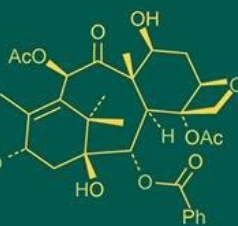
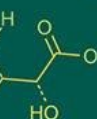


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(3): 549-560
www.biochemjournal.com
Received: 13-01-2026
Accepted: 17-02-2026

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Biotechnological advances in Silkworm (*Bombyx mori*) research: From genomics to silk biomaterials

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i3g.7921>

Abstract

The domesticated silkworm, *Bombyx mori*, has transitioned from its traditional role in sericulture to a powerful and versatile platform in modern biotechnology. This review critically synthesizes the monumental progress in silkworm research, charting its evolution from a genomic model to a source of advanced biomaterials. The completion of the silkworm genome has catalyzed a cascade of innovations, providing foundational resources for functional genomics, transcriptomics and proteomics. These multi-omics approaches have unraveled the complex molecular machinery governing silk protein synthesis, developmental biology and immune responses. We discuss the transformative impact of genetic engineering technologies, including the pioneering *piggyBac* transposon system and the revolutionary CRISPR-Cas9 platform, which have enabled precise genome editing for enhancing traits such as disease resistance and silk yield. Furthermore, these tools have repurposed the silkworm silk gland into a highly efficient bioreactor for the large-scale production of valuable recombinant proteins, including pharmaceuticals and vaccines. The unique biochemical and mechanical properties of silk proteins, particularly fibroin, have been harnessed to fabricate a new generation of biomaterials. We explore the burgeoning applications of silk fibroin in tissue engineering scaffolds, controlled drug delivery systems and biodegradable medical implants. The development of silk-based nanoparticles, hydrogels and Electrospun nanofibers further expands their utility in nanotechnology and regenerative medicine. This review concludes by addressing the current challenges, including scalability and regulatory hurdles and outlines future prospects, such as the integration of synthetic biology to design smart silk biomaterials and advance precision sericulture, underscoring the silkworm's enduring significance in science and industry.

Keywords: *Bombyx mori*, silkworm genomics, CRISPR-Cas9, silk fibroin, sericulture biotechnology, biomaterials, recombinant protein and tissue engineering

1. Introduction

The domesticated silkworm, *Bombyx mori*, represents one of the most profound examples of human-animal co-evolution, a partnership forged over 5,000 years ago for the sole purpose of producing silk (Xiang *et al.*, 2018) [79]. This lepidopteran insect, entirely dependent on human care for its survival, has been the cornerstone of the global sericulture industry, producing the “queen of fabrics” prized for its unparalleled luster, texture and strength. The economic and cultural significance of silk is immense, having shaped trade routes, driven economies and defined luxury for millennia. However, beyond its traditional role, *B. mori* has emerged in the 21st century as a formidable model organism and a versatile biotechnological workhorse. Its large size, well-characterized genetics and remarkable protein synthesis capacity make it an ideal system for fundamental research and applied science (Goldsmith *et al.*, 2005) [26].

The transition of the silkworm from an agricultural commodity to a sophisticated biotechnological tool was catalyzed by the advent of molecular biology. Classical breeding, while successful in improving traits like cocoon size and silk yield over centuries, faced limitations in introducing novel functionalities or overcoming complex challenges such as viral diseases. The development of molecular tools provided an unprecedented opportunity to understand and manipulate the silkworm's biology at the genetic level. This paradigm shift began with the establishment of foundational genomic resources. The publication of the draft genome sequence of *B. mori* by two independent groups in 2004 marked a watershed moment,

providing a comprehensive blueprint of its genetic makeup (Mita *et al.*, 2004; Xia *et al.*, 2004) [56, 81]. This initial effort laid the groundwork for a deeper exploration of the genes controlling every aspect of silkworm physiology, from development and metabolism to immunity and, most critically, silk production.

The availability of the genome sequence unlocked the potential for functional genomics. Researchers could now systematically identify and characterize the genes responsible for the synthesis of the two main silk proteins: the core structural protein, fibroin and the adhesive gum-like protein, sericin. High-throughput technologies such as microarrays and, more recently, RNA sequencing (RNA-seq) have enabled detailed transcriptomic profiling of various tissues, particularly the silk gland, revealing the intricate regulatory networks that orchestrate the massive production of silk proteins (Xia *et al.*, 2007; Ma *et al.*, 2022) [82, 53]. These studies have not only elucidated the roles of specific transcription factors and signaling pathways but have also provided a rich dataset for identifying targets for genetic improvement.

Perhaps the most transformative development in silkworm biotechnology has been the ability to directly engineer its genome. The establishment of a stable germline transformation system using the piggyBac transposon was a major breakthrough, allowing for the insertion of foreign genes into the silkworm genome for the first time (Tamura *et al.*, 2000) [67]. This technology paved the way for creating transgenic silkworms with novel traits. The subsequent development of the GAL4/UAS binary expression system provided a method for targeted, tissue-specific gene expression, enabling precise control over transgene activity (Imamura *et al.*, 2003) [32]. More recently, the advent of genome editing technologies, most notably the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system, has revolutionized the field. CRISPR-Cas9 allows for highly efficient and precise targeted mutagenesis, enabling researchers to knockout, modify, or insert genes with unprecedented ease (Wang *et al.*, 2013) [72]. These powerful tools have been applied to enhance disease resistance, improve silk quality and yield and, most excitingly, to repurpose the silkworm as a living bioreactor for producing valuable recombinant proteins.

The silk gland of *B. mori* is a protein synthesis factory of extraordinary efficiency, capable of producing grams of silk protein in a matter of days. By hijacking this machinery, scientists have successfully produced a wide range of complex proteins, from pharmaceutical antibodies and vaccines to industrial enzymes, within the silk cocoon (Ogawa *et al.*, 2007; Chen *et al.*, 2024) [57, 11]. Concurrently, the silk proteins themselves have become the focus of intense research in materials science and biomedical engineering. Silk fibroin, the primary structural component, possesses a unique combination of biocompatibility, biodegradability and remarkable mechanical strength. These properties make it an ideal material for a host of biomedical applications, including tissue engineering scaffolds that support cell growth, drug delivery vehicles that provide sustained release of therapeutics and biodegradable implants for bone and cartilage repair (Kundu *et al.*, 2013) [42]. This review will explore these multifaceted biotechnological advances, tracing the journey of the silkworm from its genomic foundations to its cutting-edge applications in creating novel biomaterials, thereby highlighting its pivotal and expanding role in modern science and technology.

2. Silkworm Genome Sequencing and Genomic Resources

The journey into the molecular era of silkworm research began in earnest with the monumental effort to sequence its genome. This endeavor culminated in 2004 with the independent publication of two draft genome sequences by Chinese and Japanese consortia, providing the first comprehensive look at the genetic blueprint of *B. mori* (Mita *et al.*, 2004; Xia *et al.*, 2004) [56, 81]. These initial assemblies, generated using whole-genome shotgun sequencing, were a landmark achievement for lepidopteran genomics. In 2008, these two datasets were merged and integrated with new BAC-end and fosmid-end sequences by the International Silkworm Genome Consortium, resulting in a significantly improved assembly with an 8.5-fold coverage of the estimated 432 Mb genome. This collaborative effort successfully anchored 87% of the scaffold sequences to the 28 silkworm chromosomes using a high-density single nucleotide polymorphism (SNP) linkage map, providing a robust framework for genetic and genomic studies (The International Silkworm Genome Consortium, 2008) [70].

A distinguishing feature of the *B. mori* genome is its high content of repetitive sequences, estimated to be around 43.6%, which are predominantly composed of transposable elements (The International Silkworm Genome Consortium, 2008) [70]. This characteristic presented significant challenges for assembly and annotation. Despite these hurdles, the 2008 assembly predicted 14,623 gene models, offering initial insights into the genetic basis of key silkworm traits. Subsequent advances in sequencing technology have led to even higher-quality genome assemblies. The use of long-read sequencing technologies, such as PacBio, has been instrumental in resolving complex repetitive regions and closing gaps. A high-quality assembly of the p50T strain was published in 2019, leveraging PacBio long reads to achieve a scaffold N50 of over 16 Mb and providing a more contiguous and accurate reference (Kawamoto *et al.*, 2019) [36]. More recently, a near-complete, telomere-to-telomere (T₂T) assembly of the improved Japanese strain Nichi01 was reported, integrating PacBio Sequel II long reads with a high-density genetic linkage map. This “gold-standard” assembly corrected several mis assemblies in previous versions and provided gap-free sequences for 18 of the 28 chromosomes, setting a new benchmark for accuracy and completeness in silkworm genomics (Kiuchi *et al.*, 2022) [38].

The proliferation of genomic data has been accompanied by the development of specialized databases that serve as critical resources for the research community. SilkDB was one of the first dedicated platforms, providing access to the genome sequence, gene models and expression data (Duan *et al.*, 2010) [21]. KAIKObase, another major resource, offers an integrated platform with a genome browser, BLAST search tools and linkage map information and has been continuously updated with the latest assemblies, including the Nichi01 genome (Yang *et al.*, 2021) [85]. SilkBase provides an integrated database for transcriptomic and genomic resources, facilitating comparative analyses across different strains and species (Arunkumar *et al.*, 2022) [3]. These databases are indispensable for gene annotation, functional prediction and comparative genomics. The initial gene prediction of 14,623 models in 2008 has been refined and expanded, with the Nichi01 assembly, for instance, predicting 18,397 coding genes based on extensive mRNA-

seq and protein sequence data (Kiuchi *et al.*, 2022)^[38]. Comparative genomics has further enriched our understanding of silkworm biology. The availability of a high-quality reference genome has enabled large-scale resequencing projects, such as the analysis of 40 domesticated and wild silkworm genomes, which provided a detailed map of genetic variation and shed light on the genetic changes that occurred during domestication (Xia *et al.*, 2009)^[83]. More recently, a massive pan-genome study involving the deep re-sequencing of 1,078 silkworms and long-read assembly of 545 representative genomes has

created a high-resolution pan-genome dataset. This resource identified over 7,000 new genes and millions of structural variations, providing unprecedented insights into the genetic basis of artificial selection, ecological adaptation and key economic traits like silk yield and fineness (Tong *et al.*, 2022)^[71]. These comprehensive genomic resources have firmly established *B. mori* as a premier model for lepidopteran genetics and have provided the essential foundation for all subsequent biotechnological advancements.

Table 1: Genomic resources and databases for *Bombyx mori*.

Resource/Database	Description	Key Features	Primary Reference
Initial Draft Genomes	First whole-genome shotgun sequences of the p50 strain.	~3-6x coverage; established the foundation for silkworm genomics.	Xia <i>et al.</i> (2004) ^[79] ; Mita <i>et al.</i> (2004) ^[56]
ISGC Assembly	Merged and improved assembly from the International Silkworm Genome Consortium.	8.5x coverage; 432 Mb genome size; 14,623 predicted genes; 87% of scaffolds anchored to 28 chromosomes.	The International Silkworm Genome Consortium (2008) ^[70]
p50T High-Quality Assembly	Assembly using PacBio long-read sequencing technology.	Highly contiguous with scaffold N50 > 16 Mb; resolved many repetitive regions.	Kawamoto <i>et al.</i> (2019) ^[36]
Nichi01 T2T Assembly	Chromosome-scale, near-complete assembly of an improved Japanese strain.	18 telomere-to-telomere (T2T) gap-free chromosomes; corrected previous mis assemblies; QV score > 50.	Kiuchi <i>et al.</i> (2022) ^[38]
Silkworm Pan-Genome	High-resolution pan-genome from re-sequencing 1,078 individuals.	Identified >7,000 new genes and >3.4 million structural variations; insights into domestication and adaptation.	Tong <i>et al.</i> (2022) ^[71]
SilkDB	A dedicated platform for silkworm genome biology.	Provides genome browser, gene models, expression data and analysis tools.	Duan <i>et al.</i> (2010) ^[21]
KAIKObase	An integrated silkworm genome database.	Hosts multiple genome assemblies (including Nichi01), annotation data, BLAST services and linkage maps.	Yang <i>et al.</i> (2021) ^[85]
SilkBase	An integrated database for transcriptomic and genomic resources.	Facilitates comparative analysis of gene expression and structure across silkworm species.	Arunkumar <i>et al.</i> (2022) ^[3]

3. Functional Genomics and Gene Expression Studies

With a robust genomic framework in place, the focus of silkworm research shifted towards functional genomics—understanding how the genetic code translates into biological function. High-throughput transcriptomic technologies, particularly microarray analysis and RNA sequencing (RNA-seq), have been pivotal in this endeavor. An early landmark study utilized a whole-genome oligonucleotide microarray to create comprehensive gene expression profiles across multiple tissues, including the silk gland, fat body, midgut and gonads, at various developmental stages (Xia *et al.*, 2007)^[82]. This work provided the first global view of tissue-specific and developmentally regulated gene expression in *B. mori*, revealing large clusters of genes with coordinated expression patterns related to specific biological processes. For example, it highlighted the massive upregulation of cuticular protein genes during molting stages, reflecting the dynamic process of exoskeleton remodeling (Liang *et al.*, 2010)^[48].

The silk gland, as the organ of primary economic importance, has been the subject of intense transcriptomic investigation. This highly specialized organ is anatomically and functionally divided into three regions: the posterior silk gland (PSG), which synthesizes the core silk protein fibroin; the middle silk gland (MSG), which produces the adhesive sericin proteins; and the anterior silk gland (ASG), which acts as a duct for spinning. RNA-seq analyses comparing these regions have revealed distinct transcriptional landscapes that mirror their specialized functions (Chang *et al.*, 2015; Wang *et al.*, 2019)^[9, 74]. The PSG shows massive expression of the genes encoding fibroin heavy chain (fib-

H), fibroin light chain (fib-L) and fibrohexamerin (P25). In contrast, the MSG is dominated by transcripts for the sericin family genes (Ser1, Ser2, Ser3). These studies also identified numerous transcription factors, signaling molecules and metabolic enzymes that are specifically enriched in each region, providing a rich list of candidate genes involved in regulating silk synthesis and secretion (Kimoto *et al.*, 2022)^[37].

The recent application of single-cell RNA sequencing (scRNA-seq) has provided an even more granular view of the silk gland's cellular heterogeneity. A comprehensive cell atlas of the developing silk gland identified 10 distinct cell types, each with unique marker genes and functions (Ma *et al.*, 2022)^[53]. This study not only confirmed the regional specialization of fibroin- and sericin-producing cells but also uncovered novel cell populations involved in processes like chitin metabolism, epithelial remodeling and stress responses. For instance, it identified specific cell clusters in the PSG (FPSs, DRRs, FPCs) responsible for fibroin synthesis and others in the MSG (SPSs, SPCs) for sericin synthesis, clarifying the cellular basis of silk production. Such high-resolution data is invaluable for understanding the precise molecular mechanisms of silk gland development and function.

Proteomic studies have complemented transcriptomic data by directly analyzing the protein composition of the silk gland and cocoon. Two-dimensional electrophoresis and mass spectrometry have been used to identify hundreds of proteins in the different silk gland compartments, confirming the high abundance of silk proteins and also revealing a complex array of chaperones, enzymes and cytoskeletal proteins involved in the synthesis, folding and

secretion process (Hou *et al.*, 2007; Li *et al.*, 2015) [31, 46]. Comparative proteomics of cocoon layers have further detailed the distribution of different sericin and non-silk proteins, which contribute to the cocoon's structural integrity and protective functions (Zhang *et al.*, 2015) [87]. Together, these functional genomics and proteomics approaches have built a detailed molecular picture of the silkworm, particularly its unparalleled silk production machinery, providing numerous targets for biotechnological manipulation to improve silk properties and yield.

4. Genetic Engineering and Genome Editing in Silkworm

The ability to manipulate the silkworm genome has been the cornerstone of its transformation into a modern biotechnological platform. The journey began with the development of reliable methods for introducing foreign DNA into the germline, leading to stable, heritable genetic modifications. The most significant breakthrough in this area was the successful application of the piggyBac transposon system for silkworm transformation (Tamura *et al.*, 2000) [67]. piggyBac, a transposable element originally discovered in the lepidopteran insect *Trichoplusia ni*, proved to be highly effective in *B. mori*. The system typically involves co-injecting a “helper” plasmid expressing the piggyBac transposase enzyme and a “donor” plasmid containing the gene of interest flanked by piggyBac's inverted terminal repeats into pre-blastoderm embryos. The transposase recognizes these repeats, excises the intervening DNA cassette and integrates it into the host genome at TTAA sequences. This method achieved stable germline transformation with efficiencies around 2-12.5%, enabling the creation of the first transgenic silkworms expressing reporter genes like Green Fluorescent Protein (GFP) (Tamura *et al.*, 2000; Li *et al.*, 2015) [67, 45]. The piggyBac system remains a workhorse in the field, widely used for generating transgenic lines for both basic research and applied purposes.

To achieve more precise control over transgene expression, researchers adapted the GAL4/UAS binary system from *Drosophila* for use in the silkworm (Imamura *et al.*, 2003) [32]. This powerful system involves two separate transgenic lines: a “driver” line that expresses the yeast transcription factor GAL4 under the control of a tissue-specific or developmental stage-specific promoter and a “responder” line that contains the gene of interest downstream of an Upstream Activating Sequence (UAS), the DNA element to which GAL4 binds. When these two lines are crossed, the progeny express the target gene only in the cells where GAL4 is present. This approach has been successfully used to drive expression specifically in the silk gland using promoters from the fibroin or sericin genes,

providing a robust method for producing recombinant proteins in a targeted manner (Tatematsu *et al.*, 2010; Imamura *et al.*, 2003) [69, 32].

Another key technology for functional genomics is RNA interference (RNAi), a mechanism for post-transcriptional gene silencing. In silkworms, RNAi has been successfully induced by microinjecting double-stranded RNA (dsRNA) or short interfering RNA (siRNA) into embryos or larvae (Quan *et al.*, 2002; Yamaguchi *et al.*, 2011) [60, 80]. This technique has been invaluable for knocking down gene expression to study gene function. However, RNAi efficiency in lepidopterans, including silkworms, can be variable and often transient. To address this, researchers have developed transgenic approaches to enhance or stabilize the RNAi effect, such as overexpressing core RNAi machinery components like Argonaute2 (BmAgo2), which significantly improved silencing efficiency in larvae (Li *et al.*, 2015) [45]. More recently, efforts have focused on developing oral delivery methods for dsRNA, for example, by encapsulating it in chitosan nanoparticles to protect it from degradation in the insect gut, which could pave the way for large-scale, non-invasive applications (Liu *et al.*, 2025) [51].

The advent of the CRISPR-Cas9 system has revolutionized genome editing in almost all organisms and the silkworm is no exception. The first successful application in *B. mori* was reported in 2013, demonstrating that co-injection of Cas9 mRNA and a single guide RNA (sgRNA) into embryos could induce targeted mutations with high efficiency (Wang *et al.*, 2013) [72]. This system has proven to be remarkably robust, achieving mutation frequencies of up to 35% in the injected generation (G0) and demonstrating heritable germline transmission (Ma *et al.*, 2014) [50]. A key advantage of CRISPR-Cas9 is its capacity for multiplexing, allowing for the simultaneous targeting of multiple genes by simply injecting a cocktail of different sgRNAs (Liu *et al.*, 2014) [49]. This has greatly accelerated functional genetic studies. CRISPR-Cas9 has been widely applied for various purposes, from basic research, such as knocking out genes to study their roles in development and metabolism (Li *et al.*, 2015; Zhang *et al.*, 2017) [47, 89], to applied biotechnology. For instance, it has been used to engineer resistance to devastating pathogens like the *B. mori* nucleopolyhedrovirus (BmNPV) by targeting essential viral genes or host susceptibility factors (Chen *et al.*, 2017; Dong *et al.*, 2019) [10, 20]. It has also been used to modify silk properties by editing the silk protein genes themselves or to create “empty” silk glands by knocking out the fibroin heavy-chain gene, turning the gland into an even more efficient bioreactor for producing purely recombinant proteins (Ma *et al.*, 2014; Zhang *et al.*, 2019) [51, 88].

Table 2: Gene editing and expression technologies used in silkworm biotechnology.

Technology	Mechanism	Key Application(s)	Landmark Reference
<i>piggyBac</i> Transposon System	Transposase-mediated integration of a DNA cassette into the genome at TTAA sites.	Stable germline transformation; creation of transgenic silkworms expressing foreign genes.	Tamura <i>et al.</i> (2000) [67]
GAL4/UAS System	Binary system where a tissue-specific promoter drives GAL4, which in turn activates a target gene via a UAS element.	Spatially and temporally controlled gene expression, especially in the silk gland.	Imamura <i>et al.</i> (2003) [32]
RNA Interference (RNAi)	Post-transcriptional gene silencing induced by dsRNA or siRNA.	Functional genomics (gene knockdown); potential for pest and disease control.	Quan <i>et al.</i> (2002) [60]; Li <i>et al.</i> (2015) [31]
CRISPR-Cas9 System	sgRNA-guided Cas9 nuclease creates a double-strand break at a specific genomic locus, leading to mutations via NHEJ or HDR.	Highly efficient and heritable gene knockout, knock-in and multiplex editing for functional analysis, trait improvement and bioreactor development.	Wang <i>et al.</i> (2013) [72]
TALENs	Transcription Activator-Like Effector Nucleases; engineered DNA-binding proteins fused to a nuclease domain create double-strand breaks.	Targeted gene knockout and knock-in; used for creating spider silk-producing silkworms.	Ma <i>et al.</i> (2018) [82]

5. Silkworm as a Bioreactor for Recombinant Protein Production

One of the most compelling applications of transgenic silkworm technology is its use as a living bioreactor for the production of valuable recombinant proteins. The silk gland of *B. mori* is a natural protein synthesis powerhouse, evolved to produce grams of silk protein per larva. By harnessing this highly efficient machinery, researchers can achieve large-scale, cost-effective production of complex proteins that require eukaryotic post-translational modifications, such as glycosylation and disulfide bond formation, which are often challenging to achieve in microbial systems like *E. coli* (Tatabe *et al.*, 2010) [69]. The silkworm expression system offers several advantages over traditional mammalian cell culture, including lower production costs, inherent safety (as it is not a host for human viruses) and simpler scalability by simply rearing more larvae.

The primary strategy for using the silkworm as a bioreactor involves driving the expression of a target protein in the silk gland, leading to its secretion and storage within the cocoon. This is typically achieved using the GAL4/UAS system or by directly fusing a strong, silk gland-specific promoter, such as that of the fibroin or sericin genes, to the gene of interest in a piggyBac-based vector. An early approach involved creating fusion proteins where the recombinant protein was linked to one of the native silk proteins, such as fibroin heavy-chain. While this ensured incorporation into the silk fiber, recovering the target protein often required harsh chemical treatments that could denature it (Tomita *et al.*, 2003) [71]. A more refined and widely adopted method is to express the recombinant protein as an independent entity that is secreted into the lumen of the middle silk gland (MSG) and incorporated into the soluble sericin layer of the cocoon. This allows for much gentler extraction conditions, typically using simple aqueous buffers, which helps preserve the protein's structure and function. Using this strategy, researchers successfully produced recombinant human serum albumin (rHSA) in the sericin layer, yielding a protein that was conformationally and functionally identical to native HSA (Ogawa *et al.*, 2007) [57].

The silkworm bioreactor has proven particularly adept at producing complex pharmaceutical proteins and vaccine antigens. For example, human type III procollagen has been successfully produced in cocoons, offering a potential source for biomedical materials (Adachi *et al.*, 2006) [1]. More recently, the platform has been leveraged for rapid vaccine development. In response to the COVID-19 pandemic, researchers used the silkworm baculovirus expression system to produce two prototype SARS-CoV-2 vaccines: a virus-like particle (VLP) vaccine and a recombinant baculovirus vector vaccine displaying the viral capsid protein. Both vaccine candidates were shown to elicit robust humoral and cellular immune responses in mice, demonstrating the immense potential of the silkworm system for rapid and scalable vaccine manufacturing (Chen *et al.*, 2024) [11]. The ability to produce properly folded and assembled multi-subunit proteins and VLPs highlights the superiority of the silkworm system for complex biologics. By optimizing expression cassettes, including the choice of promoter, signal peptide and intron sequences, production yields have been dramatically increased, with reports of up to 500 µg of protein per larva, making the silkworm a

commercially viable and attractive alternative to conventional bioreactor systems (Tatematsu *et al.*, 2010) [69].

6. Molecular Biology of Silk Proteins

The remarkable properties of silk fiber originate from the unique molecular architecture of its constituent proteins, primarily fibroin and sericin, which are synthesized in distinct compartments of the silk gland. Understanding the molecular biology of these proteins and their genes is fundamental to any biotechnological effort to modify silk. The core of the silk fiber is composed of fibroin, a complex of three proteins: the fibroin heavy chain (Fib-H), the fibroin light chain (Fib-L) and a glycoprotein called fibrohexamerin (P25, previously known as Fhx). These are encoded by the fib-H, fib-L and P25 genes, respectively, which are expressed exclusively in the posterior silk gland (PSG). Fib-H is a massive protein (~390 kDa) characterized by a highly repetitive sequence of Gly-Ala-Gly-Ala-Gly-Ser motifs that form crystalline β -sheet structures, conferring strength and toughness to the fiber. Fib-L (~26 kDa) is linked to Fib-H via a single disulfide bond and is crucial for the secretion of the fibroin complex from the PSG cells (Inoue *et al.*, 2000; Tanaka *et al.*, 1999) [33, 68]. P25 (~30 kDa) is a non-covalently associated protein believed to play a role in stabilizing the fibroin hexameric unit (Fib-H)₆(Fib-L)₆(P25)₁ within the silk gland lumen (Inoue *et al.*, 2000) [33].

Surrounding the fibroin core is a layer of sericin proteins, which act as a biological glue, holding the two fibroin filaments together and forming the cocoon structure. Sericins are a family of hydrophilic proteins encoded by three main genes, Ser1, Ser2 and Ser3, which are expressed in the middle silk gland (MSG). The Ser1 gene, expressed in the middle and posterior parts of the MSG, is the major producer of sericin and generates multiple protein isoforms through alternative splicing (Garel *et al.*, 1997) [24]. The Ser2 gene is expressed in the anterior part of the MSG and encodes an adhesive protein critical for cocoon integrity (Kludkiewicz *et al.*, 2009) [39]. The Ser3 gene, also expressed in the anterior MSG, contributes a smaller fraction of the total sericin (Takasu *et al.*, 2007) [66]. The high serine content of these proteins makes them highly hydrophilic and soluble in the silk gland, but they become insoluble upon spinning.

The synthesis and assembly of silk is a masterclass in biological engineering. Within the PSG, fibroin molecules are synthesized and secreted into the lumen, where they are stored at an incredibly high concentration (~30% w/v) in a liquid crystalline state, prevented from premature aggregation by the specific ionic environment and the presence of sericin. As this fibroin “dope” is transported through the MSG, it is coated with layers of sericin. The entire silk solution then moves into the narrow anterior silk gland (ASG) and through the spinneret. The process of silk fiber formation, or spinning, is triggered by a combination of physical and chemical cues. The shear forces generated as the liquid silk is pulled through the progressively narrowing duct, coupled with a decrease in pH and changes in metallic ion concentrations (particularly Ca²⁺ and K⁺), induce a conformational change in the fibroin molecules from a soluble random coil/alpha-helix state to insoluble, stable β -sheets (He *et al.*, 2012; Zhou *et al.*, 2021) [28, 91]. This rapid, controlled phase transition results in the formation of the

strong, water-insoluble silk fiber. The intricate regulation of this process, from gene transcription in the silk gland to the final physical extrusion, is governed by a complex interplay of transcription factors (e.g., SGF-1, Sage, Antp), hormones (e.g., 20-hydroxyecdysone) and the unique physiological environment of the silk gland (Tsubota *et al.*, 2016; Kokubo *et al.*, 2003)^[72, 41].

7. Biotechnology of Silk Biomaterials

The unique combination of properties inherent in silk fibroin-outstanding mechanical strength, toughness, biocompatibility, tunable biodegradability and aqueous processability-has positioned it as a premier biomaterial for a vast array of biomedical applications (Kundu *et al.*, 2013)^[42]. Once stripped of the immunogenic sericin protein, the remaining fibroin can be processed into various formats, including films, fibers, hydrogels and porous scaffolds, making it exceptionally versatile for tissue engineering and regenerative medicine. The ability of silk fibroin to support cell adhesion, proliferation and differentiation without eliciting a significant inflammatory response has made it a material of choice for creating scaffolds that mimic the native extracellular matrix (ECM) and guide tissue regeneration (Altman *et al.*, 2003)^[2].

In the field of bone tissue engineering, silk fibroin scaffolds have shown immense promise. Porous 3D scaffolds, often fabricated by methods like freeze-drying or salt-leaching, provide a supportive microenvironment for osteoblasts and mesenchymal stem cells (MSCs). The mechanical properties of these scaffolds can be tuned to match those of native bone. To enhance their osteoinductive potential, silk scaffolds are frequently composited with bioactive materials like hydroxyapatite (nHAp), the primary mineral component of bone (Ko *et al.*, 2017)^[40]. Studies have shown that silk/nHAp composite scaffolds promote the osteogenic differentiation of MSCs, evidenced by increased alkaline phosphatase (ALP) activity and mineralization and can accelerate bone regeneration in animal models of critical-sized bone defects (Wu *et al.*, 2019; Yan *et al.*, 2019)^[75, 84]. Injectable silk hydrogels, which can be delivered in a minimally invasive manner and conform to irregular defect shapes, have also been developed for bone repair, often loaded with growth factors like BMP-2 to further stimulate osteogenesis (Zou *et al.*, 2023)^[92].

Silk biomaterials are also transforming wound healing and skin regeneration. Silk fibroin-based dressings, in the form of films, hydrogels, or Electrospun nanofiber mats, create a moist, protective environment over the wound bed that facilitates healing. They are permeable to oxygen but protect against bacterial invasion. More than just a passive barrier, silk fibroin actively promotes healing by supporting the migration and proliferation of keratinocytes and fibroblasts (Chouhan and Mandal, 2020)^[13]. Advanced silk dressings are being developed with incorporated therapeutic agents. For example, silk hydrogels loaded with antimicrobial agents like silver nanoparticles or antibiotics can prevent infection, a major complication in chronic wounds (Sheybanikashani *et al.*, 2023)^[63]. Other “smart” hydrogels are designed to release growth factors or scavenge reactive oxygen species (ROS) to reduce inflammation and accelerate tissue repair in challenging contexts like diabetic ulcers or burn wounds (Liu *et al.*, 2025; Ramakrishnan *et al.*, 2022)^[52, 61].

The application of silk fibroin extends to cartilage and neural tissue engineering. For cartilage repair, silk hydrogels provide a hydrated, mechanically robust environment that supports chondrocyte viability and encourages the deposition of new cartilage matrix (Li *et al.*, 2021)^[48]. Bilayered silk scaffolds have been designed to regenerate osteochondral defects, with one layer optimized for bone and the other for cartilage, mimicking the native tissue interface (Jiang *et al.*, 2022)^[34]. In neural engineering, the ability to process silk into conduits and hydrogels makes it suitable for guiding nerve regeneration after injury. Conductive silk hydrogels, created by incorporating materials like MXene or polypyrrole, can be used to provide electrical stimulation to neural stem cells, promoting their differentiation into neurons and enhancing functional recovery after spinal cord or brain injury (Yang *et al.*, 2024; Jiang *et al.*, 2023)^[86, 35].

Beyond tissue scaffolds, silk fibroin is an excellent candidate for creating biodegradable medical implants and drug delivery systems. Its slow, predictable degradation rate allows it to provide mechanical support during tissue healing before being safely resorbed by the body. This has led to the development of silk-based screws, plates and sutures for orthopedic fixation (Yeon *et al.*, 2018)^[87]. As a drug delivery vehicle, silk can be formulated into nanoparticles, microparticles, or hydrogels to encapsulate therapeutic molecules, from small-molecule drugs to large proteins and nucleic acids. The crystalline structure of fibroin protects the encapsulated cargo from degradation and allows for controlled, sustained release over extended periods, which can be tuned by altering the material's processing conditions (Seib *et al.*, 2013; Kundu *et al.*, 2012)^[62, 43].

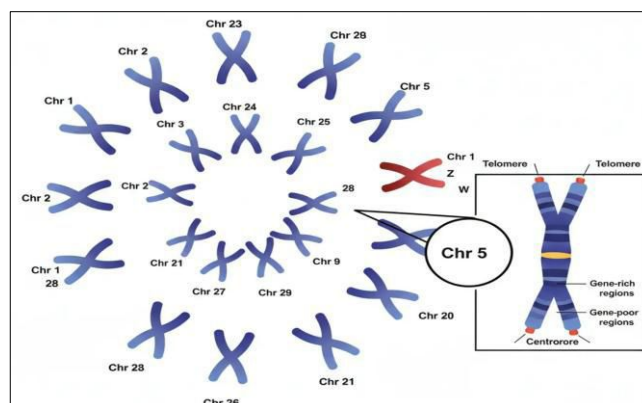


Fig 1: Genome organization of *Bombyx mori*.

Schematic representation of the *B. mori* genome structure. The genome is organized into 28 chromosomes (n=28), including the Z and W sex chromosomes. The figure highlights the high density of genes, interspersed with a large proportion (~45%) of repetitive elements, primarily transposable elements. Key genomic resources, such as the high-quality Nichi01 reference assembly with telomere-to-telomere (T₂T) chromosomes and the pan-genome derived from hundreds of resequenced individuals, are depicted. These resources provide a foundation for identifying genes, structural variants (SVs) and regulatory elements controlling important traits. (Image is a conceptual representation).

8. Nanotechnology and Advanced Silk Materials

The convergence of silk biotechnology and nanotechnology has given rise to a new class of advanced materials with unprecedented functionality. By processing silk fibroin at the nanoscale, its inherent properties can be enhanced and tailored for sophisticated applications, particularly in biomedicine. One of the most prominent examples is the fabrication of silk nanoparticles (SFNPs). These particles, typically ranging from 50 to 500 nm in size, can be produced through various methods, including desolvation, salting-out, or electrospraying (Lammel *et al.*, 2010) [44]. SFNPs serve as exceptional carriers for drug delivery. Their biocompatibility and biodegradability ensure minimal toxicity, while their proteinaceous nature allows for easy surface functionalization with targeting ligands, such as folate, to direct them to specific cells or tissues, like cancer cells (Subia *et al.*, 2014) [65]. The encapsulation of drugs within the silk matrix protects them from premature degradation and enables controlled, sustained release. Furthermore, silk nanoparticles can be engineered to be responsive to environmental stimuli, such as pH. For instance, SFNPs have been designed to release their anticancer drug payload preferentially in the acidic microenvironment of tumors, thereby increasing therapeutic efficacy and reducing systemic side effects (Seib *et al.*, 2013) [62].

Silk hydrogels represent another versatile platform in advanced materials. These are three-dimensional, water-swollen networks of crosslinked silk fibroin chains. Gelation can be induced by various physical or chemical methods, including changes in temperature, pH, sonication, or enzymatic crosslinking (Zhang *et al.*, 2011; Wang *et al.*, 2006) [90, 73]. The resulting hydrogels are highly biocompatible and possess mechanical properties that can be tuned to mimic those of soft tissues, making them ideal for tissue engineering and as injectable scaffolds for minimally invasive procedures. Silk hydrogels have been extensively used for encapsulating cells, providing a protective and supportive 3D environment that promotes cell viability and function (Wang *et al.*, 2006) [73]. They are also excellent vehicles for the localized, sustained delivery of growth factors and therapeutic drugs. Recently, “smart” silk hydrogels have been developed that can respond to specific biological cues or external stimuli, or possess self-healing capabilities, further expanding their potential in regenerative medicine and as biosensors (Sheybanikashani *et al.*, 2023) [63].

Electrospinning is a powerful technique used to produce continuous silk nanofibers with diameters ranging from nanometers to a few micrometers, creating non-woven mats that closely resemble the architecture of the natural extracellular matrix (ECM) (Min *et al.*, 2004) [54]. These Electrospun silk scaffolds exhibit high porosity, a large surface-area-to-volume ratio and excellent mechanical integrity. These features make them highly effective for tissue engineering applications, as they promote cell attachment, migration and proliferation. In wound healing, Electrospun silk mats have been shown to accelerate skin regeneration (Gholipourmalekabadi *et al.*, 2020) [25]. For bone tissue engineering, these nanofibrous scaffolds can be mineralized with hydroxyapatite to enhance osteoconductivity (Park *et al.*, 2010) [58]. The versatility of electrospinning allows for the creation of composite fibers

by blending silk with other polymers or by incorporating nanoparticles and drugs directly into the fibers, leading to multifunctional materials with combined structural and therapeutic properties.

9. Multi-Omics Approaches in Silkworm Biotechnology

The complexity of biological systems in the silkworm, from silk synthesis to immune response, cannot be fully understood by studying single molecular layers in isolation. The integration of multiple “omics” disciplines-genomics, transcriptomics, proteomics and metabolomics-has enabled a more holistic and systems-level understanding of *B. mori* biology. This multi-omics approach allows researchers to connect genetic information (genomics) to gene expression (transcriptomics), protein function (proteomics) and metabolic output (metabolomics), providing a comprehensive picture of cellular processes and their regulation.

In the study of silk production, multi-omics has been particularly insightful. Genomic data provides the catalogue of silk protein genes and their regulatory elements (The International Silkworm Genome Consortium, 2008) [70]. Transcriptomics, through RNA-seq of the different silk gland compartments, reveals when and where these genes are expressed, identifying key transcription factors that control their activity (Wang *et al.*, 2019; Ma *et al.*, 2022) [74, 53]. Proteomics then confirms the presence and abundance of the translated silk proteins and associated machinery (e.g., chaperones, enzymes) in the silk gland lumen and final cocoon (Dong *et al.*, 2013; Li *et al.*, 2015) [19, 46]. Finally, metabolomics analysis of the hemolymph and silk gland identifies the key metabolites, such as amino acids, that are mobilized and utilized for the massive synthesis of silk proteins (Zhou *et al.*, 2015) [90]. Integrating these datasets has allowed for the construction of detailed models of the silk synthesis pathway, identifying bottlenecks and potential targets for improving silk yield and quality. For example, linking transcriptomic data showing upregulation of amino acid transporters with metabolomic data showing high levels of specific amino acids in the silk gland provides a complete picture of the nutrient supply chain for silk production.

Multi-omics approaches have also been crucial in dissecting the silkworm's response to pathogens, such as the *B. mori* nucleopolyhedrovirus (BmNPV). Comparative transcriptomics of resistant and susceptible silkworm strains has identified hundreds of differentially expressed genes (DEGs) involved in immune signaling, apoptosis and metabolic pathways (Bao *et al.*, 2009) [4]. Proteomic analysis of hemolymph or specific tissues post-infection reveals changes in the levels of immune effectors like antimicrobial peptides and proteins involved in the prophenoloxidase cascade (Liu *et al.*, 2022) [50]. Metabolomic profiling can uncover shifts in energy metabolism and the production of antiviral compounds. By integrating these layers of information, researchers can identify key resistance genes and pathways. For instance, studies have linked BmNPV resistance to the upregulation of specific pattern recognition receptors, signaling components of the Toll and Imd pathways and genes involved in oxidative stress responses (Liu *et al.*, 2022; Cheng *et al.*, 2016) [50, 12]. This systems-level understanding is essential for developing strategies to breed more robust, disease-resistant silkworm strains.

Table 3: Biomedical applications of silk biomaterials.

Application Area	Biomaterial Format	Mechanism of Action and Key Features	Representative Reference
Bone Tissue Engineering	Porous 3D Scaffolds, Injectable Hydrogels	Provides mechanical support, mimics ECM, promotes osteoblast adhesion and differentiation. Often combined with hydroxyapatite (nHAp) or growth factors (BMP-2).	Yan <i>et al.</i> (2019) ^[85] ; Zou <i>et al.</i> (2023) ^[92]
Cartilage Repair	Hydrogels, Biphasic Scaffolds	Maintains chondrocyte phenotype, supports glycosaminoglycan (GAG) deposition and withstands compressive loads. Can be designed to regenerate osteochondral defects.	Li <i>et al.</i> (2021) ^[31] ; Jiang <i>et al.</i> (2022) ^[34]
Wound Healing and Skin Regeneration	Films, Nanofiber Mats, Hydrogels	Creates a moist, protective environment; promotes fibroblast and keratinocyte proliferation; can be loaded with antimicrobial agents (e.g., silver) or growth factors.	Chouhan and Mandal (2020) ^[13] ; Liu <i>et al.</i> (2025) ^[51]
Drug Delivery	Nanoparticles, Microparticles, Hydrogels	Encapsulates and protects therapeutic cargo (small molecules, proteins, nucleic acids); provides tunable, sustained release. Can be surface-functionalized for targeting.	Seib <i>et al.</i> (2013) ^[62] ; Kundu <i>et al.</i> (2012) ^[42]
Neural Tissue Engineering	Conduits, Conductive Hydrogels	Provides guidance cues for axonal regeneration. Conductive composites can deliver electrical stimulation to promote neural stem cell differentiation.	Yang <i>et al.</i> (2024) ^[86] ; Jiang <i>et al.</i> (2023) ^[35]
Biodegradable Medical Devices	Fibers, Screws, Plates	Offers high mechanical strength for temporary fixation (e.g., in orthopedics) and degrades predictably as tissue heals, eliminating the need for removal surgery.	Yeon <i>et al.</i> (2018) ^[87]

10. Challenges and Research Gaps

Despite the remarkable progress in silkworm biotechnology, several challenges and research gaps remain that must be addressed to fully realize its potential. While genome editing with CRISPR-Cas9 has been highly successful, off-target effects, though less frequent than with earlier technologies, remain a concern that requires rigorous validation, especially for commercial applications (Ma *et al.*, 2014) ^[50]. Moreover, the efficiency of homology-directed repair (HDR) for precise gene knock-in remains relatively low in silkworms, limiting the ease of inserting large or complex genetic constructs at specific loci. Developing more efficient HDR strategies or alternative editing systems is an active area of research. From a practical standpoint, the microinjection of embryos, the standard method for delivering editing reagents, is labor-intensive and requires specialized skills, posing a bottleneck for high-throughput genetic screening and large-scale production of transgenic lines.

Biosafety and public perception are significant hurdles for the commercialization of genetically modified (GM) silkworms. The release of GM insects into the environment, even if they are domesticated and cannot survive in the wild like *B. mori*, raises ecological concerns that must be addressed through robust containment strategies and thorough risk assessments. Gaining regulatory approval and public acceptance for products derived from GM silkworms, whether it be enhanced silk fibers or recombinant pharmaceuticals, requires transparent communication and comprehensive safety data. The regulatory frameworks for GM insects and their products are still evolving in many parts of the world, creating uncertainty for industrial development.

In the realm of silk biomaterials, a major challenge is the industrial scalability of production while maintaining consistent quality. The process of extracting and purifying silk fibroin from cocoons and then fabricating it into scaffolds or nanoparticles, involves multiple steps that can introduce batch-to-batch variability. Standardizing these processes and developing cost-effective, large-scale manufacturing techniques that comply with Good Manufacturing Practice (GMP) standards is crucial for clinical translation. Furthermore, while the biocompatibility of silk fibroin is well-established, its long-term degradation

profile and the host response to silk-based implants in humans need to be more extensively studied in long-term clinical trials. The complexity of recreating the intricate architecture and functionality of native tissues remains a fundamental challenge in tissue engineering and silk scaffolds alone are often insufficient without the incorporation of cells, growth factors and other bioactive cues.

11. Future Prospects

The future of silkworm biotechnology is poised for even more exciting innovations, driven by emerging technologies and a deeper understanding of molecular biology. The field of synthetic biology offers the potential to redesign the silkworm's genetic circuitry to create "smart" organisms with novel capabilities. This could involve engineering silkworms that produce silk with entirely new properties, such as embedded antimicrobial peptides, fluorescent markers, or drug-eluting capabilities, directly in the fiber. By designing and implementing novel genetic circuits, it may be possible to create silkworms that can sense environmental changes and respond by altering their silk composition or producing specific therapeutic proteins on demand.

The production of completely artificial silk proteins in microbial or plant-based systems is another promising frontier. While challenging due to the large size and repetitive nature of fibroin, success in this area could lead to the sustainable, large-scale production of silk-like materials with precisely controlled properties, independent of silkworm rearing. This would allow for the rational design of proteins with optimized mechanical strength, elasticity, or specific biological activities.

In biomaterials science, the next generation of silk-based materials will likely be "smart" and dynamic. This includes the development of 4D-printed silk scaffolds that can change shape over time in response to physiological stimuli, injectable hydrogels with self-healing properties and biosensors that can detect disease biomarkers in real-time. Integrating silk with electronics could lead to biodegradable and biocompatible electronic devices for monitoring health or delivering therapeutic stimulation.

Finally, the concept of "precision sericulture" aims to apply modern biotechnological and data-driven approaches to

optimize the entire silk production pipeline. This involves using genomic selection to rapidly breed elite silkworm strains with desired traits, developing nanoparticle-based oral delivery systems for vaccines or RNAi-based treatments to protect against disease and using multi-omics data to fine-tune rearing conditions for maximum productivity and health. These future directions promise to further solidify the silkworm's role as a cornerstone of both sustainable agriculture and advanced biotechnology.

12. Conclusion

The journey of the silkworm, *Bombyx mori*, through the landscape of modern biotechnology has been nothing short of transformative. From its ancient origins in sericulture, it has evolved into a sophisticated and multifaceted platform for scientific discovery and innovation. The breakthroughs in silkworm genomics, beginning with the initial genome sequencing and culminating in high-quality, chromosome-scale assemblies and comprehensive pan-genomes, have provided the essential blueprint for all subsequent advances. These genomic tools have enabled a deep dive into the molecular underpinnings of the silkworm's unique biology, particularly the highly efficient and complex process of silk protein synthesis.

The development and refinement of genetic engineering technologies, from the *piggyBac* transposon to the revolutionary CRISPR-Cas9 system, have empowered researchers to precisely manipulate the silkworm's genome. This has opened up avenues for enhancing agronomically important traits, such as disease resistance and silk yield and has successfully repurposed the silk gland into a cost-effective and scalable bioreactor for producing a wide range of valuable recombinant proteins for medicine and industry. Concurrently, the silk fiber itself, once prized only for textiles, has been reimagined as a high-performance biomaterial. The exceptional properties of silk fibroin-biocompatibility, mechanical robustness and tunable degradation-have been harnessed to create a new generation of materials for tissue engineering, drug delivery and regenerative medicine. As we look to the future, the integration of synthetic biology, nanotechnology and multi-omics approaches promises to unlock even greater potential, paving the way for smart biomaterials and precision sericulture. The silkworm, a creature of ancient tradition, stands firmly at the forefront of modern biotechnology, a testament to the power of integrating fundamental science with applied innovation.

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