Transcriptional and post-translational activation of AMPK α by oxidative, heat, and cold stresses in the red flour beetle, *Tribolium castaneum*

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Received: 25 April 2019 / Revised: 16 July 2019 / Accepted: 2 August 2019 / Published online: 10 August 2019
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Abstract

The AMP-activated protein kinase (AMPK) has important roles in the regulation of energy metabolism, and AMPK activity and its regulation have been the focus of relevant investigations. However, functional characterization of AMPK is still limited in insects. In this study, the full-length cDNA coding AMPK α (TcAMPK α) was isolated from the red flour beetle, *Tribolium castaneum*. The TcAMPK α gene contains an ORF of 1581 bp encoding a protein of 526 amino acid residues, which shared conserved domain structure with *Drosophila melanogaster* and mammalian orthologs. Exposure of female adults to oxidative, heat, and cold stresses caused an increase in TcAMPK α mRNA expression levels and phosphorylation of Thr-173 in the activation loop. The RNAi-mediated knockdown of TcAMPK α resulted in the increased sensitivity of *T. castaneum* to oxidative, heat, and cold stresses. These results suggest that stress signals regulate TcAMPK α activity, and TcAMPK α plays an important role in enabling protective mechanisms and processes that confer resistance to environmental stress.

Keywords *Tribolium castaneum* · AMPK · Stress resistance · Phosphorylation · RNAi

Introduction

Effective regulation of energy metabolism is essential for cellular homeostasis. The adenosine 5'-monophosphate-activated protein kinase (AMPK) is a crucial cellular energy sensor and is evolutionarily conserved across all eukaryotic species including yeast (sucrose nonfermenting1, SNF1), *Caenorhabditis elegans* (AAK-2), and plants (Snf1-related kinase1, SnRK1) (Hardie 2007; Polge and Thomas 2007). The mammalian heterotrimeric AMPK complex consists of a catalytic subunit α and two regulatory subunits, β and γ , each subunit is encoded by multiple genes (α 1, α 2, β 1, β 2, γ 1, γ 2, γ 3), and as many as 12 possible AMPK complexes are generated by different combinations of these isoforms.

However, in *Drosophila melanogaster*, each subunit is encoded by a single gene (Pan and Hardie 2002), which makes the fruit fly an appealing organism for elucidating the functional deficiency of AMPK on signaling pathways and phenotypes.

AMPK is activated in response to energy stress by sensing increases in AMP/ATP and ADP/ATP ratios, which leads to the activation of catabolic pathways that produce ATP and the inhibition of anabolic pathways that consume ATP (Hardie 2007; Rider 2016). This allosteric activation of AMPK is regulated by phosphorylation at Thr172 in the activation loop of the catalytic α -subunit in mammals. Stress factors that activate AMPK can be divided into two categories including physiological stresses, such as starvation, exercise, hypoxia, and ischemia, and environmental stresses, such as heat shock, cold environment, oxidative stress, sodium ion stress, and alkaline pH (Hardie et al. 1994; Wilson et al. 1996; Rasmussen and Winder 1997; Choi et al. 2001; Fisher et al. 2002; Platara et al. 2006; Rider et al. 2011). In *D. melanogaster*, treatment under hypoxic conditions caused a 2-fold increase in DmAMPK activity after 2 and 4 h (Pan and Hardie 2002). Additionally, inhibition of *dAMPK α* in muscle enhances sensitivity to

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12192-019-01030-x>) contains supplementary material, which is available to authorized users.

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Table 1 Oligonucleotide primers used for RT-PCR, RACE, and RT-qPCR

Primer name	Sequence (5' to 3')	Description
TcAMPK α F1	TGTGGGCAGAAGCCAATGAG	TcAMPK α RT-PCR
TcAMPK α R1	GGATTCTTATGACGCACTCT	
TcAMPK α R2	GATACAGCTTGATAATATGCGGGTG	TcAMPK α 5'-RACE
TcAMPK α R3	CGAAACAGCTTCAAGTTCTGAATCT	
TcAMPK α F2	TTAGTATGAGCCCGAGCCCAAGTCC	TcAMPK α 3'-RACE
TcAMPK α F3	GGGAAGAGTTAAGAGGGCTA AGTGG	
TcAMPK α F4	ACATCCTTGGCCAGACTTTG	TcAMPK α RT-qPCR
TcAMPK α R4	GCCGAAACAGCTTCAAGTTC	
Trps3F1	ACCGTCGTATTCGTGAATTGAC	rps3 RT-qPCR
Trps3R1	ACCTCGATACACCATAGCAAGC	
TcAMPK α iF1	GTTTCGGGGGTTGATTACTGCC	TcAMPK α dsRNA
TcAMPK α iR2	GGACTTCCTTCTCCTGCACCC	

paraquat and starvation stress (Tohyama and Yamaguchi, 2010). Nevertheless, the involvement of AMPK in response to environmental stresses in other insects is still unclear.

In this paper, we have cloned and characterized AMPK α -subunit gene, *TcAMPK α* , in the red flour beetle, *Tribolium castaneum*. The roles of *TcAMPK α* in response to oxidative, heat, and cold stresses were studied. We also examined the effects of RNA interference (RNAi)-mediated knockdown of *TcAMPK α* on stress resistance.

Materials and methods

Experimental insects

The Georgia-1 (GA-1) strain of *T. castaneum* was reared at 30 °C and 50% relative humidity in 5% yeasted flour under standard conditions (Haliscak and Beeman 1983; Li et al. 2011).

Total RNA isolation and reverse transcription

Total RNAs were extracted from the whole body of insects at various developmental stages (such as the eggs, larvae, pupae, and adults) or specific tissues (including ovary, thorax, integument, head, midgut, malpighian tubule, and fat body) of 7-day-old female adults using TRIZOL[®] Reagent (Invitrogen, Waltham, MA, USA). First-strand cDNA was synthesized from total RNA using the Primescript[™] First-Strand cDNA Synthesis kit (TaKaRa, Dalian, China), following the manufacturer's instructions.

Polymerase chain reaction and RACE

The amino acid sequence of *D. melanogaster* AMPK α (GenBank: NP_477313) was searched against the whole

genome shotgun database (WGS) of *T. castaneum* in NCBI (<https://www.ncbi.nlm.nih.gov/>), and the region with the best match was manually annotated to identify the putative transcript. Specific primer pairs were designed based on the identified sequence (Table 1). PCR reactions were performed with LA Taq[™] DNA polymerase (TaKaRa, Dalian, China). To complete the cDNA sequences of *TcAMPK α* , 5'-RACE and 3'-RACE reactions were performed using the SMART RACE cDNA Amplification Kit (Clontech, Mountain View, CA, USA), following the manufacturer's instructions.

Reverse transcription quantitative PCR

Reverse transcription quantitative PCR (RT-qPCR) reactions were performed on the Bio-Rad CFX 96 Real-time PCR system using TB Green[™] Premix Ex Taq[™] (Takara, Dalian, China) and gene specific primers (Table 1). The stably expressed gene encoding ribosomal protein S3 (rps3, GenBank: CB335975) was used as a reference gene (Arakane et al. 2008). PCR conditions were set as an initial incubation of 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 30 s, and a final melting curve analysis was performed. The mRNA levels were normalized to reference gene with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The means and standard errors for each time point were obtained from the average of at least three biologically independent sample sets.

RNA interference

Gene specific primers (Table 1) with T7 promoter were designed to synthesize the dsRNAs targeting nucleotides 844–1285 (442 bp) of the ORF region of the *TcAMPK α* using TranscriptAid[™] T7 High Yield Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The synthesized dsRNAs were

Fig. 1 Amino acid sequence alignment of TcAMPKα and representative AMPKαs from *Dastarcus helophoroides* (DhAMPKα, GenBank: ANT47199), *Drosophila melanogaster* (DmAMPKα, GenBank: NP_477313), *Bombyx mori* (BmAMPKα, GenBank: ABQ62953), and *Homo sapiens* (HsAMPKα, GenBank: AAA64745). Identical residues in the sequences are in black and similar residues are in gray. The

conserved domains including the activation loop, AIS, α-RIM, NLS, and NES motif are indicated by a solid line below the alignment and the conserved phosphorylation site of Thr172 is indicated by "▼" above the alignment. Besides, the conserved auto inhibitory domain is indicated by a dotted line below the alignment

diluted in DEPC water with a concentration of 2 μg/μL, and about 200 ng of dsRNA in 200 nL injection buffer was injected into 3-day-old pupae using a Nanoliter 2010 injector system (WPI, Sarasota, FL, USA) under a stereomicroscope. The 4-day-old female adults were used to determine the suppression of TcAMPKα transcripts by RT-qPCR as described for gene expression analysis. The buffer-injected larvae (IB group) and the uninjected wild-type larvae (CK group) were set as controls in all injection experiments. Three biologically independent replicates were carried out with at least 30 insects in each replicate.

Effect of dsTcAMPKα on survival ability of *T. castaneum*

Because the adult stage of *T. castaneum* can last for months or even more than a year, a 30-day period was used to compare the adult survival rate across different treatments (Sang et al. 2012). When the dsTcAMPKα-treated pupae entered adulthood, adults were transferred to Petri dishes with food and the daily mortality was counted within 1 month to evaluate the effect of dsTcAMPKα on survival ability of *T. castaneum* under normal conditions. Three biologically independent

Fig. 2 Phylogenetic analysis of AMPK α from *T. castaneum* and other insects and mammals. The phylogenetic tree was constructed by MEGA7 using the neighbor-joining method with 1000 bootstrap replicates

replicates were carried out with at least 30 insects in each replicate.

Stress assays

Two kinds of stress assays were conducted in this study, including stress-induced expression assay and stress tolerance assay. In stress-induced expression assay, the 2-day-old female adults were divided into several groups for *TcAMPK α* expression analysis under various environmental stresses. In the heat and cold stress treatment groups, 50 female adults for

each group were reared at 45 and 4 °C condition, respectively. To induce in vivo oxidative stress, the adult individuals were starved for 12 h and then treated with 20 mM paraquat in 1% sucrose. Three insects for each time point were collected after the insects were treated for 0, 1, 2, 4, and 12 h, respectively. In total, three biological replications were carried out for these experiments, and the expression levels of *TcAMPK α* were measured by RT-qPCR.

The treatments in stress tolerance assay were the same as those in stress-induced expression assay, except that ds*TcAMPK α* -treated insects were used, and survival rates

Fig. 3 Schematic diagrams of the genomic organization for *TcAMPK α* and other insect homologs

Fig. 4 The expression patterns of *TcAMPK α* in different developmental stages (a) and various tissues of 7-day-old female adults (b). 1-E, 1-day-old eggs; 1-L, 1-day-old larvae; 10-L, 10-day-old larvae; 20-L, 20-day-old larvae; 1-P, 1-day-old pupae; 5-P, five-day-old pupae; 1-M, 1-day-old

males; 7-M, 7-day-old males; 1-F, 1-day-old females; 7-F, 7-day-old females. Bars not sharing a common letter are significantly different ($p < 0.05$)

were investigated in 24 h for paraquat treatment, and every 12 h for heat and cold treatments until all of the insects were dead. The IB group and WT group were set as controls. Three biological replications were carried out for the experiments, and 20 beetles were used in each replicate treatment.

Western blot analysis

Total protein was extracted using tissue protein extraction reagent (ComWin Biotech, Beijing, China), and the protein concentration was determined using BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's protocol. Equal amounts of protein samples (50 μ g) were subjected to SDS-PAGE (8% polyacrylamide), and transferred onto polyvinylidene fluoride membrane (Bio-Rad, Hercules, CA, USA), followed by membrane staining using Ponceau S to visualize transfer result. Discolored membrane was incubated at room temperature for 1 h in Tris-buffered saline with Tween (TBST) containing 5% BSA blocking buffer and then at 4 °C overnight in either anti-AMPK α (Abcam, Cambridge, UK, catalogue number ab80039) or phospho-specific anti-AMPK α Thr-172 primary antibodies (Cell Signaling Technology, Danvers, MA, USA, catalogue number 2535) at 1:1000 dilution in TBS containing 1% BSA and 0.1% Tween-20. Hybridized primary antibody was detected using HRP-conjugated goat anti-rabbit IgG (Bio-Rad, CA, USA, catalogue number 170-6515) at a 1:5000 dilutions in TBS containing 1% non-fat dry milk and 0.1% Tween-20. Hybridized secondary antibody was detected by using ECL reagents and ChemiDoc system (Bio-Rad, CA, USA). α -Tubulin mouse monoclonal antibody (HuaBio, Hangzhou, Zhejiang, China, catalogue number M1501-1)

was used as a loading control. The blots were quantified with Image Lab™ software (Bio-Rad, Hercules, CA, USA) and normalized to the corresponding controls. All western blot analyses were repeated at least three times.

Statistical analysis

The data analysis was done using GraphPad Prism 6 by one-way analysis of variance, followed by Tukey's Honestly Significant Difference test. All data are presented as the mean $\pm$ SE.

Results

cDNA cloning and characterization of *TcAMPK α* in *T. castaneum*

The full-length cDNA sequence of *TcAMPK α* was amplified by RT-PCR and RACE. The *TcAMPK α* cDNA comprises 1962 bp with a 126-bp 5'-untranslated region (UTR), 1581 bp of ORF encoding a 526-amino acid residue protein with molecular mass of 59.49 kDa and an isoelectric point of 7.68, and a 255-bp 3'-UTR (GenBank accession no. MK543269, supplementary Fig. 1). An amino acid sequence alignment shows that *TcAMPK α* shares 89.6, 78.2, 71.5, and 62.9% identities with DhAMPK α in *Dastarcus helophoroides*, BmAMPK α in *Bombyx mori*, DmAMPK α in *D. melanogaster*, and HsAMPK α in *Homo sapiens*, respectively (Fig. 1). The phylogenetic tree constructed using the neighbor-joining method cleared showed that *TcAMPK α* was clustered with other coleopteran insect homologs (Fig. 2).

Fig. 5 RNA interference (RNAi) of *TcAMPKα*. **a** Expressions of *TcAMPKα* transcripts in the uninjected wild-type larvae (WT group), the buffer-injected larvae (IB group), and the dsRNA injected group. **b** Representative western blots showing the protein expression and phosphorylation levels of *TcAMPKα* in WT, IB, and the dsRNA injected groups. α -Tubulin was used as a loading control. **c** Densitometric

quantification of protein abundance of AMPK α (t-AMPK α) relative to α -tubulin. **d** Densitometric quantification of the ratios of phospho-AMPK (p-AMPK α) to α -tubulin. **e** Injection of dsTcAMPK α resulted in defective pupal-adult metamorphosis. **f** Average eclosion rates in WT, IB, and the dsRNA-injected group. Each bar represents the mean $\pm$ SE ($n = 3$) of the ratio for three independent experiments (by Student's *t* test, * $p < 0.05$)

The sequence alignments also revealed the conserved domain structure in *TcAMPKα* and AMPK α from other species (Fig. 1). The kinase domain was located in the amino-terminal half of *TcAMPKα* with a recognized activation loop structure (ADFGLSNMMSDGEFLRTSCGSPN). The activation loop contains a conservative phosphorylation site (*T. castaneum* Thr-173; *D. melanogaster* Thr-184; Human Thr-172) that plays a key role in the kinase catalytic activity of AMPK (Hawley et al. 1996; Pan and Hardie 2002). A β -subunit interaction domain (β -SID) was located in the carboxy-terminal half of *TcAMPKα*, and a NLS (nuclear localization signal) motif (Lys-(Lys/Arg)-X-(Lys/Arg)) was identified on the β -SID (Hodel et al. 2001; Suzuki et al. 2007). The carboxy-

terminus 20 amino acids of *TcAMPKα* was a NES (nuclear export signal) motif due to its closely matching sequences with leucine-rich CRM1-dependent NESs (Kazgan et al. 2010). Between the kinase domain and β -SID was a regulatory region comprising the auto inhibitory domain (AID) and a α -RIM structure which plays a key role in allosteric regulation of AMPK (Chen et al. 2013).

The genomic structure of *TcAMPKα* was predicted by comparing the cDNA sequence with the genomic sequence retrieved from contigs in the whole genome shotgun release for *T. castaneum*. The result showed that the coding region of *TcAMPKα* contains three exons connected by two introns (Fig. 3). Further analysis showed that the number, location,

Fig. 6 Effects of dsTcAMPK α treatment on the survival curve (a) and average survival time (b) within 1 month among WT, IB, and the dsRNA-injected groups (by Student's *t* test, **p* < 0.05)

and length of introns varied in different insects, indicating the diversities of AMPK α gene structure among different insects (Fig. 3).

The temporal and spatial expression patterns of TcAMPK α

The temporal expression patterns of TcAMPK α were analyzed using RT-qPCR. The result showed that TcAMPK α was expressed at all developmental stages tested. The mRNA level of TcAMPK α was the highest in the 1-day-old males, and the 10-day-old larvae exhibited the lowest mRNA level of TcAMPK α (Fig. 4a). The determination of spatial expression patterns showed that TcAMPK α was predominantly expressed in the head and thorax (Fig. 4b).

RNAi phenotypes of TcAMPK α

Injection of dsTcAMPK α during the pupal stage resulted in suppression of transcript level of TcAMPK α by $95.50 \pm 1.86\%$

(Fig. 5a) and significantly reduced protein amount as well as phosphorylation of TcAMPK α (Fig. 5b–d). Defects in pupal-adult metamorphosis were observed in dsTcAMPK α -treated insects with the wings spreading to the dorsal side and the pupal cuticles attached to the end of the body (Fig. 5e), and approximately $38.33 \pm 10.14\%$ pupae failed to eclose (Fig. 5f).

RNAi of TcAMPK α reduced survival rate of *T. castaneum*

Due to the adult stage of *T. castaneum* can last for months or even more than a year, we used a period of 30-day following pupal RNAi to assess the effect of dsTcAMPK α on adult survival. All tested adults were viable and no visible phenotype was observed. The results showed that knock down of TcAMPK α significantly decreased the survival rate within 1 month (approximately $32.24 \pm 0.12\%$ reduction compared with controls) of *T. castaneum* (Fig. 6).

Response of TcAMPK α to oxidative, heat, and cold stress

The mRNA transcript levels of TcAMPK α under various environmental stresses were quantified using RT-qPCR. The results showed that, after paraquat treatment, the mRNA expression of TcAMPK α significantly increased and reached a peak after 2 h, which was 25.15 ± 3.37 -fold higher than the control (Fig. 7). Significant increase of mRNA expression of TcAMPK α was also observed after heat and cold stress treatment (Fig. 7). In agreement with increased mRNA expression level, paraquat, heat, and cold treatment resulted in the increase of protein abundance (Fig. 8a, b).

We next determined the TcAMPK activity by measuring the amount of phosphorylation at TcAMPK α Thr173 (p-AMPK α) under different stresses. The results showed that paraquat, heat, and cold stresses significantly increased p-

Fig. 7 The mRNA expression patterns of TcAMPK α during oxidative, heat, and cold stresses. *Tribolium* ribosomal protein S3 (*rps3*) transcript with the same complementary DNA (cDNA) template served as an internal control. Asterisks indicate significant differences compared to the respective controls (by Student's *t* test, ***p* < 0.001)

Fig. 8 Oxidative, heat, and cold stresses stimulated the phosphorylation of the activation loop threonine (Thr173) of TcAMPKα in *T. castaneum*. **a** Representative western blots showing the phosphorylation and total TcAMPKα protein levels (p-AMPKα and t-AMPKα) during different stresses. α-Tubulin was used as a loading control. **b** Densitometric quantification of the ratios of total-AMPKα (t-AMPKα) to α-tubulin from

western blot signals. **c** Densitometric quantification of the ratios of phospho-AMPK (p-AMPKα) to total-AMPK (t-AMPKα) from western blot signals. Each bar in panels represents the mean ± SE ($n = 3$) of the ratio for three independent experiments (by Student's *t* test, * $p < 0.05$; ** $p < 0.001$)

AMPKα levels relative to the amount of AMPKα (p-AMPKα/t-AMPKα) with the peak observed after 1 h (gray-scale value 3.76 ± 0.83 -fold), 4 h (9.95 ± 3.46 -fold), and 2 h (1.76 ± 0.12 -fold) of treatment, respectively, when compared with the control (Fig. 8c). Collectively, these results indicated that environmental stresses increased TcAMPK activity levels through phosphorylation.

RNAi of *TcAMPKα* increased sensitivity to stress treatment

The involvement of *TcAMPKα* in the response to environmental stresses was further tested through RNAi. The results showed that, after exposure to oxidative stress-inducing 20 mM paraquat for 24 h, the survival rate of dsTcAMPKα group was $30.95 \pm 2.38\%$, which was significantly lower than the WT ($57.14 \pm 7.14\%$) and IB ($61.90 \pm 6.30\%$) groups (supplementary Fig. 2). Similarly, after heat and cold treatment, the survivorship of dsTcAMPKα-treated adults was much lower than WT and IB groups. The adults from dsTcAMPKα group could only survive for a mean ± SE of 26.00 ± 2.00 and 155.33 ± 2.91 h at 45 and 4 °C, respectively, which were much shorter than the WT group (55.00 ± 1.53 ;

242.67 ± 8.19 h) and IB group (54.83 ± 1.96 ; 222.00 ± 14.74 h) (Fig. 9).

Discussion

AMPK is a highly conserved protein kinase among most eukaryotes. As an energy sensor, AMPK plays an important role in maintaining the energy homeostasis at the cellular and whole body levels and in response to metabolic stress (Hardie et al. 1998; Kahn et al. 2005). While AMPK has been extensively studied in mammals, characterization of insect AMPK is still limited. In this study, the full-length catalytic subunit of AMPK in *T. castaneum*, *TcAMPKα*, was cloned for the first time. Sequence analysis revealed that TcAMPKα shares the similar structure with other insect and mammalian orthologs including the kinase domain to activate AMPK and the regulatory region to play a critical role in allosteric regulation of AMPK as well as the β-SID to link with β-subunit. In addition, the NES motif on the terminal of β-SID and NLS motif on the regulatory region were identified in TcAMPKα, indicating that TcAMPKα may travel back and forth between nucleus and cytoplasm in response to the upstream signal and

Fig. 9 Effects of heat (**a**) and cold (**b**) treatments on the survival curve and average survival time in WT, IB, and the dsRNA-injected groups (by Student's *t* test, ***p* < 0.001)

regulate the downstream targets (Leff 2003). Interestingly, while two NLS motifs were found in human AMPK α 2 (Suzuki et al. 2007), only one NLS motif was identified in TcAMPK α .

Besides its roles in energy homeostasis and metabolic regulation, AMPK is also involved in the response to environmental stresses (Crozet et al. 2014). In this study, we found that the oxidative, heat, and cold stresses could activate TcAMPK α not only at the mRNA level but also at the phosphorylation level, indicating the regulation of TcAMPK α activity by stress signals. Similar results have been reported in other organisms. For example, AMPK activity was increased under heat stress in rat primary hepatocytes (Corton et al. 1994). The mRNA expressions of AMPK α 1 and protein expressions of p-AMPK α^{Thr172} were also up-regulated in the small intestine epithelium cells after 7 or 14 days of heat exposure (He et al. 2018). In *Saccharomyces cerevisiae*, exposure of cells to oxidative stress caused an increase in Snf1 catalytic activity and phosphorylation of Thr210 (Hong and Carlson 2007). A recent study revealed that the phosphorylation levels of AMPK were significantly enhanced under oxidative stress induced by natural pyrethrins treatment in the HepG2 cells (Yang et al. 2018). On the contrary, heat stress inhibited AMPK activity by dephosphorylation of AMPK α in human and rodent cells (Kodiha et al. 2007; Wang et al. 2010). Down-regulation of AMPK/aak-2 was also observed during

oxidative stress induced by tert-butyl hydroperoxide (TBHP) in HEK293T cells and *C. elegans* (Kosztelnik et al. 2019). The opposite AMPK response to environmental stresses in different species suggested the functional divergence of AMPK despite the cross-species structural conservation.

The regulation of TcAMPK α activity by stress signals indicated the role of TcAMPK α in stress resistance, which was confirmed by functional analysis using RNAi. The dsTcAMPK α treatment resulted in significant increase of mortalities of female adults exposed to oxidative, heat, and cold stresses. This result was consistent with the result from yeast in which *snf1* null mutant cultures showed reduced thermo tolerance in the stationary phase (Thompson-Jaeger et al. 1991). Similarly, inhibition of dAMPK α in muscle significantly increased sensitivity to paraquat in female flies (Tohyama and Yamaguchi 2010). In cultured osteoblastoma MG63 cells, inhibition of AMPK by its inhibitor (compound C) or by shRNA-mediated knockdown dramatically enhanced H₂O₂-induced cell apoptosis (She et al. 2014). The involvement of AMPK in stress resistance might be due to the impact of AMPK on phosphorylation of transcriptional factor and the mRNA expression of stress-related genes, as has been reported that under low glucose conditions, SNF1 complex phosphorylates Hsf1 (heat shock factor 1 transcriptional factor) to response the heat shock stress signal (Hahn and Thiele 2004), and genetic deletion of the AMPK α 1 gene in mice resulted in

the down-regulation of the expression of antioxidant enzymes including Sod2, catalase, and glutathione peroxidase (GPx-1) (Wang et al. 2010). Further research is needed to determine the role of *TcAMPK* in the regulation of stress-related genes.

Author contributions JW conceived and designed research. HJ, NZ, MC, CJ, HG, and XX conducted experiments. XM, FD, LM, XY, and KQ analyzed data. HJ and JW wrote the manuscript. All authors read and approved the manuscript.

Funding information This work was supported by the National Natural Science Foundation of China under grant nos. 31572000 and 31871974.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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