Determining the cellular localization of the bacterial signal recognition particle (SRP) by constructing fluorescent protein gene fusions

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INTRODUCTION

The signal recognition particle (SRP) is a highly conserved ribonucleoprotein complex that is important for targeting proteins to cellular membranes in all three kingdoms of life (Eukaryotes, Eubacteria, and Archaea) (FIG 1). Since the Gram-negative bacteria Escherichia coli expresses the simplest version of SRP, comprised of the Ffh protein bound to a small structural RNA (4.5S RNA), it is an appealing model to study SRP function. While much is known about the biochemical activity of the SRP in E. coli, how the cellular localization of Ffh and 4.5S RNA influences their activity is not as well understood. To approach this question, we have used green fluorescent protein (GFP) and the flavin-based fluorescent protein iLOV to tag the Ffh protein at both the amino- and carboxy-termini. GFP is widely used as a reporter to investigate protein localization within the cell. Although not as extensively, the relatively small size of the iLOV polypeptide suggests it may be a useful reporter protein in cases where the larger GFP interferes with correct functionning of the target protein (2). In addition, we have also used the Broccoli fluorescent RNA (3) to localize 4.5S RNA within living cells.

FIG 1. Composition of the SRP from the 3 kingdoms of life. Protein components are labeled and their relative positions on the RNA scaffold is shown.

METHODS

Fusions between Ffh and GFP and iLOV were constructed using standard techniques of recombinant DNA. Constructs encoding GFP and iLOV were obtained as synthetic DNA (IDT, Coralville IA) and cloned into the plasmid pHfhs322, as shown in FIG 2. After ligation, transformants were selected on LB +ampicillin and screened for fluorescence under UV illumination. Plasmid DNA was extracted from transformants with the appropriate phenotype and tested for their ability to complement an ffh deletion mutant.

RESULTS

Three separate plasmids expressing Ffh-fluorescent protein fusions were constructed, as summarized in FIG 2. Transformants of each of the plasmids grew poorly in comparison to wild type controls. FIG 3 shows the results of Ffh-N-GFP expression, but similar results were observed for each of the constructs, suggesting that expression of the Ffh-GFP/iLOV proteins exerted a dominant negative effect on SRP function. This observation was further tested by microscopic examination of individual cells (FIG 4). The unusual morphology of the cells expressing the Ffh fusion proteins is characteristic of bacteria with an impaired ability for membrane protein biogenesis. As anticipated from the expression of the Ffh-GFP fusions, none of the constructs were able to complement an ffh deletion mutant (TABLE 1).

Structural predictions of the protein fusions suggest that addition of the folded GFP and iLOV domains likely interfere with the folding or function of the G domain (N-terminus) or M domain (C-terminus) known to be important for SRP function (FIG 5).

CONCLUSIONS

Fusion of GFP or iLOV protein tags to the amino- and carboxy-termini of the Ffh protein resulted in a dominant negative phenotype indicating expression of the hybrid proteins interferes with normal SRP protein function. The poor growth and atypical E. coli cell morphology is characteristic of aberrant membrane protein biogenesis. While the new constructs failed to function as wild type Ffh, they nonetheless will be useful genetic tools to perturb SRP function to better understand how it functions in membrane protein biogenesis.

We also initiated an alternative strategy to visualize SRP in bacteria by tagging 4.5S RNA (FIG 1) with Broccoli fluorescent RNA (3). Future studies will test the ability of this construct to complement an ffs (encoding 4.5S RNA) deletion mutant and to determine the cellular localization of SRP in E. coli.

REFERENCES


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TABLE 1 Results of complementation tests

<table>
<thead>
<tr>
<th>Reporter fusion</th>
<th>Complementation</th>
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<tbody>
<tr>
<td>WT (Ffh+)</td>
<td>+</td>
</tr>
<tr>
<td>Ffh-C-GFP</td>
<td>-</td>
</tr>
<tr>
<td>Ffh-N-GFP</td>
<td>-</td>
</tr>
<tr>
<td>Ffh-N-iLOV</td>
<td>-</td>
</tr>
<tr>
<td>Ffs-3’-Broccoli</td>
<td>ND</td>
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