Biochemical and Biophysical Research Communications 405 (2011) 667–672

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A gain-of-function screen identifies *wdb* and *lkb1* as lifespan-extending genes in *Drosophila*

Masabumi Funakoshi [a], Manabu Tsuda [a], Keigo Muramatsu [a], Hiroshi Hatsuda [b], Shinichi Morishita [b], Toshiro Aigaki [a,*]

[a] Department of Biological Sciences, Tokyo Metropolitan University, 1-1 Minami-osawa, Hachioji, Tokyo 192-0397, Japan
[b] Department of Computational Biology, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa 277-0882, Japan

ARTICLE INFO

Article history:
Received 22 January 2011
Available online 31 January 2011

Keywords:
Drosophila
Gain-of-function screen
Organ size
Lifespan extension

ABSTRACT

The insulin/insulin-like growth factor (IGF) and the target of rapamycin (TOR) signaling pathways are known to regulate lifespan in diverse organisms. However, only a limited number of genes involved in these pathways have been examined regarding their effects on lifespan. Through a gain-of-function screen in *Drosophila*, we found that overexpression of the *wdb* gene encoding a regulatory subunit of PP2A, and overexpression of the *lkb1* gene encoding a serine/threonine kinase, reduced organ size and extended lifespan. Overexpression of *wdb* also reduced the level of phosphorylated AKT, while overexpression of *lkb1* increased the level of phosphorylated AMPK and decreased the level of phosphorylated S6K. Taken together, our results suggest that *wdb*- and *lkb1*-dependent lifespan extension is mediated by downregulation of S6K, a downstream component of the insulin/IGF and TOR signaling pathways.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

The insulin/insulin-like growth factor (IGF) signaling pathway plays an important role in controlling metabolism, organ growth and lifespan [1,2]. In *Drosophila*, mutations in the insulin receptor substrate *chico* or overexpression of phosphatase and tensin homolog (PTEN) reduced body size and extended the lifespan [3,4]. The insulin/IGF pathway activates Akt, followed by the target of rapamycin (TOR), which promotes protein synthesis by activating S6K [2]. Mutations in *dTOR* or *dS6K* were also reported to reduce body size [5] and increase lifespan [6,7]. TOR is also regulated by AMP-activated protein kinase (AMPK) signaling pathways. AMPK is a cellular energy sensor that is activated in response to low levels of nutrition [8]. The activation of AMPK leads to downregulation of TOR signaling by stimulating the GTPase-activating protein (GAP) activity of tuberous sclerosis protein-2 [9,10] and phosphorylation of raptor, a component of the TOR complex [11]. In *Caenorhabditis elegans*, a mutation in *aak-2*, a homolog of AMPK, reduced the lifespan while the expression of the constitutive active form of AMPK extended the lifespan [12]. Therefore, AMPK could be involved in the regulation of cell growth and lifespan.

A number of genes could modify insulin/IGF and TOR signaling. However, relatively few genes have been investigated regarding their effects on lifespan. Since the lifespan is one of the most complex biological traits, experimental studies are necessary to understand the mechanisms involved in the regulation of lifespan.

In this study, we performed a gain-of-function screen to identify novel lifespan-extending genes in *Drosophila*. We generated a collection of *Drosophila* Gene Search (GS) lines, in which a *P*{*GS*} misexpression vector containing an upstream activating sequence (UAS) was inserted into the genome at random, allowing for GAL4-dependent forced expression of the vector-flanking genes [13]. Since the lifespan is often inversely correlated with body size, we first screened for insertions that reduce wing size when misexpression was induced in the developing wing discs. We also examined these insertions to determine whether they also reduced eye size when misexpressed in eye discs. Among the insertions that reduced the sizes of wings and eyes, we found two genes, *wdb* and *lkb1*, that extended lifespan when overexpressed ubiquitously. We provide biochemical evidence showing that overexpression of *wdb* reduces the level of phosphorylated AKT, while overexpression of *lkb1* increases the level of phosphorylated AMPK and reduces the level of dephosphorylated S6K. Our results suggested that *wdb*- and *lkb1*-dependent lifespan extension is mediated by downregulation of S6K, a downstream component of the insulin/IGF and TOR signaling pathway.

2. Materials and methods

2.1. Drosophila stocks and culture

Flies were raised on a standard corn meal, yeast, glucose agar medium at 25 °C. The *P*{*GS*} vector insertion lines (GS lines) were generated as previously described [13]. *hs-GAL4*, *GMR-GAL4*, *vg-*

* Corresponding author. Fax: +81 42 677 2559.
E-mail address: aigaki-toshiro@tmu.ac.jp (T. Aigaki).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.01.090

GAL4 (*P*{*vgM-GAL4*.Exel}2), *ptc-GAL4*, *UAS-Akt* and *UAS-GFP* (*P*{*UAS-GFP*^{S65T}}DC7) were obtained from the Bloomington Stock Center (Bloomington, IN).

2.2. Determination of P-element insertion sites

Ligation-mediated PCR (LM-PCR) was used to amplify the vector-flanking sequence. Genomic DNA extracted from each of the GS lines was digested with *Hha*I, followed by primer extension with a GS vector-specific primer (5′-CTGCAGTAAAGTGCAAGTTAAAGTG-3′) using EX Taq (TaKaRa Bio, Shiga, Japan). The primer extension products were ligated to double-stranded linker sequences (5′-CGGTAACCCGGGAGATCTGAATTCT-3′ and 5′-GAATTCAGATC-3′) using T4 ligase (Promega, Tokyo, Japan), amplified by PCR using the GS vector-specific primer and the linker-specific primer, and then sequenced. The resulting sequences were used to determine the insertion site in the *Drosophila* genome sequence (version 5.18).

2.3. Measurement of wing size

Wings were removed from flies with forceps and mounted on a microscopic slide with a drop of Fly Line Dressing (TIEMCO, Tokyo, Japan). Wing images were captured using a stereoscopic microscope (Leica MZ16F, Leica Microsystems, Tokyo, Japan) equipped with a DP-70 digital camera system (Olympus, Tokyo, Japan). Wing size was measured using Image J software (version 1.38, http://rsb.info.nih.gov/ij/download.html).

2.4. Real-time PCR analysis

Total RNA was extracted from 3 to 5-day-old adult flies using Trizol reagent (Invitrogen, Carlsbad, CA), treated with DNase I, and then reverse-transcribed using a Superscript VILO cDNA Synthesis kit (Invitrogen). Real-time PCR was carried out using SYBR Premix Ex Taq (TaKaRa Bio) and a Chromo 4 Detector (MJ Research, Hercules, CA). The results were normalized by the level of *Actin 5C* mRNA. The relative expression levels of *wdb* and *lkb1* mRNA in flies as *hs* > *wdb* and *hs* > *lkb1*, respectively, were calculated against that of a control (*hs* > *GFP*), and means ± standard error were calculated from triplicate assays. The primer sequences used for real-time PCR were as follows: *wdb*_F, 5′-CAAGGTTCCTCGCGCTACAGAA-3′; *wdb*_R, 5′-ACACGCAACACTGGCGCAGCTT-3′; *lkb1*_F, 5′-GCCAACCTGATTCTCGGCATGC-3′; *lkb1*_R, 5′-TGGGTATCGGTGGGCCGGTCTT-3′; *Actin5C*_F, 5′-CGGGATGGCTTTGATTCTGC-3′; *Actin5C*_R, 5′-AACTTCCACCACTCGCACTTG-3′.

2.5. Lifespan measurement

The lifespan of adult flies was measured at 25 °C, as previously described [14]. Briefly, newly eclosed males and females of the appropriate genotypes were collected and transferred to new vials containing standard food every 2–3 days until all of the flies had died. One hundred flies (20 flies/vial) were used for each genotype, and the number of dead flies was counted at the time of transfer. The logrank test was used to evaluate the difference between the two genotypes for each sex.

2.6. Western blot analysis

Adult heads were cut from 5-day-old flies, homogenized in SDS-sample buffer (12.5 mM Tris (pH 6.8), 20% glycerol, 4% SDS, 2% 2-mercaptoethanol and 0.001% bromophenol blue) and boiled for 10 min at 95 °C. The samples were separated by 10% SDS–PAGE and transferred to PVDF membranes (GE Healthcare, Buckinghamshire, UK). After blocking with 5% bovine serum albumin (Sigma–Aldrich, St. Louis, MO), the membranes were incubated with a primary antibody in Tris-buffered saline containing Tween (TBST) overnight at 4 °C and then with a secondary antibody in TBST for 1 h at 25 °C. The signals were detected with an ECL-plus kit (GE Healthcare). As primary antibodies, rabbit anti-phospho-Akt (Cell Signaling Technology, Danvers, MA), rabbit anti-phospho-p70 S6 kinase (Cell Signaling Technology) and mouse anti-α-tubulin (Sigma–Aldrich) were used at dilutions of 1:1000, 1:1000 and 1:8000, respectively. HRP-conjugated anti-rabbit IgG (Cell Signaling Technology) and HRP-conjugated anti-mouse IgG (GE Healthcare) were used as secondary antibodies at dilutions of 1:2000 and 1:1000, respectively.

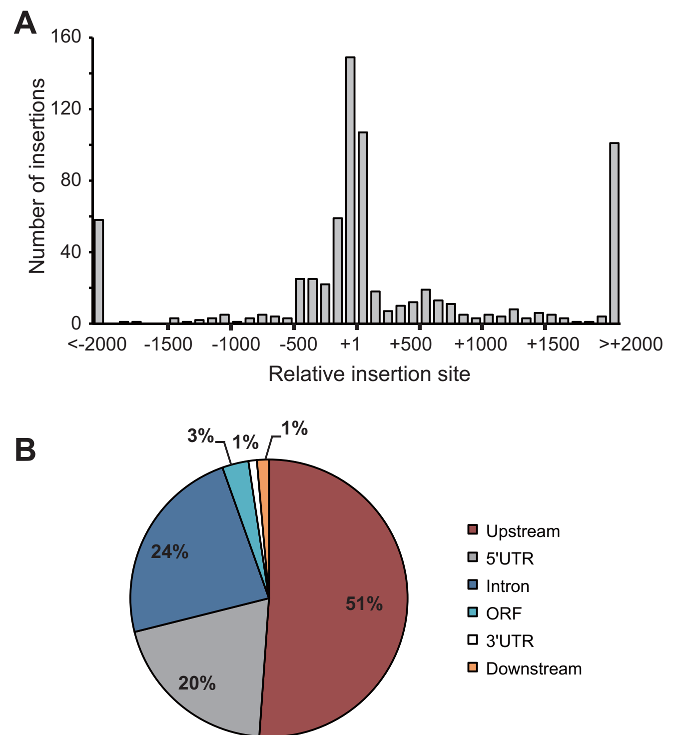

Fig. 1. Mapping of the *P*{*GS*} vector insertion sites. (A) Distribution of the *P*{*GS*} insertion sites relative to the transcription start site of the flanking genes. The transcription start site (5′-end of mRNA) was defined as +1. (B) Gene region-wise distribution of *P*{*GS*} insertion sites.

2.7. Immunohistochemistry

Wing imaginal discs were dissected from third instar larvae, immersed in TBS, and fixed for 20 min in 4% paraformaldehyde in TBS.

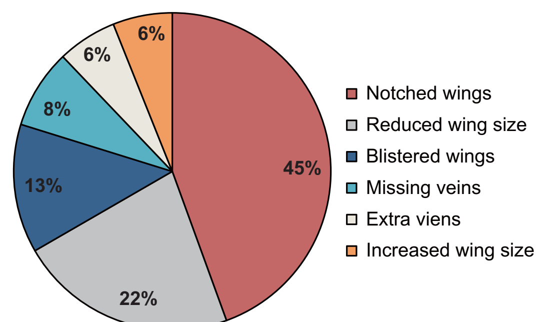

Fig. 2. Frequency of wing phenotypes in GS-misexpressing flies. Phenotypes were classified into the indicated categories.

Download English Version:

https://daneshyari.com/en/article/1931178

Download Persian Version:

https://daneshyari.com/article/1931178

Daneshyari.com