Journal of Insect Science (*2017*) 17(3): 73; 1–8
doi: 10.1093/jisesa/iex047
Research article

Expression and RNA Interference of Ribosomal Protein L5 Gene in *Nilaparvata lugens* (Hemiptera: Delphacidae)

Jiajun Zhu,[1,*] Peiying Hao,[1,2,*] Chaofeng Lu,[1] Yan Ma,[1] Yalin Feng,[1] and Xiaoping Yu[1]

[1]Zhejiang Provincial Key Laboratory of Biometrology and Inspection & Quarantine, College of Life Sciences, China Jiliang University, Hangzhou 310018, China (chuh2010@163.com; haopeiy@163.com; lcf19890107@163.com; ma_yan1989@126.com; 707794822@qq.com; yxp@cjlu.edu.cn) and [2]Corresponding author, e-mail: haopeiy@163.com

*These authors contributed equally to this work.

Subject Editor: Jake Tu

Received 16 October 2016; Editorial decision 26 April 2017

Abstract

The ribosomal proteins play important roles in the growth and development of organisms. This study aimed to explore the function of *NlRPL5* (GenBank KX379234), a ribosomal protein L5 gene, in the brown planthopper *Nilaparvata lugens.* The open reading frame of *NlRPL5* was cloned from *N. lugens* based on a previous transcriptome analysis. The results revealed that the open reading frame of *NlRPL5* is of 900 bp, encoding 299 amino acid residues. The reverse transcription quantitative PCR results suggested that the expression of *NlRPL5* gene was stronger in gravid females, but was relatively low in nymphs, males, and newly emerged females. The expression level of *NlRPL5* in the ovary was about twofolds of that in the head, thorax, or fat body. RNAi of *dsNlRPL5* resulted in a significant reduction of mRNA levels, ~50% decrease in comparison with the *dsGFP* control at day 6. Treatment of *dsNlRPL5* significantly restricted the ovarian development, and decreased the number of eggs laid on the rice (Oryza sativa) plants. This study provided a new clue for further study on the function and regulation mechanism of *NlRPL5* in *N. lugens*.

Key words: *Nilaparvata lugens*, *NlRPL5* gene, RNA interference, ovarian development

Brown planthopper, *Nilaparvata lugens* Stål, is one of the most destructive inset pests in the subtropical areas of Asia. *N. lugens* feeds on rice (Oryza sativa) crop and can cause hopperburn on rice plant, whenever its density is high enough. *N. lugens* can also transmit the grassy stunt disease, which will further reduce rice yield (Nault and Ammar 1989, Zhu et al. 2004, Bao et al. 2009). Various types of resistant rice varieties have been used to control this insect pest as an effective and environment-friendly strategy for protecting rice (Wang et al. 2015). However, several *N. lugens* strains have recovered their virulence to these resistant rice varieties due to a continuous cultivation of planthopper-resistant varieties and a high level application of nitrogen fertilizer (Tanaka 1999, Horgan et al. 2016, Rashid et al. 2016). Therefore, it is important to develop the strategy for controlling *N. lugens*, including identifying some new genes as targets.

In order to screen suitable genes as targets, we analyzed the gene expression profile of *N. lugens*, and found that the "ribosome" term in KEGG pathway showed significant difference between the Rh colony reared on resistant rice variety Rathu Heenati (RHT) and the Tn colony reared on susceptible variety TN1 (Unpublished data Hao, et al). In this pathway, a ribosomal protein gene *L5* (*NlRPL5*) was upregulated about twofolds in the Rh colony, compared with that in the Tn colony, indicating that *NlRPL5* may play a role in the interaction between *N. lugens* and resistant rice. As several ribosomal proteins including *RPL5* have been implicated in stress amelioration besides house-keeping in several species (Grewal et al. 2007, Li et al. 2010, Moin et al. 2016), *NlRPL5* gene was selected for further investigation.

Ribosomes are cellular machines essential for protein synthesis. The biogenesis of ribosomes is a highly complex and energy consuming process that initiates in the nucleolus (Goudarzi and Lindström 2016). In recent research, ribosomal proteins were found also involved in functions beyond ribosome, such as transcriptional regulation, cell development, and cell differentiation process (Ferguson et al. 2015, Orelle et al. 2015, Takada and Kurisaki 2015). Ribosomal protein L5 (RPL5) is a part of the 60S ribosomal subunit and is localized in both cytoplasm and nucleus of eukaryotic cells. Acting as a nucleocytoplasmic shuttle protein, RPL5 plays an important role in 5S rRNA intracellular transport during assembly of the large ribosomal subunit (Rosorius et al. 2000). RPL5, together with RPL11, can monitor ribosome biogenesis and control cell proliferation and growth (Liao et al. 2013). Teng et al (2013) found that the loss of RPL5 strongly suppressed cell cycle progression, and the effects on cell cycle progression stemmed from reduced ribosome content and translational capacity, which suppressed the accumulation

© The Author 2017. Published by Oxford University Press on behalf of the Entomological Society of America.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

of cyclins at the translational level. Although ribosomal proteins have been extensively studied, especially in vertebrates and plants, the functions beyond ribosome of *NlRPL5* remain largely unclear in *N. lugens*.

In this study, the complete open reading frame (ORF) of *NlRPL5* was cloned, and the physicochemical properties of the protein sequence were predicted. The reverse transcription quantitative PCR (RT-qRCR) analysis was conducted to detect the relative mRNA expression levels of *NlRPL5* in different stages and different populations of *N. lugens*. RNA interference (RNAi) was performed to explore the effect of *NlRPL5* knockdown on *N. lugens*. We aimed to explore the function of *NlRPL5* in *N. Lugens*.

Materials and Methods

Insects and Plants

Rice varieties include susceptible variety TN1 and resistant varieties ASD7 (with a brown planthopper resistance gene, *bph2*) and RHT (with a brown planthopper resistance gene, *Bph3*). Test insects were collected from the following *N. lugens* colonies: Tn colony reared on TN1 continuously for >80 generations; As colony reared on ASD7 continuously for >70 generations; Rh colony reared on RHT continuously for >70 generations; All test insects and plants were maintained in an controlled conditions room at 26 ± 2 °C, and 75–80% relative humidity, with a 16:8 (L:D) h photoperiod.

Isolation of Total RNA and Synthesis of First Strand cDNA

Total RNA was extracted using Takara MiniBEST Universal RNA Extraction Kit cot9767 (Takara, Tokyo, Japan). The RNA quality was determined with a spectrophotometer Nanodrop 2000 (Thermo, Wilmington, DE, USA) and then the integrity of RNA was checked using the 1% agarose gel electrophoresis. About 1 μg RNA was used to synthesize the first-strand cDNA, using the reverse transcription kit PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Tokyo, Japan).

The Full-Length ORF cDNA Amplification of *NlRPL5*

A sequence predicted as *RPL5* gene (particial), obtained from our previous *N. lugens* transcriptome, was used to do BLAST search in the database of the whole genome of *N. lugens* (Xue et al. 2014). As a result, a hit scaffold (scaffold3211_7) was selected and submitted to Augustus (http://bioinf.uni-greifswald.de/augustus/submission.php) for the ORF prediction, and *Acyrthosiphon pisum* was selected as reference specie. The whole ORF and the untranslated regions were predicted firstly, and then primers of *NlRPL5*-flF and *NlRPL5*-flR were designed at both ends of the ORF to test the validation of the prediction. All primers were designed using Primer Premier 5.0 software and synthesized by Sunny Biotechnology Co. Ltd. (Shanghai, China). PCR amplification was performed in 50 μl reaction volumes using the following protocol: 95 °C for 4 min, followed by 35 cycles of 95 °C for 30 s, 59 °C for 30 s, 72 °C for 100 s, and a final extension at 72 °C for 10 min. The PCR product was confirmed by electrophoresis in the 1% agarose gel and stained by ethidium bromide, and then purified by the DNA Gel Extraction Kit (Axygen, Union City,CA,USA), cloned into the pMD18-T vector (Takara, Tokyo, Japan) and sent to Sunny Company (Shanghai, China) for sequencing.

Sequence and Structure Analysis of the ORF of *NlRPL5*

The cloned cDNA sequence of *NlRPL5* was analyzed by using DNAMAN 5.0 software. The identity of Amino acid sequences was performed on NCBI by BLAST (http://www.ncbi.nlm.nih.gov/), using the ORF Finder (http://www.ncbi.nlm.nih.gov/orffinder/) to get the sequence information of ORF. Molecular mass of the predicted proteins was calculated using the Compute pI/Mw program available online (http://www.expasy.ch/tools/pi_tool.html).

Table 1. Primers used in gene cloning, real-time PCR and dsRNA synthesis

Use of primers	Primer name	Primer sequence
cDNA cloning	*NlRPL5* -fl-F	CCGTGTTCATATGGTAGGGC
	NlRPL5 -fl-R	ACAGACATTTACGATTCAGCG
Real-time PCR	Q*NlRPL5*-F	CAAGAGGCGTAGGGAAGGTAAA
	Q*NlRPL5*-R	CGATACGAGAGTAAGCGATCTGG
	Q*Actin*-F	TGCGTGACATCAAGGAGAAGC
	Q*Actin*-R	CCATACCCAAGAAGGAAGGCT
dsRNA synthesis	*dsNlRPL5*-F	GGATCCTAATACGACTCACTATAGGG ACGGCGGTCTCAACA
	dsNlRPL5-R	GGATCCTAATACGACTCACTATAGGG TTCTGGGCGATGGTG
	dsGFP-F	GGATCCTAATACGACTCACTATA CGCAACCAGAAGGACA
	dsGFP-R	GGATCCTAATACGACTCACTATA GGGCAGATTGTGTGGACAGG

RT-qPCR Analysis

Specific primer pairs (Table 1) for RT-qPCR analysis were designed based on the cloned sequence of *NlRPL5*. RT-qPCR was performed in 20 μl reactions that included 10 μl SYBR Premix EX Taq (Takara, Tokyo, Japan), 0.4 μl of each of the forward and reverse primers, 0.4 μl ROX Reference Dye (Takara, Tokyo, Japan), 6.8 μl ddH_2O, and 2 μl of cDNA template. PCR cycling conditions were as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and 55 °C for 30 s. A melting curve analysis was performed by heating the PCR product from 65 °C to 95 °C to ensure the specificity of the amplified product (Supp Fig. S1 [online only]). The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001) was used to calculate the relative quantity of *NlRPL5* mRNA, based on a pilot experiment result that the efficiencies of the PCR reactions are almost the same (*Actin*: $E = 100.3\%$, $R^2 = 0.999$; *NlRPL5*: $E = 100.6\%$, $R^2 = 0.998$; Supp Fig. S2 [online only]). The mRNA level was normalized using *Actin* gene as an endogenous control as in previous expression profile analysis (Chen et al. 2010).

RNA Interference

For RNAi, dsRNAs were synthesized using the MEGAscript T7 Transcription Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. Using the primers listed in Table 1, the cDNA for the *dsNlRPL5* synthesis was amplified, and then cloned into the pMD18-T vector (TaKaRa, Tokyo, Japan), transferred into JM109 for sequencing. The ORF of *NlRPL5* was amplified using two primers (Table 1) with a T7 promoter sequence. The clone with T7 promoter was screened out, propagated, and extracted for plasmids to prepare *dsNlRPL5* templates. The primers of *dsGFP* were designed according to the sequence of a green fluorescent protein GFP (GenBank AF234298). The *dsNlRPL5* and *dsGFP* fragments did not cover the fragments for RT-qPCR so that it will not affect the detection of *NlRPL5* mRNA after RNAi. PCR was performed under the following conditions: 94 °C for 3 min, followed by 30

cycles of 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 90 s; and a final step at 72 °C for 10 min.

Fifth-instar nymphs were used for the injection of RNAi, and each group of *dsNlRPL5* or *dsGFP* includes 120 individuals divided into three replications. In total, 0.15 μg *dsNlRPL5* or *dsGFP* of 1.5 μg μl^{-1} was injected into each individual under a microscope. After injection, the injected insects were maintained on TN1 rice plant in a controlled conditions room at 26 ± 2 °C for 2, 4, 6, 8 days and then sampled for RT-qPCR using the extracted RNA as template. The RNAi treated insects held on TN1 rice plant for 6 days in a parallel experiment were used for isolating the ovary. Five ovaries in both *dsNlRPL5* treated group and *dsGFP* control group were dissected and taken photo under a stereoscope equipped with a digital image acquisition system (Nikon SMZ1500, Tokyo, Japan). Eggs laid on TN1 rice plants were counted at day 2, 4, 6, and 8.

Data Analysis

The data of mRNA expression level were analyzed by ANOVA (LSD) for different time points of a given population or for different populations. However, data of mRNA expression level and number of eggs from different treatment groups in RNAi experiments were evaluated using *t*-test.

Results

Sequence Analysis of *NlRPL5*

A cDNA sequence of 959 bp predicted as *RPL5* was cloned from *N. lugens*, and named *NlRPL5* (GenBank KX379234). The *NlRPL5* gene contains six exons and five introns, with an ORF of 900 bp ((Figs. 1 and 2). ProtParam (http://web.expasy.org/cgi-bin/protparam/) analysis showed that the sequence encodes a 299-amino acid protein with a calculated molecular weight of 33.9 kDa and its theoretical pI is 9.84. The chemical formula of NlRPL5 protein is $C_{1526}H_{2442}N_{436}O_{427}S_8$. The total number of negatively charged residues (Asp + Glu) is 33, and the total number of positively charged residues (Arg + Lys) is 60. Homology analysis revealed that the NlRPL5 protein is highly conserved in insects, and the deduced amino acid sequence shares a high similarity with the RPL5 of other species, for example, 99% identity with *Laodelphax striatella* (ADQ73874.1) and 82% identity with *Riptortus pedestris* (BAN20290.1; Supp Fig. S3 [online only]).

Expression of *NlRPL5* in *N. lugens* at Different Developmental Stages

RT-qPCR revealed that the mRNA expression level of *NlRPL5* in gravid female of *N. lugens* was the highest among all developmental stages analyzed. When the relative expression of *NlRPL5* in first–second instar nymph was set as reference (1.0), then the expression of *NlRPL5* in gravid female was 1.95, about onefold more than that of the reference. The mRNA expressions of third–fourth instar and fifth instar nymphs were near to that of first–second instar ones. Meanwhile, the expressions of male adult and newly emerged female were relatively low, only about half of that in first–second instar nymphs (Fig. 3).

Expression of *NlRPL5* in Different Parts and Tissues of *N. lugens*

RT-qPCR revealed that the mRNA expression level of *NlRPL5* in the head, thorax and fat body showed no significant differences between each two groups. Meanwhile, the mRNA expression level of *NlRPL5* in the ovary (2.07) was relatively higher, about twofold of that in the head (1.0), suggesting that *NlRPL5* may play an important role in ovarian development (Fig. 4).

Expression of *NlRPL5* in *N. lugens* on Resistant and Susceptible Rice Varieties

As to gravid female *N. lugens*, the expression levels of *NlRPL5* on resistant rice varieties (ASD7 and RHT) were higher than that on susceptible rice variety TN1. When the relative expression of *NlRPL5* in *N. lugens* on susceptible rice varieties TN1 was set as reference (1.0), then the expressions of *NlRPL5* in *N. lugens* on resistant rice varieties were 1.66 (ASD7) and 1.85 (RHT), respectively (Fig. 5). The result suggests that *NlRPL5* might be an important responsive gene in *N. lugens* feeding on resistant rice, and the upregulation of *NlRPL5* might contribute to the adaptation of *N. lugens* to resistant rice.

Effects of RNAi on mRNA Expression of *NlRPL5* and Ovarian Development

After RNAi was initiated by injecting the insects with dsRNA solution, samples were collected every two days. RT-qPCR results revealed that the mRNA expression of *NlRPL5* showed no significant difference between day 2 and day 4, but then clearly increased from day 6 in both *dsGFP* control group and *dsNlRPL5* treated group. It also showed that RNAi with *dsNlRPL5* significantly inhibited the expression of *NlRPL5*, resulting in significantly lower mRNA expression of *NlRPL5* in *dsNlRPL5* treated groups from day 2 to day 6. At day 4 or 6, a decrease ~50% was achieved in *dsNlRPL5* injected group in contrast to that of *dsGFP* control group (Fig. 6).

N. lugens was dissected at day 6 to elevate the ovarian development situation. The results showed that the ovaries in the *dsGFP* control group were well developed, but those in *dsNlRPL5* treated group were clearly inhibited with less and smaller eggs (Fig. 7). The number of eggs laid per female of *dsNlRPL5* treated group was 14.1 at day 8, significantly less that of *dsGFP* control 41.0 (Fig. 8). The results suggest that *NlRPL5* is important to the ovarian development and female fecundity.

Discussion

Ribosomal RNAs and ribosomal proteins assemble into ribosomes, which play an important role for the maintenance of normal life activities and biological regulation of organism growth and development (Ferreira-Cerca and Hurt 2009). The regulation of ribosome biogenesis involves the coordination of multiple steps including synthesis and processing of ribosomal RNA (rRNA), synthesis of ribosomal proteins and their import into the nucleus, assembly of ribosome subunits, transport of the mature 40S and 60S subunits to the cytoplasm, and assembly of 80S ribosome in the cytoplasm (Tschochner and Hurt 2003). The ribosomal subunits subsequently bind to and translate mRNAs into polypeptides. In a vigorously growing cell, ribosome biogenesis is a major consumer of cellular energy and resource. Thus, as growth conditions change, cells must rapidly rebalance ribosome production with the availability of resources (Kressle et al. 2010, Tschochner and Hurt 2003). In this study, we found that the expression of *NlRPL5* in gravid female was higher than that of other developmental stages, and it was also higher in ovary than other parts of *N. lugens*. These results indicated that the higher expression of *NlRPL5* in the ovary of gravid female may be due to the fast cell proliferation and a large demand for protein synthesis. In this process, *NlRPL5* plays role probably in a normal way, being assembled into ribosome to translate enough proteins. Under RNA interference, the ovarian development in *dsNlRPL5* group was seriously inhibited, indicating that knockdown of *NlRPL5* may interfere the balance between ribosomal

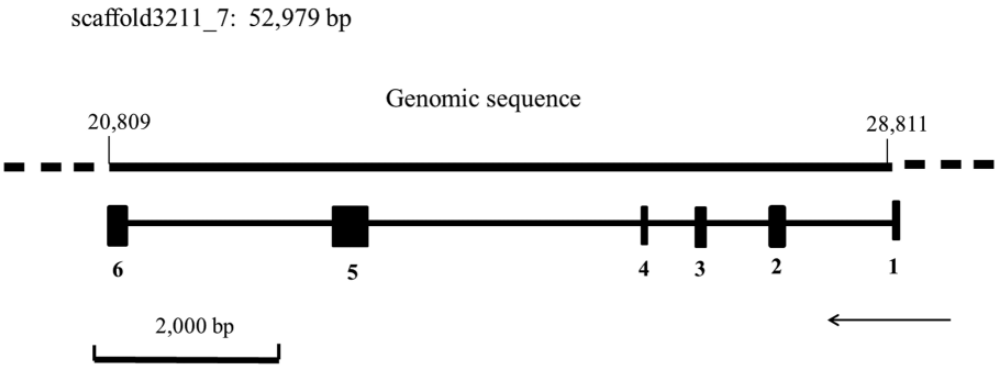

Fig. 1. Gene structure of *NIRPL5* from *Nilaparvata lugens*. Arrows indicate the direction of the gene transcription; Numbers 1–6 represent exons.

```
1     CCGTGTTCATATGGTAGGGCCTGTGAATTTTTCAATAAATTAGGATTTATC**ATG**GGTTTC
                                                          M   G   F
61    GTCAAAGTAGTAAAGAACAAGCAGTACTTCAAGAGGTACCAGGTCAAATTCAAGAGGCGT
      V   K   V   V   K   N   K   Q   Y   F   K   R   Y   Q   V   K   F   K   R   R
121   AGGGAAGGTAAAACTGATTACTACGCCAGGAAGCGTCTTATTGTCCAAGACAAAAACAAA
      R   E   G   K   T   D   Y   Y   A   R   K   R   L   I   V   Q   D   K   N   K
181   TACAACACCCCCAAGTATCGTCTTATTGTGAGGCTCTCCAACAAAGATATCACATGCCAG
      Y   N   T   P   K   Y   R   L   I   V   R   L   S   N   K   D   I   T   C   Q
241   ATCGCTTACTCTCGTATCGAAGGAGACAAGATTGTGTGTGCTGCATACAGTCATGAACTC
      I   A   Y   S   R   I   E   G   D   K   I   V   C   A   A   Y   S   H   E   L
301   CCCAAATATGGAGTCAAAGTTGGTTTGACCAATTATGCCTCAGCCTACTGTACAGGTCTC
      P   K   Y   G   V   K   V   G   L   T   N   Y   A   S   A   Y   C   T   G   L
361   CTCATTGCCCGAAGGCTGCTGAAGAAACTTGGCCTGGACCAGTTGTACGCGGGCACGACC
      L   I   A   R   R   L   L   K   K   L   G   L   D   Q   L   Y   A   G   T   T
421   GAGGTGACCGGCGAGGAGTACAATGTGGAGGCTCTGGACGAGGGTCCCGGCGCCTTCAAG
      E   V   T   G   E   E   Y   N   V   E   A   L   D   E   G   P   G   A   F   K
481   TGTTTCCTGGACGTCGGTCTGATGAAGACGTCGACCGGCGCCCGCGTGTTCGGCGCCATG
      C   F   L   D   V   G   L   M   K   T   S   T   G   A   R   V   F   G   A   M
541   AAGGGAGCCGTCGACGGCGGTCTCAACATCCCCCACAGCGTGAAGCGGTTCCCCGGCTTT
      K   G   A   V   D   G   G   L   N   I   P   H   S   V   K   R   F   P   G   F
601   GACGGCGAGTCGAAGAACTTCAACGCCGACGTGCACCGCAAGCACATCTTTGGCCTGCAC
      D   G   E   S   K   N   F   N   A   D   V   H   R   K   H   I   F   G   L   H
661   GTGAGCGAGTACATGAAGGAGCTGGCCGAGAACGACGACGAGGCCTACAAGAAGCAGTTC
      V   S   E   Y   M   K   E   L   A   E   N   D   D   E   A   Y   K   K   Q   F
721   TCGCAGTTCATCAAGAACGGCGTTCAACCGGAGGCGATCGAAGGTATCTACAAGAAGGCA
      S   Q   F   I   K   N   G   V   Q   P   E   A   I   E   G   I   Y   K   K   A
781   CATGAGGCGATCCGCGCCGACCCAGTTCTGAAGGCGAAGGCGCGCGCCAAGCCACCTGTG
      H   E   A   I   R   A   D   P   V   L   K   A   K   A   R   A   K   P   P   V
841   AAGAGGCGCTGGAACCGCAAGAAGCTGACCCTGTCCGAGCGACGCAACACCATCGCCCAG
      K   R   R   W   N   R   K   K   L   T   L   S   E   R   R   N   T   I   A   Q
901   AAGAAGGCCGCCTTCCTCAAGAAGCTCGAGGCTGGAGACGCTGAATCG**TAA**ATGTCTGT
      K   K   A   A   F   L   K   K   L   E   A   G   D   A   E   S   *
```

Fig. 2. cDNA and deduced amino acid sequence of *NILRPL5* gene in *N. lugens*. The start codon and stop codon are in boldface.

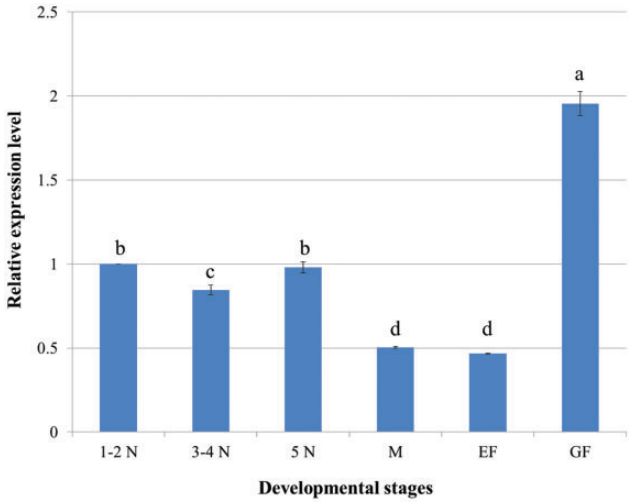

Fig. 3. Expression pattern of *NlRPL5* in different developmental stages of *N. lugens*. *N. lugens* samples were collected from Tn colony. The mRNA level of *NlRPL5* was detected using RT-qPCR. The mRNA level was normalized relative to the *Actin* transcription levels and the reference was the mRNA level of first–second instar nymph. 1–2N: first–second instar nymph; 3–4N: third–fourth instar nymph; 5N: fifth instar nymph; M: Male adult; EF: Newly emerged female adult; GF: Gravid female adult. Data were presented as mean ± SD. Symbols of a, b, c and d indicate significant differences between each two groups (ANOVA, LSD, $P < 0.05$).

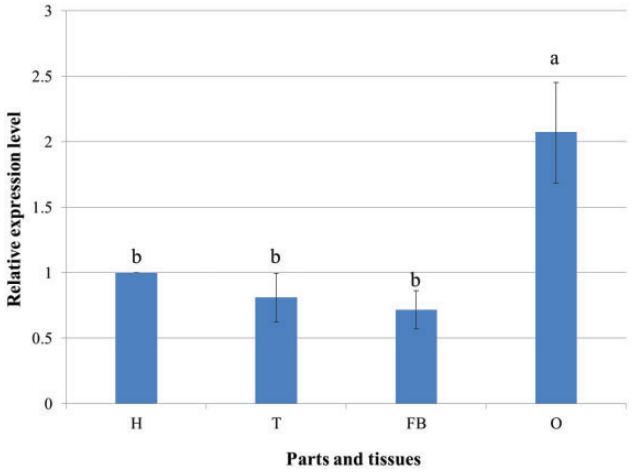

Fig. 4. Expression pattern of *NlRPL5* in different parts and tissues of *N. lugens*. The mRNA level of *NlRPL5* was detected using RT-qPCR. The mRNA level was normalized relative to the *Actin* transcription levels and the reference was the mRNA level in head of *N. lugens*. H: Head; T: Thorax; FB: Fat body; O: Ovary. Data were presented as mean ± SD. Symbols of a and b indicate significant differences between each two groups (ANOVA, LSD, $P < 0.05$).

RNA and ribosomal protein, and subsequently affect the assembling of ribosome and the translation of protein.

In the state of nutritional deficiencies, organisms will inhibit the synthesis of ribosomal RNA, which may break the balance between ribosomal proteins and ribosomal RNA, causing the ribosome stress (Grummt et al. 1976, Grewal et al. 2007, Warner and McIntosh 2009, Kim et al. 2014, Liu et al. 2014). Impaired ribosome assembly generates a feedback signal to cell cycle regulators, resulting in cell cycle arrest or apoptosis (Kressle and Bassler 2010). In this study, *NlRPL5* transcription was upregulated in *N. lugens* fed on resistant rice varieties, which seems unreasonable given that the *N. lugens* females usually show lower fecundity on the resistant rice than that

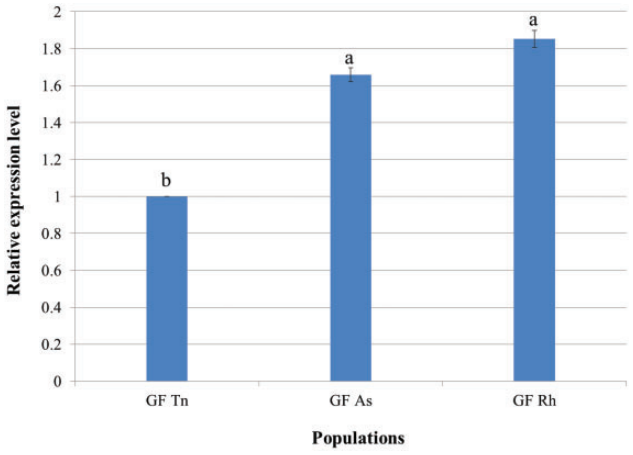

Fig. 5. Expression of *NlRPL5* in gravid female of different *N. lugens* populations. The mRNA level of *NlRPL5* was detected using RT-qPCR. The mRNA level was normalized relative to *Actin* and the reference was the mRNA level in head of *N. lugens*. H: Head; T: Thorax; FB: Fat body; O: Ovary. Data were presented as mean ± SD. Symbols of a, b, and c indicate significant differences between each two groups (ANOVA, LSD, $P < 0.05$).

on the susceptible one. However, when consider that ribosomal proteins also play roles beyond the ribosome, for example, slowing down the cell cycle to optimize allocation of resources and energy, it is reasonable to suggest that the upregulation of *NlRPL5* may contribute to the adaptation of *N. lugens* on resistant rice. When *N. lugens* feeds on resistant rice, the condition of food and nutrition is different from that on susceptible rice (Wang et al. 2012). Generally, resistant rice varieties have lower levels of free amino acids, higher levels of phenolic compounds, and lower levels of reducing sugars (Das 1976. Grayer et al. 1994, Thayumanavan 1990, Jung and Im 2005, Chen et al. 2011). Although the exact mechanism of rice resistance to *N. lugens* has not been completely demonstrated yet, at least several mainstream views below should be concerned. First, the nutrition, especially the variation in amino acid composition and abundance, may affect *N. lugens* fitness and development as well (Chino et al. 1987, Sogawa and Pathak 1970, Chen et al. 2011). On nitrogen-deficient or resistant rice plants, *N. lugens* excreted less honeydew, and most of the amino acids in honeydew were significantly decreased, compared with those fed on susceptible TN1 plants (Peng et al. 2016). On the contrary, application of a nitrogen fertilizer can increase the free amino acids available in the phloem sap, which may affect resistance of rice plants to *N. lugens* (Jairin et al. 2017). Second, the inhibiting factors in the phloem sap will also cause nutritional deficiency to *N. lugens*. Some secondary metabolites, such as flavanoids, were found different in resistant and susceptible rice varieties, and can inhibit the feeding behavior of *N. lugens* at concentrations high enough (Grayer et al. 1994, Stevenson et al. 1996). *N. lugens* feeding on the resistant variety RHT generally ingested lower quantity of phloem sap, which indicated that the *N. lugens* was in nutritional deficiency states (Ghaffar et al. 2011, Peñalver et al. 2011, Xiao et al. 2016). Third, the endosymbionts are suggested to have evolved to complement the amino acids needs of their host, so that *N. lugens* can survive on a nutritionally imbalanced food source (Chen et al. 2011, Xue et al. 2014). On the other hand, if the endosymbionts were inhibited by some components in resistant rice, *N. lugens* may be in amino acids starvation. Therefore, when *N. lugens* feeds on resistant rice varieties, it will probably be faced to nutrition deficiency directly or indirectly.

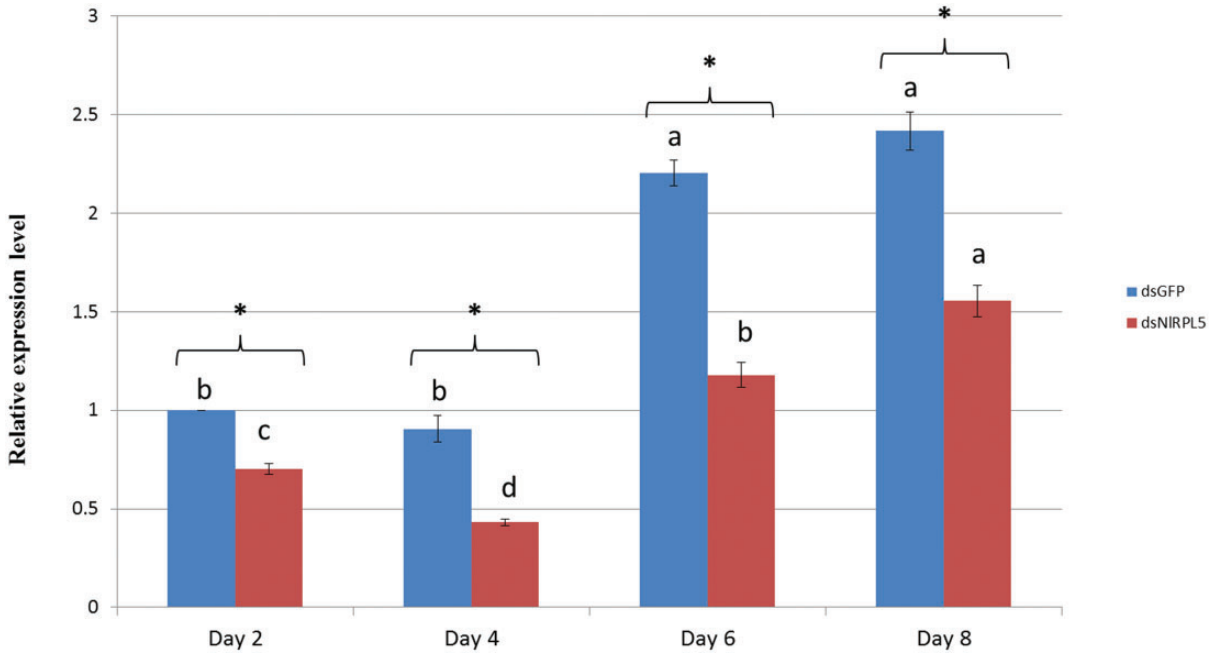

Fig. 6. Effects of RNAi with *NlRPL5* on mRNA expression. The mRNA level of *NlRPL5* was detected using RT-qPCR. The mRNA level was normalized relative to the *Actin* transcription levels and the reference was the mRNA level of *dsGFP* injected *N. lugens* on day 2. *dsGFP*: RNAi treatment of *dsGFP* (1.5 μg μl^{-1}, total 0.15 μg). *dsNLRPL5*: RNAi treatment of *dsNLRPL5* (1.5 μg μl^{-1}, total 0.15 μg). Data were presented as mean ± SD. Symbols of a, b, c indicate significant differences between each two groups (ANOVA, LSD, $P < 0.05$). The statistical significance of the gene expression between two different processing samples was evaluated using *t*-test. Asterisks represent significant differences between controls and treatments at $P < 0.05$.

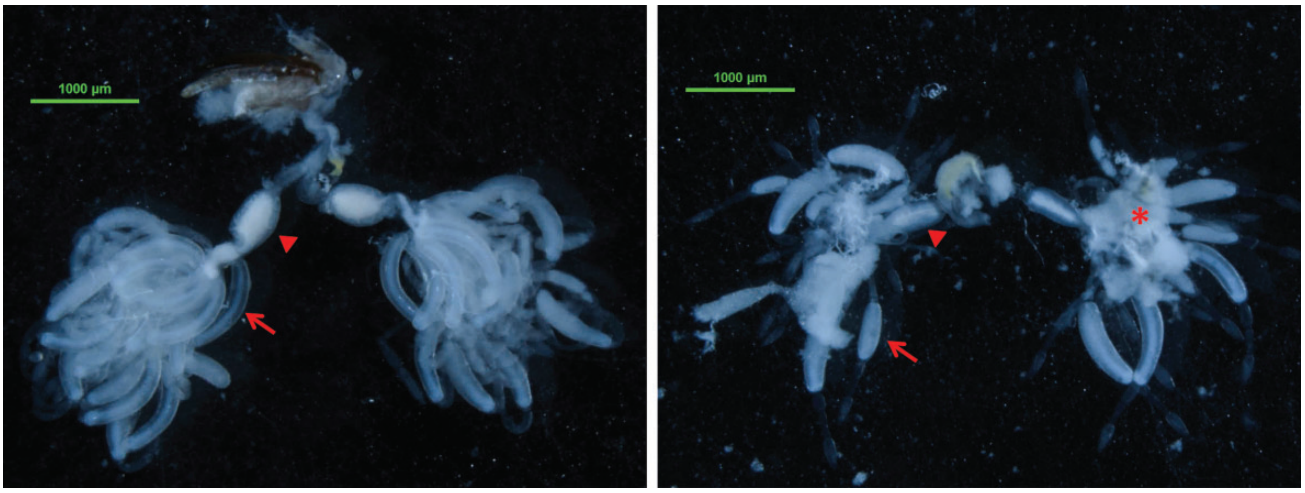

Fig. 7. Effects of *NlRPL5* RNAi on ovarian development. Left, *dsGFP* treated group; Right, *dsNlRPL5* control group. Arrows indicate eggs in ovarian tubules. Arrow heads indicate lateral oviduct. Asterisks indicate fat body tissue.

Under the conditions of nutrition deficiency or stress, such as feeding on the resistant rice varieties, *N. lugens* may be encountered with a ribosome stress, and up-regulates the expression of *NlRPL5* gene, so that it can meet the energy and resource demand for survival. In this process, *NlRPL5* should play its role in a different way, by regulating adaptation-related genes expression rather than translating more protein as it does in the ovarian development. Similarly, in *Drosophila*, it was found that starvation for dietary amino acids leads to dynamic changes in transcript levels of ribosome pathway (Li et al. 2010). Ribosomal genes also enhanced in the resistance of the whitefly *Bemisia tabaci* to phenylproapnoids/flavonoids pathway (Alon et al. 2012). Lior et al. (2014) found when the cell was faced to the ribosome stress, the expression of RPL5 also upregulated. Although, at present, there is very few information available about *RPL5* participates in regulating the adaptation of insect to its resistant host, there still are some clues for us to suggest that *NlRPL5* may play roles in: (1) monitoring the ribosome biogenesis and inhibiting the ovarian development to reduce the energy and resource consumption, probably by miRNAs just as the ribosomal proteins L5 and L11 do, co-operatively inactivate the transcription factors Myc via RNA-induced silencing complex (Liao et al. 2013); (2) playing a moonlighting role in the unfolded protein response (UPR) signaling to maintain cellular protein homeostasis and reutilize amino acids (Anshu and Dey 2015); (3) mobilizing the resources

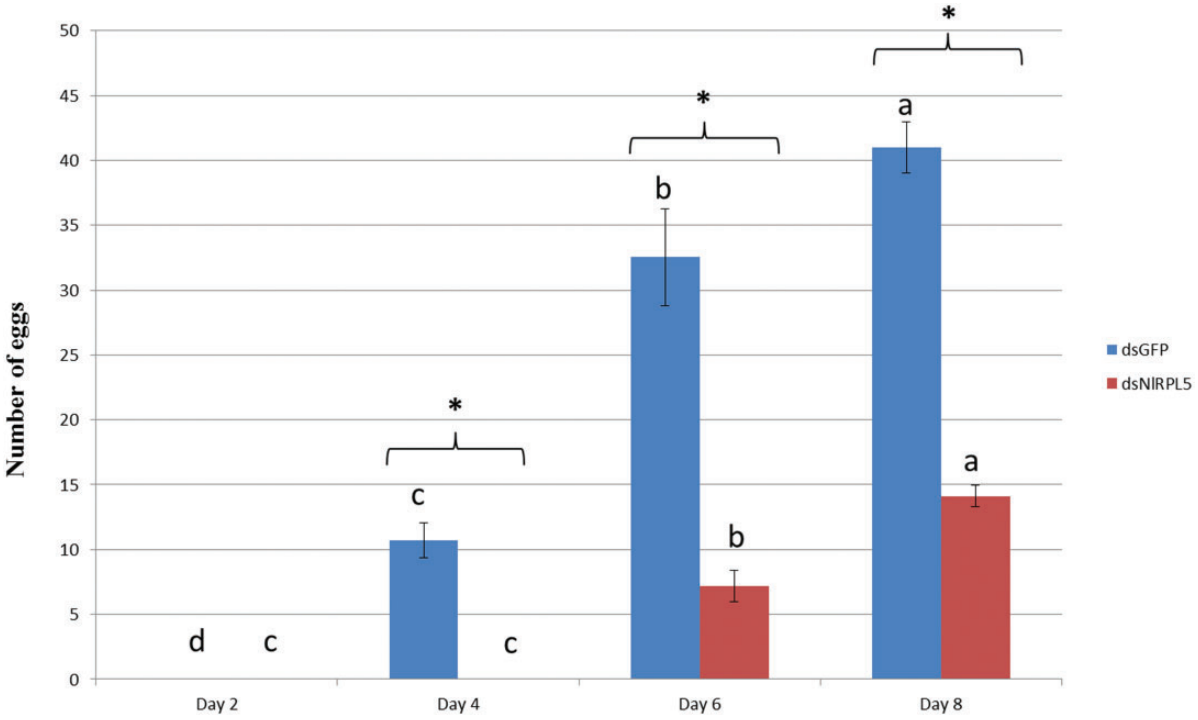

Fig. 8. Eggs laid on TN1 rice plants. Data were presented as mean ± SD. Symbols of a, b, c indicate significant differences between each two groups (ANOVA, LSD, $P < 0.05$). The statistical significance of the egg number between two different processing samples was evaluated using *t*-test. Asterisks represent significant differences between controls and treatments at $P < 0.05$.

and energy, for example, as it was reported that free ribosomal proteins play roles in promoting lipolysis, enhancing β oxidation of mitochondrial fatty acids, and activating ATP production (Liu et al. 2014). Take together, the results of this work suggest that nutritional status of the resistant rice potentially modulate the molecular biology and biology of the *N. lugens*, in turn, *NIRPL5* participates in the regulation of *N. lugens* adaptation to resistant rice. This should have profound ramifications in terms of insect biology and pest control on both the basic and applied levels.

Future work need to be undertaken to demonstrate that *NlRPL5* actually contributes to the adaptation of *N. lugens* to resistant rice varieties, and to reveal how *NlRPL5* plays its role in this process. Therefore, NlRPL5 protein and its antibody need to be prepared, so that effects on *NIRPL5* at the protein level can be investigated in order to establish a functional relationship between NIRPL5 and ovarian development. It is also necessary to identify the possible nutrition/resistant factors in rice which can induce *NlRPL5* upregulation. For this purpose, feeding experiments need to be conducted by changing the amount of certain amino acid in the artificial diet, or adding potential metabolites/flavonoids into the diet. Currently, it is difficult to generate genetically modified insect of *N. lugens* to explore whether the overexpression of *NlRPL5* contributes to the ability to overcome resistant rice varieties, alternatively, overexpressing *NIRPL5* in the culture cell should be more practicable. With cell culture systems, further investigations, such as subcellular location of NlRPL5 protein, cell division, UPR signaling, activity of mitochondria, and ATP production, are able to be conducted, and thus the regulation mechanism of *NlRPL5* will be better understood.

In this study, we cloned the cDNA of *NlRPL5* from *N. lugens* and analyzed it functions by using RT-qPCR and RNAi. The results suggest that the *NlRPL5* might play roles in regulating the development of *N. lugens*, and participating in the adaptation to the host. From this point of view, the *NlRPL5* gene has some potential to be developed as a target for controlling *N. lugens*. In the future, RNAi of *NlRPL5* may be undertaken by dsRNA-transgenic plant-mediated RNA interference (Zha et al. 2011), so that a more effective and lasting RNAi to *N. lugens* can be achieved.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Acknowledgments

This work was supported by the National Natural Science Foundation of China [grant numbers 31672026, 31171860, 31501632]; the 973 Program for Key Basic Research of the Ministry of Science and Technology, China [grant numbers 2012CB114100, 2011CB111602]; and the Research on Public Welfare Technology Application Projects of Zhejiang Province, China [grant number 2012C22041].

References Cited

Alon, M., M. Elbaz, M. M. BenZvi, E. Feldmesser, A. Vainstein, and S. Morin. 2012. Insights into the transcriptomics of polyphagy: Bemisia tabaci adaptability to phenylpropanoids involves coordinated expression of defense and metabolic genes. J. Insect Biochem. Mol. Biol. 42: 251–263.

Anshu, F. A., and M. Dey. 2015. Ribosomal protein L5 plays a moonlighting role in HAC1 mRNA. FASEB J. 29: 1–2.

Bao, H. B., L. S. Hua, G. J. Hua, W. X. Zhe, L. X. Liang, and L. Z. Wen. 2009. Sublethal effects of four insecticides on the reproduction and wing formation of brown planthopper, *Nilaparvata lugens*. J. Pest Manage. Sci. 65: 170–174.

Chen, J., D. Zhang, Q. Yao, J. Zhang, X. Dong, H. Tian, J. Chen, and W. Zhang. 2010. Feeding-based RNA interference of a trehalose phosphate

synthase gene in the brown planthopper, *Nilaparvata lugens*. Insect Mol. Biol. 19: 777–786.

Chen, Y. H., C. C. Bernal, J. Tan, F. G. Horgan, and M. A. Fitzgerald. 2011. Planthopper "adaptation" to resistant rice varieties: changes in amino acid composition over time. J. Insect Physiol. 57: 1375–1384.

Chino, M., H. Hayashi, and T. Fujumorita. 1987. Chemical composition of rice phloem sap and its fluctuation. J. Plant Nutr. 10: 1651–1661.

Das, Y. T. 1976. Some factors of resistance to Chilo-suppressalis in rice varieties. J. Entomol. Exp. Appl. 20: 131–134.

Ferguson, A., W. L. Yi, A. Roger, T. S. Daniel, J. F. Manuel, B. J. Benjamin, A. L. Jose, D. A. Randall, P. M. Matthew, V. C. Theresa, et al. 2015. Functional dynamics within the human ribosome regulate the rate of active protein synthesis. J. Mol. Cell. 60: 475–486.

Ghaffar, M.A.B., J. Prichard, and B. Ford-Lloyd. 2011. Brown planthopper (*N. lugens* Stal) feeding behaviour on rice germplasm as an indicator of resistance. J. PLoS ONE. 6: e22137.

Goudarzi, K. M., and M. S. Lindström. 2016. Role of ribosomal protein mutations in tumor development. Int. J. Oncol. 48: 1313–1324.

Grayer, R. G., F. M. Kimmins, P. C. Stevenson, J. B. Harborne, and M. Wijayagunesekera. 1994. Phenolics in rice phloem sap as sucking deterrents to the brown planthopper, (*Nilaparvata lugens*). J. Acta Horticult. 381: 691–694.

Grewal, S. S., J. R. Evans, and B. A. Edgar. 2007. Drosophila TIF-IA is required for ribosome synthesis and cell growth and is regulated by the TOR pathway. J. Cell Biol. 179: 1105–1113.

Grummt, I., V. A. Smith, and F. Grummt. 1976. Amino acid starvation affects the initiation frequency of nucleolar RNA polymerase. J. Cell. 7: 439–445.

Horgan, F. G., T. S. Srinivasan, B. S. Naik, A. F. Ramal, C. C. Bernal, and M.L.P. Almazan. 2016. Effects of nitrogen on egg-laying inhibition and ovicidal response in planthopper-resistant rice varieties. Crop Protect. 89: 223e230.

Jairin, J., Teangdeerith, S.P.K. Leelagud, A. Phengrat, Vanavichit, and T. Toojinda. 2007. Physical mapping of *Bph3*, a brown planthopper resistance locus in rice. 01: 166–177.

Jung, J. K., and D. J. Im. 2005. Feeding inhibition of the brown planthopper, *Nilaparvata lugens* (Homoptera: Delphacidae) on a resistant rice variety. J. Asia-Pacific Entomol. 8: 301–308.

Kim, T. H., P. Leslie, and Y. Zhang. 2014. Ribosomal proteins as unrevealed caretakers for cellular stress and genomic instability. J. Oncotarget. 5: 860–871.

Kressle, D., E. Hurt, and J. Bassler. 2010. Driving ribosome assembly. J. Biochim. Biophys. Acta. 1803: 673–683.

Li, L., B. A. Edgar, and S. S. Grewal. 2010. Nutritional control of gene expression in Drosophila larvae via TOR, Myc and a novel cisregulatory element. J. BMC Cell Biol. 11: 1–13.

Liao, J., M. X. Zhou, A. Gatignol, and H. Lu. 2014. Ribosomal proteins L5 and L11 co-operatively inactivate c-Myc via RNA-induced silencing complex. J. Oncogene. 33:4916–4923.

Lior, G., V. Sinisa, and O. Moshe. 2014. p53 and ribosome biogenesis stress: the essentials. J. FEBS Lett. 588: 2571–2579.

Liu, Y., Y. Z. He, J. Aiwen, P. T. Andrey, L. S. Zhou, A. T. Laura, L. Patrick, T. Kim, O. Lei, A. C. Rosalind, et al. 2014. Ribosomal protein-Mdm2-p53 pathway coordinates nutrient stress with lipid metabolism by regulating MCD and promoting fatty acid oxidation. Proc. Natl. Acad. Sci. U. S. A. 111: 2414–2422.

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. J. Methods. 25: 402–408.

Moin, M., A. Bakshi, A. Saha, M. Dutta, S. M. Madhav, and P. B. Kirti. 2016. Rice ribosomal protein large subunit genes and their spatio-temporal and stress regulation. J. Front. Plant Sci. 7: 1284.

Nault, L. R., and E. D. Ammar. 1989. Leafhopper and planthopper transmission of plant viruses. J. Annu. Rev. Entomol. 34: 503–529.

Orelle, C., E. D. Carlson, T. Szal, T. Florin, M. C. Jewett, and A. S. Mankin. 2015. Protein synthesis by ribosomes with tethered subunits. J. Nat. 7563: 119–124.

Peñalver, C. A., A. Arida, K. L. Heong, and F. G. Horgan. 2011. Aspects of brown planthopper adaptation to resistant rice varieties with the *Bph3* gene. J. Entomol. Exp. Appl. 141: 245–257.

Peng, L., Y. Zhao, H. Wang, J. Zhang, H. Song, X. Shangguan, L. Zhu, and G. He. 2016. Comparative metabolomics of the interaction between rice and the brown planthopper. J. Metab. 12: 1–15.

Rashid, M. M., M. Jahan, and K. S. Islam. 2016. Response of adult brown planthopper *Nilaparvata lugens* (Stål) to rice nutrient management. Neotrop. Entomol. 45: 588–596.

Rosorius, O., B. Fries, R. H. Stauber, N. Hirschmann, D. Bevec, and J. Hauber. 2000. Human ribosomal protein L5 contains defined nuclear localization and export signals. J. Biol. Chem. 275: 12061–12068.

Ferreira-Cerca, S., and E. Hurt. 2009. Cell biology: arrest by ribosome. J. Nat. 459: 46–47.

Sogawa, K., and M. D. Pathak. 1970. Mechanisms of brown planthopper resistance in Mudgo variety of rice (Hemiptera: Delphacidae). J. Appl. Entomol. Zool. 5: 145–158.

Stevenson, P. C., F. M. Kimmins, R. J. Grayer, and S. Raveendranath. 1996. Schaftosides from rice phloem as feeding inhibitors and resistance factors to brown planthoppers, *Nilaparvata lugens*. J. Entomol. Exp. Appl. 80: 246–249.

Takada, H., A. Kurisaki. 2015. Emerging roles of nucleolar and ribosomal proteins in cancer, development, and aging. J. Cell. Mol. Life Sci. 72: 4015–4025.

Tanaka, K. 1999. Quantitative genetic analysis of biotypes of the brown planthopper *Nilaparvata lugens*: heritability of virulence to resistant rice varieties. J. Entomol. Exp. Appl. 90: 279–287.

Teng, T., A. M. Carol, H. Philip, T. George, and F. Stefano. 2013. Loss of tumor suppressor RPL5/RPL11 does not induce cell cycle arrest but impedes proliferation due to reduced ribosome content and translation capacity. J. Mol. Cell. Biol. 33: 4660–4671.

Thayumanavan, B. 1990. Phenolic compounds reducing sugars and free amino acids in rice leaves of varieties resistant to rice thrips. J. Int. Rice Res. Newslett. 11: 14–15.

Tschochner, H., and E. Hurt. 2003. Pre-ribosomes on the road from the nucleolus to the cytoplasm. J. Trends Cell Biol. 13: 255–263.

Wang, X., M. Zhang, F. Feng, and R. He. 2015. Differentially regulated genes in the salivary glands of brown planthopper after feeding in resistant versus susceptible rice varieties. J. Arch. Insect Biochem. Physiol. 89: 69–86.

Wang, Y. B., H. M. Guo, H. C. Li, H. Zhang, and X. X. Miao. 2012. Identification of transcription factors potential related to brown planthopper resistance in rice via microarray expression profiling. J. BMC Genomics. 13: 687.

Warner, J. R., and K. B. McIntosh. 2009. How common are extraribosomal functions of ribosomal proteins? J. Mol. Cell. 34: 3–11.

Xiao, C., J. Hu, Y. T. Ao, M. X. Cheng, G. J. Gao, Q. L. Zhang, G. C. He, and Y. Q. He. 2016. Development and evaluation of near-isogenic lines for brown planthopper resistance in rice cv.9311. J. Sci. Rep. 6: 38159.

Xue, J. X., Zhou, C. X. Zhang, L. L. Yu, H. W. Fan, Z. Wang, H. J. Xu, Y. Xi, Z. R. Zhu, W. W. Zhou, et al. 2014. Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. J. Genome Biol. 15: 521.

Zha, W., X. Peng, R. Chen, B. Du, L. Zhu, and G. He. 2011. Knockdown of midgut genes by dsRNA-transgenic plant-mediated RNA interference in the hemipteran insect *Nilaparvata lugens*. PLoS ONE. 6: e20504.

Zhu, Z. R., J. A. Cheng, M. X. Jiang, and X. X. Zhang. 2004. Complex influence of rice variety, fertilization timing and insecticide on population dynamics of *Sogatella furcifera* (Horvath), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae) and their natural enemies in rice in Hangzhou, China. J. Pest Sci. 77: 65–74.