

Unraveling Chlamydia abortus: Targeting the SRP-Dependent Pathway to Neutralize Pathogenicity

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References



Abstract

This case study focuses on a DNA fragment set from an unknown bacterium, which was identified as belonging to the *Chlamydia abortus* species through an **nBLAST search**. Using theoretical, genomic, and proteomic analyses, the genes present, the proteins they encode, and their roles in pathogenicity, protein interactions, and cellular functions were examined, as well as strategies for managing infections. These analyses were performed using tools such as **NCBI**, **KBase**, **AlphaFold** and **Benchling**.

Introduction

The “*Tree of Life*” is a fundamental concept in Biology which divides all life into three major groups, one of which is Bacteria. Bacteria are highly abundant, constantly evolving, and remain a major focus of study.

In this case study, a DNA fragment from an unknown bacterium was identified as ***Chlamydia abortus*** and analyzed using theoretical and bioinformatics tools, focusing on genes and proteins involved in pathogenicity.

Chlamydia abortus is a non-motile, obligate intracellular, Gram-negative bacterium that primarily infects small ruminants but can also infect pregnant women, making it a zoonotic pathogen (1). Transmission occurs mainly through **ingestion or aerosol inhalation** of contaminated abortion material (2).

It has a **biphasic life cycle**, alternating between the infectious elementary body (EB) and the replicative reticulate body (RB) (3). Several EB surface proteins, including **polymorphic membrane proteins (Pmps)**, function as autotransporters essential for host adhesion (4).

Their correct localization depends on **the signal recognition particle (SRP) pathway**, with ffh gene encoding the SRP protein responsible for directing membrane proteins to their proper location (5, 6).

The potential neutralization of pathogenicity was explored through **gene overexpression** using an in-silico design.

Methods and materials

Organism and gene identification

NCBI nucleotide blast was used to identify from which organism DNA fragment set nine was from (see table 1). Standard database (nr, etc.) (core_nt) was used and optimisation of highly similar sequences (megablast). **KBase** was used for efficient and comprehensive gene annotations (see table 2) by Annotate Assembly and Re-annotate Genomes with Prokka - v1.14.5 Type: KBaseGenomes.Genome-11.1 Version: 1.

Literature research on pathogenicity

To understand the transmission and infection mechanisms of Chlamydia abortus, a **literature review** was conducted focusing on its origin, host targets, modes of infection, and pathogenicity, with particular attention to the SRP pathway and the roles of ffh, ftsY, and FtsY.

Structure visualization of FtsY

The protein structure of the signal recognition particle receptor (FtsY) was found and visualized in **AlphaFold** (EMBL-EBI, AlphaFold PSD). The three domains were highlighted with the “domain” feature in cartoon presentation mode after which the A domain was further highlighted.

In silico design of the ftsY expression vector

The nucleotide sequence of ftsY was found on **NCBI** and imported in **Benchling**. **ThermoFisher** was used to find the sequence for the pBAD/His vector and Primers were designed in **NCBI Primer-BLAST**. Digestion and ligation were performed and analysis of recombinant DNA was done to check that final construct is correct.

Results

Organism identification

Scientific Name	Total Score	Query Cover	E value	Per. Ident	Accession
Chlamydia abortus S26/3	36934	100%	0	100%	CR848038.1
Chlamydia abortus	36928	100%	0	100%	CP024084.1
Chlamydia abortus	36767	100%	0	9.986%	LN554883.1
Chlamydia abortus	36767	100%	0	9.986%	CP070224.1
Chlamydia abortus	36767	100%	0	9.986%	LN554882.1
Chlamydia abortus	36767	100%	0	9.986%	CP021996.1
Chlamydia abortus LLG	36322	100%	0	9.946%	CP018296.1
Chlamydia abortus	36265	100%	0	9.941%	CP158101.1

Table 1: nBLAST results for organism identification showing the scientific name, total score, query cover, E-value, percentage identity, and accession number. The total score reflects alignment strength, query cover shows sequence overlap, the E-value indicates match significance, and percentage identity measures sequence similarity. The table identifies bacterium Chlamydia abortus as the organism fragment set 9 belongs to.

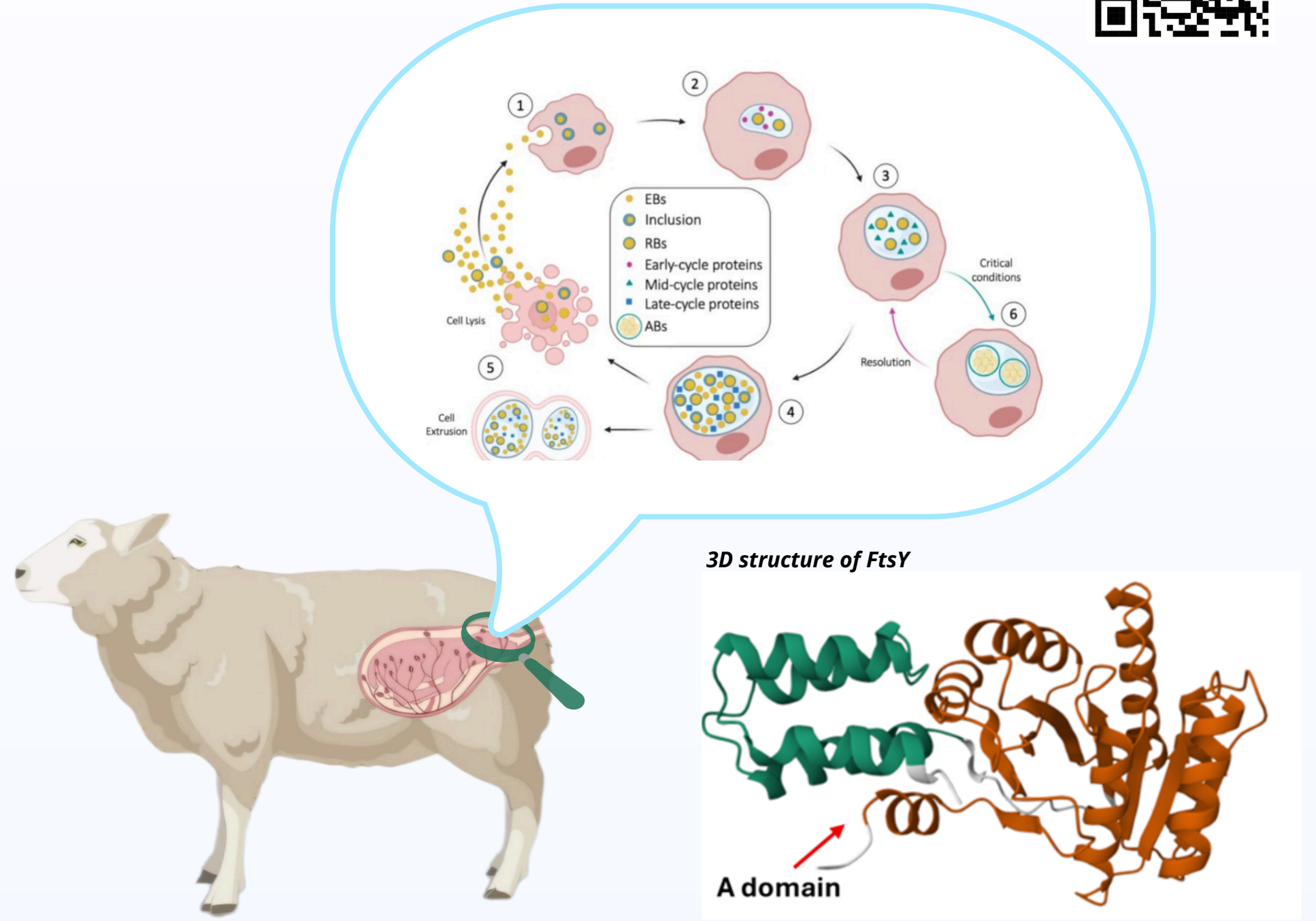


Figure 1: Cartoon representation of a pregnant sheep showing the biphasic developmental cycle of Chlamydia abortus, illustrating the attachment and entry of elementary bodies (EBs) into susceptible host cells, their conversion to reticulate bodies (RBs), and subsequent release as EBs (6).

KBase genome annotation

Fragment num.	Type	Start position	Length (nt)	Strand (-/+)	Gene synonym	Full name of product
fragment_2	gene	4764	5390	-	atpD	V-type ATP synthase subunit D
fragment_2	gene	9313	9939	-	atpE	V-type proton ATPase subunit E
fragment_1	gene	6324	7190	+	COQ2	4-hydroxybenzoate polyprenyltransferase, mitochondrial
fragment_1	gene	8234	9334	+	ecdB	putative UbiX-like flavin prenyltransferase
fragment_3	CDS	19390	19995	-	ffh	Signal recognition particle protein
fragment_3	gene	19390	19995	-	ffh	Signal recognition particle protein

Table 2: Table summary of 6 Kbase Prokka genome annotation results from all 3 fragments of Fragment_set_09 with hypothetical proteins and regions containing no product removed. The CDS and gene of ffh (with product signal recognition particle protein) highlighted, as this gene and its protein is used for further analysis.

Biological process of SRP-dependent pathway

1. **The signal recognition particle SRP** is a ribonucleoprotein, consisting of Ffh-protein and 4.5S RNA, binds to signal sequences of translating **virulence proteins (Pmps)** emerging out of ribosomes.
2. RNC-SRP/FtsY GTPases **dimerization** at the NG domains
3. Pmps are passed through the translocation pore on the inner plasma membrane of the bacteria by the **hydrolysis of GTP**.
4. **Disassociation** of the RNC-SRP/FtsY complex (7).

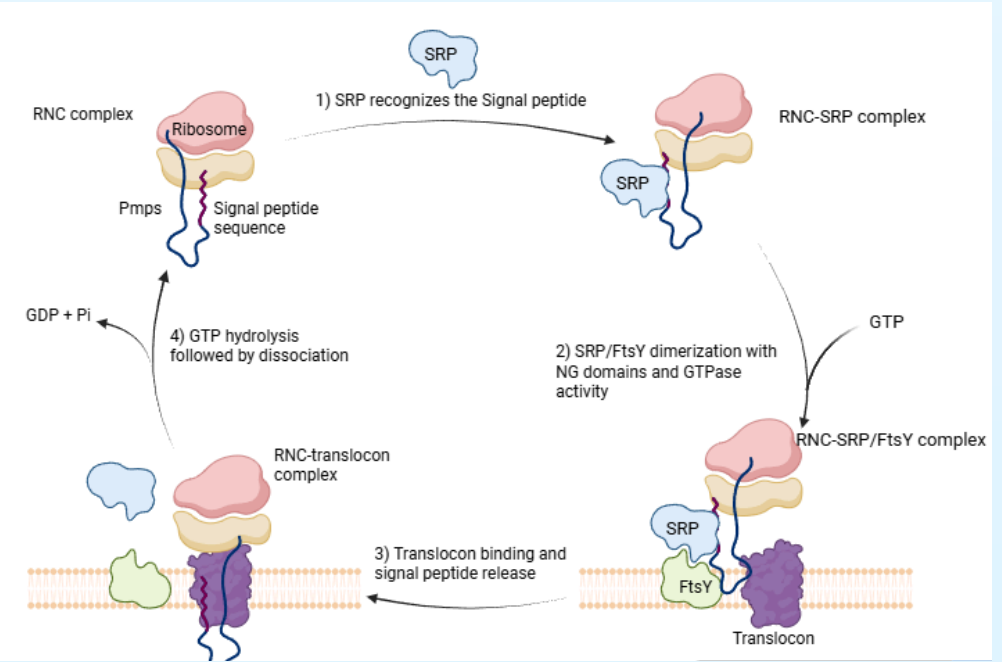


Figure 2. The four steps of the signal recognition particle (SRP) dependent pathway. The pathway starts with recognition of the signal peptide sequence on the Pmp by SRP, SRP/FtsY dimerization, translocon binding and dissociation of the SRP/FtsY complex. Created with biorender.

3D structure of FtsY

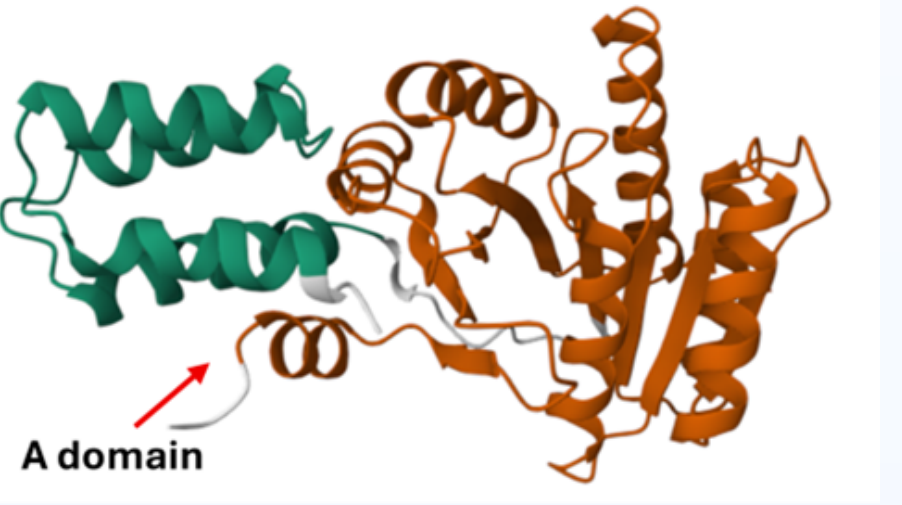


Figure 3: AlphaFold protein structure of the FtsY receptor is divided into three domains: the NG domains (N = orange, G = green) and the A domain (highlighted in the picture). The NG domain is divided into a nucleotide binding domain (N) and a GTPase domain (G) that contains nucleoside triphosphate hydrolases whereas the A-domain anchors the protein to the membrane.

In Silico Design overexpression ftsY gene

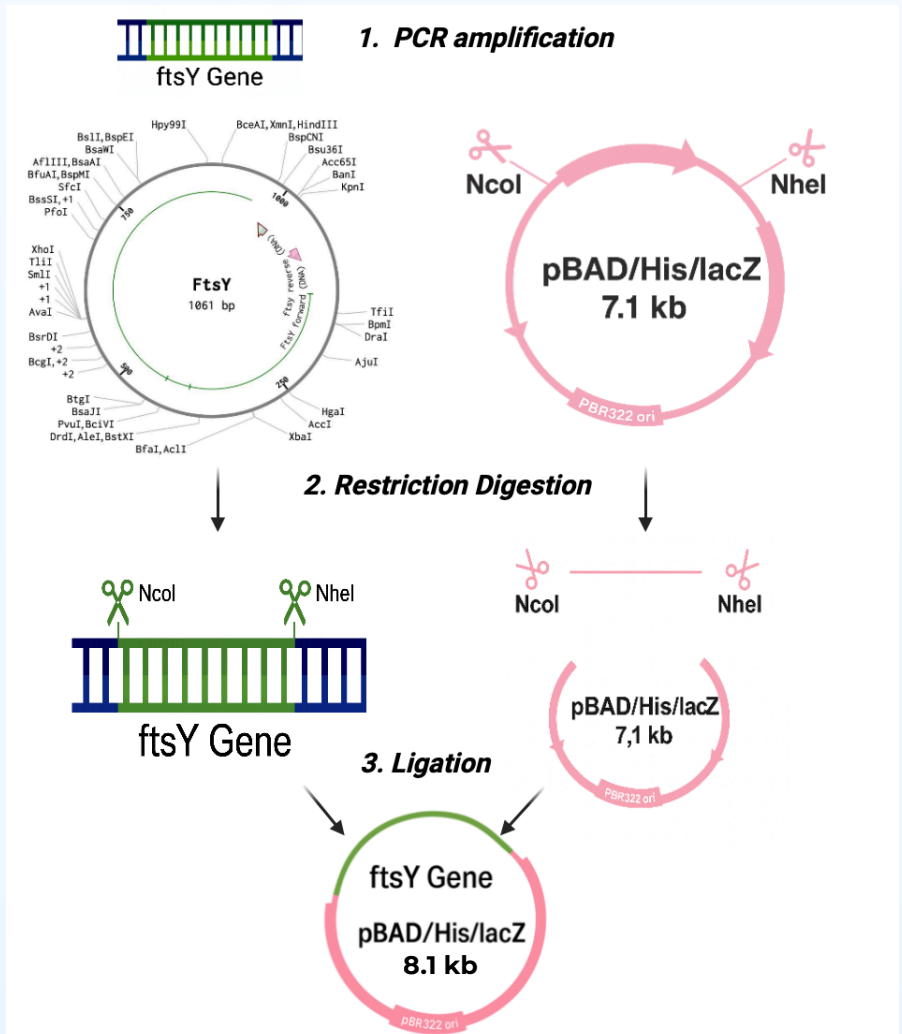


Figure 4: in Silico Design for cloning FtsY gene in pBAD vector. It shows pBAD vector used to clone FtsY receptor gene from Chlamydia abortus S26/3 strain using NcoI and NheI restriction enzymes. The recombinant plasmid has a length of 8083 bp.

Conclusion

Nucleotide BLAST analysis of fragment set 9 showed the sequence belonged Chlamydia abortus. The ffh gene from 57 KBase annotated genes was selected for further study because it encodes a signal recognition particle protein (SRP) involved in the correct localization of polymorphic membrane proteins (Pmps) that play a role in the **organism's pathogenicity**.

The protein structure of the SRP receptor FtsY showed that FtsY is anchored to the inner membrane with its A domain and that the **SRP/FtsY complex** is made possible by the NG domains dimerizing.

The overexpression of the FtsY receptor will stimulate premature binding to the Ffh-SRP preventing the **translocation of virulence protein Pmps**.

Further optimization of the outlined mechanism could consider post translational modification by **cleavage of N-terminus** at the A domain. This would lead to increased solubility of the FtsY receptor after overexpression ensuring selective accumulation of FtsY in the cytosol, promoting premature binding of Ffh-SRP (8).

Ultimately, regulation of FtsY-Ffh interactions could enable **new antimicrobial therapeutic treatments** against Chlamydia Abortus infections in affected organisms.