

Genetic Evidence for Functional Interaction of the *Escherichia coli* Signal Recognition Particle Receptor with Acidic Lipids *in Vivo**

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The mechanism underlying the interaction of the *Escherichia coli* signal recognition particle receptor FtsY with the cytoplasmic membrane has been studied in detail. Recently, we proposed that FtsY requires functional interaction with inner membrane lipids at a late stage of the signal recognition particle pathway. In addition, an essential lipid-binding α -helix was identified in FtsY of various origins. Theoretical considerations and *in vitro* studies have suggested that it interacts with acidic lipids, but this notion is not yet fully supported by *in vivo* experimental evidence. Here, we present an unbiased genetic clue, obtained by serendipity, supporting the involvement of acidic lipids. Utilizing a dominant negative mutant of FtsY (termed NG), which is defective in its functional interaction with lipids, we screened for *E. coli* genes that suppress the negative dominant phenotype. In addition to several unrelated phenotype-suppressor genes, we identified *pgsA*, which encodes the enzyme phosphatidylglycerophosphate synthase (PgsA). PgsA is an integral membrane protein that catalyzes the committed step to acidic phospholipid synthesis, and we show that its overexpression increases the contents of cardiolipin and phosphatidylglycerol. Remarkably, expression of PgsA also stabilizes NG and restores its biological function. Collectively, our results strongly support the notion that FtsY functionally interacts with acidic lipids.

Membrane-bound ribosomes are responsible for the biosynthesis of many integral membrane proteins that insert into the membrane in a co-translational manner (1, 2). Targeting of ribosomes to the cytoplasmic membrane in *Escherichia coli* requires the signal recognition particle (SRP)³ receptor, FtsY (3).

Many reports have suggested that FtsY functions as a membrane-bound receptor (4, 5), despite the fact that it has no known membrane anchor partner homologous to the mam-

malian β -subunit of the SRP receptor (SR- β). In agreement with this, previous studies showed that FtsY interacts with membrane-bound ribosomes (6) and the translocon (7) and that its membrane localization is required for its function (4, 5, 8). In addition to convincing evidence for FtsY-membrane protein interaction(s), other studies demonstrated unequivocally that the receptor also interacts with lipids (9, 10) and more specifically acidic lipids (11, 12). However, the identified interactions probably do not dictate docking of the receptor (13). In direct support of a *functional* interaction between FtsY and lipids, our recent studies revealed that the receptor contains a short and conserved lipid-binding amphipathic α -helix at the N-terminal edge of the N domain, which affects the enzymatic behavior of FtsY upon interaction with lipids (13, 14), and recent studies further confirmed our conclusions (15). Modeling of the lipid-binding helix of FtsY and its similarity to the MinD membrane targeting sequence (14, 16) suggest that the short helix interacts with acidic lipids, as proposed previously (11). Through the following *in vivo* studies, we obtained genetic evidence that lends support to this notion.

EXPERIMENTAL PROCEDURES

Materials—Antibodies to FtsY were described previously (6). India HisProbe-horseradish peroxidase was purchased from Pierce. Arabinose and isopropyl β -thiogalactoside (IPTG) were purchased from Sigma. *n*-Dodecyl β -D-maltopyranoside was from Anatrace. Nickel-nitrilotriacetic acid beads were from Qiagen.

Strains and Plasmids—*E. coli* Top10 (Invitrogen) was used for the propagation and preparation of various plasmid constructs as well as screening for genes suppressing the NG effect. *E. coli* K12 BW25113 (Δ (*araD-araB*)567, Δ (*lacZ4787*::rrnB-3), λ^- , *rph-1*, Δ (*rhaD-rhaB*)568, *hsdR514*) and Top10 were used for *in vivo* suppression assays. For complementation assays, we used *E. coli* IY28 (BW25113-Kan-*araCFUP*-FtsY), in which the endogenous promoter of FtsY was replaced by the arabinose promoter.⁴ *E. coli* MG1655 was used for the construction of genomic library. The PgsA mutant T60P and insertion of a 6-histidine (His₆) tag into PgsA were constructed by PCR.

Construction of a Chromosomal Library—Genomic DNA of *E. coli* MG1655 was partially digested with Sau3AI. Following

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³ The abbreviations used are: SRP, signal recognition particle; IPTG, isopropyl β -thiogalactoside.

⁴ I. Yosef and E. Bibi, unpublished data.

size selection (2–4 kb), the digested DNA was ligated into BamHI-digested plasmids pCV3 or pT7-5.

Screening Strategy—*E. coli* Top10 harboring either pT7-5-*tacP-NG* or pCV3-*araP-NG* was transformed with the genomic library and plated on LB plates containing ampicillin (200 $\mu\text{g}/\text{ml}$) and kanamycin (30 $\mu\text{g}/\text{ml}$), in the absence (as a control) or presence of a lethal concentration of the NG inducer (0.1 mM IPTG or 0.5% arabinose). Viable colonies from plates with the NG inducer were further analyzed.

Growth Experiments, NG Toxicity, and FtsY Complementation Studies—For growth experiments with NG and FtsY, cells were co-transformed with the compatible plasmids pCV3-*araP-NG* and pT7-5-*tacP-FtsY*. For growth experiments with NG and PgsA and for purification of NG, cells were co-transformed with pT75-*tacP-NG* and either pCV3, pCV3-*pgsA* (+tRNAs, see under “Results”), or pCV3-*pgsA(T60P)* (+tRNAs). For growth experiments with NG and PgsA cloned under the regulation of the *lac* promoter (*lacP His₆-pgsA*) (Aska clone JW1897) (17), cells were co-transformed with pCV3-*araP-NG* and either pCA24N or pCA24N-*His₆-pgsA*. Cultures were grown overnight at 37 °C in LB medium and supplemented with either ampicillin (200 $\mu\text{g}/\text{ml}$) or chloramphenicol (30 $\mu\text{g}/\text{ml}$) and kanamycin (30 $\mu\text{g}/\text{ml}$). Cells were diluted to A_{600} 0.015 induced at A_{600} 0.03 with either 0.2–0.5 mM IPTG or 1% arabinose. Cultures were then grown for 5 or 2 h for protein expression or purification studies, respectively. For FtsY complementation experiments in broth, cells harboring pT7-5-*tacP-NG* and either pCV3 or pCV3-*pgsA* (+tRNAs) were grown overnight at 37 °C in LB, supplemented with ampicillin (200 $\mu\text{g}/\text{ml}$), kanamycin (30 $\mu\text{g}/\text{ml}$), and arabinose (0.2%), the inducer of the chromosomal *ftsY*. Cells were washed four times and diluted in LB broth to A_{600} 0.0005 without arabinose, with or without IPTG (10 μM) and grown for 10 h.

Cell Fractionation—Harvested cultures were washed in 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA and resuspended in the same buffer. The cell suspensions were sonicated, and cell debris was removed by centrifugation (10 min at 10,000 $\times g$). Membranes were collected by ultracentrifugation (45 min at 150,000 $\times g$) and resuspended in the same buffer. The supernatant was also collected and used for analysis of soluble proteins.

Protein Purification from Membrane and Cytosolic Fractions—Cells were resuspended in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl (buffer S), supplemented with 8% sucrose and 1 mM phenylmethylsulfonyl fluoride, and sonicated. Cell debris was removed by centrifugation (10 min at 10,000 $\times g$). Membranes were isolated by ultracentrifugation (45 min at 150,000 $\times g$). Membranes were solubilized by 1% *n*-dodecyl β -D-maltopyranoside in buffer S, and insoluble materials were removed by ultracentrifugation. For the purification of membrane-bound NG, 0.05% *n*-dodecyl β -D-maltopyranoside was added to all the buffers. Cytosolic proteins and solubilized membrane proteins were incubated with pre-equilibrated nickel-nitrilotriacetic acid beads in buffer S supplemented with 5 mM imidazole for 1 h at 4 °C with agitation. The beads were washed once with 10 column volumes of 20 mM Tris-HCl, pH 8.0, 500 mM NaCl (buffer B) supplemented with 5

mM imidazole and two more times with 10 column volumes of buffer B supplemented with 10 mM imidazole. Bound proteins were eluted using 1 column volume of buffer S containing 150 mM imidazole.

Membrane Interaction Studies Using Liposome Flotation—Flotation experiments were performed as described previously (14). Briefly, purified NG and NG+1 proteins (20 μg) were incubated with large unilamellar vesicles of different composition in assay buffer for 20 min at 37 °C. Details of the individual lipid composition are given in the legend to Fig. 6D.

Determination of Acidic Lipids—To analyze changes in the content of various phospholipids, cells were grown in LB medium containing 1 $\mu\text{Ci}/\text{ml}$ [^{32}P]phosphoric acid for 3 h at 37 °C. For pCA24N (empty vector) and pCA24N-*His₆-PgsA*, 50 μM IPTG was included in the incubation media. The cultures (25 ml) were harvested by a 15-min centrifugation at 5000 $\times g$, and the pellet was resuspended in 0.8 ml of PBS, after which 2 ml of methanol and 1 ml of chloroform were added with thorough mixing. After a 1-h incubation at room temperature, PBS and chloroform (1 ml each) were added and mixed thoroughly. Following a brief, low speed centrifugation, the resulting chloroform phase was analyzed by thin layer chromatography with Silica Gel 60 (20 \times 20 cm) (Merck), utilizing chloroform, methanol, water, 25% NH_4OH (120:75:5.6:2.4, v/v) as the developing solvent. Phospholipids were identified on the chromatogram by phosphorimaging (Fuji FLA7000), and the resulting bands were quantified by densitometry using ImageJ software (rsb.info.nih.gov). Phospholipid identities were confirmed by comparison with known reference lipids: dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidylglycerol (DOPG), and tetraoleoyl cardiolipin (TOCL) (Avanti Polar Lipids), which were visualized by 20% phosphomolybdic acid solution.

N-terminal Amino Acid Sequence Analysis—Purified NG proteins were subjected to SDS-PAGE, electroblotted to a polyvinylidene fluoride (PVDF) membrane, and sequenced by Protein Sequencer Procise 491 (Applied Biosystems).

SDS-PAGE and Immunoblotting—Membrane or cytosolic fractions (10–15 μg of protein) were subjected to 12% SDS-PAGE. Proteins were electroblotted to nitrocellulose membranes and probed with rabbit anti-FtsY antibodies or His-Probe-HRP-conjugated goat anti-rabbit immunoglobulin antibodies.

RESULTS AND DISCUSSION

An Unbiased Genetic Search for Factors That Interact with FtsY—Previous studies have shown that the biological activity of *E. coli* FtsY is mediated through its C-terminal domain (termed NG+1) (18). When NG+1 is truncated by one amino acid in its N terminus (NG), it loses activity and becomes highly toxic upon overexpression (13).⁵ To test the possibility that NG confers a dominant negative effect, we co-expressed wild-type FtsY and NG. The results show that FtsY expression in *trans* relieves the toxicity of NG (Fig. 1), suggesting that the nonfunctional NG mutant might compete with the wild-type receptor for binding to an unknown site. To search for pro-

⁵ A. M. Zelazny and E. Bibi, unpublished data.

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teins that functionally interact with NG (and FtsY), we utilized a genetic screen that is based on NG toxicity. Briefly, we anticipated that overexpression of any protein that interacts with NG might dilute the amount of free NG and thus relieve its toxicity. *E. coli* harboring a plasmid encoding NG under a strong promoter (either *tacP* or *araP*) was transformed with a chromosomal library, and the transformants were plated on LB agar containing the NG inducer, IPTG, or arabinose. In the absence of phenotype suppressors, no colonies were formed under conditions of IPTG induction (Fig. 1). In contrast, in plates with the library-transformed cells, several colonies appeared under these conditions. Following re-evaluation of the results by plasmid preparation and retransformation, the positive clones were sequenced, and the results are shown in Table 1. Not surprisingly, clones harboring putative sugar efflux transporters were identified. Although this assumption was not fully validated by deletion analysis, it is plausible that

these transporters would rescue the cells by extruding the NG inducer IPTG, which is a sugar analog (e.g. 19), or arabinose. In sharp distinction to the efflux transporters that one would have expected to identify, the fourth suppressor clone was both surprising and potentially relevant and informative. This clone harbored the *pgsA* gene, which encodes the enzyme phosphatidylglycerophosphate synthase (PgsA) (Fig. 2A, *GS100-3*). PgsA is an integral membrane protein that catalyzes the committed step in the biosynthesis of acidic phospholipids (20). The importance of PgsA and consequently of acidic lipids in recruiting and regulating the activity of peripheral membrane enzymes has been investigated extensively by Dowhan and co-workers (20–22).

Characterization of the Effect of PgsA Expression on NG Toxicity—The increase in anionic phospholipids in cells overexpressing PgsA raised the possibility that the NG toxicity is alleviated by an excess of acidic lipids. As shown in Fig. 2, B and C, induction of NG expression alone abolished growth, whereas cells co-expressing NG and various constructs harboring *pgsA* grew relatively well. It is interesting that cells tolerated PgsA expression only when the *pgsA* gene was followed by the flanking 3' region (Fig. 2, *pCV3-pgsA(+tRNAs)*). A similar observation was made in the past where cells tolerated plasmid-borne *pgsA* only with an unrelated insert at its 3' end that differs from the tRNAs sequences found here (20, 23). This suggests a nonspecific polar effect of 3' sequences on the expression of the *pgsA* gene. To examine further if the 3' tRNA sequences are functionally essential, we repeated the experiments with a plasmid encoding His₆-PgsA under the regulation of the *lac* promoter (*lacP His₆-PgsA*) (Aska clone JW1897) (17). This clone does not contain the 3' tRNA region. Fig. 2D shows that NG-overexpressing cells that simultaneously express His₆-PgsA under the regulation of *lacP* did restore growth, although not as well as the chromosomal clone (compare the right plates in Fig. 2, B and D). To investigate why the expression of His₆-PgsA from the *lac* promoter seems to impose toxicity (Fig. 2D, right plate) we examined the PgsA expression level in cells transformed with plasmids encoding either of the three tested constructs as follows: PgsA-His₆ under its endogenous promoter, with and without the flanking tRNAs 3' region, and PgsA-His₆ under the *lac* promoter (Fig. 2E). The results suggest that only moderate expression of PgsA (from *pgsA-His₆+tRNAs*) can fully restore the NG toxicity, whereas low expression (from *pgsA-His₆*) could not suppress NG toxicity, and very high expression (from *lacP His₆-pgsA*) seems to be toxic, as observed in the past with other PgsA-encoding high copy number plasmids (see below) (23). Notably, the PgsA-His₆ protein migrated

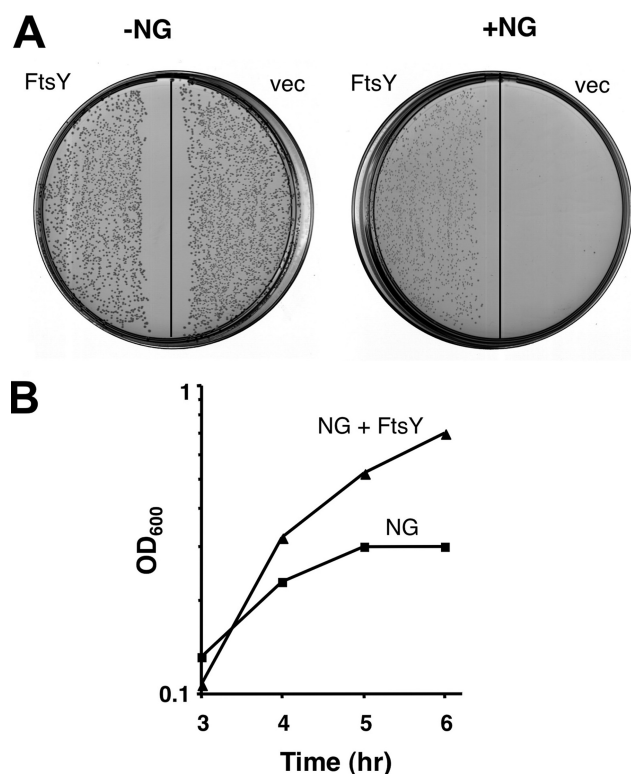


FIGURE 1. FtsY relieves the toxic effect of NG. *A*, *E. coli* cells harboring *pCV3-araP-NG* were co-transformed either with plain vector (*vec*) or *pT75-tacP-FtsY* (FtsY). The transformants were plated on LB agar without (as a control) or with 0.5% arabinose for NG induction. *B*, overnight cultures were diluted and grown in LB broth. At A_{600} (OD_{600}) of 0.03, the cultures were induced with 1% arabinose and grown for additional 4 h. Growth was followed by measuring the optical density of the cultures.

TABLE 1
Proteins identified as suppressors of the NG toxicity

Protein	Inducer used ^a	Cellular location	Function
PgsA ^b	IPTG/arabinose	Inner membrane	Biosynthesis of acidic phospholipids
MdfA ^c	IPTG	Inner membrane	Multidrug transporter
SetA ^c	IPTG	Inner membrane	Sugar efflux transporter
YdeA ^c	IPTG/arabinose	Inner membrane	Sugar efflux transporter

^a The screening was performed in two different systems, in which the NG was expressed under different inducible promoter (*tac* and *ara* promoter) utilizing different inducers (IPTG or arabinose, respectively).

^b Protein was identified.

^c Protein was uncharacterized.

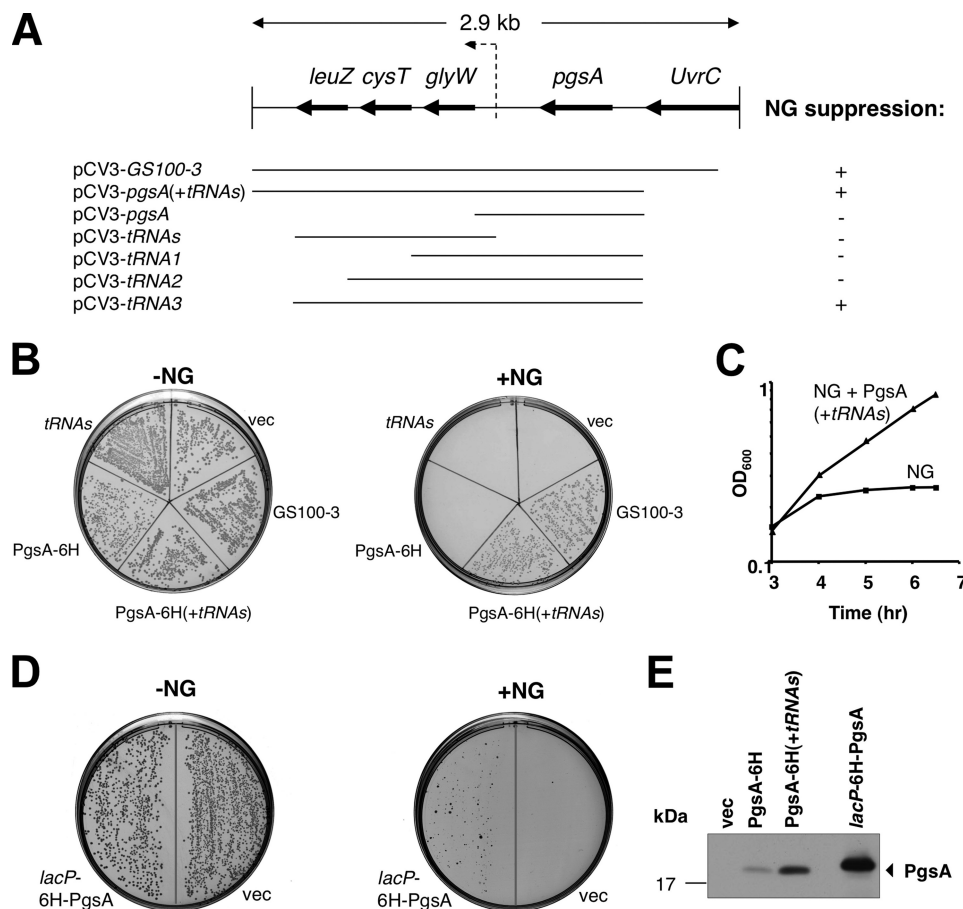


FIGURE 2. Deletion and functional analysis of clones harboring the *pgsA* gene. *A*, schematic representation of several deletion constructs that were characterized. *B*, plasmids harboring the deletion constructs shown in *A*, containing 6 histidine tags at the C terminus of PgsA, were transformed into *E. coli* Top10/pT7-5-*tacP*-NG and examined for their ability to suppress the NG toxicity. *C*, overnight cultures of cells co-expressing NG and either plain vector (NG) or PgsA (NG + *pgsA*(+tRNAs)) were diluted and grown in LB broth. At A_{600} (OD_{600}) of 0.03, the cultures were induced with 0.2 mM IPTG and grown for additional 5 h. Growth was followed by measuring the optical density of the cultures. *D*, Aska clone JW1897 (25) harboring the *His*₆-*pgsA* gene under the *lacP* promoter (*lacP*) (without the *tRNAs* operon) was transformed into *E. coli* Top10/pCV-5-*araP*-NG and plated on LB agar plates with 0.5% arabinose and without IPTG, the PgsA inducer. *E*, *E. coli* cells co-expressing NG and either plain vector (*vec*), PgsA-*His*₆, PgsA-*His*₆ (+tRNAs), or *lacP*-*His*₆-PgsA (Aska clone JW1897) were grown in LB broth. Membrane fractions were isolated, and protein expression was analyzed by Western blotting using India HisProbe-HRP (for *His*₆-tagged PgsA identification). *His*₆-PgsA expressed under the regulation of *lacP* is larger than PgsA-*His*₆ expressed under its endogenous promoter due to addition of 15 amino acids derived from insertion of *Sfi*I restriction sites (25).

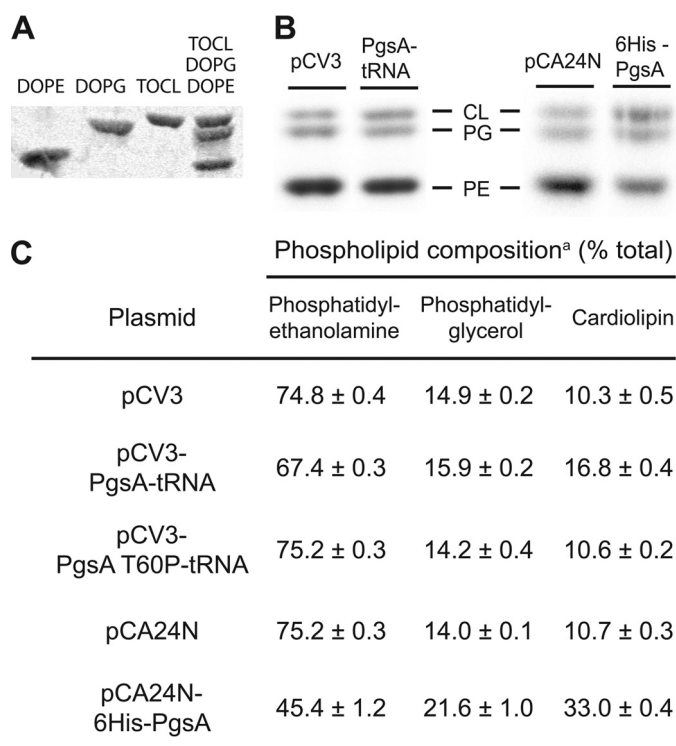
slowly on the gel (Fig. 2*E*) because it harbors additional 15 amino acids encoded by the insertion of *Sfi*I restriction sites (17). These results demonstrate that PgsA alone is sufficient for suppressing the NG toxicity. The 3' gene sequences in our studies (*tRNAs*) and others (20, 23) probably affect the level of expression by an uncharacterized mechanism (such as mRNA stability or translation regulation). For consistency, we used the PgsA clone with the flanking 3' *tRNAs* region throughout most of the experiments.

PgsA Overexpression Changes the Phospholipid Composition—Of special relevance to our studies are previous reports on the importance of acidic lipids that participate in the regulation of pathways that involve peripheral membrane proteins such as MinD (16, 24). It was shown that overexpression of PgsA alters cellular phospholipid composition, as manifested by an increase of about 10% in the concentrations of the acidic phospholipids phosphatidylglycerol and cardiolipin (23, 25). To verify that overexpression of PgsA indeed increased the concentration of acidic phospholipids in our expression systems, we examined the phospholipid compositions of *E. coli*

harboring empty vectors or plasmids encoding various PgsA constructs. Fig. 3*C* clearly shows that all the tested active PgsA constructs increased the amount of acidic phospholipids in the cells compared with cells harboring empty vectors or the inactive mutant PgsA(T60P) (see below). It is likely that the toxicity observed with *His*₆-PgsA (Fig. 2*D*) is caused by the dramatic increase in the content of anionic phospholipids, in addition to problems associated with PgsA overexpression in cells harboring pCA24N-*lacP*-*His*₆-*pgsA* (Fig. 3*C*). Hence, this construct could only partially restore the growth of NG-overexpressing cells.

Enzymatic Activity of PgsA Is Required for Suppressing NG Toxicity—PgsA may affect the toxic phenotype of NG through its enzymatic activity by elevating the level of acidic lipids (Fig. 3) or by direct or indirect physical interaction with NG. To distinguish between these possibilities, we mutated PgsA and examined how the inactive PgsA mutant (PgsA(T60P)) (26) affects the NG toxicity. As shown in Fig. 4*A*, cells expressing NG did not grow when co-transformed with a plasmid expressing PgsA(T60P). By utilizing a 6-histi-

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^aThe results are the averages of three to six experiments

FIGURE 3. Effect of PgsA overexpression on lipid composition *in vivo*. *A*, thin layer chromatography separation of phospholipid standards. *B*, *E. coli* Top10 harboring plasmid-encoded variants of PgsA or empty vectors were labeled with [³²P]phosphoric acid at a final concentration of 1 μCi/ml and grown for 3 h at 37 °C. Lipids were extracted and separated by thin layer chromatography. *C*, quantitation of the phospholipid composition (moi % of lipid phosphorus calculated from radioactivity of each spot, see "Experimental Procedures"). The data shown are mean values, and the standard deviations are calculated from two or three different batches of cultures.

dine tag, we confirmed that the mutant is expressed (Fig. 4*B*), suggesting that the enzymatic activity of PgsA is required for the rescue of NG-expressing cells. Taken together, the results strongly support the notion that NG toxicity can be relieved by increasing the abundance of acidic lipids.

PgsA Expression Restores the Biological Activity of NG—How do elevated levels of anionic phospholipids suppress the toxicity of NG? One possible explanation is that acidic lipids bind and sequester NG, leaving a crucial membrane-attachment site free to accommodate the functional wild-type FtsY. Alternatively, it is also possible that an excess of acidic lipids might restore the function of the lipid-binding element at the N terminus of NG and thereby revive its biological activity. To examine the latter, we used cells depleted of FtsY, which co-expressed NG and PgsA. In the absence of FtsY, such cells would grow only if NG functions properly as an SRP receptor. Fig. 5*A* shows that in the presence of the FtsY inducer (arabinose), all the transformants formed colonies on agar plates. However, in the absence of arabinose, only cells co-expressing NG and PgsA were able to grow. This phenomenon was also observed when cells were grown in liquid media (Fig. 5*B*). The results indicate that the nonfunctional NG is activated by elevated amounts of anionic phospholipids.

PgsA Expression Affects NG Stability—What is the mechanism underlying the restoration effect of acidic lipids on NG

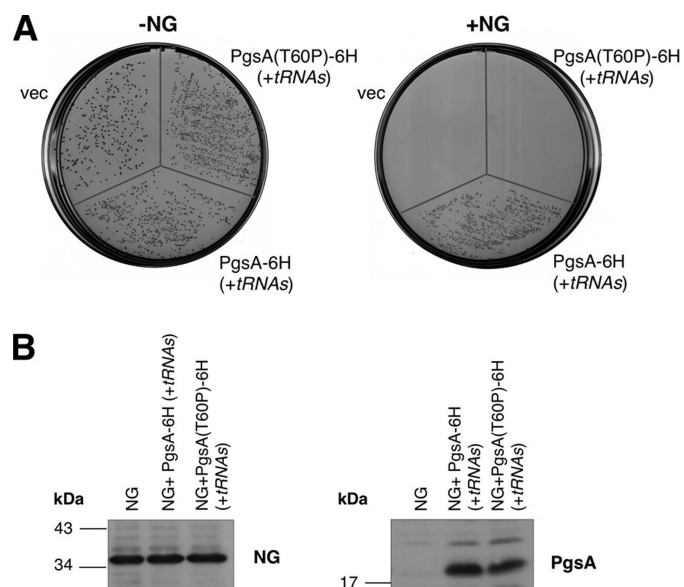


FIGURE 4. Inactive PgsA mutant does not suppress NG toxicity. *A*, inactive PgsA mutant (T60P) was examined for its ability to suppress the NG toxic effect as in Fig. 2. *vec*, vector. *B*, *E. coli* co-expressing NG and either plain vector, PgsA-His₆ (+*tRNAs*) or PgsA(T60P)-His₆ (+*tRNAs*) were grown in LB broth. Membrane fractions were isolated, and protein expression was analyzed by Western blotting using antibodies against FtsY for NG identification (*left panel*) or India HisProbe-HRP for PgsA identification (*right panel*).

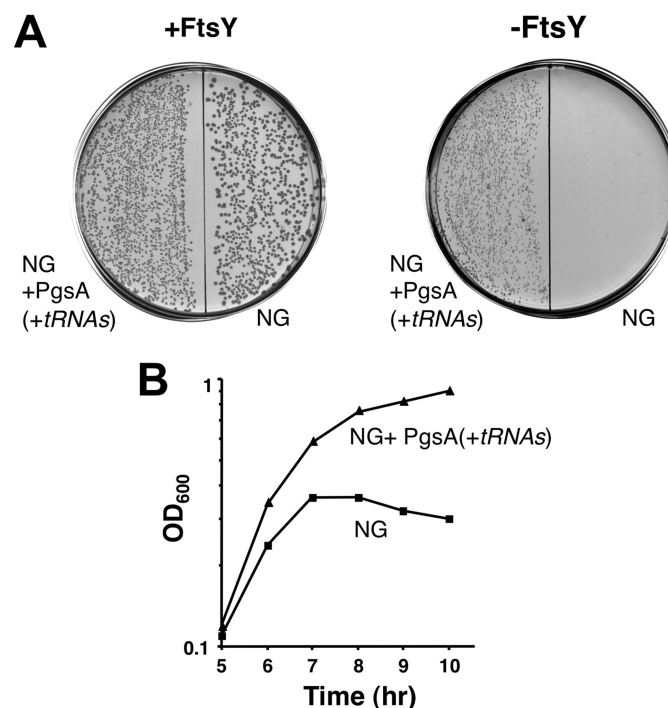


FIGURE 5. NG activation by PgsA. *A*, *E. coli* IY28-pT75-tacP-NG cells were transformed either with a plain vector (NG) or PgsA (NG+*pgsA* (+*tRNAs*)) and plated with (as a control) or without arabinose, the FtsY inducer. *B*, overnight cultures were grown with arabinose, washed four times, and diluted in LB broth without arabinose. Growth was followed by measuring the optical density of the cultures.

activity? It was previously shown that the addition of one amino acid to NG at its N terminus abolished its toxicity and restored its function (18). The crystal structure of the active mutant (NG+1) (14) revealed that it has an ordered N terminus that folds as an amphipathic helix. In contrast, the N ter-

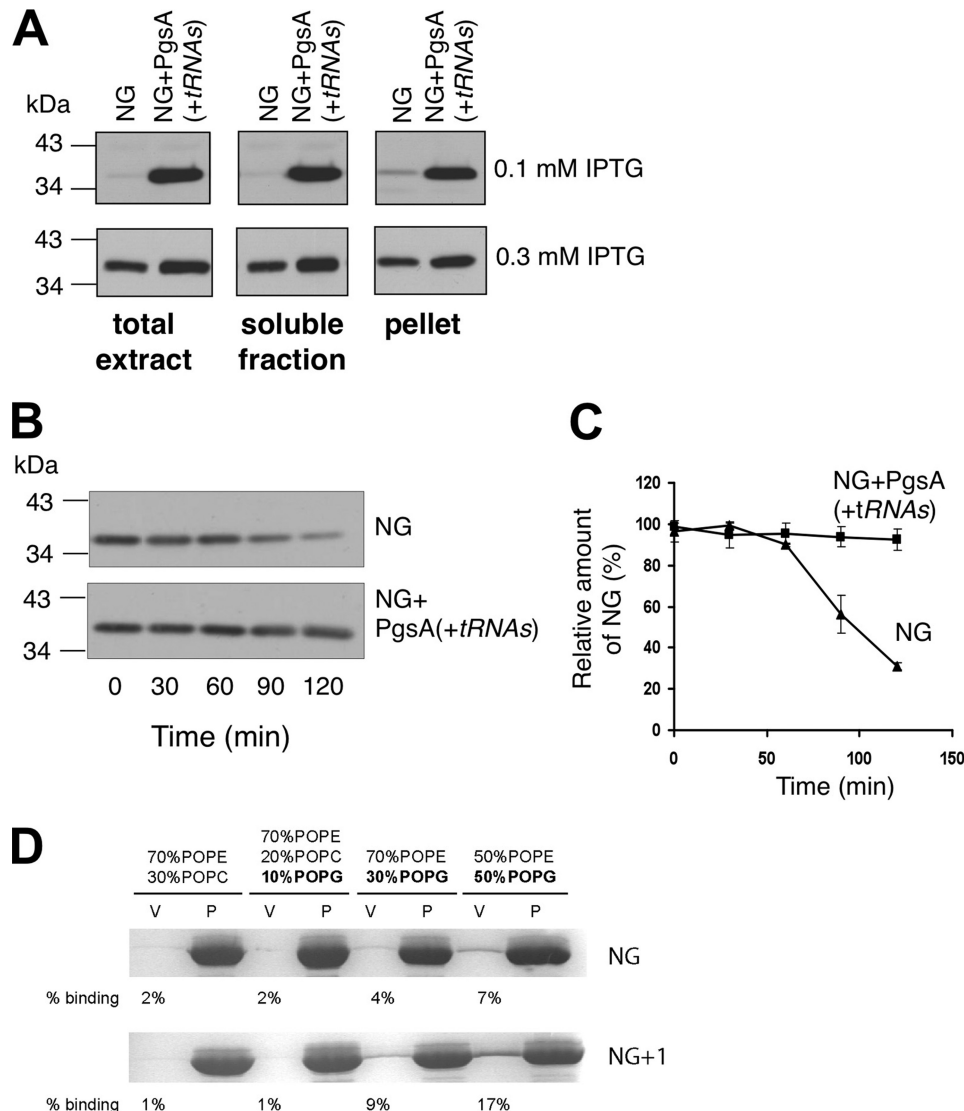


FIGURE 6. PgsA expression stabilizes NG and restores membrane localization. *A*, *E. coli* BW25113 cells expressing NG alone (NG) or with PgsA (NG+PgsA (+tRNAs)) were induced using 0.1 or 0.3 mM IPTG for 2 h. *B*, cells were induced using 0.5 mM IPTG for 30 min, rinsed four times to remove the inducer, and grown again. Samples were taken at different time points, and cells were fractionated. NG expression was analyzed by Western blotting using antibodies against FtsY. *C*, amount of NG was quantified by densitometry, and the average of three independent experiments is shown, with error bars representing standard deviations. *D*, flotation experiments with anionic phospholipids. In presence of zwitterionic phospholipids (phosphatidylethanolamine/phosphatidylcholine ratio of 70:30), no interaction was seen. Lipid interaction could be restored with increasing amounts of anionic phospholipids (10–50% phosphatidylglycerol). The vesicle fraction is marked by “v” and the pellet fraction by “p.” POPE, palmitoyl-oleoylphosphatidylethanolamine; POPG, palmitoyl-oleoylphosphatidylglycerol; POPC, palmitoyl-oleoylphosphatidylcholine.

minus of NG is disordered (27). The N-terminal amphipathic helix of NG+1 was found to be essential for the lipid-stimulated GTPase activity of the receptor in the context of its complex with the SRP (13, 14). Interestingly, analysis of the purified NG protein revealed that it is further processed *in vivo* and consequently lacks its first methionine (Met-195) (14). Therefore, the so-called toxic NG protein actually lacks two amino acids at its N terminus compared with NG+1 and is consequently unable to form an amphipathic helix. We hypothesized that if acidic lipids protect the N-terminal Met-195 residue of NG, the protein would be able to partially re-establish the formation of a helix in its N terminus and consequently its biological activity. To test this, NG was expressed alone or in the presence of PgsA and purified from the cytoplasmic and membrane fractions (data not shown).

The N-terminal amino acid sequences of the purified NG proteins were determined. The results of this experiment rule out the suggestion that the NG N terminus is protected by access of acidic lipids because even when NG was expressed in the presence of PgsA, it lacks Met-195, indicating that Met-195 processing was not prevented by anionic phospholipids (data not shown).

During our studies, we noted that NG is usually better expressed in cells co-expressing PgsA (Fig. 6A). Similarly, previous studies showed that when expressed under identical conditions, the amount of NG was substantially lower than that of the active mutant NG+1, suggesting that, in addition to its functional role, the N-terminal helix of NG+1 may also have a stabilizing effect. To test the possibility that an excess of acidic lipids (in cells expressing PgsA) stabilizes NG, we per-

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formed the following experiment. *E. coli* cultures harboring plasmid-borne NG, alone or together with a constitutively expressed PgsA, were induced for NG expression, rinsed several times to remove the inducer, and grown again. Samples were taken at different time points, and cells were fractionated. Fig. 6, B and C, shows that the level of NG fell in time when expressed alone. However, when NG was co-expressed with PgsA, its amount remained constant throughout the experiment. Importantly, in the absence of PgsA, NG is unable to complement FtsY-depletion even when highly induced.⁶

Because in the presence of PgsA, the amount of NG at the membrane remained constant, we tested the ability of NG and NG+1 to associate with membranes containing different amounts of anionic phospholipids in an *in vitro* binding assay (Fig. 6D, upper panel) (14). The association of NG with large unilamellar vesicles increases with increasing amounts of phosphatidylglycerol. Interaction was not observed with zwitterionic phospholipids. Therefore, our data support a crucial role of anionic phospholipids in functional membrane localization of NG. Importantly, when NG+1 containing an intact lipid-binding α -helix was tested in this experiment (Fig. 6D, lower panel), the efficiency of lipid association was significantly higher, as described previously (14). Taken together, the results presented here imply that anionic phospholipid-enriched membranes can induce structural changes in NG that not only restore its biological function at the membrane but also stabilize the protein. In light of these results, it is surprising that *pgsA* null mutants of *E. coli* are viable in the absence of phosphatidylglycerol and cardiolipin (28). Other studies, however, offered a likely explanation as they showed that other acidic species accumulate in membranes of *pgsA*-deleted cells (29, 30). These observations indirectly suggest that the functional interaction of FtsY with acidic lipids is probably not specific for a certain lipid.

In summary, our studies offer strong genetic support for the notion that acidic lipids play a crucial role in the function of the *E. coli* SRP receptor *in vivo*, probably at a defined step during the SRP pathway that requires GTP hydrolysis by the SR-SRP complex, when ribosomes translating membrane proteins should be transferred to and assembled on the translocon.

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