International Foundation for Biotechnology Research









Tenebrio molitor Stored Product Insect Image Database (Flinn and Oppert)

Why study Cry3Aa intoxication in *T. molitor*?

- One of the primary interfaces of an insect with its environment is through the gut.
- Understanding gene expression profiles in the insect gut can provide understanding of interactions with the environment as well as identify novel control methods for pests.
- The first sequence of a coleopteran genome was from the genetic model *Tribolium castaneum*, and our microarray study provided expression data for gut transcripts (Morris et al., 2009).
- However, *Tenebrio molitor* lacks a sequenced genome, so we obtained gene expression data via 454 pyrosequencing and microarray analysis of the larval gut transcriptome.

RNA-Seq Design

- *T. molitor* larvae (approximately 1 mo old) were placed on control diet (85%) wheat germ, 10% wheat flour, 5% brewers yeast) or the same diet containing 0.1% Cry3Aa toxin (Bt *tenebrionis*). After 24 h, 23-26 larvae were sacrificed and guts were collected in RNA later.
- Gut tissues were processed to obtain total RNA (RNeasy, Invitrogen, Carlsbad, CA) and samples were shipped to 454 Life Sciences for processing. Poly+RNA was purified (Invitrogen) and fragmented. First strand cDNA was prepared with Superscript II (Invitrogen), and directional adaptors were ligated for clonal amplification and sequencing on the Genome Sequencer FLX pyrosequencing system.
- All reads from control and treated larvae were combined into individual dataets for analyses. Sequences were compared to other insect genomes; tentative functions were assigned to gene products; RNA-Seq (i.e., counting numbers of reads associated with each gene product) was used to compare gene expression in control and Cry3Aa-treated larvae.

Microarray Design

- A custom *T. molitor* microarray was developed using the uniESTs generated from the second assembly, with eArray software used for probe design (Agilent Technologies, Inc., Santa Clara, CA USA).
- Of the 25,201 uniESTs, 23,671 oligos were selected as unambiguous (with the program selecting specific oligos representative of each contig) and were arrayed in duplicate or triplicate on a custom array chip (4x44K, Agilent Technologies, Inc.), incorporating standards supplied by eArray.
- Newly molted *T. molitor* larvae (approximately 1 mo old and mean mass of 5–6 mg) were selected from a standard laboratory colony (reared on 50% rolled oats, 2.5% brewer's yeast, 47.5% flour) and were starved overnight.
- Larvae were placed on a diet of 85% stabilized wheat germ, 10% flour, and 5% brewer's yeast with a 3% dilution of a concentrated solution of FD&C Blue #1 and 97% of a mixture of 85% stabilized wheat germ, 10% flour, 5% brewer's yeast with or without 0.1% (w/w) Cry3Aa (pre-equilibrated at 28°C, 75% R.H. over saturated sodium chloride).
- Larvae were monitored for blue guts every 30 min, and those with blue guts (containing either protoxin or not) were selected and reared further on diet containing protoxin or control diet, respectively, for 6, 12, or 24 h. Each corresponding time interval had a separate control and treatment. For each treatment, midguts from four larvae were dissected under and into RNA*later*[®] (Qiagen, Valencia, CA USA). A biological replicate was with larvae from a different oviposition tray.
- mRNA was obtained from each treatment and was labeled with colored dyes and hybridized to the custom array.
- Hybridized probes were measured for relative intensity, and treatment groups were compared.

Snapshots of Gene Expression in Cry3Aa-intoxicated *Tenebrio molitor* larvae

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Number of genes significantly enriched (left) or repressed (right) in intoxicated T. molitor larvae exposed to Cry3Aa for 6, 12, or 24 h.



Comparison of increased gene expression in Cry3Aa-intoxicated T. molitor larvae, as determined by microarray and RNA-Seq (24 h Cry3Aa exposure).



Principle Component Analysis of microarray data demonstrated that gene expression in larvae exposed to Cry3Aa for 24 h was more similar to control, whereas 12 h-exposed larvae were most different from all treatments.





3921 DUF1077 2915_aquaporin 4644 hypothetical protein 19513 peroxiredoxin

(p<0.05).

Relative expression of CBD3 transcripts in Cry3Aa-treated *T. molitor* larvae.

In microarray and RNA-Seq analyses, transcripts encoding proteins with a chitin-binding domain 3 (CBD3) were the most enriched and repressed in 24 h Cry3Aa-intoxicated larvae. Therefore, we used quantiative PCR to confirm that contigs representing these transcripts with both increased (Contig_12590) and decreased (Contig_16411) when larvae were exposed to Cry3Aa (upper left panel). CBD3 proteins are highly conserved among bacteria, plants and animals, but their function is unknown. In the related tenebrionid Tribolium castaneum, there are nine CBD-3 genes, seven in tandem on chromosome 7, which have homology to T. molitor CBD-3 transcripts at the gene (lower left) and predicted protein level (below).



Conclusions

•As transcriptome sequencing is becoming more economical, we have demonstrated that it can be combined with gene expression to address biological questions.

Conservation

•RNA-Seq and microarray analysis can be used for validation.

•Cry3Aa induces multiple changes in the *T. molitor* transcriptome.

Future Directions

Transcriptome sequencing will be used to provide additional data to understand gut physiology in coleopteran pests. •Questions to address:

•What are these prevalent and highly conserved CBD3 proteins in the insect gut? •Why are they differentially expressed in response to Cry3Aa intoxication?



ANOVA of differential gene expression in *T. molitor* larvae exposed to Cry3Aa for 6, 12, or 24 h

Relative Fold Difference

■6 h □12 h ■24 h

				CBD3					
20		40	-	0000		80		100	
	LLLSGLVAK-		- ISGHGMMLE	PPNRSSLWRF					35
	VVFCLLVEK-		- I SGHGMMME	PPNRSSLWRF					40
	LVEGLEVEK-		- I SGHGMLME	PPNRSSLWRF	**********				35
	AISSILLKE-		- ISGHGMMLE	PPNRSSLWRY					35
									-
			IF	PPNRSSIWRE					13
	VALC-YLEE-		-ASCHGMMLE	PSNRASLWRF	DWKQPSNYND	MGYFCGGVKF	GLVTIATMVS	KEVTVVLALV	79
RMFLGPLA	FLFVFFLPR-		- ISCHGRLID	PPSRASAWRY	GF				50
DLDIKSYS	KFLEMLVPLS	RFFILLHLYN	FVRGHGRLMD	PPARNSMWRF	GFPN				64
	-VLC-LVEK-		- I SGHGMMLE	PPNRSSLWRF	*********	********			
120	1	140		160		180		200	
	DPTAPVN	YNDNQNYCGG	ASSMDG	KCGVCGDPYN	APHPQENENT	GKYGQGKIVR	QYSPGSVVDI	QVSLTTNHLG	108
	DPTAPPN	YDDNQNFCGG	VAVQWKQFNG	KCGVCGDIYD	APHPQDNENT	GTYGQGKVVR	TYNSGSVVDI	TINLTANHMG	117
	NPNAPVN	YNDNONYCGG	AGVOWASLGG	KCGLCGDKYD	DPHPOANENT	GKYGOGIIAA	EYKAGSALDV	EVLLTTNHKG	113
	DKSALPN	YQDNQNFCGG	YYVQWELNGG	RCGVCGDTYS	DPHPQDNENS	GKYGSGKIVR	TYEAGSVINV	DVWLSKNHLG	112
	KTATPN	YQDNQNFCGG	SYVQWSLNGG	KCGVCGDSYT	DAHPODNENT	GKFGQGKIVR	TYKAGSVIDV	EVLLTKNHLG	76
	NSSAPIN	YNDNONFCGG	ESVOWGKEGG	KCGPCGDKYD	DPHPOANENG	GKYGRGEVVA	EYKAGSVIDV	OVKLTANHLG	90
DPTNRASR	WRVDPKAPIN	YDDNAFFCGG	FAVQYEQNGG	KCGVCGDNYA	DPVPRSNENT	GKFGNGVISK	TYTAGSIITA	NVTLTANHLG	179
	DTPHN	YNDHELYCGG	FTRQWVKNEG	KCGVCGDAWD	SKIPRAHEFG	GTYGQGVIVR	KYTAGSVINI	RVELTANHFG	125
	DETAPEN	YNDNELFCGG	YAVQWEQNNG	KCGLCGDPHH	VKEPRPHEAG	GLYAKGIISR	HYSVGQEIDI	EVELTANHYG	137
	DFTAPPN	TNDNQNFCGG	FAVQWEQNGG	REGVEGDATD	DPHPQANENI	GRTGQGATVR	ATTAGSVIDY	AVALIANHLU	
				V				$\sim \sim \sim$	
220		240		260		280		300	
SAPESGEE	CFQPI	SLANG-DDRY	NVTFSERT	VNTQVKLP	DGLTCDRCVL	RWHY I GGNNW	GQCEDGSYQE	GCGPQENFRS	198
NAPESCEE	CFQPI	TLANG-EPRY	YIQSTDKT	LIVDTQVKLP	DGLKCDRCVL	RWHYNCGNSW	GQCDDGSYAE	GCGPQETFRS	209
NAPESGED	CFOPL	KLANG-DKQY	NVVTGEK	-TINTKVQLP	SGLTCDRCVL	RWHYQAGNNW	GOCEDGSYDQ	GCGPOETFRS	200
NAPESGEE	CFKVL	PLADG-SQQY	NVSAGETD	ITVALQLP	KGKSCAHCVL	RWHYRAGNNW	GDCGDGTWAK	GCGSQETFRT	202
NAPESGEN	CFKVL	PLADG-SSQY	NVVANESE	VKVALRLP	EGKTCEOCVL	RWQYTAGNNW	GDCGNGTWDK	GCGPQETFRT	166
NAPESGED	CFKPL CFMPL	KLVDG-SVKY	TLPNKQEQGS	YLIKNKIKIP	TRIKCOREOL	RWHYTVGNSW	GLCDDGTSAV	GCGPQETFRS	145
TKPET-ED	CFVDL	PLADG-SSKY	PVSADEYE	I VNQVQLP	AGVTCDRCVL	RWHYKSGNSW	GICTDGTQRM	GCGAQETERS	268
DFKRATQK	CLDKHVLKLV	KPQEGVDHHH	STRYYPKEGN	KVYEMKYRLP	KA-TCDHCLF	QWRYIAGNNW	GTCPNGTGAV	GCGPQEEFRA	220
PN-QVATQD	CFDRYPL	YLSGTRNFVY	NIPEDGKKKA	-IFRYKVQLP	PYVTCTQCVL	QWSYYTGNQW	GTCPNGTEAQ	GCGKSETFRN	232
NAPESCEX	CFKPL	XLADG-SSXY	NVPATERE	INTKVKLP	DGLTCDRCVL	RWHTTAGNNW	GQCEDGTTAE	GCGPQETFRS	
				~~~	$\sim$ $\sim$				
320	)	340		360		380		400	
									204
									215
									209
									208
									167
					*********				151
						*********			274
REMEEDTTE	VEVEVATTTV	AAPPSGEETH	SPITALVLSL	VSFLLVFLIL	SLLYIHFYQV	GKQLKSWLKG	DKDKEQAPMP	PPRTRRARNV	320
		TSAG	SAVPPLEVGV	NNPYLLYYKD	FSKPAPYNVY	PLVVREQVCV	PNSLYKSIPG	VNEWCQSNCL	303
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