Open Access

Edited by:
Wei Guo,
Chinese Academy of Sciences (CAS), China

Reviewed by:
LinQuan Ge,
Yangzhou University, China
Junzheng Zhang,
China Agricultural University, China

*Correspondence:
Hai-Jun Xu
haijunxu@zju.edu.cn

Specialty section:
This article was submitted to Epigenomics and Epigenetics, a section of the journal Frontiers in Genetics

Received: 20 July 2020
Accepted: 12 October 2020
Published: 03 November 2020

Citation:
Chen H-H, Liu Y-L, Liu X-Y, Zhang J-L and Xu H-J (2020) Functional Analysis of Nuclear Factor Y in the Wing-Dimorphic Brown Planthopper, Nilaparvata lugens (Hemiptera: Delphacidae). Front. Genet. 11:585320. doi: 10.3389/fgene.2020.585320

Introduction

Nuclear transcription factor Y (NF-Y) is an evolutionarily conserved transcription factor that exists in almost all organisms, from prokaryotes to eukaryotes (Dorn et al., 1987; Laloum et al., 2013). NF-Y exerts differential regulation on a wide variety of genes through binding to a CCAAT box, one of the most ubiquitous elements in eukaryotic promoters (Li et al., 1992; Mantovani, 1998; Suzuki et al., 2001). NF-Y consists of three subunits, NF-YA, NF-YB, and NF-YC, all required for DNA-binding (McNabb et al., 1995; Sinha et al., 1995; Bi et al., 1997; Mantovani, 1999). Accumulated evidence indicates that animal NF-Y is essential for numerous biological processes involved in proliferation and apoptosis, cancer and tumorigenesis, stress responses, growth, and development (Li et al., 2018). For instance, the deletion of the mouse NF-YA homolog causes early embryo lethality (Bhattacharya et al., 2003). Down-regulation of mouse NF-YA was found to reduce the expression of several cell cycle control genes in differentiated muscle cells, suggesting NF-YA...
is involved in specifying myogenic fate (Gurtner et al., 2003). Knockdown or overexpression of the Drosophila melanogaster NF-Y homolog in all tissues results in lethality at the larval stage, indicating that a certain level of NF-YA is necessary for viability in flies (Yoshioka et al., 2007). However, he contribution of NF-Y homologs to the life-history traits of other insect species has yet to be reported.

The wing-dimorphic brown planthopper (BPH), Nilaparvata lugens (Hemiptera: Delphacidae), is the most destructive pest of rice in Asia (Xue et al., 2014), causing a loss in rice production of more than $300 million annually in Asia. It feeds exclusively on the phloem sap of the rice plant and transmits plant viruses such as rice ragged stunt virus and rice grassy stunt virus, resulting in loss of plant vigor and reduced yield (Backus et al., 2005; Otuka, 2013). The BPH is a hemimetabolous insect, the newly hatched first-instar nymphs look like miniature adults except for the absence of wings and sexual immaturity, and grow gradually within increasing stages. The BPH has five nymphal stages, and wing buds grow with each of these stages, but short- and long-winged morphs are externally indistinguishable until the adults emerge (Xu and Zhang, 2017). Long-winged BPH adults have well-developed forewings, hindwings, and indirect flight muscles (IFM), whereas, short-winged adults have undeveloped forewings and rudimentary hindwings (Xu and Zhang, 2017; Zhang et al., 2019). Wing dimorph and high fecundity are the two most important biological features contributing to the ecological success of the BPH (Zhang et al., 2019). Recently, facilitated by the development of genetic tools (Xu et al., 2013; Xue et al., 2018) and genomic information (Xue et al., 2014), the molecular basis underlying wing dimorphism (Xu et al., 2015) and population resurgence (Wu et al., 2020) are emerging, which offers a basis for the design of new control agents to combat BPH infestation.

In the present study, we identified the BPH NF-YA (NlNF-YA), NF-YB (NlNF-YB), and NF-YC (NlNF-YC) homologs by searching the BPH genomic and transcriptomic databases. RNA interference (RNAi)-mediated gene silencing showed that NlNF-YA, NlNF-YB, and NlNF-YC all played pivotal roles in nymph growth, wing formation, IFM development, and reproduction. In addition, we found that NF-Y regulated adult eclosion most likely through linking to the ecdysone signaling pathway. Our findings deepen our understanding of the function of NF-Y in hemipteran insects.

**MATERIALS AND METHODS**

**Insects**
The BPH strain was originally collected from a rice field in Hangzhou, China. Insects were reared on rice seedlings (rice variety: Xiushui 134) in a walk-in chamber at 26 ± 0.5°C, with a light: dark photoperiod of 16: 8 h and relative humidity of 50 ± 5%.

**Gene Identification and Sequence Analysis**
The amino acid sequences of Drosophila NF-Y homologs were used to screen BPH genomic and transcriptomic databases for homologs. Total RNA was isolated from BPH nymphs using RNAiso plus (Takara #9109) according to the manufacturer’s protocol. For cDNA syntheses, 450 ng of total RNA was reversely transcribed in a 10 μL reaction with HiScript QRT SuperMix (Vazyme #R223-01). The NlNF-YA, NlNF-YB, and NlNF-YC sequences were amplified from cDNA using fidelity DNA polymerase (Toyobo #930700) with NlNF-YA-F/R, NlNF-YB-F/R, and NlNF-YC-F/R primer pairs, respectively (Supplementary Table 1). The PCR product was cloned and the sequence was determined by Sanger sequencing.

To identify the exon-intron construction of NlNF-Y, open reading frames (ORFs) of NlNF-YA, NlNF-YB, and NlNF-YC were used to search a BPH genomic database (Xue et al., 2014). For phylogenetic analysis, 39 NF-Y sequences from 13 species including D. melanogaster, N. lugens, Acyrthosiphon pisum, Apis cerana, Bemisia tabaci, Bombyx mori, Cryptotermes secundus, Halyomorpha halys, Monomorium pharaonis, Nasonia vitripennis, Niphalopus vestiploides, Tribolium castaneum, and Zootermopsis nevadensis were used for sequence alignment. A phylogenetic tree was constructed with maximum-likelihood method and 1000 bootstraps using the MEGA 7 program (Kumar et al., 2016).

**Spatio-Temporal Expression Pattern of NlNF-Y**
Total RNA was isolated from eggs (n = 100), first-instar (n = 100), second-instar (n = 50), third-instar (n = 50), fourth-instar (n = 30), fifth-instar nymphs (n = 15), and adult females (n = 15), which were laid or ecdysed within 24 h. To examine tissue distribution, we dissected the head (n = 50), antenna (n = 200), tergum (n = 50), leg (n = 100), fat body (n = 30), cuticle (n = 50), and gut (n = 100) from four-instar nymphs for RNA extraction. First-strand cDNA was synthesized from total RNA (450 ng) using HiScript QRT SuperMix (Vazyme #R223-01). The synthesized cDNAs were 10-fold diluted and used as templates for quantitative real-time PCR (qPCR). The qPCR primers for NlNF-Y (Supplementary Table 1) were designed using Primer-Blast.1 The qPCR was conducted on a CFX96TM real-time PCR detection system (Bio-Rad) using the following conditions: denaturation for 3 min at 95°C, followed by 40 cycles at 95°C for 10 s, and 60°C for 30 s. The ribosomal protein S11 gene (rps11) was used as the internal reference gene (Yuan et al., 2014). The 2−ΔΔCt method (ΔCt represents the cycle threshold) was used to measure the relative expression level (Livak and Schmittgen, 2001). Three independent biological replicates with three technical replicates were conducted for each experiment.

**RNAi and Microinjection**
The dsRNAs were synthesized using a T7 High Yield Transcription Kit (Vazyme #TR101-02) according to the manufacturer’s instructions with primers containing the T7 RNA polymerase promoter at both ends (Supplementary Table 1). A dsRNA injection was carried out as in our previous study (Xu et al., 2015). Briefly, fourth-instar nymphs were anesthetized with carbon dioxide for 10–15 s. Approximately

1https://www.ncbi.nlm.nih.gov/tools/prime-blast/
150 ng dsRNA was microinjected into the mesothorax using a FemtoJet microinjection system (Eppendorf). To investigate whether disruption of NlNF-YA affected ecdysone signaling activity, fifth-instar nymphs (n = 10 for each of three replicates) were collected to examine the expression levels of NlIE74A and NIE75B by qPCR using corresponding primers (Supplementary Table 1). To investigate RNAi efficiency, BPHs (n = 5 for each of three replicates) at 24 h after adult eclosion were collected for qPCR assay.

**Forewing Size and Hind Tibiae Length**

Fourth-instar nymphs (36–48 h after ecdysis, hAE) were microinjected with dsRNAs targeting NlNF-YA (dsNlNF-YA), NlNF-YB (dsNlNF-YB), NlNF-YC (dsNlNF-YC), or GFP (dsGFP). After adult eclosion (24 h), images of the forewings and hind tibiae were captured with a DFC320 digital camera attached to a Leica S8AP0 stereomicroscope using a LAS digital imaging system (v. 3.8). Digital images of forewings (n = 20) and hind tibias (n = 20) were collected for the measurement of forewing size and hind tibia length using ImageJ (v. 1.47).

**Microinjection With 20-hydroxyecdysone (20E)**

Fourth-instar nymphs (36–48 h AE) were microinjected with corresponding dsRNAs targeting each gene, and then nymphs were maintained on fresh rice seedings until the fifth-instar stage (within 48 h). Fifth-instar nymphs were microinjected with 20E (1 mg/ml) or distilled water, and the adult eclosion rate was calculated.

**Transmission Electron Microscopy**

Fourth-instar nymphs (36–48 h AE) were microinjected with dsRNAs targeting NlNF-YA, NlNF-YB, NlNF-YC, or GFP. The thoraxes were dissected from adult females (24 h after emergence) for Transmission Electron Microscopy (TEM) as in our previous study (Xue et al., 2013). Briefly, samples were fixed in 2.5% glutaraldehyde overnight at 4°C. After fixation, samples were post-fixed in 1% osmium tetroxide for 1.5 h. Then, samples were dehydrated in a standard ethanol/acetone series, infiltrated and embedded in Spurr medium, and then superthin sections were cut. The sections were stained with 5% uranyl acetate followed by Reynolds’ lead citrate solution and observed under a JEM-1230 transmission electron microscope (JEOL).

**Fecundity Assay**

Newly emerged females and males (within 12 h after eclosion) were microinjected with dsNF-YA, ds-NF-YB, dsNF-YC, or dsGfp. Then each female was allowed to match with two males in a glass tube. Insects were removed at 5 days after matching, and eggs were counted under a Leica S8AP0 stereomicroscope.

**Data Analysis**

Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software). Means were compared using a two-tailed Student’s t-test and log-rank (Mantel-Cox) test at a significance level set at \( ^* P < 0.05 \), \( ^{**}P \leq 0.01 \), and \( ^{***}P \leq 0.001 \).

**RESULTS**

**NlNF-YA, NlNF-YB, and NlNF-YC Sequence Analysis**

We identified three genes encoding BPH NF-Y homologs, NlNF-YA, NlNF-YB, and NlNF-YC, in a BLAST search against BPH genomic (Xue et al., 2014) and transcriptome databases using the D. melanogaster NF-Y proteins as query sequences. The ORFs of NlNF-YA, NlNF-YB, and NlNF-YC were 1002-, 609-, and 1011-bp in length, encoding 333, 202, and 336 amino acid residues, respectively (Supplementary Figures 1–3). Exon-intron construction analysis showed that the NlNF-YA, NlNF-YB, and NlNF-YC ORFs consist of eight, five, and eight exons, located in scaffolds 6450/1657, 3414, and 1809, respectively. A phylogenetic analysis based on NF-Y homologs from 13 species showed that NlNF-YA, NlNF-YB, and NlNF-YC together with their counterparts formed three distinct clusters (Figure 1), indicating that the BPH NF-Y genes we identified were authentic NF-Y homologs.

**Spatio-Temporal Analysis of NlNF-YA, NlNF-YB, and NlNF-YC**

Spatio-temporal expression of NlNF-YA, NlNF-YB, and NlNF-YC was examined by qPCR. NlNF-YA, NlNF-YB, and NlNF-YC transcripts were readily detected from egg to adult stages,
and relatively high levels were detected in eggs laid within 24 h (Egg-24 h) (Figure 2A), indicating that they might play important functions in egg development. Tissue distribution analysis showed that NINF-YA, NINF-YB, and NINF-YC were evenly expressed in head, antenna, tergum, leg, fat body, cuticle, and gut of fourth-instar nymphs (Figure 2B), indicating NINF-Y genes might be important for BPH growth and development.

**Knockdown of NINF-Y Genes Leads to Small and Malformed Wings**

To investigate whether NINF-YA, NINF-YB, and NINF-YC played any roles in BPH development, fourth-instar short-wing-destined nymphs were microinjected with corresponding dsRNAs targeting each gene. At 48 h after microinjection, RNAi efficiency was examined by qPCR, which showed that dsRNA treatments significantly down-regulated the expression levels of NINF-YA, NINF-YB, and NINF-YC by 63.7, 99.9, and 82.7%, respectively, relative to the GFP RNAi treatment (Figure 3A). Notably, NINF-YA RNAi, NINF-YB RNAi, and NINF-YC RNAi caused high mortality, leading to approximately 60, 60, and 40% of nymphs died before adult eclosion (Figure 3B), respectively. The remaining nymphs could successfully molt into adults. However, these adults had moderately smaller and slightly malformed forewings relative to GFP RNAi BPHs.

![FIGURE 2](https://example.com/figure2.png) Spatio-temporal expression of NINF-Y genes. (A) Developmental profile of NINF-YA, NINF-YB, and NINF-YC. Total RNAs were isolated from eggs (n = 100), first-instar (n = 100), second-instar (n = 50), third-instar (n = 50), fourth-instar (n = 30), fifth-instar nymphs (n = 15), and adult females (n = 15). (B) Tissue distribution of NINF-Y. Total RNAs were extracted from the head (n = 30), antenna (ant, n = 200), tergum (n = 50), leg (n = 100), fat body (n = 30), cuticle (n = 50), and gut (n = 100) from fourth-instar nymphs. First-strand cDNA was synthesized using random primers, and qPCR was conducted using specific primers corresponding to each gene. The relative expression level was normalized by the ribosomal S11 gene (rps11). Bars represent s.d. derived from three independent biological replicates.
(Figures 4A,B). NINF-YARNAi, NINF-YBRNAi, and NINF-YCRNAi specifically reduced forewing size but had marginal roles on hind tibiae length (Figure 4C). These data indicated that NINF-YA, NINF-YB, and NINF-YC were essential for normal wing growth in BPHs.

Knockdown of NINF-Y Genes Leads to Defective IFM

Because short-wing BPHs lack hindwings and IFM, we used long-wing BPHs to investigate whether NINF-YA, NINF-YB, and NINF-YC had any effect on IFM development. For this,
fourth-instar long-wing-destined nymphs were microinjected with corresponding dsRNAs targeting each gene. $NINF-YA^{RNAi}$, $NINF-YB^{RNAi}$, and $NINF-YC^{RNAi}$ gave rise to adults with curved forewings and hindwings (Figure 5A). In line with the phenotype in short-winged BPHs, smaller forewings and hindwings were observed in adults previously treated with either $NINF-YA^{RNAi}$, $NINF-YB^{RNAi}$, or $NINF-YC^{RNAi}$ (Figure 4B), although there was no discernable effect on hind tibia length (Figure 4C). Next, these adults were collected for IFM ultrastructure examination using TEM. In GFP$^{RNAi}$ adults, sarcomeres were clearly divided by Z discs and well-organized (Figure 6). In contrast, $NINF-YA^{RNAi}$, $NINF-YB^{RNAi}$, or $NINF-YC^{RNAi}$ led to defective sarcomeres, with deformed Z discs and weakly organized myofibrillar. These events indicated that the $NINF-Y$ complex might be essential for IFM assembly in BPH.

**Knockdown of $NINF-Y$ Genes Impairs Nymphal Growth and Adult Molting**

Given that $NINF-Y$ genes were evenly expressed across nymphal stages (Figure 2A), we investigated whether knockdown of $NINF-Y$ genes would affect nymph growth. For this purpose, third-instar nymphs were microinjected with corresponding dsRNAs targeting each gene. Nymphs treated with $NINF-YA^{RNAi}$, $NINF-YB^{RNAi}$, or $NINF-YC^{RNAi}$ had an extended fifth-instar duration compared with those treated with GFP$^{RNAi}$ (Figure 7A), with $NINF-YA^{RNAi}$ showing the most profound effect (Figure 7A). In addition, the majority of $NINF-YA^{RNAi}$-treated nymphs died before adult emergence (Figure 7B), indicating that the disruption of the NF-Y complex impaired nymph-to-adult ecdysis.

Given that ecdysone is a hormone that initiates all major developmental transitions from the egg, to larva, to pupa, to adult in insects (Gilbert et al., 2002; Dubrovsky, 2005), we asked whether it mediated the effects of $NINF-Y$ on adult molting. For this purpose, we first assessed the expression levels of two early ecdysone response genes, $NIE74A$ and $NIE75E$ (Dubrovsky, 2005), in the context of $NINF-YA$ knockdown. $NINF-YA^{RNAi}$ significantly reduced both $NIE74A$ and $NIE75E$ transcripts compared with GFP$^{RNAi}$ (Figure 7C), indicating that knockdown of $NINF-YA$ might impair ecdyson signaling activity. Following this observation, we asked whether the addition

---

**FIGURE 5 | Knockdown of $NINF-Y$ reduces forewing size in long-winged BPHs.** (A) Morphology of dsRNA-treated long-winged BPHs. (B) The size of forewings and hindwings after dsRNA treatment. (C) Relative wing size and tibia length in BPHs with $NINF-Y$ or GFP knockdown. Each dot represents the wing size and tibia length derived from an individual female ($n = 20$). Bar represents mean ± s.d. derived from independent biological replicates ($n = 20$). Statistical comparisons between two groups were performed using a two-tailed Student’s t-test (n.s., non-significant).
of ecdysone could rescue the NINF-YA RNAi defect. To this end, fourth-instar nymphs were microinjected with dsNINF-YA, followed by microinjection with 20E when nymphs proceeded to the fifth-instar at 48 h after eclosion. The addition of 20E could partially restore the molting defect caused by NINF-YA RNAi (Figure 7B) compared with the addition of water, indicating that NINF-YA RNAi decreased adult eclosion at least partially through the ecdysone signaling pathway.

**Knockdown of NINF-Y Genes Impairs Reproduction**

Given that relatively high amounts of NINF-YA, NINF-YB, and NINF-YC transcripts were detected at the early egg stage (Figure 2A), we asked whether NINF-YA, NINF-YB, and NINF-YC contributed to BPH reproduction. Female and male adults at 12 h after eclosion were microinjected with corresponding dsRNAs targeting each gene, and then allowed to mate and to lay eggs for 5 days. BPHs treated with NINF-YA RNAi, NINF-YB RNAi, and NINF-YC RNAi laid substantially fewer eggs than those treated with GFP RNAi (Figure 8A). In addition, the eggs laid by BPHs previously treated with NINF-YA RNAi, NINF-YB RNAi, or NINF-YC RNAi failed to develop eye pigmentation, a characteristic hallmark of egg development, indicating NINF-Y genes might be essential for embryogenesis (Figure 8B).

**DISCUSSION**

In this study, we investigated the functions of individual NINF-Y members in the BPH during nymphal development, adult molting, wing growth, IFM development, and reproduction. The results demonstrated that NINF-Y played pivotal roles in these processes. Depletion of NINF-YA, NINF-YB, and NINF-YC resulted in extended nymphal duration, affected nymph-adult molting progress, reduced wing size, disrupted IFM assembly, reduced egg production, and impaired egg development. These findings provide an impetus to understand the function of NF-Y in life-history traits of insects.

The CCAAT box is one of the most common cis-acting elements found in the promoter and enhancer regions of various genes in eukaryotes (Li et al., 1992). An analysis of a large database of 1,031 human promoters indicated that the CCAAT box is present in 63% of them (Suzuki et al., 2001). NF-Y is the major CCAAT box recognizing protein that binds to DNA with high specificity and affinity (Bi et al., 1997; Mantovani, 1998). Knockdown or overexpression of the Drosophila NF-YA homolog with different GAL4 also drives lethality in the Drosophila pharate adult stage, possibly by influencing disc specification (Yoshioka et al., 2007; Ly et al., 2013). A similar phenomenon was observed in BPH,
FIGURE 7 | Nymphal duration and adult eclosion of dsRNA-treated BPHs. (A) Third-instar nymphs (0–24 hAE) were microinjected with dsRNAs targeting NlNF-YA (n = 660), NlNF-YB (n = 435), NlNF-YC (n = 480), or Gfp (n = 442). The curve in different developmental stages indicates the molting ratio. (B) The nymph-adult eclosion rate of BPHs. Fourth-instar nymphs (36–48 hAE) were microinjected with dsRNAs targeting NlNF-YA or Gfp, and then 20E or distilled water was microinjected when nymphs proceeded the fifth-instar stage. (C) The expression of NlE74A and NlE75B in context of NlNF-YA knockdown. The relative expressions of NlE74A and NlE75B were normalized to the expression of rps11. Statistical comparisons between two groups were performed using two-tailed Student’s t-test (*P < 0.05, and **P < 0.01).

FIGURE 8 | Fecundity of adults with gene knockdown and phenotype of eggs produced. (A) Newly emerged adults (0–12 hAE) were treated with dsRNAs targeting each gene. The dsRNA-treated females and males were allowed to mate and laid eggs for 5 days. Each circle represents eggs produced by an individual female (n = 6). Bars represent the mean ± s.d. Statistical comparisons between two groups were performed using a two-tailed Student’s t-test (***P < 0.001). (B) Morphology of eggs deposited after 5 days by dsRNA-treated BPHs. The eye pigmentation is indicated by an arrow head.

depleting NlNF-YA, NlNF-YB, and NlNF-YC delayed fifth-instar development and led to high lethality, indicating that the CCAAT box might also be a common cis-acting element in the BPH. In addition, we noticed that depletion either NlNF-YA, NlNF-YB, or NlNF-YC led to small and malformed forewings and hindwings. As a wing-dimorphic insect, fifth-instar BPH nymphs have the ability to develop into either short-winged or long-winged adults. A recent finding showed that cells of short wing pads are largely in the G2/M phase of the cell cycle, whereas those of long wings are largely in G1, indicating that cell cycle progression is necessary for wing morph determination (Lin et al., 2020). These observations are in line with previous reports that cell cycle-related genes are the main target genes of NF-Y (Zwicker et al., 1995; Bolognese et al., 1999; Farina et al., 1999; Kao et al., 1999; Korner et al., 2001; Manni et al., 2001). In addition, the depletion of NlNF-Y not only affected wing size, but also led to an IFM defect, the latter tissue is only present in long-winged adults. Based on these events, we speculate that NF-Y might be tightly involved in regulating alternative wing morphs in the BPH although this needs to be further confirmed experimentally.

It is of interest that the depletion of NlNF-YA significantly decreased the adult molting rate, and this defect could be partially rescued by the addition of 20E. In addition, the depletion of NlNF-YA significantly reduced the expression levels of NIE74A and NIE75B, the downstream genes of the ecdysone pathway. These findings indicated that NlNF-Y might affect the ecdysone pathway although the underlying mechanism remains unknown.
Although there remains much to be done, our findings provided a first glimpse of the function of NF-Y in hemipteran insects.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

H-HC and H-JX designed the experiment and wrote the manuscript. H-HC, Y-LL, X-YL, and J-LZ conducted the experiments. H-JX managed and directed the project. All authors contributed to the article and approved the submitted version.

REFERENCES

Backus, E. A., Serrano, M. S., and Ranger, C. M. (2005). Mechanisms of hopperburn: an overview of insect taxonomy, behavior, and physiology. Annu. Rev. Entomol. 50, 125–151. doi: 10.1146/annurev.ento.49.061802.123310

Bhattacharya, A., Deng, J. M., Zhang, Z., Behringer, R., de Crombrugghe, B., and Maity, S. N. (2003). The B subunit of the CCAAT box binding transcription factor complex (CBF/NF-Y) is essential for early mouse development and cell proliferation. Cancer Res. 63, 8167–8172.

Bi, W., Wu, L., Coustry, F., de Crombrugghe, B., and Maity, S. N. (1997). DNA binding specificity of the CCAAT-binding factor CBF/NF-Y. J. Biol. Chem. 272, 26562–26572. doi: 10.1074/jbc.272.42.26562

Bolognese, F., Wasner, M., Dohna, C. L., Gurtner, A., Muller, H., Manni, I., et al. (1999). The cyclin B2 promoter depends on NF-Y, a trimer whose CCAAT-binding activity is cell-cycle regulated. Oncogene 18, 1845–1853. doi: 10.1038/sj.onc.1202494

Dorn, A., Bollekins, J., Staub, A., Benoist, C., and Mathis, D. (1987). A multiplicity of CCAAT box-binding proteins. Cell 50, 863–872. doi: 10.1016/0092-8674(87)90513-7

Dubrovsky, E. B. (2005). Hormonal cross talk in insect development. Trends Endocrinol. Metab. 16, 6–11. doi: 10.1016/j.tem.2004.11.003

Farina, A., Manni, I., Fontemaggi, G., Tlain, M., Cenciarelli, C., Bellorini, M., et al. (1999). Down-regulation of cyclin B1 gene transcription in terminally differentiated skeletal muscle cells is associated with loss of functional CCAAT-binding NF-Y complex. Oncogene 18, 2818–2827. doi: 10.1038/sj.onc.1202472

Gilbert, L. I., Rybczynski, R., and Warren, J. T. (2002). Control and biochemical nature of the ecdysteroidogenic pathway. Annu. Rev. Entomol. 47, 883–916. doi: 10.1146/annurev.ento.47.091201.145302

Gurtner, A., Manni, I., Fuschi, P., Mantovani, R., Guadagni, F., Sacchi, A., et al. (2003). Requirement for down-regulation of the CCAAT-binding activity of the NF-Y transcription factor during skeletal muscle differentiation. Mol. Biol. Cell. 14, 2706–2715. doi: 10.1091/mbc.e02-09-0060

Kao, C. Y., Tanimoto, A., Arima, N., Sasaguri, Y., and Padmanabhan, R. (1999). Transactivation of the human cdc2 promoter by adenovirus E1A. E1A induces the expression and assembly of a heteromeric complex consisting of the CCAAT binding factor, CBF/NF-Y, and a 110-kDa DNA-binding protein. J. Biol. Chem. 274, 23043–23051. doi: 10.1074/jbc.274.33.23043

Korner, K., Jerome, V., Schmidt, T., and Muller, R. (2001). Cell cycle regulation of the murine cdc25B promoter: essential role for nuclear factor-Y and a proximal repressor element. J. Biol. Chem. 276, 9662–9669. doi: 10.1074/jbc.M008696200

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. doi: 10.1093/molbev/msw054

Laloum, T., De Mita, S., Gamas, P., Baudin, M., and Niebel, A. (2013). CCAAT-box binding transcription factors in plants: Y so many? Trends Plant Sci. 18, 157–166. doi: 10.1016/j.plants.2012.07.004

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (31772158 and 31972261), and the National Natural Science Foundation of China for Excellent Young Scholars (31522047). The authors declare that they have no competing interest. We thank the International Science Editing company for polishing this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2020.585320/full#supplementary-material

Supplementary Table 1 | Primers used in this study.

Li, G., Zhao, H., Wang, L., Wang, Y., Guo, X., and Xu, B. (2018). The animal nuclear factor Y: an enigmatic and important heterotrimeric transcription factor. Am. J. Cancer Res. 8, 1106–1125.

Li, X. Y., Mantovani, R., Hooft van Huijsduijnen, R., Andre, I., Benoist, C., and Mathis, D. (1992). Evolutionary variation of the CCAAT-binding transcription factor NF-Y. Nucleic Acids Res. 20, 1087–1091. doi: 10.1093/nar/20.5.1087

Lin, X., Gao, H., Xu, Y., Zhang, Y., Li, Y., Lavine, M. D., et al. (2020). Cell cycle progression determine wing morph in the polyphagen insect Nilaparvata lugens. iScience 23, 10040.101040. doi: 10.1016/j.isci.2020.101040

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25, 402–408. doi: 10.1006/meth.2001.1262

Ly, L. L., Suyari, O., Yoshioka, Y., Sue, T. N., Yoshida, H., and Yamaguchi, M. (2013). dNF-YB plays dual roles in cell death and cell differentiation during Drosophila eye development. Gene 520, 106–118. doi: 10.1016/j.gene.2013.02.036

Manni, I., Mazzaro, G., Gurtner, A., Mantovani, R., Haugwitz, U., Krause, K., et al. (2001). NF-Y mediates the transcriptional inhibition of the cyclin B1, cyclin B2, and cdc25C promoters upon induced G2 arrest. J. Biol. Chem. 276, 5570–5576. doi: 10.1074/jbc.M006052200

Mantovani, R. (1998). A survey of 178 NF-Y binding CCAAT boxes. Nucleic Acids Res. 26, 1135–1143. doi: 10.1093/nar/26.5.1135

Mantovani, R. (1999). The molecular biology of the CCAAT-binding factor NF-Y. Gene 239, 15–27. doi: 10.1016/S0378-1119(99)00368-6

McNab, D. S., Xing, Y., and Guarente, L. (1995). Cloning of yeast HAP5: a novel subunit of a heterotrimeric complex required for CCAAT binding. Genes Dev. 9, 47–58. doi: 10.1101/gad.9.1.47

Otuka, A. (2013). Migration of rice plant hoppers and their vectorized re-emerging and novel rice viruses in East Asia. Front. Microbiol. 4:309. doi: 10.3389/fmicb.2013.00309

Sinha, S., Lu, J., Maity, S. N., and de Crombrugghe, B. (1995). Recombinant rat CBF-C, the third subunit of CBF-NF-Y, allows formation of a protein-DNA complex with CBF-A and CBF-B and with yeast HAP2 and HAP3. Proc. Natl. Acad. Sci. U.S.A. 92, 1624–1628. doi: 10.1073/pnas.92.5.1624

Suizuki, Y., Tsumoda, T., Sese, J., Taira, H., Mizushima-Sugano, J., Hata, H., et al. (2001). Identification and characterization of the potential promoter regions of 1031 kinds of human genes. Genome Res. 11, 677–684. doi: 10.1101/gr.1640

Wu, J., Ge, L., Liu, F., Song, Q., and Stanley, D. (2020). Pesticide-induced planthopper population resurgence in rice cropping system. Annu. Rev. Entomol. 65, 409–429. doi: 10.1146/annurev-ento-011019-025215

Xu, H. J., Chen, T., Ma, X. F., Xue, J., Pan, P. L., Zhang, X. C., et al. (2013). Genome-wide screening for components of small interfering RNA (siRNA) and micro-RNA (miRNA) pathways in the brown planthopper, Nilaparvata lugens (Hemiptera: Delphacidae). Insect Mol. Biol. 22, 635–647. doi: 10.1111/imb.12091
Chen et al. Planthopper NF-Y

Xu, H. J., Xue, J., Lu, B., Zhang, X. C., Zhao, J. C., He, S. F., et al. (2015). Two insulin receptors determine alternative wing morphs in planthoppers. *Nature* 519, 464–467. doi: 10.1038/nature14286

Xu, H. J., and Zhang, C. X. (2017). Insulin receptors and wing dimorphism in rice planthoppers. *Philos. Trans. R. Soc. Lond B Biol. Sci.* 372:20150489. doi: 10.1098/rstb.2015.0489

Xue, J., Zhang, X. Q., Xu, H. J., Fan, H. W., Huang, H. J., Ma, X. F., et al. (2013). Molecular characterization of the flightin gene in the wing-dimorphic planthopper, *Nilaparvata lugens*, and its evolution in Pancrustacea. *Insect Biochem. Mol. Biol.* 43, 433–443. doi: 10.1016/j.ibmb.2013.02.006

Xue, J., Zhou, X., Zhang, C. X., Yu, L. L., Fan, H. W., Wang, Z., et al. (2014). Genomes of the rice pest brown planthopper and its endosymbionts reveal complex complementary contributions for host adaptation. *Genome Biol.* 15:251. doi: 10.1186/s13059-014-0521-0

Xue, W. H., Liu, Y. L., Jiang, Y. Q., He, S. F., Wang, Q. Q., Yang, Z. N., et al. (2018). CRISPR/Cas9-mediated knockout of two eye pigmentation genes in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). *Insect Biochem. Mol. Biol.* 93, 19–26. doi: 10.1016/j.ibmb.2017.12.003

Yoshioka, Y., Suyari, O., Yamada, M., Ohno, K., Hayashi, Y., and Yamaguchi, M. (2007). Complex interference in the eye developmental pathway by *Drosophila* NF-YA. *Genesis* 45, 21–31. doi: 10.1002/dvg.20260

Yuan, M., Lu, Y., Zhu, X., Wan, H., Shakeel, M., Zhan, S., et al. (2014). Selection and evaluation of potential reference genes for gene expression analysis in the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae) using reverse-transcription quantitative PCR. *PLoS One* 9:e86503. doi: 10.1371/journal.pone.0086503

Zhang, C. X., Brisson, J. A., and Xu, H. J. (2019). Molecular mechanisms of wing polymorphism in insects. *Annu. Rev. Entomol.* 64, 297–314. doi: 10.1146/annurev-ento-011118-112448

Zwicker, J., Lucibello, F. C., Wolfram, L. A., Gross, C., Truss, M., Engeland, K., et al. (1995). Cell cycle regulation of the cyclin A, cdc25C and cdc2 genes is based on a common mechanism of transcriptional repression. *EMBO J.* 14, 4514–4522. doi: 10.1002/j.1460-2075.1995.tb00130.x

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Chen, Liu, Zhang and Xu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.