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Research Article

Cloning and Expression of SCAMP3 in Transgenic Silkworm (*Bombyx mori*)

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Abstract: SCAMP3 (Secretory Carrier Membrane Protein 3) is a crucial component of vesicular trafficking pathways and plays important roles in endocytosis and protein recycling. Previous studies demonstrate SCAMP3 dysregulation in various cancers, suggesting potential applications as a biomarker and therapeutic target. Understanding SCAMP3 expression and post-translational processing provides insights into its biological functions and potential applications in cancer diagnostics and therapy. In this study, we utilized transgenic *Bombyx mori* (silkworm) with the Bac-to-Bac expression system to produce recombinant SCAMP3. Protein expression was analyzed using Western blot and enzyme-linked immunosorbent assay (ELISA) techniques. Both methods confirmed successful SCAMP3 expression in transgenic silkworms. Western blot analysis revealed multiple protein products, indicating proteolytic processing during or following expression. This cleavage pattern may reflect functional protein maturation or processing, providing insights into SCAMP3 structural dynamics and functional domains. These findings demonstrate the utility of *B. mori* as a robust eukaryotic expression system for producing and studying proteins requiring complex post-translational modifications. The transgenic silkworm platform offers advantages for investigating structure-function relationships and generating proteins for potential therapeutic applications.

Keywords: SCAMP3, *Bombyx mori*, Transgenic Silkworm, Bac-to-Bac System, Recombinant Protein Expression, Post-Translational Modification, Cancer Biomarker, Vesicular Trafficking

Introduction

Secretory Carrier Membrane Proteins 3 (SCAMP3), a member of the SCAMP protein family (SCAMP 1-5), are integral membrane proteins that are ubiquitously expressed across species and are primarily prevalent in secretory membranes (Brand et al., 1991; Laurie et al., 1993; Hubbard et al., 2000). All SCAMPs have a highly conserved tetraspanning transmembrane core known as the SCAMP domain, which is centrally located and composed of four hydrophobic transmembrane domains (TMDs) with three inter-TMD-flanking sequences. (Hubbard et al., 2000). This 38 kDa protein differs from the other SCAMP proteins in that it has a proline-rich domain near its N-terminus.

SCAMP3 is widely distributed in membranes between the cell surface and internal compartments, such as early and late endosomes, Golgi-derived vesicles, membranes that circulate various transporters, and secretory granules and vesicles in hematopoietic cells (Hubbard et al., 2000). SCAMP3's localization suggests that it may play an important role in vesicular transport processes such as vesicle trafficking, membrane fusion and recycling, endocytosis and exocytosis control, and cellular signaling (Singleton et al., 1997).

Homologs of SCAMPs have been detected in humans, metazoans, plants, and certain fungi. In addition to normal cells, SCAMP3 is expressed in cancer cells as well. A previous study has shown that SCAMP3 is overexpressed in hepatocellular carcinoma tissues and associated with hepatocellular carcinoma progression (Zhang et al., 2017). Increasing expression of SCAMP3 in glioma patients were found to enhance tumor growth through a pathway reliant on mTORC1/EGFR signaling, correlating with unfavorable prognosis and decreased survival (Li et al., 2020). Knockdown SCAMP3 was also shown to inhibit melanoma cells and breast cancer cells proliferation and invasion (Tseng et al., 2019, Acevedo-Diaz et al., 2022). Previous studies on SCAMP3 expression in cancer suggest that SCAMP3 has the potential biomarker for cancer and a target for cancer therapy.

The cloning and expression of the SCAMP3 gene aim to produce proteins that will be used for diagnostic and therapeutic purposes. Recombinant DNA technology enables the production of large quantities of proteins that are otherwise scarce or difficult to obtain from natural sources. Among the many hosts available for recombinant protein production, the silk worm *Bombyx mori* (*B. mori*) stands out as a highly efficient system capable of yielding large quantities of complex proteins.

One of the key advantages of *B. mori* as an expression system is its ability to perform eukaryotic post-translational modifications which are essential for the proper folding and functionality of many proteins. Unlike bacterial systems (e.g., *Escherichia coli*), silkworms ensure that recombinant proteins maintain their native structure and biological activity. This feature is particularly important for therapeutic and diagnostic applications, where functional integrity is crucial. Compared to mammalian cell expression systems, silkworm-based expression is more cost-effective and scalable, as it does not require expensive culture media or sophisticated bioreactor setups. Furthermore, silkworm larvae can be directly injected with recombinant bacmids, eliminating the need for complex transfection procedures, which further simplifies production (Kato et al., 2009). The first production of foreign protein (recombinant human α-interferon) in silkworm was first reported by Maeda et al. (1985) using the *B. mori* nucleopolyhedrovirus (BmNPV) expression system. BmNPV, which belongs to the family of Baculoviridae, is a type of virus that infects the domesticated silkworm B. mori. The Baculoviruses were initially utilized as biopesticides for insect control due to their limited host range. Subsequently, the baculovirus-based expression vector system (BEVS) has gained popularity for overexpressing cloned foreign genes in insect cells and larval caterpillars. Most eukaryotic proteins synthesized in insect cells undergo post-translational processing in a manner comparable to native proteins and are physiologically active (Vaitsopoulou et al., 2022).

In this study, we successfully cloned and expressed SCAMP3 in silkworm, utilizing the baculovirus expression system. We optimized the transformation, transfection, and protein expression processes to achieve high yield and functional protein production. The expressed SCAMP3 protein was characterized through Western blotting and ELISA to confirm its integrity and activity. The study is novel as it demonstrates SCAMP3 expression in silkworms, a system not previously reported for this protein This work demonstrates the potential of *B. mori* as a cost-effective and scalable platform for recombinant protein production with applications in functional studies of SCAMP3 involved in intracellular trafficking and membrane dynamics.

Materials and Methods

Silkworm Growth Condition

Silkworm (*Bombyx mori*) strain PS1 procured by Rumah Sutera farm, Indonesia, was reared on a natural diet of Mulberry leaves at 26°C. The silkworm larvae on the 7th day of the 5th instar were prepared for the microinjection.

Recombinant Plasmid Construction

The synthetic gene encoding SCAMP3 was designed using SnapGene software version 8.0. The pFastBac plasmid was selected as the expression vector due to its compatibility with the Bac-to-Bac baculovirus system. The *SCAMP3* gene, flanked by restriction enzyme recognition sites compatible with the MCS (Multiple Cloning Site) of the pFastBac plasmid, was virtually ligated into the plasmid backbone. The resulting construct included the *SCAMP3* gene in-frame with the 8x His tag.

Transforming of SCAMP3 Gene

The protocol of transforming the *SCAMP3* gene following the Bac-to-Bac Baculovirus Expression System by Invitrogen. 10 ng of *SCAMP3* synthetic gene has been transformed to DH10Bac competent cells by heat shock to induce the DNA uptake. Cells were incubated at 37°C with shaking (200 rpm) for 1 hour to allow recovery and expression of antibiotic resistance genes. The recovered culture was plated onto LB agar plates containing kanamycin (50 µg/mL), gentamicin (7 µg/mL), tetracycline (10 µg/mL), IPTG (Isopropyl β-d-1-thiogalactopyranoside) (40 µg/mL), and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (100 µg/mL), then was incubated at 37°C for 16–18 hours. Following incubation, white colonies (indicative of successful transformation) were selected for further analysis, as blue colonies result from non-recombinant bacmids.

Analyzing recombinant bacmid DNA by PCR

A single colony of recombinant bacmid DNA was verified by PCR using the M13 Forward and Reverse primers as below:

M13 Forward

5'-CCCAGTCACGACGTTGTAAAACG-3'

M13 Reverse

5'-AGCGGATAACAATTTCACACAGG-3'

The PCR product was analyzed by 1% agarose gel electrophoresis.

Isolating Recombinant Bacmid DNA

A single white bacterial colony was inoculated into 500 ml LB medium with kanamycin (50 µg/mL), gentamicin (7 µg/mL), tetracycline (10 µg/mL) and incubated at 37°C in a shaking water bath at 200 rpm overnight. The high quality Bacmid DNA from DH10Bac E. coli was isolated based on The PureLinkTM HiPure Plasmid DNA Miniprep Kit.

Injection to Silkworm

A solution of recombinant bacmid DNA (containing the SCAMP3 gene) at a concentration of 100 – 500 ng/µL in 1x PBS was injected into hemocoel of each larva through the intersegmental membrane. The injected larvae are transferred to a clean rearing container and maintained at 26-28ºC with optimal humidity (approximately 70%). Collection of tissue

The hemolymph and fat body were harvested from the larvae 5 days post-injection. Hemolymph collection is performed by cutting the tip of the larva's leg, while fat body collection is carried out by dissecting the larva. All hemolymph and fat body were placed in 100 µL sterile PBS.

Western Blot Analysis

A Western blot was carried out to verify the expression of recombinant SCAMP3 protein. A 5 µL of samples and negative control at a concentration of 10 µg/mL were applied. Using a semi-dry electroblotting device (Bio-Rad), the protein was initially separated by SDS-PAGE and then transferred to a nitrocellulose membrane. After that, the membrane was placed in a blocking buffer (5% BSA in PBS buffer with 0.1% Tween 20, PBS-T) overnight at room temperature. After PBS-T washing, the membrane was incubated for 4 hours at room temperature with a primary antibody against His-tag (1:4000) and α-SCAMP3 (1:500) separately in the blocking solution. The membrane was treated with a secondary anti-mouse antibody (1:20000) and anti-rabbit antibody (1:5000) for 1 hour at room temperature following repeated washing processes with PBS-T.

ELISA Analysis

The expressed SCAMP3 in silkworm larvae was quantified using ELISA. 2 µL of samples at a concentration of 6 µg/mL was coated by 100 µL of 0.05 M carbonate-bicarbonate buffer (pH 9.6). Each well received 100 µL of the antigen solution and was incubated overnight at 4°C. After coating, the wells were washed three times with PBS containing 0.05% Tween-20 (PBS-T) to remove unbound antigen. To block nonspecific binding sites, 200 µL of blocking buffer (1% BSA in PBS-T) was added to each well, and the plate was incubated for 1 hour at room temperature. Following blocking, the wells were washed three times with PBS-T 0,1%. The protein was then incubated by primary antibody anti-His (1:1000) for overnight at room temperature after repeated washing process with the washing buffer. 100 µL of substrate (0.1 mg/ml 3,3',5,5'-tetramethylbenzidine 21 (TMB) in 100 mM sodium acetate, pH 6.0, with 0.2% (v/v) of 30% hydrogen peroxidase) was added to each well and left at room temperature for blue color change development. The reaction was stopped by adding 100 µL of 0,5 N H2SO4 solution.

Results

Verification of the recombinant plasmid

The construction of the pFastBac-SCAMP3 synthetic recombinant is illustrated in Fig. 1.

The recombinant of pFastBac-SCAMP3 construct was designed to include an 8x His tag at the C-terminal of the SCAMP3 protein to allow for easy purification using nickel-affinity chromatography and detection via anti-His antibodies in Western blot assays.

Recombinant pFastBac-SCAMP3 plasmids were transformed into chemically competent E. coli DH10Bac cells containing the bacmid backbone and a helper plasmid system. The cells were plated onto LB agar containing specific antibiotics (kanamycin, gentamicin, tetracycline), X-gal, and IPTG to enable blue-white screening. Successful recombination between the pFastBac-SCAMP3 donor plasmid and the bacmid disrupted the lacZ gene, resulting in the formation of white colonies. Non-recombinant bacmids retained an intact lacZ gene, producing blue colonies upon hydrolysis of X-gal. After 48 hours of incubation at 37°C, white colonies were selected as putative recombinant bacmids (Fig. 2).

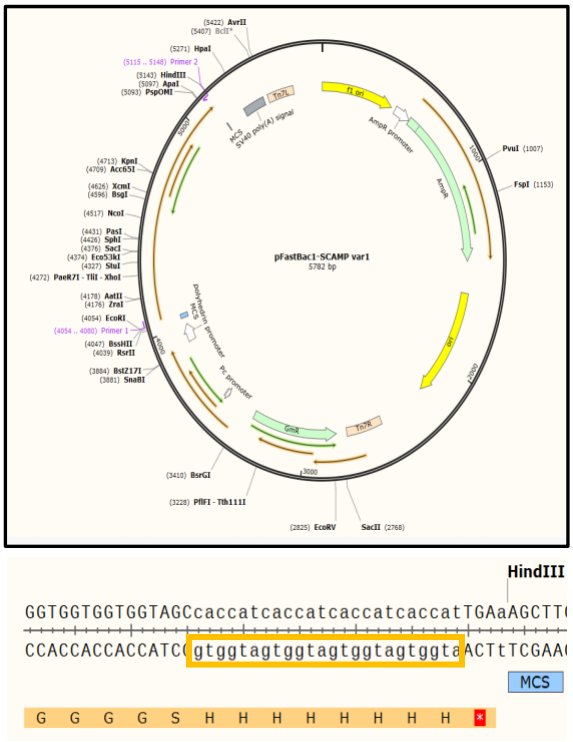

Fig. 1: (Above) P lasmid map of pFastBac1-SCAMP3 variant 1 (5712 bp). The plasmid contains an ampicillin resistance gene (AmpR) for selection, a Tn7 transposon region for site-specific integration into the baculovirus genome, and a multiple cloning site (MCS) for insertion of the SCAMP3. (Below) pFastBac-SCAMP3 construction including the 8x-His addition of 8x His in C-terminal

Confirmation of the Recombinant Bacmid

White colonies were picked and cultured in LB medium, and recombinant bacmid DNA was isolated using an alkaline lysis method. The verification of the recombinant bacmid by PCR using M13 primers, which produced an expected band size of 3869 bp as shown in Fig. 3, confirmed that the gene had been successfully inserted into the bacmid. These recombinant bacmids were subsequently used for silkworm injection.

A concentration of 200 ng of SCAMP3 recombinant bacmid DNA was injected into the hemocoel of fifth-instar silkworm larvae to facilitate protein expression in this organism.

Following injection, the larvae were monitored for physiological changes, feeding behavior, and overall survival. No significant differences were observed between the recombinant bacmid-injected larvae and the control group injected with sterile buffer.

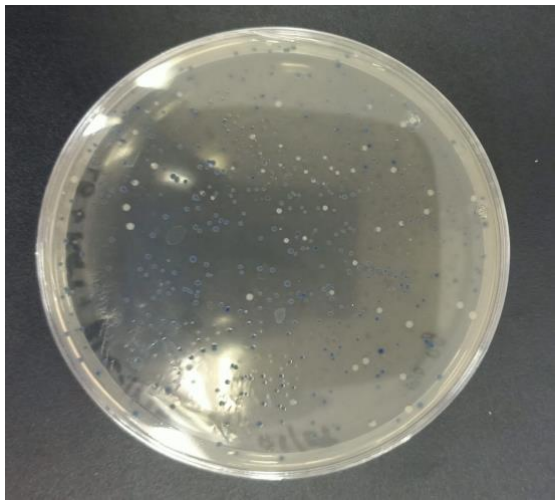

Fig. 2: Blue-white screening of recombinant bacterial colonies on an LB agar plate supplemented with X-Gal and IPTG. The blue colonies suggest the presence of non-recombinant bacteria. The white colonies indicate successful ligation of the SCAMP3 gene into the plasmid

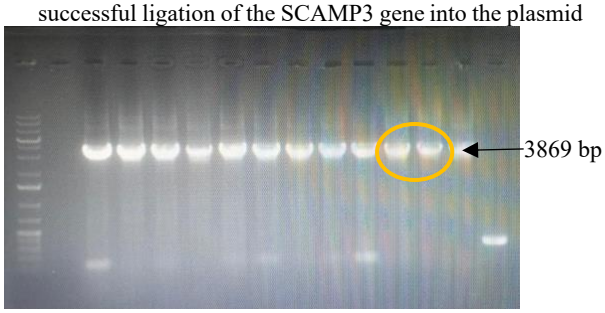

Fig. 3: Agarose gel electrophoresis analysis of PCR using M13 primer for recombinant bacmid verification. The expected band size of 3869 bp (in yellow circle) confirmed the recombinant bacmid

At 5 days post-injection, hemolymph and fat body tissues were collected for analysis. Total protein was extracted from these samples and analyzed by SDS-PAGE and Western blotting with α-His and α-SCAMP3 as primary antibody. The SCAMP3 protein was successfully expressed, as indicated by the appearance of distinct faint bands on the gel (Fig. 4) and membrane.

The analysis of SCAMP3 protein using anti-SCAMP3 revealed four bands at sizes of 38 kDa, 31 kDa, 27 kDa, and 21 kDa (Fig. 5.).

In contrast, analysis with anti-His showed only a single band at 38 kDa (Fig. 6)

To determine whether SCAMP3 is naturally expressed in *E. coli* and mammals, we performed Western blot analysis on *E. coli* pET samples induced and uninduced by IPTG, as well as MCF7 cell line samples representing mammals (Fig. 7).

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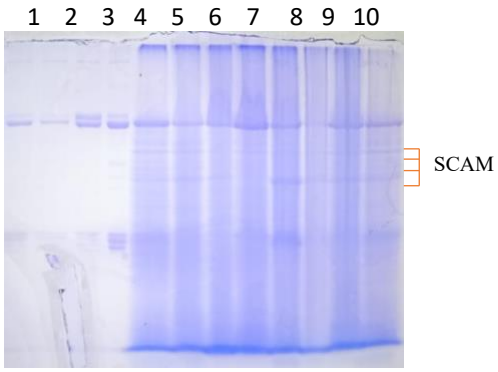

Fig. 4: SDS-PAGE analysis of protein expression in recombinant samples. The gel was stained with Coomassie Brilliant Blue. Lane 1-2: negative control; 3-4: SCAMP3 from silkworm's haemolymph; 5-12: SCAMP3 from silkworm's fat bodies. Four distinct faint bands on the lane 12 indicate SCAMP3 protein which undergoes proteolytic cleavage

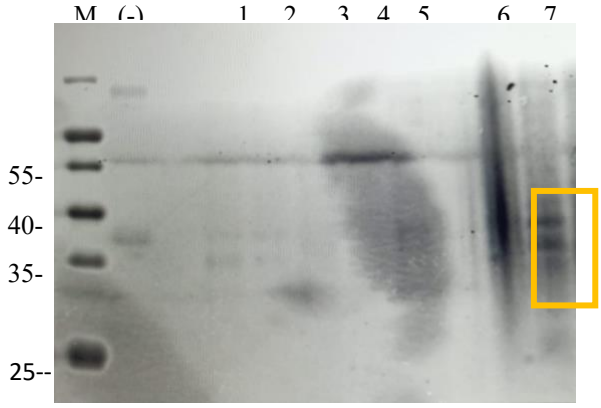

Fig. 5: Western blot analysis of recombinant SCAMP3 protein expression. Protein samples were separated by SDS-PAGE and transferred onto a PVDF membrane for immunodetection using an anti-SCAMP3 primary antibody and an anti-rabbit secondary antibody. Lane M is protein ladder marker. Lane (-) is negative control. Lane 1-9 are the samples with various dilution. The highlighted region on the right (orange box) indicates the detected protein bands, suggesting the presence of SCAMP3 or its cleaved fragments

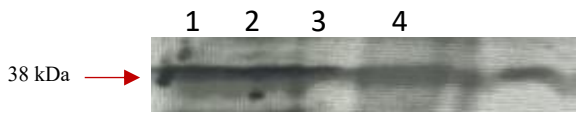

Fig. 6: Western blot analysis of recombinant SCAMP3 protein expression. Protein samples were separated by SDS-PAGE and transferred onto a PVDF membrane for immunodetection using an anti-His primary antibody and an anti-mouse secondary antibody. Lane 1-4 are the samples with various dilution. A band of 38kDa in size indicated SCAMP3 (pointed by red arrow). Protein analyzes by Western blot using α-His 1:4000 (1-4: samples with various dilution)

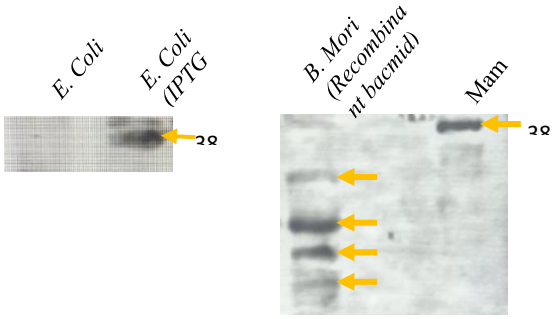

Fig. 7: Western blot analysis of SCAMP3 expression in different expression systems. SCAMP3 expression in *E. coli and Mammals* showing a single band at the expected molecular weight. SCAMP3 expression in B. mori fat body showing multiple bands, indicating possible proteolytic cleavage

To support the data that SCAMP3 is expressed in *B. mori*, an ELISA was performed using fat body samples from injected silkworms and untreated silkworms as a negative control. The mean ELISA OD values were significantly higher in recombinant larvae compared to the negative control. A t-test analysis confirmed a statistically significant difference ($p < 0.05$), supporting the successful expression of SCAMP3 in *B. mori*. (Fig. 8).

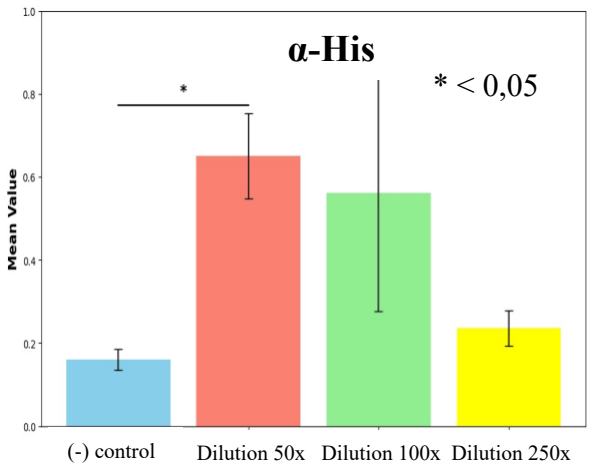

Fig 8: Detection of SCAMP3 expression in the fat body of bacmid-transformed *B. mori* larvae using ELISA with an α-His (Histidine) antibody. Mean ELISA OD values (± standard error) are shown for different sample dilutions: negative control (untreated larvae, 0.20 ± 0.02), 50× dilution (0.75 ± 0.08), 100× dilution (0.60 ± 0.15), and 250× dilution (0.30 ± 0.05). The 50× dilution group exhibited the highest OD value, indicating the strongest SCAMP3 expression. A significant difference ($p < 0.05$, indicated by *) was observed between the 50× dilution group and the negative control

Discussion

Research on SCAMP3 expression in mammals has been extensively conducted, yet there is no previous evidence of its expression in silkworms. The cloning and expression of SCAMP3 in silkworms represent a significant advancement in the development of alternative protein expression systems, offering both efficiency and scalability. In this study, we achieved to clone and express the SCAMP3 in *B. mori*, which is an important proof of concept for utilizing insect models in the production of functional recombinant proteins.

The selection of the pFastBac plasmid as the vector for SCAMP3 expression was based on its compatibility with the baculovirus expression system in *B. mori*. This vector enables efficient recombinant protein production in insect cells by driving high levels of transcription through the highly active polyhedrin promoter in baculovirus-infected cells. Furthermore, the pFastBac vector supports proper folding and post-translational modifications, which are essential for the functional expression of eukaryotic proteins like SCAMP3. Utilizing the baculovirus system with *B. mori* as a host offers an economical and scalable solution for producing recombinant proteins (Felberbaum, 2015).

The successful cloning and expression of SCAMP3 in *B. mori* was confirmed through Western blot analysis. Western blot analysis using α-SCAMP3 antibody revealed the presence of four distinct bands, suggesting that SCAMP3 undergoes cleavage into multiple fragments. This pattern indicates that SCAMP3 may be processed into smaller isoforms or fragments after expression. This resembles the process observed in SCAMP1, where sequential cleavage takes place in the N- and C-terminal regions, which is probably related to protein folding (Hubbard et al., 2000). One possible reason for the cleavage of recombinant SCAMP3 in silkworms is the presence of endogenous proteases in the *B. mori* fat body. Previous studies have reported significant proteolytic activity in the fat body, with an optimal pH of 4.0, particularly in 5- to 6-day-old female pupae (Yaginuma and Ushizima, 1991). These endogenous proteases may cleave recombinant proteins during expression, potentially leading to degradation or fragmentation, which could impact protein stability and yield.

When the same sample was probed with α-His antibody, a single band was observed. This result indicates that the His-tagged SCAMP3 protein, irrespective of cleavage, is being detected as a whole, with the His-tag serving as a reliable marker for the protein. The difference in band patterns observed with the two antibodies emphasizes the specificity of α-SCAMP3 in detecting different forms of the protein, while α-His provides an overall detection of the His-tagged recombinant protein.

The significant difference in SCAMP3 expression levels, as determined through ELISA using anti-His antibodies, demonstrates the successful expression of SCAMP3 in the silkworm system. ELISA assays are commonly employed in clinical and research settings to analyze proteins, peptides, and small molecules because of their high specificity, simplicity, stability, and rapid results (Chen et al., 2018). By targeting the His-tag, which was engineered as part of the recombinant SCAMP3 construct, the assay provided direct evidence of SCAMP3 production in the fat body of recombinant bacmid-injected silkworms. In contrast, negative controls (non-injected silkworms) consistently showed negligible signal, confirming the absence of SCAMP3 expression and ruling out background interference or nonspecific antibody binding. The observed differences were statistically significant, with SCAMP3 levels in recombinant silkworms being 6-7-fold higher than in the controls. These results confirm the functionality of the bacmid expression system and the capacity of silkworms to effectively produce recombinant SCAMP3 protein.

SCAMP3 was not naturally expressed in *E. coli* or *B. mori*, but it was expressed in mammals (Singleton et al, 1997). In our preliminary study, SCAMP3 was successfully expressed in *E. coli* upon induction with IPTG. Despite its advantages such as rapid growth, cost-effectiveness, and high protein yield the lack of post-translational modifications (PTMs) in *E. coli* could influence SCAMP3's native structure and function. In contrast, yeast systems offer improved folding and some PTMs, but they still lack the complex processing mechanisms found in higher eukaryotes.

Furthermore, the expression of SCAMP3 in silkworms often involves the baculovirus expression system, which might influence how the protein is folded or processed. In yeast and mammalian cells, the molecular mechanisms of protein folding within the ER have been well-characterized. However, these processes remain less understood in insects. While the folding of heterologous proteins in insect cells is more similar to mammalian cells compared to bacteria and yeast, it is not entirely identical (Liu et al., 2013).

The recombinant bacmid was injected into 5th instar larvae, the final developmental stage before transitioning into pupae. During the larva-to-pupa transformation in silkworm, significant proteolytic activity occurs, enabling the degradation and remodeling of larval tissues to form pupal structures. This critical process for metamorphosis involves various proteases that actively break down

proteins in the midgut (Muniv and Bhawane, 2012) and other tissues. These proteases play a crucial role in protein degradation, supporting tissue remodeling and the successful completion of metamorphosis

The explanation of proteolytic cleavage in SCAMP3 in silkworm has not been fully elucidated but it could possibly be related to its role in cellular protein transport and trafficking (Law et al., 2011). This post-translational modification might serve as a mechanism to modulate SCAMP3's activity or generate fragments that participate in different stages of the trafficking process, which is important for vesicle formation, cargo sorting, or membrane recycling in silkworm cells. However, a key question remains regarding how these cleavages affect SCAMP3's functionality in *B. mori* compared to its mammalian counterpart. Proteolytic cleavage could either enhance SCAMP3's role in membrane trafficking, similar to the G-protein Coupled Receptor Proteolytic Site (GPS) cleavage in Polycystin-1, which facilitates ER-to-Golgi transport (Su et al., 2015), or it could lead to functional loss if essential domains are disrupted. This raises the possibility that silkworm-specific proteases may alter SCAMP3's trafficking function in a way that differs from mammalian systems.

While *B. mori* offers several advantages as an expression system, such as high protein solubility and cost-effective large-scale production, there are also inherent limitations, particularly in the purification process. The presence of endogenous silkworm proteins in the fat body extracts can complicate purification, requiring additional steps to isolate the target recombinant protein effectively. Moreover, proteolytic degradation by endogenous proteases may result in fragmented or partially degraded proteins, further challenging downstream purification

Our findings reveal that SCAMP3 undergoes cleavage in *B. mori* provide new insights into the post-translational modifications and stability of heterologous proteins expressed in silkworm systems. Further investigation into the identity and specificity of the proteases responsible for SCAMP3 cleavage could enhance our understanding of protein processing in *B. mori* and inform strategies to improve the stability and yield of recombinant proteins. Additionally, the protein must be purified, or optimization strategies for protein purification should be implemented to ensure the production of high-quality SCAMP3.

The use of silkworms for heterologous protein expression holds great potential in biomedical research, offering a cost-effective, scalable, and sustainable alternative to traditional expression systems. Their ability to perform eukaryotic post-translational modifications ensures the correct folding and biological activity of complex proteins, making them suitable for producing therapeutic proteins, monoclonal antibodies (Ebihara et al., 2021), and vaccines (Masuda, 2023). Future advancements could enable personalized medicine, where silkworms produce patient-specific proteins, and on-site biomanufacturing, allowing rapid, localized drug production in remote areas or during pandemics.

Conclusion

The successful expression of SCAMP3 in *B. mori* demonstrates the effectiveness of the *B. mori* expression system for synthesizing heterologous proteins. This research supports the potential of *B. mori* as a dependable bioreactor for recombinant protein production, especially for eukaryotic proteins like SCAMP3 that require accurate folding and post-translational modifications. Nonetheless, the detected cleavage of SCAMP3 during expression indicates active proteolytic activity, emphasizing the need for further improvements to boost protein stability and yield.

SCAMP3 plays a key role in cellular processes like membrane trafficking and signal transduction, with its dysregulation linked to diseases such as cancer. Recombinant SCAMP3 production enables detailed structural and functional studies, critical for understanding its role in disease and evaluating its potential as a therapeutic target. The ability to produce SCAMP3 in large amounts lays the groundwork for developing monoclonal antibodies against the protein. These antibodies could be used as diagnostic tools or therapeutic agents to modulate SCAMP3-related pathways. Together, these findings contribute to advancing our understanding of SCAMP3 and its role in disease, potentially guiding new diagnostic and therapeutic strategies.

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Author's Contributions

Selly S. Rajagukguk: Conducted the research, acquired funding for the study, drafted the manuscript.
Sabar Pambudi: Conceptualized the research methods and gave final approvement to be submitted
Astari Dwiranti: Reviewed and edited the manuscript
Doddy I.S. Utomo: Elaborated the research methods, searched of silkworm supply, reviewed the discussion section of the manuscript
Retno Lestari: Examined the research plan, reviewed the manuscript
Upi. C. Nisa: Contributed additional research methods
Fadhillah: Contributed the research idea
Anom Bowolaksono: Supervised the research ansd gave final approvement to be submitted

Ethics

This study is entirely original and has not been submitted or published anywhere. The datasets generated and/or examined in this investigation are accessible from the corresponding author upon a reasonable request.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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