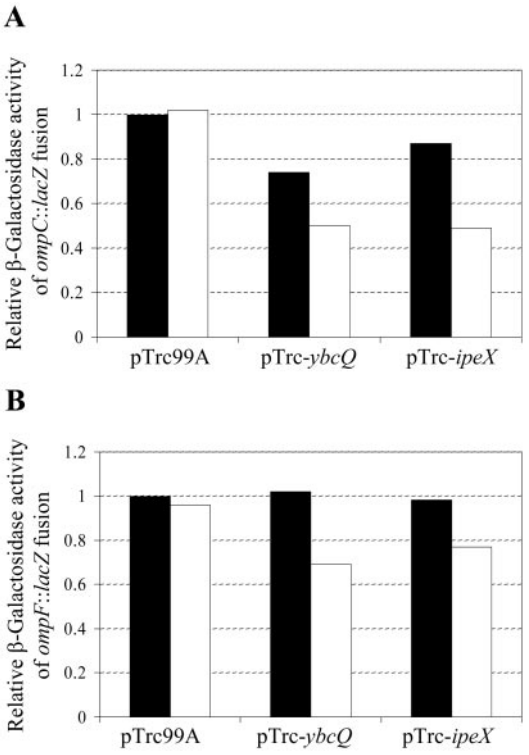

FIG. 5. Transcriptional activities of *ompC* (A) and *ompF* (B) as analyzed through assaying β-galactosidase activities of the *ompC′*::*lacZ*$^+$ and *ompF′*::*lacZ*$^+$ operon fusion constructs, respectively. Cultures were grown in the absence (solid bars) or presence (empty bars) of IPTG. Enzymatic activities are relative to those obtained from pTrc99A-containing cells grown without IPTG. Names of plasmids present in the bacterial strains are shown.

fected (Fig. 6B). *ompF* transcripts were also reduced by *ipeX* overexpression but to a lesser extent than that of *ompC* (data not shown). These results showed that porin mRNA production and/or stability is lowered when *ipeX* is overexpressed.

We determined the half-life of *ompC* mRNA to gain a better insight into the *ipeX*-mediated inhibition mechanism. Since the overexpression of *ipeX* severely lowered *ompC* mRNA levels, making it difficult to evaluate half-lives, experiments were performed under conditions of modest *ipeX* expression (without IPTG induction) that still produced a negative effect on *ompC* mRNA levels (Fig. 6A). Cultures were grown to mid-log phase, and rifampin was added to prevent new rounds of transcription initiation. Cells were collected immediately after the addition of rifampin (time zero) and 2, 4, 8, and 16 min thereafter. Cells withdrawn at various times were instantly frozen to minimize mRNA decay. *ompC* and *ompA* (control) mRNAs were analyzed by RT-PCR (Fig. 7). The results clearly show that only when cells are expressing *ipeX* do we see a significant reduction in the half-life of *ompC* mRNA (Fig. 7B). Consistent with the protein data, the half-life of *ompA* mRNA was unaffected by *ipeX* (Fig. 7B).

Regulatory regions of *ompC* required for *ipeX*-mediated inhibition. Overexpression of *ipeX* from an IPTG-inducible promoter effectively inhibited OmpC when it was produced from a compatible plasmid (pSC101 replicon) containing the entire *ompC* coding region under the control of *ompC*'s native promoter and the untranslated region (UTR) between the promoter and initiation codon (Fig. 8A). On other hand, when the *ompC* coding region was placed under the transcriptional and translational control sequences of the pTrc99A vector plasmid, OmpC synthesis was unaffected when *ipeX* was expressed from the arabinose-inducible promoter of pBAD33 (data not shown). These results suggested that *ipeX*-mediated inhibition requires the noncoding region of the *ompC* gene. To narrow down this region, we constructed two *ompC-phoA* chimeras. In one chimera, PhoA was synthesized under the transcriptional and translational controls of *ompC* and used OmpC's signal sequence (Fig. 8B). The other chimera had the *ompC* promoter and UTR of *ompC* but incorporated PhoA's native signal sequence instead of OmpC's (Fig. 8C). Both chimeras synthesized PhoA that could be readily detected by Western blot analyses using PhoA antibodies (Fig. 8B and C). However, PhoA synthesis was inhibited when *ipeX* was overproduced (Fig. 8B and C). These results showed that the 248-bp sequence encompassing the promoter and UTR of *ompC* is sufficient to observe the *ipeX*-mediated inhibition of *ompC* expression.

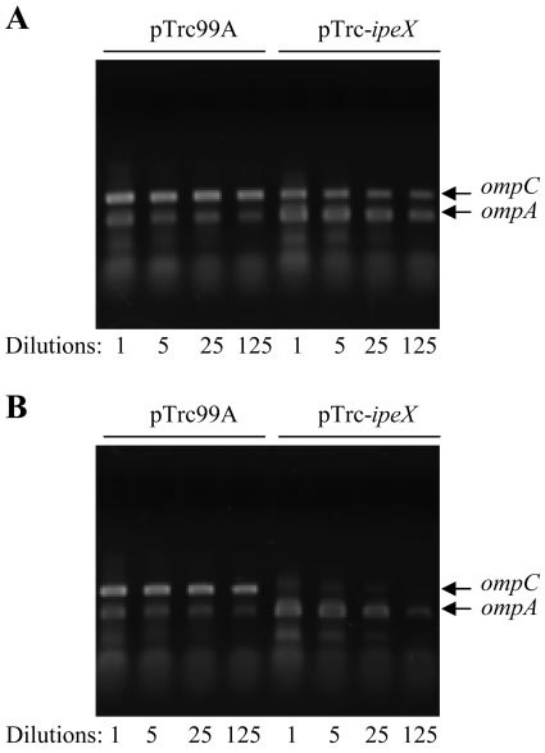

FIG. 6. Semiquantitative analysis of OmpC and OmpA RNAs through RT-PCR. RNA was isolated from cultures grown in the absence (A) or presence (B) of IPTG. Undiluted (1-fold) and 5-, 25-, and 125-fold diluted cDNAs were amplified by PCR using *ompC*- and *ompA*-specific primers. Both *ompC* and *ompA* PCRs were carried out in the same tube. Amplified DNAs were analyzed on agarose gels.

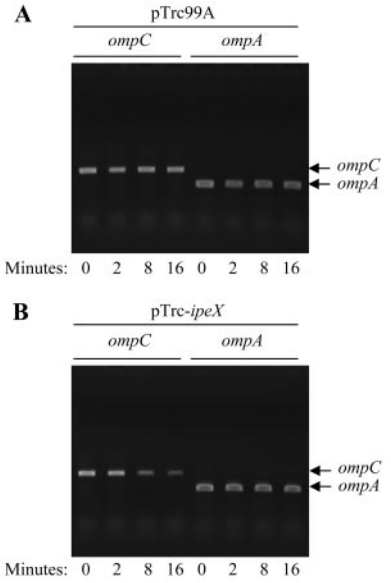

FIG. 7. Half-life determination of *ompC* and *ompA* RNA by RT-PCR. RNA was isolated from bacterial cultures treated with rifampin for various time durations without (pTrc99A) (A) or with (pTrc-*ipeX*) (B) *ipeX* expression. One-in-ten diluted cDNA products were used in separate PCRs using *ompC*- or *ompA*-specific primers. Amplified DNAs were analyzed on agarose gels.

***hfq* is not required for the *ipeX*-mediated inhibition of *ompC* expression.** It is well documented that several small regulatory RNAs that act by base pairing to their target mRNA require Hfq, a RNA chaperone (24, 37). Both *micC* and *micF* RNAs bind to Hfq (7, 37) and *micC* is shown to require Hfq for its action (7). We asked whether Hfq is also required for the *ipeX*-mediated regulation of *ompC* and *ompF* expression. Our results showed no change in the *ipeX*-mediated inhibition of OmpC expression when Hfq was absent from the cell (Fig. 9A). However, *ipeX* could not inhibit OmpF expression in cells lacking Hfq (Fig. 9A). It should be noted that we introduced the *hfq*-null allele into the $ompC_{1Cys}$ strain because $ompC_{2Cys}$ *hfq*-null cells produced extremely small and heterogeneous colonies. Lastly, even though elevated expression of *ipeX* in an *hfq*-null background severely reduced *ompC* mRNA levels, *ompA* mRNA levels remained unaffected (Fig. 9B).

Suppression by the plasmid clone containing the 76-minute chromosomal region. Western analysis of envelope proteins revealed that the presence of the suppressor plasmid significantly reduced $OmpC_{2Cys}$ levels compared to that observed in an $ompC_{2Cys}$ $degP^+$ strain (data not shown). As with the *ipeX* clone, the effect of this suppressor was not specific to $OmpC_{2Cys}$, as the levels of $OmpC_{1Cys}$ and wild-type OmpC were also reduced by the plasmid. Nucleotide sequence analysis revealed that the insert DNA contained a contiguous chromosomal region encompassing the 3′-truncated *ompR*, *greB*, and *yhgF* and 5′-truncated *feoA* genes (Fig. 10A). We suspected that the expression of the 3′-truncated *ompR*, lacking the last 75 nucleotides of the 717-bp *ompR* ORF, is likely responsible for the reduced OmpC level. This assertion was based on the previous characterization of a specific class of

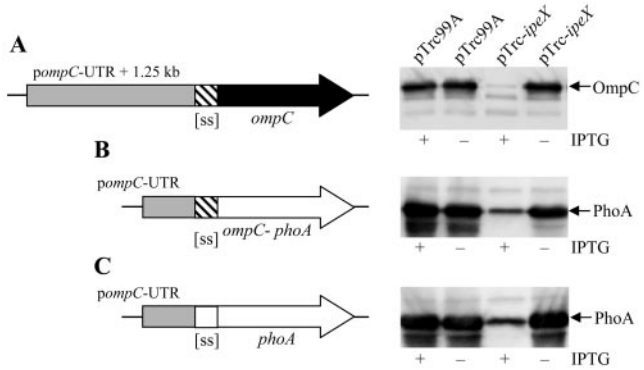

FIG. 8. Effects of *ipeX* overexpression on OmpC and PhoA synthesis from various plasmid constructs. *ipeX* was expressed from an IPTG-inducible promoter of pTrc99A. *ompC* was expressed from a 1.5-kb noncoding region of *ompC* which contains its native 248-bp promoter and UTR (gray box) (A). This *ompC* clone was present on a plasmid replicon (pSC101) compatible with the ColE1 replicon of pTrc99A. *phoA* clones were present on pACYC184, which is also compatible with pTrc99A. PhoA synthesis was placed under the control of the 248-bp *ompC* promoter and UTR sequences (gray box) (B and C). Filled and open arrows show mature protein-coding regions of *ompC* and *phoA*, respectively. *phoA* constructs used either *ompC*'s (striped box) (B) or *phoA*'s (open box) (C) signal sequence-coding region (ss). Plus and minus signs refer to cultures with or without the IPTG inducer, respectively. OmpC and PhoA proteins were visualized from whole cells by Western blot analyses with OmpC (A) and PhoA (B and C) antibodies.

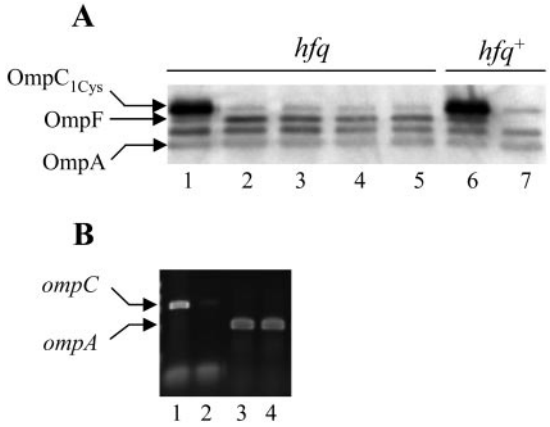

FIG. 9. Effects of Hfq on *ipeX*-mediated inhibition of OmpC, OmpF, and OmpA expression. (A) OmpC, OmpF, and OmpA were analyzed from envelopes of *hfq*-null or *hfq* wild-type strains carrying either the pTrc99A vector (lanes 1 and 6) or the *ipeX* clone (lanes 2 to 5 and 7). OMPs were detected by Western blot analyses using OmpC polyclonal antibodies that also recognize OmpF and OmpA. Due to an apparent growth defect of the strain carrying the *hfq*-null allele, multiple (four) independent cultures (lane 2 to 5) were analyzed to assess the effect of *ipeX* on porin expression. (B) *ompC* and *ompA* transcripts were analyzed by RT-PCR from *hfq*-null strains carrying the vector plasmid (lanes 1 and 3) or the *ipeX* clone (lanes 2 and 4). *ipeX* expression from the resident plasmid was induced by IPTG.

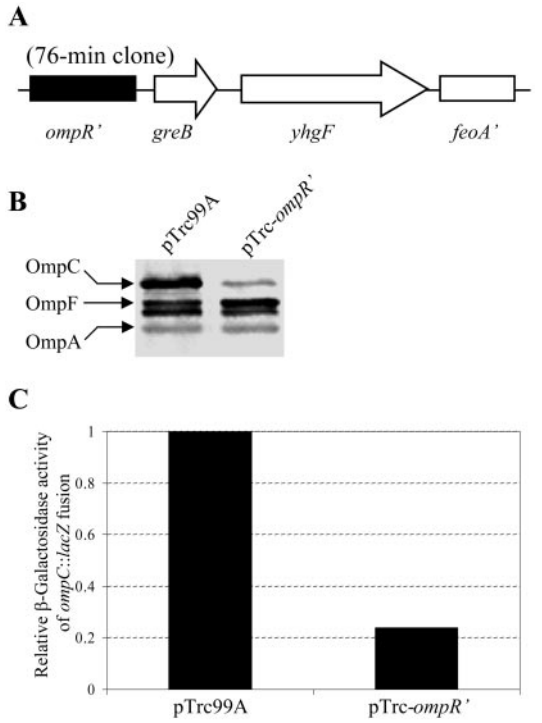

FIG. 10. (A) A schematic diagram of the 76-min clone that suppresses $OmpC_{2Cys}$-mediated lethality. The *ompR* and *feoA* genes are truncated at their 3′ and 5′ ends, respectively. (B) Western blot analysis of OmpC, OmpF, and OmpA from envelopes of strains carrying the plasmid vector or the truncated *ompR* clone lacking the last 25 codons of the *ompR* gene. The orientation of the *ompR′* gene in the pTrc99A clone is opposite to that of the pTrc99A promoter, thus *ompR′* was expressed from its indigenous promoter. OMPs were detected by Western blot analyses using OmpC polyclonal antibodies that also recognize OmpF and OmpA. (C) Effect of the truncated *ompR* clone on *ompC* transcription relative to the pTrc99A vector. OmpC expression was measured by assaying β-galactosidase activities of an *ompC′*::*lacZ*$^+$ operon fusion construct.

ompR mutants, referred to as *ompR2* (12, 13), which displays an $OmpF^+$ $OmpC^-$ phenotype and produces either C terminus-truncated OmpR (1) or full-length OmpR with alterations at the C terminus (26). To test this, a plasmid clone containing all but the last 75 nucleotides (i.e., the last 25 codons) of the *ompR* gene was created and transformed into strains expressing various *ompC* alleles. The presence of the *ompR′* plasmid substantially reduced OmpC levels (Fig. 10B), thus confirming that the expression of C terminus-truncated OmpR from the suppressor plasmid is responsible for the reduced OmpC expression. These results also showed that the plasmid-encoded truncated OmpR mutant is dominant to the chromosomally encoded wild-type protein. Finally, since the effect of the OmpR2 mutants is exerted at the transcription level, we examined the effect of the *ompR′* clone on *ompC* transcription by assaying β-galactosidase activities from an *ompC′*::*lacZ*$^+$ operon fusion. Compared to the control strain containing just the vector plasmid, the presence of the *ompR′* clone inhibited the β-galactosidase activity by almost 80% (Fig. 10C), thus demonstrating that the inhibitory effect of the plasmid clone on *ompC* is primarily exerted at the transcription level. Taken together, these data showed that the expression of the C terminus-truncated OmpR protein from the suppressor plasmid reduces $ompC_{2Cys}$ transcription, thus eliminating the toxic effect of $OmpC_{2Cys}$ in a *degP*-null background.

DISCUSSION

The search for multicopy suppressors of the assembly-defective and lethal $OmpC_{2Cys}$ mutant protein yielded two plasmid clones that rescued lethality by reducing OmpC expression. One plasmid produced a C terminus-truncated OmpR protein, which exerted its effect by lowering *ompC* transcription, resulting in an $OmpF^+$ $OmpC^-$ phenotype typical of previously characterized OmpR2 mutants (1, 12, 13, 26). The other plasmid clone also reduced OmpC expression but exerted its effect mainly at the posttranscription level. Further analysis of this latter plasmid led to the discovery of a regulatory strategy, possibly employed by certain temperate phages, by which OmpC expression is down-regulated concomitant to the synthesis of a phage-encoded porin.

Over 30 years ago, Schnaitman's lab reported that the lysogenization of *E. coli* strains by a lambdoid phage, PA-2, resulted in the production of a phage-encoded membrane protein called Lc and the inhibition of synthesis of OmpC, which had been shown to serve as the cell surface receptor for PA-2 (28). Subsequently, it was determined that Lc is highly homologous to the classical porins, OmpC and OmpF (2). Although the exact reason for the Lc expression-mediated inhibition of OmpC was unknown, it was speculated that reduced levels of OmpC on the cell surface either render cells ineffective in neutralizing the phage progeny generated when the lysogen is induced or prevent superinfection of lysogens (2, 28).

The data presented in this work showed that transcription from a 247-bp-long clone containing the 3′ end of the phage-

encoded porin *nmpC′* gene produced the inhibitory effect on OmpC expression. The *E. coli* K-12 strains used in our laboratory are not PA-2 lysogens but contain a remnant of the cryptic lambdoid phage, *qsr′* (DLP12), genome incorporated at the 12-min region of the chromosome (14). The *qsr′* phage genome contains a segment encompassing the NmpC porin gene that is identical to the PA-2 phage genome (14). The NmpC porin from the *qsr′* phage is not expressed due to the presence of an insertion element (IS*5*) separating the last 19 codons of *nmpC* from the rest of its reading frame (2). We have determined that the 247-bp DNA located at the 3′ end of the *nmpC* gene contains an inhibitor of porin expression, *ipeX*, which is transcriptionally inactive in our *E. coli* strains. Expression of *ipeX* from the original suppressor plasmid occurred presumably from the vector plasmid promoter; *ipeX* from subsequent smaller clones was expressed exclusively from an IPTG-inducible pTrc99A promoter. Thus, *ipeX* was discovered serendipitously through our efforts to seek multicopy suppressors of the toxic $OmpC_{2Cys}$ protein.

The 247-bp *qsr′* phage sequence encompassing *ipeX* is identical to the DNA sequence present downstream from the PA-2 phage's *lc* gene. Therefore, it is reasonable to assume that in PA-2 lysogens, transcription of the *lc* gene from its promoter also transcribes the immediately downstream region corresponding to *ipeX*, which in turn inhibits *ompC* expression. The first stem-loop of *ipeX* commences only 16 nucleotides downstream from the translation stop codon of the *lc* and *nmpC* genes and all four *ipeX* stem loops are sandwiched between the stop codon and the predicted Rho-independent transcription terminator (referred to as SL-5) of these genes (Fig. 2A). We suspect that the location of the *ipeX* stem-loops at the end of the *lc*/*nmpC* transcript ensures that the expression of the host *ompC* porin gene is inhibited only when the phage porin gene is completely transcribed in lysogens.

Data presented here suggest that the *ipeX* sequence does not code for a protein; rather, it is transcribed only into a RNA molecule. Since *ipeX* and the targeted porin genes do not share significant sequence complementarities, it is likely that the *ipeX*-mediated regulation is different from that mediated by *micC* and *micF*. There is a region of short complementarity between the loop 2 sequence of *ipeX* and the SD sequence of *ompC* and *ompF*, but this region is not critical for overall inhibition or specificity. This is because the inhibitory effect of *ipeX* on *ompC*, with five nucleotides complementary to its SD region, is significantly greater than that on *ompF*, in which all 6 nucleotides of loop 2 are complementary to the *ompF* SD region. Moreover, elimination of complementary nucleotides between the loop 2 of *ipeX* and *ompC* SD region only slightly weakened the mutant *ipeX*'s ability to inhibit *ompC* expression. Thus, unlike with *micC* and *micF*, extended base pairing between *ipeX* and the porin RNA molecule does not appear to be involved in, or critical for, inhibition.

It is interesting that whereas the inhibitory actions of *ipeX* and *micF* on OmpF and *micC* on OmpC are dependent on an RNA chaperone, Hfq, the *ipeX*-mediated inhibition of *ompC* is independent of Hfq. The reason for this anomaly is unclear but may possibly reflect different mechanisms or strengths of RNA-RNA interactions. A reduced half-life of *ompC* mRNA by *ipeX* fits well with the *ompC-lacZ* fusion data and together they show that the inhibitory action of *ipeX* on *ompC* expression is largely posttranscriptional. Our data also narrowed down where *ipeX* acts to the 248-bp promoter and UTR of *ompC*. However, since there is no obvious base pairing involved between *ipeX* and *ompC* UTRs, we are unable to pinpoint a precise site of action. Additional work is needed to find this and the underlying molecular mechanism by which *ipeX* inhibits porin gene expression.

We find it interesting that *ipeX*, *micC*, and *micF* sequences are located adjacent to a porin gene. Close proximity is presumably important in the coordinated regulation of porin genes. A fascinating aspect of the *ipeX* location is that unlike *micC* and *micF*, which are synthesized as independent units from their own promoters, we have shown that *ipeX* is synthesized from the phage's porin gene promoter as part of the phage porin transcript at the 3′ terminus. Our experiments also demonstrated that the inhibitory action of *ipeX* does not require that it be physically attached to the porin gene transcript. Thus, it is possible that the *ipeX* portion of the *lc-ipeX* transcript is cleaved after synthesis. We hypothesize that the coupling of the porin gene and *ipeX* to the same transcript ensures that the *ipeX*-mediated inhibition of *ompC* expression occurs only when the phage-encoded porin gene is expressed in lysogens.

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