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Data Article

Dataset on the effect of Rubicon overexpression on polyglutamine-induced locomotor dysfunction in *Drosophila*

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ABSTRACT

The accumulation of pathogenic misfolded proteins is believed to be a common mechanism of generation of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and polyglutamine (polyQ) diseases. The autophagy–lysosome degradation system has been considered as a potential therapeutic target against these disorders, as it is able to degrade large protein aggregates. Previously, we focused on Rubicon, a negative regulator of autophagy, and demonstrated that knockdown of the *Drosophila* homolog of Rubicon (dRubicon) suppressed locomotor dysfunction in a fly model of polyQ disease. This suppression was associated with increased autophagic activity and a marked reduction in the number of polyQ inclusion bodies [1]. We generated transgenic fly lines expressing hemagglutinin-tagged dRubicon wild-type (WT) or dRubicon in which the RUN [after RPIP8 (RaP2 interacting protein 8), UNC-14 and NESCA (new molecule containing SH3 at the carboxyl-terminus)] domain was deleted (ΔRUN). We provide data regarding the effect of WT and ΔRUN dRubicon co-expression on polyQ-induced locomotor dysfunction in *Drosophila*.

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Specifications Table

Subject	Biology
Specific subject area	Molecular neurobiology, neurodegenerative disease
Type of data	Graph Figure
How data were acquired	Immunoblotting, locomotor function analysis
Data format	Raw Analyzed
Parameters for data collection	Immunoblotting and locomotor function analysis in adult *Drosophila melanogaster*
Description of data collection	The expression of dRubicon was assessed by immunoblotting. The locomotor function analysis was performed using flies expressing MJDtrQ78 (a truncated form of MJD1 with a 78 glutamine repeat), with or without dRubicon.
Data source location	Diabetic Neuropathy Project, Department of Disease and Infection, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
Data accessibility	With the article
Related research article	S. Nakamura, M. Oba, M. Suzuki, A. Takahashi, T. Yamamuro, M. Fujiwara, K. Ikenaka, S. Minami, N. Tabata, K. Yamamoto, S. Kubo, A. Tokumura, K. Akamatsu, Y. Miyazaki, T. Kawabata, M. Hamasaki, K. Fukui, K. Sango, Y. Watanabe, Y. Takabatake, T.S. Kitajima, Y. Okada, H. Mochizuki, Y. Isaka, A. Antebi, T. Yoshimori. Suppression of autophagic activity by Rubicon is a signature of aging. Nat. Commun. 10(2019) 847 https://doi.org/10.1038/s41467-019-08729-6

Value of the Data

- The present data support the importance of Rubicon in polyglutamine (polyQ)-induced locomotor dysfunction, and provide information about the functional domain of Rubicon modulating the toxicity in *Drosophila*.
- Rubicon knockdown extends the lifespan and suppresses age-associated changes in worms, flies, and mice. The data are valuable to researchers investigating the molecular mechanisms of autophagy, neurodegenerative diseases, and aging.
- The present data might be used for the development of further experiments investigating the way in which an increase in Rubicon exacerbates the toxicity of the polyQ protein.

1. Data Description

Alignment of Human and *Drosophila* Rubicon sequences by CLUSTAL W [2] and domain search using InterPro (http://www.ebi.ac.uk/interpro/) showed that both human and *Drosophila* Rubicon bear N-terminal RUN [after RPIP8 (RaP2 interacting protein 8), UNC-14, and NESCA (new molecule containing SH3 at the carboxyl-terminus)] domain and the C-terminal really interesting new gene (RING)-type zinc finger domain (Fig. 1). We established transgenic fly lines expressing hemagglutinin (HA)-tagged dRubicon wild-type (WT), or with a deletion of the RUN domain (ΔRUN) using the GAL4/upstream activating sequence (UAS) system. We used *Daughterless* (*da*)-*GAL4*, which directs ubiquitous expression, for immunoblotting analysis, and *embryonic lethal abnormal vision* (*elav*)-*GAL4*, which directs pan-neuronal expression, for locomotor function assays. We prepared whole-body lysates from *Canton-S* (WT fly strain), *da-GAL4/UAS-WT dRubicon-HA*, and *da-GAL4/UAS-ΔRUN Rubicon-HA* flies, and performed immunoblotting using an

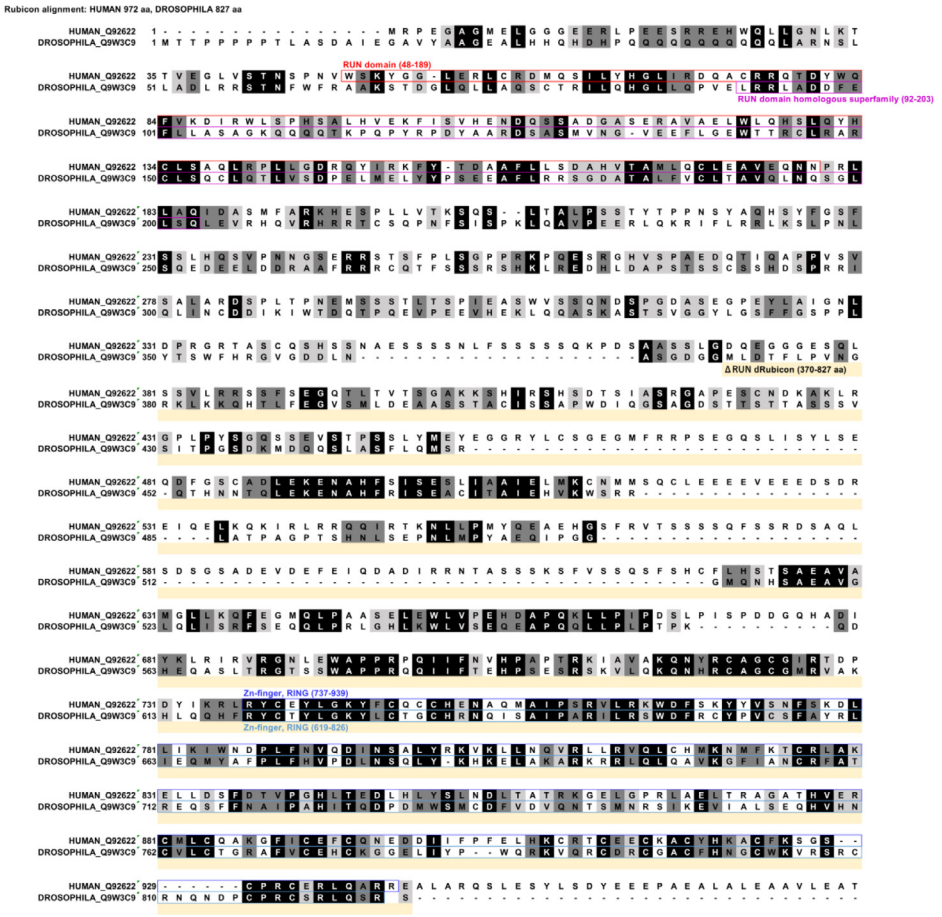

Fig. 1. Amino acid alignment of human and *Drosophila* Rubicon.
Amino acid alignment of the human and *Drosophila* homolog of Rubicon performed using CLUSTAL W [2]. The amino acid sequences of the UniProt accession number indicated after the species name were used for the analysis. Domain information was taken from the InterPro database (http://www.ebi.ac.uk/interpro/). RUN domain, after RPIP8 (RaP2 interacting protein 8), UNC-14 and NESCA (new molecule containing SH3 at the carboxyl-terminus) domain; ΔRUN, deletion of the RUN domain; RING, really interesting new gene.

antibody against HA and Actin (Fig. 2). We then analyzed the effect of dRubicon co-expression on the locomotor dysfunction induced by the expression of a polyQ protein, MJDw (Fig. 3, see Supplementary Tables for primary raw data, summary data, and results of statistical analyses).

2. Experimental Design, Materials and Methods

2.1. Generation of dRubicon transgenic flies

The WT dRubicon fragment was amplified using polymerase chain reaction (PCR) from RH61467 (*Drosophila* Genomics Research Center, IN, USA) using the following primer pair: forward primer 5′-CACCATGACCACGCCCCCGCCA-3′ and reverse primer 5′-GCTGGCACGGCTTTGAAGGC-3′. Similarly, the ΔRUN dRubicon fragment was amplified

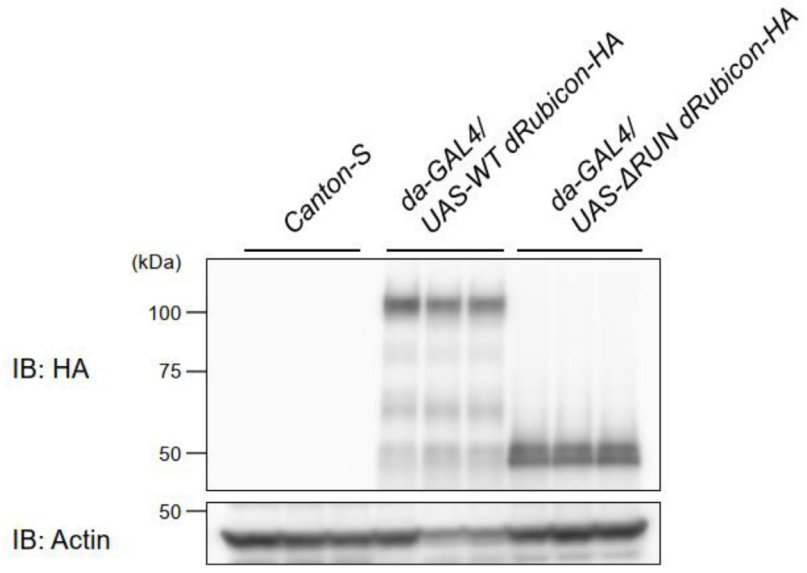

Fig. 2. Immunoblotting analysis of transgenic dRubicon.
Whole-body lysates were prepared from *Canton-S* (WT fly strain), *da-GAL4/UAS-WT dRubicon-HA*, and *da-GAL4/UAS-ΔRUN Rubicon-HA* flies two to three days after eclosion. The ubiquitous expression driver *da-GAL4* was used for transgene expression.

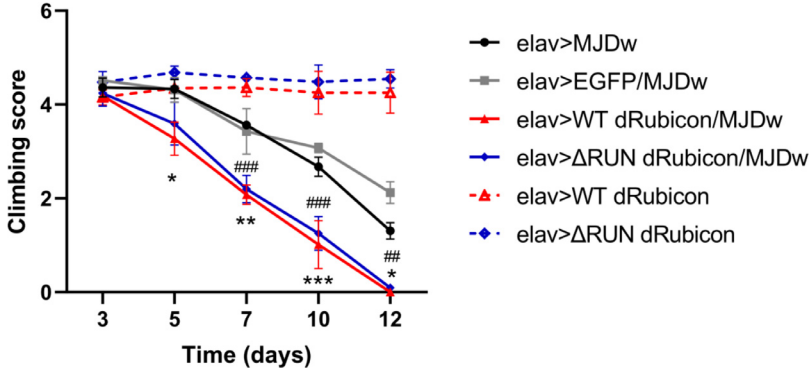

Fig. 3. Locomotor function analysis of flies expressing dRubicon and a polyQ protein.
WT or ΔRUN dRubicon was co-expressed with MJDw, a C-terminal fragment of the human MJD/SCA3 protein with a 78 repeat polyQ tract, in neurons under control of the *elav-GAL4* driver. Locomotor function was assessed by the climbing assay from 3 to 12 days after eclosion. elav>MJDw, *elav-GAL4/+;+/+;UAS-MJDw/+*; elav>EGFP/MJDw, *elav-GAL4/+;UAS-EGFP/+;UAS-MJDw/+*; elav>WT dRubicon/MJDw, *elav-GAL4/+;+/+;UAS-WT dRubicon-HA/UAS-MJDw*; elav>ΔRUN dRubicon/MJDw, *elav-GAL4/+;+/+;UAS-ΔRUN dRubicon-HA/UAS-MJDw*; elav>WT dRubicon, *elav-GAL4/+;+/+;UAS-WT dRubicon-HA/+*; elav>ΔRUN dRubicon, *elav-GAL4/+;+/+;UAS-ΔRUN dRubicon-HA*. Data represent mean ± s.e.m. *P* values were obtained using two-way ANOVA followed by Tukey's *post hoc* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for elav>WT dRubicon/MJDw vs. elav>MJDw. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ for elav>ΔRUN dRubicon/MJDw vs. elav>MJDw.

using the primer pair: forward primer 5′-CACCATGTTGGACACCTTCC-3′ and reverse primer 5′-GCTGGCACGGCTTTGA-3′. The fragments were inserted into the pENTR vector, and then transferred to pTWH using the LR recombination reaction system (Invitrogen, CA, USA). Transgenic fly lines were generated using w^{1118} flies as a host strain (BestGene Inc., CA, USA).

2.2. Fly stocks and culture conditions

Flies were grown on a standard cornmeal-agar-yeast-based medium at 25°C. Transgenic fly lines bearing *UAS-MJDtr-Q78w* (BDSC_8141), *da-GAL4* (BDSC_55849), and *elav-GAL4^{c155}* (BDSC_458) were obtained from the Bloomington *Drosophila* Stock Center (IN, USA). Female flies were used for all the experiment.

2.3. Immunoblotting

Two whole fly bodies were lysed in 100 μl of 2 $\times$ sodium dodecyl sulfate (SDS) sample buffer (4% SDS, 125 mM Tris-HCl, pH 6.8, 20% Glycerol, 0.01% bromophenol blue, and 10% 3-mercapto-1,2-propanediol, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) 2–4 days after eclosion. The lysates were heated at 99 °C for 10 min. After centrifugation at 15,000 $\times$ g for 10 min, the supernatants were collected. The protein lysates were separated on 5–12% polyacrylamide gels and transferred to polyvinylidene fluoride membranes (Merck Millipore, Darmstadt, Germany). After blocking with 5% skim milk in phosphate-buffered saline (PBS) containing 0.1% Tween 20, the following antibodies were used: anti-HA (3F10, 1:500, Sigma-Aldrich Japan, Tokyo, Japan), anti-Actin (JLA20, 1:2000, Developmental studies Hybridoma Bank, IA, USA), horseradish peroxidase (HRP)-conjugated anti-rat IgG (1:10000, Jackson ImmunoResearch Laboratories, PA, USA), and HRP-conjugated anti-mouse kappa light chain (1:10000, Novus Biologicals, CO, USA). The antibodies were diluted in Can Get Signal® (TOYOBO, Osaka, Japan) immunoreaction enhancer solution. The bands were visualized with ImmunoStar Zeta or LD (FUJIFILM Wako Pure Chemical Industries, Osaka, Japan), and images were captured using an LAS-4000 imaging system (FUJIFILM Wako Pure Chemical Industries, Tokyo, Japan).

2.4. Locomotor function assay

Negative geotaxis climbing assays were performed to assess locomotor function, according to a previously published protocol [3], with slight modifications. Experimental flies were collected over a 48 h period after eclosion, and a total of 10–20 flies were assigned to each vial containing the standard medium. Every two or three days after assignment, the flies were placed in a conical glass vial (length, 15 cm; diameter, 2.5 cm, AGC techno glass, Shizuoka, Japan) without anesthesia. Ten seconds after tapping the flies to the bottom of the vial, the number of flies in each vertical area was counted, and scored according to the distance climbed, as follows: score 0 (0–2 cm), 1 (2–3.9 cm), 2 (4–5.9 cm), 3 (6–7.9 cm), 4 (8–9.9 cm), and 5 (10–15 cm). Three trials with 20 s intervals were performed for each group. The climbing index was calculated as follows: each score, multiplied by the number of flies, was divided by the total number of flies. The mean score of the three trials was calculated. Results are presented as the mean $\pm$ s.e.m. of the scores obtained from three to six independent populations. Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test, using GraphPad Prism (GraphPad Software, CA, USA).

Ethics Statement

All experiments comply with the ARRIVE guidelines and were performed under the Guidelines for the Care and Use of Animals of Tokyo Metropolitan Institute of Medical Science. All authors declare that the paper provides entirely original work. The policies outlined in the Guide for Authors were considered during the preparation of the paper.

CRediT Author Statement

Masaki Oba: Investigation, Writing - Original Draft; **Koji Fukui**: Supervision; **Kazunori Sango**: Supervision, Project administration; **Mari Suzuki**: Conceptualization, Methodology, Writing - Review & Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2021.107222.

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