

## SecG Function and Phospholipid Metabolism in *Escherichia coli*

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**SecG is an auxiliary protein in the Sec-dependent protein export pathway of *Escherichia coli*. Although the precise function of SecG is unknown, it stimulates translocation activity and has been postulated to enhance the membrane insertion-deinsertion cycle of SecA. Deletion of *secG* was initially reported to result in a severe export defect and cold sensitivity. Later results demonstrated that both of these phenotypes were strain dependent, and it was proposed that an additional mutation was required for manifestation of the cold-sensitive phenotype. The results presented here demonstrate that the cold-sensitive *secG* deletion strain also contains a mutation in *glpR* that causes constitutive expression of the *glp* regulon. Introduction of both the *glpR* mutation and the *secG* deletion into a wild-type strain background produced a cold-sensitive phenotype, confirming the hypothesis that a second mutation (*glpR*) contributes to the cold-sensitive phenotype of *secG* deletion strains. It was speculated that the *glpR* mutation causes an intracellular depletion of glycerol-3-phosphate due to constitutive synthesis of GlpD and subsequent channeling of glycerol-3-phosphate into metabolic pathways. In support of this hypothesis, it was demonstrated that addition of glycerol-3-phosphate to the growth medium ameliorated the cold sensitivity, as did introduction of a *glpD* mutation. This depletion of glycerol-3-phosphate is predicted to limit phospholipid biosynthesis, causing an imbalance in the levels of membrane phospholipids. It is hypothesized that this state of phospholipid imbalance imparts a dependence on SecG for proper function or stabilization of the translocation apparatus.**

The Sec-dependent translocation of secretory proteins across the inner membrane of *Escherichia coli* is catalyzed by the action of translocase, a complex of cytoplasmic and inner membrane proteins. The core components of translocase are the integral membrane proteins SecY and SecE and the peripheral membrane protein, SecA. SecY and SecE interact to form a protein-conducting channel that translocates secretory proteins across the membrane, while SecA is an ATPase that binds to the SecYE complex. SecA undergoes cycles of membrane insertion and deinsertion coupled to ATP binding and hydrolysis that are believed to drive the segmental translocation of the secretory protein (13, 15, 18, 32).

A number of genetic and biochemical studies have demonstrated that SecY, SecE, and SecA are necessary and sufficient for preprotein translocation (2, 4, 9, 10, 34). However, translocation with only these three components of translocase does not achieve optimal efficiency; the interaction of additional inner membrane proteins with the SecYE complex enhances translocation activity. SecG is a small protein that copurifies with SecYE and stimulates translocation activity in reconstituted membrane vesicles (8, 35), while SecD, SecF, and YajC form a heterotrimeric complex that also interacts with SecYE (16). Duong and Wickner (16) demonstrated that SecYE can associate with either SecG or SecDFYajC to generate full translocation activity. It appears that both SecG and SecDFYajC modulate the SecA cycle of insertion and deinsertion, but they do so by subtly different mechanisms. SecG stimulates the insertion of SecA after initiation of translocation, while

SecDFYajC increases SecA insertion and stabilizes the inserted state. Thus, SecG and SecDFYajC appear to have overlapping roles in fully activating the translocation process (16).

Neither SecG nor SecDFYajC is required for translocation activity, although each stimulates activity in vitro (16, 35). In keeping with these observations, none of these genes are essential for viability of *E. coli* (19, 33, 36). However, depletion of SecDF has a profound effect on cell growth and protein export. Null mutants of *secDF* form only minute colonies at 37°C and are unable to form colonies at all at 30°C or lower (36). In contrast, deletion of *secG* results in a less severe export defect and a cold-sensitive phenotype that is manifest only at very low temperatures (20°C) (33). Importantly, both the cold sensitivity and the severity of the  $\Delta secG$  export defect have been shown to be strain dependent. Indeed, only the initial deletion strains (including KN370) containing the *secG* deletion are cold sensitive; no other strain could be constructed that produced this phenotype (6, 19). We previously proposed that the cold sensitivity of KN370 was due to the combination of  $\Delta secG::kan$  with another unidentified mutation (19). In this report, that hypothesis is verified and the mutation in KN370 that acts in concert with  $\Delta secG::kan$  to produce a cold-sensitive phenotype is identified.

### MATERIALS AND METHODS

**Bacterial strains, plasmids, and media.** All bacterial strains used are *E. coli* K-12 derivatives and are listed in Table 1. Bacteria were grown in Luria-Bertani (LB) or M63 minimal media, with kanamycin (50 mg/liter), ampicillin (125 mg/liter), or tetracycline (25 mg/liter) added when appropriate (40). The DL- $\alpha$ -glycerophosphate (Sigma) was a generous gift from John Cronan (University of Illinois at Urbana-Champaign). Plasmid pBAD18 has been described elsewhere (23). Plasmids pAF69 and pAF70 were constructed by PCR amplification of *glpE* and *glpR*, respectively, from the chromosome of MG1655, using primers with restriction sites near the termini (glpE-1, GCAATGCCCGGGTACCCTAAAGA AAGAGAGACGCATG; glpE-2, CCTAGCCTGCAGAAGCCTTGACAGTAT

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TABLE 1. Bacterial strains used in this study

Strain	Relevant characteristics	Source	Reference
KN370	FS1576 (C600 <i>recD1009</i> ) $\Delta$ <i>secG::kan</i>	Lab stock	33
MC4100	F <sup>-</sup> <i>araD139</i> $\Delta$ ( <i>argF-lac</i> ) <i>U169 rpsL150 relA1 fliH5301 fruA25 deoC1 thi rbsR22</i>	Lab stock	11
Lin205	<i>fhuA22</i> $\Delta$ <i>phoA8 ompF627 glpA24 fadL701 pit-10 spoT1 relA1 glpD26 glpR8</i> (Const) <i>glpR7?</i> <sup>a</sup>	<i>E. coli</i> Genetic Stock Center	12
MG1655	F <sup>-</sup> <i>rph-1</i>	Lab stock	22
AF538	KN370 <i>zgi-203::Tn10 secG</i> <sup>+</sup>	Lab stock	19
AF539	KN370 <i>zgi-203::Tn10</i>	Lab stock	19
AF634	KN370 <i>yzgL::Tn10 glpR</i> <sup>+</sup>	This study	
AF635	MC4100 <i>glpR200 yzgL::Tn10</i>	This study	
AF636	MC4100 <i>yzgL::Tn10</i>	This study	
AF637	MC4100 $\Delta$ <i>secG::kan glpR200 yzgL::Tn10</i>	This study	
AF638	MC4100 $\Delta$ <i>secG::kan Tn10</i>	This study	
AF645	KN370 <i>zhe-3084::Tn10</i>	This study	
AF646	KN370 <i>glpD26 glpR8</i> (Const) <i>glpR7? zhe-3084::Tn10</i>	This study	

<sup>a</sup> According to the *E. coli* Genetic Stock Center, *glpR7* may or may not be present in this strain.

AAAGCGTTACGC; *glpR-1*, GCAATGCCCGGGTACCATTCCAGGGATTTATAAATG; and *glpR-2*, CCTAGCCTGCAGAAAGCTTGCACAGCTCCAGT TGAATAT; *KpnI* and *HindIII* sites are underlined), followed by digestion with *KpnI* and *HindIII* and ligation with pBAD18 that had been digested with *KpnI* and *HindIII*. Preparation of competent cells, transformation, and genetic manipulations were performed as described previously (37, 40).

**Growth curves.** Growth curves were performed with a Bioscreen C Microbiology Reader from Labsystems. The Bioscreen C is a computer-controlled shaker-incubator-reader. Customized microplates hold up to 200 individual 400- $\mu$ l samples. The Bioscreen maintains the samples at the desired temperature, shakes the samples, and measures the optical density at set intervals. Bacterial dilutions were prepared in LB medium prior to inoculation into the microplates, and cell density was determined by viable plate counts from the diluted cultures. For the experiments described here, the temperature was held at 20°C, shaking occurred once every minute for 10 s, and the  $A_{600}$  was measured and recorded every 30 min. The growth curves were continued until all cultures reached stationary phase, usually in 5 days. Data were exported to a Microsoft Excel worksheet for analysis.

**Construction of the mini-Tn10 library.** The mini-Tn10 library, constructed in strain MG1655, was a generous gift from Majda Valjavec-Gratian and Thomas Hill (University of North Dakota). P1-mediated transduction was used to introduce the library into strain KN370, with selection for tetracycline-resistant transductants. Following transduction, 200 individual colonies were inoculated into LB medium containing 10 mM sodium citrate and 12 mg of tetracycline/liter. The colonies were inoculated into 20 tubes, with 10 inocula per tube. The mixed cultures were grown overnight at 37°C and then diluted to a starting concentration of approximately 500 CFU/ml. Duplicate samples (400  $\mu$ l) of each culture were inoculated into microwell plates, and growth rates were analyzed in the Bioscreen C as described above.

**Inverse PCR.** Inverse PCR was performed as described previously (31). In short, chromosomal DNA from fast-growing transductants was digested with various restriction enzymes that do not cleave within the mini-Tn10 sequence (*Bam*HI, *Pst*I, *Kpn*I, or *Xho*I). The digested DNA was ligated in a dilute reaction mixture and then used as a template for PCR amplification. The primers used for PCR were derived from mini-Tn10 sequences (Tn10T-645, GAACCATTTTCA GTGATCCATTGCTGTTGAC and Tn10T-3358, AAAGTGATGATAAAAAG GCACCTTTGG) and were oriented such that the resultant PCR product would include the chromosomal DNA. DNA from the samples with the most easily detectable PCR product (from the *Kpn*I digest) were purified and used for DNA sequence analysis.

**DNA sequencing.** All PCR DNA to be sequenced was purified by agarose gel electrophoresis followed by Qiaex II gel extraction (Qiagen). DNA sequencing was performed by the Macromolecular Resources Sequi-Net Department at Colorado State University (Fort Collins).

## RESULTS

**Characterization of the  $\Delta$ *secG::kan* cold-sensitive phenotype.** Previous studies demonstrated that the presence of the  $\Delta$ *secG::kan* allele resulted in a cold-sensitive phenotype only in

certain strains, specifically the original deletion strain, KN370, and its derivatives (6, 19, 33). It was also noted that the cold sensitivity manifested by these strains was a leaky phenotype (19). That is, the cold-sensitive strains were able to form single colonies at 20°C, albeit at a slower rate than wild-type strains. After streaking on solid media and incubation at 20°C, the primary streaks grew normally while a marked defect was apparent in streaks further out on the plate. These results suggested that the cold-sensitive strains had a defect in single colony formation but were able to grow well at high cell density (in the initial streak).

To characterize the  $\Delta$ *secG::kan*-related growth defect more carefully, growth rates in liquid media were determined for the isogenic strains AF538 (*secG*<sup>+</sup>) and AF539 ( $\Delta$ *secG::kan*), both derivatives of KN370. Surprisingly, in a standard growth curve experiment in which overnight cultures were diluted to a starting  $A_{600}$  of 0.1, there was no discernable difference in the growth rates of the two strains, even at 20°C (data not shown). Because the growth pattern on plates suggested that the cold-sensitive phenotype was exacerbated when cells were more dilute, the effect of increasing dilution on the growth rate in liquid media was examined (Fig. 1). Overnight cultures were diluted such that the starting concentration of bacteria ranged from 3 to 4 CFU/ml to 37,500 CFU/ml in 10-fold increments. At the highest bacterial concentration (37,500 CFU/ml), there was a slight difference in the growth curve (Fig. 1), with AF539 ( $\Delta$ *secG::kan*) growing more slowly in early exponential growth phase and then at the same rate as AF538 (*secG*<sup>+</sup>) in late exponential phase. As the cultures were diluted further, a delay before the onset of exponential growth became more pronounced (Fig. 1). In all cases, however, the maximal growth rate of AF539 was indistinguishable from that of AF538. Therefore, the cold sensitivity is a concentration-dependent defect in the ability of these  $\Delta$ *secG::kan* cells to enter exponential growth at the maximal rate.

To ensure that the eventual wild-type growth of AF539 was not due to acquisition of a suppressor mutation, AF539 was allowed to grow to saturation at 20°C from the most dilute sample, and then serial dilutions were performed and the growth rate was measured again. The pattern of dilution-dependent extension of lag phase was completely reproducible,

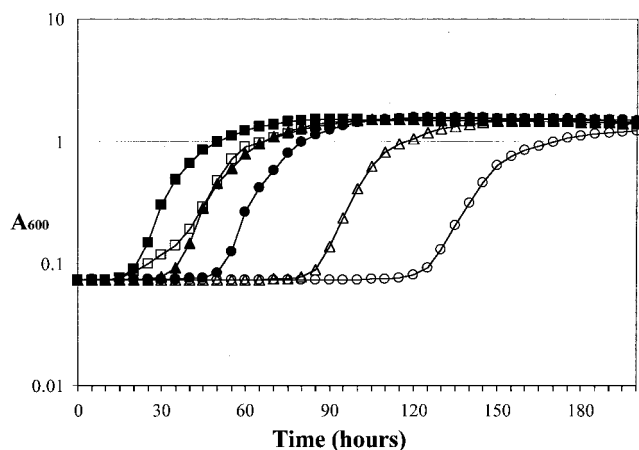


FIG. 1. Growth curves of AF538 and AF539. Strains were diluted from overnight cultures to starting concentrations of 37,500 CFU/ml (squares), 375 CFU/ml (triangles), or 3 to 4 CFU/ml (circles) and grown at 20°C in LB. AF538 is represented by closed symbols and AF539 is shown by open symbols. Although absorbance was measured every 30 min, for clarity only readings for each 5-h increment are shown.

indicating that this growth pattern is an inherent characteristic of the strain (data not shown). To determine whether the defect was due to an inability to emerge from lag phase, or if it was a concentration-dependent growth defect regardless of growth phase, bacteria in exponential growth at 20°C were diluted and the growth rates were measured. Again, the more dilute the cells, the longer the apparent lag phase before exponential growth began (data not shown). These results indicated that AF539 exhibits a cold-sensitive growth defect that manifests as an inability to grow at maximal rate when the cell culture is dilute.

#### The growth defect of KN370 can be rescued by a single gene.

The observed growth defect of AF539 was clearly dependent on the deletion of *secG*, as its isogenic partner, AF538, did not exhibit this cold sensitivity. To confirm our previous hypothesis that a second mutation contributes to this phenotype (19) and to identify the second mutation, the presumed mutation was replaced with a wild-type allele, thereby restoring normal growth characteristics to the strain. To this end, a mini-Tn10 library was constructed from a wild-type strain (MG1655) and transduced into KN370 (AF539 could not be used because it is tetracycline resistant). If the growth defect is due to a single other mutation in combination with  $\Delta secG::kan$ , then some transductants should receive a wild-type copy of that gene and thereby regain normal growth characteristics.

Preliminary investigation revealed that mixing nine colonies of AF539 (slow grower) and one colony of AF538 (fast grower) would yield a mixed culture that grew with a phenotype intermediate to the two, but that was easily distinguishable from AF539; that is, one fast grower in a mix of 10 colonies will overtake the culture quickly enough that its phenotype will predominate. Accordingly, transductants were pooled into 20 cultures with 10 colonies each (200 total individual transductants) and incubated at 37°C. After overnight growth, the pooled cultures were diluted and growth rates at 20°C were measured to identify pools that contained fast growers. Six such cultures were identified. Although each culture repre-

sented a mix of transductants, the fast grower should have predominated. Therefore, each culture was plated for single colonies, and one colony was isolated for further analysis.

There are two ways a colony could become a fast grower in this experiment. One is the replacement of the unidentified mutant gene with a wild-type copy, and the other is replacement of  $\Delta secG::kan$  with wild-type *secG*. To eliminate the latter isolates, colonies from each of the fast-growing pools were isolated and examined for kanamycin resistance. Two of the six had become kanamycin sensitive, demonstrating that  $\Delta secG::kan$  had been replaced by a wild-type copy of *secG*. However, the four remaining colonies were kanamycin resistant, indicating that the  $\Delta secG::kan$  allele was still present. These four isolates were examined in pure culture for their growth characteristics. Three cultures repeated the fast-growth phenotype, while one did not. Therefore, in these three isolates, wild-type growth characteristics were restored while the strain retained the *secG* deletion.

To ensure that the enhanced growth of these isolates was due to replacement of a single mutant gene in KN370, the mini-Tn10 from each isolate was transduced back into KN370 and tetracycline resistance was selected. Fifty colonies from each transduction were patched on solid media at 20°C and examined for cold sensitivity. The majority (>90%) of colonies from each group of transductants appeared to be cold resistant. As this test is not as sensitive as the growth curves, a colony of each was grown in LB media, diluted, and analyzed for growth. All three were found to be fast growers. These results confirmed our hypothesis that a single mutant gene in combination with  $\Delta secG::kan$  was responsible for the cold-sensitive phenotype of KN370 and its derivatives.

**Cold sensitivity can be rescued by *glpR*<sup>+</sup>.** To identify the gene that contributes to the cold sensitivity of KN370, inverse PCR was used to locate the site of insertion of two of the three mini-Tn10s. Both mapped to approximately 76.5 min on the *E. coli* chromosome, one in *glpG* and the other in *yzgL* (5). Cotransduction frequencies indicated that the gene that rescues the growth defect was approximately 95 to 98% linked to each of the mini-Tn10s. Cloning of chromosomal DNA fragments demonstrated that a 6-kb *KpnI* fragment, which contains only five intact genes, *glpR*, *glpG*, *glpE*, *glpD*, and *yzgL*, was able to complement the cold sensitivity of KN370. As the mini-Tn10s were located in two of these genes, only three candidate genes remained, *glpD*, *glpE*, and *glpR*. The *glpE* and *glpR* wild-type genes were amplified from MG1655 by PCR and subcloned into pBAD18. Recombinant plasmids were transformed into KN370, and growth was examined at 20°C in the presence and absence of arabinose. The plasmid containing *glpR* rescued growth of KN370 in the presence of arabinose. This result suggested that a mutation in *glpR* contributed to the growth defect in KN370 and, further, that the *glpR* mutation was recessive because the growth defect was complemented by plasmid-borne wild-type *glpR*.

**KN370 has a constitutive mutation in *glpR*.** The *glpR* gene encodes the repressor for the *glp* regulon, which is involved in metabolism of glycerol phosphate (38). KN370 is a derivative of C600 (3), which is known to have a mutation in *glpR*, *glpR200* (17). To confirm that KN370 did in fact contain the *glpR200* mutation, *glpR* was PCR amplified from KN370 as well as from fast-growing derivatives, and DNA sequence analysis

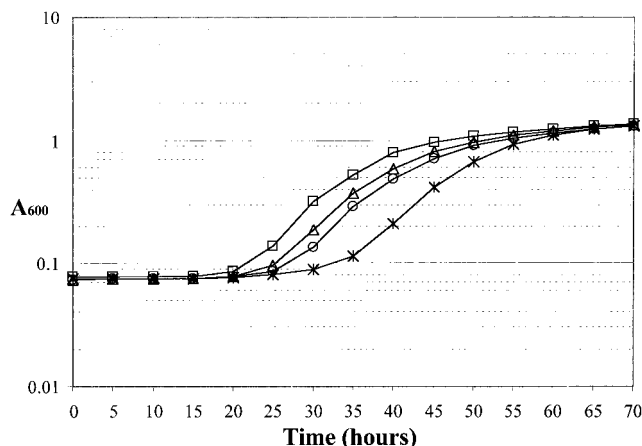


FIG. 2. Growth curves for MC4100 containing  $\Delta secG::kan$  and  $glpR200$ . Strains were diluted from overnight cultures to a starting concentration of approximately 400 CFU/ml and grown in LB medium at 20°C. Symbols: AF636 ( $secG^+ glpR^+$ ), open triangles; AF638 ( $\Delta secG::kan$ ), open squares; AF635 ( $glpR200$ ), open circles; AF637 ( $\Delta secG::kan glpR200$ ), asterisks. Absorbance readings for each 5-h increment are shown.

was performed. KN370  $glpR$  had a mutation in amino acid 55 from Gly to Ala, while the fast-growing derivatives did not contain this mutation. Because KN370 was complemented by the wild-type copy of  $glpR$ , it seemed likely that this mutation resulted in an inactive GlpR repressor and constitutive synthesis of the  $glp$  regulon. Indeed, the original characterization of  $glpR200$  in C600 demonstrated that it does result in constitutive expression of the regulon (17). Further, this same amino acid alteration has been identified independently as  $glpR2$  and characterized as a constitutive mutation (Donald Pettigrew, Texas A&M University, personal communication).

**The combination of  $\Delta secG::kan$  and  $glpR200$  results in a cold-sensitive phenotype.** To verify that the  $glpR200$  mutation in combination with  $\Delta secG::kan$  results in cold sensitivity, both mutations were introduced into MC4100 by P1-mediated transduction. As shown previously,  $\Delta secG::kan$  alone does not produce a cold-sensitive phenotype in MC4100 (Fig. 2). Furthermore,  $glpR200$  alone does not result in cold sensitivity (Fig. 2). However, the two mutations in combination did cause a cold-sensitive growth defect in the MC4100 background (Fig. 2). The lag in growth was not as striking as it was in KN370, but it was reproducible and was detectable by streaking as well as by growth curve analysis. The growth defect was more apparent when the length of time required to reach mid-log phase ( $A_{600} = 0.3$ ) was calculated. The parent and singly mutant strains reached this point in 30 to 35 h, while the doubly mutant strain required 42.5 h to reach the same absorbance. This result explains the puzzling prior observations that  $\Delta secG::kan$  resulted in cold sensitivity in some strains but not in others. Only when  $glpR200$  and  $\Delta secG::kan$  were both present in the same strain was a growth defect observed.

**Growth of KN370 is improved by increasing the intracellular concentration of glycerol-3-phosphate.** Why does the combination of  $glpR200$  and  $\Delta secG::kan$  result in cold sensitivity? One possible explanation is that constitutive expression of the  $glp$  regulon results in depletion of the intracellular pool

of glycerol-3-phosphate, due to overexpression of  $glpD$  (the aerobic glycerol-3-phosphate dehydrogenase) and subsequent channeling of glycerol-3-phosphate into metabolic pathways (29). It is possible that this depletion limits the glycerol-3-phosphate available for phospholipid biosynthesis, leading to perturbations in the phospholipid levels (Fig. 3). Under these circumstances, the function of SecG may become critical. If this hypothesis is correct, the growth defect should be alleviated either by addition of glycerol-3-phosphate to the media or by introduction of a null mutation in  $glpD$ .

This hypothesis first was tested by growing AF538 ( $glpR200$ ), AF539 ( $\Delta secG::kan, glpR200$ ), and AF634 ( $\Delta secG::kan$ ) at 20°C with increasing concentrations of glycerol-3-phosphate and monitoring growth (Fig. 4). At low levels of glycerol-3-phosphate (<2 mM), growth of the doubly mutant strain was greatly improved, with the length of time required to reach mid-log phase ( $A_{600} = 0.3$ ) dropping from 96 to 68 h (Fig. 4). However, increasing the glycerol-3-phosphate concentration above 2 mM resulted in an inhibition of growth. This inhibition occurred in the  $glpR200$  single mutant as well (Fig. 4), while growth of the  $glpR^+ \Delta secG::kan$  strain was unaltered at any concentration of glycerol-3-phosphate (Fig. 4). The growth inhibition in a  $glpR$  mutant strain at higher levels of glycerol-3-phosphate is not surprising, as it is known that excess intracellular glycerol-3-phosphate is detrimental to the bacterial cell (1, 20) and that the  $glpR200$  mutation will result in constitutive expression of GlpT, the transporter for glycerol-3-phosphate, thereby allowing the uptake of excess glycerol-3-phosphate (21, 29). The observation that is important to these studies is that glycerol-3-phosphate did rescue the growth defect of the double mutant, supporting the hypothesis that intracellular pools of glycerol-3-phosphate are limiting in the  $glpR$  mutant strain.

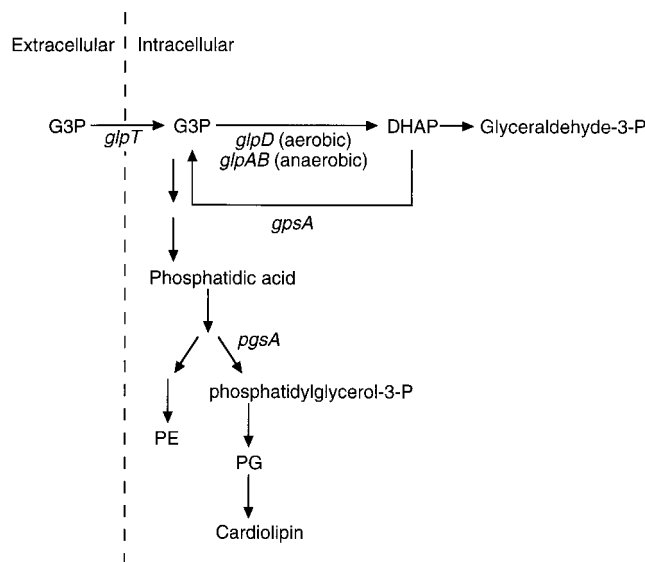


FIG. 3. Pathways for glycerol-3-phosphate and phospholipid metabolism. Abbreviations are as follows: G3P, glycerol-3-phosphate; DHAP, dihydroxyacetone phosphate; PE, phosphatidylethanolamine; PG, phosphatidylglycerol. Genes encoding relevant proteins are indicated. Not all steps of phospholipid biosynthesis are shown. This figure is adapted from previous reviews (14, 29).

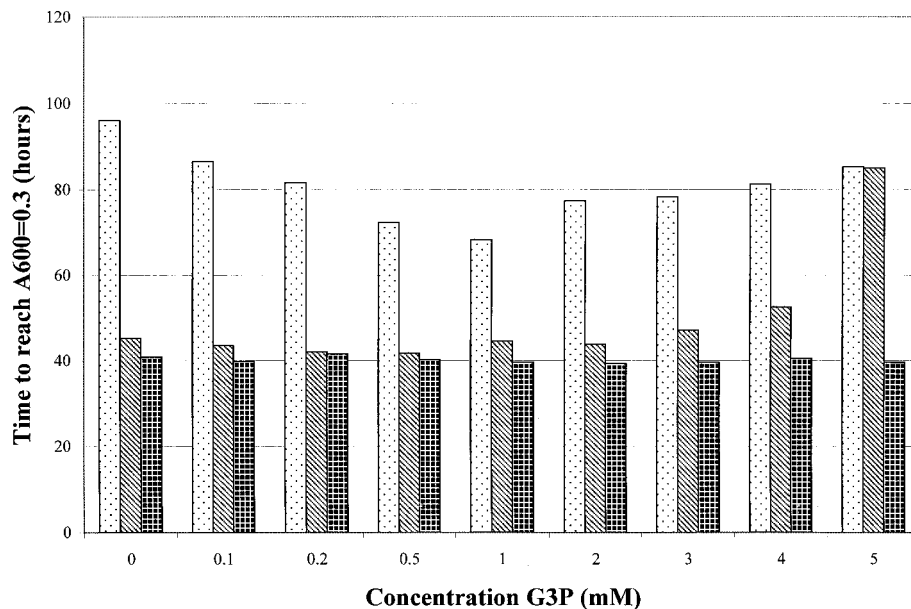


FIG. 4. Growth characteristics in the presence of glycerol-3-phosphate. Strains were grown in LB with the indicated amounts of glycerol-3-phosphate (G3P) at 20°C. Strains were diluted from overnight cultures to a starting concentration of approximately 400 CFU/ml. The bars represent the number of hours required for each culture to reach mid-log phase ( $A_{600} = 0.3$ ). AF539 ( $\Delta secG::kan glpR200$ ), stippled bars; AF538 ( $glpR200$ ), striped bars; AF634 ( $\Delta secG::kan$ ), cross-hatched bars. All assays were performed in duplicate, and the standard error never exceeded  $\pm 5\%$ .

To further test the hypothesis that the growth defect was attributable to constitutive expression of *glpD*, a *glpD* mutation was introduced into KN370 and growth at 20°C was assessed. According to this hypothesis, the *glpD* mutation should prevent the excessive channeling of glycerol-3-phosphate to metabolic pathways, thereby promoting synthesis of phospholipids and alleviating the growth defect. Indeed, strain AF646 containing  $\Delta secG::kan$ , *glpR8* (another *glpR* constitutive allele), and *glpD26* was cold resistant (Fig. 5), while the isogenic *glpD*<sup>+</sup> strain (AF645) remained cold sensitive. This result directly demonstrated that overexpression of *glpD* was contributory to the growth defect of KN370.

A final demonstration of the relationship between glycerol-3-phosphate metabolism and the growth defect of KN370 was the analysis of the effect of different carbon sources on growth at 20°C. This hypothesis predicts that AF539 should be cold sensitive on glucose minimal medium or other carbon sources unrelated to glycerol-3-phosphate metabolism, while glycerol or glycerol-3-phosphate should permit growth by increasing the intracellular pool of glycerol-3-phosphate. This in fact was the case (Fig. 6). Strain AF539 (*glpR200*  $\Delta secG::kan$ ) was cold sensitive on glucose minimal medium and cold resistant on glycerol and glycerol-3-phosphate media, demonstrating that providing the precursor metabolites for phospholipid biosynthesis alleviated the growth defect.

## DISCUSSION

Early observations of the phenotypes associated with the  $\Delta secG::kan$  mutation were perplexing because the observed cold sensitivity and the severity of the export defect were strain dependent and few strains other than the original *secG* deletion strain could be constructed that demonstrated these phe-

notypes (6, 16, 19). To reconcile these data, we proposed that an additional mutation was required for the cold sensitivity of  $\Delta secG::kan$  (19), and the results presented here support that hypothesis. The original *secG* deletion strain, KN370, contains a mutation in *glpR*; replacement of the mutant allele with wild-type *glpR* resulted in abrogation of the cold-sensitive phenotype. Further, introduction of both  $\Delta secG::kan$  and *glpR200* into another strain background, MC4100, also produced a cold-sensitive phenotype. Clearly, the phenotype of the *secG* deletion mutation varies with the genetic background. Understanding this variation is crucial to understanding the function of SecG.

Several genes were identified previously as high-copy suppressors of the cold sensitivity of KN370. The *Bacillus subtilis* *pgsA* and *scgR* genes were both identified by selection of cold-resistant transformants from a plasmid-borne genomic library (26, 27); the *E. coli* *pgsA* and *gpsA* genes encoded on high-copy-number plasmids also suppressed the cold-sensitive phe-

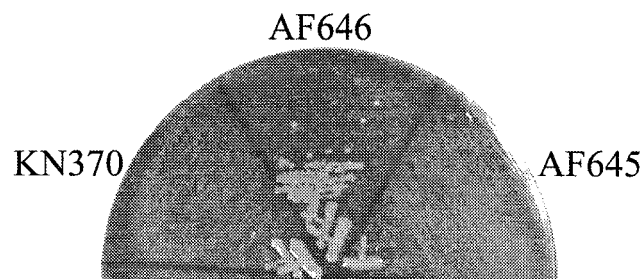


FIG. 5. Effect of *glpD26* on growth. Strains were grown on LB plates at 20°C. AF645 is  $\Delta secG::kan glpR200$ , while AF646 is  $\Delta secG::kan glpR8 glpD26$ .

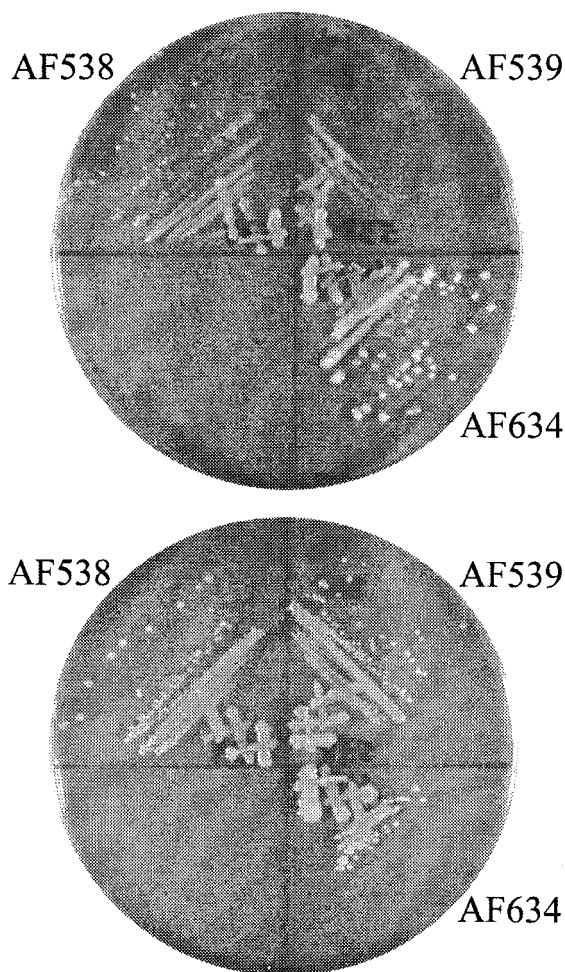


FIG. 6. Effect of alternate carbon sources on growth of  $\Delta secG::kan$  strains. Strains were grown on either glucose minimal (top) or glycerol minimal (bottom) medium at 20°C.

notype (27, 39). The identification of these high-copy-number suppressors led to a hypothesis that the effect of *secG* deletion can be ameliorated by an increase in the level of acidic phospholipids (39, 41).

The evidence presented here suggests an alternative interpretation of those previous results. The current data demonstrate that deletion of *secG* has a detrimental effect on the cell only in the presence of a *glpR* constitutive mutation. We propose that the *glpR200* mutation leads to constitutive expression of *glpD*, thereby shunting glycerol-3-phosphate to dihydroxyacetone phosphate which, in turn, limits the amount of glycerol-3-phosphate available for phospholipid biosynthesis (Fig. 3). Consistent with this prediction, the cold-sensitive growth defect of the *secG* deletion strain was alleviated by addition of glycerol-3-phosphate to the growth media or by introduction of a *glpD* null mutation. In this scheme, therefore, the previously observed suppression by overexpression of *pgsA*, *gpsA*, or *scgR* is explained not because the amount of acidic phospholipids increases above wild-type levels, but rather because expression of these genes reestablishes normal phospholipid biosynthetic patterns.

The proposed alteration to phospholipid levels is very subtle.

It was demonstrated previously that steady-state phospholipid ratios in the *secG* deletion strain, KN370, are essentially identical to that of MC4100 (39). Our results are consistent with this finding, as we found that KN370 and its derivatives grow at a rate indistinguishable from wild-type strains, once logarithmic growth begins. The growth defect is apparent only in dilute cultures prior to onset of exponential growth. Therefore, we predict that the *glpR200* mutation confers a defect in phospholipid metabolism that is not detectable by measurement of steady-state levels. It is possible that there is an alteration in the flux of the pathways, particularly during the transition from stationary phase to log phase. Along these lines, it is interesting to note that phospholipid metabolism is growth-phase regulated, with an increase in cardiolipin and a decrease in phosphatidylglycerol as cells enter stationary phase (14). Additionally, it remains unclear why cell density affects the phenotype of the *glpR200*  $\Delta secG::kan$  strain, but there is evidence that population density affects the global pattern of cellular metabolites (30), perhaps explaining the density dependence of the cold sensitivity observed here.

Acidic phospholipids (phosphatidylglycerol and cardiolipin) enhance translocation at several steps: they promote the interaction of SecA with the inner membrane (7, 28, 43) and with SecYE (24), they are required for SecA translocation ATPase activity (28, 42), they are involved in the interaction of the signal sequence with the inner membrane (25), and they are important in achieving the proper orientation of membrane proteins (44). SecA insertion and deinsertion is tightly linked to the phospholipid content of the membrane, and it has been shown that an increase in acidic phospholipids stimulates both the insertion and deinsertion steps of the SecA cycle in  $\Delta secG::kan$  or *secAcsR11* strains (41). Although the precise function of SecG remains unknown, the present studies suggest that SecG may be involved in the function and/or stabilization of the translocation apparatus under conditions that alter the phospholipid content of the membrane.

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