

Biomimetic Manufacturing of Fibers

M98-C5

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Biotechnology provides the tools to clone and express designed synthetic protein fibers in simple organisms. Our objectives are

- to identify the fundamental molecular biology of clonal production of fiber forming protein polymers by genetic expression in yeast and plants,
- to understand arachnid biology of silk production to lay the foundation for the development of biomimetic protein fiber

We are exploiting recombinant DNA and plant transgenic technologies to create and produce novel protein polymers in significant quantities for fiber spinning.

spinning.

Spider dragline silk is spidroin, a strong, elastic, waterproof, stretchable, biodegradable, β -sheet natural protein polymer. The dragline silk of the spider *Nephila clavipes* is the archetype for study of these materials. We have used spidroin 1 and spidroin 2 oligonucleotide sequences to design synthetic genes corresponding to repeat units of these two spidroin genes. Our ultimate goal is to express these synthetic genes in transgenic organisms in order to obtain sufficient quantities of recombinant protein for fiber and film production. We are currently designing spinning technologies based on biological systems and are investigating the role that various protein primary structural components play in fiber production. We introduced one of the synthetic spidroin 2 genes in yeast for protein production.

To test the importance of the polyalanine repeats present in these spidroin proteins and their impact on the physical and mechanical properties of the resulting fiber, we are making several spidroin 1 gene constructs:

- Normal genes encoding for the normal spidroin 1 protein,
- Modified gene constructs encoding for proteins having no alanine runs, or less alanine runs than native spidroin 1.

The genes are sequenced and are being cloned into yeast for protein production and characterization.

We have also constructed a heteropolymer of spidroin 2 and collagen, using parts of the α -helical (Gly-X-Y) repeat from a nematode (*Meloidogyne incognita*) cuticle collagen. This synthetic collagen/spidroin gene was introduced and expressed in yeast. Large-scale production of the heteropolymer collagen-spidroin protein was done and the purified protein will be tested for its ability to form a fiber.

In order to express this fibrous protein on a more significant scale, we engineered this same gene in a plant vector for transgenics experiment. We completed the first several rounds of tobacco transformation using *Agrobacterium tumefaciens*, a

species of bacteria that is capable of incorporating foreign genes into the plant genome and are now selecting transgenic tissue. Once we have identified transgenic plants, we will examine expression at both the RNA and protein levels in various tissues. Fiber extracted from such plants has the potential to be used by the textile industry to make many types of products. In addition, this could create a new and much safer use for tobacco, allowing farmers to continue to cultivate a familiar crop.

Ultimately, we believe that plants that produce high protein seeds might provide a good system for high-level expression. To specifically target the synthetic protein production into the transgenic plant seeds, we decided to fuse these synthetic genes to a seed specific promoter (regulatory sequence) that we are currently characterizing in peanuts (peanut omega 9 desaturase gene promoter). We now have clones of this seed-enhanced promoter and are about to test them for promoter activity (GUS assay) in developing peanut seeds.

There is evidence that structural proteins exhibit *in vivo* self-assembly. For this reason, we are examining self-assembly processes in conjunction with the formation of spider silk. Examination of the biology of the *Nephila clavipes* spider and other spider species may discern the molecular events during spider silk spinning. Our studies in the complementary areas of self-assembly and spider biology will be used to further refine the design of our material production system.

We have begun a study of the piezoelectric properties of silk, including silk from *B. mori*. In conjunction with this work, we are conducting Raman and X-ray diffraction studies of silk; these structure and property studies are designed to help us better understand process-structure-property relationships in natural spider silks and biomimetic analogs. These studies will provide the foundation for engineering the genes to produce proteins specific to desired end uses.

Industry interactions: 2

Project Web Site Address:

<http://hubcap.clemson.edu/~ellison/Biomimetic%20Fibers/index.htm>

For Further Information

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