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Resistance is non-futile: Resistance to Cry5B in the nematode *Caenorhabditis elegans*

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Abstract

The nematode, *Caenorhabditis elegans*, can be mutated to resistance to the Cry5B toxin of *Bacillus thuringiensis*. By cloning and characterization of these *C. elegans* resistance genes, we have determined that a major mechanism by which *C. elegans* resists Cry5B is by loss of function mutations in any one of four gylcosyltransferase genes that glycosylate glycolipids specific to arthropods. Without correct gylcosylation, binding of Cry5B is greatly impaired in *C. elegans*. That these specific arthroseries glycolipids do not occur in vertebrates potentially helps explain why Cry toxins are specific for arthropods.

Keywords: *Caenorhabditis elegans*; Cry5B resistance; Cry toxin specificity; Mutations in glycotransferase genes; Glycolipid Cry toxin receptors; Arthroseries glycolipids; Mechanism of Cry toxin resistance

1. Introduction

In the year 2005, over 26 million hectares of crops were planted around the world that expressed crystal (Cry) proteins derived from the bacterium *Bacillus thuringiensis* (Bt) (James, 2005). These Cry proteins are used to control insect pests that eat various crops, e.g., corn and cotton. This large-scale use, although beneficial for agriculture and the environment due to the reduction in chemical insecticides, is in effect a large-scale selection screen for insects resistant to Cry proteins. In addition, Cry proteins have recently been shown to have potential therapeutic use against parasitic nematodes, in particular those that infect humans (Cappello et al., 2006).

2. Experimental system

For the above reasons, we have undertaken detailed studies of the genes in the nematode *Caenorhabditis elegans* that mutate providing resistance to Cry proteins.

The main Cry protein studied in our laboratory is Cry5Ba—a three-domain protein phylogenetically related to the Cry proteins used in transgenic crops and in vector control. Exposure of *C. elegans* to the mutagen ethylmethane sulfonate (EMS) allowed us to perform forward-directed genetic screens for animals resistant to Cry5B (Marroquin et al., 2000).

3. Summary of results

Mutations in five distinct loci were identified in these screens, and the genetics suggested that loss of function in any one was sufficient for Cry5B resistance. These genes were called the "*bre*" genes for *Bacillus*-toxin resistant." We went on to show that four of these five genes (*bre-2*, *-3*, *-4*, and *-5*) encode glycosyltransferase genes (Fig. 1), and hypothesized based on various data that these genes were involved in production of a carbohydrate receptor present on the *C. elegans* intestine that bound Cry5B (Griffitts et al., 2001,2003). Indeed, we were then able to show that *bre* genes were involved in the production of carbohydrate structures on arthroseries glycolipids (Fig. 2), and that these glycolipids bind Cry5B (Griffitts et al., 2005).

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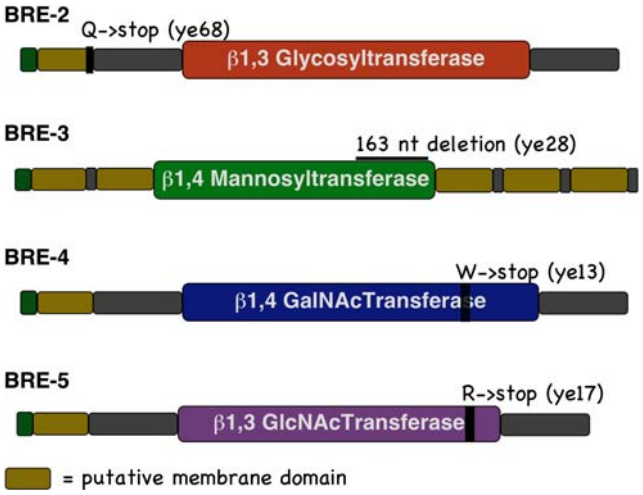

Fig. 1. Schematic representation of *bre-2*, *bre-3*, *bre-4*, and *bre-5* glycosyltransferase genes, along with molecular defects associated with various alleles responsible for resistance.

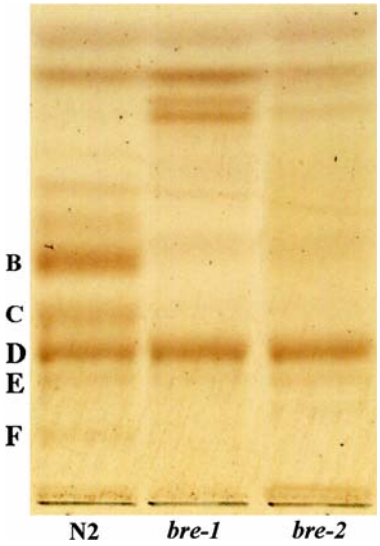

Fig. 2. Thin-layer chromatography (TLC) analysis of glycolipids from N2 wild-type, *bre-1(ye4)*, and *bre-2(ye31)* of the nematode, *C. elegans*. Upper phase glycolipids were purified, resolved, and visualized with the carbohydrate stain orcinol. For details of the methodology and glycolipid defects associated with other *bre* mutants, as well as for designation of glycolipid species (B, C, D, E, and F), see (Griffitts et al., 2005).

Furthermore, studies in the laboratory of Michael Adang at the University of Georgia indicate that insecticidal proteins in the Cry1A family also bind insect glycolipids (Griffitts et al., 2005). Thus, arthroseries glycolipids appear to be conserved receptors for Bt Cry proteins. Our data may partly explain why these proteins are specific for insects and nematodes, as arthroseries glycolipids are found in invertebrates such as insects, but and not in other organisms, for example, vertebrates.

More recently, we have cloned and characterized the *bre-1* gene (Barrows et al., 2007). Nematodes with *bre-1* mutant animals, although resistant to Cry5B (Fig. 3), are less resistant than mutants in the other four cloned *bre* genes (Barrows et al., 2007; Marroquin et al., 2000). We cloned the *bre-1* gene and found it was represented by the open reading frame C53B4.7. C53B4.7 encodes the worm homolog of GDP-mannose 4,6-dehydratase, which is required for the *de novo* synthesis of GDP-fucose. Since many of the glycolipids that bind Cry5B contain fucose, we hypothesized that *bre-1* mutant animals might be defective in the production of these glycolipids. Subsequently, we showed this to be true—*bre-1* mutant animals are defective in the production of glycolipids (Fig. 2) and exhibit defects

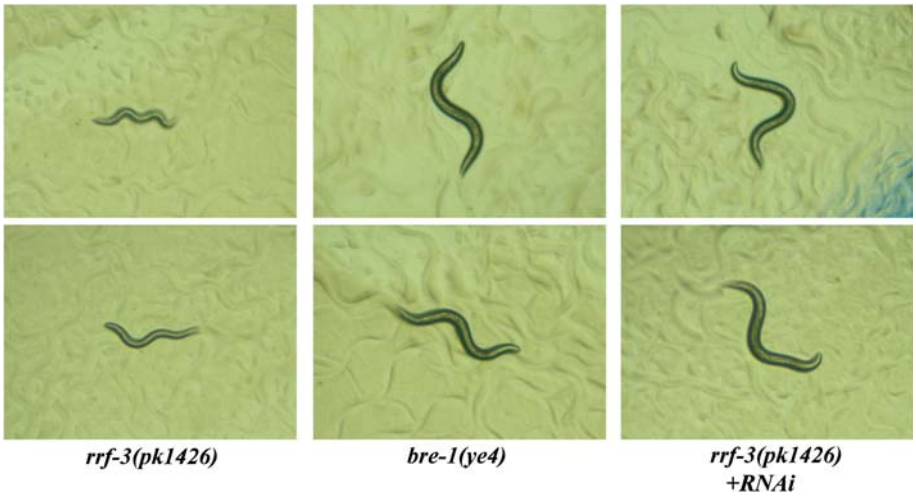

Fig. 3. *Caenorhabditis elegans bre-1* mutant resistance by mutation and by RNAi. (Left) *rrf-3(pk1426)* control animals on Cry5B toxin. These nematodes, useful for RNAi studies, behave the same as wild type on toxin (Huffman et al., 2004) and are small and sick when exposed to Cry5B. (Middle) *bre-1(ye4)* animals under identical Cry5B toxin conditions. Animals are much healthier than controls. (Right) progeny from *rrf-3(pk1426)* animals injected with double-stranded RNA from the *bre-1* gene (C53B4.7) demonstrating that RNAi of the *bre-1* gene mimics the *ye4* resistance phenotype. For experimental details, see (Barrows et al., 2007).

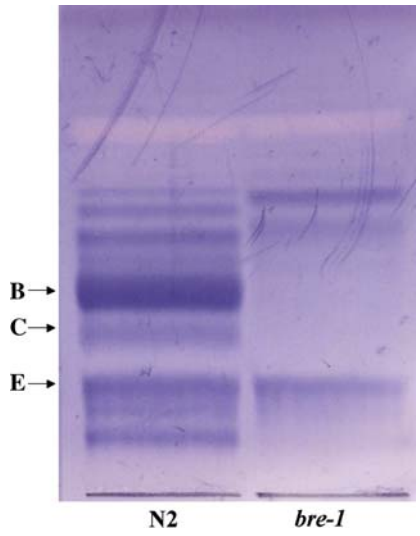

Fig. 4. TLC overlay in which glycolipids from N2 wild-type (left lane) and *bre-1(ye4)* (right lane) animals were resolved by TLC and then probed with biotinylated Cry5B to detect Cry5B-binding glycolipids. For details, see (Barrows et al., 2007).

in the binding of Cry5B to glycolipids (Fig. 4). Furthermore, we found that *bre-1* mutant animals contained no detectable fucose (Barrows et al., 2007). These data indicate that resistance to a crystal protein can develop from the loss of a carbohydrate biosynthetic pathway and that *C. elegans* has evolved to survived with little fucose.

4. Conclusions

Thus, the *C. elegans*—Cry protein system has proven to be fruitful on two different fronts: (1) the molecular genetics of *C. elegans* continues to increase our understanding of Bt toxin receptors and resistance, and (2) Bt toxins continue to increase our understanding of nematode biology.

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