Neuroscience Letters 648 (2017) 8–13

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Knockdown of APPL mimics transgenic Aβ induced neurodegenerative phenotypes in *Drosophila*

Sandeep Kumar Singh, Saurabh Srivastav, Amarish Kumar Yadav, Saripella Srikrishna*

Cancer and Neurobiology Laboratory, Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi 221005, India

HIGHLIGHTS

- APPL-RNAi mimics Aβ induced phenotypes like eye degeneration, reduced longevity and motor deficit functions.
- Neuronal tissue specific loss of APPL show reduced longevity comparable to Aβ expressing flies.
- APPL is required for motor neuron activity and eye development in *Drosophila*.

ARTICLE INFO

Article history:
Received 3 December 2016
Received in revised form 14 March 2017
Accepted 17 March 2017
Available online 20 March 2017

Keywords:
Drosophila
Alzheimer's disease
APP
APPL-RNAi
Longevity
Motor neuron function
Eye degeneration
SEM

ABSTRACT

A variety of *Drosophila* mutant lines have been established as potential disease-models to study various disease mechanisms including human neurodegenerative diseases like Alzheimer's disease (AD), Huntington's disease (HD) and Parkinson's disease (PD). The evolutionary conservation of APP (Amyloid Precursor Protein) and APPL (Amyloid Precursor Protein-Like) and the comparable detrimental effects caused by their metabolic products strongly implies the conservation of their normal physiological functions. In view of this milieu, a comparative analysis on the pattern of neurodegenerative phenotypes between *Drosophila* APPL-RNAi line and transgenic *Drosophila* line expressing eye tissue specific human Aβ (Amyloid beta) was undertaken. Our results clearly show that *Drosophila* APPL-RNAi largely mimics transgenic Aβ in various phenotypes which include eye degeneration, reduced longevity and motor neuron deficit functions, etc. The ultra-structural morphological pattern of eye degeneration was confirmed by scanning electron microscopy. Further, a comparative study on longevity and motor behaviour between Aβ expressing and APPL knockdown lines revealed similar kind of behavioural deficit and longevity phenotypes. Therefore, it is suggested that APPL-knockdown approach can be used as an alternative approach to study neurodegenerative diseases in the fly model. To the best of our knowledge this is the first report showing comparable phenotypes between APPL and Aβ in AD model of *Drosophila*.

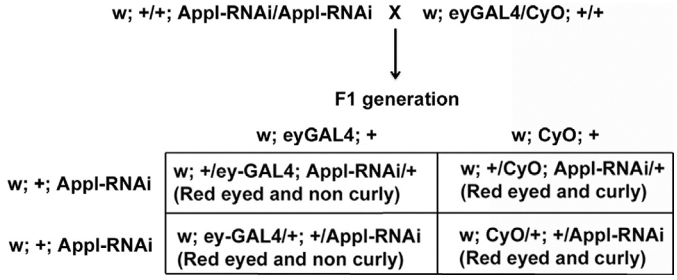
1. Introduction

Amyloid beta peptide, the main culprit of AD, is generated by the proteolytic processing of the APP via amyloidogenic pathway [1] by the combinatorial action of β- and γ-secretases [2]. Besides δ-secretase mediated processing of APP is also known to regulate the AD pathogenesis [3]. Earlier reports show that APP is well conserved across species, from APL-1 in *Caenorhabditis elegans* [4], APPL in case of *Drosophila* [5], APP747 in *Xenopus* [6], to APP, APLP1, and APLP2 in mammals [7–9]. Thus, it can be suggested that APP may have some common essential physiological functions. In the case of humans, three different protein isoforms (APP695, APP751, and APP770) are known to be generated by the alternate splicing of mRNA encoded by Human *APP* gene [10–12]. APP interacts with various conserved signalling protein molecules and can be used as a potential therapeutic target [13,14]. Also, recent studies based on computational approaches have suggested some common targets for the treatment of neurodegenerative diseases [15,16]. In Drosophila, APPL shows high sequence similarity and structural homology to APP and function as human APP [5]. *Drosophila* homolog of the APP is also known to serve as a conserved modulator of Wnt-PCP pathway [17]. Mutational analysis of Appl indicates that it is not a vital gene in *Drosophila* [18]; however, the behavioural deficits exhibited by mutant flies lacking Appl gene ($Appl^{d}$) imply that APPL protein is

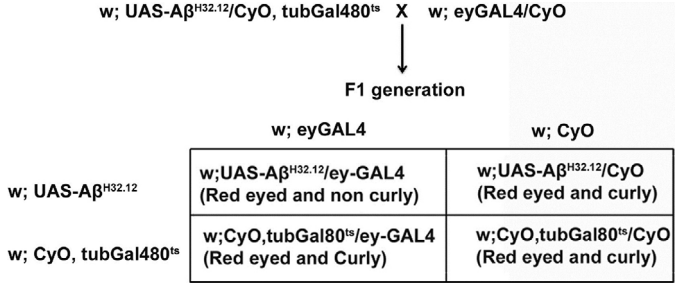
Abbreviations: Aβ, Amyloid beta peptide; AD, Alzheimer's disease; APP, Amyloid Precursor Protein; APPL, Amyloid Precursor Protein-Like; CPD, critical point drying; SEM, scanning electron microscopy; UAS, Upstream Activating Sequence.

* Corresponding author.
E-mail addresses: skrishna@bhu.ac.in, sskrishna2000@yahoo.com (S. Srikrishna).

http://dx.doi.org/10.1016/j.neulet.2017.03.030
0304-3940/



Scheme 1. The F1 generation with red eyed and non-curly wing flies carrying the APPL-RNAi insert (w; +ey-GAL4; APPL-RNAi/+ or w; ey-GAL4/+; +APPL-RNAi) was selected for further experiments.



Scheme 2. The F1 generation with red eyed and non-curly flies carrying the UAS-Aβ insert (w; UAS-Aβ$^{H32.12}$/ey-GAL4) was selected for further experiments.

necessary for the proper functioning of the nervous system. Previous study by Goguel et al., suggested that APPL is also required for long term memory in *Drosophila* [19]. The evolutionary conservation of APPL and APP in the similarities in their processing and downstream effects caused by Aβ peptides strongly suggest the conservation of normal physiological functions. However, the cellular processes by which these proteins engage in the nervous system remain poorly understood. Therefore, in vivo studies in *Drosophila* might provide valuable insights into the normal function of human APP proteins. In this context, we have employed *Drosophila* APPL-RNAi and transgenic Aβ expressing fly lines to study pathophysiological aspects between these two strains. We found that these two strains mimic AD like symptoms and cause neurodegeneration strikingly in similar manner.

2. Methods

2.1. Drosophila *stocks and their rearing*

Stocks, (w[*];P{w[+mC]=UAS-Dab.W}2,P{w[+mC]=GAL4-ninaE.GMR}12/CyO) and y[1]v[1];P{y[+t7.7] v[+t1.8]=TRiP.JF02878}attP2 obtained from Bloomington stock centre and w; UAS–Aβ$^{H32.12}$/CyO, tubGal80^{ts} having Aβ transgene under the control of UAS promoter was generously gifted by Dr. M. Konsolaki (Department of Genetics, Rutgers, The State University of New Jersey, USA). These stocks have been used for the genetic experiments and abbreviated in the text as ey-GAL4 and APPL-RNAi and UAS-Aβ respectively. All crosses and stocks were maintained at 24 ± 1 °C on normal food consisted of standard cornmeal, yeast and sugar [20].

2.2. Genetic cross

The GAL4/UAS system was used for the tissue specific expression of UAS transgene in *Drosophila* [18,21]. Virgin female flies collected from the APPL-RNAi and UAS-Aβ lines were separately crossed with male flies carrying the ey-GAL4 driver balanced with curly (CyO) balancer on 2nd chromosome (Schemes 1 and 2). Adult flies of F1 progeny were collected up to 10 days and kept them in separate vials to avoid the mixing of offsprings from the next generation. The F1 generation was having both curly as well as non-curly winged flies. Ey-GAL4 driven APPL-RNAi and UAS-Aβ were selected as shown in Scheme 1 and Scheme 2, respectively. The flies were observed under binocular microscope for eye degeneration phenotypes.

2.3. Scanning electron microscopy

The method of Tanya Wolff [22], with minor modification was followed for the SEM analysis. Around 8–10 non-curly flies from the F1 progeny of each group of Scheme 1 (normal, mild, severe and highly severe) and Scheme 2 were etherized and heads were detached and placed them into a 1.5 ml eppendorf tube. To this 1.5 ml of fixative (0.1 M PBS, 25% glutaraldehyde and dH_2O) was added in each vial followed by 1–2 drops of Tween20. Tubes were kept at 4 °C for overnight on a shaker. Tissues were washed once with PBS and then twice with distilled water for 15 min each. Dehydration was done by using 25%, 50%, 75% and 100% alcohol serially for about 3 h. Finally, the tissues were washed with 100% alcohol three times for 15 min each. Then CPD (critical point drying) was done for the preservation of structure and removing any moisture present in the sample. After CPD coating was done with gold and images of sample from each group were captured by Scanning Electron Microscope (Hitachi S-3400N).

2.4. Climbing assay for motor activity

This assay has been performed to test the comparative locomotor activity of $A\beta_{42}$ and APPL-RNAi-expressing flies. Test vials of 10 cm length containing each of the three classes (Gal4-elavC^{155}, elav-Appl and elav-$A\beta_{42}$ flies) were taken for the measurement of comparative motor neuron ability by using climbing assay experiment. Twenty flies were placed in each plastic vial and gently tapped to the bottom. The number of flies at the top of the vial was counted after 18 s. The percentage of flies climbed up to the top of the vial was calculated over time. Each experiment included 15 vials with 20 flies in each vial, to the total of 300 flies for each condition. The climbing assay was repeated 3 times. Statistical analysis was done by using Prism3, One-way ANOVA.

2.5. Longevity assay

Ten flies each expressing APPL-RNAi, $A\beta_{42}$ peptide and *Gal4-elavC^{155}* was cultured at 29 °C. Ten vials containing each group ($n = 100$) have been taken for longevity assay. *Gal4-elavC^{155}* flies reared in normal food were taken as control and flies expressing $A\beta_{42}$ and APPL-RNAi were compared with control for their survival ability. Viable transgenic (Aβ and APPL-RNAi driven) and control (*Gal4-elavc155*) flies were counted on day to day basis. Experiment was repeated for 3 times. Differences in survival were analyzed using the Kaplan–Meier analysis.

3. Results

3.1. Comparative statistical analysis of eye phenotypes between APPL-RNAi and UAS-Aβ

The comparative analysis of neurodegenerative eye phenotypes was done between UAS-Aβ and APPL-RNAi expressing flies in triplicate. Three different eye phenotypes were found in case of APPL-RNAi ranging from mild, severe to highly severe with complete loss of ommatidia and shortening of the eye. While in case of UAS-Aβ only two types of eye phenotype were observed (mild