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The diversity of Bt resistance genes in species of Lepidoptera

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Abstract

Although the mode of action of Cry1A toxins produced by *Bacillus thuringiensis* is fairly well understood, knowledge of the molecular mechanisms by which lepidopteran species have evolved resistance to them is still in its infancy. The most common type of resistance has been called "Mode 1" and is characterized by recessive inheritance, >500-fold resistance to and reduced binding by at least one Cry1A toxin, and negligible cross-resistance to Cry1C. In three lepidopteran species, *Heliothis virescens*, *Pectinophora gossypiella*, and *Helicoverpa armigera*, Mode 1 resistance is caused by mutations in a toxin-binding 12-cadherin-domain protein expressed in the larval midgut. These mutations all interrupt the primary sequence of the protein and prevent its normal localization in the membrane, presumably removing a major toxic binding target of the Cry1A toxins. In *Plutella xylostella*, however, Mode 1 resistance appears to be caused by a different genetic mechanism, as Cry1A resistance is unlinked to the cadherin gene. Mapping studies in *H. virescens* have detected an additional major Cry1A resistance gene, which on the basis of comparative linkage mapping is distinct from the one in *P. xylostella*. An additional resistance mechanism supported by genetic data involves a protoxin-processing protease in *Plodia interpunctella*, and this is likely to be different from the genes mapped in *Plutella* and *Heliothis*. Thus, resistance to Cry1A toxins in species of Lepidoptera has a complex genetic basis, with at least four distinct, major resistance genes of which three are mapped in one or more species. The connection between resistance genes and the mechanisms they encode remains a challenging task to elucidate.

Keywords: *Heliothis virescens*; *Plutella xylostella*; *Helicoverpa armigera*; *Pectinophora gossypiella*; *Bacillus thuringiensis*; Cry1A toxin; Genetics; Resistance

1. Introduction

Insecticide resistance is an evolutionary phenomenon, involving changes in allele frequencies of specific genes over time. Insecticidal toxins of the Cry1A family produced by *Bacillus thuringiensis* (Bt) used in agricultural pest control have exerted a strong selective effect on populations of insects. For at least three species of Lepidoptera, prolonged applications for pest control in the granary, open field, or greenhouse have led to the appearance of resistance [Indianmeal moth (McGaughey, 1985), diamondback moth (Tabashnik et al., 1990; Shelton et al., 1993), and cabbage looper (Janmaat and Myers, 2003)]. Bt-resistant strains have been developed by laboratory selection in many other species of insects. The widespread adoption of transgenic maize and cotton expressing Bt toxins has greatly increased the opportunity for resistance selection in the field. Anticipating, and preventing or delaying this process in other target pest species that have not yet evolved Bt resistance in the field requires an understanding of which genes are subject to Bt selection and how they confer resistance. A genetic approach to analyzing Bt resistance is thus a necessary complement to the biochemical and physiological

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approaches widely utilized in the field. Some clues as to the likely identities of these genes are suggested by the complex mode of action of the Cry1A-type toxins on lepidopteran larvae.

2. Cry protein mode of action

Although some aspects of Bt-toxin mechanism of action are still poorly understood and controversial, the major steps are generally agreed upon (Knowles, 1994; Pietrantonio and Gill, 1996; Rajamohan et al., 1998; Schnepf et al., 1998). The site of action is the larval midgut, and the toxin must be ingested for lethality. The toxin is packed into proteinaceous parasporal crystalline inclusions. When Bt spores are consumed, they germinate and the crystals associated with spores dissolve in the alkaline lumen of the midgut. The soluble protoxin, whether derived from crystals or the cytoplasm of a transgenic plant producing the Bt toxin, is then cleaved in stages from the carboxy- and amino-termini by insect digestive proteases to produce the active, protease-resistant toxin core protein. Some of the proteolytic processing may have occurred in the tissue of the transgenic plant as well, prior to or during ingestion. The toxin crosses the peritrophic matrix to reach the ectoperitrophic space bordered by the apical membranes of the midgut cells. Toxin molecules undergo various interactions with molecules in the epithelial membranes, including reversible and irreversible binding, which is generally accepted as being crucial to toxicity. The toxin then participates in the formation of pores in the bilayer lipid membrane. Aggregation of toxin into oligomers happens prior to or during this step. The disruption of membrane integrity eventually kills the cells; in this process the mechanism of "colloid-osmotic lysis" (Knowles and Ellar, 1987) has received the most attention. If enough toxin has been ingested, these steps may occur within minutes or hours, usually accompanied by a complete cessation of feeding behavior. Eventual death of the larva may take several hours or even days, and is generally attributed to starvation which is likely exacerbated by proliferation of Bt and other microorganisms in the damaged midgut (Broderick et al., 2006).

3. Possible mechanisms of resistance

Even if this scenario is incomplete or erroneous in some of the details, it illustrates the sequential procession of events leading to toxicity, and the fact that resistance to Bt toxin by the insect may develop by any of several mechanisms that block the sequence at any point (Heckel, 1994, 2002). Inhibition of germination could confer resistance to spores. Failure of crystal dissolution would prevent passage through the peritrophic matrix and result in eventual excretion. Formation of the active toxin could be blocked by failure to completely process the protoxin (Oppert, 1999). Even if activated toxin is provided by the transgenic plant or another source, it could be further degraded by a protease with increased activity (Shao et al., 1998), sequestered by precipitation (Milne et al., 1998) or coagulation (Ma et al., 2005) or trapped by binding sites within the peritrophic matrix. Once present in the ectoperitrophic space, modification of the binding targets or molecules that otherwise interact with the toxin could reduce or prevent the irreversible binding believed to be crucial to toxicity (van Rie et al., 1990; Ferré et al., 1991). Binding targets could be shed from the midgut epithelium (Valaitis, 1995). Pore formation could be interfered with (Shai, 2001) or pores could be plugged. Replacement of dead midgut cells could be accelerated by increased activity of stem cells (Loeb et al., 2001).

4. Genetic analysis for identification of Cry resistance genes

This diversity of potential mechanisms suggests that mutations in a large number of genes could potentially cause resistance. The goals of a genetic analysis of a given resistant strain are to determine the number of genes involved, to measure the relative potency of each gene in conferring resistance and how they interact with one another, to evaluate known genes as candidates for resistance genes, and to facilitate positional cloning of unknown genes. Genetic analysis can address these questions without making any assumptions about the mechanism of resistance, although a full understanding is only achieved when the mechanisms are also known. The basic approach relies on analysis of the patterns of toxin-induced mortality in the F_1, F_2, and backcross generations resulting from crosses between resistant and susceptible strains, and this is achievable with a good bioassay irrespective of the mechanism of resistance. Mortality bioassays producing a dose–mortality response are analyzed by probit analysis, and growth bioassays relying on the concentration-dependent growth inhibition by sublethal amounts of toxin can be employed in QTL (quantitative trait locus) analysis.

Genetic analysis is even more powerful when marker loci and a genetic linkage map are used. Genetic linkage between marker loci and resistance genes causes a correlation between resistance level and marker genotype that can be measured. Complete coverage of a species' genome with marker genes ensures that all potential resistance genes will be near a marker and hence detectable by linkage. Probit analysis measures the combined action of different resistance genes but lacks the ability to distinguish among them. However, once localized on a linkage map, map position becomes an identifying property of a resistance gene independent of the mechanism it encodes. Candidate genes, encoding putative receptors for example, can be localized on the map and tested for linkage to resistance genes. Rejection of candidates is useful as it focuses future effort on a subset of genes; and acceptance of a candidate by linkage is the first step to more definitive studies including positional cloning. Finally, identification by linkage map locations enables strain or species comparisons even if the identities of the resistance genes are unknown.