Insect science

Insect Science (2021) 28, 1582–1590, DOI 10.1111/1744-7917.12879

ORIGINAL ARTICLE

The receptor tyrosine kinase torso regulates ecdysone homeostasis to control developmental timing in *Bombyx mori*

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Abstract Insect growth and development are precisely controlled by hormone homeostasis. The prothoracicotropic hormone (PTTH) receptor, Torso, is a member of the receptor tyrosine kinase family in insects. Activation of Torso by PTTH triggers biosynthesis and release of the steroid hormone in the prothoracic gland (PG). Although numbers of genes functioning in steroid hormone synthesis and metabolism have been identified in insects, the PTTH transduction pathway via its receptor Torso is poorly understood. In the current study, we describe a loss-of-function analysis of Torso in the silkworm, *Bombyx mori*, by targeted gene disruption using the transgenic CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/RNA-guided Cas9 nucleases) system. Depletion of *B. mori Torso* (*BmTorso*) did not eventually affect larval ecdysis and metamorphosis processes. Instead, *BmTorso* deficiency resulted in significant extension of developing time during larval and pupal stages with increased pupa and cocoon sizes. The ecdysteriod titers in the hemolymph of *BmTorso* mutants sharpy declined. Transcriptional levels of genes involved in ecdysone biosynthesis and ecdysteroid signaling pathways were significantly reduced in *BmTorso*-deficient animals. Additionally, RNA-Seq analysis revealed that genes involved in the longevity pathway and protein processing in the endoplasmic reticulum pathway were affected after *BmTorso* deletion. These results indicate that Torso is critical for maintaining steroid hormone homeostasis in insects.

Key words *Bombyx mori*; CRISPR/Cas9; developmental timing; Torso

Introduction

During the development of multicellular organisms, tissue growth must be timed appropriately to ensure the emergence of adults with correct body size (Nijhout, 2015). Body size cannot be changed once the adult stage has been reached, which indicates that adult size is completely fixed by the growth that takes place during the juvenile stages (Nijhout *et al*., 2014). In holometabolous insects, the growth of larva is clearly punctuated by several times of molting process before metamorphosis and the transition to reproductively mature adults (Yamanaka *et al*., 2013). A variety of neuropeptides and hormones play distinct roles in the control of developmental transition (Roller *et al*., 2008; Nassel & Winther, 2010). In addition, the precise regulation of hormone titers is also critical in the process of molting and metamorphosis (Warren *et al*., 2006; Riddiford, 2012).

Steroid hormones are the primary endocrine molecules that regulate the developmental transition (Gilbert *et al*., 2002). In insects, the timing of hatching, molting and metamorphosis are modulated by a steroid hormone 20-hydroxyecdysone (20E). The precursor of 20E, ecdysone, is synthesized primarily in the prothoracic gland (PG) and secreted into the hemolymph (Yamanaka *et al*., 2015).

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Production and release of ecdysone begins with the secretion of prothoracicotropic hormone (PTTH), a neuropeptide produced by two pairs of dorsolateral neurosecretory cells in the brain (McBrayer *et al*., 2007). Subsequently, PTTH signaling promotes ecdysone biosynthesis by stimulating the transcription of several Halloween genes including *neverland* (*nvd*), *spookier* (*spok*), *phantom* (*phm*), *shadow* (*sad*) and *disembodied* (*dib*) (Gilbert, 2004). Then, ecdysone is converted to the active form, 20E, in peripheral tissues (Petryk *et al*., 2003). In the model insect *Drosophila*, genetic ablation of PTTH-producing neurons strongly delayed larval development because of low ecdysone titers (McBrayer *et al*., 2007).

Recently, a receptor tyrosine kinase (RTK) called Torso, which serves as a master control of terminal pattern during early embryogenesis (Schoppmeier & Schroder, 2005), was identified as the receptor of PTTH in *Drosophila* (Rewitz *et al*., 2009). After early embryogenesis, Torso is expressed specifically in the PG. During the larval stage, PTTH binds to Torso and activates ecdysone synthesis via the MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) pathway, resulting in the activation of ERK (Lin & Gu, 2007; Rewitz *et al*., 2009). In *Drosophila*, loss of Torso in PG not only delayed the onset of metamorphosis, but also resulted in larger fly owing to excessive growth during the prolonged duration of the larval stage (Rewitz *et al*., 2009).

In the lepidopteran model insect *Bombyx mori*, Torso has been identified as a PTTH receptor *in vitro* (Rewitz *et al*., 2009). As in *Drosophila*, BmTorso is predominantly expressed in PG during the larval stage. BmPTTH specifically stimulates ERK phosphorylation through BmTorso, rather than other receptors (Rewitz *et al*., 2009). The Torso activation by PTTH is maintained by unusual intermolecular disulfide in the transmembrane region (Konogami *et al*., 2016). Although the physiological roles and activation mechanism for Torso have been extensively studied in *B. mori*, the comprehensive function of Torso have yet to be revealed *in vivo*. Recent advance in genome editing tools, such as the CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/RNA-guided Cas9 nucleases) system, have provided a promising approach for loss of functional analysis (Hsu *et al*., 2014; Xu *et al*., 2017; Zeng *et al*., 2017). In *B. mori*, these techniques have been established, including the transgenic CRISPR/Cas9 systems (Li *et al*., 2015). The transgenic CRISPR/Cas9 would be risky, because it could not knock out the target gene completely, with mild phenotype. However, this somatic mutagenesis system still provides an efficient platform to perform gene loss-of-function analysis in *B. mori*.

In the current study, we established *BmTorso* mutants by using the CRISPR/Cas9 system. The mutants show significant developmental delay during the larval and pupal stages. The prolonged development period especially in feeding larval stage results in larger pupae. The ecdysteriod titers at wandering stage and pupal stage were sharply reduced. Furthermore, we found that transcription levels of Halloween genes in ecdysone synthesis were reduced. RNA-Seq analyses revealed that longevity pathway and protein processing in the endoplasmic reticulum were affected by *BmTorso* deletion. Taken together, our results indicate that Torso regulates the developmental timing from larval to pupal stages and determines the final body size by controlling the ecdysteriod production in silkworm.

Materials and methods

Silkworm strains

A multivoltine and non-diapausing silkworm strain, Nistari, was used in all experiments. Larvae were fed on fresh mulberry leaves under standard conditions at 25 °C and 75% relative humidity.

Plasmid construction

To target *BmTorso* gene, the plasmid *pBac[IE1-DsRed2-U6-BmTorso-sgRNAs]* (*Torso-sgRNAs*) expressing *BmTorso*-specific single guide RNA (sgRNA) was constructed as previously described (Zhang *et al*., 2017). The primer sequences used for plasmid construction are listed in Table S1.

Silkworm germline transformation

A mixture of *Torso-sgRNAs* plasmid and *piggyBac* helper plasmids was injected into preblastoderm embryos (G_0). G_0 adults were sib-mated or crossed with wild-type (WT) to obtain G_1 progeny. G_1 positive progeny expressing red fluorescent protein were screened under a fluorescence microscope (Nikon AZ100).

RNA isolation and complementary DNA (cDNA) synthesis

Total RNA was extracted from PG and fat body at wandering stage using TRIzol reagent (Invitrogen) and treated with DNase I (Invitrogen) to digest genomic DNA. cDNA was synthesized using the ReverAid First

Strand cDNA Synthesis Kit (Fermentas) in accordance with the manufacture's protocol.

Quantitative real-time polymerase chain reaction (q-RT-PCR) analysis

q-RT-PCR was performed using SYBR Green Real-time PCR Master Mix (Toyobo). The thermal cycling conditions were as follows: initial incubation at 95 °C for 5 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Three independent biological replicates were performed in quantitative messenger RNA (mRNA) measurements, and data were normalized to *Bmrp49*. The primers used in q-RT-PCR are listed in Table S1.

Genomic DNA extraction and mutagenesis analysis

Genomic DNA of WT and *BmTorso* mutants at adult stage were extracted as previously described (Zhang *et al*., 2017). The DNA fragments including the sgRNA targeting site were amplified using KOD-Plus polymerase (Toyobo). The PCR conditions were as follows: 94 °C for 2 min, 35 cycles of 94 °C for 15 s, 55 °C for 30 s, and 68 °C for 60 s, followed by a final extension period of 68 °C for 5 min. The amplified products were sub-cloned into pJET1.2 vectors (Fermentas) and subsequently sequenced. The primers used for mutagenesis analysis are listed in Table S1.

Determination of ecdysteriod titers

Hemolymph of WT and *BmTorso* mutants at wandering and pupal stages respectively were collected. The samples were extracted with methanol as previously described (Warren *et al*., 2006). The extracts were evaporated and re-dissolved in enzyme immunoassay (EIA) buffer (Cayman), and EIA kit (Cayman Chemical, MI, USA) as described before (Zeng *et al*., 2017). Anti-20-hydroxyecdysone EIA antiserum, acetylcholinesterase (AchE)-conjugated 20E, and standard 20E (Sigma) were used in competitive assay to quantify 20E titers. The AchE activity was quantified by Ellman's buffer and measured at 405 nm by using a Multiskan FC microplate photometer (Thermo).

Feeding rescue experiments

A piece of mulberry leaf (10 cm^2) was smeared with 400 μL of 20E (50 μg/mL). For rescue experiments, newly molted 5th instar (L5) larvae were first reared on fresh mulberry leaves without 20E solution. On day 4 of the 5th instar, they were transferred to the mulberry leaves smeared with 20E solution until spinning.

Immunoblot analysis

PG proteins from WT and *BmTorso* mutants at wandering stage were extracted by using TRIzol reagent (Invitrogen) according to the manufacturer's instructions and subsequently were quantified using a BCA kit (Thermo). Extracted proteins were separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Thermo). The polyclonal rabbit anti-phospho-ERK primary antibody was used to detect phospho-ERK (1 : 500 dilution; Cell Signaling Technology). And β-Actin detected by the rabbit anti-β-Actin primary antibody (1 : 5000 dilution; Vazyme Biotech) was used as the control. Horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (1 : 5000 dilution; Beyotime) was used as the secondary antibody. The ECL Plus Western Blotting Detection Kit (GE Healthcare) was applied to detect the protein signal.

RNA-Seq analysis

Total RNA were extracted from the PG dissected from WT and *BmTorso* mutants with the methods described above. The cDNA libraries were generated by using the Illumina TruSeq^{TM} RNA Sample Preparation Kit (Illumina) following the manufacturer's recommendations. The cDNA libraries were then sequenced using the Illumina HiSeq 2000 platform (BGI). The raw data were qualified, filtered and mapped to the silkworm genome database (http://www.silkdb.org/silkdb/). Differentially expressed genes (DEGs) between WT animals and *BmTorso* mutants were functionally annotated by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes.

Results

Generation of BmTorso mutant using the transgenic CRISPR/Cas9 system

To genetically assess *BmTorso* function, we employed the binary transgenic CRISPR/Cas9 system that has been described in our previous studies (Li *et al*., 2015). This system included two independent lines, which expressing Cas9 and gene-specific sgRNAs. The Cas9-expressed line was established as described (Xu

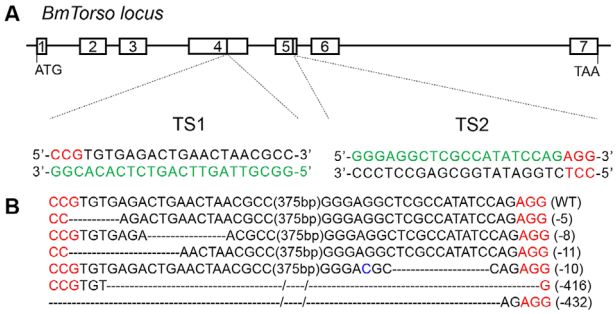


Fig. 1 CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/RNA-guided Cas9 nucleases) system mediated disruption of *BmTorso*. (A) Schematic diagram of the *BmTorso* locus structure and single guide (sgRNA)-targeted sites. Two sgRNA targeting sites, TS1 and TS2, are located at the anti-sense strand of exon 4 and the sense strand of exon 5, respectively. The sequences of sgRNA-targeted sites are in green. The red letters show the protospacer adjacent motif (PAM) sequences. (B) Sequence of mutations at TS1 and TS2 induced by CRISPR/Cas9. The dashes in every sequence line represent deleted residues and the detailed indel size is shown on the right. The base of point mutation is shown in blue.

et al., 2017). To establish the *BmTorso*-specific sgRNAs expressing lines, we identified two sgRNA targeting sites located on exons 4 and 5 of *BmTorso* (Fig. 1A) and constructed the transgenic plasmid *pBac[IE1-DsRed2-U6-BmTorso-sgRNAs]*. The *BmTorso*-specific sgRNA expressing lines (*BmTorso-sgNRAs*) were established by transposon-mediated germ line transformation.

nos-Cas9 and *BmTorso-sgNRAs* lines were both viable and fertile, which indicated that the expression of neither Cas9 nor *BmTorso*-specific sgRNAs had deleterious effects on silkworm physiology. In heterozygous *nos-cas9:BmTorso-sgRNAs* (△*Torso*) offspring, genomic deletions at the two target sites were detected in all examined animals by PCR analysis (Fig. 1B), demonstrating that this somatic mutagenesis system was effective.

Developmental delay in BmTorso mutants

The previous study revealed that homozygous mutants of PTTH showed severe delay in developmental timing in *B. mori* (Uchibori-Asano *et al*., 2017). To investigate whether Torso also affects the developmental timing, we investigated the life span of WT and △*Torso*. We found that the duration of each larval instar extended in △*Torso* animals (Fig. 2A). The time span in 1st larval instar of △*Torso* was not different from WT (Fig. 2A). The developmental time of the 2nd instar from △*Torso* increased ∼1 day compared to WT (Fig. 2A). Intriguingly, during the 3rd instar and 4th instar, the development time in △*Torso* larvae only increased ∼12 h compared to WT (Fig. 2A). However, during the last instar, the developmental time of △*Torso* larvae increased ∼5.5 d compared to WT (Fig. 2A). After pupation, the time to eclosion for △*Torso* increased ∼8.5 d compared to WT (Fig. 2A). These results suggest that the deletion of *BmTorso* induces extreme life cycle expansion from larval to pupal stages. Compared to WT, the pupae and cocoons of △*Torso* were both increased sharply (Fig. 2B). Male mutant whole cocoons were 167% heavier than WT (Fig. 2C). Female mutant whole cocoons were 162% heavier than WT (Fig. 2C). The male mutant cocoon ratio increased by 5.4% and female mutant cocoon ratio increased by 5.7% (Fig. 2D). As expected, the depletion of *BmTorso* resulted in larger adults (Fig. S2).

Ecdysone titers in BmTorso mutants

Since PTTH is the ligand of Torso, and PTTH is thought to be the primary factor regulating the production and release of ecdysone (Rewitz & O'Connor, 2011). Thus, we measured the ecdysteriod titers in the hemolymph of WT and △*Torso* larvae at the wandering stage. We found that the ecdysteriod titers in △*Torso* larvae decreased ∼60% at wandering stage (Fig. 3A). To investigate whether developmental delay of △*Torso* was the result of a defect in ecdysone biosynthesis, we fed 20E to △*Torso* larvae from day 4 of the 5th instar. As expected, feeding △*Torso* larvae with mulberry leaves smeared with 20E solution completely rescued the developmental delay (Fig. S1). These results indicate that reduced synthesis of ecdysone in the PG is responsible for an extensive delay in the onset of pupal metamorphosis in △*Torso* larvae, demonstrating that Torso modulates the timing of pupal metamorphosis in the last instar through the control of ecdysteriod titers.

The precise regulation of ecdysteriod is required for pupal–adult development (Yamanaka *et al*., 2013). The ecdysteriod titers reached the peak concentration 2 d after pupation in *B. mori* (Mizoguchi *et al*., 2001). Then the titers decreased to undetectable levels before eclosion. Thus, we hypothesized that the prolonged pupal stage of △*Torso* animals might be determined by abnormal ecdysteriod titers. Thus, we measured the ecdysteroid titers during the pupal stage and we found that ecsyteriod titers in the hemolymph collected from WT reached the peak concentration at P3 (Fig. 3B). However, the ecdysteriod titers of *BmTorso* mutants remained at a very low level from P1 to P9 (Fig. 3B). Taken together, these results demonstrate that deletion of *BmTorso* disrupts the

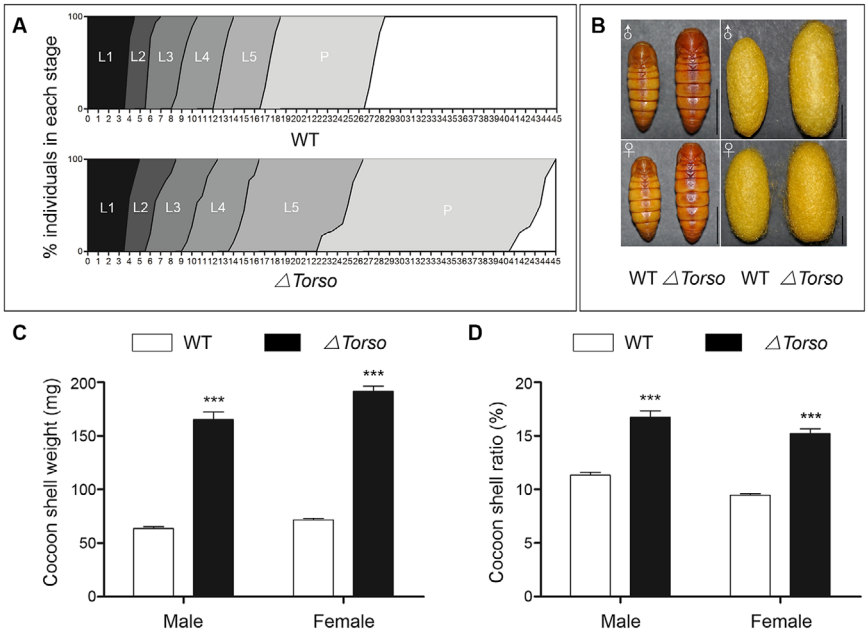


Fig. 2 Developmental delay and increased body size in △*Torso* animals. (A) The stages of larval and pupal development in WT (wild-type) and △*Torso* (L1, 1st instar; L2, 2nd instar; L3, 3rd instar; L4, 4th instar; L5, 5th instar; P, pupae). (B) The upper graph shows male pupae and cocoons for the indicated genotype. The down graph showed female pupae and cocoons for the indicated genotype. (C) The cocoon shell weight of WT and △*Torso*. (D) The cocoon shell ratio of WT and △*Torso*. The data are shown as the mean ± SEM (n = 30). ***$P < 0.001$ according to two-tailed t-test.

ecdysteriod titers, leading to an increase in the duration of larval and pupal stages. Moreover, western blotting using p-ERK antibody revealed a decrease in protein level in the △*Torso* larvae (Fig. S3), indicating that *BmTorso* knockout disrupts the MAPK/ERK pathway in the PG. Overall, *BmTorso* knockout disrupted ecdysone synthesis and caused developmental delay.

q-RT-PCR and RNA-seq analysis in BmTorso mutants

To investigate the reason for the low ecdysteriod titers in *BmTorso* mutants, we measured the expression of the ecdysteriod biosynthesis genes in the PG. mRNA levels of *nvd*, *spok*, *phm*, *sad*, and *dib* were analyzed by q-RT-PCR at the wandering stage. Compared to WT, the transcription levels of *nvd*, *spok*, *phm*, *sad*, and *dib* in △*Torso* animals were reduced to 34%, 10%, 16%, 10%, 16% and 16% respectively (Fig. 3C). We also investigated the transcription expression of *shd* in fat body, another member of the Halloween genes which converted ecdysone into 20E in the peripheral tissues. Interestingly, the relative mRNA level of *shd* up-regulated 1.6-fold (Fig. 3C).

We then investigated the mRNA expression of three 20E regulated genes: *E75A*, *E75B*, *HR3*. Significant down regulation of *E75A*, *E75B* and *HR3* were detected in △*Torso* animals (Fig. 3D). To further explore the molecular mechanisms of how *BmTorso* regulates the developmental timing, we performed RNA-Seq analysis by using PG at wandering stage between WT and △*Torso*. A total of 1699 DEGs (543 up-regulated and 1153 down-regulated) were identified. GO functional classification showed that DEGs were mainly involved in cellular and metabolic processes, and binding and catalytic activities (Fig. 4A). KEGG enrichment analysis revealed that longevity regulating pathway, protein processing in endoplasmic reticulum pathways and ABC transporters were in the top five affected pathways. (Fig. 4B) We further investigated the expression levels of genes known to play critical roles in longevity regulating pathway by using q-RT-PCR. Four positively regulated genes including *PI3K*, *AKT* and *S6K* were down-regulated to 80%, 87% and 60%, respectively (Fig. 4C). Two negatively regulated genes: *FOXO* and *4EBP* were up-regulated by 1.4 and 2.2-fold when compared to the levels in WT (Fig. 4C). Two key genes, *UGT* and *MANI*, of protein processing in endoplasmic reticulum pathway, were down-regulated to 63% and 53%, respectively. (Fig. 4C). Since Atet

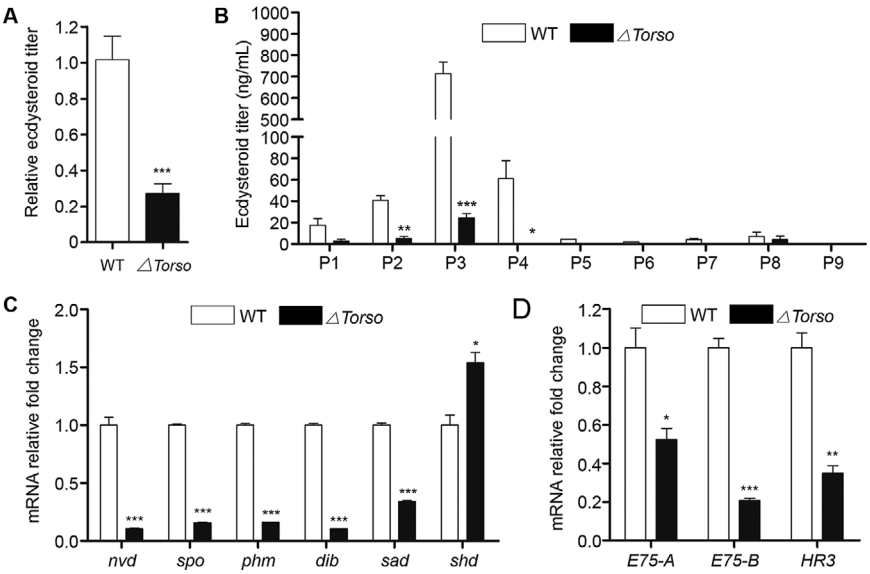


Fig. 3 The ecdysteroid titers and expression differences of ecdysone pathway genes in △*Torso* animals. (A) Relative ecdysteriod titers in the hemolymph at wandering stage of wild-type (WT) and △*Torso*. Hemolymph was collected from 10 larvae, and the pooled sample was used to determine ecdysteriod titers. (B) Hemolymph ecdysteroid titers of WT and △*Torso* during pupal stage. (C). Expression change of Halloween genes in prothoracic gland (PG) at wandering stage. (D) Expression change of ecdysone responsive genes in fat body (FB) at wandering stage. The data are shown as mean ± SEM ($n = 3$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ according to two-tailed *t*-test.

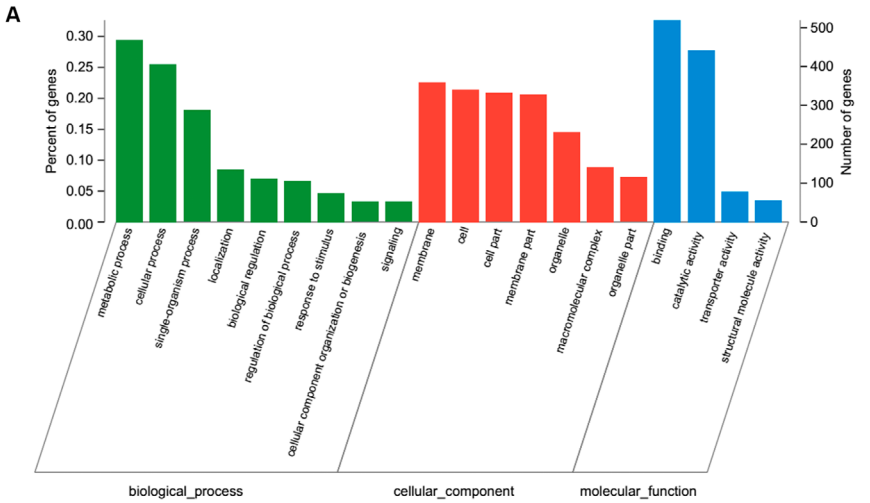
has been identified as the ABC transporter required for ecdysone secretion in the PG (Yamanaka *et al*, 2015), we detected the expression levels of two homologous genes of *Atet*. *Atet1* and *Atet2* were both significantly down-regulated (Fig. S4).

Recent study showed that ecdysone dose-dependently affects *PTTH* transcription, promoting its expression at lower concentrations and inhibiting it at higher concentrations (Christensen *et al*., 2020). The ecdysteriod titers in △*Torso* animals were dramatically reduced (Fig. 3A). Thus, we hypothesized that the expression levels of *PTTH* could be affected. As expected, the relative mRNA level of *PTTH* up-regulated 1.4-fold in △*Torso* animals (Fig. S4).

Discussion

Torso is a RTK that plays distinct roles in different developmental process. During embryonic development, activation of Torso is required for correct terminal pattern (Schoppmeier & Schroder, 2005). After embryonic development, Torso is predominantly expressed in PG, which acts as the receptor of PTTH. Torso mediates PTTH signaling through the activation of the ERK pathway and regulates ecdysone biosynthesis (Rewitz *et al*., 2009). Our data presented here indicated that Torso is required for ecdysone synthesis, and it plays a vital role in regulating developmental timing and body size in *B. mori*.

Previous study in *B. mori* showed that *PTTH* mutants showed a developmental delay during the larval and pupal stages (Uchibori-Asano *et al*., 2017). The developing timing of each larval stage showed significant difference among PTTH mutant individuals (Uchibori-Asano *et al*., 2017). The majority of *PTTH* mutants stopped developing at the second larval stage. Some larvae undergo precocious metamorphosis and pupating at the end of the 4th instar. The rest of the larvae could complete the five larval instars. The diversity of phenotypes emerged in *PTTH* mutants was different from the phenotypes observed in *BmTorso* mutants. The depletion of *BmTorso* showed also developmental delay throughout the larval and pupal stages. However, all *BmTorso* mutants undergo the five larval instars without developmental arrest or precocious metamorphosis. The phenotypic difference between *PTTH* homozygous mutants (Uchibori-Asano *et al*., 2017) and *BmTorso* mutants could be explained by: (1) different silkworm strains were used; (2) transcription activator-like effector nuclease-mediated mutagenesis was complete knock out while CRISPR/Cas9-mediated mutagenesis was incomplete; and (3) PTTH and



B

Pathway	Pathway ID	*P*-value
Insulin pathway	Map04213	0
Protein processing in endoplasmic reticulum	Map04141	0
Endocytosis	Map04144	0.0051
ABC transporters	Map02010	0.0076
Spliceosme	Map03040	0.0688

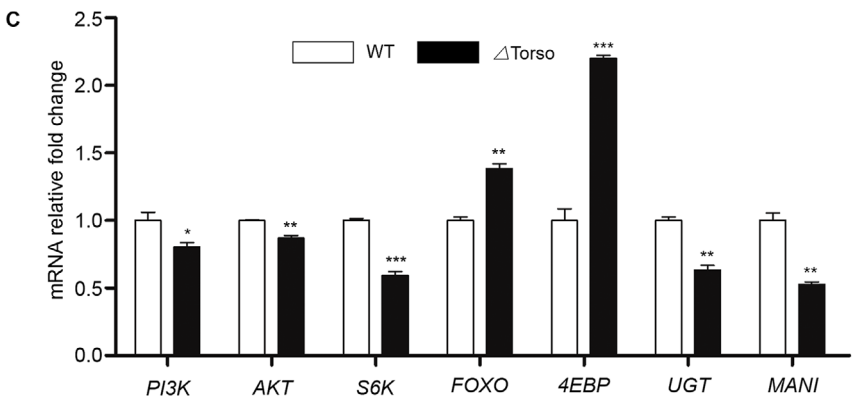


Fig. 4 RNA-Seq analysis reveals the differentially expressed genes (DEGs) between wild-type (WT) and △*Torso*. (A) Gene Ontology functional classification of the DEGs. (B) The top five enriched Kyoto Encyclopedia of Genes and Genomes pathways. (C) Quantitative real-time polymerase chain reaction validation for the genes associated with the regulation of longevity and protein processing signaling pathway. The data are shown as mean ± SEM ($n = 3$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ according to two-tailed *t*-test.

its receptor Torso function differently in the PTTH signaling pathway. In addition, the high levels of PTTH were detected in △*Torso* animals (Fig. S4), supporting the idea that PTTH exerts an additional role independent of Torso.

An intriguing finding in the present study is that the life cycle was extremely extended in *BmTorso* mutants. The larval growth period of *BmTorso* mutants was extended ~5.5 days. In addition, the mutants took ~8.5 days longer to complete the pupal stage. And final body size was also increased. Low ecdysone synthesis by PG can delay developmental timing and induce overgrowth in insects (Colombani *et al*., 2005). We hypothesized, therefore, that the low ecdysteriod titers due to *BmTorso* depletion is likely responsible for this phenotype. In order to assess if Torso can regulate developmental timing and body size by reducing the production of ecdysone in *B. mori*, we measured the ecdysteriod titers of hemolymph at the wandering stage and pupal stage. As expected, the ecdysteriod titers were declined sharply in *BmTorso* mutants. The mRNA levels of ecdysone biosynthetic genes were

all down-regulated in △*Torso* animals. However, p-ERK levels were only reduced slightly in △*Torso* PG. This is in agreement with previous experiments in *Drosophila* (Rewitz *et al*., 2009), which demonstrate that other signals might be responsible of ERK phosphorylation. A recent study shown that the epidermal growth factor receptor (EGFR) signaling is the major signaling that induced the phosphorylation of ERK and regulated ecdysone biosynthesis (Cruz *et al*., 2020). Taken together, these results showed that Torso signaling might act synergistically only to increase the ERK pathway activity and regulate developmental timing in *B. mori*.

RNA-Seq and subsequent q-RT-PCR analysis revealed that expressions of genes in the longevity pathway were inactivated in △*Torso* animals, suggesting that low ecdysteriod titers in △*Torso* could negatively modulate the longevity pathway. As a result, the transcription level of positively and negatively regulated genes were down-regulated and up-regulated in PG, respectively. Previous studies showed that PG was not only a tissue producing ecdysone, but also a size-assessing tissue by using longevity dependent PG cell growth to determine the final body size in insects (Mirth *et al*., 2005). The impaired longevity pathway only in PG resulted in low ecdysteriod titers and increased body size. Our data revealed that ecdysteriod titers could in turn control body size by regulating the longevity signaling, especially in PG. The present study thus provides the first genetic evidence that *BmTorso* affects the ecdysone synthesis to regulate growth and development in the silkworm.

Acknowledgments

This work was supported by grants from National Postdoctoral Program for Innovative Talents Grant (BX20190350) and China Postdoctoral Science Foundation Grant (2019M661654).

Disclosure

The authors declare no conflict of interest.

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Manuscript received June 21, 2020
Final version received September 11, 2020
Accepted September 24, 2020

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1 20-hydroxyecdysone (20E)-feeding experiments.

Fig. S2 Photos of adults of wild-type (WT) and △*Torso*. Scale bars: 5 mm.

Fig. S3 Immunoblot analysis of phospho-extracellular signal-regulated kinase (p-ERK) protein.

Fig. S4 Messenger RNA (mRNA) expressions of *PTTH* and genes involved in ecdysteroid transport pathway.

Table S1 Oligonucleotide primers used in this study.