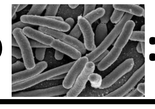
From  to  : Construction of Synthetic Genes of the Acinoform Spider Silk Protein (AcSp1)

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Introduction

Spider silks have remarkable physical properties due to a combination of strength and elasticity. In addition, spider silks are biocompatible and biodegradable. Our laboratory has shown that the strength products, such as fibers, produced with other silk proteins correlates with the size of the silk protein. The aciniform silk (AcSp1), has been shown to produce the thinnest and strongest fibers of all the natural spider silks. Aciniform silk is composed of a nonrepetitive amino-terminal region, 14 repeats of approximately 200 amino acids each, and a nonrepetitive carboxy-terminal region. We have been able to produce different versions of these genes encoding for 8, 10, 12, and 14 repeats. In addition, we were able to express these large proteins in *E. coli*.

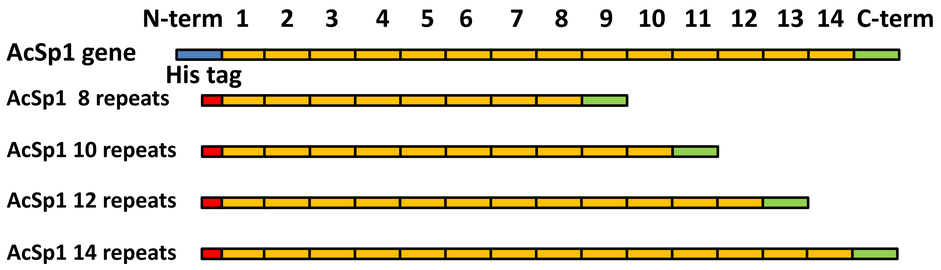
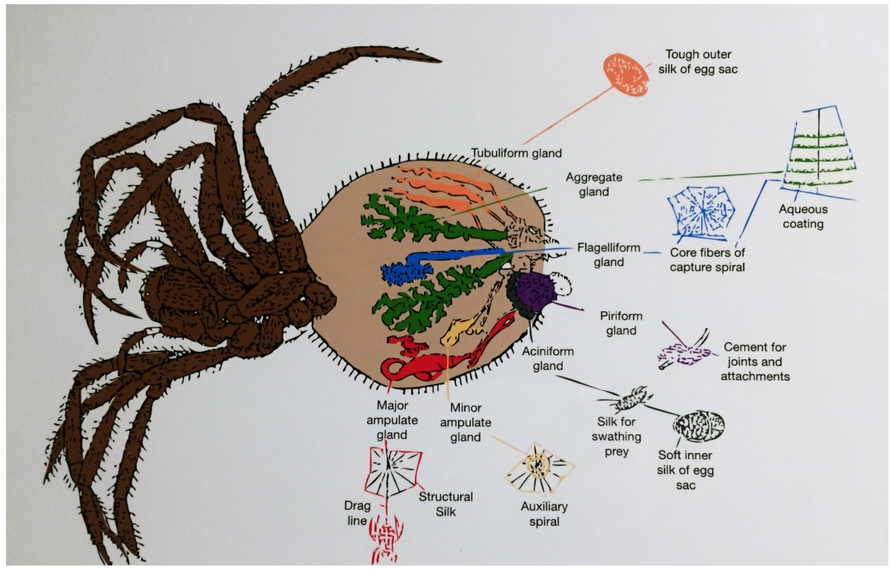


Figure 1. Schematic representation of AcSp1 genes



Orb Weaving Spider Silks. The aciniform gland produces AcSp1 responsible for wrapping the prey and protecting the eggs.

Techniques and Strategies

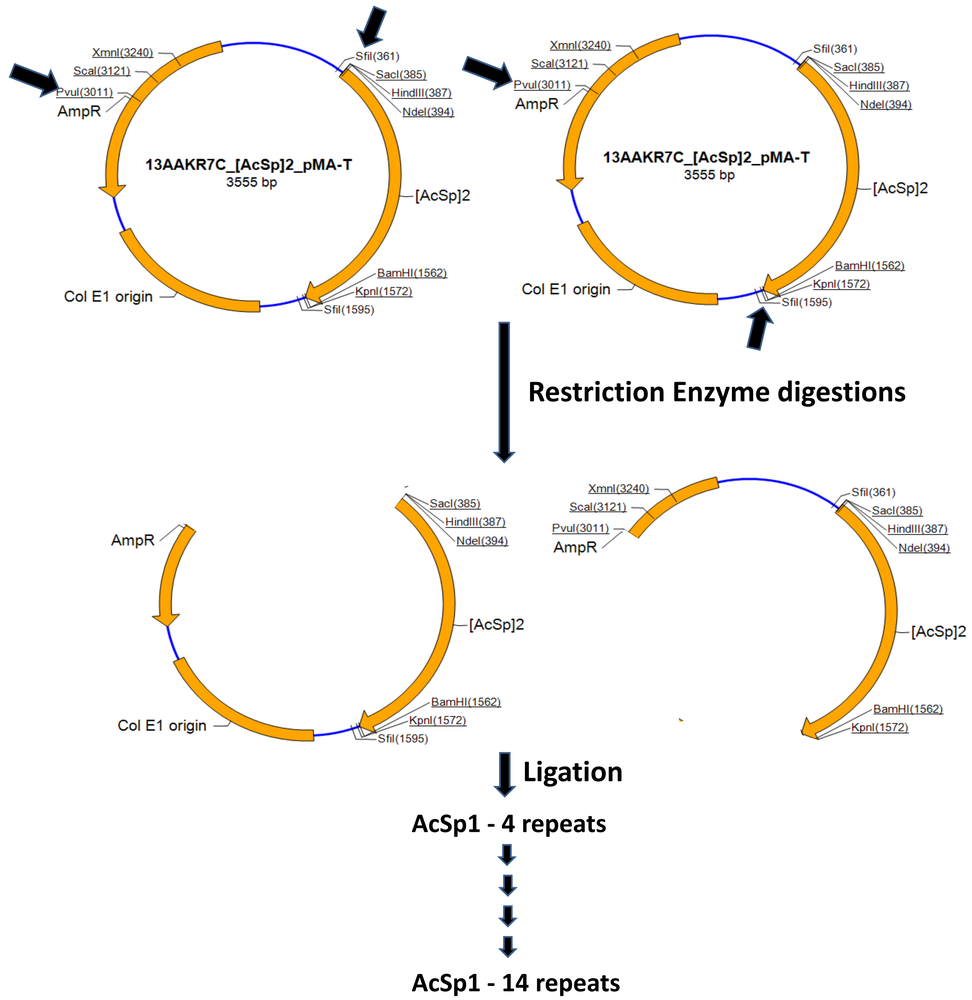


Figure 2. A synthetic gene was designed containing two repeats of AcSp1. This gene was inserted in a plasmid that contains the ampicilin resistant gene (AMP^r)[1]. This plasmid contains several restriction sites that disrupt the AMP^r, the region encoding for the amino-terminal region and the carboxy-terminal region. This plasmid was used for the construction of all the AcSp1 genes. The ligation results in the restoration of the AMP^r.

Results

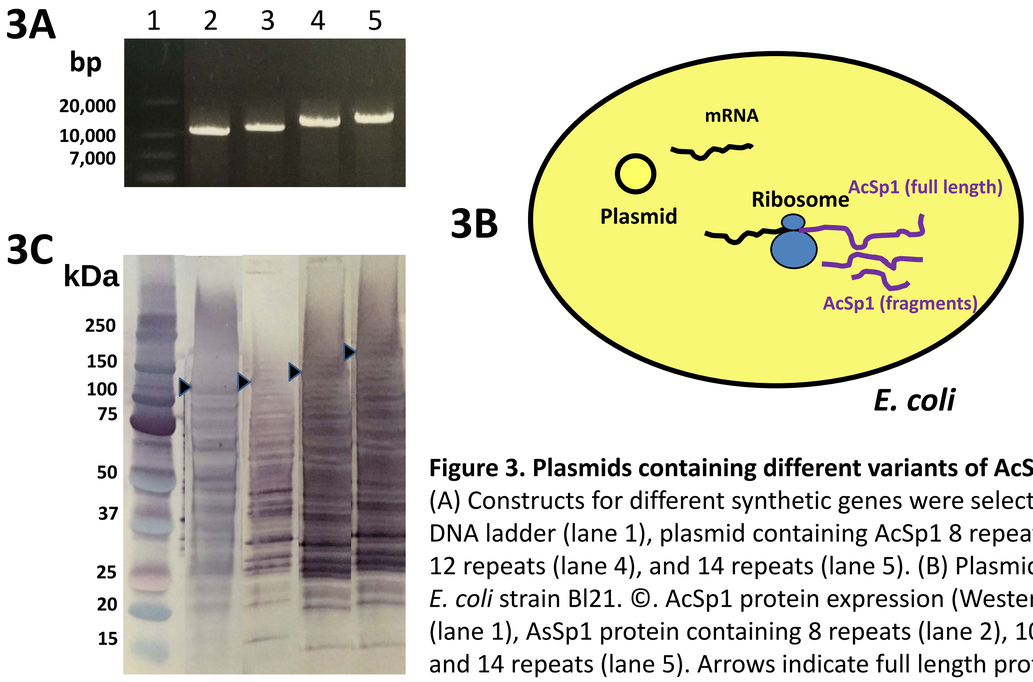


Figure 3. Plasmids containing different variants of AcSp1 and protein expression. (A) Constructs for different synthetic genes were selected by DNA size and sequenced. DNA ladder (lane 1), plasmid containing AcSp1 8 repeats (lane 2), 10 repeats (lane 3), 12 repeats (lane 4), and 14 repeats (lane 5). (B) Plasmids were introduced in the expression *E. coli* strain Bl21. ©. AcSp1 protein expression (Western blot analysis). Protein standards (lane 1), AsSp1 protein containing 8 repeats (lane 2), 10 repeats (lane 3), 12 repeats (lane 4), and 14 repeats (lane 5). Arrows indicate full length proteins.

Conclusions and Future Work

- Synthetic genes were successfully constructed
- Genes are capable of expressing the corresponding proteins
- Gene sequences need to be optimized to reduce/eliminate synthesis of protein fragments

References

E. Coli electronmicrograph: http://www.explainthatstuff.com/electronmicroscopes.html
[1]Hayashi CY, Blackledge TA, **Lewis RV**. Mol Biol Evol. **2004** Oct;21(10):1950-9.