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Multicopy Suppression of Cold-Sensitive *sec* Mutations in *Escherichia coli*

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Mutations in the secretory (*sec*) genes in *Escherichia coli* compromise protein translocation across the inner membrane and often confer conditional-lethal phenotypes. We have found that overproduction of the chaperonins GroES and GroEL from a multicopy plasmid suppresses a wide array of cold-sensitive *sec* mutations in *E. coli*. Suppression is accompanied by a stimulation of precursor protein translocation. This multicopy suppression does not bypass the Sec pathway because a deletion of *secE* is not suppressed under these conditions. Surprisingly, progressive deletion of the *groE* operon does not completely abolish the ability to suppress, indicating that the multicopy suppression of cold-sensitive *sec* mutations is not dependent on a functional *groE* operon. Indeed, overproduction of proteins unrelated to the process of protein export suppresses the *secE501* cold-sensitive mutation, suggesting that protein overproduction, in and of itself, can confer suppression. This multicopy suppression is reminiscent of the previously characterized suppressors of *sec* mutations which compromise protein synthesis and the observation that low levels of protein synthesis inhibitors can suppress as well. In all cases, the mechanism of suppression is unrelated to the process of protein export. We suggest that the multicopy plasmids also suppress the *sec* mutations by compromising protein synthesis.

Genetic analyses have identified six *sec* genes (*secA*, *-B*, *-D*, *-E*, *-F* and *-Y*), whose products are required for efficient protein translocation across the cytoplasmic membrane of *Escherichia coli* (3, 29). Because protein translocation is an essential process, only conditional-lethal loss-of-function *sec* mutations are obtainable by genetic means. At the nonpermissive temperature, strains containing either cold-sensitive or temperature-sensitive mutations in *secA*, *-D*, *-E*, *-F*, or *-Y* accumulate cytoplasmic precursor proteins and eventually die (3, 29). However, even at such nonpermissive temperatures, the conditional-lethal alleles only partially block translocation, indicating that they are not complete null mutations (for examples, see references 1, 12, 23, 26, and 31). The nature of these partial defects is best illustrated by the cold-sensitive alleles of the *secE* locus. The cold-sensitive *secE15* [*secE15*(Cs)] and *secE501*(Cs) mutations do not lie within the *secE* coding region. Instead, they alter the untranslated leader of the *secE* mRNA and reduce the steady-state level of wild-type SecE (30). Hence, the cold sensitivity conferred by these *secE* mutations implies that the cell requires wild-type levels of the secretory apparatus for viability at low temperatures. Consistent with this notion, Pogliano and Beckwith (24) have demonstrated that protein translocation is itself an inherently cold-sensitive process. Although the cold-sensitive mutations in *secD*, *-F* and *-Y* have not been as thoroughly characterized, it has been suggested that they act by decreasing the overall amount of functional secretory apparatus, in similar fashion to those in *secE* (4).

Several studies have sought to identify extragenic suppressors of the conditional-lethal *sec* mutations with the hope that the suppressor mutations would identify genes encoding interacting proteins involved in protein secretion. Instead, suppressor analysis has yielded secondary mutations that act by compromising protein synthesis (6, 11, 18, 22, 26). Indeed, low levels of chloramphenicol and other protein synthesis inhibitors suppress the lethality and secretion defects associated with the *sec* conditional mutants. Although the precise mechanism of this suppression is unknown, it has been suggested that decreased synthesis of precursor proteins relieves a lethal burden placed on the mutant Sec machinery (18).

In addition, previous studies have shown that high-level synthesis of the chaperonins GroES and GroEL suppresses the temperature-sensitive *secA51* [*secA51*(Ts)] and *secY24*(Ts) mutations (38, 39). On the basis of the folding activities of the GroE proteins, it was suggested that the suppression was the result of an increased frequency of proper folding of thermolabile SecA and SecY proteins (39). In this study, we have analyzed the ability of different genes cloned in multicopy vectors, including *groES* and *groEL*, to suppress *sec*(Cs) mutations. Our results reveal that functional chaperonin genes are not required for suppression. Rather, it is the actual process of protein overproduction which functions to suppress the *sec*(Cs) mutations.

MATERIALS AND METHODS

Media and reagents. Media were prepared as described by Silhavy et al. (35). Standard microbiological techniques were used for strain construction and bacterial growth (35).

Strains. All strains used in this study are derivatives of MC4100 or MC1000 (35). The conditional-lethal alleles listed in Table 1 have been described elsewhere: *secD1* (12); *secE15* and *secE501* (30); *secF62* (13); *secY39* and *secY40* (1); *secA51* (32); *secY24* (34); *prlF1* (16); and *hisH8606* (39). Each conditional allele was introduced into the appropriate strain background by cotransduction with a linked Tn*10* insertion mutation. The *secD* and *secF* alleles were cotransduced with the *zaj*::Tn*10* mutation (36). The *secE* alleles were cotransduced with either the *argE*::Tn*10* mutation (36) or *zijRK498*(Tn*5*) (24). The *secY* mutations were cotransduced with a linked Tn*10* described by Bieker and Silhavy (4). The *secA* alleles were cotransduced with a *leu*::Tn*10* mutation. The *prlF1* allele was cotransduced with an *argG*::Tn*10* mutation. Each mutation was transduced into the appropriate strain background at the permissive temperature (30°C for temperature-sensitive strains and 37°C for cold-sensitive strains) and subsequently

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TABLE 1. Multicopy suppression of *sec*(Cs) mutations by the *groE* operon

Mutation	Growth[a]						Reference(s)
	pOF39 (GroES$^+$L$^+$)	pND1 (GroES$^+$L′)	pND5 (GroES$^+$L$^-$)	pND6 (GroES′L$^-$)	pBR325 (control)	pBR322 (control)	
secA51(Ts)	+/−	−	−	−	−	−	38, 39, this study
secY24(Ts)	+	−	−	−	−	−	39, this study
secY39(Cs)	+/−	+/−	+/−	+/−	−	−	This study
secY40(Cs)	+	+	+	+	+/−	−	This study
secE15(Cs)	+/−	+/−	+/−	+/−	−	−	This study
secD1(Cs)	+	+	+	+	+/−	−	This study
secE501(Cs)	+	+	+	+	+/−	−	This study
secF62(Cs)	+	+	+	+	+/−	−	This study
prlF1(Cs)	−						This study
hisH8606(Cs)	−						39
Δ*secE*	−						This study

[a] +, growth comparable to that of a wild-type control strain; +/−, growth is significantly slower than that of a wild-type control strain; −, no growth.

scored for the conditional allele at the nonpermissive temperature (42°C for temperature-sensitive strains and 23°C for cold-sensitive strains). All mutant strains were compared with isogenic strains containing the appropriate Tn*10* insertion linked to a wild-type allele of the gene in question.

Analysis of multicopy suppression of *sec*(Cs) and *sec*(Ts) mutations. Strains were grown on L agar containing 125 μg of ampicillin per ml at 23°C for cold-sensitive mutations and 42°C for temperature-sensitive mutations. Suppression was measured by colony formation after 48 h. A score of + in Table 1 indicates that the colonies of the experimental strain were as large as those of the isogenic wild-type control strain; a score of +/− indicates that the colonies of the experimental strain were smaller than those of the isogenic wild-type control strain; a score of − indicates that no colonies formed with the experimental strain, whereas the isogenic wild-type control strain formed colonies. Colonies scored +/− were no more than 50% of the size of colonies scored +.

Analysis of multicopy suppression of the *secE* deletion. Suppression of Δ*secE* by the various plasmids (see Table 1 and Results for details) was tested in the strain CM100 (MC4100, Δ*secE19-111*, pCM10). The Δ*secE19-111* allele results in a deletion of codons 19 to 111 of the chromosomal *secE* open reading frame (30). This deletion is complemented by plasmid pCM10 (20), an ampicillin-resistant derivative of pBR322 containing a complementing copy of *secE* flanked by the counterselectable genes *lacY* and *rpsL*, which confer *o*-nitrophenyl-β-D-thiogalactoside (tONPG) (2) and streptomycin sensitivity, respectively. CM100 was transformed to tetracycline (2 μg/ml) resistance with pBR322 or pOF39, and then growth (i.e., indicative of the loss of pCM10 and thus *secE*) was tested on minimal succinate plates containing 1.5 mg of streptomycin per ml and 1 mM tONPG (21). Growth of these strains was not observed after 10 days on the selective plates, whereas CM100 transformed with plasmid pJS51 (*secE*$^+$ [31]) plasmids grew after 2 days on the same plates.

Radiolabeling and immunoprecipitation. Strain KJ184 [MC1000, *phoR araD139* Δ(*ara-leu*)*7679* Δ*lacX74 galE galK rpsL thi secF62 zaj*::Tn*10* (24)] was transformed with pBR322, pCGSH1 (*secDF*$^+$ [13]), or pOF39. Overnight cultures grown at 37°C in M63 minimal medium plus thiamine (1 μg/ml), amino acids (50 μg/ml, without methionine), and maltose (0.4%) were passaged at 1:50 into the same medium. After reaching an optical density at 600 nm of 0.3, these cultures were placed at the nonpermissive temperature of 23°C for 30 min. Cells (3 ml) were added to preequilibrated tubes containing 150 μCi of [^{35}S]methionine. After 30 s, 300 μl of 0.5% methionine was added; a 1-ml aliquot of cells was removed and placed in an ice water bath immediately and at 1 and 4 min after labeling. Samples were immunoprecipitated with antisera to maltose-binding protein (MBP; prepared by Kathy Strauch) and OmpA (a generous gift from Carol Kumamoto) as described previously (25). Precursor and mature forms of MBP and OmpA were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% polyacrylamide gel) and autoradiography.

Plasmids. pOF39 carries a 2.2-kb fragment containing the *groE* operon inserted into the *Eco*RI-*Hin*dIII site of plasmid pBR325. pOF39 generates high-level synthesis of the chaperonins GroES and GroEL (10). Plasmids pBR322 and pBR325 have been described elsewhere (5). pND1 was created by deleting the small *Eco*RV fragment of pOF39 and religating the large fragment. pND1 contains the entire *groES* gene and approximately 60% of the 5′ end of the *groEL* open reading frame. The following single-stranded DNA primers were used to create plasmids pND5 and pND6: GroE5′, 5′ CACATTCTTGCCCGCCTGAT GAATGC 3′; Mopdelc, 5′ CGTCTTTAGCTGCCATAAGCTTTATTCCTT AAATTC 3′; and Deltas1, 5′ CATTGTCGATCTTCTAAGCTTTCACACCGT AGC 3′. pND5 was created by amplifying DNA from pOF39 by PCR (28), using the GroE5′ and Mopdelc primers. The resulting PCR product and pOF39 were each digested with *Eco*RI and *Hin*dIII, and the PCR product was ligated with the large fragment generated by the pOF39 digestion. The resulting plasmid, pND5, contains the *groES* gene and is deleted of the entire *groEL* open reading frame. The *groE* chromosomal DNA contained in pND5 spans from nucleotides 1 to 467 in the published sequence (14). Nucleotide 467 is located in the intergenic region between the *groES* and *groEL* open reading frames, 40 nucleotides downstream of the *groES* stop codon. pND6 was created by amplifying pOF39 DNA by PCR, using the GroE5′ and Deltas1 primers. The resulting PCR product and pOF39 were each digested with *Eco*RI and *Hin*dIII, and the PCR product was ligated with the large fragment generated by the pOF39 digestion. The resulting plasmid, pND6, is deleted of the entire *groEL* open reading frame and the final 22 codons of the *groES* open reading frame. The first 74 codons (approximately 76%) of the *groES* open reading frame remain.

Plasmids that overexpressed various cytoplasmic proteins were analyzed for the ability to suppress the conditional growth defects conferred by the *secE501* mutation. pNF2 is a P_{rm}-driven derivative of the LacZ-encoding plasmid p1109 that expresses 1,500 U of β-galactosidase (16a, 17). This plasmid was tested in strain KJ188 [isogenic to strain KJ184 except that it contains *secE501 zijRK498*(Tn*5*) instead of *secF62 zaj*::Tn*10*] at 23°C. pSPER1-E is a derivative of pSPER1 (15) that is deleted for part of the gene encoding RspA, thus inactivating its overexpression phenotype of stationary-phase gene repression. The RspA fragment produced from this plasmid is highly overexpressed (reference 14a and data not shown). This plasmid was tested in strain PS265 (MC4100, *secE501 zijRk498*[Tn*5*]) at 23°C.

RESULTS

A GroES/EL-overproducing plasmid suppresses *sec*(Cs) mutations. We initially observed that plasmid pOF39, which overexpresses the chaperonin genes *groES* and *groEL* (10), suppressed the *sec*(Cs) mutations listed in Table 1. This suppression of cold sensitivity appears to be specific to *sec* mutations, since cold-sensitive mutations in two non-*sec* genes (*prlF* and *hisH*) are not suppressed (Table 1). Although the observed suppression is specific to mutations in the *sec* pathway, we were surprised by the large array of mutations within this pathway which were suppressed by overexpression of *groE*. Indeed, overexpression of *groE* suppresses mutations in all *sec* genes in which cold-sensitive mutations have been found. Because high-level synthesis of the GroE proteins suppressed this large class of *sec* mutations, it seemed that an analysis of this suppression could provide insights into the actions of the GroE proteins and/or the nature of the lethality conferred by the *sec*(Cs) mutations.

Overproduction of GroES/EL enhances the rate of protein export in *sec*(Cs) mutants. The mechanism of GroE-mediated suppression was examined by analyzing the effects of GroES/L overproduction on the secretion defects associated with the *sec* mutants. Analysis of the kinetics of signal sequence cleavage of precursor MBP and precursor OmpA in a *secF62*(Cs) background indicated that secretion was enhanced significantly when the GroES/EL-overexpressing plasmid was present. Figure 1 shows that in a *secF62*(Cs) strain transformed with pBR322, the majority of newly synthesized MBP and OmpA

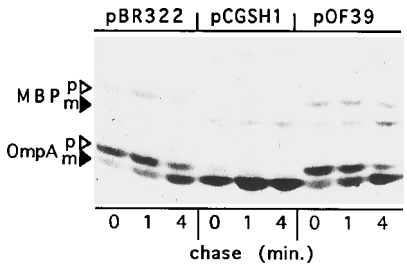

FIG. 1. Plasmids expressing SecD and SecF or GroES/L suppress the secretion defect conferred by the *secF62*(Cs) allele. Strain KJ184 containing plasmid pBR322, pCGSH1, or pOF39 was grown at 23°C for 30 min and then subjected to pulse-chase, SDS-PAGE and autoradiography as described in Materials and Methods to examine signal sequence processing of MBP and OmpA. m, mature species; p, precursor species. Chase times are indicated below the lanes. The percent mature protein (corrected for the number of methionines in the precursor and mature forms) at the pulse, 1-min, and 4-min time points are as follows: for KJ184/pBR322, 0, 0, and 52% for mature MBP and 32, 45, and 74% for mature OmpA; for KJ184/pCGSH1, 100% at all points for mature MBP and mature OmpA; for KJ184/pOF39, 49, 50, and 74% for mature MBP and 45, 59, and 88% for mature OmpA.

exists as an unprocessed precursor species. Transformation of the same strain with a complementing *secF* plasmid (pCGSH1) completely relieves the secretion defect observed in the pBR322-transformed strain. Finally, transformation of the *secF62* mutant with pOF39 significantly enhances translocation over the pBR322 control strain, even though suppression of the processing defect is not as strong as observed with the complementing plasmid pCGSH1. A similar effect was also observed when the *groE* operon was overexpressed in cells lacking SecD and SecF (25a). This result suggests that overproduction of GroES/EL suppresses the lethality of the *sec* mutants by enhancing the rate of protein translocation.

There are a number of possible mechanisms by which high-level synthesis of the GroE proteins could suppress these *sec* mutations and enhance protein export. For example, it is possible that overexpression of *groE* bypasses the need for the Sec pathway altogether. However, overexpression of the *groE* operon does not suppress a deletion of the *secE* gene (Table 1), indicating that this is not the case. Alternatively, it is possible that overexpression of the *groE* operon increases the frequency of proper folding of cold-sensitive mutant Sec proteins. This model has been used to explain the suppression of the *sec*(Ts) mutations by high-level synthesis of GroES/L (39). However, this model fails to explain the suppression of the *secE*(Cs) mutations, because these mutations confer cold sensitivity not by altering the primary amino acid sequence of SecE but by reducing the levels of SecE protein (30). Thus, these models cannot account for the GroE-mediated suppression of the *sec* mutations. Another model posits that overproduction of GroES/EL suppresses the *sec* mutations by enhancing the rate of translocation of an essential protein(s) which is present in insufficient quantities in the bacterial envelope in a *sec* mutant. Such a stimulation in the translocation of an essential protein(s) could be accomplished via a protein unfolding activity of GroES/EL. Indeed, this explanation seems likely given the ability of the GroE proteins to suppress aggregation of unfolded proteins in vitro (40). However, evidence presented below indicates that the observed suppression is not related to the specific functions of the GroE proteins. Rather, the suppression is related to protein overproduction per se.

Deletion derivatives of a GroES/EL-overproducing plasmid suppress *sec*(Cs) mutations. In an attempt to determine whether the suppression of the *sec*(Cs) mutations was dependent on overproduction of GroEL, we constructed a plasmid that contains only *groES* and 60% of the 5′ end of the *groEL* open reading frame (see Materials and Methods for details). This plasmid, pND1, suppressed the *sec*(Cs) mutations as well as pOF39 (Table 1). There are a number of explanations for the observed suppression by pND1: (i) suppression may be solely dependent on GroES, (ii) the amino-terminal fragment of GroEL produced by pND1 may retain the function(s) required for suppression, or (iii) suppression is not related to the functions of the GroE proteins but is instead dependent on the protein overproduction itself. Plasmid pND5 was created to further distinguish between these possibilities. pND5, which overexpresses *groES* alone, was sufficient to suppress the *sec*(Cs) mutations (Table 1; Fig. 2f), albeit to a lesser extent than observed with pOF39 and pND1. This result could indicate that the multicopy suppression of the *sec*(Cs) mutations was mediated primarily by GroES. To test this, we deleted approximately 25% of the 3′ end of the *groES* coding region from plasmid pND5, creating pND6. pND6 produces a nonfunctional GroES protein, as assayed by its inability to complement the temperature sensitivity and phage λ growth defect of the chromosomal *groES619* mutation (data not shown). Surprisingly, pND6 was able to suppress a number of the *sec*(Cs) mutations (Table 1; Fig. 2g). Even more striking was the finding that pBR325, the parent plasmid for pOF39, pND5, and pND6, weakly suppresses certain *sec* mutations (Table 1; Fig. 2d). pBR322, which is the parent plasmid of pBR325, does not suppress any *sec* mutation tested (Table 1; Fig. 2c). pBR325 differs from pBR322 in that it encodes the chloramphenicol acetyltransferase (*cat*) gene (5). We suggest that pBR325 has partial suppressor activity due to expression of this *cat* gene. Importantly, the *cat* gene itself is not specifically responsible for suppression in the pOF39, pND5, and pND6 derivative plasmids, because in each of these plasmids the *groE* operon is inserted within the *cat* gene (10).

The results presented above indicated that the suppression observed with pOF39 was not dependent on the actual functions of the overproduced chaperonins. Rather, it appeared that protein overproduction, per se, was mediating suppression. This model makes a clear prediction. If such a method of

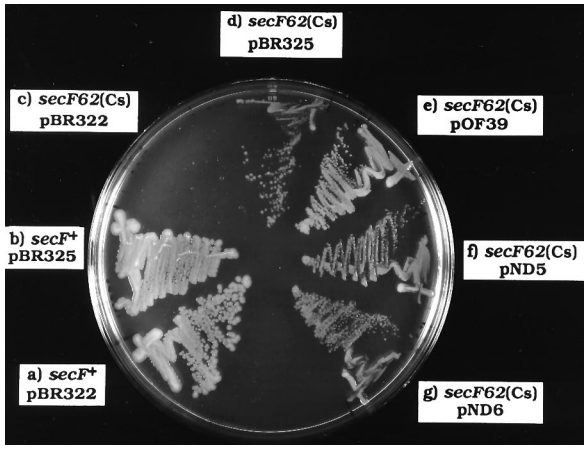

FIG. 2. Multicopy suppression of the *secF62*(Cs) mutation. (a and b) Strain PND11 (MC4100, *secF*$^+$ *zaj*::Tn*10*); (c through g), strain PND10 (MC4100, *secF62 zaj*::Tn*10*). The strains were transformed with plasmids pBR322 (control for pBR325) (a), pBR325 (control for pOF39, pND5, and pND6) (b), pBR322 (c), pBR325 (d), pOF39 (overproduces GroES and GroEL) (e), pND5 (overproduces GroES) (f), and pND6 (overproduces a truncated form of GroES) (g). Strains were grown on L agar containing 125 μg of ampicillin per ml for 48 h at 23°C.

suppression were occurring, then overproduction of any cytoplasmic protein could potentially suppress *sec*(Cs) mutations.

Overexpression of proteins unrelated to the protein export process suppress the *secE501*(Cs) mutation. Two proteins that have no known role in protein secretion were analyzed for the ability to suppress the *secE501* mutation when overexpressed: (i) a nonfunctional derivative of RspA (RspA-E), a protein involved in the expression of stationary-phase genes (15), and (ii) β-galactosidase, a protein involved in lactose metabolism (17, 19). Both plasmids pSPER1-E (which overproduces RspA-E) and pNF2 (which overproduces β-galactosidase) suppressed the cold-sensitive phenotype of the *secE501* allele.

DISCUSSION

Our data support the notion that overexpression of genes unrelated to the process of protein export suppresses the *sec*(Cs) mutations. This multicopy suppression is similar to the suppression of *sec* mutations by ribosomal protein mutations and protein synthesis inhibitors (19). In all cases, suppression is not directly related to the process of protein export. We suggest that the multicopy suppressors act by inhibiting translation and in this way indirectly suppress the *sec* mutations. For example, large amounts of transcript produced from a gene on a multicopy vector could compete with host chromosomal transcripts for the translational apparatus. This would create a situation similar to that observed when protein synthesis is compromised. This model predicts that decreasing the length of the suppressing gene would reduce the strength of suppression. Consistent with this prediction, the progressive deletion derivatives of the *groE* plasmid pOF39 are also progressively weaker multicopy suppressors of the *sec* mutations (Table 1). In support of this model, Dong et al. (8) have shown that overproduction of β-galactosidase or a truncated form of EF-Tu causes a decrease in growth rate and a concomitant destruction of rRNA molecules.

Presently, the mechanism by which translational inhibitors suppress *sec* mutations is unknown. Lee and Beckwith (18) have suggested that the *sec* mutations are suppressed when translation is compromised because the synthesis of presecretory proteins is reduced. This reduction allows the defective Sec machinery to better manage the demands for secretion. Alternatively, physiological states which compromise translation in *E. coli* could increase the relative levels of Sec proteins, thus conferring suppression. For example, Dennis (7) has noted that transcription of the *spc* operon, which includes *secY*, is induced when translation is compromised. In either case, it is clear that *sec* suppressor analysis often identifies genes whose products are not directly involved in protein translocation.

An important point is raised from these findings. Certain *sec* conditional alleles can be suppressed effectively by overproduction of a variety of proteins, whereas other alleles are not suppressed at all. This may be explained by considering that the mutations are not of equal strength (24). For instance, the *secE501*(Cs) allele is weaker than *secE15*; strains containing the former mutation can grow at 30°C, whereas strains containing the latter cannot grow at this temperature (31). Thus, the *secE501* mutation is partially suppressed by the relatively weak multicopy suppressor, pBR325, whereas the *secE15* mutation is not (Table 1). Analyzing suppression of stronger alleles at an intermediate or semipermissive temperature might also lead to observable suppression. Alternatively, it is possible that suppression of stronger alleles by the functional GroES/EL-overproducing plasmid is actually due to the functions of these chaperonins.

For example, studies by Van Dyk et al. (39) and Ueguchi and Ito (38) have shown that overproduction of the GroE proteins suppresses *sec*(Ts) mutations. Our analysis confirms this observation. Moreover, the deletion derivatives of the *groE* plasmids do not suppress the *sec*(Ts) mutations (Table 1). Van Dyk and colleagues (39) and Ueguchi and Ito (38) have suggested that overproduction of the GroE proteins suppresses these *sec*(Ts) mutations by enhancing the frequency of proper folding of the thermolabile mutant Sec proteins. However, it is also possible that the suppression of the *sec*(Ts) mutations is not dependent on the function of the *groE* gene products. In this case, the dependence of suppression on the entire length of the *groE* operon would be due to the quantity of overexpressed protein needed for suppression. That is, the amount of overproduced protein required for suppression is achieved only when both GroES and GroEL are overexpressed. Consistent with this possibility, Ueguchi and Ito (38) have found other multicopy suppressors of the *secY24*(Ts) mutation. These loci specify products that do not appear to function in protein translocation (see below). This observation is qualitatively similar to the results described in this study and is consistent with the model that the suppression of the *sec*(Ts) mutations by overproduction of the GroE proteins is not dependent on the actual functions of the GroE proteins. A more detailed analysis is required to determine the precise mechanism of GroE-mediated multicopy suppression in this case.

Several other studies have used multicopy suppression in an attempt to identify other proteins involved in protein secretion. Esnault et al. (9) have examined a yeast *secY* homolog, *sec61*. The *sec61-2* temperature-sensitive mutation confers a translocation defect for proteins entering the lumen of the endoplasmic reticulum. The temperature sensitivity conferred by *sec61-2* can be partially suppressed by multicopy vectors expressing either the *SSS1* or *SSS2* gene. *SSS1* encodes an essential protein which is required for protein translocation. The *SSS2* suppressor, however, is allelic with *TDH3*, encoding glyceraldehyde-3-phosphate dehydrogenase (9). *TDH3* is not involved in protein translocation. Rather, its normal function is in intermediary metabolism. Esnault et al. (9) suggested that the high-level expression of *TDH3* from a multicopy plasmid indirectly suppresses *sec61-2* in a manner similar to that observed for multicopy suppression of the *sec*(Cs) mutations.

In *E. coli*, several genes have been identified as multicopy suppressors of *sec* conditional mutations. These include *ssyG*, which is allelic with the *infB* gene and encodes translation initiation factor 2 (33); *htpG*, which encodes a heat shock protein; *hns*, which encodes a highly expressed histone-like protein involved in chromosome compaction; *msyB*, which specifies a highly acidic protein of unknown function (38); *YSY6*, a yeast gene of unknown function (27); *ydr*, a suppressor of a dominant lethal *secY* allele; and *yajC* (also referred to as *ORF3* or *ORF12*), an open reading frame of unknown function located in the *secDF* operon (37). In several of these studies, deletion or mutation of the gene responsible for multicopy suppressor activity did not lead to defects in secretion, suggesting that these genes are not directly involved in the export process. In light of our findings, we propose that high-level synthesis of these proteins may compromise translation by competition for factors required for protein synthesis.

Although we do not dispute the overall utility of multicopy suppression, our analysis highlights some of the pitfalls which may be encountered in this type of analysis. Ideally, before initiation of multicopy suppressor analysis, the starting mutation must be carefully characterized, and the precise nature of the phenotype conferred by this mutation should be determined. Also, any physiological change brought about by over-

expressing proteins in the cell that could indirectly affect the process being studied must be carefully ruled out as the cause for suppression. Ultimately, one must demonstrate that the multicopy suppressor specifies a product which is directly involved in the process in question.

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