Transcriptional Profiling of Ivermectin Resistant *Cooperia punctata* Using Deep Sequencing

Jess A. Millar[1,2], Samantha N. McNulty[2], Dante S. Zarlenga[3], Makedonka Mitreva[2,4]

[1]Department of Biology, Portland State University, Portland, OR 97201
[2]The Genome Institute, Washington University School of Medicine, St. Louis, MO 63108
[3]US Department of Agriculture, Animal Parasitic Disease Laboratory, Beltsville, MD 20705
[4]Division of Infectious Diseases, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63108

Abstract

Intestinal parasites pose a burden to the livestock industry throughout the world. Since the discovery of macrocyclic lactones, Ivermectin has been a popular choice to combat nematode (roundworm) infectious due to its broad spectrum of action and high efficacy. Widespread use of this drug has led to the development of resistance in many parasitic nematodes, including the highly prevalent cattle intestinal parasite *Cooperia punctata*. Ivermectin is known to bind and activate glutamate-gated chloride channels, thereby disrupting neurotransmission and paralyzing the worms. We hypothesize that Ivermectin treatment of drug-resistant *C. punctata* will lead to changes in the expression pattern of genes related to the drugs mode of action. We used high throughput sequencing to study the adult transcriptome of drug-resistant *C. punctata* and to assess Ivermectin's impact on gene expression. RNAseq datasets from treated and untreated Ivermectin resistant adult worms were assembled into 110,772 unique transcripts corresponding to 66,788 genetic loci. 18,585 loci showed evidence of alternative splicing, with an average of 4 transcripts per alternatively spliced locus. Fragmentation rate was estimated at 18.1% by comparison with *C. elegans* CDSs. 101,411 CDSs, representing some 69% of the complete *C. punctata* gene repertoire, were predicted from our dataset. The predicted CDSs are associated with 4,738 unique InterPro protein domains, 3,013 unique Kyoto Encyclopedia of Gene and Genomes (KEGG) terms, and 524 unique Gene Ontology (GO) terms. 5,308 predicted CDSs were up-regulated in Ivermectin treated worms while 4,047 were down-regulated. Thirteen GO terms were statistically over-represented among up-regulated CDSs, including gated channels and metabolism. This suggests that functions related to Ivermectin's mode of action are up-regulated in response to treatment. These results provide insights into potential mechanisms of Ivermectin resistance and possible genetic markers that may be used to monitor the spread of resistant *C. punctata* in cattle and prevent treatment failures.

Introduction

Cooperia punctata

- *Cooperia* ssp. most prevalent internal parasite in U.S. cattle[1]
- Free living (L1 - L3i) and parasitic (L3act - adult) stages
- Causes damage to intestines, decreased nutrient uptake

Ivermectin

- Broad spectrum anthelmintic drug
- Binds to glutamate-gated chloride channels (GluCl)
- Causes paralysis leading to starvation
- Widespread use in cattle since 1980's
- Ivermectin resistance is becoming a problem[2]

Study Design

- USDA juvenile cattle were infected with Ivermectin resistant *C. punctata*
- One calf treated with Ivermectin, the other left untreated
- Samples of *C. punctata* taken when cows reach adult stage
- Create Illumina RNA-Seq data

Specific Aims

- Assemble draft transcriptome for *C. punctata*
- Gene prediction and assessment of differential gene expression

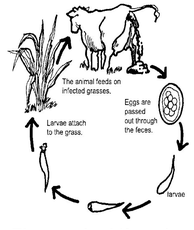

Figure 1: Lifecycle of *C. punctata*

Methods

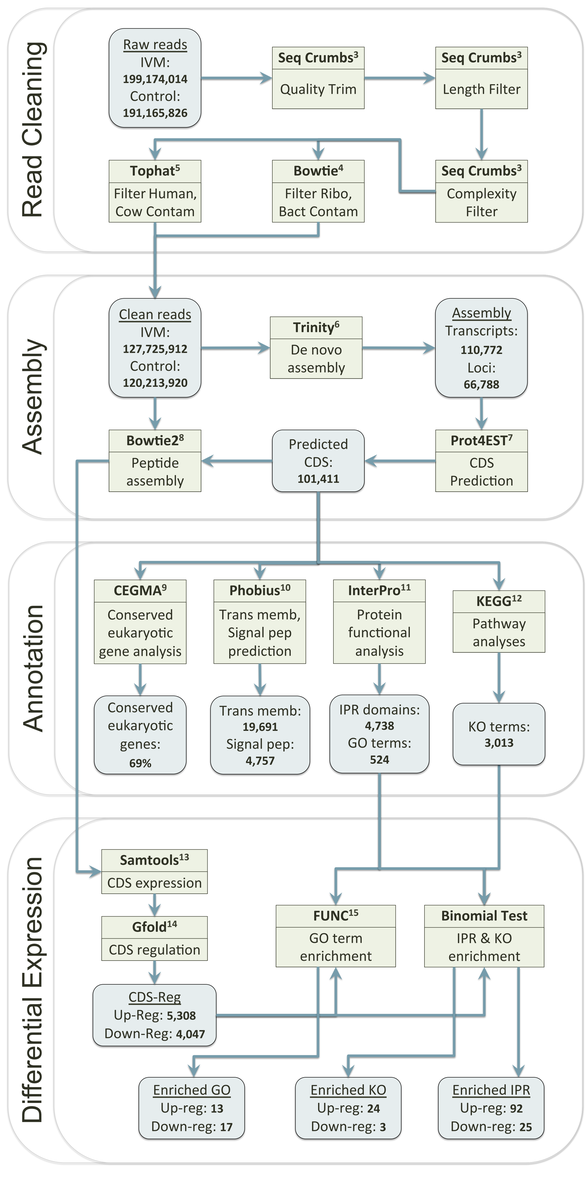

Figure 2: Bioinformatic workflow includes four main steps: read cleaning, *de novo* transcriptome assembly and CDS prediction, CDS annotation, and assessment of differential expression. Square tan boxes include software name and their function. Blue rounded boxes include type of data generated and relevant tallies.

Results

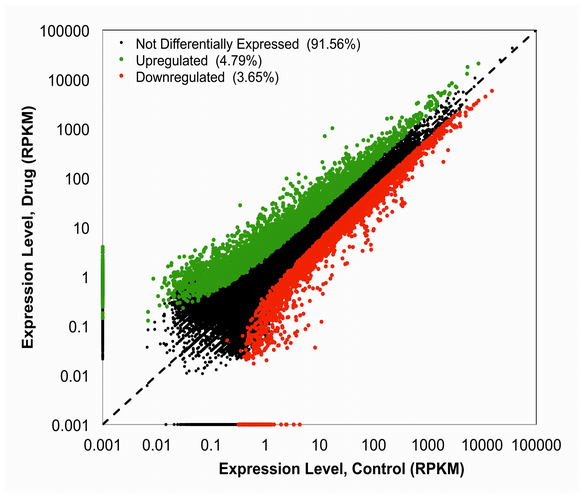

Figure 3: Differential expression of *C. punctata* CDSs in response to drug treatment. Genes were chosen based on significant ($p<10^{-5}$) Gfold values, with a threshold cut off 1 standard deviation above or below the mean.

(A) InterPro Domains

IPR identifier	IPR descriptor	*p* value
IPR021109	Peptidase aspartic	<1.00E-15
IPR009007	Peptidase aspartic, catalytic	<1.00E-15
IPR014782	Peptidase M1, membrane alanine aminopeptidase, N-terminal	<1.00E-15
IPR001930	Peptidase M1, alanine aminopeptidase/leukotriene A4 hydrolase	<1.00E-15
IPR002213	UDP-glucuronosyl/UDP-glucosyltransferase	<1.00E-15
IPR003599	Immunoglobulin subtype	<1.00E-15
IPR003598	Immunoglobulin subtype 2	<1.00E-15
IPR013320	Concanavalin A-like lectin/glucanase, subgroup	<1.00E-15
IPR013098	Immunoglobulin I-set	<1.00E-15
IPR007110	Immunoglobulin-like	<1.00E-15
IPR013783	Immunoglobulin-like fold	<1.00E-15
IPR001461	Peptidase A1	6.34E-13
IPR017452	GPCR, rhodopsin-like, 7TM	5.67E-12
IPR024571	Domain of unknown function DUF3358	7.23E-12
IPR006201	Neurotransmitter-gated ion-channel	1.95E-11
IPR016187	C-type lectin fold	<1.00E-15
IPR016186	C-type lectin-like	<1.00E-15
IPR001304	C-type lectin	<1.00E-15
IPR001314	Peptidase S1A, chymotrypsin-type	<1.00E-15
IPR018114	Peptidase S1/S6, chymotrypsin/Hap, active site	<1.00E-15
IPR009003	Peptidase cysteine/serine, trypsin-like	<1.00E-15
IPR001254	Peptidase S1/S6, chymotrypsin/Hap	<1.00E-15
IPR008962	PapD-like	1.58E-12
IPR009072	Histone-fold	1.77E-12
IPR000535	Major sperm protein	3.92E-10
IPR014044	CAP domain	3.98E-10
IPR016040	NAD(P)-binding domain	1.15E-09
IPR002486	Nematode cuticle collagen, N-terminal	4.76E-09
IPR008160	Collagen triple helix repeat	8.41E-09
IPR007125	Histone core	1.22E-08

(B) KEGG Orthologies

KO identifier	KEGG descriptor	*p* value
K06233	Low density lipoprotein-related protein 2	<1.00E-15
K11140	Aminopeptidase N	<1.00E-15
K05677	ATP-binding cassette, subfamily D (ALD), member 3	1.06E-12
K10352	Myosin heavy chain	4.41E-12
K00281	Glycine dehydrogenase	1.98E-10
K00164	2-oxoglutarate dehydrogenase E1 component	3.47E-09
K05315	Voltage-dependent calcium channel alpha 1	1.42E-07
K06277	Rap guanine nucleotide exchange factor (GEF) 1	2.29E-07
K01187	Alpha-glucosidase	2.29E-07
K11997	Tripartite motif-containing protein 2/3	7.01E-07
K05392	Transient receptor potential cation channel subfamily M	1.07E-06
K09267	Transcription factor SOX1/3/14/21 (SOX group B)	1.14E-06
K00815	Tyrosine aminotransferase	1.98E-06
K13218	Polypyrimidine tract-binding protein 1	1.98E-06
K07188	Hormone-sensitive lipase	1.98E-06
K06107	Erythrocyte membrane protein band 4.1	1.98E-06
K04958	Inositol 1,4,5-triphosphate receptor type 1	1.98E-06
K04426	Mitogen-activated protein kinase kinase kinase 5	1.98E-06
K00901	Diacylglycerol kinase (ATP dependent)	1.33E-06
K05312	Nicotinic acetylcholine receptor	1.40E-06
K01254	Leukotriene-A4 hydrolase	2.09E-06
K14616	Cubilin	3.32E-06
K05850	$Ca2+$ transporting ATPase, plasma membrane	5.55E-06
K05637	Laminin, alpha 1/2	5.66E-06
K14564	Nucleolar protein 56	3.52E-06
K12890	Splicing factor, arginine/serine-rich 1/9	3.53E-06
K11674	Microsphere protein 1	9.14E-06

Figure 4: **(A)** InterPro domains (IPR) and **(B)** KEGG Orthologies (KO) significantly ($p<10^{-5}$, FDR-corrected binomial dist. test) enriched among genes up/down-regulated in Ivermectin resistant *C. punctata* in response to drug treatment. Green=up-regulated; red=down-regulated.

Results

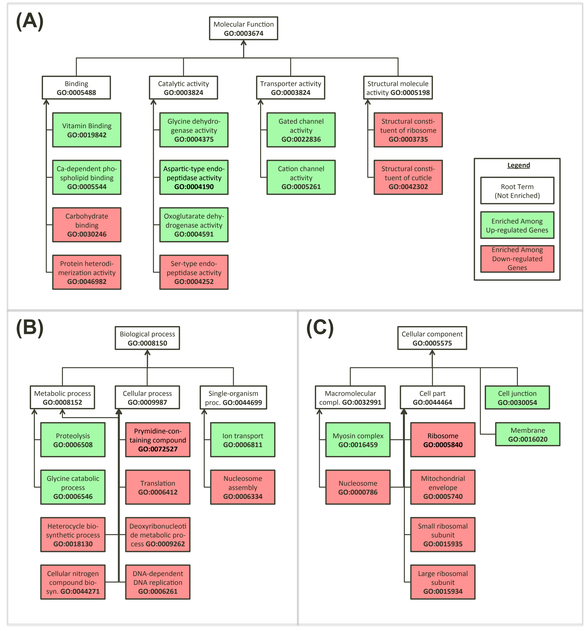

Figure 5: Gene Ontology (GO) terms significantly ($p<10^{-5}$, using FUNC, FDR-corrected) enriched among genes up/down-regulated related to **(A)** Molecular Functions, **(B)** Biological Processes, and **(C)** Cellular Components in Ivermectin resistant *C. punctata* in response to drug treatment.

Discussion

- Transporter activity related to Ivermectin's mode of action was up-regulated. This also may be related to P-glycoprotein up-regulation, shown to facilitate Ivermectin resistance in nematodes[16]
- Metabolism was up-regulated, possibly due to withstanding starvation during paralysis (also observed in *Caenorhabditis elegans*)[17]

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Acknowledgments

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