Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Overexpression of Larp4B downregulates dMyc and reduces cell and organ sizes in *Drosophila*

Masabumi Funakoshi [a, 1], Manabu Tsuda [b, 1], Keigo Muramatsu [a], Hiroshi Hatsuda [c], Shinichi Morishita [c], Toshiro Aigaki [a, *]

[a] Department of Biological Sciences, Tokyo Metropolitan University, 1-1 Minami-osawa, Hachioji-shi, Tokyo 192-0397, Japan
[b] Department of Liberal Arts and Human Development, Kanagawa University of Human Services, 1-10-1, Heiseicho, Yokosuka-shi, Kanagawa 238-8522, Japan
[c] Department of Computational Biology, Graduate School of Frontier Sciences, University of Tokyo, Kashiwa 277-0882, Japan

ARTICLE INFO

Article history:
Received 13 February 2018
Accepted 17 February 2018
Available online xxx

Keywords:
RNA-Binding protein
Larp4B
dMyc
Organ size
Cell death
Drosophila

ABSTRACT

Regulation of cell and organ sizes is fundamental for all organisms, but its molecular basis is not fully understood. Here we performed a gain-of-function screen and identified *larp4B* whose overexpression reduces cell and organ sizes in *Drosophila melanogaster*. Larp4B is a member of La-related proteins (LARPs) containing an LA motif and an adjacent RNA recognition motif (RRM), and play diverse roles in RNA metabolism. However, the function of Larp4B has remained poorly characterized. We generated transgenic flies overexpressing wild-type Larp4B or a deletion variant lacking the LA and RRM domains, and demonstrated that the RNA-binding domains are essential for Larp4B to reduce cell and organ sizes. We found that the *larp4B*-induced phenotype was suppressed by *dMyc* overexpression, which promotes cell growth and survival. Furthermore, overexpression of *larp4B* decreased dMyc protein levels, whereas its loss-of-function mutation had an opposite effect. Our results suggest that Larp4B is a negative regulator of dMyc.

1. Introduction

RNA-binding proteins are associated with RNAs to form ribonucleoprotein (RNP) complexes and play important roles in post-transcriptional control of RNAs, such as splicing, polyadenylation, mRNA stabilization, mRNA localization and translation [1]. The La-related proteins (LARPs) are RNA-binding proteins containing an LA motif and an adjacent RNA recognition motif (RRM) [2,3]. The LARP proteins have diverse roles in RNA metabolism. In mammals, there are five LARP subfamilies, LARP1 (paralogues LARP1a and 1b), LARP3, LARP4 (paralogues LARP4a and 4b), LARP6, and LARP7 [2,3]. Members of LARP3 and LARP7 subfamilies bind to RNA polymerase III (pol III) transcripts and regulate their stability and processing. LARP3 is the most abundant RNA-binding protein in eukaryotic species studied [4]. It has a high affinity to the 3′-terminal uridines and interacts with every RNA pol III transcripts to confer correct folding and to provide protection against exonuclease digestion [4]. LARP7 binds to 7SK RNA specifically and stabilizing its RNP complex [5,6]. The 7SK RNP includes to positive transcription elongation factor b (P-TEFb) and sequesters it from Pol II [5,6]. Thus LARP7 indirectly regulates transcriptional efficiency.

Larp1, Larp4, and Larp6 subfamilies are associated with mRNAs and modulate translation directly. LARP1 forms the RNP complex with poly(A)-binding protein (PABP) and eIF4E and regulates mRNA stability and translation [7,8]. LARP1 also associate with the terminal oligopyrimidine (TOP) motifs at 5′ terminal of mRNAs and represses their translation when mTORC1 is inactive [9]. Interestingly, TOP mRNAs mostly encode proteins involved in translation machinery [10]. Therefore, LARP1 is thought to play a crucial role in connecting mTORC1 pathway with protein synthesis. LARP4 has been thought to interact with cytosolic PABP and ribosome-associated receptor for activated C kinase1 (RACK1) to promote mRNA stabilization and translation of a broad spectrum of genes [11,12]. LARP6 interacts with stem loop structures located in the 5′ untranslated region of collagen mRNA specifically and stimulates translation [13]. LARP6 also forms inhibitor of differentiation (Id) transcription factor with CASK-Cd and functions upstream of transcription factor MyoD in myoblast [14,15].

* Corresponding author.
E-mail address: aigaki-toshiro@tmu.ac.jp (T. Aigaki).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.02.148
0006-291X/

In *Drosophila,* Larp, Larp4B, and Larp7 have been identified so far, which are members of LARP1, LARP4, and LARP7 subfamilies, respectively. Larp has been shown to form an RNP complex with PABP and eIF4E and be essential for proper mitosis, cell survival and migration [16]. *Drosophila* Larp7 is appeared to play the conserved roles in 7SK RNP complexes that required for *Drosophila* development [17]. The *CG11505* gene encodes *Drosophila* Larp4B, which has conserved La and RRM domains, but lacks the *N*-terminal PAM2 domain that is required for the interaction with PABP. However, the function of *Drosophila* Larp4B has not been studied yet.

Here we performed a gain-of-function screen and identified *Drosophila larp4B* as a gene reducing cell and organ sizes. We found that the *larp4B*-induced phenotype was suppressed by overexpression of *dMyc*. In addition, overexpression of *larp4B* decreases dMyc protein levels, while, conversely, reducing *larp4B* expression increases dMyc levels, suggesting that Larp4B regulates dMyc negatively. Since expression of *larp4B* is upregulated upon starvation, Larp4B is likely to be involved in nutrition-dependent growth regulation.

2. Materials and methods

2.1. Drosophila stocks

The *y Df(1)w*^{67C23} (*y w*) strain was used as a control unless otherwise stated. The *GMR-Gal4*, *ey-Gal4* (*P{ey3.5-Gal4*.Exel}2), *sd-Gal4* (*P{GawB}*), *vg-Gal4* (*P{vgM-Gal4*.Exel}2), *ppl-Gal4* (*P*{w[+mC] = *ppl-Gal4.P*}2), UAS-*dm* (*P{w*[+mC] = *UAS-dm.Z*}132) [18], *UAS-InR* (*P{UAS-InR*.K1409A*}*), and *chico*^{1} [19] strains were obtained from the Bloomington Stock Center. The *P{GawB}CG11505*^{NP2535} (*larp4B*^{NP2535}) and *PBac{WH}CG11505*^{f07116} (*larp4B* ^{f07116}) strains were obtained from the Kyoto Stock Center. The *P{GS}* vector insertion line *GS9719* [20] was used to overexpress *larp4B*. For generation of somatic clones, *y w, hsFLP/Y; neoFRT80B, ry*^{506}, *larp4B* ^{f07116}*/neoFRT80B,* and *Ubi-GFP* strain larvae were heat-shocked for 1 h at 37 °C at 2 days after egg laying. The *UAS-akt* [21], *akt1* [21], *InR*^{304} [19], and *UAS-PTEN* [22] strains were gifts from Dr. E. Hafen. The *PTEN*^{dj189} strain was obtained from Dr. D. Pan [23]. Unless otherwise stated, all experiments were carried out at 25 °C, including those with *heat shock protein 70* (*hs*)*-Gal4*.

2.2. Measurement of organ and cell sizes

Images of wings, eyes from five day-old flies and fat bodies from third instar larvae were captured using a stereomicroscope (Leica MZ16F) equipped with a digital camera (Olympus DP50) and a confocal microscope (Nikon C1Si), respectively. Sizes were measured using Image J 1.38 (http://rsb.info.nih.gov/ij/download.html). At least 3 wings and eyes, and 5 cells were measured for each genotype. Female flies were used to for measurement of organ size.

2.3. Western blot

Five-day-old adult flies or adult heads overexpressing Larp4B or GFP (control) were used for western blot to quantify dMyc and α-Tubulin levels. Samples were homogenized in SDS-sample buffer (12.5 mM Tris at 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 primary antibody, rabbit anti-dMyc (Santa Cruz Biotechnology, Santa Cruz, CA) or mouse anti-α-Tubulin (Sigma-Aldrich) at 1:500 and 1:5000 dilutions, respectively. HRP-conjugated anti-rabbit IgG (GE Healthcare) and anti-mouse IgG (GE Healthcare) were used as secondary antibodies at dilutions of 1:1000 and 1:2000, respectively. Signals were generated using an ECL-plus kit (GE Healthcare). Chemiluminescence imaging was performed using a ChemiDoc XRS System (Bio-Rad Laboratories, Hercules, CA) and the signal intensity of each protein band was quantified using Image J 1.38.

2.4. Scanning electron microscopy

Scanning electron microscopy was performed using a Carry Scope JCM-5100 (Nippon Densi, Tokyo, Japan), and images were processed with Adobe Photoshop.

2.5. Immunohistochemistry

Fat body cells were stained with Phalloidin conjugated to Alexa Fluor 568 (Thermo Fisher scientific, San Jose, CA, USA) at a dilution of 1:1000, and mounted with Vectashield Mounting medium with DAPI (Vector laboratories, CA, USA). To stain dMyc protein in the fat body cells, mouse anti-dMyc antibody [24] was used as a primary antibody at a dilution of 1:5 and Alexa Fluor 568-conjugated goat anti-mouse IgG (H + L; Thermo Fisher scientific) was used as a secondary antibody at a dilution of 1:500. Images were captured using a laser scanning confocal microscope (Nikon, C1si).

2.6. DNA constructs and fly transformation

Total RNA was extracted from adult flies using Trizol Reagent (Thermo Fisher scientific) and then reverse-transcribed into complementary DNA (cDNA) using the Superscript III First-Strand Synthesis System (Thermo Fisher scientific). cDNAs encoding Larp4B were amplified by PCR with primers, *larp4B*—F (5′-AGATCTATCACCATGGAATACTTCCTAGGAGG-3′) and *larp4B*-R (5′-TCTAGATTAAGAGTTGTTGGCCCTGGAACT-3′), and subcloned into the *Eco*RV site of pBluescript SK+ (Stratagene, La Jolla, CA, USA). A cDNA encoding a Larp4B variant lacking both the LA and RRM domains (Larp4BΔLA-RRM) was generated as follows: Two DNA fragments encoding the *N*-terminal and the *C*-terminal regions were respectively amplified by PCR using two sets of primers, larp4B—F and *larp4B*-ΔLA-RRM-R (5′-GGACAAGCTCGGCGGCGGCAAGCCCAAGTCGTTTATCAA-3′), and *larp4B*-ΔLA-RRM-F (5′-ACTTGGGCTTGCCGCCGCCGAGCTTGTCCAGCGGTATGT-3′) and *larp4B*-R. The resulting two fragments were used as templates for second PCR with primers *larp4B*—F and *larp4B*-R to generate the deletion variant cDNA. Both wild-type and the variant cDNAs were digested with *Bgl*II and *Xba*I, and subcloned into the corresponding sites of the pUASTattB transgenesis vector [25]. Transgenic flies were generated using φC31 integrase-mediated site-specific germline transformation [25]. The recipient strain was *y w; attP40*.

2.7. Quantitative RT-PCR analysis

Total RNA was extracted from adult whole flies or heads overexpressing Larp4B or GFP (control), and reverse-transcribed using the Superscript VILO cDNA Synthesis kit (Thermo Fisher scientific). Quantitative PCR (qPCR) was carried out using SYBR Premix Ex Taq (TaKaRa Bio, Shiga, Japan) and a Chromo 4 Detector (MJ Research, Hercules, CA, USA). Expression levels were normalized by the level of *rp49* mRNA. Relative values were calculated against that of the control, and mean ± SE values were calculated from technical triplicate. At least two independent tests were carried out to assess the reproducibility. The sequences of primers are listed in Table S1.

Please cite this article in press as: M. Funakoshi, et al., Overexpression of Larp4B downregulates dMyc and reduces cell and organ sizes in *Drosophila*, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.02.148