Biochemical and Biophysical Research Communications 394 (2010) 112–118

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A critical role of *SNF1A/dAMPKα* (*Drosophila AMP-activated protein kinase α*) in muscle on longevity and stress resistance in *Drosophila melanogaster*

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ARTICLE INFO

Article history:
Received 10 February 2010
Available online 23 February 2010

Keywords:
AMPK
Longevity
Stress resistance
Drosophila melanogaster

ABSTRACT

Energy homeostasis and stress resistance are closely linked on aging and longevity. AMPK (AMP-activated protein kinase) is a sensor of cellular energy status activated by metabolic stress that accelerates AMP/ATP ratio, regulating cell polarity, metabolic homeostasis and sensitivity to stress resistance. AMPK could be therapeutic targets for cancer, diabetic mellitus and obesity, providing a possible link to metabolic syndrome. However, little is known how functional deficiency of AMPK affects longevity and stress resistance *in vivo* due to its redundancy and lethality in null-mutant. *SNF1A/dAMPKα* (*CG3051*) is a single orthologue for its mammalian counterparts in *Drosophila melanogaster*. Using time- and tissue-specific RNAi system in *D. melanogaster*, we found that adult-onset inhibition of *dAMPKα* especially in muscle shortens lifespan. In addition, inhibition of *dAMPKα* in muscle enhances sensitivity to paraquat and starvation stress. Real-time PCR analysis showed that inhibition of *dAMPKα* in muscle affected the transcriptional regulation of various genes in response to starvation. These results raise the possibility that muscle is one of major tissues in which AMPK plays a critical role on longevity and stress resistance and the intervention to activate AMPK in muscle could be a prominent treatment strategy for longevity.

1. Introduction

AMPK (AMP-activated protein kinase) is AMP-activated serine/threonine kinase conserved across species, playing a crucial role in systemic and cellular energy homeostasis through balancing anabolism and catabolism. AMPK is a heterotrimeric enzyme complex consisting of a catalytic subunit α and two regulatory subunits β and γ, and exerts functions as an energy gauge through modulating downstream effectors via phosphorylation in response to AMP/ATP ratio [1]. The three subunits of the kinase have a similar domain structure in all eukaryotes [2]. Since each subunit has numerous variants that are possibly redundant [3], little is known how AMPK affects longevity and stress resistance in whole organism. *Drosophila melanogaster SNF1A/dAMPKα* (*CG3051*), a catalytic subunit of *dAMPK*, is a single orthologue for its mammalian and yeast counterparts [4]. Deletion of this gene in *D. melanogaster* results in lethality with severe abnormalities in cell polarity and mitosis [4].

Excess nutrients could lead to metabolic diseases such as obesity and diabetes, resulting in shorter lifespan [5]. Recently AMPK is recognized as a therapeutic target for these diseases and called "metabolic master switch", influencing various metabolic pathways including carbohydrate metabolism, lipid metabolism and protein synthesis [6].

Metabolic changes often influence lifespan in model organisms such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *D. melanogaster* and rodents. For instance dietary restriction (DR), which is restriction of food intake without malfunction, extends lifespan in these model organisms [7–10]. Inhibition of the TOR pathway by genetic or pharmacological intervention extends lifespan across species including mouse [11,12]. Further inhibition of mRNA translation which leads to suppression of global protein synthesis shows lifespan extension in *C. elegans* [13,14].

Accumulating evidences have revealed a strong correlation between lifespan extension and resistance to environmental stress [15]. While most genetic mutations that extend lifespan confer stress resistance, many genetic mutations that enhance stress resistance result in lifespan extension [16]. Environmental stressor such as a high-temperature pulse that increases the AMP/ATP ratio extends lifespan in *C. elegans* [17]. *aak-2*, one of *C. elegans AMPKα* homologs, functions to mediate the lifespan extension of *daf-2/Insulin-like receptor* mutants in parallel to *daf-16/FOXO*, conserved forkhead transcription factor involved in aging and longevity [17]. However, it remains obscure whether AMPK affects longevity across species or which tissues are important for AMPK on longevity and stress resistance *in vivo*. To circumvent the lethality of null-mutant in *D. melanogaster* [4], we examined the spatiotemporal influences of RNAi-mediated knock-down for *dAMPKα* on lifespan and stress resistance using well-established GAL4/UAS system [18] and spatiotemporal targeting gene expression system GeneSwitch [19].

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doi:10.1016/j.bbrc.2010.02.126

2. Materials and methods

2.1. Drosophila strains and maintenance

w^{1118}, *C564-GAL4(w[1118];P{w[+mW.hs]=GawB}c564)*, *da-GAL4 (P{GAL4-da.G32}UH1)*, *elav-GAL4(P{GAL4-elav.L}2)*, *24B-GAL4(P{w+ mW.hs=GawB}24B)*, *Act5c-GS(P{Act5C(-FRT)GAL4.Switch.PR}3)*, *S106-GS (P{Switch1}106)* were all obtained from Drosophila Stock Center (Bloomington, IN). *Drosophila* transgenic RNAi line for *CG3051* (*UAS-dAMPKα^{RNAi}*) was obtained from Vienne Drosophila Resource Center (Transformant ID #1827). *MHC-GS* and *elav-GS* line were kindly provided by Dr. Haig Keshishian (Yale University). The *Drosophila* strain *w^{1118}* was used in all control crosses and as the background for generation of transgenic lines. All flies were raised on a standard mixture of agar, cornmeal, sucrose and yeast at 25 °C.

2.2. Lifespan assay

Crosses were set up between 10 virgin females and 20 males of various strains. Progeny were collected for 2–4 days after adult emergence by separating males and females under a brief (<2 min) carbon dioxide anesthesia, and transferred into fresh vials containing 2% agar, 10% yeast and 10% sucrose and maintained at 25 °C. For each lifespan experiment, 10–30 flies were put in a single vial. They were transferred into fresh vials within 4 days and survival rates were scored. In this system, expression of UAS-transgenes can be induced by feeding with RU486. For the induction of RNAi, 50 μg/ml RU486 (Mifepristone, Sigma–Aldrich) in 60% ethanol was added to the food (RU+) while the controls received the vehicle (RU−). F1 males and females were examined for their lifespan. Survival curves were analyzed with the Graphpad Prism 4 software, yielding *p* values for a *log-rank* test. All *log-rank* test *p* values are calculated to compare the lifespans; RU486 (RU+) vs vehicle (RU−) in the case of GeneSwitch system; the over-expressor strain vs the driver or transgenic line alone in the case of GAL4/UAS system.

2.3. Stress resistance assay

Newly eclosed flies were collected on the standard medium containing 2% agar, 10% yeast and 10% sucrose. Ten to 30 flies were put in a single vial. In the case of GeneSwitch system, flies were maintained with 50 μg/ml RU486 (Mifepristone, Sigma–Aldrich) in 60% ethanol for 5 days, while the controls received the vehicle (RU−) for 5 days. *Oxidative stress*: For oxidative stress assays, paraquat (*N*,*N*′-dimethyl-4,4′-bipyridinium dichloride) was used to induce oxidative stress. Flies were transferred into new vials containing a filter paper with a solution of 20 mM paraquat in 1% sucrose and examined for their survival within every 12 h. *Starvation stress*: Flies were transferred into new vials containing 1% agar and checked for their survival within every 12 h. Each experiment was repeated at least twice.

2.4. Real-time polymerase chain reaction (PCR) analysis

Newly eclosed flies were maintained at 25 °C in new food vials containing 2% agar, 10% yeast and 10% sucrose for 4 days. For the induction of RNAi, 50 μg/ml RU486 in 60% ethanol was added to the food (RU+) while the controls received the vehicle (RU−) for 5 days. Total RNA was extracted from 10 male flies by using TRIZOL (Invitrogen). Extracted RNA was used for synthesis of cDNA by using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems) and ABI 7300 real-time PCR system (Applied Biosystems), and relative levels were assessed using the $\Delta\Delta C_t$ method according to the manufacturer's protocol. mRNA level of RP49(RpL32, CG7939) was used for normalization. Primer sequences were designed using Primer ExpressSoftware v3.0 (Applied Biosystems).

3. Results

3.1. Effect of dAMPKα on longevity in a tissue-specific manner

To investigate the effects of AMPK on longevity *in vivo*, we used *D. melanogaster*, one of well-established model organisms for aging/longevity research. *Drosophila SNF1A/dAMPKα* (*CG3051*), a catalytic subunit of AMPK, is a single orthologue for mammalian and yeast counterparts [4]. The deletion in this gene leads to lethality before the mid-pupal stage [4]. To circumvent this, we obtained a *Drosophila* transgenic RNAi line for *dAMPKα* (*UAS-dAMPKα^{RNAi}*) from Vienne Drosophila Resource Center. This line carries the transgene constructed by cloning the fragment of *dAMPKα* as inverted repeats (IRs) into a modified *pUAST* vector, which drives the expression of a hairpin RNA of *dAMPKα* depending on GAL4 [20] Crossing *UAS-dAMPKα^{RNAi}* line with various tissue-specific *GAL4* drivers, we can perform RNAi-mediated knock-down in a tissue-specific manner.

We first test the efficacy of RNA knock-down of *dAMPKα* in our system. *UAS-dAMPKα^{RNAi}* line was crossed with *daughterless-GAL4* (*da-GAL4*) driver, a ubiquitous expressed driver line. In consistent with the phenotype of *dAMPKα* null-mutant previously reported [4], *da-GAL4/UAS-dAMPKα^{RNAi}* animals were lethal during pupal stage (Fig. 1A). To further examine the extent of RNAi knock-down of *dAMPKα*, we performed a real-time PCR assay preparing total RNA from third instar larvae of *da-GAL4*/UAS-*dAMPKα^{RNAi}* and controls (*da-GAL4/+* and *+*/UAS-*dAMPKα^{RNAi}*) (The plus symbol indicates the parental strain *w^{1118}*). Expression levels of *dAMPKα* mRNA in *da-GAL4/UAS-dAMPKα^{RNAi}* animals were reduced to approximately 20% of corresponding controls (Fig. 1B) (relative expression level of *dAMPKα* mRNA; *da-GAL4/+*, 1; *+/UAS-dAMPKα^{RNAi}*, 0.82 ± 0.21; *da-GAL4/UAS-dAMPKα^{RNAi}*, 0.19 ± 0.03).

We then investigated the effects of RNAi-mediated knock-down for *dAMPKα* on longevity using various tissue-specific drivers. We crossed *UAS-dAMPKα^{RNAi}* line with three drivers (*24B-GAL4, C564-GAL4* and *elev-GAL4*), enhancer traps that are predominantly expressed in muscle, fat body and neuron, respectively [21,22]. All of these crossed lines were viable and showed no visible phenotype. Among them, *24B-GAL4/UAS-dAMPKα^{RNAi}* animals resulted in extremely significant shorter lifespan (approximately 90% reduction of mean lifespan compared with controls) (Fig. 1C and D) [mean lifespan (male); *24B-GAL4/+*, 48 d (n = 153); *+*/UAS-*dAMPKα^{RNAi}*, 56.1 d (n = 120), *24B-GAL4/UAS-dAMPKα^{RNAi}*, 5.4 d (n = 47); *log-rank* test $p < 0.0001$] [mean lifespan (female); *24B-GAL4/+*, 74.1 d (n = 132); *+*/UAS-*dAMPKα^{RNAi}*, 69.0 d (n = 137), *24B-GAL4/UAS-dAMPKα^{RNAi}*, 5.9 d (n = 167); *log-rank* test $p < 0.0001$]. Since *24B-GAL4* is an enhancer-trap line expressing GAL4 in all embryonic and larval somatic muscles [23], we cannot rule out the possibility that *dAMPKα* in muscle during development could play a critical role on longevity. RNA knock-down of *dAMPKα* using neuron-specific *elav-GAL4* driver did not remarkably shorten mean lifespan (4% reduction of mean lifespan compared with *+/elav-GAL4*) (Fig. 1E) [mean lifespan (male); *elav-GAL4/+* male, 63.1 d (n = 45); *+/UAS-dAMPKα^{RNAi} male*, 56.1 d (n = 120), *elav-GAL4*/UAS-*dAMPKα^{RNAi}* male, 60.7 d (n = 79), *log-rank* test $p = 0.0330$]. Further RNA knock-down of *dAMPKα* using fat body-specific *C564-GAL4* driver had no significant effect on male mean lifespan (Fig. 1F) [mean lifespan (male); *C564-GAL4/+*, 49.5 d (n = 125); *+/UAS-dAMPKα^{RNAi}*, 56.1 d (n = 120), *C564-GAL4*/UAS-*dAMPKα^{RNAi}*, 60.7 d (n = 45), *log-rank* test $p = 0.3811$].

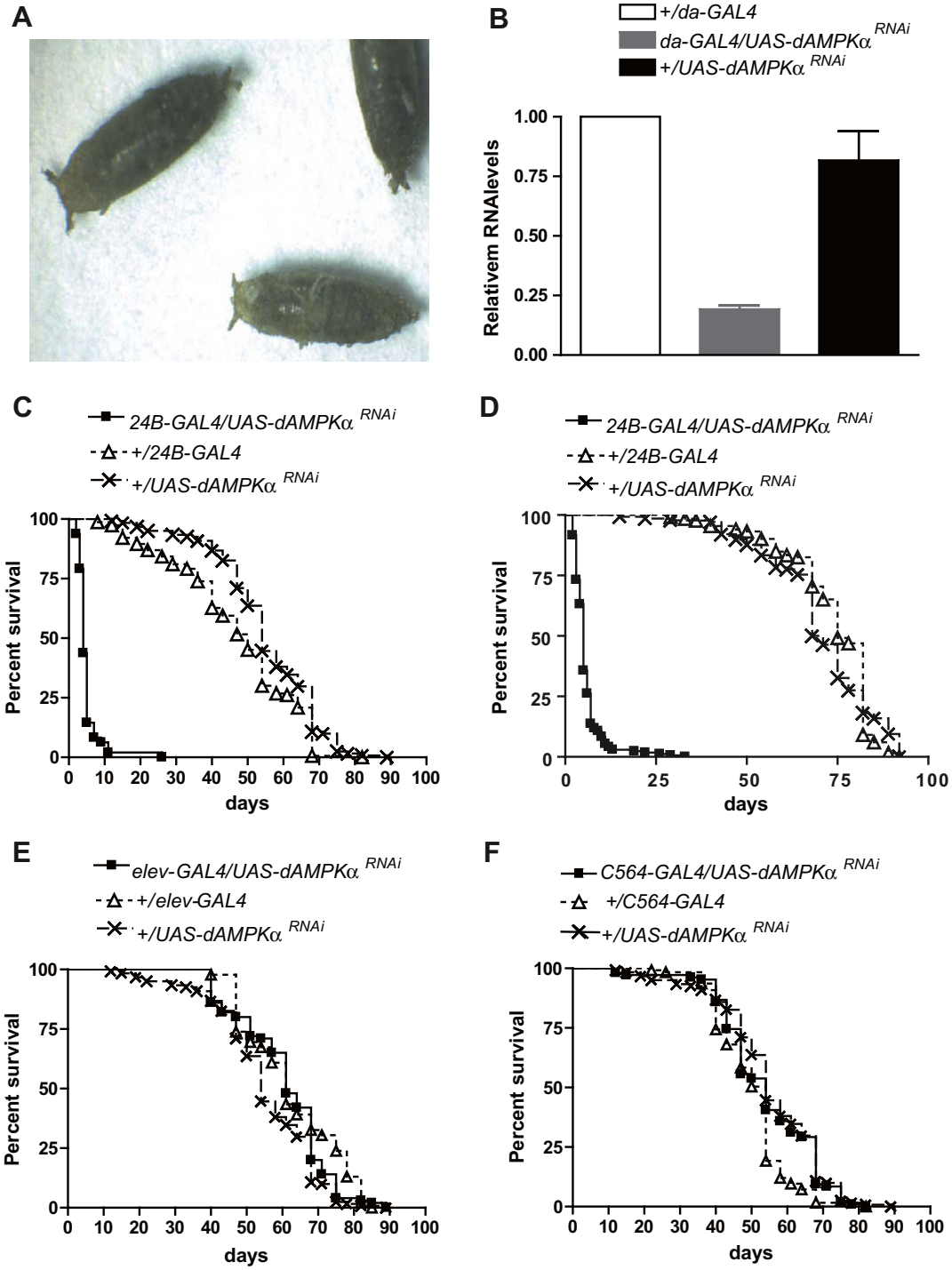

Fig. 1. *dAMPKα* RNAi in muscle shortens lifespan. (A) Representative image of developmental lethality during pupal stage in *da-GAL4/UAS-dAMPKα*^{RNAi} line. (B) Real-time PCR was performed to quantify the relative level of *AMPKα* transcript using total RNA from third instar larvae of *da-GAL4/+*, *UAS-dAMPKα*^{RNAi}*/+* or *da-GAL4/UAS-dAMPKα*^{RNAi} animals. Mean and standard deviation of the relative *dAMPKα*/*RP49* ratios based on two independent trials are shown. In each trial, biological duplicate samples were assayed. Survival curves for *24B-GAL4/+* (open triangles), *+/UAS-dAMPKα*^{RNAi} (crosses) or *24B-GAL4/UAS-dAMPKα*^{RNAi} (closed squares) [(C) male, (D) female]. (E) Male survival curves for *elav-GAL4/+* (open triangles), *+/UAS-dAMPKα*^{RNAi} (crosses) or *elav-GAL4/UAS-dAMPKα*^{RNAi} (closed squares). (F) Male survival curves for *C564-GAL4/+* (open triangles), *+/UAS-dAMPKα*^{RNAi} (crosses) or *C564-GAL4/UAS-dAMPKα*^{RNAi} (closed squares).

3.2. Adult-onset inhibition of dAMPKα in muscle shortens lifespan

The GeneSwitch system is a powerful tool allowing us to perform time- and tissue-specific transgene expression in *Drosophila* [19]. Since this system uses conditional RU486 (mifepristone)-dependent GAL4 protein (GeneSwitch), we can activate GAL4 protein on the application of RU486 to perform adult-onset RNAi for *dAMPKα*. We first tested the efficacy of GeneSwitch (GS)-mediated RNAi using ubiquitously expressed GS driver (*Act5c-GS*). Crossing *UAS-dAMPKα*^{RNAi} line with *Act5c-GS* driver, adult-onset RNAi was initiated by transferring adult *Act5c-GS/UAS-dAMPKα*^{RNAi} flies onto food with RU486. Five days treatment with RU486 resulted in approximately 50% reduction of *dAMPKα* mRNA level in adult *Act5c-GS/UAS-dAMPKα*^{RNAi} flies compared with control (Fig. 2A)

(relative expression level of *dAMPKα* in *Act5c-GS/UAS-dAMPKα*^{RNAi}; RU (−), 1; RU (+), 0.55 ± 0.0018).

We then investigated which tissue is crucial during adulthood for *dAMPKα* on longevity using three tissue-specific GS drivers (muscle-specific *MHC-GS*, neuron-specific *elav-GS* and adult fat body-specific *S106-GS*). As in Fig. 2B and C, adult-onset inhibition of *dAMPKα* in muscle (*MHC-GS/UAS-dAMPKα*^{RNAi}) resulted in significant shorter lifespan (approximately 20–40% reduction of mean lifespan compared with controls) [mean lifespan of *MHC-GS/UAS-dAMPKα*^{RNAi}(male); RU (−), 45.3 d (n = 159), RU (+), 26 d (n = 150); *log-rank* test $p < 0.0001$] [(female); RU (−), 41.7 d (n = 139), RU (+), 32.5 d (n = 140); *log-rank* test $p < 0.0001$]. In contrast, adult-onset inhibition of *dAMPKα* in neuron (*elav-GS*) or fat body (*S106-GS*) had no significant effect on male lifespan (Fig. 2D and E). To rule out the possibility that RU486 affects longevity, we confirmed that RU486 had no effect on the lifespan of *MHC-GS/+* line (Supplemental Fig. 1). In contrast RU486 had a toxic effect on the lifespan of *Act5c-GS/+* line (data not shown), we therefore did not perform lifespan assay on *Act5c-GS/UAS-dAMPKα*^{RNA} line.

3.3. Effect of dAMPKα RNAi in muscle on stress resistance

Since RNAi inhibition of *dAMPKα* especially in muscle shortens lifespan, we then examined whether this effect is linked to stress resistance. Inhibition of *dAMPKα* in muscle (*24B-GAL4/UAS-dAMPKα*^{RNAi}) significantly increased sensitivity to paraquat in female flies

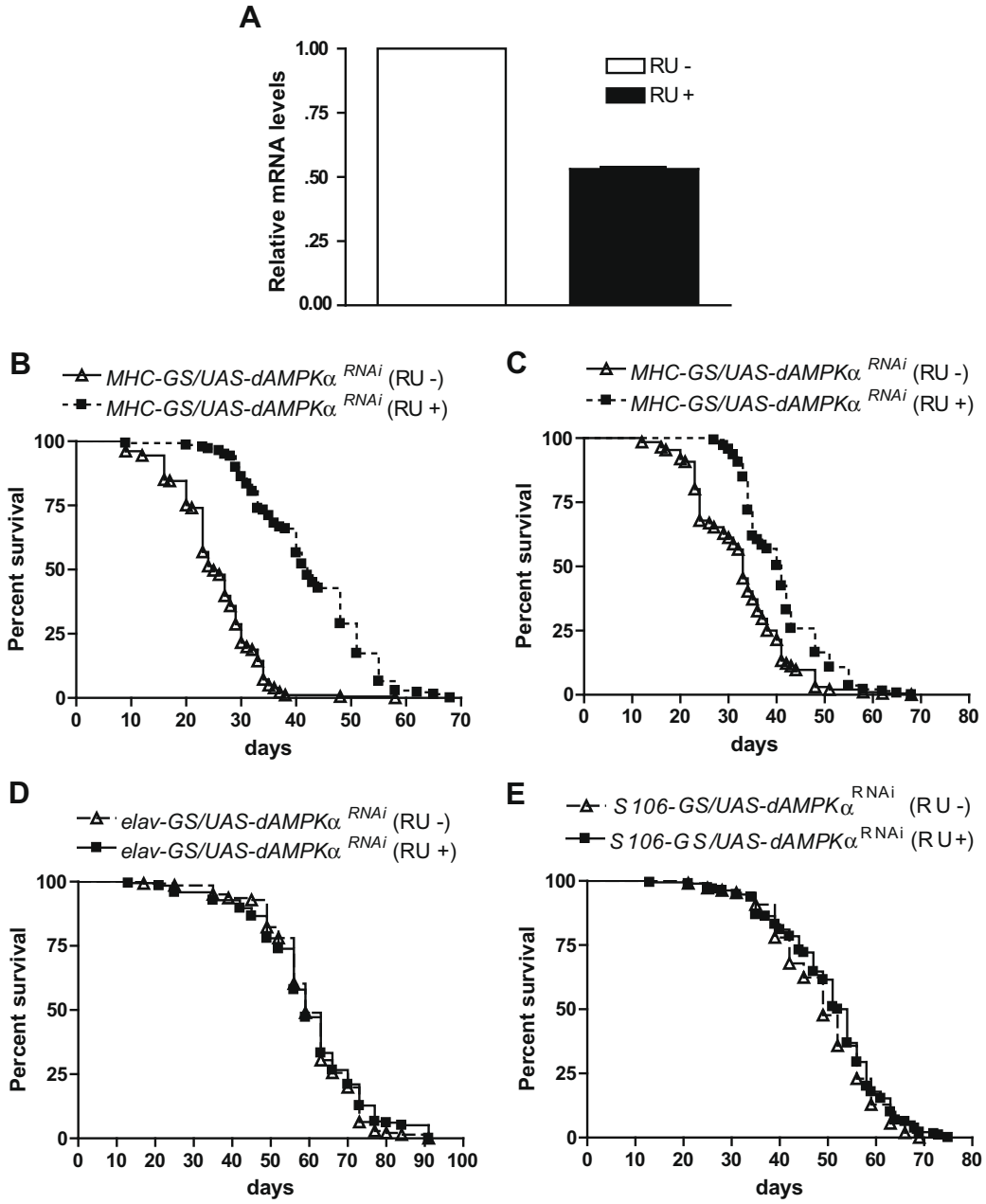

Fig. 2. Adult-onset inhibition of *dAMPKα* in muscle shortens lifespan (GeneSwitch system). (A) Real-time PCR assay was performed to quantify the relative level of *dAMPKα* transcripts preparing total RNA from male *Act5c-GS/UAS-dAMPKα*^{RNAi} flies in the presence (RU+) or absence (RU−) of RU486 for 5 days. Mean and standard deviation of the relative *dAMPKα/RP49* ratios based on two independent trials are shown. In each trial, biological duplicate samples were assayed. Survival curves for *MHC-GS/UAS-dAMPKα*^{RNAi} treated with RU486 (RU+) or mock (RU−) during adulthood [(B) male, (C) female]. Male survival curves for *elav-GS/UAS-dAMPKα*^{RNAi} (D) and *S106-GS/UAS- dAMPKα*^{RNAi} (E) in the presence (RU+) or absence (RU−) of RU486 during adulthood.

(Fig. 3A and B) [mean viability on paraquat (male); *24B-GAL4*/+, 36.3 h ($n = 49$); +/UAS-*dAMPKα*^{RNAi}, 40.0 h ($n = 42$), *24B-GAL4*/UAS-*dAMPKα*^{RNAi}, 34.1 h ($n = 45$); *log-rank* test $p = 0.5071$] [mean viability on paraquat (female); *24B-GAL4*/+, 63.0 h ($n = 43$); +/UAS-*dAMPKα*^{RNAi}, 63.3 h ($n = 40$), *24B-GAL4*/UAS-*dAMPKα*^{RNAi}, 31.2 h ($n = 18$); *log-rank* test $p = 0.01657$]. Further inhibition of *dAMPKα* in muscle (*24B-*

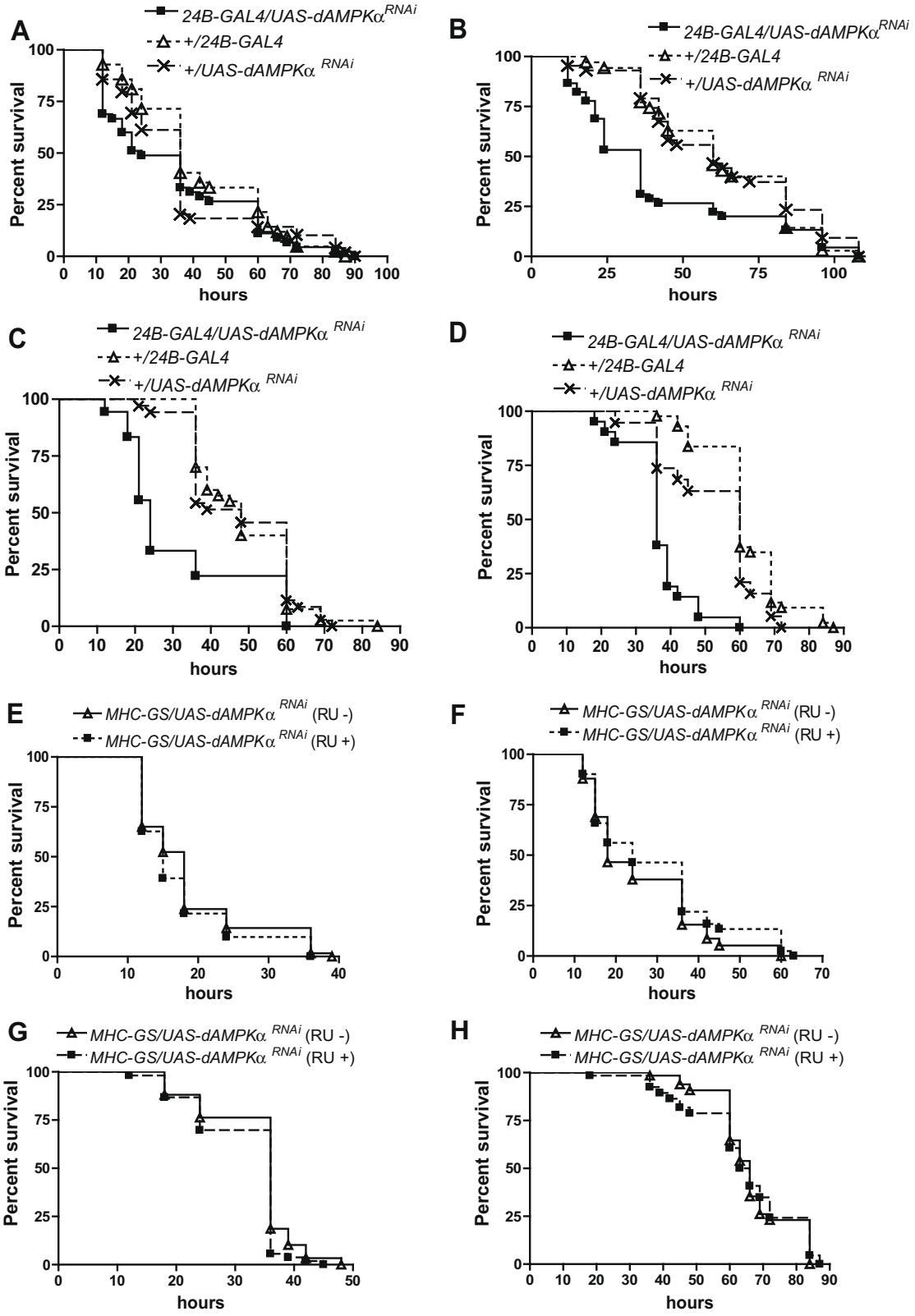

Fig. 3. RNAi-mediated knock-down of *dAMPKα* in muscle increases sensitivity to stresses. (A,B) Survival curves on paraquat challenge. *24B-GAL4*/UAS-*dAMPKα*^{RNAi} flies and their genetically matched controls were exposed to paraquat [(A) male, (B) female]. (C,D) Survival curves on starvation challenge. *24B-GAL4*/UAS-*dAMPKα*^{RNAi} flies and their genetically matched controls were exposed to starvation [(C) male, (D) female]. (E,F) Survival curves on paraquat challenge. Adult *MHC-GS*/UAS-*dAMPKα*^{RNAi} flies were exposed to paraquat after the treatment with RU486 (RU+) or mock (RU−) for 5 days [(E) male, (F) female]. (G,H) Survival curves on starvation challenge. Adult *MHC-GS*/UAS-*dAMPKα*^{RNAi} flies were exposed to starvation after the treatment with RU486 (RU+) or mock (RU−) for 5 days [(G) male, (H) female].

GAL4/UAS-dAMPKα^{RNAi}) significantly increased sensitivity to starvation (Fig. 3C and D) [mean viability on starvation (male); *24B-GAL4/+*, 47.8 h (n = 35); +/UAS-*dAMPKα^{RNAi}*, 49.3 h (n = 40), *24B-GAL4/UAS-dAMPKα^{RNAi}*, 31.2 h (n = 18); *log-rank* test p = 0.0049] [mean viability on starvation (female); *24B-GAL4/+*, 52.8 h (n = 19); +/UAS-*dAMPKα^{RNAi}*, 61.8 h (n = 43), *24B-GAL4/UAS-dAMPKα^{RNAi}*, 36.8 h (n = 42); *log-rank* test $p < 0.0001$]. Next we tested whether adult-onset inhibition of *dAMPKα* affects sensitivity to stress resistance. Crossing *UAS-dAMPKα^{RNAi}* line with *MHC-GS* driver, adult-onset RNAi was initiated by transferring adult *MHC-GS/UAS-dAMPKα^{RNAi}* flies onto food with RU486. In contrast to the results in *24B-GAL4/UAS-dAMPKα^{RNAi}*, adult-onset inhibition of *dAMPKα* had no effect on sensitivity to paraquat (Fig. 3E and F) and starvation (Fig. 3G and H). Taken together, these results suggest that inhibition of *dAMPKα* in muscle during development could have more effects on the sensitivity to stressors as well as on longevity.

3.4. Effect of dAMPKα RNAi in muscle on the transcriptional regulation in response to stress

Mammalian muscle has the ability to adapt to exercise training by up-regulating metabolic enzymes and mitochondria content, a part of these adaptations in protein expression results from a transient increases in transcription on exercise [24]. Activated AMPK influences metabolic pathways including carbohydrate metabolism, lipid metabolism and protein synthesis. In addition, AMPK is activated in response to environmental stressor that increases the AMP/ATP ratio in *C. elegans* [17].

We then investigated whether inhibition of *dAMPKα* in muscle affects the transcriptional regulation of the genes by real-time PCR assay, including stress resistant genes [*GSTD1*(*glutathione S-transferases 1*), *GSTD2*(*glutathione S-transferases 2*), *hsp68*, *sod1*, *catalase*, *l(2)efi* (*small heat-shock protein*), *fer1*(*ferritin subunit*)] and metabolic genes [*Glt1(glucose transporter)*, *CG5966*(*triacylglycerol lipase*), *HexA(Hexokinase-A)*, *thor(4E-BP)*]. *UAS-dAMPKα^{RNAi}*, *24B-GAL4* or *w^{1118}* flies were crossed with each other, and progeny of *24B-GAL4/+*, *+/UAS-dAMPKα^{RNAi}* or *24B-GAL4/UAS-dAMPKα^{RNAi}* were exposed to starvation. We prepared total RNA from male animals in normal or starvation condition. As in Fig. 4A and B, inhibition of *dAMPKα* in muscle (*24B-GAL4/UAS-dAMPKα^{RNAi}*) remarkably affected the transcriptional regulation of several genes in response to starvation, including *CG5966*, *GSTD2* and *thor* compared with corresponding controls (*24B-GAL4/+* and *+/UAS-dAMPKα^{RNAi}*). [*CG5966* relative expression level in starvation; *24B-GAL4/+*, 1; *+/UAS-dAMPKα^{RNAi}*, 2.55 ± 0.41; *24B-GAL4/UAS-dAMPKα^{RNAi}*, 4.64 ± 2.72] [*GSTD2* relative expression level in starvation; *24B-GAL4/+*, 1; *+/UAS-dAMPKα^{RNAi}*, 3.15 ± 0.64; *24B-GAL4/UAS-dAMPKα^{RNAi}*, 9.22 ± 0.75] [*thor* relative expression level in starvation; *24B-GAL4/+*, 1; *+/UAS-dAMPKα^{RNAi}*, 0.83 ± 0.34; *24B-GAL4/UAS-dAMPKα^{RNAi}*, 3.95 ± 0.59].

4. Discussion

This study shows that adult-onset inhibition of *dAMPKα* especially in muscle shortens lifespan. Inhibition of *dAMPKα* in muscle enhances sensitivity to paraquat and starvation stress. Further this affected the transcriptional regulation of various genes in response to starvation. These results shed a light on muscle as one of major tissues in which dAMPK could play a pivotal role on longevity and stress resistance.

What is the physiological significance of AMPK in muscle on longevity and stress resistance? As described above, mammalian muscle adapts to exercise by up-regulating metabolic enzymes and mitochondria content, a part of these adaptations result from a transcriptional increases on exercise. AMPK is activated in response to endurance exercise in skeletal muscle [24]. AMPKα2-isoform translocates to the nucleus in human skeletal muscle in response to exercise [25], supporting that AMPK has been suggested as a molecule to transmit a signal into the nucleus on exercise [25]. In fact, AMPK inactivation in muscle exerts an impact on gene expression leading to changes in the transcription of many genes, including energy metabolism and intracellular signaling in mouse [26]. Further mice expressing mutant AMPKα2 in muscle showed a decrease of the voluntary activity [26], and are associated with impaired glycogen synthesis in skeletal muscle [27]. In addition, an elevated metabolic rate as a result of exercise can dramatically increase oxygen consumption in muscle as well as other tissues [28]; exercise may cause oxidative stress and tissue damage in muscle [28], including free radical generation [28], increases in oxidative damage biomarkers [29] and mitochondrial dysfunction [29,30]. Therefore, inhibition of AMPK activity in muscle could dis-

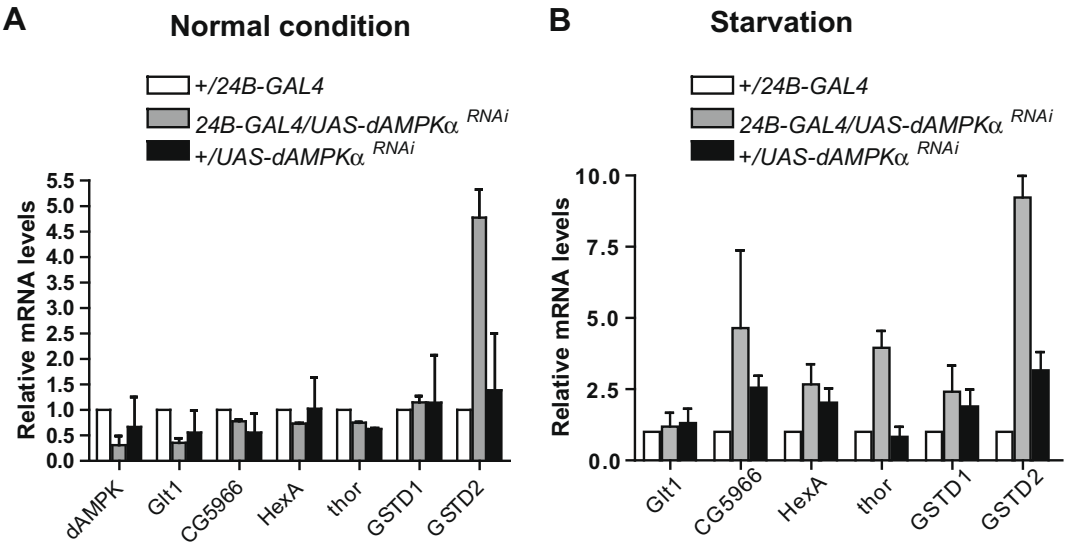

Fig. 4. Inhibition of *dAMPKα* in muscle altered the transcriptional regulation of various genes. Total RNAs were prepared from male *24B-GAL4/+*, *+/UAS-dAMPKα^{RNAi}* or *24B-GAL4/UAS-dAMPKα^{RNAi}* flies in normal condition (A) or starvation (B). Real-time PCR was performed to quantify the relative expression levels of genes, including *dAMPK*, *Glt1*, *CG5966*, *HexA*, *thor*, *GSTD1* and *GSTD2*. Mean and standard deviation of the relative ratios to *RP49* based on two independent trials are shown. In each trial, biological duplicate samples were assayed.

rupt the energy homeostasis as well as stress-resistant systems especially to oxidative stress, leading to shorter lifespan across species.

One of possible key players in AMPK-mediated effects on longevity and stress resistance is forkhead transcriptional factor FOXO [31]. FOXO is thought to mediate longevity promoting signals by trans-activating genes involved in stress resistances [32]. Since activation of AMPK modulates the transcriptional activity of FOXO through direct phosphorylation [31,33], it is possible AMPK activation in muscle could enhance a gene expression program to induce cellular stress resistance and longevity through FOXO.

In summary, this study showed that muscle could be one of tissues in which AMPK plays a pivotal role on longevity and stress resistances. These results would support the idea that intervention to activate AMPK in muscle could be a prominent treatment strategy for longevity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.02.126.

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