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Full Length Article

Core RNAi Machinery and Three Sid-1 Related Genes in *Spodoptera litura* (Fabricius)

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Abstract

Our previous works confirmed that RNAi efficiency can be achieved by injection of dsRNA into the fourth-instar larva (Zhao *et al*., 2013) or pupa (Dong *et al*., 2013) in *Spodoptera litura*. However, it has been considered that the RNAi efficiency is refractory in lepidopteran insects. In the present study, we focused on the RNAi machinery in *S. litura* by using an our previously constructed transcriptome (GenBank TSA: GBBY00000000) to detect genes predicted to be involved in the RNAi response, including the *R2d2*, *Loqs*, *Ago1*, *Ago2*, *Dic1* and *Dic2*. By using quantitative PCR, we found that these genes were highly expressed in the development stage of adult and in the tissue of fat body. We also found three homologues of the sid1-like gene responsible for dsRNA transport inside the cell in the *S. litura* transcriptome. These genes were preferentially expressed in the pupal stage and in the tissue of midgut. Z-test of neutral evolution analysis showed that only between *Sl-sid1* and *Sl-sid3* have the probability of rejecting the null hypothesis of strict-neutrality (dN = dS, P< 0.05), indicating no evolutionary pressure between *Sl-sid1* and *Sl-sid3*. Our data support that *S. litura* may have a conserved RNAi mechanism, but that different tissues and/or developmental stages may differ in the efficacy of the RNAi response. © 2015 Friends Science Publishers

Keywords: *Spodoptera litura*; RNAi; RNAi machinery; Sid; Systemic RNAi response

Introduction

RNA interference (RNAi) was firstly described in petunias at the early 1990s, in which genes were over expressed for pigment production, but presenting white flowers that resulted from pigment synthesis inhibition, caused by coordinated gene silencing of both transgene and endogenous gene (Napoli *et al*., 1990). This phenomenon was known as "co-suppression" and also observed in other species of plants, fungi and insects, but the mechanism leading to gene silencing is still not fully understood, especial in lepidopteran insects.

Currently there is no ideal experimental strategy for successful application of RNAi in the study of insect functional genes, probably due to the limited understating of the molecular basis of the RNAi mechanism in insects (Scott *et al*., 2013). The core RNAi machinery in some organisms, such as *Drosophila melanogaster* consists of two RNAi pathways: the small interfering RNA (siRNA) and the micro RNA (miRNA) pathways. Two major functions of the RNAi pathways in some organisms have been confirmed: (1) to regulate gene expression and control critical cellular and developmental processes; (2) to protect from viral infection (Ding and Voinnet, 2007; Castel and Martienssen, 2013). A number of key protein components involved in the RNAi pathways have been reported in plant and invertebrates mainly including Dicer (Dic), R2D2, Loquacious (Loq) and Argonaute (Argo) (Liu *et al*., 2003; Liu *et al*., 2006; Makarova *et al*., 2009; Swevers *et al*., 2011; Wu *et al*., 2013). As reported in *D. melanogaster* that the proteins Dic1, Loq and Ago1 are involved in the miRNA pathway whereas Dic2, R2d2 and Ago2 are involved in the RNAi pathway (Mukherjee and Hanley, 2010). Through these proteins, the stem-loop hairpin transcripts of endogenous genes are processed to generate miRNAs or long dsRNA precursors (either exogenous or endogenous) that are cut to siRNAs. Both of the miRNA and siRNA are incorporated into the RNA-induced silencing complex (RISC) to generate miRISC and siRISC, respectively. The effectors (miRISC and siRISC) serve as guide RNA for sequence-specific cleavage and/or translational repression of complementary mRNA (Bernstein *et al*., 2001; Hannon, 2002; Richter *et al*., 2013). However, the core RNAi machineries not include genes for a systemic RNAi response, when the RNAi effect spreads to the whole body from the initial silenced cell, as it was found in

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Caenorhabditis elegans. Several genes have been identified in *C*. *elegans* that are crucial for systemic spread of dsRNA, but not for the interference itself, such as *sid-1*, encoding a multi-trans membrane domain protein that fucntions as a channel for dsRNA (Winston *et al*., 2002; Winston *et al*., 2007). Interestingly, a systemic RNAi response does not exist in *D. melanogaster*, which may be caused by the absence of *sid-1* in this species (Tomoyasu *et al*., 2008). Insect Sid-1 genes were studied in the preliminary stage, although its homologs have been found in various insect orders (Tian *et al*., 2009; Luo *et al*., 2012; Bansal and Michel, 2013).

The tobacco cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is one of the most destructive insect pests of agricultural crops, such as cotton, vegetables, oilseed and fiber crops. In recent years, its frequent outbreaks in the Asian have caused considerable agricultural economic losses and long-term use of synthetic pesticides that have resulted in serious resistance of *S. litura* against various insecticides (Tuan *et al*., 2013). Therefore, *S. litura* has been paid close attention with the studies extensive on its insecticidal toxicology (Gong *et al*., 2014; Meng *et al*., 2013), molecular mechanisms of its insecticide resistance (Rajagopal *et al*., 2002; Zhou *et al*., 2012) and new alternatives for its integrated management (Arasu *et al*., 2013; Huang *et al*., 2013; Zhao *et al*., 2013). In some of these cases, it was proofed that RNAi is a useful tool for studying genes function in *S. litura*. In addition, our previous works confirmed as well that RNAi efficiency can be achieved by injection of dsRNA into the fourth-instar larva (Zhao *et al*., 2013) and the pupa (Dong *et al*., 2013) in *S. litura*. However, it was known that the RNAi efficiency is refractory in lepidopteran insects (Swevers *et al*., 2013). In the present study, with the aim to search the limiting factors that may affect the efficiency of RNAi in *S. litura*, we explored the RNAi machinery and *sid-1* homologs from our previously constructed *S. litura* transcriptome. We found that the homologues of RNAi machinery are highly conserved in *S. litura* compared to alternative invertebrate systems.

Materials and Methods

Insect Strain and Samples Collection

A population of *Spodoptera litura* that has been reared in our lab for more than 10 years was kept under the conditions as previously described (Tu and Zheng, 2010). Four insect samples from different developmental stages including eggs, larvae (the third instar), pupae and adults (male and female) were collected during 24 h after metamorphosis or molt. Tissue samples including midgut, cuticle and fat body were dissected from the fifth stage larvae of *S. litura*. All the samples were kept in -80°C until to be used.

Annotation and Bioinformatics Analysis of *Dcr*, *R2d2*, *Loq*, *Ago* and *Sids* in *S. litura* Transcriptome

cDNAs coding for Dcr, R2d2, Loq, Ago and Sids were retrieved from the functional annotation of *S. litura* transcriptome (GenBank TSA: GBBY00000000). Each contig displaying significant similarity to *Dcr* (*Dcr1* and *Dcr2*), *R2d2*, *Loq*, *Ago* (*Ago1* and *Ago2*) and *Sid* (*Sid1*, *Sid2* and *Sid3*) homologs was further performed ORF (Open Reading Frame) search at NCBI on-line service (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). More sequence analysis was performed by several on-line tools, such as multiple sequence alignment was carried out with the online service at http://www.ebi.ac.uk/Tools/msa/clustalw2/; Domain architecture was predicated by Scan-Prosite (http://prosite.expasy.org/scanprosite/) (De *et al*., 2006); prediction of trans membrane helices was performed by on-line serves of TMpred program (http://www.ch.embnet.org/software/TMPRED_form.html) (Ikeda *et al*., 2003). The phylogenetic tree was constructed using MEGA 4.0 software with Neighbor-joining method (Tamura *et al*., 2007). Z-test of neutral evolution analysis among the nucleotide sequence of three *S. litura sids* was computed using the Nei-Gojobori method (Nei and Gojobori, 1986).

Quantitative RT-PCR Analysis of Core RNAi Machineries and Three SID-related Genes in *S. litura* Different Tissues and Developmental Stages

Quantitative RT-PCR (qRT-PCR) was performed to verify the expression profile of the selected genes in tissues of the firth stage larva of *S. litura* including midgut, cuticle and fat body, as well as different developmental stages including egg, the third instar larva, pupa and adult. Experiments including RNA extraction, DNase treatment, cDNA synthesis and qPCR reactions were performed as described previously (Gong *et al*., 2011; Gong *et al*., 2013).

qRT-PCR reactions were performed by three technical replicates on a BioRad iQ5 real-time PCR detection system using 200 ng of cDNA, 0.2 μM of primers and SYBR Premix Ex Taq (TaKaRa). The primers were listed in Table 1. Amplification conditions consisted of an initial denaturation at 95°C for 30s followed by 40 cycles of 95°C for 5s, 58°C for 30 s and a dissociation step was added as the end. After the reaction was completed, analysis of the amplification and melting curves was performed according to the manufacturer's instructions. The relative amounts of the transcripts were first normalized to the endogenous reference gene and then normalized relative to the transcripts level in stage of egg or in the tissue of midgut of *S. litura* according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical Analysis

qRT-PCR data are represented by the mean SEM.

Statistical Analysis was performed based on t-test with $p<0.05$ representing significance using Sigma Plot 12.0 software (Systat Software Inc.).

Results

Identification of Three *SID*-related Genes and Phylogenetic Relation Analysis in *S. litura*

Three unigenes coding *SID*-related genes were retrieved from the *S. litura* transcriptome (Table 2). The Comp16495 contig (GenBank KF717090) was 2,097 bp long, with an open reading frame encoding 680 amino acids. Sequence analysis indicates that this transcript shares 88% and 84% similarity to *Helicoverpa armigera sid-1* (KC292008) and *Bombyx mori sid-3* (NM_001113265), respectively. The Comp16844 contig GenBank KF717089) was 2,373 bp long, with an open reading frame encoding 789 amino acids. Sequence analysis indicates that this transcript shares 75% similarity to *Bombyx mori sid-1* (NM_001113264). On the other hand, contig Comp18252 (GenBank KF717088) was 2,649 bp long and has an open reading frame encoding 873 amino acids. Sequence analysis indicated that this transcript shares 73% similarity to *Bombyx mori sid-2* (XM_004930678). The prediction graphics of the three Sl-SIDs showed the number of transmembrane regions are 13, 11 and 10, respectively in SL-SID1, SL-SID2 and SL-SID3 (Supplementary file 1). Codon-based test of neutrality analysis among three *sid-1* orthologs of *S. litura* showed that the only probability of rejecting the null hypothesis of strict-neutrality occurs between *Sl-Sid1* and *Sl-Sid3* ($d_N = d_S$; $P<0.05$), suggesting no evolutionary pressure between the sequences of *Sl-Sid1* and *Sl-Sid3*. A phylogenetic tree was generated using 18 code regions of SID-related proteins from 11 species including vertebrates, insects and *C. elegans* (Fig. 2). The phylogenetic tree clearly shows that SID-related proteins have long-time independent evolutionary history.

Characterizations of Two *dicer* genes in *S. litura*

Contig Sl-Dicer-1 (GenBank KF717091, Table 2) was 4,140 bp and encoded a 1,362 amino acid protein. Sequence alignment shows that this contig has 53.6% and 29.52% similarity to *Bombyx mori Dicer-1* (XP_004922366) and *Drosophila melanogaster Dicer-1* (ABD61603), respectively. We found a highly conserved region among these three species, which ranges from 825-944 in Sl-Dicer-1, but no function has been reported about this region yet (Supplementary file 2A). A second contig, Sl-Dicer-2 (GenBank KF717092, Table 2), was 5,112 bp in length and encoded a protein of 1,685 amino acids. Sequence alignment shows that SL-Dicer-1 has 63.74% and 27.83 similarity to *Bombyx mori Dicer-2* (NP_001180543) and *Drosophila melanogaster Dicer-2* (ABB54749). We found a dsRNA-specific ribonuclease region (amino acids 1207-1273)

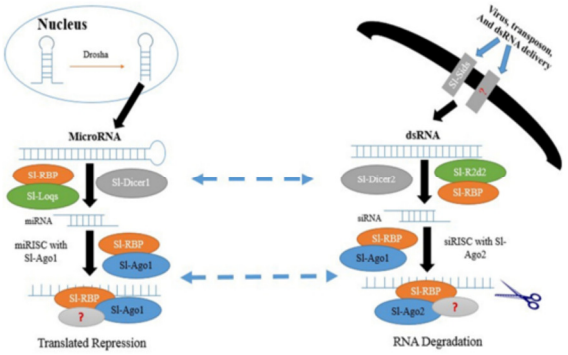

Fig. 1: Diagram of the pathways leading to RNA interference by microRNAs and siRNAs in *S. litura*. (siRISC: RNA Induced Silencing Complex associated with siRNA; miRISC: miRNA associated RISC; Ago: Argonaute; Dcr: Dicer; Loquacious: Loqs; RBP: RNA-binding protein)

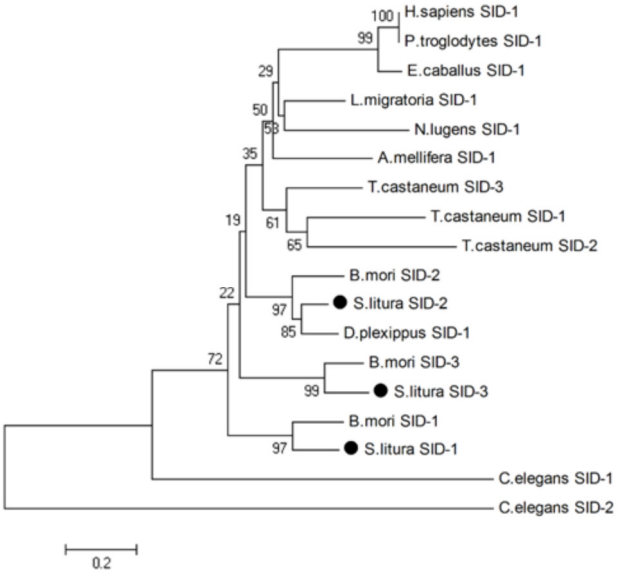

Fig. 2: Unrooted phylogenetic tree of SIDs from vertebrates, insects and C.elegans generated by the Neighbor-joining method using the software package MEGA4 (Tamura *et al.*, 2007)

The SIDs included in the analysis were *Homo sapiens* (NP_060169); *Pan troglodytes* (XP_526266); *Equus caballus* (XP_001917509); *Locusta migratoria* (AFQ00936); *Nilaparvata lugens* (AEI25514); *Apis mellifera* (XP_395167); *Tribolium castaneum* SID-1 (NP_001099012); *Tribolium castaneum* Sid-2 (NP_001103253); *Tribolium castaneum* SID-3(NP_001099128); *Bombyx mori* SID-1 (NP_001106735); *Bombyx mori* SID-2 (BAF95807); *Bombyx mori* SID-3 (NP_001106736); *Danaus plexippus* (EHJ72579); *Caenorhabditis elegans* SID-1(NP_504372); *Caenorhabditis elegans* SID-2 (NP_499823)

in *B. mori Dicer-1* (Marchler-Bauer *et al*., 2013), which was highly conserved in *S. frugiperda* Sl-Dicer-2 (^{1218}LGDSF^{1223} and ^{1254}SNRNL^{1257}), indicating that they may represent active sites of dsRNA-specific ribonuclease in SL-Dicer-2 (Supplementary file 2B). A phylogenetic tree based on

Table 1: Primers used in qRT-PCR

Name/Primer Sequence	size(bp)
SL-Dcr	
SL-Dcr1_Fw: 5' TGCTGTATGCGATAGTATCA 3'	473
SL-Dcr1_Rv: 5'CTCTTCCGTATGTTGGTATATG 3'	
SL-Dcr2_Fw: 5' GGACGAGTATGTAGAGGATG 3'	212
SL-Dcr2_Rv: 5' CAGTGTTGACCAGGAAGAA 3'	
SL-Ago SL-Ago1_Fw: 5'ACTTCGCCACAGACCTTA 3'	478
SL-Ago1_Rv: 5'CCGCCTCCAACTTGATAC 3'	
SL-Ago2_Fw: 5' AGTCGTGCTTCGTCTCTA 3'	461
SL-Ago2_Rv: 5' CCGTCTGATTCCGTAATAGT 3'	
SL-R2D2 R2D2_Fw: 5'GAGCTGTGCGAGGAATAC 3'	336
R2D2_Rv: 5'AATGAGTGCGAGGTGGTA 3' SL-Loq SL-Loq_Fw: 5' ACAAGTCACAGCCACAAG 3'SL-Loq_Rv: 5' AAGTCCTGAGCCATAGCA 3'	255
SL-sid	
SL-sid1_Rv: 5'TCCTCTCAGACTTCAACCA3'	267
SL-sid1_Fw: 5' CCATCACCAACACTGCTAT3'	
SL-sid2_Rv: 5'GCTACTTCAACTTCCTATGC3'	133
SL-sid2_Fw: 5'TGATGCGTATTATGTCTCCT3'	
SL-sid3_Fw: 5' AACGCACTCAACTTCCAA 3'	192
SL-sid3_Rv: 5'GAACACGCTCCAGAACAA3'Reference geneS. litura actin_Fw:5' GCCAACAGGGAGAAGATG 3'S. litura actin_Rv:5' ATGAGGTAGTCGGTCAAGT3'	230

Table 2: Description of RNAi machinery and three sid-1 related genes in *S. litura*

Name	cDNA (bp)	ORF	Accession numbers	amino acid(aa)	pI	MM (kDa)	Match	Identities
Sl-R2d2	1029	22-109	KF717086	335	6.004	36.695	Bombyx mori(NP_001182007)	50%
Sl-Loqs	1188	31-1188	KF717087	385	8.766	42.511	Danaus plexippus(EHJ63609)	92%
Sl-Ago1	2805	19-2805	KF717084	928	9.024	103.22	Danaus plexippus (EHJ71131)	94%
Sl-Ago2	3273	88-3273	KF717085	1061	9.27	119.39	Bombyx mori(NP_001036995)	55%
Sl-Dic1	4140	55-4140	KF717091	1362	5.008	153.977	Bombyx mori(XP_004922366)	61%
Sl-Dic2	5112	55-5112	KF717092	1685	6.288	192.769	Bombyx mori(NP_001180543)	64%
Sl-Sid1	2373	4-2373	KF717089	789	7.843	88.471	Bombyx mori(NP_001106735)	63%
Sl-Sid2	2649	28-2649	KF717088	873	6.498	99.456	Danaus plexippus (EHJ69120)	66%
Sl-Sid3	2097	25-2067	KF717090	680	7.93	75.388	Bombyx mori(NP_001106736)	63%

Note: ORF, Open reading frame; MM, Molecular mass; p*I*, Isoelectric Point

amino acid sequences of *Dicer* genes was constructed using neighbor-joining method with 1,000 replications (Supplementary file 2C), which showed two groups reflecting the functional divergence of *Dicer-1* for producing MicroRNA and *Dicer-2* for producing siRNA as in *D. melanogaster* (Lee *et al*., 2004).

Identification of Two Argonaute (Argo) Genes in *S. litura*

The contig Sl-Ago1 (GenBank KF717084, Table 2) was 2,805 bp long, with an open reading frame encoding 928 amino acids. Multiple sequence alignment shows that SL-Ago 1 has 96.6%, 95.9% and 85.1% similarity to *B. mori* (NP_001095931), *D. plexippus* (EHJ71131) and *D. melanogaster* (NP_523734) *Argo1*, respectively. In contrast, contig SL-Argo2 (GenBank KF717085, Table 2) was 3,273 bp and encoded 1,061 amino acids. Multiple sequence alignment shows that Sl-Argo2 has 53.6%, 64.9% and 31.4% similarity to *B. mori* Argo 2 (NP_001036995), *D. plexippus* (EHJ72821) and *D. melanogaster* (ABB54726), respectively.

Sequence Analysis of *R2d2* and *loquacious* in *S. litura*

A 1,029 bp full-length cDNA of *R2D2* (GenBank KF717086, Table 2) was found in the *S. litura* transcriptome, which has a code region of 1,008 bp encoding 335 amino acids. Multiple sequence alignment shows that Sl-R2d2 has 46.9% and 20% similarity to *B. mori R2d2* (NP_001182007) and *Tribolium castaneum R2d2* (NP_001128425), respectively. The architecture of Sl-R2d2 indicates two regions with the function of double stranded RNA-binding, which are from the amino acids 3 (T) to 70 (R) and from 103 (S) to 171 (E) (Fig. 4, Supplementary file 3). A 1,188 bp full-length cDNA of *Loqs* (GenBank KF717087, Table 2) was also found in the *S. litura* transcriptome, which has a code region of 1,158 bp encoding 385 amino acids. Multiple sequence alignment shows that Sl-Loqs has 83.5% and 58.1% similarity to *B. mori Loqs* (NP_001182008) and *Tribolium castaneum Loqs* (XP_966668), respectively. The architecture of Sl-Loqs indicates three regions with the function of double stranded RNA-binding, which are from the amino acids 75 (T) – 146 (G), 175 (N) – 247 (D) and 313 (N) – 381 (I) (Fig. 4, Supplementary file 3).

Expression Profiles of *S. litura* SID-related Genes in Different Tissues and Developmental Stages of *S. litura*

We found that three *S. litura* Sid-related genes were all

expressed in tissues of the fifth instar larvae, including midgut, cuticle and fat body. However, the highest levels of expression were detected in the midgut and the lowest were detected in the cuticle (Fig. 5B). For example, expression of *S. litura sid-3* in midgut was > 1,000-fold and 47.6-fold than that detected in the cuticle and fat body, respectively. This observation indicates a possible role of *Sl-Sid3* with the function of uptake of dsRNA in *S. litura* midgut. Expression of these genes were tested in different developmental stages, showing that the highest expression occurs in pupa. Moreover, the expression of *Sl-sid1* and *Sl-sid2* were significantly higher in the egg stage than in other stages (Fig. 5D).

Expression Profiles of Genes Associated with the RNAi Machinery in Different Tissues and Developmental Stages of *S. litura*

We found that all the selected contigs (*Sl-R2d2*, *Sl-Ago, Sl-Loqs* and *Sl-Dic*) were expressed in tissues of midgut, cuticle and fat body. The highest expression levels for all of them were detected in the fat body, followed by in the cuticle, except that *SL-Dcr2* was higher expressed 3.2-fold in the midgut than the in cuticle (Fig. 5A). The expression in developmental stages including larva, pupa and adult, showed that their expression increased from larva to pupa, and pupa to adult. For example, the expression of *Sl-Loq* was 1.6-fold and 5.6-fold higher in the pupa and adult stages, respectively, than the expression in the larva stage (Fig. 5C).

Discussion

In the present study, the core RNAi machineries and *sid-1* related genes were found in *S. litura* transcriptome, indicating that *S. litura* possesses all of the core RNAi machinery genes including *R2d2*, *Loqs*, *Ago1*, *Ago2*, *Dic1* and *Dic2*, as well as three *sid* homologs for the possible function of spreading double-strand RNA among cells or/and tissues. Our data support that *S. litura* may have systemic RNAi response, but different tissues and/or developmental stages may differ in the efficiency of the RNAi response. Our work also the first point out that two RNA pathways (siRNA and miRNA pathways, Fig. 1) exist in *S. litura*, suggesting that *S. litura* may have a conserved RNAi mechanism.

General speaking, Sl-Dic1, Sl-Loqs and Sl-Ago1 are involved in the miRNA pathway whereas Sl-Dic2, Sl-R2d2 and Sl-Ago2 are involved in the siRNA pathway. But it should be noted that there is extensive interaction and overlap between the two branches (Represented by dotted arrows in Fig. 1). The protein architectures of both Sl-Argo1 and Sl-Argo2 consisted of PAZ and PIWI domains (Fig. 3A), which are typical of proteins involved in post-transcriptional gene silencing (PTGS) through RNAi (Cerutti *et al*., 2000). Although, Sl-Argo1 and Sl-Argo2 are

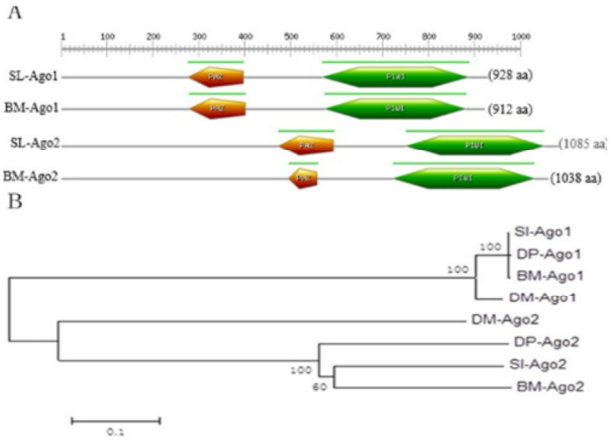

Fig. 3: Protein architectures (A) and phylogenetic analysis (B) of Sl-Ago1 and Sl-Ago2. Abbreviations are DP, *Danaus plexippus*; BM, *Bombyx mori*

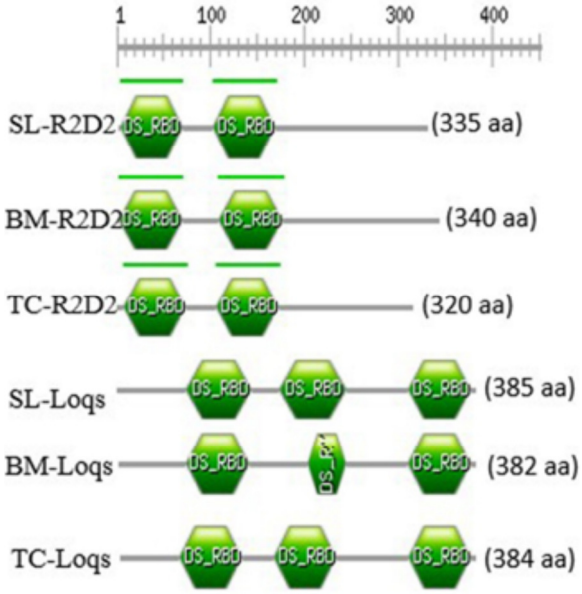

Fig. 4: Protein architectures of Sl-R2d2 and Sl-Loqs. Abbreviations are TC, *Tribolium castaneum*; BM, *Bombyx mori*

highly conserved in PAZ and PIWI domains, the phylogenetic analysis indicates they belong to two different groups within Lepidoptera (*S. litura*, *B. mori* and *D. plexippus*) (Fig. 3B), suggesting both are especial binding modules that accommodate the small RNA components, such as microRNAs and siRNAs (Meister and Tuschl, 2004). Both R2d2 and loqs are functional in dsRNA-binding, but they perform different biochemical functions, for example, Loqs is required for processing of miRNA precursors by Dcr-1, while R2d2 is required for Dcr-2 dependent loading of siRNAs into Ago2 (Marques *et al*., 2010). All of the core RNAi machineries were highly expressed in the fat body, which may be the main tissue

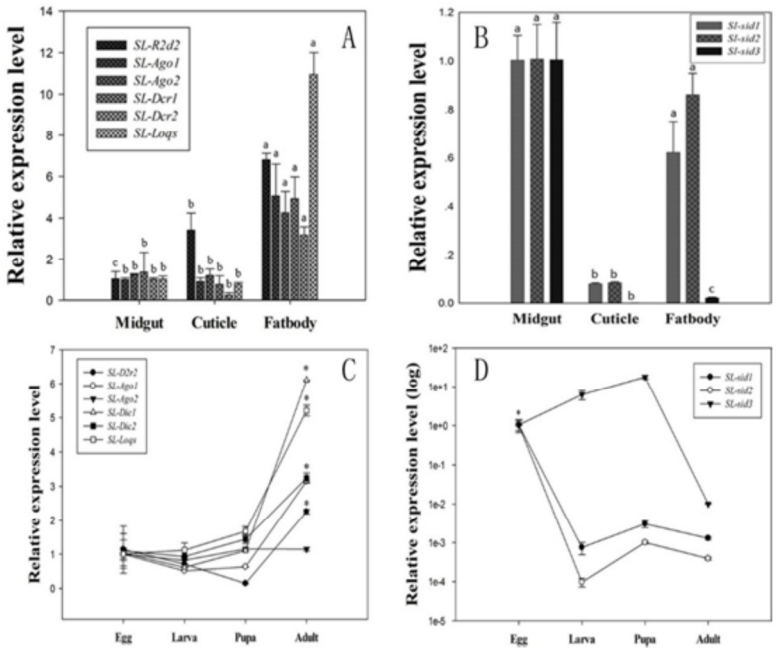

Fig. 5: Relative transcript quantification of gens involved in the RNAi response in different developmental stages and different tissues. Bars with same letter are not significantly different from each other at P<0.05 based on T-test (* p<0.05)

for producing proteins involved in the RNAi response. On the other hand, a limited amount of selected genes were also detected in midgut and cuticle, indicating the fifth instar *S. litura* larvae may possess the RNAi machinery in the whole-body. In the present study, the adult is the high expression stage of the RNAi machineries among different developmental stages, however, in the case of *A. glycines*, the core RNAi Machineries, such as *Dcr2*, *Ago2* and *R2d2* were high expression in the early development stages (Bansal and Michel, 2013). In four reproductive morphs of *Acyrthosiphon pisum*, expression levels of four genes (two Argonaute genes and two dicer genes) were the highest in parthenogenetic sexuparae female compared to parthenogenetic virginoparae female, sexual oviparae female and sexual male (Ortiz-Rivas *et al*., 2012).

We supposed that the SID-related proteins may evolve from one common ancient gene, but there was diverged during the evolutionary history. For example, sid-1 participates in the systemic RNAi in *C. elegans* (Grishok, 2005) but this function has been lost following the evolution in *Locusta migratoria* (Luo *et al*., 2012). Lepidopterna insects (*S. litura* and *B. mori*) seem to be located in the upstream of the evolution indicating a possible role in the systemic RNAi response (Fig. 2). However, because of the small amount of data available, it is difficult to analyze the evolution and duplication of *sid-1*-like genes in different organisms. The sid-1 gene or thologous were reported in many different insect orders, but few studies focus on its expression and functionality (Huvenne and Smagghe, 2010). In a study of *Aphis glycines sid1*, which was not significant differences in the expression level in different tissues, such as epidermis, gut and fatbody (Bansal and Michel, 2013). However, our work confirmed that three sid-1 gene or thologs in *S. litura* are significantly high expression in the midgut, indicating a possible role of the sid1-like genes in uptake dsRNA in the midgut lumen of *S. litura*. Although *sid-1* homologs were found in many different insect species, no conclusion could be drawn regarding these gene orthologs in the involvement of dsRNA uptake. For example, three sid-1 or thologs of *Tribolium castaneum* were silenced individually or all together, but no influence of RNAi in this species was found (Tomoyasu *et al*., 2008). Our data for the first time provide the genes sequence information of three *sid-1* or thologs in *S. litura*, which will facilitate the detailed function research of *sid-1* or thologs in a non-model organism of *S. litura*.

Conclusion

We studied the RNAi machinery in *S. litura* by using a transcriptome to detect genes predicted to be involved in the RNAi silencing response, including the *R2d2*, Loqs, *Ago1*, *Ago2*, *Dic1* and *Dic2* genes. All these genes were highly expressed in the tissue of the adult fat body. Our data support that *S. litura* may have a conserved RNAi mechanism, but that different tissues and/or developmental stages may differ in the efficacy of the RNAi response. We also found three homologues of the SID-1 gene responsible for dsRNA transport inside the cell in *S. litura* transcriptome. These genes were preferentially expressed in the midgut tissue, indicating *S. litura* might possess systemic RNAi response. By Z-test of neutral evolution

analysis, there is no evolutionary pressures occurred between *Sl-sid1* and *Sl-sid3*. Based on their preferentially expressed tissue and developmental stage, our data are conclusive that injecting of dsRNA into pupa or adult of *S. litura* that is more possible to obtain the efficacy of the RNAi response in this species.

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References

Arasu, M.V., N.A. Al-Dhabi, V. Saritha, V. Duraipandiyan, C. Muthukumar and S.J. Kim, 2013. Antifeedant, larvicidal and growth inhibitory bioactivities of novel polyketide metabolite isolated from Streptomyces sp. AP-123 against *Helicoverpa armigera* and *Spodoptera litura*. *BMC Microbiol*., 13, doi:10.1186/1471-2180-13-105

Bansal, R. and A.P. Michel, 2013. Core RNAi Machinery and Sid1, a Component for Systemic RNAi, in the Hemipteran Insect, Aphis glycines. *Int. J. Mol. Sci*., 14: 3786–3801

Bernstein, E., A.A. Caudy, S.M. Hammond and G.J. Hannon, 2001. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature*, 409: 363–366

Castel, S.E. and R.A. Martienssen, 2013. RNA interference in the nucleus: roles for small RNAs in transcription, epigenetics and beyond. *Nat. Rev. Genet*., 14: 100–112

Cerutti, L., N. Mian and A. Bateman, 2000. Domains in gene silencing and cell differentiation proteins: the novel PAZ domain and redefinition of the Piwi domain. *Trends Biochem. Sci*., 25: 481–482

De, C.E., C.J.A. Sigrist, A. Gattiker, V. Bulliard, P.S. Langendijk-Genevaux, E. Gasteiger, A. Bairoch and N. Hulo, 2006. ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. *Nucleic Acids Res*., 34: W362–W365

Ding, S.W. and O. Voinnet, 2007. Antiviral immunity directed by small RNAs. *Cell*, 130: 413–426

Dong, X.L., G.H. Zhong, M.Y. Hu, X.Yi, H.M. Zhao and H.D. Wang, 2013. Molecular cloning and functional identification of an insect odorant receptor gene in *Spodoptera litura* (F.) for the botanical insecticide rhodojaponin III. *J. Insect Physiol*., 59: 26–32

Gong, L., H.D. Wang, J.F. Huang, M.Y. Hu, Z. Hu and G.H. Zhong, 2014. Camptothecin- induced expression of programmed cell death gene 11 in *Spodoptera litura*. *Pest Manage. Sci*., 70: 603–609

Gong, L., X.Q. Yang, B.L. Zhang, G.H. Zhong and M.Y. Hu, 2011. Silencing of Rieske iron-sulfur protein using chemically synthesised siRNA as a potential biopesticide against *Plutella xylostella*. *Pest Manage. Sci*., 67: 514–520

Gong, L., Y. Chen, Z. Hu and M.Y. Hu, 2013. Testing insecticidal activity of novel chemically synthesized siRNA against *Plutella xylostella* under laboratory and field conditions. *PLoS One*, 8: e62990

Grishok, A., 2005. RNAi mechanisms in *Caenorhabditis elegans*. *FEBS Lett*., 579: 5932–5939

Hannon, G.J., 2002. RNA interference. *Nature*, 418: 244–251

Huang, S.H., J.D. Xian, S.Z. Kong, J.H. Xie, J. Lin, J.N. Chen, H.F. Wang and Z.R. Su, 2013. Insecticidal activity of pogostone against *Spodoptera litur*a and *Spodoptera exigua* (Lepidoptera: Noctuidae). *Pest Manage. Sci*., doi: 10.1002/ps.3635

Huvenne, H. and G. Smagghe, 2010. Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: A review. *J. Insect Physiol*., 56: 227–235

Ikeda, M., M. Arai, T. Okuno and T. Shimizu, 2003. TMPDB: a database of experimentally-characterized transmembrane topologies. *Nucleic Acids Res*., 31: 406–409

Lee, Y., M. Kim, J. Han, K.H. Yeom, S. Lee, S.H. Baek and V.N. Kim, 2004. MicroRNA genes are transcribed by RNA polymerase II. *Embo J*., 23: 4051–4060

Liu, Q.H., T.A. Rand, S. Kalidas, F.H. Du, H.E. Kim, D.P. Smith and X.D. Wang, 2003. R2D2, a bridge between the initiation and effector steps of the *Drosophila* RNAi pathway. *Science*, 301: 921–1925

Liu, X., F. Jiang, S. Kalidas, D. Smith and Q.H. Liu, 2006. Dicer-2 and R2D2 coordinately bind siRNA to promote assembly of the siRISC complexes. *RNA*, 12: 1514–1520

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods*, 25: 402–408

Luo, Y., X.H. Wang, D. Yu and L. Kang, 2012. The SID-1 double-stranded RNA transporter is not required for systemic RNAi in the migratory locust. *RNA Biol*., 9: 663–671

Makarova, K.S., Y.I. Wolf, J. Van der Oost and E.V. Koonin, 2009. Prokaryotic homologs of Argonaute proteins are predicted to function as key components of a novel system of defense against mobile genetic elements. *Biol. Direct*, 4: 29

Marchler-Bauer, A., C.J. Zheng, F. Chitsaz, M.K. Debyshire, L.Y. Geer, R.C. Geer, N.R. Gonzales, M. Gwadz, D.I. Hurwitz, C.J. Lanczycki, F. Lu, S.N. Lu, G.H. Marchler, J.S. Song, N. Thanki, R.A. Yamashita, D.C. Zhang and S.H. Bryant, 2013. CDD: conserved domains and protein three-dimensional structure. *Nucleic Acids Res*., 41: 348–352

Marques, J.T., K. Kim, P.H. Wu, T.M. Alleyne, N. Jafari and R.W. Carthew, 2010. Loqs and R2D2 act sequentially in the siRNA pathway in *Drosophila*. *Nat. Struct. Mol. Biol*., 17: 24–30

Meister, G. and T. Tuschl, 2004. Mechanisms of gene silencing by double-stranded RNA. *Nature*, 431: 343–349

Meng, X., J.J. Hu, X.X. Xu, Z.Q. Wang, Q.B. Hu, F.L. Jin and S.X. Ren, 2013. Toxic tffect of destruxin A on abnormal wing disc-like (SLAWD) in *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *PLoS ONE*, 8: e57213

Mukherjee, S. and K. Hanley, 2010. RNA interference modulates replication of dengue virus in *Drosophila melanogaster* cells. *BMC Microbiol*., 10: 127

Napoli, C., C. Lemieux and R. Jorgensen, 1990. Introduction of a chimeric chalcone synthase gene into petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell*, 2: 279–289

Nei, M. and T. Gojobori, 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. *Mol. Biol. Evol*., 3: 418–426

Ortiz-Rivas, B., S. Jaubert-Possamai, S. Tanguy, J.P. Gauthier, D. Tagu and R. Claude, 2012. Evolutionary study of duplications of the miRNA machinery in aphids associated with striking rate acceleration and changes in expression profiles. *BMC Evol. Biol*., 12: 216

Rajagopal, R., S. Sivakumar, N. Agrawal, P. Malhotra and R.K. Bhatnagar, 2002. Silencing of midgut aminopeptidase N of *Spodoptera litura* by double-stranded RNA establishes its role as Bacillus thuringiensis toxin receptor. *J. Biol. Chem*., 277: 46849–46851

Richter, H., L. Randau and A. Plagens, 2013. Exploiting CRISPR/Cas: interference mechanisms and applications. *Int. J. Mol. Sci*., 14: 14518–14531

Scott, J.G., K. Michel, L.C. Bartholomay, B.D. Siegfried, W.B. Hunter, G. Smagghe, K.Y. Zhu and A.E. Douglas, 2013. Towards the elements of successful insect RNAi. *J. Insect Physiol*., 59: 1212–1221

Swevers, L., J. Liu, H. Huvenne and G. Smagghe, 2011. Search for limiting factors in the RNAi pathway in silkmoth tissues and the Bm5 cell line: the RNA-binding proteins R2D2 and Translin. *Plos One*, 6: e20250

Swevers, L., J.V. Broeck and G. Smagghe, 2013. The possible impact of persistent virus infection on the function of the RNAi machinery in insects: a hypothesis. *Front. Physiol*., 4: 319

Tamura, K., J. Dudley, M. Nei and S. Kumar, 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol*., 24: 1596–1599

Tian, H.G., H. Peng, Q. Yao, H.X. Chen, Q. Xie, B. Tang and W.Q. Zhang, 2009. Developmental control of a lepidopteran pest *Spodoptera exigua* by ingestion of bacteria expressing dsRNA of a non-midgut gene. *PLoS One*, 4: e6225

Tomoyasu, Y., S.C. Miller, S. Tomita, M. Schoppmeier, D. Grossmann and G. Bucher, 2008. Exploring systemic RNA interference in insects: a genome-wide survey for RNAi genes in *Tribolium*. *Genome Biol*., 9: R10

Tu, Y.G. and J.H. Zeng, 2010. A method for artificial rearing of common cutworm, *Spodoptera litura*. *Acta Agric. Jiangxi*, 22: 87–88

Tuan, S.J., C.C. Lee and H. Chi, 2014. Population and damage projection of *Spodoptera litura* (F.) on peanuts (*Arachis hypogaea* L.) under different conditions using the age-stage, two-sex life table. *Pest Manage. Sci*., 70: 805–813

Winston, W.M., C. Molodowitch and C.P. Hunter, 2002. Systemic RNAi in *C. elegans* requires the putative transmembrane protein SID-1. *Science*, 295: 2456–2459

Winston, W.M., M. Sutherlin, A.J. Wright, E.H. Feinberg and C.P. Hunter, 2007. *Caenorhabditis elegans* SID-2 is required for environmental RNA interference. *P. Natl. Acad. Sci. USA*, 104: 10565–10570

Wu, J.Y., W.Z. Huang and Z.Y. He, 2013. Dendrimers as Carriers for siRNA Delivery and Gene Silencing: A Review. *Sci. World J*., Article ID 630654,doi: 10.1155/2013/630654

Zhao, H.M., X. Yi, Z. Hu, S.H. Chen, X.L. Dong and L. Gong, 2013. RNAi-mediated knockdown of catalase causes cell cycle arrest in SL-1 cells and results in low survival rate of *Spodoptera litura* (Fabricius). *PLoS One*, 8: e59527

Zhou, J.L., G.R. Zhang and Q. Zhou, 2012. Molecular characterization of cytochrome P450 CYP6B47 cDNAs and 5′-flanking sequence from *Spodoptera litura* (Lepidoptera: Noctuidae): Its response to lead stress. *J. Insect Physiol*., 58: 726–736

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