Development of a visual complementation system: application to the essential *ffh* gene of *Escherichia coli*

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INTRODUCTION

Bacterial complementation tests have revealed insights into gene functions through the characterization of mutations. However, challenges remain when characterizing essential bacterial genes because a functional copy of the gene must always be provided. We have developed a new visual genetic system able to conduct complementation tests that characterize essential genes in bacteria. We have applied this system to characterize the essential *ffh* gene of *Escherichia coli*, which encodes a subunit of the signal recognition particle (SRP). The SRP is responsible for targeting and insertion of membrane proteins into the cytoplasmic membrane (1). Specifically, we have applied this system to assess the importance of the methionine residues in the Ffh protein M domain (FIG 1). The Ffh M domain is important for binding the hydrophobic signal sequences of nascent membrane proteins (2).

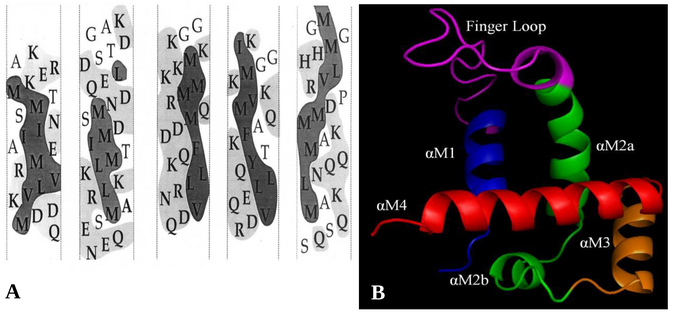


FIG 1 Ffh protein M domain. A: Ffh M domain 2D amino acid structure showing the concentration of methionines in the M domain helices (2). B: Ffh M domain 3D structure showing methionine-rich M domain helices and finger loop region (3).

METHODS

We constructed an *E. coli* mutant, AC101 were a deletion of *ffh* (4) was complemented by a derivative of the plasmid pCLIP (pCLIP-*ffh*-CP) encoding *ffh*$^+$ and a purple chromoprotein from the coral *Acropora millepora* (CPamill) (5) (FIG 2). Since this plasmid lacks an active partitioning system, it is easily lost from *E. coli* in the absence of selection.

Using standard methods of DNA recombination, we also created alleles of *ffh* in which each methionine of the M domain, except the Met383 that has previously been shown to be necessary for Ffh function (6), was converted to other hydrophobic amino acids, including tryptophan, valine, phenylalanine, tyrosine, or isoleucine (TABLE 1). Each allele was expressed by the compatible ColE1 plasmid pFfh322 (FIG 3) for subsequent transformation into AC101. Transformants were screened for *ffh* complementation, as shown in FIG 4.

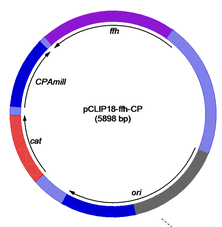


FIG 2 Map of pCLIP-*ffh*-CP used for complementation tests. This plasmid, encoding *ffh*$^+$ and purple chromoprotein (CPamil), was used to complement the *ffh*$^-$ genome of AC101. *ori*: origin of replication; *cat*: chloramphenicol resistance gene.

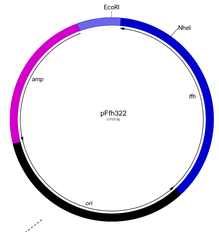


FIG 3 Map of pFfh322 used for complementation tests. This plasmid, encoding the *ffh* M domain alleles, was transformed into AC101 for complementation tests. *ori*: ColE1 origin of replication; *Eco*RI, *Nhe*I: restriction enzyme sites flanking the M domain of *ffh* used for plasmid construction.

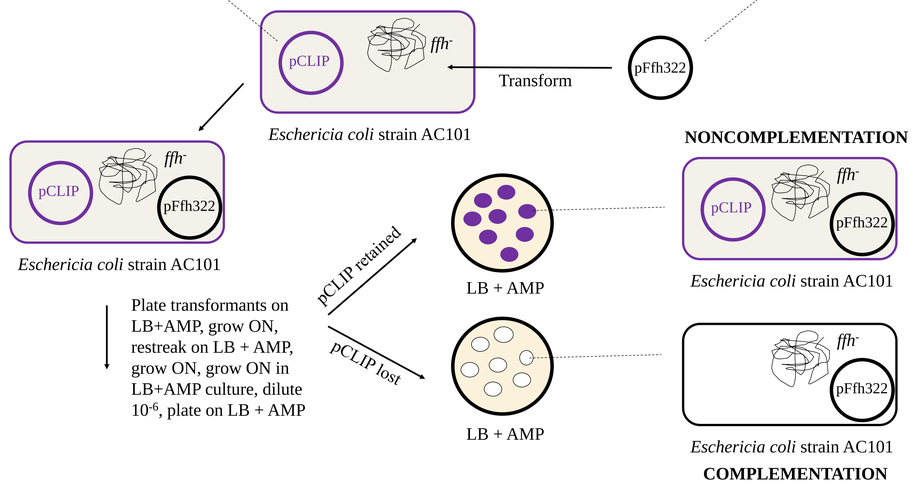
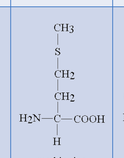
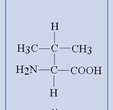
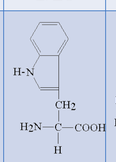
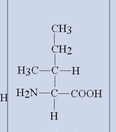
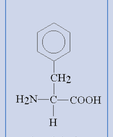
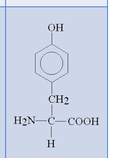


FIG 4 Scheme of visual genetic complementation system used to analyze the *E. coli ffh* gene products. As shown, AC101 was transformed with pFfh322 carrying different M domain alleles of *ffh* (7). Purple colony color resulted if pCLIP-*ffh*-CP was retained, indicating noncomplementation. In contrast, white colony color resulted from the loss of the plasmid, indicating complementation. LB: Luria broth agar media; AMP: ampicillin; ON: overnight.

TABLE 1. Summary of *ffh* alleles and results of complementation tests. In all mutant alleles, 19 of the 20 methionines in the M domain were replaced with the indicated amino acid. The structures of the amino acids used to replace methionine are shown.

Ffh Allele	Met	Val	Trp	Ile	Phe	Tyr
Complement	+	+/-	-	-	+/-	-
# of Mets	20/20	1/20	1/20	1/20	1/20	1/20
Amino acid structure	 methionine	 valine	 tryptophan	 isoleucine	 phenylalanine	 tyrosine

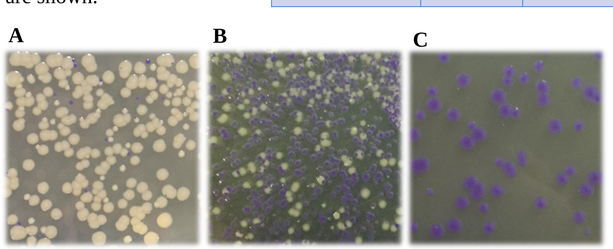


FIG 5 Results of visual complementation system. Shown are colonies of AC101 transformed with A: pFFh322 *ffh*$^+$ (white colonies indicate complementation). B: pFfh322 Val (mixed white and purple colonies are an example of complementation with low efficiency). C: pFfh322 Trp (purple colonies reveal noncomplementation).

RESULTS

To first confirm the effectiveness of the complementation test system summarized in FIG 4, we compared the ability of pFfh322 *ffh*$^+$ and pFfh322 *ffh*$^-$ to complement the *ffh* deletion in AC101. Examples of a both positive and negative complementation test results are shown in FIG 5. After demonstrating its effectiveness with control plasmids, we used the complementation system to assess the importance of the methionine residues of the Ffh protein M domain.

The results of the M domain mutant complementation tests are summarized in Table 1. We determined that only valine and phenylalanine could substitute for all of the methionine residues throughout the Ffh M domain, apart from the methionine residue at position 383 previously established to be necessary for Ffh function. However, they did not complement at the same efficiency as *ffh*$^+$.

CONCLUSION

We have successfully developed a new visual genetic system to conduct complementation tests to characterize essential bacterial genes. This genetic system has several advantages over traditional complementation systems because it can characterize essential genes, it is independent of growth temperature and media, and it is quantifiable. In our application of this system to analyze the *E. coli* Ffh protein, we determined that only valine and phenylalanine could substitute for all of the methionine residues throughout the Ffh M domain, apart from a methionine residue at position 383. Future work will characterize additional *ffh* mutant alleles. This genetic system should be amenable to isolate and characterize mutant alleles of many other essential bacterial genes.

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