matic gene silencing. Whether Arg 3 methylation helps the recruitment of specific histone acetyltransferases, such as p300, remains to be determined. As new HMTs responsible for the methylation of different histone arginine or lysine residues are identified, the functions of histone methylation on transcription and other processes involving chromatin will be revealed.

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16. Recombinant H4 was purified as described (*24*) and used as substrates for PRMT1 methylation (*10*) in the presence of excess amounts of unlabeled SAM. Complete methylation was verified by the lack of further incorporation of [^{3}H]SAM. Acetylation was performed in a 20-μl volume containing 50 mM Hepes (pH 8.0), 5 mM DTT, 5 mM PMSF, 10 mM sodium butyrate, 10% glycerol, 2 μl [^{3}H]acetyl-CoA, and 2 μl of p300. Reaction mixture was incubated for 1 hour at 37°C and terminated by the addition of SDS sample buffer.
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23. One 100-mm plate of 293T cells (about 1.5×10^6) were transfected with 4 μg of empty pCDNA vector or pCDNA-PRMT1 by using the Effectene transfection reagent (Qiagen). Forty-eight hours after transfection, nuclei were isolated and core histones were purified by acid extraction and trichloroacetic acid precipitation.
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Identification of a Gene Associated with Bt Resistance in *Heliothis virescens*

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Transgenic crops producing insecticidal toxins from *Bacillus thuringiensis* (Bt) are widely used for pest control. Bt-resistant insect strains have been studied, but the molecular basis of resistance has remained elusive. Here, we show that disruption of a cadherin-superfamily gene by retrotransposon-mediated insertion was linked to high levels of resistance to the Bt toxin Cry1Ac in the cotton pest *Heliothis virescens*. Monitoring the early phases of Bt resistance evolution in the field has been viewed as crucial but extremely difficult, especially when resistance is recessive. Our findings enable efficient DNA-based screening for resistant heterozygotes by directly detecting the recessive allele.

Field populations of the tobacco budworm *H. virescens*, a key pest of cotton and other crops in the Americas, have developed resistance to most classes of chemical insecticides. This species is the primary target of recently commercialized transgenic Bt cotton, which protects itself from insect damage by producing the insecticidal Cry1Ac toxin from *B. thuringiensis*. Concerns about Bt resistance led the U.S. Environmental Protection Agency to mandate a management plan, the "high-dose/refuge strategy" (*1*). It assumes that Bt cotton produces enough toxin to kill heterozygotes (with just one resistance allele) as well as

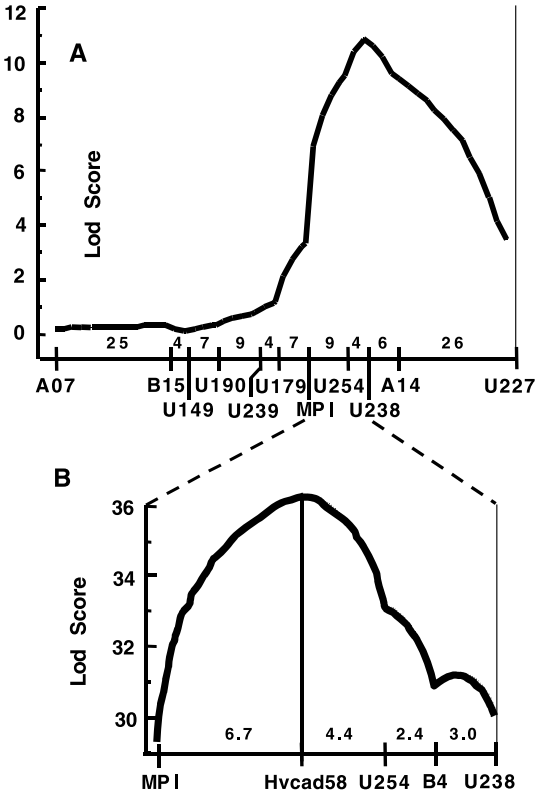

Fig. 1. QTL mapping of Cry1Ac resistance on linkage group 9 of *H. virescens*. (**A**) Resistance QTL lod (logarithm of the odds ratio for linkage) profile for initial scan of 105 cM on LG 9 spanned by 11 markers, based on 48 progeny of segregating backcross family D6. Marker order and spacing (in cM) was calculated by Mapmaker EXP 3.0 (*16*) and lod scores by Mapmaker QTL 1.9 (*17*). (**B**) Lod profile for fine-scale QTL mapping over the 16-cM region between MPI and U238, based on 268 progeny of nine segregating backcross families. The maximum lod score of 35.9 occurs at Hvcad58, which accounts for 46% of the trait variance. The resistance trait is the log of larval weight after 10 days of growth on 0.032 μg of Cry1Ac toxin per milliliter of diet (*3*).

susceptible homozygotes (with none). To counterbalance selection for Bt-resistant insects, farmers growing Bt cotton are also required to grow a non–Bt cotton "refuge" from selection, intended to produce a large number of homozygous susceptibles. These are expected to mate with any homozygous resistant survivors of Bt cotton (carrying two resistance alleles), producing heterozygous progeny that cannot survive the Bt toxin dose.

Population models predict that this strategy should greatly retard the spread of resistance, but the difficulty of measuring allele frequencies in the field has made verification problematic. Conventional bioassay-based monitoring methods are too insensitive, especially when resistance is rare and recessive, because of the extreme rarity of resistant homozygotes. A DNA-based method of detecting resistant alleles directly in heterozygotes, where most of them occur initially, would be more efficient.

Although Bt-resistant populations of *H. virescens* have not yet been observed in the field, resistant strains have been developed in the laboratory by selection with toxin-impregnated diet. One of these, YHD2, exhibits high-level (resistance ratio = 10,128), recessive resistance to the Cry1Ac toxin (*2*). A single major gene (*BtR-4*) is responsible for 40 to 80% of Cry1Ac resistance levels in YHD2; the remainder is controlled by a combination of environmental factors and other genes of minor effect. We previously assigned *BtR-4* to linkage group 9 (LG 9) (*3*).

To further localize *BtR-4* on LG 9, we developed 11 polymorphic markers that spanned a total genetic length of 105 centimorgans (cM). These markers were scored on a segregating backcross family of 48 progeny of a hybrid male and a YHD2 female. When LG 9 was scanned for resistance QTLs (quantitative trait loci) using interval mapping (*4*), a single highly significant peak indicated that the most likely location of *BtR-4* was between markers MPI and A14 (Fig. 1A).

Bt resistance in some species is accompanied by a loss of toxin binding to the midgut epithelium (*5, 6*). As candidates for *BtR-4*, we tested genes encoding Bt-binding midgut proteins to see whether they mapped to LG 9. Two types of Bt-binding proteins are known

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```
                                 SIG <-                                               -> CR 1
HevCaLP   MAVDVRILTAAVLILAAHLTVAQDCSYMVAIPRPERPDFPNQNFEGVPWSQNPLLPAEDREDVCMNAFDPSALNPVTVIF    80
r1        -----------------N--F-----------------------------------------------------------    80

HevCaLP   MEEEIEGDVAIARLNYRGTNTPTVVTPFNFGTFHLLGPVIRRIPEQGGDWHLVITQRQDYETPNMQQYIFNVRVEDEPQE   160
r1        --------------------------------------------------------------------------------   160

                         -> CR 2
HevCaLP   ATVMLIIVNIDDNAPIIQMFEPCDIPEHGETGTTECKYVVSDADGEISTRFMTFQIESDRNDEEYFELVRENIQGQWMYV   240
r1        ---------------M---------------------S----------------E-------------------------   240
                                                               -> CR 3
HevCaLP   HMRLILNKPLDYEENPLHLFRVTALDSLPNVHTVTMMVQVENIESRPPRWMEIFAVQQFDEKTAQAFRVRAIDGDTGIDK   320
r1        -----------------------------------------------------------------S--------------   320

HevCaLP   PIFYRIETEESEKDLFSVETIGAGREGAWFKVAPIDRDTLEKEVFHVSLIAYKYGDNDVEGSPSFESKTDIVIIVNDVND   400
r1        --------------------------------------------------------------------------------   400

              -> CR 4
HevCaLP   QAPVPFRPSYYIEIMEEAAMTLNLEDFGFHDRGLGPHAQYTVHLESISPAGAHEAFYIAPEVGYQRQSFIVGTQNHHMLD   480
r1        ----------F------T--------------D--------------H--------------------------------   480
                                                    -> CR 5
HevCaLP   FEVPEFQKIQLRAVAIDMDDPRWVGIAIININLINWNDELPIFEHDVQTVTFKETEGAGFRVATVLAKDRDIDDRVEHSL   560
r1        ------------V-------------------------------------------------------------------   560
                                                               td
HevCaLP   MGNAVNYLSIDKDTGDILVTIDDAFNYHRQNELFVQIRADDTLGEPYNTNTAQLVIQLQDINNTPPTLRLPRTTPSVEEN   640
r1        ----------------------------------------------------CSGLSHRAGL                     622
            -> CR 6
HevCaLP   VPDGFVIPTELHATDPDTTAELRFSIDWDTSYATKQGRDADAEEFVNCIEIETVYPNLNDRGTAIGRVVVREIREHVTID   720
                                                    -> CR 7
HevCaLP   YEMFEVLYLTVRVTDLNTVIGDDYDISTFTIIIIDMNDNPPLWVEGTLTQEFRVREVAASGVVIGSVLATDIDGPLYNQV   800

HevCaLP   RYTITPRLDTPEDLVEIDFNSGQISVKKHQAIDADEPPRQHLYYTVVASDKCDLLSVDVCPPDPNYFNTPGDITIHITDT   880
               -> CR 8
HevCaLP   NNRVPRVEEDKFEEIVYIYEGAEDGEHVVQLFASDLDRDEIYHKVSYQINYAINPRLRDFFEVDLETGLVYVNNTAGEKL   960
                                                            -> CR 9
HevCaLP   DRDGDEPTHRIFFNVIDNFYGEGDGNRNQDETQVLVVLLDINDNYPELPEGLSWDISEGLLQGVRVTPDIFAPDRDEPGT  1040

HevCaLP   DNSRVAYDIVSLSPTDRDITLPQLFTMITIEKDRGIDQTGELETAMDLRGYWGTYEIHVKAYDHGVPQRISYEKYPLVIR  1120
                -> CR 10
HevCaLP   PYNFHDPVFVFPQPGMTIRLAKERAVVNGVLATVDGEFLERIVATDEDGLHAGVVTFSISGDDEALQYFDVFNDGVNLGA  1200
                                                          -> CR 11
HevCaLP   LTITQLFPEDFREFQVTIRATDGGTEPGPRSTDCTITVVFVPTQGEPVFETSTYTVAFIEKDAGMEERATLPLAKDPRNI  1280
                                                                                         -> MPR
HevCaLP   MCEDDCHDTYYSIVGGNSMGHFAVDPQSNELFLLTPLERAEQETHTLIIGASDSPSPAAVLQASTLTVTVNVREANPRPV  1360

HevCaLP   FQSALYTAGISTLDTINRGLLTLHATHSEGLPVTYTLVQDSMEADSTLQAVQETAFNLNPQTGVLTLNFQPTASMHGMFE  1440

HevCaLP   FDVMATDTVGETARTEVKVYLISDRNRVFFTFMNTLEEVEPNEDFIAETFTLFFGMRCNIDQALPASDPATGAARDDQTE  1520
                                                                       -> TM
HevCaLP   VRAHFIRDDLPVPAEEIEQLRGNPTLVATIQNALQEENLNLADLFTGETPILGGEAQARAVYALAAVAAALALLCVVLLI  1600
              -> CYT
HevCaLP   LFFIRTRALNRRLEALSMTKYSSQDSGLNRVGLAAPGTNKHAVEGSNPIWNETLKAPDFDALSEQSYDSGLIGIEDLPQF  1680

HevCaLP   RNDYFPPDEESSMRGVVNEHMPGANSVANHNNNFGFNATPFSPEFANSQLRR                              1732
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Fig. 2. Conceptual translation of HevCaLP and predicted product of *r1* allele. For the latter, only residues differing from HevCaLP are shown. SIG, signal peptide; CR 1 to CR 11, cadherin repeats; MPR, membrane-proximal region; TM, transmembrane domain; CYT, cytoplasmic domain; td (underlined), region of target sequence duplication and insertion of Hel-1 (*18*). Single-letter abbreviations for amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

in insects: aminopeptidases (APNs) and cadherins. Bt-binding APNs have been isolated from several lepidopteran species, including two from *H. virescens*. A 170-kD APN (Receptor A) binds Cry1A toxins with high affinity and mediates Bt toxin–induced pore formation when reconstituted into phospholipid vesicles in vitro (*7*). We amplified a portion of the gene for Receptor A, and we found that it mapped to LG 12 by restriction fragment length polymorphism analysis. A second, 120-kD Cry1Ac-binding APN (BTBP1) (*8*) maps to LG 23. This eliminates both of these APNs as candidates for *BtR-4*.

The other Bt-binding proteins are members of the cadherin superfamily (*9*), but none had previously been isolated from *H. virescens.* BTR1 from *Manduca sexta*, when expressed on the surface of transfected human cells, bound to Cry1Ab with high affinity (*10*). BtR175 from *Bombyx mori*, when expressed on the surface of insect cells, mediated binding and cell lysis by Cry1Aa. Moreover, testing of various truncated forms of BtR175 enabled localization of the Cry1Aa-binding domain to the membrane-proximal region and the nearest cadherin repeat (*11*). We sought to amplify a homolog of these cadherins from *H. virescens* midgut cDNA using the polymerase chain reaction (PCR). Primers designed from the membrane-proximal region of BtR175 yielded a product (Hvcad58) with high similarity to BtR175. Hvcad58 mapped to LG 9, between the markers MPI and U238. QTL mapping at a finer scale, with additional markers and progeny, produced a highly significant likelihood peak directly above Hvcad58 (Fig. 1B), making it a strong candidate for *BtR-4*.

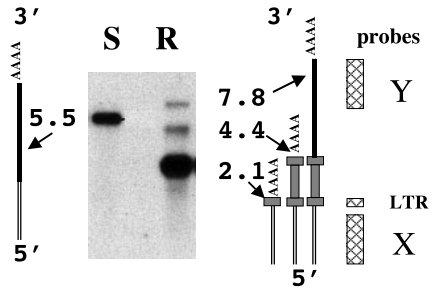

Fig. 3. Northern analysis of susceptible (S) and resistant (R) strains showing HevCaLP transcripts. One S and three R transcripts are seen when mRNA isolated from fifth instar larval midgut is probed with segment X from the 5′ end. (Not shown: When the filter was stripped and probed with the LTR segment, only the three R transcripts were seen; when probed with segment Y from the 3′ end, only the S transcript and the 7.8-kb R transcript were seen.) The Hel-1 element is shaded, with LTRs at both ends. Cross-hatched regions show segments of DNA used as probes.

Hvcad58 was used to screen larval midgut cDNA libraries made from resistant (YHD2) and susceptible strains. A representative allele (*s1*) cloned from a susceptible strain produces a 5.5-kb transcript with a predicted protein product of 1732 amino acids, which we term HevCaLP (*Heliothis virescens* cadherin-like protein, Fig. 2). HevCaLP is 70% identical overall to BtR175, sharing a signal sequence, 11 extracellular cadherin-type repeats, a noncadherin membrane-proximal region, a transmembrane region, and a cytoplasmic domain.

Expression of the mRNA encoding HevCaLP in susceptible and resistant larval midguts was studied by Northern analysis (Fig. 3) and sequencing. Susceptible larvae had a single transcript of 5.5 kb. Resistant YHD2 larvae showed three transcripts. The rarest (7.8 kb) was similar to the susceptible transcript, except for a 2.3-kb insert we denote as Hel-1. The middle transcript (4.4 kb) was truncated at the end of Hel-1 by a polyadenylated tail. The third, highly abundant transcript (2.1 kb) was truncated at the beginning of Hel-1 by a polyadenylated tail.

Hel-1 shows two hallmarks of LTR-type retrotransposons (*12*): It has long terminal repeat sequences (LTRs) of about 255 bases at both ends, and it is flanked by an 8-base duplication of the host target sequence ACACTGCC encoding the amino acids Asn-Thr-Ala (Fig. 4). Hel-1 is much shorter than known functional retrotransposons and lacks identifiable *gag*, *pol*, or *env* genes, which suggests that it is an internally deleted copy of a full-length retrotransposon (*13*).

As the result of an in-frame stop codon occurring 30 bases into the first LTR of Hel-1, conceptual translation of the three different YHD2 transcripts produces the same truncated 622–amino acid protein sharing 601 residues with the NH_2 terminus of HevCaLP (Fig. 2). Multiple stop codons in all reading frames of the LTR prevent translation of a larger protein containing the COOH terminus of HevCaLP. Thus, the predicted protein product of the *r1* allele (for which YHD2 is homozygous) would have the same signal sequence as HevCaLP (possibly directing its secretion into the midgut lumen) but no predicted transmembrane or toxin-binding domain.

Southern blots probed with the LTR of Hel-1 show 10 to 15 copies in the genome of both YHD2 and susceptible insects (Fig. 5). Apparently, mobilization of a Hel-1 element and insertion into the gene encoding HevCaLP has created the novel knockout *r1* allele. This could have occurred in the laboratory during the Bt resistance selection protocol that produced YHD2, or may have already been present in one of the 490 field-collected founders of the selection line. Because these founders were not preserved, the only way to obtain evidence for the latter possibility is to recover the *r1* allele from new field collections.

To illustrate detection of the *r1* allele, we designed a PCR assay with two primers flanking the insertion point and a third primer internal to the left LTR (Fig. 4A). Primers 1 and 3 produced a 71–base pair (bp) band from the *r1* allele. Primers 1 and 2 amplified a 99-bp band from susceptible alleles lacking the Hel-1 insert. Both bands were seen in heterozygotes. Although bioassay cannot distinguish heterozygotes from homozygous susceptibles because resistance is recessive, the PCR assay can directly detect the resistance allele in heterozygotes. It correctly predicted the genotype of all individuals in a sample of 86 known *r1* homozygotes, heterozygotes, and susceptible homozygotes.

We propose that the gene encoding HevCaLP is identical to *BtR-4*, the major resistance gene in YHD2. Recessivity of the *r1* resistance allele can be explained by Hel-1 inactivation of HevCaLP. HevCaLP appears to be a "lethal target" of Bt toxin, because two copies of the disrupted allele are required for high resistance. Heterozygotes still present a lethal target to Cry1Ac because they have one copy of the susceptible allele. The normal physiological function of HevCaLP is unknown, although other cadherins are in-

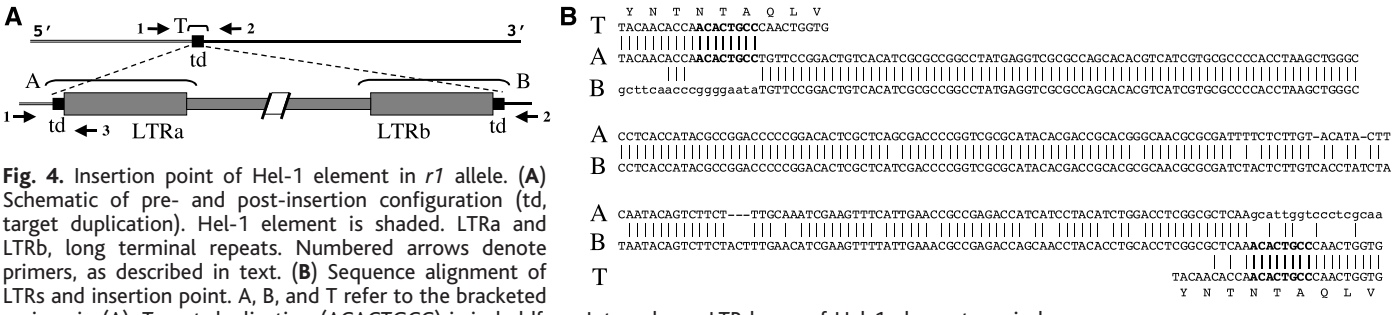

Fig. 4. Insertion point of Hel-1 element in *r1* allele. (**A**) Schematic of pre- and post-insertion configuration (td, target duplication). Hel-1 element is shaded. LTRa and LTRb, long terminal repeats. Numbered arrows denote primers, as described in text. (**B**) Sequence alignment of LTRs and insertion point. A, B, and T refer to the bracketed regions in (A). Target duplication (ACACTGCC) is in boldface. Internal non-LTR bases of Hel-1 element are in lowercase.

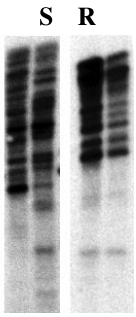

Fig. 5. Southern blot of susceptible (S) and resistant (R) strain individuals showing multicopy occurrence of Hel-1. Genomic DNA was digested with Apa LI, electrophoresed in a 0.8% agarose gel, blotted to nylon membranes, and probed with radiolabeled Hel-1 LTR.

volved in cell adhesion (*14*). Whatever its function, it is not essential for life, because YHD2 is viable and fertile under laboratory conditions despite being a "natural knockout" for HevCaLP. Whether its absence confers a fitness disadvantage in the field has important implications for resistance management, and this question can now be addressed with the information developed here.

These results suggest a new interpretation of our previous estimate of 0.0015 for the frequency of YHD2-type resistance alleles in field populations of *H. virescens* before widespread planting of Bt cotton (*15*). In that study, field-caught males were individually mated to homozygous resistant YHD2 virgin females, and their progeny were tested at a discriminating dose of Cry1Ac-containing diet. The majority of males were homozygous susceptible, as expected, producing only heterozygous progeny that did not grow on Cry1Ac because the resistance-conferring effect of *r1* is recessive. However, 3 of 1025 males were heterozygous, producing some progeny that did grow on the Cry1Ac diet because they inherited the *r1* allele from their YHD2 mother and a field-derived resistance allele from their father.

Our previous interpretation implicitly assumed that the paternally contributed resistance allele was also *r1*. But it is now evident that any other allele with a molecular lesion somewhere in HevCaLP preventing it from functioning as a lethal target would give the same result, because *r1* is a null allele. Thus, 0.0015 actually represents a frequency estimate of the entire class of such defective HevCaLP alleles. This statement applies even if *r1* itself does not occur in the field but arose in the lab. Thus, the development of efficient DNA-based methods to detect other types of mutants at *BtR-4* should be a high priority. Screening solely for the Hel-1 insert detects *r1* but may underestimate the total frequency of resistance alleles in the field.

Monitoring resistance allele frequencies in field populations will enable a direct test of whether the high-dose/refuge strategy is succeeding. If it starts to fail, detection of increasing heterozygote frequencies will indicate that a problem is looming, well before resistant homozygotes become frequent enough to cause uncontrollable outbreaks. This may allow enough time for the strategy to be adjusted to reverse the increase. We thus suggest that allele frequency monitoring be incorporated into resistance risk assessment. At the very least, preservation of DNA samples should accompany existing bioassay-based monitoring programs. Even if other Bt resistance genes are later discovered in *H. virescens*, any delay in initiating *BtR-4* allele monitoring erodes the opportunity to make informed modifications to a strategy that could sustain the use of Bt transgenics and prolong their environmental benefits of reducing dependency on conventional insecticides.

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Bt Toxin Resistance from Loss of a Putative Carbohydrate-Modifying Enzyme

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The development of resistance is the main threat to the long-term use of toxins from *Bacillus thuringiensis* (Bt) in transgenic plants. Here we report the cloning of a Bt toxin resistance gene, *Caenorhabditis elegans bre-5*, which encodes a putative β-1,3-galactosyltransferase. Lack of *bre-5* in the intestine led to resistance to the Bt toxin Cry5B. Wild-type but not *bre-5* mutant animals were found to uptake toxin into their gut cells, consistent with *bre-5* mutants lacking toxin-binding sites on their apical gut. *bre-5* mutants displayed resistance to Cry14A, a Bt toxin lethal to both nematodes and insects; this indicates that resistance by loss of carbohydrate modification is relevant to multiple Bt toxins.

Crystal toxins produced by *B. thuringiensis* are used worldwide in transgenic crops to control caterpillars and beetles, are an important tool of organic farming, and have made important contributions to the control of insect-borne diseases such as African river blindness. Once ingested by an insect, Bt toxins are proteolytically activated in the midgut and bind to membrane gut receptors, leading to pore formation and death (*1, 2*). Although Bt toxins are safe to vertebrates and are considered beneficial to the environment relative to chemical pesticides, Bt toxin effectiveness is threatened in the long term by the development of insect resistance (*3*). Bt-resistant variants of the diamondback moth have been identified in the field, and resistant strains of at least 11 insect species have been documented in the laboratory (*4, 5*). Understanding the molecular mechanism of toxin action and identifying the genes that can mu-

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