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Silkworm: A Promising Model Organism in Life Science

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Abstract

As an important economic insect, silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) has numerous advantages in life science, such as low breeding cost, large progeny size, short generation time, and clear genetic background. Additionally, there are rich genetic resources associated with silkworms. The completion of the silkworm genome has further accelerated it to be a modern model organism in life science. Genomic studies showed that some silkworm genes are highly homologous to certain genes related to human hereditary disease and, therefore, are a candidate model for studying human disease. In this article, we provided a review of silkworm as an important model in various research areas, including human disease, screening of antimicrobial agents, environmental safety monitoring, and antitumor studies. In addition, the application potentiality of silkworm model in life sciences was discussed.

Key words: silkworm, model organism, drug screening, antimicrobial drug, disease model

Animal models are widely used in life science and other fields for deeper, and more fully understanding specific scientific problems. In many cases, higher organisms such as mammals are used in these studies. However, an extensive use of mammals may raise issues not only limited to biosafety, but also lead to animal emotions, animal rights, and many other bioethical issues (Levy 2012). Therefore, the choice of a suitable model animal to replace mammals or reduce the use of mammals becomes a concern in current life science research. Studies showed that about 80% of pathogen infection experiments that use mammals could be replaced with insects, and using lower animals instead of higher has been accepted and suggested by many experts (Renwick and Kavanagh 2007).

Silkworm *Bombyx mori*, a typical representative of lepidoptera insects, is of great agricultural and economic importance. Having short generation time, clear genetic background, rich genetic resources, and a considerable number of genes that are homologous to human, silkworm has widely been used in various life science studies. In 2003, China and Japan launched the silkworm genome project and completed the draft map, fine map, and multistrain genome re-sequencing of the silkworm (Xia and Yang 2004, Xia et al. 2009), which greatly promoted the development of sericulture science, and expedited the use of silkworm as an ideal model organism for scientific research. Currently, the application of silkworm model has gradually emerged in the field of life science (Nwibo et al. 2015), such as antipathogenic drug screening, treatment evaluation, and environmental safety supervision. This model organism showed very promising future, which will be discussed in detail below.

The Advantages of Silkworm as a Model Organism

Silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae) has big progeny size and short generation time with larval stage lasting about 25–30 d (Fig. 1). It has 28 pairs of chromosomes, which is far more than those of *Drosophila melanogaster* (four pairs), rich in genetic traits. Silkworm genome is about 450 million bp, which is 1/6 of the human genome and four times more than the genome of *D. melanogaster*. In addition, silkworm has diverse mutant strains and morphological mutations. Its death, either genetic or imposed, does not involve any bioethical issues (Chen et al. 2014). In addition, silkworm has moderate body size and can be easily dissected to obtain many tissues and organs such as midgut, fat body, silk gland, and hemolymph. Furthermore, silkworm can be used to perform oral administration and intravenous injection experiments similar to those of mammals. With the completion of the silkworm genome project and the establishment of silkworm genomic database and protein database, silkworm begin to stand out as a valuable model in scientific research (Mita 2008).

Silkworm as a Model Organism in Life Science

Using silkworm as a model animal to study human microbial toxicology and pathology has experienced rapid development over the past decade. Studies found that silkworm was highly sensitive to human pathogenic microorganisms, pathogenic fungi, antibiotics, and pesticides. Silkworm models for bacterial infection, fungal infection, virus infection, and natural immune stimulation have been
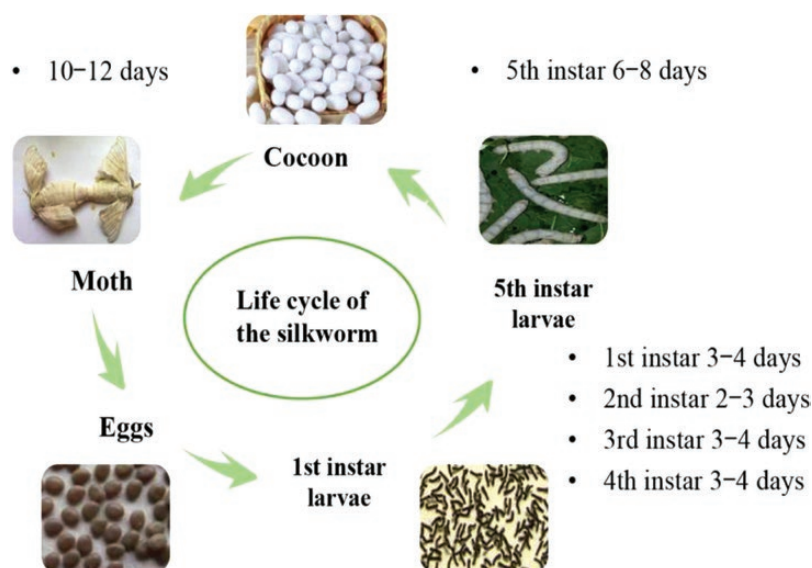
Fig. 1. Generation cycle pattern of the silkworm (*Bombyx mori*).

Table 1. Silkworm models in life science

Silkworm models	Test systems	Using of test systems	References
Disease silkworm models	Bacterial infection model	*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholera*	Hamamoto et al. (2004), Fujiyuki et al. (2012), Miyazaki et al. (2012)
	Fungi infection model	*Candida albicans*, *Candida tropicalis*, *Cryptococcus neoformans*	Matsumoto et al. (2012), Ueno et al. (2011)
	Virus infection model	Human herpesvirus or Cytomegalovirus	Wang and Schweizer (2008)
	Diabetes (hyperglycemia) model	Type II diabetes	Matsumoto et al. (2014), Matsumoto et al. (2015)
	Antibiotic drug screening model	Nosokomycins	Kaito et al. (2002)
Natural immunity stimulation test	Muscle contraction	β-Glucan, yeast β-1,6-glucan, green tea	Ishii et al. (2008), Fujiyuki et al. (2012)
Safety test	Pathogenicity test	*S. aureus*, *P. aeruginosa*, *V. cholera*	Hamamoto et al. (2004), Fujiyuki et al. (2012), Miyazaki et al. (2012)
	Toxicity test	Ethanol, *m*-cresol, sodium azide	Hamamoto et al. (2008)
Drug kinetics test	Gastrointestinal absorbability test	Chloramphenicol, tetracycline, vancomycin	Hamamoto et al. (2004)

established (Kaito and Sekimizu 2007, Ishii et al. 2015a) (Table 1). Thus, the use of silkworm as a model organism for studying human tumor, degenerative disease, and metabolic disease has become a current research focus.

The Application of Silkworm in the Screening of Antimicrobial Drugs

Silkworm has received extensive attention as a model for human antimicrobial drug screening. Kaito et al. (2002) used silkworm to the study of antimicrobial drugs and demonstrated that silkworm could replace mammals for bacterial pathogenicity experiments. Therefore, investigating the defense mechanism of silkworms against pathogenic microbes may be a feasible solution to the development of antimicrobial drugs. Studies have showed that latrogenic pathogens, such as *Staphylococcus aureus* (Hanada et al. 2011), *Canidida*, *Aspergillus* (Hamamoto et al. 2004), can cause death of the silkworm. Hamamoto et al. (2004) evaluated the efficacy and toxicity of several antibiotics, such as vancomycin, tetracycline, abecellin, flucloxacillin, and linezolid using the model of silkworm. These results showed that the ED_{50} (median effective dose) and LD_{50} (median lethal dose) of certain

human pathogens and fungi in silkworm are consistent with those in mouse (Table 2). Usui et al. (2016) verified that an acute oral toxicity test in silkworms is a useful way to evaluate the toxicity of compounds in mammals. Panthee et al. (2017) utilized silkworm bacterial infection model to screen the therapeutic effectiveness of various microbial culture broths and successfully identified a therapeutically effective novel antibiotic, lysocin E, which has a novel mode of action of binding to menaquinone, an important membrane molecule for the bacterial electron transport chain, as the lysocin E target, thus leading to membrane damage and bactericidal activity. Study confirms that silkworm antibiotic screening model is very effective for identifying novel antibiotics. These studies imply that silkworm and mammals may have very similar metabolic pathway for antibiotics.

Silkworm has a simple but efficient immune system. Its innate immune response is similar to that of the mammals (Fujiyuki et al. 2012, Ishii et al. 2013). Studies found that there were similar drug metabolic features between silkworm and rat as well as other mammals, and the pharmacodynamics results obtained in silkworm and mammals were consistent (Inagaki et al. 2012). It was also found that silkworm had innate immune response to β-glucan, yeast

β-1,6-glucan, green tea extract, and other stimulants (Dhital et al. 2011, Fujiyuki et al. 2012). In silkworms, over-stimulation of innate immunity induces tissue damage, leading to the death of the host, a symptom similar to human sepsis-induced multiple organ failure (Ishii et al. 2015b). These findings demonstrate the successful use of silkworm as an animal model for screening natural immune activator.

The Application of Silkworm in Human Disease Model

Because of the high degree of homology between silkworm genes and certain hereditary disease genes in human, many silkworm disease models have been established. Adenylate protein kinase signaling pathway plays an important role in the regulation of human blood glucose, although this signaling pathway also regulates hemolymph glucose in the silkworm (Yusuf et al. 2011). The insulin-like peptide encoded by the silkworm gene was found to have about 40% similarity to human insulin (Zhang et al. 2015). Based on this, a silkworm diabetes model was established by expressing human insulin receptor (hIR) with transgenic silkworm. Human insulin was administered to transgenic silkworm expressing hIR to reduce hemolymph glucose levels and promote Akt phosphorylation in the fat body. The inhibition of human insulin-induced hypoglycemia in the transgenic silkworm expressing hIR was blocked by co-injection of wortmannin (phosphatidylinositol 3-kinase inhibitor). Application of bovine insulin, an hIR ligand, also effectively reduces the transgenic silkworm sugar content. This study using the GAL4/UAS system established a transgenic silkworm strain expressing the hIR and indicate that functional hIRs that respond to human insulin were successfully induced in the transgenic silkworms (Matsumoto et al. 2014). Studies also found that the occurrence of gout is directly related to hyperuricemia, which is mainly caused by purine metabolic disorders and uric acid excretion decrease (Choi et al. 2005). Silkworm has similar purine metabolism pathway to that of human with uric acid the metabolic end products (Hayashi 1962). It was found that urate granules were mainly deposited in the dermis layer of silkworm, making the body surface white. The color of the silkworm epidermis could change obviously from white to transparent upon treatment with a gout drug (Yokoyama 1976). Thus the gout drug efficacy can be evaluated using a silkworm model by observing color changes of the silkworm skin.

The silkworm mutant strain albino (*al*) is caused by mutations of *BmPTS* gene, which blocked the synthesis of tetrahydro biopterin (BH4), a cofactor involved in the synthesis of melanin. Interestingly, the pathogenesis of *al* silkworm is similar to that of phenylketonuria in patients lacking BH4, so the *al* silkworm is considered to be a potential model for human phenylketonuria (Bonafé et al. 2001, Blau and Bonafe 2001). Another mutant strain lemon (*lem*) is caused by loss of *BmSPR* gene, which leads to difficulty in BH4 synthesis and abnormal melanin metabolic pathways. This has resulted in the accumulation of xanthine B_1 in the silkworm dermal cells, a symptom that is similar to human sepiapterin reductase deficiency (SRD), an autosomal-recessive disease which results indopa-responsive dystonia. So far, only *lem* silkworm was found to have similar pathological pathway to this human disease, making it one and the only model for this type of disease (Meng et al. 2009).

p-Translucent (*op*) silkworm is caused by downregulation of *PARK7/DJ-1* gene, resulting in decrease in xanthine oxidase synthesis and increase in body oxidative stress response, which in turn lead to oxidative damage to the nerves and tissues (Wang et al. 2013). Therefore, p-translucent silkworm mutant was regarded as human Parkinson's models (Chen et al. 2016). *BmBLOS2* and *Bmdysb* are homologous genes to biogenesis of lysosome-related organelles complex-2 (BLOC2) and dysbindinhue, which encode human protein complex subunit biogenesis of lysosome-related organelles complex-1(BLOC1) (Fujii et al. 2010, Wang et al. 2013). *BmHPS5* is the homologous gene to human protein complex BLOC2 subunit *HPS5* (Fujii et al. 2012). *BmBLOS2*, *Bmdysb*, *BmHPS5* gene mutations in silkworm resulted in d-translucent (*od*), mottled translucent of var (*ov*), and aojuku translucent (*oa*) silkworm mutants, respectively. These mutations lead to difficulty in the formation of urate particles and make silkworm body transparent. In humans, BLOCK1 and BLOCK2 protein complex abnormalities can cause Hermansky–Pudlak syndrome, a type of albinism syndromes (Wei 2006). Accordingly, all three mutants were considered a candidate disease model for Hermansky–Pudlak syndrome (Wei 2006). These studies indicate that silkworm mutants have great potential in the establishment of human disease models (Table 3).

Recently, CRISPR/Cas9-mediated gene editing has become a powerful gene editing tool, and has widely been used in life sciences

Table 2. ED_{50} of antifungal agents in a silkworm model with *Candida tropicalis* or *Candida albicans* (Hamamoto et al. 2004, Ishii et al. 2017)

Antifungal agent	True fungus	ED_{50} in silkworm (μg/g)	MIC μg/ml	ED50/MIC ratio in	
				Silkworm	Mouse
Amphotericin B	*C. tropicalis*	1.8	3.2	0.6	0.2
	C. albicans	4.1	1.6	2.6	1.3
Fluconazole	*C. tropicalis*	1.8	1.6	1.1	7.4
	C. albicans	1.8	0.4	4.5	8.6

MIC (minimum inhibitory concentration).

Table 3. The application of silkworm as human disease model

Silkworm strain	Silkworm gene	Silkworm models	References
Albino (*al*)	*BmPTS*	Human phenylketonuria	Blau and Bonafe (2001), Bonafé et al. (2001)
Lemon (*lem*)	*BmSPR*	Human sepiapterin reductase deficiency (SRD) model	Meng et al. (2009)
p-Translucent (*op*)	PARK7/DJ-1	Human Parkinson's models	Chen et al. (2016)
d-Translucent (*od*)	*BmBLOS2*	Hermansky–Pudlak syndrome	Wei (2006), Fujii et al. (2010)
Mottled translucent of var (*ov*)	*Bmdysb*		
Aojuku translucent (*oa*)	*BmHPS5*		

(Life Technologies, Carlsbad; QIAGEN,Duesseldorf, GER) (Bassett et al. 2013, Auer et al. 2014). It is reported that using the CRISPR/Cas9 technique, *BmBLOS2* gene was knocked out by injecting mRNA and sgRNA complexes in the egg phase of the silkworm, and silkworm skin became translucent (Liu et al. 2014). In addition, *Bm-ok*, *BmKMO*, *BmTH*, *Bmtan*, and other silkworm genes were successfully knocked out, thus targeted gene editing in the silkworm becomes possible (Wang et al. 2013, Ma et al. 2014), which provides new paths to pest prevention and treatment as well as to investigating gene functions. Therefore, CRISPR/Cas9 presented as a method for creation of human disease model in silkworm.

The Application of Silkworm in Environmental Monitoring

Rapid economic development has brought environmental problems such as heavy metal pollution and pesticides residues. A key to the assessment of ecological environment safety is to find a model animal that can be used in environment monitoring. Silkworm is sensitive to environmental pollutions, especially to pesticides, heavy metals, and other harmful chemicals (Sekimura 2005, Hamamoto et al. 2009). It was found that silkworm survival rate dramatically decreased when the addition of Cd^{2+} reached 10 ppm in silkworm artificial food (Sekimura 2005). Another study found that addition of low concentration nano-TiO_2 to the silkworm feedstuff increased the weight of silkworm (Li et al. 2016), while at the transcriptional level, the expression of detoxification genes such as *BmCYP6ae22*, *BmGSTol*, *Bmcce* were upregulated (Tian et al. 2016). Tian et al. also observed, at the individual level, growth-inhibiting and toxic effects of Ag nanoparticles (AgNPs) on silkworms. AgNPs expose has influenced the functions of the metabolic cycle, as well as signal transduction, apoptosis, and ion transport, silkworm as a model organism is used to assess the potential hazards of nanomaterials (Meng et al. 2017). In addition, the organophosphorus pesticide MEP emulsion could poison the silkworm even after 5,000 times dilution (Sugiyama and Emori 1980). Thus, the silkworm model can be used to monitor toxic substances that have negative effect on soil, water quality, medical environment, and so on.

The Application of Silkworm in Pest Control

Kodama et al. (1972) first established a silkworm larvae virus infection model for drug therapy and insect defense research. Injecting the virus into the haemolymph of silkworm, the results showed that nalidixic acid could inhibit the proliferation of flacherie virus and nuclear polyhedrosis virus (*Bm*NPV) and prevent silkworms from related virus infection. Flufenoxuron, an insect growth regulator, promoted infection of *Bm*NPV in fifth instar *B. mori* larvae, when the chemical was dissolved in acetone and incorporated into the larval diet. And the LD_{50} of *Bm*NPV was decreased as the concentration of flufenoxuron increased. However, undissolved flufenoxuron did not exert such an effect when it was incorporated into the diet as a powder form (Arakawa et al. 2002). Studies have shown that flufenoxuron can be used as auxiliaries in the use of insect viruses to control agricultural pests.

Other Applications of the Silkworm Model

In addition to being an experimental model, silkworm is an important information carrier in cutting-edge science and technology. Epigenetics, a branch of genetics, studies genetic variation at the level of modifications of DNA and chromatin in the absence of differences in the sequence of the gene (Häfner et al. 2016). In 2010, the first silkworm methylation map was constructed. DNA methylation, as an important marker of epigenetics, plays an important role in gene regulation. It was found that about 0.11% of genomic cytosine in silkworm was methylated, which was less than 1/50 of that of mammals and plants. At the same time, methylation of transposable elements, promoters, and ribosomal RNAs was low, and methylation regions were mainly concentrated in the gene region and positively correlated with the gene expression level. This phenomenon shows that methylation plays an active role in gene transcription, and the construction of methylation spectrum of silkworm provides a reference for the study of insect epigenetic regulation (Xiang et al. 2010).

Porphyromonas gingivalis is a pathogen of human periodontal tissue inflammation (Darveau et al. 2012). Ishii et al. (2010) conducted a study on the periodontal disease model of silkworm. The study found that the bacteria injected into the silkworm hemolymph can quickly kill the silkworm. Injection of the pathogen causes the silkworm blood to be blackened, and the activity of caspase in the silkworm tissue is increased. However, injections of antioxidants (glutathione, catalase), inhibitors of blackening (phenylthio and serine protease inhibitors), and caspase inhibitors can delay the death of silkworms. Periodontal disease mildly infected silkworm model, the results show that *P. gingivalis* excessive stimulating activation of natural immune induced silkworm death. Similarly, the human infection of periodontal disease can also cause death (Ishii et al. 2010). It is generally accepted that the death of a patient with periodontal pathogens is caused by cytokine storm, similar to the periodontal disease model of silkworm.

In recent years, silkworm bioreactors has been rapidly developed and widely used, including silk gland bioreactor, nuclear polyhedrosis virus (NPV) expression system bioreactor, transgenic silkworm reactor, and so on (Wu 2014). Silkworm bioreactors are widely used in the field of biology, medicine, and food. For example, bioreactors have been successfully used to express exogenous proteins, such as human recombinant DNA polymerase and high-fidelity recombinant proteins (Zhou et al. 2011, Park et al. 2015).

Summary and Prospect

The silkworm model has been successfully used in various aspects of life science research and has greatly promoted scientific development in this field. However, there are still many challenges ahead, and the application of silkworm model in many areas is just at the initial stage, which lacks sufficient animal experiments and clinical trial data. Silkworm may be used to study drug efficacy in place of mammals in the near future. However, there are several disadvantages associated with silkworm model. It is not applicable using silkworm as a model in genetic disease study because silkworm is not susceptible to human genetic diseases, such as neurological disorders or neurodegenerative diseases. Also silkworm does not exhibit emotional disorders such as depression or anxiety. Although silkworm can not replace mammals completely, it plays a complementary and supplementary role. In conclusion, promoting the establishment of silkworm models in scientific studies will provide new solutions and new insights into traditional views of problem-solving and greatly benefit science as well as the society.

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References Cited

Arakawa, T., and M. Sugiyama. 2002. Promotion of nucleopolyhedrovirus infection in larvae of the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae) by an antibiotic, nikkomycin Z. Appl.Entomol. Zool. 37: 393–397.

Auer, T. O., K. Duroure, C. A. De, J. P. Concordet, and B. F. Del. 2014. Highly efficient CRISPR/Cas9-mediated knock-in in *Zebrafish* by homology-independent dna repair. Genome Res. 24: 142–153.

Bassett, A. R., C. Tibbit, C. P. Ponting, and J. L. Liu. 2013. Highly efficient targeted mutagenesis of *Drosophila* with the CRISPR/Cas9 system. Cell Rep. 4: 1178–1179.

Blau, N., and L. B. Bonafe. 2001. Minireview tetrahydrobiopterin deficiencies without hyperphenylalaninemia: diagnosis and genetics of dopa-responsive dystonia and sepiapterin reductase deficiency. Mol. Genet. Metab. 74: 172–185.

Bonafé, L., B. Thöny, J. M. Penzien, B. Czarnecki, and N. Blau. 2001. Mutations in the sepiapterin reductase gene cause a novel tetrahydrobiopterin-dependent monoamine-neurotransmitter deficiency without hyperphenylalaninemia. Am. J Hum. Genetics. 69: 269–277.

Chen, K. P., J. T. Huang, and Q. Yao. 2014. Modle organism *Bombyx mori*. Phoenix Science Press, Nan Jing, China.

Chen, M., J. B. Song, L. I. Zhi-Quan, D. M. Tang, X. L. Tong, and F. Y. Dai. 2016. Progress and perspective of silkworm as a model of human diseases for drug screening. Acta Pharm. Sin. 51: 690–697.

Choi, H. K., D. B. Mount, A. M. Reginato, American College of Physicians; American Physiological Society. 2005. Pathogenesis of Gout. Ann. Intern. Med. 143: 499–516.

Darveau, R. P., G. Hajishengallis, and M. A. Curtis. 2012. *Porphyromonas gingivalis* as a potential community activist for disease. J. Dental Res. 91: 816–820.

Dhital, S., H. Hamamoto, M. Urai, K. Ishii, and K. Sekimizu. 2011. Purification of innate immunostimulant from green tea using a silkworm muscle contraction assay. Drug Discov. Ther. 5: 18–25.

Fujii, T., T. Daimon, K. Uchino, Y. Banno, S. Katsuma, H. Sezutsu, T. Tamura, and T. Shimada. 2010. Transgenic analysis of the BmBLOS2 gene that governs the translucency of the larval integument of the silkworm, *Bombyx mori*. Insect Mol Biol. 19: 659–667.

Fujii, T., Y. Banno, H. Abe, S. Katsuma, and T. Shimada. 2012. A homolog of the human hermansky-pudluck syndrome-5 (HPS5) gene is responsible for the *oa* larval translucent mutants in the silkworm, *Bombyx mori*. Genetica. 140: 463–468.

Fujiyuki, T., H. Hamamoto, K. Ishii, M. Urai, K. Kataoka, T. Takeda, S. Shibata, and K. Sekimizu. 2012. Evaluation of innate immune stimulating activity of polysaccharides using a silkworm (*Bombyx mori*) muscle contraction assay. Drug Discov. Ther. 6: 88–93.

Häfner, S. J., and A. H. Lund. 2016. Great expectations – epigenetics and the meandering path from bench to bedside. Biomed. J. 39: 166–176.

Hamamoto, H., K. Kurokawa, C. Kaito, K. Kamura, I. M. Razanajatovo, H. Kusuhara, T. Santa, and K. Sekimizu. 2004. Quantitative evaluation of the therapeutic effects of antibiotics using silkworms infected with human pathogenic microorganisms. Antimicrob. Agents Chemother. 48: 774–779.

Hamamoto, H., A. Tonoike, K. Narushima, R. Horie, and K. Sekimizu. 2009. Silkworm as a model animal to evaluate drug candidate toxicity and metabolism. Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 149: 334–339.

Hanada, Y., K. Sekimizu, and C. Kaito. 2011. Silkworm apolipophorin protein inhibits *Staphylococcus aureus* virulence. J. Biol. Chem. 286: 39360–393609.

Hayashi, Y. 1962. Some properties of the xanthine dehydrogenase of the silkworm, *Bombyx mori*, L. Biochim. Biophys. Acta. 58: 351–352.

Inagaki, Y., Y. Matsumoto, K. Kataoka, N. Matsuhashi, and K. Sekimizu. 2012. Evaluation of drug-induced tissue injury by measuring alanine aminotransferase (alt) activity in silkworm hemolymph. BMC Pharmacol. Toxicol. 13: 1–7.

Ishii, K., H. Hamamoto, M. Kamimura, and K. Sekimizu. 2008. Activation of the silkworm cytokine by bacterial and fungal cell wall components via a reactive oxygen species-triggered mechanism. J. Biol. Chem. 283: 2185–2191.

Ishii, K., H. Hamamoto, K. Imamura, T. Adachi, M. Shoji, K. Nakayama, and K. Sekimizu. 2010. *Porphyromonas gingivalis* peptidoglycans induce excessive activation of the innate immune system in silkworm larvae. J. Biol. Chem. 285: 33338–33347.

Ishii, K., T. Adachi, H. Hamamoto, T. Oonish, M. Kamimura, K. Imamura, and K. Sekimizu. 2013. Insect cytokine paralytic peptide activates innate immunity via nitric oxide production in the silkworm *Bombyx mori*. Dev. Comp. Immunol. 39: 147–153.

Ishii, K., H. Hamamoto, and K. Sekimizu. 2015a. Studies of host-pathogen interactions and immune-related drug development using the silkworm: interdisciplinary immunology, microbiology, and pharmacology studies. Drug Discov. Ther. 9: 238–246.

Ishii, K., H. Hamamoto, and K. Sekimizu. 2015b. Paralytic peptide: an insect cytokine that mediates innate immunity. Arch. Insect Biochem. Physiol. 88: 18–30.

Ishii, M., Y. Matsumoto, I. Nakamura, and K. Sekimizu. 2017. Silkworm fungal infection model for identification of virulence genes in pathogenic fungus and screening of novel antifungal drugs. Drug Discov. Ther. 11: 1–5.

Kaito, C., and K. Sekimizu. 2007. A silkworm model of pathogenic bacterial infection. Drug Discov. Ther. 1: 89–93.

Kaito, C., N. Akimitsu, H. Watanabe, and K. Sekimizu. 2002. Silkworm larvae as an animal model of bacterial infection pathogenic to humans. Microb. Pathog. 32: 183–190.

Kodama, R., and Y. Nakasuji. 1972. Bacteria isolated from silkworm larvae: X. Inhibition of development of viral diseases in gnotobiotic silkworm by nalidixic acid. J. Insect Biotechnol. Sericol. 41: 7–41.

Levy, N. 2012. The use of animal as models: ethical considerations. Int. J. Stroke. 7: 440–442.

Li, Y., M. Ni, F. C. Li, H. Zhang, K. Z. Xu, X. M. Zhao, J. H. Tian, J. S. Hu, B. B. Wang, W. D. Shen, et al. 2016. Effects of TiO$_2$, NPS on silkworm growth and feed efficiency. Biol. Trace Elem. Res. 169: 1–5.

Liu, Y., S. Ma, X. Wang, J. Chang, J. Gao, R. Shi, J. Zhang, W. Lu, Y. Liu, P. Zhao, et al. 2014. Highly efficient multiplex targeted mutagenesis and genomic structure variation in *Bombyx mori*, cells using CRISPR/Cas9. Insect Biochem. Mol. Biol. 49: 35–42.

Ma, S., J. Chang, X. Wang, Y. Liu, J. Zhang, W. Lu, J. Gao, R. Shi, P. Zhao, and Q. Xia. 2014. CRISPR/Cas9 mediated multiplex genome editing and heritable mutagenesis of Bmku70 in *Bombyx mori*. Sci. Rep. 4: 4489.

Matsumoto, Y., S. Miyazaki, D. H. Fukunag, K. Shimizu, S. Kawamoto, and K. Sekimizu. 2012. Quantitative evaluation of cryptococcal pathogenesis and antifungal drugs using a silkworm infection model with *Cryptococcus neoformans*. J. Appl. Microbiol. 112: 138–146.

Matsumoto, Y., M. Ishii, K. Ishii, W. Miyaguchi, R. Horie, Y. Inagaki, H. Hamamoto, K. Tatematsu, K. Uchino, T. Tamura, et al. 2014. Transgenic silkworms expressing human insulin receptors for evaluation of therapeutically active insulin receptor agonists. Biochem. Biophys. Res. Commun. 455: 159–164.

Matsumoto, Y., M. Ishii, Y. Hayashi, S. Miyazaki, T. Sugita, E. Sumiya, and K. Sekimizu. 2015. Diabetic silkworms for evaluation of therapeutically effective drugs against type II diabetes. Sci. Rep. 5: 10722.

Mita, K. 2008. The genome of a lepidopteran model insect, the silkworm *Bombyx mori*. Insect Biochem. Mol. Biol. 38: 1036–1045.

Meng, Y., S. Katsuma, T. Daimon, Y. Banno, K. Uchino, and H. Sezutsu. 2009. The silkworm mutant lemon (lemon lethal) is a potential insect model for human sepiapterin reductase deficiency. J. Biol. Chem. 284: 11698–11705.

Meng, X., N. Abdlli, N. Wang, L. Peng, Z. C. Nie, X. Dong, S. Lu, and K. P. Chen. 2017. Effects of Ag nanoparticles on growth and fat body proteins in silkworms (*Bombyx mori*). Biol. Trace Elem. Res. 1–11.

Miyazaki, S., Y. Matsumoto, K. Sekimizu, and C. Kaito. 2012. Evaluation of *Staphylococcus aureus* virulence factors using a silkworm model. FEMS Microbiol. Lett. 326: 116–124.

Nwibo, D. D., H. Hamamoto, Y. Matsumoto, C. Kaito, and K. Sekimizu. 2015. Current use of silkworm larvae (*Bombyx mori*) as an animal model in pharmaco-medical research. Drug Discov. Ther. 9: 133–135.

Park, S., I. W. Hwang, T. Kato, E. Park, and A. Terzic. 2015. High-fidelity recombinant protein production in a silkworm bioreactor. Protein Sci. 112–112.

Panthee, S., A. Paudel, H. Hamamoto, and K. Sekimizu. 2017. Advantages of the silkworm as an animal model for developing novel antimicrobial agents. Front. Microbiol. 8: 1–8.

Renwick, J., and K. Kavanagh. 2007. Insects as models for studying the virulence of fungal pathogens of humans, pp. 45–67. New insights in medical mycology. Springer, Dordrecht, the Netherlands.

Sekimura, T. 2005. The effect of heavy metal cadmium on growth, survival rate, and genetics of silkworm. Annu. Rep. Coll. Biosci. Biotechnol. 4: 15–20.

Srisailam, S., A. I. Arunkumar, W. Wang, C. Yu, and H. M. Chen. 2000. Conformational study of a custom antibacterial peptide cecropin b1: implications of the lytic activity. Biochim. Biophys. Acta. 1479: 275–285.

Sugiyama, H., and T. Emori. 1980. Pesticide residues of MEP, MPP and PAP in mulberry stumps and its effect on the silkworm (*Bombyx mori* L.). J. Pestic. Sci. 5: 423–425.

Tian, J. H., J. S. Hu, F. C. Li, M. Ni, Y. Y. Li, B. B. Wang, K. Z. Xu, W. D. Shen, and B. Li. 2016. Effects of TiO$_2$ nanoparticles on nutrition metabolism in silkworm fat body. Biol. Open. 5: 764–769.

Ueno, K., Y. Matsumoto, J. Uno, K. Sasamoto, K. Sekimizu, Y. Kinjo, and H. Chibana. 2011. Intestinal resident yeast, candida glabrata, requires Cyb2p-mediated lactate assimilation to adapt in mouse intestine. PLoS One. 6: 65.

Usui, K., S. Nishida, T. Sugita, T. Ueki, Y. Matsumoto, H. Okumura, and K. Sekimizu. 2016. Acute oral toxicity test of chemical compounds in silkworms. Drug Discov. Ther. 10: 57–61.

Wang, J., and H. C. Schweizer. 2008. A silkworm baculovirus model for assessing the therapeutic effects of antiviral compounds: characterization and application to the isolation of antivirals from traditional medicines. J. General Virol. 89: 188–194.

Wang, Y., Z. Li, J. Xu, B. Zeng, L. Ling, L. You, Y. Chen, Y. Huang, and A. Tan. 2013. The crispr/cas system mediates efficient genome engineering in *Bombyx mori*. Cell Res. 23: 1414–1416.

Wang, L., T. Kiuchi, T. Fujii, T. Daimon, M. Li, Y. Banno, S. Katsuma, and T. Shimada. 2013. Reduced expression of the dysbindin-like gene in the *Bombyx mori* ov mutant exhibiting mottled translucency of the larval skin. Genome. 56: 101–108.

Wei, M. L. 2006. Hermansky-pudlak syndrome: a disease of protein trafficking and organelle function. Pigment Cell Melanoma Res. 19: 19–42.

Wu, X. F., and Y. Z. Zhang. 2014. Silkworm bioreactor. Science and Technology of China Press, Beijing, China.

Xiang, H., J. Zhu, Q. Chen, F. Dai, X. Li, and M. Li. 2010. Single base-resolution methylome of the silkworm reveals a sparse epigenomic map. Nat. Biotechnol. 28: 516–520.

Xia, Q., and H. Yang. 2004. A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). Science. 306: 1937–1940.

Xia, Q., Y. Guo, Z. Zhang, D. Li, Z. Xuan, Z. Li, F. Dai, Y. Li, D. Cheng, R. Li, et al. 2009. Complete resequencing of 40 genomes reveals domestication events and genes in silkworm (*Bombyx*). Science. 326: 433–436.

Yokoyama, T. 1976. On the influence of paramidine, a drug for gout, on silkworm. Reports of the Silk Science Research Institute, Tokyo, Japan.

Yusuf, M., Q. Fariduddin, S. Hayat, and A. Ahmad. 2011. An invertebrate hyperglycemic model for the identification of anti-diabetic drugs. PLoS One. 6: 589.

Zhang, X. L., R.Y. Xue, G. L. Cao, Z. H. Pan, X. J. Zheng, and C. L. Gong. 2015. Silkworms can be used as an animal model to screen and evaluate gouty therapeutic drugs. J. Insect Sci. 12: 4.

Zhou, Y., H. Chen, X. Li, Y. Wang, K. Chen, S. Zhang, X. Meng, E. Y. Lee, and M. Y. Lee. 2011. Production of recombinant human DNA polymerase delta in a *Bombyx mori* bioreactor. PLoS One. 6: e22224.