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Bt Toxin-Receptor Interactions: Advances in Understanding Insect Specificity

Ming Li, Shusheng Liu, Minsheng Lin
Hainan Tropical Agricultural Resources Research Institute, Tropical Microbial Resources Research Center, Sanya, 572025, Hainan, China
Corresponding author: minsheng.lin@hitar.org

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Abstract The Bt toxin produced by *Bacillus thuringiensis* (Bt) has been widely used in agricultural pest management because of its efficient insect targeting and relatively low impact on environmental and non-target organisms. These toxins are able to specifically bind to the receptors of insect intestinal cells, triggering insect death. With increasing reports of pests developing resistance to existing Bt crops, studying toxin-receptor interactions will not only help us design new or improved Bt toxins, but also help us predict and manage the development of pest resistance to these toxins. This study synthesizes the latest understanding of Bt toxin-receptor interactions, focusing on the structural and functional aspects of these proteins and their insect targets. The diversity and complexity of these interactions in different insect groups are highlighted through several case studies, particularly in Lepidoptera and Coleoptera. This study aims to uncover the molecular mechanisms that affect the specificity of these interactions and explore their practical applications in pest management. The significance of this research is not only to promote the in-depth research of basic science, but also to guide the practice of agricultural pest management, especially in the development of new Bt biopesticides and the development of pest resistance management strategies.

Keywords Bt toxin; Insect receptor; Toxin-receptor interaction; Insect specificity; Pest management

1 Introduction

Bacillus thuringiensis (Bt) is a bacterium known for its insecticidal properties, primarily due to the production of Cry and Cyt toxins. These toxins have been extensively utilized in agricultural biotechnology, particularly in the development of transgenic crops that express Bt toxins to control insect pests. The three-domain Cry (3d-Cry) toxins are among the most studied, with their mode of action involving binding to specific receptors in the insect midgut, leading to pore formation and cell death (Pardo-López et al., 2013). The effectiveness of Bt toxins has significantly reduced the reliance on chemical insecticides, promoting a more sustainable approach to pest management (Pardo-López et al., 2013; Wang et al., 2019).

Understanding the interactions between Bt toxins and their insect receptors is crucial for several reasons. Firstly, it helps in elucidating the mechanism of action of these toxins, which is essential for improving their efficacy and specificity (Liu et al., 2022). Secondly, knowledge of these interactions can aid in managing and mitigating the development of resistance in insect populations. Resistance to Bt toxins, such as mutations in cadherin, APN, and ABC transporter genes, poses a significant threat to the long-term effectiveness of Bt crops (Heckel, 2012). By identifying and characterizing new receptors and understanding their role in toxin binding, researchers can develop novel Bt toxins or modify existing ones to overcome resistance (Li et al., 2017; Chen et al., 2021).

The study aims to provide a comprehensive overview of the current understanding of Bt toxin-receptor interactions, with a focus on the advances made in identifying and characterizing these receptors. We will discuss the different types of receptors involved, such as cadherins, ABC transporters, and other midgut proteins, and their role in the mode of action of Bt toxins. Additionally, we will explore the mechanisms of resistance that insects have developed and the strategies being employed to counteract this resistance, including the development of new Bt toxins and the use of chimeric proteins. By synthesizing the latest research findings, this review aims to

highlight the critical aspects of Bt toxin-receptor interactions and their implications for sustainable pest management in agriculture.

2 Overview of Bt Toxins

Bacillus thuringiensis (Bt) is a bacterium known for producing crystal proteins, commonly referred to as Cry toxins, which are highly specific insecticidal agents. These toxins are widely used in biological insecticides and genetically modified crops to control pest populations. The specificity and safety of Bt toxins make them valuable alternatives to chemical pesticides (Likitvivatanavong et al., 2011; Vachon et al., 2012; Mendoza-Almanza et al., 2020).

2.1 Types of Bt toxins

Bt produces several types of toxins, each targeting different biological organisms. The most well-known are the Cry proteins, which are toxic to various insect larvae, including those affecting important crops. Other types include Cyt toxins, which primarily target mosquito larvae, and parasporins, which can kill mammalian cancer cells (Mendoza-Almanza et al., 2020). Additionally, Bt strains produce Vip (Vegetative insecticidal proteins) and Sip (Secreted insecticidal proteins) during their vegetative growth phase, which also exhibit insecticidal activity (Mendoza-Almanza et al., 2020).

2.2 Structure and function of Bt toxins

Cry toxins are composed of three distinct domains that play crucial roles in their insecticidal activity. Domain I is involved in pore formation in the insect midgut cells, while Domains II and III are responsible for binding to specific receptors on the insect midgut epithelium (Gómez et al., 2003). The binding of Cry toxins to these receptors is highly specific, involving interactions with proteins such as cadherin, alkaline phosphatase, and aminopeptidase-N (Bretschneider et al., 2016; Yuan et al., 2017). The structural determinants of these interactions have been studied extensively, revealing that specific loops and epitopes in the toxin domains are critical for binding to the receptors (Liu et al., 2018).

2.3 Mechanism of action in target insects

The mechanism of action of Cry toxins involves several steps. Initially, the protoxin is ingested by the insect and activated by proteolytic cleavage in the midgut, converting it into an active toxin (Liu et al., 2018). The active toxin then binds to specific receptors on the midgut epithelial cells, such as cadherin, ABCC2, and alkaline phosphatase (Bretschneider et al., 2016; Yuan et al., 2017). This binding triggers a series of events, including toxin oligomerization and insertion into the cell membrane, leading to pore formation (Vachon et al., 2012). The formation of pores disrupts the osmotic balance of the cells, causing cell swelling, lysis, and ultimately, insect death (Figure 1).

Recent studies have also suggested that Cry toxins may activate intracellular signaling pathways, such as the adenylyl cyclase/PKA pathway, leading to cell death without the need for pore formation (Zhang et al., 2006). This dual mechanism of action highlights the complexity of Bt toxin interactions with insect receptors and underscores the importance of understanding these interactions to develop more effective and sustainable pest control strategies.

Figure 1 shows the mechanism of action of Cyt protein. After interacting with phosphatidylcholine, phosphatidylethanolamine and sphingomyelin, Cyt toxin undergoes conformational changes, prompting six Cyt monomers to assemble into an “open umbrella” structure to form pores, resulting in increased membrane permeability and ultimately death of the larvae. At high concentrations, Cyt toxin binds to the lipid bilayer on the surface of the cell membrane through a detergent-like effect and destroys the membrane structure. These two models are not mutually exclusive. The detergent-like effect works at high concentrations of Cyt toxin, while the pore formation model works at low concentrations of Cyt toxin. In general, Cyt protein effectively destroys insect cell membranes through these two mechanisms to exert its insecticidal effect.

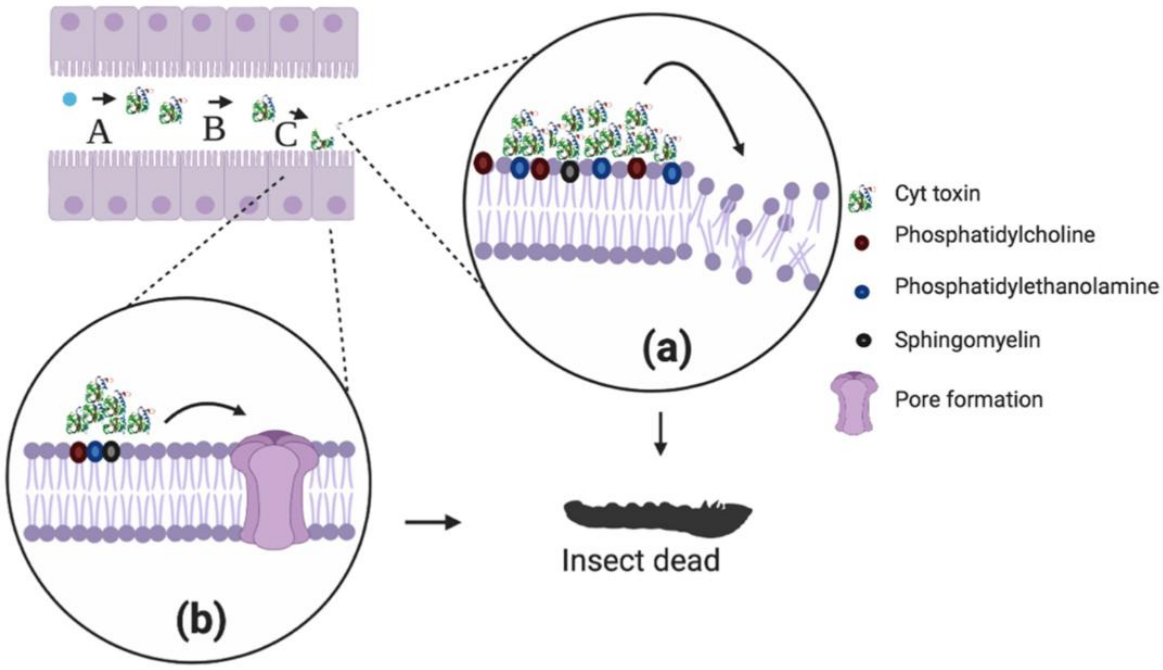

Figure 1 Mechanism of action of Cyt protein (Adopted from Mendoza-Almanza et al., 2020)
Image captions: (a) Pore-forming model; (b) Detergent effect model (Adopted from Mendoza-Almanza et al., 2020)

3 Insect Receptors for Bt Toxins

3.1 Types of insect receptors

Insect receptors for *Bacillus thuringiensis* (Bt) toxins are diverse and include cadherin-like proteins, aminopeptidase N (APN), and ATP-binding cassette (ABC) transporters. Cadherin-like proteins, such as BT-R1 in *Manduca sexta*, are primary receptors for Cry1A toxins and play a crucial role in the insecticidal process (Gómez et al., 2003; Adegawa et al., 2017; Liu et al., 2022). APNs, like the 106-kDa APN in *Anopheles gambiae*, also serve as important receptors for various Cry toxins, including Cry11Ba (Zhang et al., 2008). ABC transporters, such as ABCC2, have been identified as significant receptors for Cry1A toxins in species like *Bombyx mori* (Chen et al., 2015)

3.2 Receptor structure and function

Cadherin-like receptors are single-membrane-spanning α-helical proteins that regulate intercellular adhesion and signaling activities. The BT-R1 receptor in *M. sexta*, for instance, binds Cry1A toxins tightly and triggers a signaling pathway leading to insect death (Liu et al., 2022). APNs are glycosylphosphatidylinositol (GPI)-anchored proteins with zinc-binding motifs and glycosylation sites, which facilitate their role in binding Cry toxins and initiating insecticidal activity. ABC transporters, such as ABCC2, are involved in the transport of various molecules across cellular membranes and have been shown to interact with Cry toxins, contributing to their cytotoxic effects (Adegawa et al., 2017).

3.3 Binding sites and specificity

The binding sites on insect receptors for Bt toxins are highly specific and involve distinct epitopes. For example, the binding domain of BT-R1 for Cry1A toxins is localized in the 12th cadherin repeat (EC12), with a highly conserved 94-amino acid polypeptide designated as the toxin-binding site (TBS). In *M. sexta*, the Cry1Ab toxin interacts with specific epitopes in domain II loops and domain III of the toxin, which are crucial for binding to cadherin and APN receptors (Gómez et al., 2016). The specificity of Cry1A toxins also involves multiple structural determinants, such as the loop regions in domain II, which interact with both cadherin-like receptors and ABC transporters (Adegawa et al., 2017; Liu et al., 2022).

4 Mechanisms of Toxin-Receptor Interactions

4.1. Binding affinity and specificity

The binding affinity and specificity of Bt toxins to their receptors are critical for their insecticidal activity. The Cry1A toxins (Cry1Aa, Cry1Ab, and Cry1Ac) produced by *Bacillus thuringiensis* (Bt) bind specifically to the cadherin receptor BT-R1 in the midgut of *Manduca sexta* with high affinity (Kd = 1.1 nM) (Liu et al., 2022). This high-affinity binding is essential for the initiation of the insecticidal process. The specificity of the interaction is demonstrated by the fact that Cry1A toxins compete for the same binding site on BT-R1, and the inhibition patterns of insecticidal activity are similar for all three toxins (Liu et al., 2022). Additionally, the receptor BT-R1 has been shown to exhibit high-affinity binding to Cry1A toxins when expressed in heterologous cell cultures, further confirming its specificity.

4.2 Conformational changes upon binding

Upon binding to their receptors, Bt toxins undergo conformational changes that are crucial for their function. The binding of Cry1Ab toxin to BT-R1 triggers a Mg^{2+} dependent signaling pathway, which involves the stimulation of the G-protein α-subunit and subsequent signaling cascades. This binding event is highly specific and involves the formation of a heterodimeric complex between Cry1Ab and the 12th ectodomain region (EC12) of BT-R1, with extremely high affinity (Kd = 19.5 ± 1.6 nM) (Liu et al., 2018). The interaction between Cry1Ab and specific epitopes on BT-R1, such as the 368RRPFNIGINNQQ379 region, is critical for the toxin's specificity and function.

4.3 Cellular responses and signal transduction

The binding of Bt toxins to their receptors initiates a series of cellular responses and signal transduction pathways that lead to insect cell death. The Cry1Ab toxin, upon binding to BT-R1, activates a previously undescribed signaling pathway involving the stimulation of G protein (Gαs) and adenylyl cyclase, leading to increased cAMP levels and activation of protein kinase A (PKA). This signaling cascade results in cytological changes such as membrane blebbing, appearance of ghost nuclei, cell swelling, and lysis, ultimately causing cell death (Hu et al., 2018). Additionally, the binding of Cry1Ab monomer to BT-R1 correlates with cell death, whereas oligomeric forms of the toxin do not produce lytic pores or kill insect cells (Stevens et al., 2017). The involvement of cadherin receptors and other proteins, such as ATP-binding cassette transporters, further modulates the cytotoxic effects of Bt toxins.

5 Advances in Research Techniques

5.1. Structural biology methods

Structural biology methods have significantly advanced our understanding of Bt toxin-receptor interactions. Techniques such as X-ray crystallography and homology modeling have been pivotal in elucidating the three-dimensional structures of both toxins and their receptors. For instance, the three-dimensional structure of the Cry1Ac toxin-binding region in *Plutella xylostella* cadherin-like receptor was constructed using homology modeling, which revealed the specific domains involved in binding (Rathinam et al., 2019). Additionally, X-ray crystallography has been used to resolve the structure of the toxin-binding site of the cadherin G-protein-coupled receptor BT-R1, providing insights into the molecular determinants of toxin binding (Liu et al., 2022).

5.2 Molecular docking and simulation

Molecular docking and simulation techniques have been instrumental in predicting and validating the interactions between Bt toxins and their receptors. These computational methods allow for the exploration of binding affinities and the stability of toxin-receptor complexes. For example, molecular docking studies have shown that the Cry1Ac toxin interacts with specific regions of the cadherin-like receptor in *Plutella xylostella*, with hydrogen bonding and hydrophobic interactions playing crucial roles (Hu et al., 2018). Furthermore, molecular dynamics simulations have substantiated the stability of interactions between the chimeric Cry1AcF toxin and aminopeptidase1 receptors from *Helicoverpa armigera* and *Spodoptera litura*, demonstrating the broad-spectrum efficacy of the engineered toxin (Rathinam et al., 2019).

5.3 Biochemical and biophysical assays

Biochemical and biophysical assays have provided empirical evidence to support the findings from structural and computational studies. Techniques such as enzyme-linked immunosorbent assay (ELISA), ligand blotting, and site-directed mutagenesis have been used to validate the binding interactions and identify key residues involved (Zhang et al., 2014). For instance, binding assays using Cry1Ab toxin and a fluorescently labeled EC12 fragment of the BT-R1 receptor demonstrated high specificity and affinity, confirming the critical role of conserved sequence motifs in toxin binding (Liu et al., 2018). Additionally, site-directed mutagenesis and binding assays have identified hot spot residues in both Cry1Ac and its receptor, further elucidating the molecular basis of their interaction (Hu et al., 2018).

6 Case Studies of Toxin-Receptor Interactions

6.1 Interaction studies in lepidoptera

Lepidopteran insects, such as *Spodoptera litura* and *Spodoptera frugiperda*, have been extensively studied to understand the interactions between Bt toxins and their receptors. Cry toxins, particularly Cry1A and Cry2A, bind to specific receptors in the midgut of these insects, leading to cell lysis and insect death (Figure 2). Key receptors include cadherin, aminopeptidase N (APN), and alkaline phosphatase (ALP) (Bravo et al., 2011; Li et al., 2021; Dutta et al., 2022). For instance, ALP2 in *Spodoptera exigua* has been identified as a functional receptor for Cry2Aa, playing a crucial role in the insect's susceptibility to this toxin (Yuan et al., 2017). Additionally, RNAi-mediated silencing of receptor genes such as CAD, ABCC2, ALP1, and APN in *S. frugiperda* and *S. litura* has shown that these receptors are essential for Cry1AcF toxicity (Dutta et al., 2023). These studies highlight the importance of specific receptor interactions in determining the efficacy of Bt toxins against lepidopteran pests.

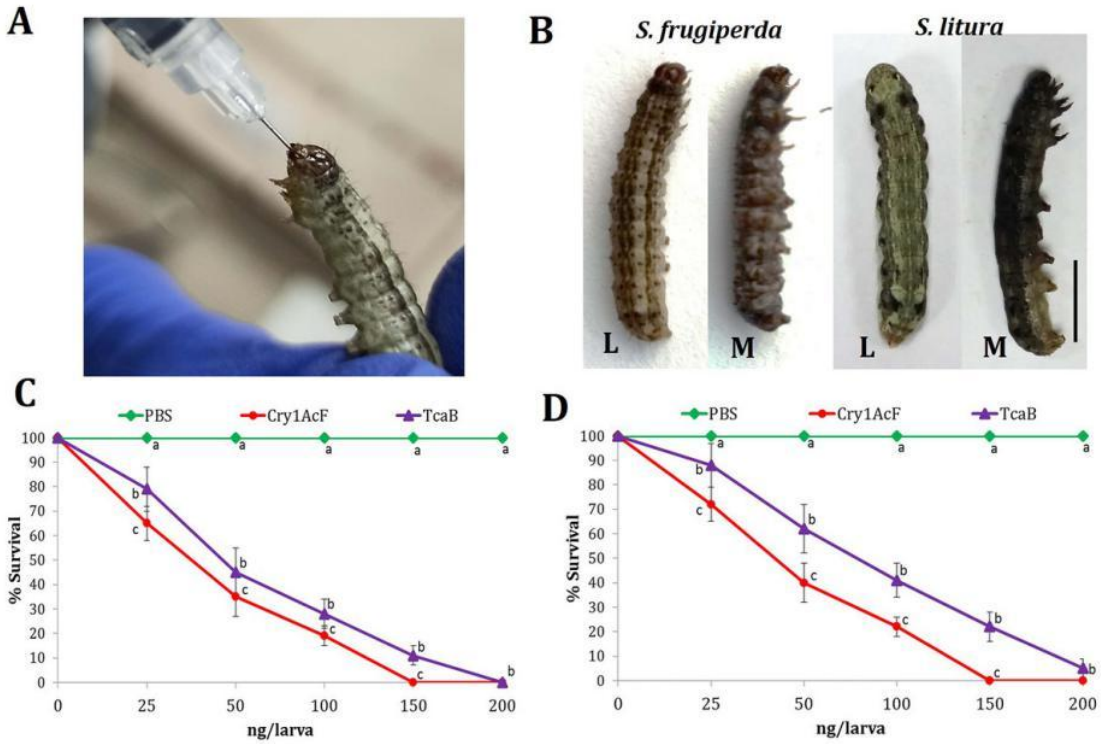

Figure 2 Insecticidal activity of Cry1AcF toxin in *S. frugiperda* and *S. litura* (Adopted from Dutta et al., 2023)

Image cation: (A) Oral ingestion of the toxin using hypodermic needle in starved *S. frugiperda* fourth-instar larvae. (B) At 24 h after inoculation. Dose-response curves depict the percent survival of *S. frugiperda* (C) and *S. litura* (D) at 24 h after toxin ingestion (Adopted from Dutta et al., 2023)

Dutta et al. (2023) demonstrated the potent insecticidal activity of Cry1AcF toxin against larvae of *Spodoptera frugiperda* and *Spodoptera litura*. A precise method of delivering the toxin to hungry fourth-instar *S. frugiperda*

larvae via the oral route was used. Significant physical deterioration of the larvae after toxin injection was highlighted, including melanization of the hemolymph and extended abdominal feet, while control larvae appeared normal. Dose-response curves (C and D) showed that the survival of both insects decreased with increasing doses, with Cry1AcF being more effective than TcaB. Statistical analysis confirmed the significance of these findings, highlighting the potential of Cry1AcF as an effective biological control agent for these agricultural pests.

6.2 Studies in coleoptera

Research on coleopteran insects, such as the cottonwood leaf beetle (*Chrysomela scripta*), has revealed that Bt toxins like Cry1Ba1 exhibit significant toxicity against these pests. The interaction between Cry toxins and midgut receptors in coleopterans is less understood compared to lepidopterans, but it is known that similar receptor families, including cadherin, APN, and ALP, are involved (Zhong et al., 2000; Domínguez-Arrizabalaga et al., 2020). For example, Cry1Ba1 has been shown to bind specifically to receptors in the midgut of coleopteran larvae, leading to mortality at low to moderate concentrations (Zhong et al., 2000). This indicates that while the specific interactions may vary, the overall mechanism of receptor-mediated toxicity is conserved across different insect orders.

6.3 Comparative analysis of different insect orders

Comparative studies across different insect orders, including *Lepidoptera*, *Coleoptera*, and *Diptera*, have shown that Bt toxins interact with a conserved set of midgut receptors, such as cadherin, APN, and ALP (Bravo et al., 2011; Likitvivatanavong et al., 2011; Li et al., 2021). For instance, a genome-wide analysis of Cry toxin receptor families in seven insect species revealed that ALPs and APNs are divided into multiple clades, with certain clades containing multiple paralogs within each species. This suggests a conserved evolutionary mechanism for Cry toxin susceptibility. Additionally, the identification of multiple receptors in mosquitoes, such as *Aedes aegypti*, further supports the idea that Cry toxin-receptor interactions are a common theme across diverse insect taxa (Likitvivatanavong et al., 2011). These comparative analyses provide valuable insights into the broad-spectrum efficacy of Bt toxins and the potential for cross-order resistance management strategies.

7 Implications for Insect Specificity

7.1. Determinants of insect specificity

The specificity of *Bacillus thuringiensis* (Bt) toxins towards different insect species is primarily determined by the interaction between the toxins and specific receptors in the insect midgut. For instance, the Cry1Ab toxin interacts with the cadherin-like receptor Bt-R1 in *Manduca sexta* through specific epitopes, such as the (865) NITIHITDTNN (875) sequence and loop 2 of domain II in the toxin (Gómez et al., 2003). Additionally, the role of ATP-binding cassette (ABC) transporters, particularly ABCC2 and ABCC3, has been highlighted in various studies. These transporters are crucial for the binding and subsequent toxicity of Cry1 toxins in insects like *Bombyx mori* and *Plutella xylostella* (Guo et al., 2019; Wang et al., 2021). The presence or absence of these receptors and transporters in different insect species determines the specificity and effectiveness of Bt toxins.

7.2 Evolution of receptor specificity

The evolution of receptor specificity is a dynamic process influenced by the continuous interaction between Bt toxins and insect receptors. Insects can develop resistance through mutations in these receptors, which alter the binding affinity of the toxins. For example, mutations in the cadherin gene in *Manduca sexta* and other insects have been linked to reduced susceptibility to Cry1Ab toxin (Figure 3) (Soberón et al., 2007). Moreover, the evolution of Bt toxins themselves, such as the development of Cry1Ac variants with higher affinity for non-native receptors, demonstrates an adaptive response to overcome insect resistance (Badran et al., 2016). This co-evolutionary arms race between Bt toxins and insect receptors underscores the importance of understanding receptor specificity to develop more effective pest control strategies.

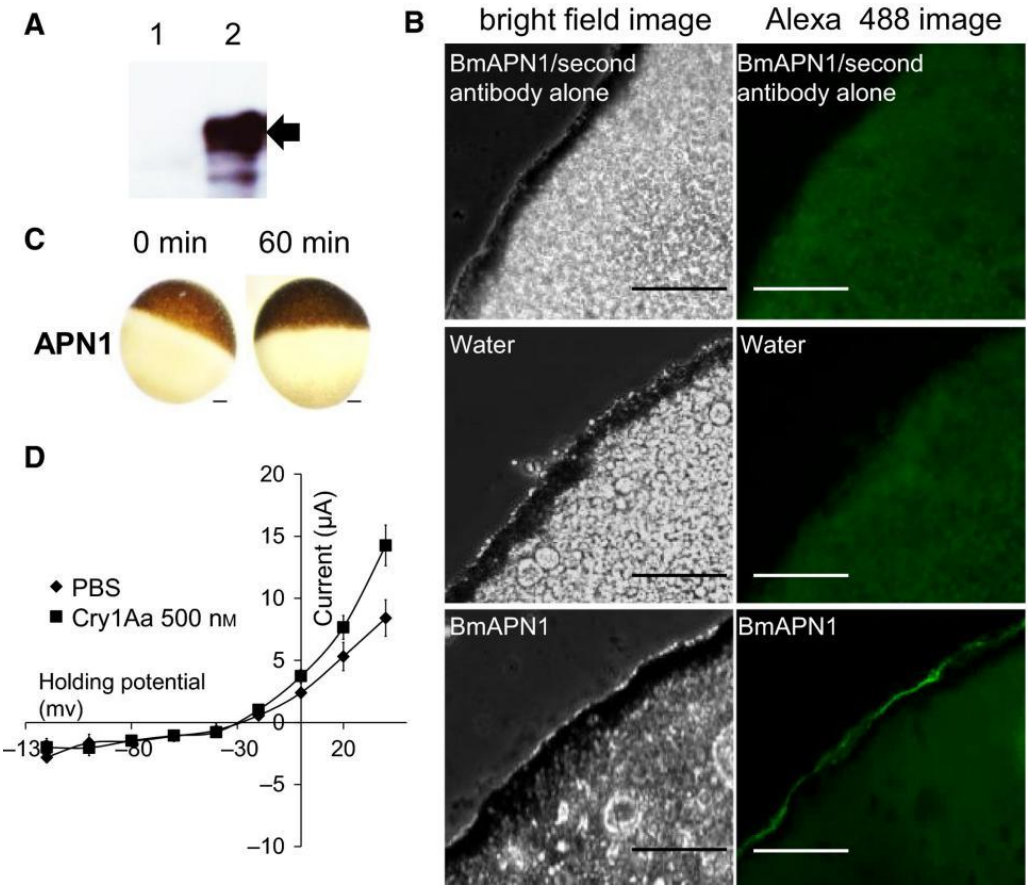

Figure 3 I-V curves plotted from the results of voltage clamp analysis of *Xenopus* oocytes expressing BmAPN1 treated with Cry1Aa toxin (Adopted from Tanaka et al., 2016)

Image captions: (A) Western blot analysis of *Xenopus* oocytes expressing BmAPN1. (B) Immunostaining of oocytes expressing BmAPN1. (C) Observation of morphological changes in oocytes expressing BmAPN1. (D) Oocytes expressing BmAPN1 were incubated with PBS or PBS containing 500nM Cry1Aa for 60 minutes, and two-electrode voltage clamp analysis was performed to measure ionic currents across the cell membrane (Adopted from Tanaka et al., 2016)

Tanaka et al. (2016) demonstrated the mechanism of action of Cry1Aa toxin on Xenopus oocytes expressing BmAPN1. The expression of BmAPN1 in oocytes was confirmed by Western blotting analysis. Part B shows the immunostaining results of BmAPN1, confirming its localization on the cell membrane. The morphological changes of BmAPN1 oocytes after 60 minutes of treatment with 100 nm Cry1Aa showed obvious cell morphological changes. The changes in ion currents of BmAPN1 oocytes after treatment with Cry1Aa toxin were measured by two-electrode voltage clamp experiments, and the results showed that the current increased significantly after treatment. These results indicate that Cry1Aa toxin changes the ion permeability of the cell membrane by interacting with BmAPN1, leading to cell dysfunction.

7.3 Role in resistance development

The development of resistance to Bt toxins in insects is a significant concern for the sustainability of Bt-based pest control methods. Resistance mechanisms often involve alterations in the receptors that Bt toxins target. For instance, the knockout of *ABCC2* and *ABCC3* genes in *Plutella xylostella* using CRISPR/Cas9 technology has been shown to confer high-level resistance to Cry1Ac toxin (Heckel, 2021). Similarly, RNA interference (RNAi) studies have demonstrated that silencing cadherin and ABC transporter genes in various insect species reduces their susceptibility to Bt toxins (Huang et al., 2020; Dutta et al., 2023). These findings highlight the critical role of receptor proteins in the development of resistance and emphasize the need for continuous monitoring and management strategies to mitigate resistance evolution.

8 Applications in Pest Management

8.1. Development of targeted Bt crops

The development of genetically engineered crops that produce *Bacillus thuringiensis* (Bt) toxins has revolutionized pest management by providing a targeted approach to controlling insect pests. Bt crops, such as corn and cotton, produce insecticidal proteins that are highly specific to certain pests, reducing the need for broad-spectrum insecticides and minimizing harm to non-target organisms (Huang et al., 2020; Gassmann and Reisig, 2022). The success of Bt crops in managing pests has been demonstrated in various regions, leading to regional suppression of pest populations and increased profits for farmers (Tabashnik et al., 2008). However, the evolution of resistance in some pest populations poses a significant challenge to the long-term efficacy of Bt crops (Tabashnik et al., 2009).

8.2 Strategies to overcome resistance

To address the issue of resistance, several strategies have been proposed and implemented. One approach is the use of pyramided Bt crops, which produce multiple Bt toxins with different modes of action. This strategy aims to reduce the likelihood of pests developing resistance to all toxins simultaneously (Carrière et al., 2015). Additionally, modified Bt toxins that can overcome resistance mechanisms, such as those involving mutations in cadherin receptors, have been developed. These modified toxins have shown effectiveness against resistant pest populations (Soberón et al., 2007). Another strategy involves the use of refuges, which are areas planted with non-Bt crops to maintain a population of susceptible pests. This approach helps to delay the evolution of resistance by promoting the survival of susceptible individuals that can mate with resistant ones, thereby diluting resistance alleles in the population (Tabashnik, 2015).

8.3 Integration with other pest control methods

Integrating Bt crops with other pest control methods can enhance the sustainability and effectiveness of pest management programs. Integrated pest management (IPM) strategies that combine Bt crops with biological control agents, crop rotation, and the use of conventional insecticides can provide a more comprehensive approach to pest control (Gassmann and Reisig, 2022). For example, increasing the prevalence of refuges and using IPM practices have been recommended to delay resistance and improve the durability of Bt crops (Gassmann and Reisig, 2022). Additionally, understanding the genetic and ecological factors that influence resistance can inform the development of more effective management strategies (Carrière et al., 2010). By combining multiple control methods, it is possible to reduce the selection pressure for resistance and achieve more sustainable pest management outcomes.

9 Concluding Remarks

The research on Bt toxin-receptor interactions has significantly advanced our understanding of insect specificity. Key findings include the identification of specific receptor binding sites and the molecular mechanisms underlying these interactions. For instance, the *Manduca sexta* Bt-R1 receptor interacts with Cry1A toxins through specific epitopes, highlighting the importance of structural determinants in toxin binding. Multiple receptors, such as cadherin, alkaline phosphatase, and aminopeptidase-N, have been identified as targets for Cry toxins in mosquitoes, demonstrating the complexity of these interactions. Additionally, the role of ATP-binding cassette (ABC) transporters, such as ABCC2 and ABCC3, in mediating Cry toxin susceptibility has been elucidated, with transcription factors like FOXA playing a regulatory role. The identification of a 106-kDa aminopeptidase as a receptor for Cry11Ba in *Anopheles gambiae* further expands the repertoire of known Bt toxin receptors.

Continued research in this field is crucial for several reasons. Understanding the molecular basis of Bt toxin-receptor interactions can aid in the design of new toxins to overcome insect resistance, a growing problem in pest management. The identification of multiple receptors and their roles in toxin binding and insect mortality can lead to the development of more effective and targeted biopesticides. Moreover, insights into the regulatory

mechanisms of receptor expression, such as the role of FOXA in upregulating ABCC2 and ABCC3, can provide new strategies for enhancing the efficacy of Bt toxins. Finally, the potential application of Bt toxins in targeting human cancer cells, as seen with parasporins, opens new avenues for biomedical research.

Future studies should focus on several key areas. There is a need to further characterize the structural determinants and binding epitopes involved in Cry toxin-receptor interactions across different insect species. Research should explore the potential for engineering Bt toxins with modified binding specificities to target a broader range of insect pests or to overcome existing resistance mechanisms. Studies should investigate the signaling pathways activated by toxin-receptor interactions to better understand the mechanisms of cytotoxicity and resistance. Additionally, the development of cell-based systems expressing various Bt toxin receptors can provide valuable models for studying the cytotoxic effects and mechanisms of action of different Cry toxins. Interdisciplinary approaches combining structural biology, molecular genetics, and bioinformatics can accelerate the discovery of new targets and the design of next-generation Bt toxins.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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