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A restricted level of PQBP1 is needed for the best longevity of Drosophila

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Abstract

A number of neurological diseases are caused by mutations of RNA metabolism-related genes. A complicating issue is that whether under- or overfunction of such genes is responsible for the phenotype. Polyglutamine tract binding protein-1, a causative gene for X-linked mental retardation, is also involved in RNA metabolism, and both mutation and duplication of the gene were reported in human patients. In this study, we first report a novel phenotype of dPQBP1 (drosophila homolog of Polyglutamine tract binding protein-1)-mutant flies, lifespan shortening. We next address the gene dose-phenotype relationship in lifespan shortening and in learning disability, a previously described phenotype. The 2 phenotypes are rescued by dPQBP1 but in different dose-phenotype relationships. Either insufficient or excessive expression of dPQBP1 does not recover lifespan, while excessive expression recovers learning ability. We finally address the mechanism of lifespan shortening. Tissue-specific expression of dPQBP1-RNA interference construct reveals both neural and nonneural dPQBP1 contribute to the lifespan, while the latter has a dominant effect. Gene expression profiling suggested retinophilin/MORN repeat containing 4, a gene promoting axonal degeneration, to contribute to lifespan shortening by neural dPQBP1. Systems biology analysis of the gene expression profiles revealed indirect influence of dPQBP1 on insulin-like growth factor 1, insulin receptor, and peroxisome proliferator-activated receptorα/γ signaling pathways in nonneural tissues. Collectively, given that dPQBP1 affects multiple pathways in different dose-dependent and tissue-specific manners, dPQBP1 at a restricted expression level is needed for the best longevity.

Keywords: Lifespan; Longevity; PQBP1; RNA; Drosophila

1. Introduction

A number of causative genes for neurodegeneration are involved in RNA metabolism. For instance, ataxin-2 causing spinocerebellar ataxia type 2 associates with an RNA binding protein with RNA binding motif ataxin 2-binding protein 1 (Shibata et al., 2000) and interacts with RNA helicase DEAD (Asp-Glu-Ala-Asp) box helicase 6 (Nonhoff et al., 2007); ataxin-1 causing spinocerebellar ataxia type 1 indirectly associates with RNA through RNA binding motif protein 17; trans activation responsive region (TAR) DNA-binding protein (TDP) 43 (TDP43) for frontotemporal lobar degeneration (FTLD) possesses 2 RNA recognition motif (Buratti and Baralle, 2001) and it is implicated in pre-m(messenger)RNA splicing, mRNA stability, and mRNA transport (Buratti and Baralle, 2008); Fused In Sarcoma (FUS)/Translocated In Sarcoma (TLS), an FTLD gene, has an RNA recognition motif and actually interacts with RNA (Crozat et al., 1993). In the case of mental retardation (MR), the causative gene for fragile X syndrome (FMRP) is an RNA-

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binding protein with K homology (KH) domains and Arg-Gly-Gly repeats motifs and negatively regulates translation (Laggerbauer et al., 2001; Siomi et al., 1994). PQBP1, polyglutamine tract binding protein-1 (Waragai et al., 1999), which is defined as a polyglutamine stretch binding protein, involved in RNA splicing is another example whose mutation cause MR.

In such disorders, expression level of the causative gene is an issue to complicate understanding of the pathology. Overexpression of mutant TDP43 in transgenic mice causes FTLD and motor neuron disease phenotypes (Wegorzewska et al., 2009). Meanwhile, depletion of TDP43 causes neuronal degeneration in a culture model (Iguchi et al., 2009). Homozygous TDP43 knockout mice were embryonic lethal while heterozygous knockout mice were almost normal (Sephton et al., 2010). Overexpression of normal TDP43 also causes neurodegeneration in vivo (Uchida et al., 2012). Knockdown of valosin-containing protein, another causative gene for FTLD, induces functional disturbances and cell death in Hela or U2OS cells (Dalal et al., 2004; Mimnaugh et al., 2006; Wójcik et al., 2004), while expression of mutant valosin-containing protein causes impaired autophagy and mislocalization of TDP43 in the other model (Ju et al., 2009). In fragile X mutations, FMRP level is decreased usually. Meanwhile, intermediate elongation of nonmethylated CGG repeats increases expression of FMRP mRNA though RNA-mediated toxicity contributes to the pathology (Hagerman et al., 2011). Although the extent of understanding of the dose-phenotype relationship is different in each case, molecular mechanisms for such discrepancies are largely unknown.

PQBP1 was originally found as a binding protein to the polyQ tract sequence in yeast 2-hybrid screening (Imafuku et al., 1998; Waragai et al., 1999), and subsequently identified as a causative gene of X chromosome-linked MR (Kalscheuer et al., 2003; Lenski et al., 2004). An increasing number of reports indicated that the frequency of PQBP1-MR is rather high (Stevenson et al., 2005) and might be equivalent to that of Rett syndrome (Poirier et al., 2006). Clinical manifestations include microcephaly, short stature, and microorchidism (Stevenson et al., 2005). Correspondingly, PQBP1 mRNA was expressed ubiquitously in multiple tissues (Waragai et al., 1999), while PQBP1 protein was dominantly expressed in the brain (Qi et al., 2005). Most reported mutations lead to truncation of C-terminal domain (Kalscheuer et al., 2003; Kleefstra et al., 2004; Lenski et al., 2004) or reduction of mRNA by nonsense decay (Kalscheuer et al., 2003). PQBP1 interacts with RNA polymerase II through the domain with 2 conserved Trp (W) residues (Okazawa et al., 2002) and splicing-related proteins like U5-15kD (Waragai et al., 2000) and WW domain binding protein 11/splicing factor that interacts with PQBP-1 and PP1 (Llorian et al., 2004) through the unique C-terminal domain. Therefore, mutations had been expected to cause functional impairment of PQBP1. However, some recent reports showed MR patients associated with gene duplication including the *PQBP1* region (Flynn et al., 2011; Honda et al., 2010).

Drosophila possesses dPQBP1, a homologue of PQBP1. We previously generated dPQBP1-mutant flies that exhibit pure learning impairment (Tamura et al., 2010) and found that learning disturbance was based on inadequate expression of NMDA (N-methyl-D-aspartate) receptor subunit 1 (NR1) at the synapse of projection neurons (Tamura et al., 2010). The similar pathological role of NR1 was suggested in cognitive impairment of PQBP1 knockdown mouse (Ito et al., 2009).

In this study, we first report a new phenotype of the dPQBP1-mutant fly, lifespan shortening. We next address relationship between gene dose and the 2 phenotypes by using dPQBP1-mutant flies and their rescue lines. We finally ask molecular mechanisms responsible for the lifespan shortening. The results reveal different dose-phenotype relationships based on molecular mechanisms specific for each phenotype, which consequently limit the expression level of dPQBP1 for the best life of fly. Our data provide the idea that a narrow range of physiological expression level of RNA metabolism-related genes is critical for totally normal phenotypes.

2. Methods

2.1. Fly stocks and rearing conditions

All flies were raised on a corn meal medium without propionic acid and were maintained at 25 °C and 60% humidity under a 12:12 hour light-dark cycle. The dPQBP1-mutant and rescued flies are described previously (Tamura et al., 2010). The dPQBP1-mutant, in which a transposable element, *piggyBac* was inserted to the third chromosome, was outcrossed more than 6 generations with w1118 strain, w(CS10) using the yellow fluorescent protein marker. Cantonized w1118 strain was used as the wild type control in this study. The UAS-dPQBP1 transgenic fly, carrying a 0.7 kb full-length cDNA of CG11820 on second chromosome was developed from w1118 parental strain. Elav-Gal4 fly, GAL4elav.PLu was described previously (Tamura et al., 2010).

2.2. Lifespan assay and pharmacological rescue

For measurement of lifespan, 20 virgin females were reared in each food vial and transferred to fresh food vials every 2 or 3 days. Numbers of dead flies were counted every 1 to 3 days. Data were analyzed by log-rank test using SPSS 16.0 (Chicago, IL, USA). For pharmacological rescue, newly emerged flies were fed fly food containing 5 mM phenylbutylate (PBA). PBA solution was prepared in 2 ways.

First, 10× stock solution of PBA neutralized by NaOH was prepared and diluted 10 times in regular fly food. Second, 5000× stock solution of PBA in 100% dimethyl