Composition and function of the Signal Recognition Particle of the Gram-Positive Bacterium, *Bacillus subtilis*

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Introduction

The Signal Recognition Particle (SRP) of *B. subtilis* is an essential cell component involved in the early stages of the protein secretion pathway commonly known as Sec pathway.[1] The SRP plays a major role in the pathway by recognising the N-terminus (signal peptide) of membrane and secretory proteins as they emerge from the ribosome, and delivering them to the membrane-bound Sec translocase. The translocase is then responsible for either inserting the proteins into the membrane or transporting them to the outside of the membrane.[2]

The SRP consists of an RNA backbone to which a number of proteins are attached. In the case of *E. coli*, the scRNA is short and a single protein, Ffh, is attached to the "S" domain. The *B. subtilis* scRNA is longer and, according to the current literature binds two protein; Ffh to the "S" domain and HBsu to the "Alu" domain. More recent work in Newcastle indicates that Hbsu is not a component of the SRP but instead YlxM bind to the "Alu domain.

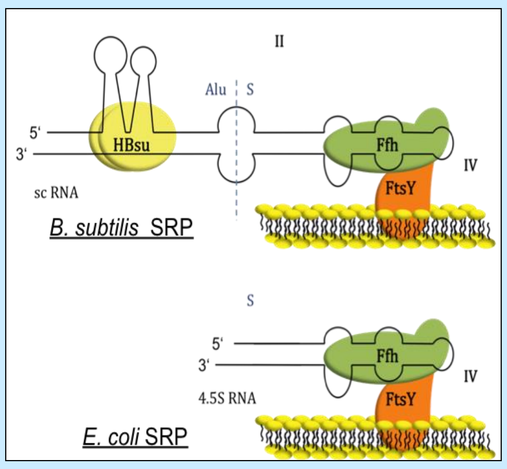

Fig.1: Published structures of the *B. subtilis* and *E. coli* SRPs.

Aims and Objectives

This project was a component of a larger project aimed at redefining the structure of the *B. subtilis* SRP. It identified a protein of unknown function, YlqC, that is encoded by the same operon as YlxM and Ffh. YlqC has an RNA binding domain, and the aim of this project was to purify YlqC and to analyze its interaction with other proteins that are either components of the SRP or that interact with these proteins. These proteins included:

- HBsu, a histone-like DNA binding protein
- YlxM, a SRP component that bind specifically to the Alu domain
- EF Tu, a ribosomal protein throught to interact with YlxM to inhibit translation

Methodology

Polymerase Chain Reaction (PCR) and Agarose Gel Electrophoresis

The *ylqC* gene was cloned into an expression vector (pET28a) and a combination of PCR and agarose gel electrophoresis was used to confirm that the was present in the plasmid.

SDS-PAGE

YlqC fused to a His affinity tag was overexpressed in *E. coli* and purified using nickel beads. Then imidazole was used to elute the bound YlqC from the beads and the eluted fractions analyzed by 12% SDS-PAGE gels.

Native Gel Electrophoresis

Protein-protein interaction between YlqC, YlxM, Hbsu and EF Tu were analysed using native gel electrophoresis.

Electromobility Shift Assays (EMSA)

EMSA was carried out to analyze interactions between scRNA and potential protein components of the SRP. This involved the *in vitro* preparation of scRNA fragments, interacting them with YlqC, YlxM and HBsu and using gel electrophoresis to determine any changes in the mobility of the scRNA in the presence of the proteins.

Results

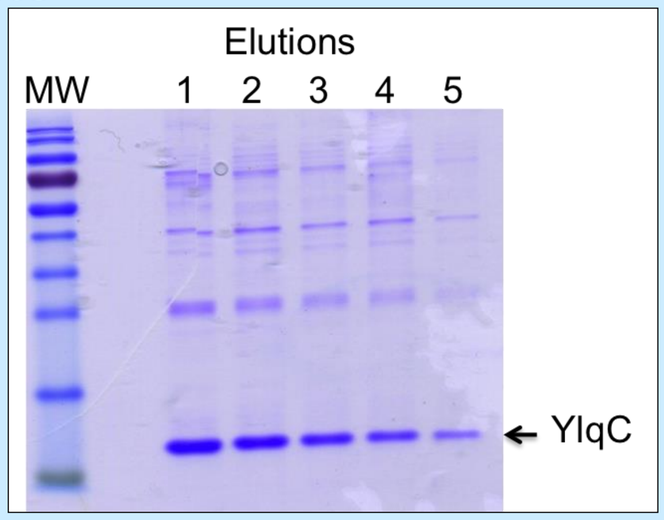

Fig. 2: SDS-PAGE (12%) showing bands of YlqC protein after various elution steps.

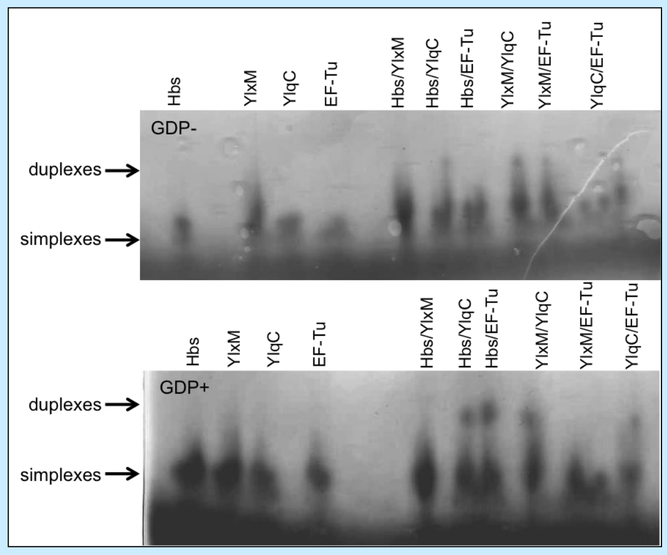

Fig. 3: Native gel showing bands of proteins with or without GDP. The tracks that have duplex band have proteins that interact with each other.

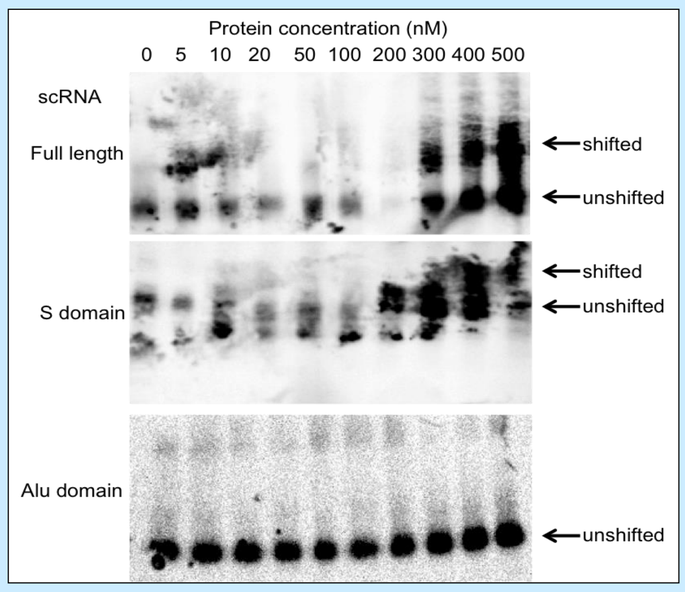

Fig. 4: Image of EMSA showing the binding between the YlqC protein and the full-length scRNA and S domain, but not the Alu domain.

Discussion and Conclusion

From Figure 3, duplexes are seen for all the different protein combination except Ef-Tu/YlqC and HBsu/YlxM. This suggests that protein interaction are found between HBsu/Ef-Tu, HBsu/YlqC, YlqC/Ef-Tu and YlqC/YlxM, but not for Ef-Tu/YlqC and Hbsu/YlxM. The presence of GDP makes the binding more stronger thus the bands appeared more prominent as compared to the one without GDP. From Figure 4, shifted bands are observed with the full length scRNA and S domain, but not with Alu domain as the protein concentration increases. This could indicate that the YlqC protein binds to the S domain of the scRNA, but does not bind with the Alu domain. In conclusion, these results suggest that the protein that we are interested in, YlqC, does interact with Hbsu and YlxM, but not with Ef-Tu. From the EMSA, we can implicate that YlqC interacts the S domain of SRP, rather than with Alu domain. These results provide a better understanding of the structural organization of the SRP and its components, but further research is warranted to get a clearer and better understanding of how YlqC function.

References:
1. Susanne Pohl, Colin R. Harwood (2010). *Heterologous Protein Secretion by Bacillus Species: From the Cradle to the Grave*. Burlington: Elsevier Inc. Academic Press. p1-25.
2. Colin R. Harwood, Rocky Cranenburgh. (2010). Bacillus protein secretion: an unfolding story. *Trends in Microbiology*. 16 (2), p73-79.