Genome Editing of *Bombyx Mori* (Silkworm) with the Cas9/CRISPR System for Potential Host of Synthetic Spider Silk Gene

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**Introduction**

Spider silk has attracted great interest in recent years due to the reported superior mechanical properties for biomedical, military and other potential applications [1,2]. However, the territorial and cannibalistic nature of the spiders has ruled out farming as a practical production method. Alternative approaches have been developed in to clone and express spider silk genes in various hosts, such as *E. coli*, plants, and in animals such as goats. These protein producing hosts could normally provide a quantity that will satisfy the demands, but the quality of the products cannot match that of natural spider silk due to the inability of the systems to assemble spider silk proteins into fibers. A new approach is now being developed by replacing the silkworm silk gene with synthetic spider silk DNA.

CRISPR (clustered regularly interspaced short palindromic repeats)/cas9 system, the next generation gene customization tool, is a new technique developed in the past couple of years. It is being utilized for gene-specific modification in this study, to allow more accurate and precise integration and replacement of the silkworm silk gene with a synthetic spider silk gene.

**Techniques**

The latest tool in genome editing – CRISPR/Cas9 – allows for specific genome disruption and replacement in a flexible and simple system resulting in high specificity and low toxicity. The CRISPR/Cas9 genome editing system requires the co-expression of a Cas9 protein with a guide RNA expressed from the U6 polymerase III promoter [4].

**Results**

Cas9-generated double-strand breaks (DSBs) in eGFP are repaired by non-homology end joining (NHEJ) and homology-based recombination (HR) in silkworm BmN cells *in vitro*. (A) (B) Flow cytometry data showed ~50% decrease in fluorescence due to CRISPR/cas9 cutting of eGFP. (C) PCR results confirmed the deletion of the fragment by double DSBs from two CRISPR/cas9 targeting sites in FibH. The white arrow points to the DNA bands without deletion, while the blue arrow points to the bands with deletions. (C) CRISPR/cas9 induced the deletion of FibH fragments which likely contributes to the observed weakened silks in cocoons.

**Conclusions and Future Work**

In this study, CRISPR/cas9 system, a recently developed gene editing tool, has been applied to cons the part of tract transgenic silkworms for spider silk production. The technique has shown its efficiency in *gene editing through NHEJ and HR in silkworm *in vitro* and *in vivo*. In the next step, proper donor DNAs will be constructed to allow the complete replacement of the repeat region of FibH with synthetic spider silk DNA. With this success, we are hoping to make a new generation of silkworms with spider silk in their cocoons.

**References**


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