Human therapeutic proteins from silkworms

An age-old technology holds promise for recombinant protein production.

Florian M. Wurm

Chinese legend has it that almost 5,000 years ago, while Princess Xi Ling Shi was preparing a cup of tea, a silkworm cocoon from a mulberry tree fell into the hot water and its silk thread started unraveling. This episode might well be considered the starting point for an industry often regarded as one of the oldest biotech activities on our planet—the production of silk from the larval envelope of the silkworm *Bombyx mori*. In this issue, Tomita *et al.*[1] report the generation of transgenic silkworms that spin recombinant human type III procollagen as a component of the cocoon, an achievement with considerable implications for the mass production of recombinant proteins.

Bombyx mori has two silk glands that constitute approximately one-quarter of the worm's mass and produce liquid silk. This polymer is composed of a 350 kDa fibroin heavy chain (H-fibroin), a 25 kDa fibroin light chain (L-fibroin), and a family of proteins called sericins that bind the two threads together as they emerge from the glands and harden in contact with the air. The silk thread is pulled from the gland by a figure-eight movement of the worm's head and can attain a length of more than a kilometer.

Specialized cells at the posterior end of the silk glands devote 85% of their protein synthesis activity to silk production. The genome of these cells is amplified several thousandfold, resulting in a single nucleus that contains the equivalent of 400,000 haploid genomes[2]. The specific protein synthesis activity in the posterior gland cells is remarkable: a total of approximately 1,000 cells produce up to 300 mg of protein per gland in 4 days. This corresponds to a specific productivity of about 80 μg protein/cell/day. By comparison, the best recombinant high-yield mammalian cell culture systems only produce about 50 pg protein/cell/day[3].

The technology to produce silk has been optimized over hundreds of years and involves simple techniques that are easy to implement in poor and non-industrialized countries. Worldwide, some 60,000 tons of silk are produced annually in a process that converts a low-value substrate, the leaves of the mulberry tree, to a high-value protein-based product, silk.

The paper by Tomita *et al.* is the first report to show stable, long-term expression of a human recombinant protein in the silk glands of *B. mori* larvae. Previously, germline transmission and silk-gland expression of a chimeric L-fibroin–green fluorescent protein (GFP) gene, integrated into the L-fibroin gene by homologous recombination, had been demonstrated following gene delivery with a recombinant baculovirus[4]. Here, Tomita *et al.* approach the challenge from a different angle by taking advantage of recent developments in insect transgenesis. Vectors derived from the transposable element *piggyBac*, originally discovered in the lepidopteran *Trichoplusia ni*[5], allowed the first, and surprisingly efficient germline transformation of the lepidopteran *B. mori* by a transposon-based method[6]. Earlier attempts to transform lepidopterans with transposons from dipterans, the only insect transposons available, had failed.

The authors use the *piggyBac* vector to express a chimeric protein comprised of L-fibroin, human type III procollagen with an internal deletion, and GFP under the control of the silk gland–specific L-fibroin promoter[1]. As a screening marker, the researchers also included a red fluorescent protein (DsRed) gene under the control of an eye and nervous tissue–specific promoter[6]. They injected embryos with the vector, mated mature moths, and detected successful germline transfer to the offspring by the occurrence of red fluorescence in the ocelli (small larval eyes) early in embryo development. Expression of the chimeric gene was confirmed by the presence of green fluorescence in the silk glands and cocoons (see Fig. 1).

Recombinant protein production remains a major bottleneck in the biotechnology industry. Although significant progress has been made with all available expression

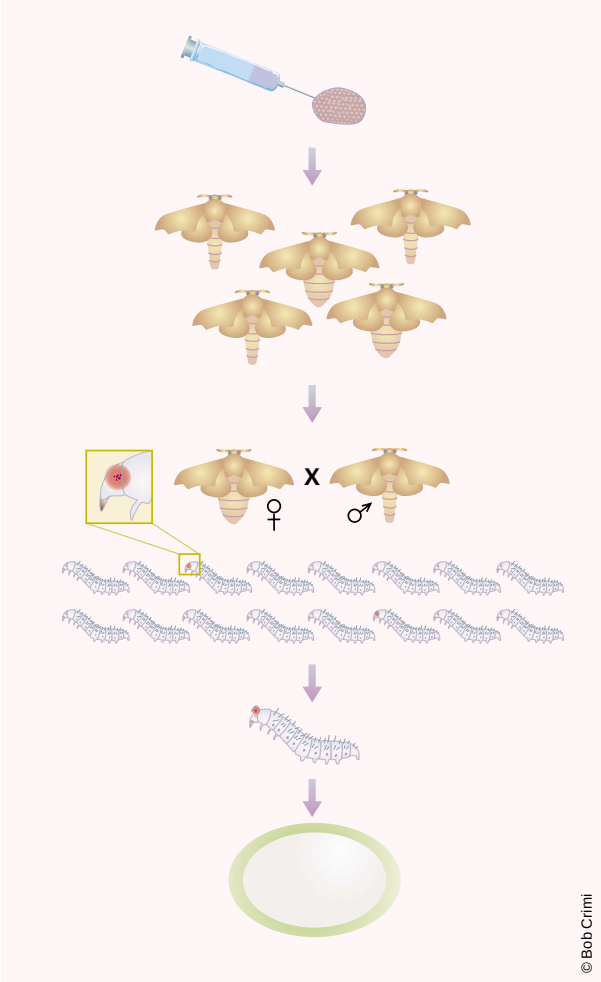

Figure 1. Transposon-mediated germline transformation in *B. mori*. A vector derived from a lepidopteran transposon is generated to deliver, by injection into early embryos, a fusion gene for a chimeric silk protein joined to human procollagen sequences and to green fluorescent protein under the control of a silk gland–specific promoter. The vector also carries the red fluorescent protein gene driven by an eye-specific promoter for use as a screening marker. The resulting adult moths are mated, and their larvae screened for germline transmission of the recombinant genes. Identification of germline transgenesis is facilitated by the appearance of red fluorescence in the ocelli of early-stage larvae; late-stage larvae with high productivity for human collagen sequences are identified by the green fluorescence of the cocoon (Art after a design by Daniele Fraboulet).

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technologies (prokaryotes, yeast, cultivated mammalian cells, and transgenic animals and plants), production yields are still insufficient for a large number of proteins. For example, 1.2 tons of human serum albumin are needed annually in a small country like Switzerland. Producing this amount requires the processing of 50,000 liters of human plasma.

Other proteins needed in large quantities include collagen and thrombin as well as therapeutics, such as recombinant antibodies or antibody-like molecules that have attained annual market demands of several hundred kilograms, if not tons. Attaining a sufficient supply of some of these drugs, which are produced in mammalian cells at facilities with reactors of 10,000 or more liters, is becoming a major concern for the pharmaceutical industry in general and for biotech companies in particular. Worldwide, only a small number of facilities with such capabilities exists, and the construction, validation, and final approval of new production facilities take many years and require substantial investments in capital and human resources.

It is obvious that the protein production capacity of silkworms exceeds that of any other industrial system in use today. In addition, the glands of these animals produce an almost pure product. Purification of recombinant protein from cocoons seems to be a rather simple process despite silk-fibroin synthesis being maintained in the insect. The authors indicate that even with the "low" yields reported, 5 kg of pure collagen can be produced on a surface area of 300 m^2 with five workers caring for 1.5 million silkworms.

Concerns arising from potential contamination by adventitious agents (such as viruses or prion) of recombinant proteins obtained from transgenic animals or cultured mammalian cells are substantially less, if not totally absent, in the case of insect-derived products for human use. The only remaining issue is protein quality. Constitutively, insects are capable of high-mannose protein glycosylation[7], but recent papers have shown that the expression of mammalian glycosyltransferase genes in insect cells can broaden their capacity for glycosylation[8,9].

In conclusion, systems based on *B. mori* could become a major technology for the production of high-value proteins, especially in the area of pharmaceutical bulk production. By relying on know-how accumulated over thousands of years of rearing silkworms, the implementation of a low-cost, high-yield protein "spinning" production system should be straightforward.

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Antibody jabs for plant enzymes

Potatoes expressing single-domain antibodies in their plastids inhibit a starch-branching enzyme and produce high-amylose starch.

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Metabolic engineering focuses on developing plant varieties with greater yields of specific products (such as carbohydrates, proteins, or oils), improved tolerance to environmental stress, or higher resistance to attack by pathogens. Modulation of *in vivo* enzyme activities plays an essential role in these strategies, which aim to direct metabolic flux toward key biochemical pathways[1]. Immunomodulation is a technique that can be used to manipulate cellular metabolism, signal transduction, or pathogen infectivity by ectopic expression of genes encoding specific antibodies or antibody fragments that can alter the function of targeted molecules. In this issue, Jobling *et al*.[2] describe the first application of immunomodulation to efficiently inhibit enzyme activity in transgenic plants.

Enzyme function in plants has traditionally been modulated using approaches such as mutagenesis or homology-dependent gene silencing. In the former approach, enzyme activity is modulated through an alteration in the function of regulatory or structural genes that have been disrupted by mutagenesis. In the latter, activity is modulated by the plant's adaptive defense mechanism against viruses and transposable elements, which silences cellular genes in response to the introduction of homologous exogenous transgenes (Fig. 1).

Because many cellular functions are performed by enzymes encoded by small or large gene families, mutagenesis has proven of limited utility in modulating enzyme activity. Often, mutagenesis of a gene will not result in a loss-of-function mutant because the presence of other genes with redundant function compensate for disruption of the gene of interest. In contrast, gene silencing via antisense, sense, or double-stranded RNA interference (dsRNAi) can precisely target one or more genes of interest, making it the preferred method of downregulating enzymes and their pathways (Fig. 1). Homology-dependent gene silencing not only is more targeted, but also allows the time- and organ-specific blockage of gene products through the selection of appropriate promoter sequences[3].

Now, Jobling *et al*. have taken an entirely different approach, demonstrating the first successful application of immunomodulation for the manipulation of a plant metabolic pathway—in this case, starch biosynthesis (see Fig.1). Their paper builds on previous work in which immunomodulation was successfully applied to study phytohormone functions and to improve pathogen resistance in transgenic plants[4].

Earlier antisense studies of starch biosynthesis had shown that synthesis of high-amylose potato starch requires simultaneous inhibition of starch-branching enzymes A and B (SBE A and SBE B). Jobling *et al.* set about determining whether an anti-SBE A single-domain antibody targeted to the plastids of transgenic potato plants deficient in SBE B could achieve the same effect. They found that immunomodulation increased the amylose content of starch granules from about 20% (the wild-type level) to up to 74%, exceeding the levels of amylose achieved by conventional antisense technologies.

A critical step in the work was the production of a single-chain antibody that could successfully target and penetrate an enzyme active site to achieve immunomod-

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