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mediate IRIF formation by RAP80 and conjugated ubiquitin. Indeed, RNF8 was essential for IRIF formation in both cases (Figs. 3F and 4A, and fig. S10A). Because RAP80 is not required for 53BP1 focus formation after IR (14) and 53BP1 is not needed for RAP80 IRIF (fig. S10B), these results indicated that RNF8 acts downstream of MDC1 to promote at least two types of IRIF: those containing 53BP1 and those containing BRCA1. In support of this model, mutation of the TQXF motifs in MDC1 also impaired BRCA1 and conjugated ubiquitin IRIF (fig. S11).

RNF8 can bind to multiple E2-conjugating enzymes to catalyze both K63-linked and K48-linked ubiquitin chains (23, 25). When we screened a panel of 13 E2 enzymes by RNAi and quantitative microscopy, only UBC13 depletion markedly impaired 53BP1 IRIF formation (fig. S12). UBC13 depletion also impaired IRIF by conjugated ubiquitin (Fig. 4B). Entirely consistent with our data, genetic ablation of UBC13 in DT40 cells abrogate BRCA1 and ubiquitin IRIF (26). As UBC13 is the only known E2-conjugating enzyme that catalyzes K63-linked polyubiquitination, these findings are consistent with data indicating that ubiquitin IRIF form in part through K63-linked polyubiquitination (15, 26). Moreover, as UBC13 physically interacts with RNF8 to catalyze K63-linked ubiquitin chains (27), the available data imply that RNF8 is responsible for formation of K63-linked ubiquitin chains at DSB sites.

Consistent with RNF8 playing an important function in the DDR, we found that RNF8 de-

pletion caused IR hypersensitivity in clonogenic cell-survival assays (Fig. 4C). Furthermore, a greater proportion of RNF8-depleted cells progressed into M phase after irradiation than did cells transfected with a control esiRNA, which indicates that RNF8 enforces the G₂/M DNA-damage checkpoint (Fig. 4D).

Our results identify mammalian RNF8 as an important component of the DDR. Specifically, RNF8 binds to ATM-target motifs on MDC1, thus recruiting RNF8 to DSB sites. RNF8 then triggers the formation of ubiquitin conjugates that promote recruitment of the RAP80-BRCA1 complex and 53BP1 to DSB sites, thereby enhancing DNA-damage checkpoint events and promoting cell survival (Fig. 4E).

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28. We thank members of the Durocher and Jackson laboratories for input on the manuscript and M. Vojvodic for experimental assistance. We also thank A. C. Gingras for the stable YFP-RNF8 line, KuDOS Pharmaceuticals for providing inhibitors and reagents, and Abcam for the MDC1 pT719 antibody. This work was supported by grants from the Canadian Institutes of Health Research (CIHR) to D.D. and by funding from Cancer Research UK and the European Union (S.P.J.). M.K.K. is a CIHR postdoctoral fellow and an alumnus of the Excellence in Radiation Research for the 21st Century training program; F.D.S. holds a Terry-Fox studentship from the National Cancer Institute of Canada; S.N. is a Gail-Posluns Fellow and is supported by the Mitsubishi Pharma Research Foundation. D.D. is a Canada Research Chair (Tier II) in Proteomics, Functional Genomics, and Bioinformatics.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1150034/DC1
Materials and Methods
Figs. S1 to S14
References

4 September 2007; accepted 5 November 2007
Published online 15 November 2007;
10.1126/science.1150034
Include this information when citing this paper.

Engineering Modified Bt Toxins to Counter Insect Resistance

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The evolution of insect resistance threatens the effectiveness of *Bacillus thuringiensis* (Bt) toxins that are widely used in sprays and transgenic crops. Resistance to Bt toxins in some insects is linked with mutations that disrupt a toxin-binding cadherin protein. We show that susceptibility to the Bt toxin Cry1Ab was reduced by cadherin gene silencing with RNA interference in *Manduca sexta*, confirming cadherin's role in Bt toxicity. Native Cry1A toxins required cadherin to form oligomers, but modified Cry1A toxins lacking one α -helix did not. The modified toxins killed cadherin-silenced *M. sexta* and Bt-resistant *Pectinophora gossypiella* that had cadherin deletion mutations. Our findings suggest that cadherin promotes Bt toxicity by facilitating toxin oligomerization and demonstrate that the modified Bt toxins may be useful against pests resistant to standard Bt toxins.

The toxins produced by *Bacillus thuringiensis* (Bt) kill some major insect pests such as mosquitoes and crop-eating caterpillars but are harmless to vertebrates and most other organisms (1). Transgenic corn and cotton

producing Bt toxins grew on more than 32 million hectares worldwide in 2006 (2). The primary threat to the long-term efficacy of Bt toxins is the evolution of resistance by pests (3–5). Many insects have been selected for resistance to Bt toxins in the laboratory, and two crop pests (*Plutella xylostella* and *Trichoplusia ni*) have evolved resistance to Bt sprays outside of the laboratory (3–13). The most widely used Bt toxins are crystal toxins in the Cry1A family, particularly Cry1Ab in transgenic Bt corn and Cry1Ac in transgenic Bt

cotton, which kill caterpillars (lepidopteran larvae) (7). Cry1A toxins bind to the extracellular domain of cadherin proteins that traverse the insect larval midgut membrane (14). Disruption of Bt toxin binding to midgut receptors is the most common mechanism of insect resistance (6). Mutations in the midgut cadherins that bind Cry1Ac are linked with and probably cause resistance in at least three lepidopteran pests of cotton (5, 10, 12).

Two hypotheses proposed to explain how Cry1A toxins function are the pore-formation model and the signaling model (15, 16). These theories share initial steps: Cry1A protoxins are ingested, solubilized in the gut, and cleaved by midgut proteases such as trypsin to yield activated 60-kD monomeric toxins that bind to cadherin with high affinity (14). The signaling model, derived from studies of insect cell cultures, suggests that after protease-activated monomeric toxins bind to cadherin, initiation of a magnesium-dependent signaling pathway causes cell death (16). In contrast, on the basis of results from in vitro experiments and bioassays, the pore-formation model proposes that protease-activated monomers bind to cadherin to facilitate protease cleavage of the N terminus of the toxin, including helix α -1 of domain I (17, 18). This cleavage induces the assembly of oligomeric forms of the toxin, which have increased binding affinity to secondary receptors,

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including glycosylphosphatidylinositol-anchored proteins, aminopeptidase N, and alkaline phosphatases (15, 19). After oligomers bind to secondary receptors, they create pores in the midgut membrane that cause osmotic shock and cell death (15).

According to the pore-formation model, the binding of protease-activated toxin to cadherin is essential for the removal of helix α -1, which in turn promotes oligomerization. Therefore, we hypothesized that modified Cry1Ab and Cry1Ac toxins lacking helix α -1 (referred to hereafter as Cry1AbMod and Cry1AcMod) could form oligomers without cadherin. To test this hypothesis, we compared oligomerization of native and modified Cry1Ab and Cry1Ac in the presence and absence of toxin-binding cadherin fragments. Toxins were activated by trypsin to mimic the protease activation that occurs in the insect gut. Previous work shows that trypsin-activated Cry1Ab forms 250-kD oligomers in the presence of *Manduca sexta* cadherin fragments containing toxin-binding regions corresponding to cadherin repeats 7 and 11 or a single-chain antibody (scFv73) that mimics these cadherin toxin-binding regions (17, 20). We found that trypsin-activated Cry1Ab and Cry1Ac formed oligomers in the presence of a protein fragment corresponding to *M. sexta* cadherin repeat 12 (CADR12), an important toxin-binding region (21), but not without cadherin or with cadherin repeat 9 (CADR9), a region that does not bind toxin (21) (Fig. 1). In contrast, trypsin-activated Cry1AbMod and Cry1AcMod formed oligomers without cadherin, although not as efficiently as the wild type with CADR12 (Fig. 1).

We used RNA interference (RNAi) to reduce production of cadherin protein (Fig. 2 and fig. S1) and decrease the susceptibility of *M. sexta* larvae to Cry1Ab. Larvae injected with either 1 μ g of cadherin double-stranded RNA (dsRNA) or water

only (control) ate a diet treated with 20 ng of Cry1Ab protoxin/cm². After 3 days, survival was 92% for 48 RNAi-treated larvae versus 0% for 48 control larvae (chi-square test, $P < 0.001$).

The next experiment showed that cadherin-silenced *M. sexta* larvae were much more susceptible to Cry1AbMod than to Cry1Ab (fig. S2). Confirming the results described above, the survival of larvae fed a diet treated with 20 ng of Cry1Ab protoxin/cm² was higher for 48 RNAi-treated larvae

(92%) than for 48 control larvae (2%) (chi-square test, $P < 0.001$). However, on a diet with 5 ng of Cry1AbMod protoxin/cm², 48 RNAi-treated larvae had only 2% survival, which is significantly lower than the aforementioned 92% survival of 48 RNAi-treated larvae fed a diet treated with 20 ng of Cry1Ab protoxin/cm² (chi-square test, $P < 0.001$).

We also tested modified and native Cry1A toxins against larvae of a resistant strain (AZP-R) and a susceptible strain (APHIS-S) of the cotton

Fig. 2. Effect of RNAi on cadherin protein expression in *M. sexta* larvae. Western blots were tested for the *M. sexta* cadherin protein (BT-R₁) and for an 80-kD brush-border membrane vesicle protein (BBMV). Lanes 1 to 5: Control larvae injected with water only and fed a diet without toxin. Lanes 6 to 12: Larvae injected with 1 μ g of BT-R₁ dsRNA and fed a diet with 20 ng of Cry1Ab/cm².

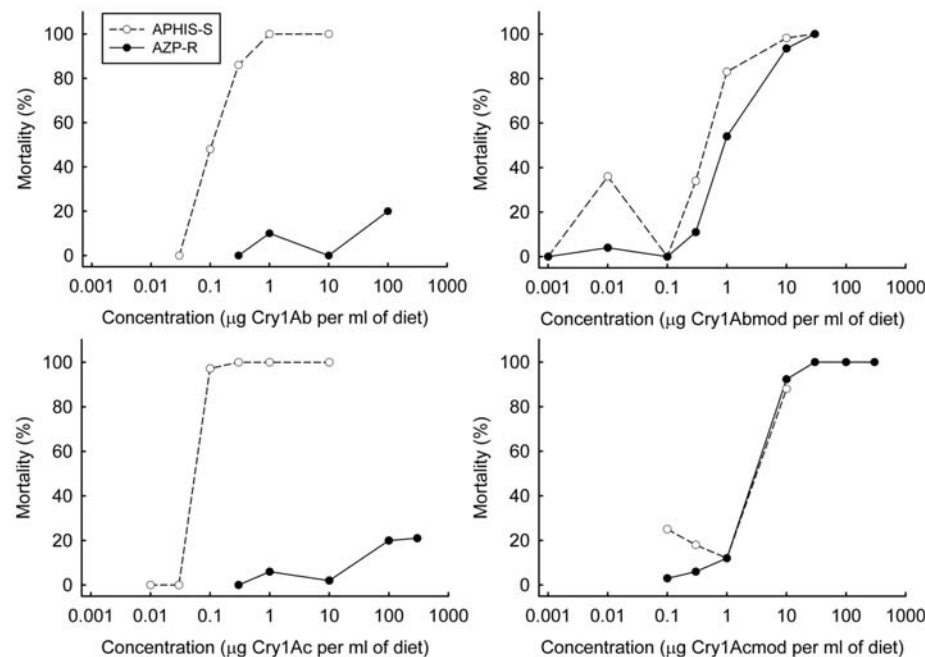
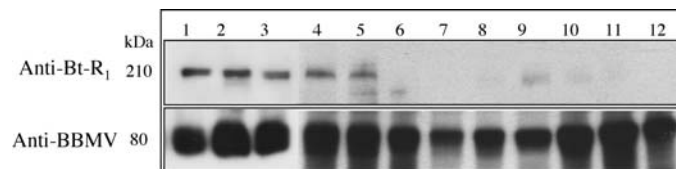


Fig. 3. Responses of susceptible (APHIS-S) and resistant (AZP-R) pink bollworm larvae to native toxins [Cry1Ab and Cry1Ac (left)] and modified toxins [Cry1AbMod and Cry1AcMod (right)].

Table 1. Modified toxins Cry1AbMod and Cry1AcMod kill pink bollworm larvae resistant to Cry1Ab and Cry1Ac.

Toxin	Insect strain ^a	<i>n</i>	LC ₅₀ (95% FL) ^b	Resistance ratio ^c
Cry1Ab	Resistant	320	>100 ^d	>910
	Susceptible	320	0.11 (0.09 – 0.14)	
Cry1AbMod	Resistant	400	1.1 (0.73 – 1.6)	2.8
	Susceptible	400	0.39 (0.28 – 0.51)	
Cry1Ac	Resistant	340	>300 ^e	>3700
	Susceptible	400	0.079	
Cry1AcMod	Resistant	460	2.8 (1.8 – 3.8)	0.41
	Susceptible	320	6.8	

^aThe resistant strain was AZP-R; the susceptible strain was APHIS-S. ^bLC₅₀ of larvae in micrograms of protoxin per milliliter of diet; 95% fiducial limits (FL) are shown in parentheses when available. ^cLC₅₀ of resistant strain divided by LC₅₀ of susceptible strain. ^dThe highest concentration tested (100 μ g of protoxin/ml of diet) killed only 20% of larvae. ^eThe highest concentration tested (300 μ g of protoxin/ml of diet) killed only 21% of larvae.

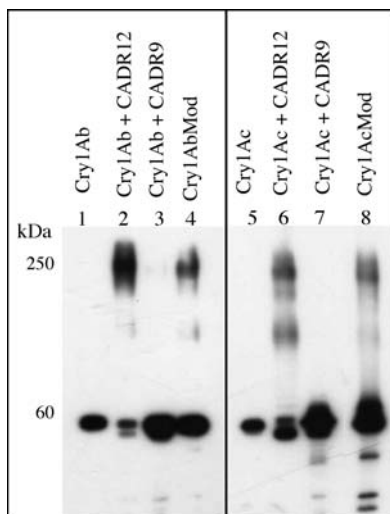


Fig. 1. Oligomer formation by native and modified Bt toxins. Cadherin fragments added correspond to regions that bind toxin (CADR12, lanes 2 and 6) and do not bind toxin (CADR9, lanes 3 and 7). Western blots were probed with polyclonal antibodies to Cry1Ab (lanes 1 to 4) or to Cry1Ac (lanes 5 to 8).

pest pink bollworm (*Pectinophora gossypiella*). Larvae from AZP-R, but not APHIS-S, survived on Bt cotton producing CryIAc (8, 9). This resistance to CryIAc in AZP-R is linked with deletion mutations in the cadherin receptor gene (10). CryIAbMod and CryIAcMod reduced or overcame resistance in the AZP-R strain (Fig. 3 and Table 1). On the basis of the concentration that killed 50% (LC₅₀) for AZP-R relative to APHIS-S, AZP-R was >910-fold resistant to CryIAb and >3700-fold resistant to CryIAc (Table 1). In contrast, AZP-R was only 2.8-fold resistant to CryIAbMod and was not resistant to CryIAcMod (Table 1). Against resistant larvae, the LC₅₀ was more than 100 times higher for CryIAc than for CryIAbMod or CryIAcMod (Table 1). Conversely, against susceptible larvae, the native toxins were more potent than the modified toxins. This implies that, relative to native toxins against susceptible larvae, modified toxins had lower stability in the midgut, reduced oligomer-forming ability (Fig. 1), or reduced ability of oligomers to ultimately cause mortality.

The results suggest that in two species of Lepidoptera, cadherin receptor protein in the larval midgut mediates the toxicity of CryIAc toxins by facilitating removal of helix α -1, which promotes toxin oligomerization. The modified toxins CryIAbMod and CryIAcMod lacking helix α -1 formed oligomers in vitro without cadherin, whereas native CryIAb and CryIAc did not. The modified toxins killed insects with greatly reduced susceptibility to native CryIAc toxins caused by RNAi silencing of the cadherin gene or by mutations in the cadherin gene. These results support the pore-formation model (15) and not the signaling model, which does not include removal of helix α -1 or toxin oligomerization (16).

If the results seen here with the pink bollworm extend to other lepidopterans, CryIAbMod and CryIAcMod could be broadly useful for

countering or delaying pest resistance to CryIAc toxins. However, we do not know whether CryIAcMod toxins kill insects with mechanisms of resistance unrelated to cadherin, such as the disruption of other receptors or decreased protease activation (22). Many Bt toxins have structural topology similar to CryIAc, form oligomers, and induce pores (17, 23–28), suggesting that they share a similar mode of action. It remains to be determined whether, parallel to results with CryIAc toxins, other Cry toxins lacking helix α -1 can kill resistant insects that have altered receptors. In addition, insects can probably evolve resistance to modified Bt toxins lacking helix α -1. Nonetheless, along with native Bt toxins such as Cry2 and Vip3 (29) that have not been used as extensively as CryIAc toxins, the modified toxins broaden the options for pest control.

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- We thank L. Cabrera, C. Unnithan, and R. Biggs for technical assistance in rearing and bioassays; T. J. Dennehy and the Extension Arthropod Resistance Management Laboratory staff for permitting use of the resistant pink bollworm strain they isolated from Arizona cotton fields; A. Gassmann for comments and graphical assistance; and L. Masson, X. Li, and M. Trejo for comments. This work was supported by CONACyT grants 48631-Q, 46176-Q, J46B29-Q, and J45B63-Q; NIH grant 1R01 AI066014; the National Research Initiative; the Cooperative State Research, Education, and Extension Service; and U.S. Department of Agriculture grants 2007-35607-17780, 2003-01469, and 2006-35302-17365.

Supporting Online Material

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Materials and Methods
Figs. S1 to S4
References

13 June 2007; accepted 19 September 2007

Published online 1 November 2007;

10.1126/science.1146453

Include this information when citing this paper.

Genetically Determined Differences in Learning from Errors

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The role of dopamine in monitoring negative action outcomes and feedback-based learning was tested in a neuroimaging study in humans grouped according to the dopamine D2 receptor gene polymorphism DRD2-TAQ-IA. In a probabilistic learning task, A1-allele carriers with reduced dopamine D2 receptor densities learned to avoid actions with negative consequences less efficiently. Their posterior medial frontal cortex (pmFC), involved in feedback monitoring, responded less to negative feedback than others' did. Dynamically changing interactions between pmFC and hippocampus found to underlie feedback-based learning were reduced in A1-allele carriers. This demonstrates that learning from errors requires dopaminergic signaling. Dopamine D2 receptor reduction seems to decrease sensitivity to negative action consequences, which may explain an increased risk of developing addictive behaviors in A1-allele carriers.

“You learn from your mistakes,” people say. We usually learn from both positive and negative action

outcomes, which induce reinforcement of successful and avoidance of erroneous behavior, respectively (1). The relative amount of learning

from successes and errors varies across individuals as a result of disease or pharmacological intervention (2). Can even our genetic makeup influence the way we learn from errors? An important factor in the use of negative and positive feedback for learning seems to be the neurotransmitter dopamine (3–5). A human genetic polymorphism (DRD2-TAQ-IA) is known to modulate dopamine D2 receptor density. The A1 allele is associated with a reduction in D2 receptor density by up to 30% (6–8). This reduction has been linked to multiple addictive and compulsive behaviors (9, 10), which suggests some insensitivity to negative consequences of self-destructive behavior. This might be linked to

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