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Transgenic silkworm for the production of recombinant protein: A review

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Abstract
Transgenic silkworms (*Bombyx mori*) have emerged as promising bioreactors for the production of recombinant proteins, offering an effective alternative to conventional microbial and mammalian expression systems. Their innate ability to secrete large amounts of silk proteins, combined with robust post-translational modification machinery, enables the synthesis of complex therapeutic proteins with structural fidelity and biological activity. Advances in genetic engineering—particularly the use of piggyBac transposons, CRISPR/Cas9, and synthetic biology approaches—have facilitated the stable integration and high-level expression of heterologous genes in silkworm silk glands. This review highlights recent progress in transgenic silkworm technology, including silk protein engineering for biomaterials, scalable recombinant protein production, and applications in pharmaceuticals, vaccines, and tissue engineering. The unique biosafety of domesticated silkworms, their cost-effectiveness, and scalability further reinforce their potential for industrial biomanufacturing. Future perspectives emphasize the integration of genome editing and computational modeling to enhance protein yield, functionality, and therapeutic specificity, positioning transgenic silkworms as a sustainable and versatile platform in biotechnology and global health.

Keywords: Transgenic silkworm, *Bombyx mori*, recombinant proteins, silk protein engineering,

Introduction
The development of genetic engineering has completely changed the biotechnology industry by making it possible to precisely modify organisms for a variety of uses, such as the manufacturing of valuable recombinant proteins and medications (Shakweer *et al.*, 2023) [18]. Because of their strong protein synthesis machinery, scalability, and capacity for intricate post-translational modifications, transgenic silkworms have become a promising platform among the many expression systems available. As such, they are perfect bioreactors for the production of biopharmaceuticals (Ji *et al.*, 2025) [7]. This review thoroughly investigates the developments in using transgenic silkworms to produce recombinant proteins and pharmaceutical compounds in an efficient and economical manner, describing the molecular mechanisms and biotechnological approaches used (Ji *et al.*, 2025) [7]. The distinctive biology of the silkworm, *Bombyx mori*—notably its capacity to secrete large amounts of silk proteins—has been harnessed to engineer advanced genetic constructs that drive the expression of heterologous proteins in silk glands or other tissues (DS *et al.*, 2024) [6]. This site-specific targeting greatly simplifies product recovery and purification, offering a clear advantage over conventional bioreactor-based systems (Baci *et al.*, 2021) [2]. Moreover, the silkworm's long-standing domestication—refined over more than 5,000 years—ensures its complete reliance on human care for survival. As a result, any accidental release of genetically modified silkworms poses no ecological risk, reinforcing their value as a safe, contained platform for bioproduction (Tatematsu, 2012). The economic importance of "Bombyx mori" is underscored by its ancient domestication for silk production, a process that has also facilitated extensive research into its genetics and physiology (Yuan *et al.*, 2023) [27] (Chen *et al.*, 2018) [3]. This rich history of study provides a foundational understanding critical for its application in advanced biotechnological endeavors (Rahman, 2020) [17]. The capacity of the silkworm to produce over 0.5 grams of silk protein, combined with its established genetic manipulation techniques, positions it as an exceptional bioreactor for large-

scale recombinant protein synthesis (Tatemastu, 2012) [22]. This capability is particularly significant given that "Bombyx mori" offers the necessary post-translational modifications for the proper structure and functionality of recombinant proteins (Baci *et al.*, 2021) [2].

Silk protein genetic engineering and their implications for biomedicine

Effective recombinant protein production has been achieved by taking advantage of the silkworm larva's ability to synthesise more than 0.5 g of silk protein during its last instar andexpel it as a cocoon silk filament (Tatemastu, 2012) [22]. For complex protein therapeutics, this feature, along with the silkworm's capacity for extensive post-translational modifications, makes it a desirable substitute for conventional expression systems (Baci *et al*., 2021 and Tatemastu, 2012) [2, 22]. As demonstrated by the successful production of a variety of human, animal, and virus-derived proteins, Bombyx mori has the potential to be a versatile bioreactor with wide-ranging applications in biomedicine (Baci *et al.*, 2021) [2]. The inherent scalability and cost-effectiveness of silkworm rearing, coupled with the high yield of recombinant proteins from their silk glands, position this system as a viable industrial platform for pharmaceutical manufacturing (Wang *et al*., 2015) [24]. One notable advantage of the silkworm system is its capacity to produce significant quantities of recombinant protein, with yields reaching up to 4 mg per silkworm in the middle silk gland system, a remarkable output compared to other protein expression platforms. Furthermore, the biological activity and stability of complex therapeutic proteins are guaranteed by the fact that the post-translational modifications, specifically glycosylation patterns, in silkworms are more similar to those present in mammalian systems than in bacterial or plant expressions (Tatemastu, 2012) [22]. Transgenic silkworms are a better option for some biopharmaceutical applications than microbial hosts because of their similar post-translational modifications, especially glycosylation, which is essential for the biological activity, half-life, and immunogenicity of therapeutic proteins.

However, while natural silk proteins exhibit excellent properties, their inherent characteristics may not always align with the specific demands of all applications, necessitating genetic modification to engineer novel silk proteins with enhanced performance characteristics (Ji *et al.*, 2025) [7]. For instance, the modification of silk proteins can introduce functional domains that enhance their mechanical strength, biocompatibility, or drug-binding capabilities, thereby broadening their utility in advanced biomaterials and drug delivery systems. The challenges associated with traditional mammalian cell lines, such as Chinese hamster ovary cells, including issues with protein clipping, undesired non-human post-translational modifications, and difficulties in producing highly glycosylated proteins for structural studies, further underscore the appeal of silkworm bioreactors (Sun *et al.*, 2023) [20]. The ease of generating transgenic silkworms using the "piggyBac" transposon system further enhances their utility as a rapid and efficient bioproduction platform (Tatemastu, 2012) [22]. The ability to incorporate foreign genes into the silkworm genome, coupled with the high expression levels achieved, makes this system particularly attractive for large-scale production of complex proteins that require precise folding and post-translational processing.

The stable integration of various transgenes made possible by this genetic manipulability makes it possible to consistently produce complex recombinant proteins, including ones with important pharmaceutical applications (Kim *et al.*, 2021) [8]. A major area of development that capitalises on the versatile qualities of silk is the creation of genetically modified silk for biomaterial applications, such as scaffolds for tissue engineering and medical implants, in addition to pharmaceutical uses. A complex spatial control over protein synthesis within the silkworm is demonstrated by the fact that the anterior silk gland and middle silk gland are separate compartments that have been designed for the production of modified silk and recombinant proteins, respectively (Tatemastu, 2012) [22]. This precise compartmentalization allows for specialized protein processing, optimizing the yield and functionality of the desired biomolecules. This engineering precision facilitates the production of novel silk materials with tailored properties, such as enhanced mechanical strength or improved biocompatibility, critical for advanced biomedical applications (Lovett *et al.*, 2010) [10]. Furthermore, the intrinsic properties of native silk, such as its biocompatibility, biodegradability, and impressive mechanical strength, render it an ideal scaffold for tissue regeneration, drug delivery, and wound healing, which can be further optimized through genetic engineering. The capacity to precisely modify silk at the genetic level allows for the incorporation of diverse functional domains, leading to the creation of advanced biomaterials with precisely tuned mechanical, chemical, and biological properties. This capacity for precise genetic modification allows for the development of smart biomaterials that can integrate therapeutic functions, such as targeted drug delivery or enhanced cellular integration (Mottaghitalab *et al*., 2015) [13].

Production and Characterization of recombinant proteins

To generate and characterize transgenic silkworms and the production of recombinant proteins encompasses the methodologies for transgene construction, microinjection into silkworm embryos, screening for successful germline transformation, and subsequent characterization of recombinant protein expression and functionality within the silk glands and secreted silk (Tatemastu, 2012) [22]. The intricate processes involved in gene editing, such as CRISPR/Cas9 applications, and vector design for optimized expression are also elaborated, providing a comprehensive understanding of the molecular tools utilized. The application of genetic engineering technologies, including CRISPR/Cas9, enables precise modification of silk protein genes to alter their properties, while transgenic expression technology facilitates mass production in heterologous systems (Ji *et al.*, 2025) [7]. This integration of advanced molecular techniques underpins the development of robust platforms for synthesizing complex biomolecules. Furthermore, synthetic biology approaches are increasingly employed to design de novo genetic circuits for inducible or tissue-specific protein expression, thereby enhancing control over the production process and tailoring protein yields. The advent of CRISPR/Cas9 technology, in particular, has revolutionized the precision and efficiency of genetic modifications in silkworms, allowing for targeted integration or knockout of genes to influence silk production and quality (DS *et al.*, 2024) [6].

This advanced gene editing ability makes it easier to create silkworm lines that are best suited for producing functionalised silk materials or high-yield production of particular therapeutic proteins (DS *et al*., 2024) [6]. Beyond these specific changes, CRISPR-Cas9 systems also facilitate multiplexed genetic engineering, which enables the simultaneous modification of several genes to produce complex phenotypic changes, like

improved protein stability or altered post-translational modifications (Xu *et al.*, 2014 and Tavakoli *et al*., 2021) [26, 23]. This increases the potential applications of recombinant proteins and silk by enabling researchers to refine their biochemical characteristics. By enabling effective and precise targeted breaks in DNA, the CRISPR/Cas9 system has transformed genome engineering and is a vital tool for gene editing and a variety of biomedical applications. (Martínez-Lage *et al*., 2017) [11]. The development of transgenic organisms, such as silkworms, for a range of biotechnological applications has been greatly accelerated by its adaptability and simplicity of use (Wang & Doudna, 2023) [25]. In agricultural biotechnology, where CRISPR/Cas9 enables crop improvement through targeted genetic modifications, this accuracy is crucial (Li *et al.*, 2020) [9]. Beyond agriculture, CRISPR-Cas systems are used in a variety of industries, including industrial biotechnology and therapeutics, providing a versatile and easy-to-use molecular platform for precise genomic control (Parsaeimehr *et al.*, 2022) [15]. Since its introduction in 2012, the CRISPR-Cas9 system has quickly emerged as the leading genetic editing technique, bringing with it previously unheard-of control over genomic alterations (Critchley *et al.*, 2019) [4]. This technology, initially identified as a bacterial immune system component, employs a guide RNA to direct the Cas9 nuclease to specific DNA sequences, enabling highly precise gene editing (Doudna and Charpentier, 2014) [5] and Ansori *et al.*, 2023) [, 1]. This system's ability to create site-specific DNA cleavage with only an RNA guide sequence and a DNA endonuclease has been instrumental in generating targeted modifications in various organisms, including silkworms (Munshi, 2016) [14]. However, despite its immense potential, challenges such as off-target effects and the complexity of the silkworm genome still need to be addressed to optimize its application in silk protein genetic engineering. The CRISPR/Cas9 system, utilizing its unique "scissors" function to design specific single-guide RNAs, precisely cleaves silk protein genes, enabling targeted mutations, deletions, or insertions.

This precise genetic manipulation offers unprecedented opportunities for enhancing the properties of silk, leading to novel applications in biomedicine and materials science (Ji *et al.*, 2025) [7]. The ongoing optimization of CRISPR/Cas9 delivery methods and off-target effect mitigation strategies will further enhance its utility in generating transgenic silkworms with superior recombinant protein production capabilities and functionalized silk materials (Song *et al.*, 2020 and DS *et al.*, 2024) [19, 6]. Furthermore, advancements in synthetic biology are poised to integrate with CRISPR/Cas9 technology, allowing for the precise design of regulatory elements and gene circuits that can finely control the expression of recombinant proteins in silkworms, thereby optimizing yield and functionality. This synergistic integration promises to unlock the full potential of silkworms as bioreactors, enabling the production of highly tailored and potent biopharmaceuticals with unprecedented efficiency and specificity (Ji *et al.*, 2025) [7]. This level of control also facilitates the creation of bespoke silk-based biomaterials for personalized medicine, addressing individual patient needs through custom-tailored genetic or medical profiles (DS *et al*., 2024) [6]. This bespoke approach extends to the development of novel drug delivery systems, where functionalized silk can precisely release therapeutic agents in a controlled and targeted manner. These advancements are further bolstered by the ability to incorporate various peptides and proteins, such as collagen or fibronectin-derived sequences, into the silk matrix, thereby enhancing its cell-adhesive properties for tissue engineering applications (DS *et al.*, 2024) [6].

Advancements in transgenic technology

It addresses the various genetic modification techniques, the wide range of therapeutic proteins that have been successfully expressed, and the benefits of using silkworms as bioreactors. Insights regarding the commercial viability and future course of this cutting-edge platform are provided by the critical evaluation of the opportunities and difficulties related to large-scale production, purification, and regulatory concerns. By contrasting transgenic silkworm technology with conventional mammalian and microbial expression systems, the review also examines the economic and environmental sustainability of this technology and evaluates its overall influence on the production of biopharmaceuticals. Transgenic silkworms could become a major platform for the production of biopharmaceuticals, according to the review's conclusion, especially as gene editing technologies advance and become more scalable. This includes optimizing gene expression cassettes and developing more efficient and targeted transgenesis methods. These advancements will facilitate the development of novel therapeutic proteins and biomaterials, addressing critical needs in medicine and biotechnology. Future research will undoubtedly explore the potential of combining transgenic silkworm technology with artificial intelligence and machine learning to predict optimal gene constructs and expression patterns, further accelerating the development of novel therapeutic proteins (Popova *et al*., 2023 and Ji *et al*., 2025) [16, 7]. The integration of advanced computational modeling with genetic engineering promises to revolutionize the efficiency and specificity of recombinant protein production in silkworms, leading to higher yields and reduced manufacturing costs. Furthermore, the silkworm's inherent ability to perform complex post-translational modifications, similar to mammalian systems, ensures the production of highly functional and biologically active proteins, making it an attractive alternative to bacterial and plant expression systems (Tatemastu, 2012) [22].

Advantages of employing transgenic silkworms as bioreactors

This section elaborates on the intrinsic advantages of employing transgenic silkworms as bioreactors for the scalable and cost-effective production of a diverse range of recombinant proteins, encompassing therapeutic antibodies, vaccines, and industrial enzymes (Baci *et al*., 2021 and Tatemastu, 2012) [2, 22]. The discussion also emphasises how silkworms provide subtle post-translational modifications that closely resemble mammalian systems, guaranteeing the structural integrity and biological activity of complex protein therapeutics. This ability sets silkworms apart from prokaryotic expression systems, which frequently lack the tools necessary for appropriate folding and glycosylation, and is especially important for the development of highly specific and effective biopharmaceuticals. The silkworm system's adaptability also makes it possible to express complex multi-subunit proteins, or fusion proteins, which are frequently difficult to make on other platforms. Additionally, when compared to costly mammalian cell culture systems, the scalability of silkworm rearing and the comparatively low cost of feed and maintenance make this platform an economically appealing option for industrial-scale protein production (Tatemastu, 2012) [22]. Additionally, the ease of handling and the robust nature of silkworms contribute to a simplified biomanufacturing pipeline, reducing the need for elaborate sterile facilities.

Conclusion

The rapidly developing field of transgenic silkworms for the production of pharmaceuticals and recombinant proteins is well-positioned to meet important unmet needs in biotechnology and global health. Silkworms are a revolutionary platform for the production of biopharmaceuticals due to their special qualities, which include their ability to produce high-yield proteins, their strong post-translational modification machinery, and their innate biosafety (Tatemastu, 2012) [22]. In addition to providing a cost-effective substitute for conventional expression systems, this novel method overcomes present accessibility and cost barriers to produce complex protein therapies and vaccines on a large scale in a sustainable manner (Mon, 2012) [12]. Future research will undoubtedly focus on refining genetic engineering techniques to enhance expression levels further and broaden the spectrum of proteins that can be successfully produced in silkworms, thereby solidifying their role as a cornerstone in biopharmaceutical innovation (Sun *et al*., 2023) [21]. This includes optimizing the integration and expression of transgenes to prevent off-target effects and ensure stable protein production over multiple generations. Moreover, ongoing advancements in CRISPR-Cas9 and other gene-editing technologies are expected to facilitate more precise and efficient genomic modifications, enabling the targeted insertion of complex gene constructs and the fine-tuning of expression profiles.

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