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dXNP/DATRX increases apoptosis via the JNK and dFOXO pathway in *Drosophila* neurons

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ABSTRACT

Mutation of the *XNP/ATRX* gene, which encodes an SNF2 family ATPase/helicase protein, leads to ATR-X syndrome and several other X-linked mental retardation syndromes. Although XNP/ATRX is a chromatin remodeler, the molecular mechanism by which mental retardation occurs in patients with ATR-X has yet to be determined. To better understand the role of XNP/ATRX in neuronal development, we expressed *Drosophila XNP* (*dXNP/DATRX*) ectopically in *Drosophila* neurons. Neuronal expression of *dXNP/DATRX* resulted in various developmental defects and induced strong apoptosis. These defects and apoptosis were suppressed by *Drosophila* inhibitor of apoptosis protein 1. Expression of *dXNP/DATRX* also increased JNK activity and the levels of *reaper* and *hid* transcripts, which are pro-apoptotic factors that activate caspase. Furthermore, *dXNP/DATRX*-induced rough eye phenotype and apoptosis were suppressed by *dFOXO* deficiency. These results suggest that dXNP/DATRX is involved in caspase-dependent apoptosis in *Drosophila* neurons *via* regulation of the JNK and *dFOXO* pathway.

Mutation of the *XNP/ATRX* gene, which encodes a member of the SNF2 family of proteins with ATPase and helicase domains, is associated with several mental retardation syndromes, including X-linked α-thalassemia/mental retardation (ATR-X) syndrome [1–4]. ATR-X syndrome is a pleiotropic disorder characterized by facial dysmorphism, urogenital defects, α-thalassemia, and mental retardation [5,6]. A major symptom of this syndrome is profound mental retardation, including various psychomotor retardations [7]. Patients also show speechlessness, microcephaly, spasticity, or seizures [8].

Consistent with the symptoms in human patients, XNP/ATRX orthologs have been implicated in neuronal development in animal models. *Atrx* overexpression in transgenic mice was associated with neural tube defects [9], and the loss of ATRX protein in the developing mouse forebrain resulted in widespread hypocellularity in the neocortex and hippocampus [10]. In *Drosophila*, dXNP/DATRX, a *Drosophila* homolog of human ATRX, has been identified as a functional interacting partner of Jing, a transcription factor required for central nervous system (CNS) midline formation [11]. *dXNP/DATRX* is expressed at high levels in embryos, the larval CNS, and the adult head [11,12]. Cell-specific knockdown of *dXNP/DATRX* gene expression results in defects of connective formation in longitudinal axons [11].

Several cellular processes have been proposed as underlying mechanisms of *XNP/ATRX*-deficiency-related defects [9,10,13–16]. The pattern of DNA methylation is altered in the rDNA Y-specific repeats and subtelomeric repeats in the lymphocytes of patients with ATR-X syndrome [13]. Transcriptional repression activity of ATRX has been demonstrated by reporter gene assay using the GAL–ATRX fusion protein and GAL-TK-luciferase reporter [14]. In conditional knockout mice, loss of the ATRX protein caused widespread hypocellularity in the CNS due to increased neuronal apoptosis [10], and elimination of *p53* in double-knockout mice rescued cell death in the embryonic telencephalon [15]. Most recently, ATRX was suggested to have a mitotic function [16]. The transition from prometaphase to metaphase is prolonged in ATRX-depleted cells, and loss of ATRX in the embryonic mouse brain induces mitotic defects in neuroprogenitors [16].

Previously, we reported that overexpression of *dXNP/DATRX* in developing tissues of *Drosophila* induced apoptosis *via* activation of JNK [12]. However, the detailed molecular mechanism of dXNP/DATRX-induced apoptosis is unclear, as is the role of dXNP/DATRX in neuronal development of *Drosophila*. Here we

Abbreviations: ATRX, α-thalassemia X-linked mental retardation; DATRX, *Drosophila* ATRX; dXNP, *Drosophila* XNP; JNK, Jun-N-terminal kinase; XNP, X-linked nuclear protein.

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show that the ectopic expression of *dXNP/DATRX* in various developing neuronal cells results in developmental defects, induction of apoptosis, JNK activation, and increased levels of *reaper* and *hid* transcripts. Furthermore, we report that *dFOXO* mutations suppress dXNP/DATRX-induced apoptosis. These results suggest that dXNP/DATRX regulates caspase-dependent apoptosis in *Drosophila* neurons *via* the JNK and dFOXO pathway.

Materials and methods

Fly strains. *elav-GAL4*, *tyrosine hydroxylase* (*TH*)*-GAL4*, *Dopadecarboxylase* (*Ddc*)*-GAL4*, *glass multimer reporter* (*GMR*)*-GAL4*, *UAS-p53*, *UAS-DIAP1*, *UAS-2× enhanced green fluorescent protein* (*EGFP*), *dXNP*^{1} (*EP635*), and *dXNP*^{2} (*UY3132*) were obtained from the Bloomington *Drosophila* Stock Center. The *dFOXO* mutants *dFOXO*^{21} and *dFOXO*^{25} were gifts from E. Hafen (University of Zürich, Switzerland). In *dFOXO*^{21} and *dFOXO*^{25}, the codons for W95 and W124 within the forkhead domain are mutated to stop codons, respectively. Although they are assumed to be null alleles of *dFOXO*, *dFOXO*^{21} and *dFOXO*^{25} are homozygous viable and display no obvious phenotype under normal culture conditions [17].

Ectopic gene expression using UAS-GAL4 system. For ectopic expression of *dXNP/DATRX*, we used the same modular misexpression system as in our previous study [12]. *elav-GAL4*, *TH-GAL4*, *Ddc-*

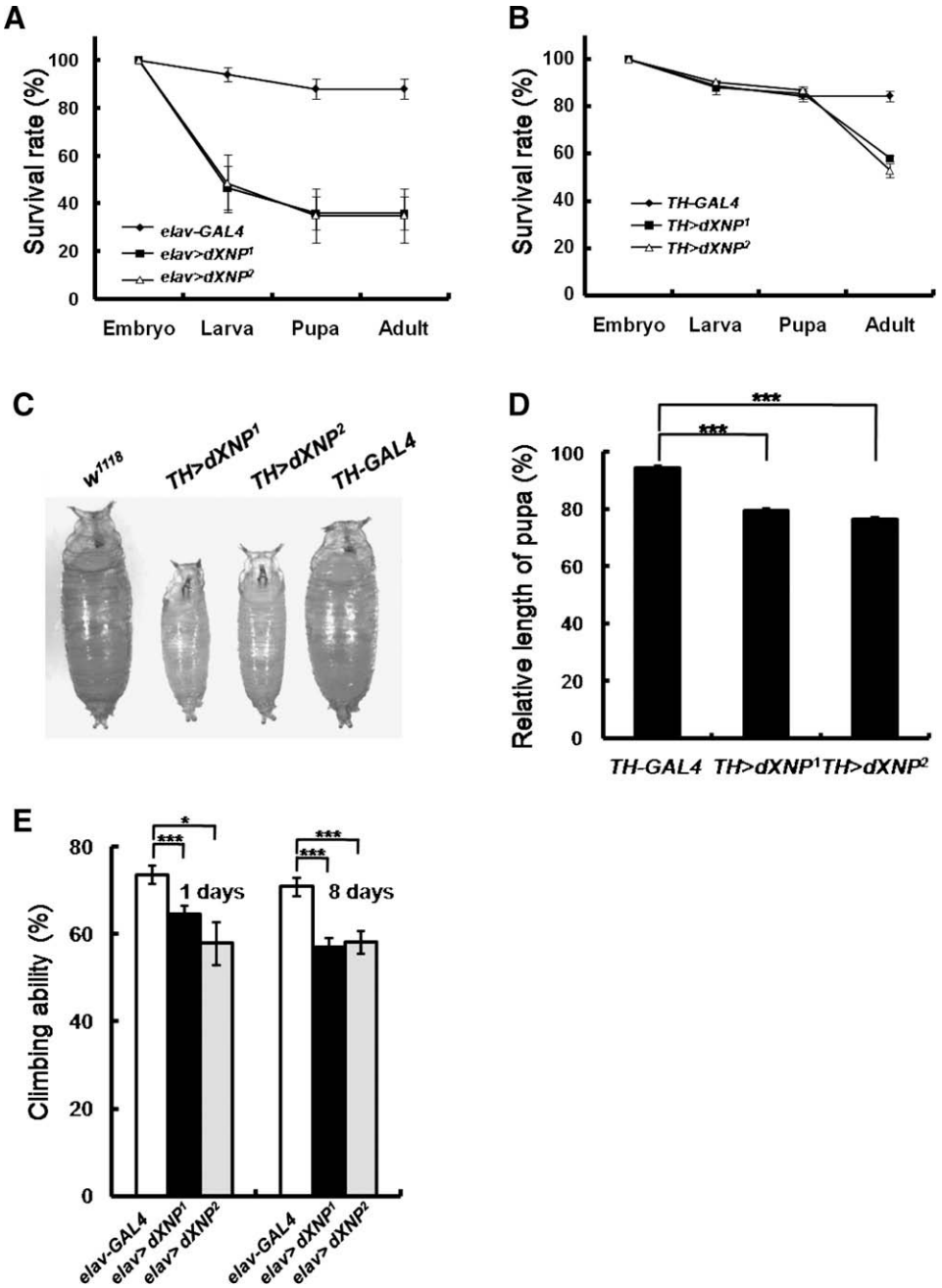

Fig. 1. Phenotypes of *dXNP/DATRX*-overexpressing flies. (A) Pan-neuronal overexpression of *dXNP/DATRX* using the *elav-GAL4* driver resulted in decreased survival among flies ($n = 200$). (B,C) Overexpression of *dXNP/DATRX* in the dopaminergic neurons using the *TH-GAL4* driver resulted in pupal lethality ($n = 200$) (B) and reduction of pupal size (C). (D) Statistical analysis of (C) ($^{***}P < 0.001$, $n \geqslant 58$, Student's t test). Error bars represent ± SE. (E) Climbing ability was measured as described in Materials and methods. ($^{***}P < 0.001$, $^{*}P < 0.05$, $n \geqslant 13$, Student's t test.)