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Localized Depletion of Drosophila *Upf1* and *Upf2* in the Central Nervous System Leading to Anatomical Defects of the CNS of the Embryo and Larva

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Abstract

Nonsense-mediated mRNA decay (NMD) is a quality control pathway that degrades mRNAs with premature termination codons, (PTC), which otherwise will result into a truncated protein with a potential dominant-negative effect. NMD is also an RNA turnover regulatory mechanism for controlling a significant percentage of wild type mRNAs. The pathway functions via coordination of some proteins known as NMD factors. The core NMD factors are Upf1, Upf2, and Upf3, which, in coordination with other important factors tag and mobilize an mRNA for degradation. The role of NMD in the architectural development of central nervous system (CNS) in different organisms has been studied. However, these *in vivo* studies of the involvement of NMD presented some challenges due to the lethality of Upf1 and Upf2. In this work, we studied the CNS localized loss of function and considered the effect of depletion of Upf1, Upf2, and Upf3 at an early and late *Drosophila* embryogenesis on the overall anatomical structures of the CNS in the embryo and the larvae. We found that depletion of Upf1 and Upf2 at early embryogenesis caused a significant reduction in the hatching and viability of the embryos. Also, the area of the ventral nerve cord in relation to the total area of the embryo was significantly reduced. However, the Upf3 depleted embryos exhibited normal hatching rates, viability, and ventral nerve cord area when compared with the control. Additionally, CNS localized depletion of Upf1 and Upf2 but not Upf3 at the late stage of embryogenesis resulted in the reduction of the optic lobe of the third instar larval brain. Our findings suggest that NMD factors Upf1 and Upf2 are necessary during the early and late *Drosophila* CNS development. Their absence in the CNS interferes with its developmental processes.

KeyWords: Nonsense-mediated mRNA decay, NMD factors, Central nervous system, Drosophila melanogaster, Embryogenesis

1. Introduction

RNA is the central conduit via which gene information is expressed. Consequently, controlling the half-life of RNA (RNA turnover) as an essential way of regulating the expression of gene information and the level of protein (Serin *et al.*, 2001; Bicknell and Ricci, 2017; Cheng *et al.*, 2017). A major RNA turnover mechanism is nonsense-mediated mRNA decay (NMD). NMD is a highly conserved surveillance pathway that cleans the system from aberrant mRNA harboring premature termination codon (PTC), which, if translated, will lead to possibly undesirable proteins with dominant-negative effect (Ghosh and Jacobson, 2010; Hug *et al.*, 2016; Nickless *et al.*, 2017). Beyond the cleaning task, NMD regulates a considerable percentage of wild-type mRNAs, having thus a gene regulatory function in addition to the quality assurance function (Wittkopp *et al.*, 2009; Nasif *et al.*, 2017). The essential player proteins of the NMD (NMD factors) in *Saccharomyces cerevisae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and vertebrates are Up-frameshift suppressor (Upf) proteins (Upf1, Upf2, and Upf3) (Peccarelli and Kebaara, 2014; Fatscher *et al.*, 2015; Son *et al.*, 2017). These core NMD factors in coordination with other factors in rather controversial modes activate and execute NMD functions.

NMD has demonstrated significance in the general development and architectural arrangement of CNS in different organisms (Laumonnier *et al.*, 2010; Barone and Bohmann, 2013). For example, NMD is essential in the development and maintenance of synapse structure and function in *Drosophila* (Metzstein and Krasnow, 2006; Long *et al.*, 2009; Giannandrea *et al.*, 2013). Similarly, NMD is indispensable for the proper development of the brain in zebrafish (Wittkopp *et al.*, 2009). In humans, Upf3B-NMD was associated with proper nervous system function (Tarpey *et al.*, 2007; Chan *et al.*, 2009). Furthermore, Upf3 and Upf2 NMDs were associated with neurophysiological abnormalities in humans, mice, and flies (Huang *et al.*, 2018; Johnson *et al.*, 2019; Jega *et al.*, 2020). *In vitro* studies with mouse cell lines have shown that the Upf1-NMD function is critical for neural differentiation (Lou *et al.*, 2014), and Upf3 was associated

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with an increase in the self-renewal of primary neural progenitor cells at the expense of their differentiation (Jolly *et al.*, 2013). In another *in vitro* study, Upf3B has shown an influence on the hippocampal neurite growth. The knockdown mutants were found to have altered expression of SIX3 and ROBO1 genes, a master regulator of cortical development and axon guide genes respectively (Nguyen *et al.*, 2012; Alrahbeni *et al.*, 2015).

Nevertheless, developmental processes are diverse and intricate with different requirements of gene products across the developmental stages and organism classes. Typically, the requirement of NMD factors was also variable across organisms and physiological processes (Avery *et al.*, 2011; Fatscher *et al.*, 2015). For instance, in *Drosophila*, Upf1 and Upf2 mutations have shown lethality independent of Upf3 by arresting cell division and survival. On the contrary, Upf3 and SMG1 (another NMD factor) lack any essential role in growth and development (Avery *et al.*, 2011). Depletion of Upf3 orthologue SMG4 in *C. elegans* has proven to only mildly affect the NMD target degradation during development. However, no noticeable effect was seen in the development and differentiation in upf1-3 mutants *S. cerevisae* (Metzstein and Krasnow, 2006; Vicente-Crespo and Palacios, 2010; Alex *et al.*, 2014). In contrast, the loss of Upf proteins in mice was accompanied by severe implications on growth and development (Takahashi and Yamanaka, 2006; Schweingruber *et al.*, 2013). The three Upf proteins and SMG1 are known to be required for NMD in mammalian cells (Serin *et al.*, 2001). Although NMD factors play a role in mammalian neuronal differentiation and survival of neural progenitors *in vitro* (Lou *et al.*, 2014) and synaptic architecture and synaptic vesicle cycle efficacy (Long *et al.*, 2009), testing its *in vivo* role and the stepwise involvement of the NMD factors in nervous system development and function has presented challenges due to the requirement for organism viability of most essential NMD factors. In this research, we used functional genetics and other Drosophila manipulation tools and techniques to study the effect of the loss of function of the core NMD factors in the development of CNS in the early and late embryogenesis.

2. Materials and Methods

2.1. Flies Used

Bloomington Stock number is designated as BM followed by a number. Initials stand for the donors of the flies: MM, AG, and FC for Mark Metzstein, University of Utah Salt City; Acaimo Gonzalez Reyes; and Fernando Casares, Centro Andaluz de Biologia del Desarrollo, Sevilla, Spain (CABD) respectively. The fly stocks used in this work are as follows:

yw GFP hsFLP neoPFRT19A/ yw neoFRT19A (AG), ovo D FRT19A/ c (1) Dx; FLP12 (BM 23880), yw FRT19A Upf1^{13D}/FM7c (MM), yw FRT19A Upf2^{29AA}/FM7c (MM), w; Upf3^{1}/SM6a (MM), Upf3[Df]/SM6 (BM 9424), SP/cyo; UASGFP/TM6B (FC), w;;SimGal4 (FC), neoPFRT19A/ovoDhsFLPFRT19A,Upf1^{13D}FRT19A/ovoD hsFLPFRT19AUpf2^{29AA}FRT19A/ovoDhsFLPFRT19A, SimGal4;UASGFP, Sim Gal4;UAS-Upf1i, Sim Gal4;UAS-Upf2i, SimGal4;UAS-Upf3i.

2.2. Generation and analysis of mutant embryos of Upf1 and Upf2

To analyze the effect of complete loss of *Upf1* or *Upf2* from early embryogenesis, mutant embryos of *Upf1* or *Upf2* were generated from the germline of the sterile mothers of *ovoD* (Avery *et al.*, 2011). Mutant flies were obtained following classical mating scheme (Roote & Prokop, 2013). The mutant virgin female flies Upf1 or Upf2 genes which are on chromosome X - were crossed with mutant males of ovoD also on chromosome X. The chromosomes were inherited following Mendelian rules, and the appropriate mutations were selected against the balancer chromosomes, FM7 for chromosome X. The embryos were collected hourly for 12hours, stained with mouse anti-Repo (1:100) and anti-mouse Cy3 (1:100) as primary and secondary antibodies respectively, and then the CNS was viewed and imaged under fluorescent microscope LEICA DM6000 (Cao *et al.*, 2006).

2.3. Generation of embryos with CNS localized loss of function of NMD factors

To generate wild type embryos with a localized absence of NMD factors in the nervous system, Male and female flies (1-3 days old) in a ratio of 1:1 were collected from each of the following: *Sim-Gal4>UAS-Upf1RNAi, Sim-Gal4>UAS-Upf2RNAi, Sim-Gal4>UAS-Upf3RNAi* (experiments) *and Sim-Gal4>UAS* (control) and placed in a food vial with dry yeast for two days at 18^{o}C. The flies were then placed in an apple juice agar for egg collection. For the early expression of RNAi, the embryos were placed in a 25^{o}C incubator (activation temperature of the Gal4>UAS recombination) 1hr after laying. Meanwhile, for the late expression of the RNAi, the Gal4>UAS recombination was supposedly activated at 25^{o}C; thus, the embryos were first kept at 18^{o}C (inactive temperature for Gal4>UAS) until after 14 hours of laying; they were then transferred to 25^{o}C (active temperature for Gal4>UAS). This was a way of circumventing the activation of the Gal4>UAS recombination until at the late stage of embryogenesis. The embryos were then analyzed for hatching and CNS development.

2.4. Viability and Antibody staining of the embryos

For viability, the hatching and survival index of the embryos were analyzed. To study the CNS structure, embryos were stained with a glial marker [Rabbit anti-repo (1:100)], as a primary antibody to examine the distribution of glial cells as a representation for the overall shape of the CNS and [Rabbit Cy3 (1:100)] was used as a secondary antibody (Cao *et al.*, 2006).

2.5. Immunostaining of the third Instar Larval Brain

To study the larval brain, third instar larvae were selected from the RNAi embryos in which the SimGal4 was expressed at the late stage of embryogenesis among the control (SimGal4>UAS), SimGal4>UAS-*Upf1i*, SimGal4>UAS-*Upf2i* and SimGal4>UAS-*Upf3i*. The larvae were dissected and the brains were collected and stained with a primary antibody [rabbit anti-Dachshund (dac) (1:500)] and a secondary antibody [Cy2 rabbit 1:100] (Cao *et al.*, 2006).

2.6. Measurement of VNC versus the embryo area

The image was converted to gray scale 8bit then measurement and scale were set to area and mm^2

respectively. The VNC was bordered with a free selection tool and the area of the VNC was displayed. Subsequently, the whole embryo was selected in the same way and the area of the embryo was displayed. The area proportion of the VNC was obtained by dividing the area of the VNC by the total area of the embryo multiplied by a hundred

2.7. Microscopy and image analysis

Stack images of Brains were obtained under the Leica SPE DM2500 confocal microscope using Obj. imm x20 with a Zoom Factor of 1 and a pinhole size of 1 Airy unit. Brain images were scanned and stored in a series of 76 images with a vertical spacing of 3µm (Z step). To analyze the stacked images obtained from the confocal microscope, Image J software was used. Z-projects were constructed for each brain. The central brain, optic lobe, and ventral nerve code areas were measured and compared against the control.

3. Results

To assess the role of core NMD factors in the development of the nervous system, we studied the effect of the loss of function of those factors in CNS development. For complete embryonic loss of function, mutant embryos of Upf1 or Upf2 were generated from the germline of a sterile female fly carrying the ovoD mutation. By doing this, it was ensured that any egg laid by these flies was maternally mutant for the selected Upf gene. These upf1 and upf2 maternal mutant eggs showed incompatibility with life as very few hatched (5.35% and 8.35% for Upf1 and Upf2 respectively), and only 0.82% and 1.78% for Upf1 and Upf2 respectively developed to second instar larval stage and none developed to the third instar larval stage. Many of these embryos are characterized by having irregular dorsal appendages, (94.02% and 90.82% for Upf1 and Upf2 respectively) and abnormally high yolk (Table 1).

Similarly, the ventral nerve cord failed to be established in most of the embryos, 96.02% and 89.3% for Upf1 and Upf2 respectively (Figure 1).

Table 1. Development analysis of the maternally mutant embryos

Average hatching, presence of regular dorsal appendages and shape, the yolk condition, and survival to the second instar larval stage of the maternally mutant embryos. FRT19A /ovoD FRT19A (control), Upf1 [13D] FRT19A /ovoD FRT19A (Upf1 mutants) and Upf2 [29AA] FRT19A /ovoD FRT19A (Upf2 mutants).* indicate significant difference compared to control ($p<0.001$, ANOVA, and Tukey's pairwise). Data are presented as means ± SEM

Genotype (no of female flies, N= 20)	Average No. of eggs laid	% Embryos hatched	% Embryos with regular dorsal appendages and shape	% Embryos with abnormally high yolk	% Embryos survived to 2nd instar
FRT19A /ovoD FRT19A	115 ± 4.16	90.5 ± 1.09	98.61 ± 1.91	1.31 ± 0.29	74.02 ± 2.11
Upf1 [13D] FRT19A /ovoD FRT19A	179 ± 21.7	5.35 ± 0.73*	3.98 ± 0.46*	94.02 ± 1.69*	0.82 ± 0.71*
Upf2 [29AA] FRT19A /ovoD FRT19A	118 ± 6.69	8.35 ± 0.89*	10.7 ± 0.70*	90.82 ± 1.61*	1.78 ± 0.32*

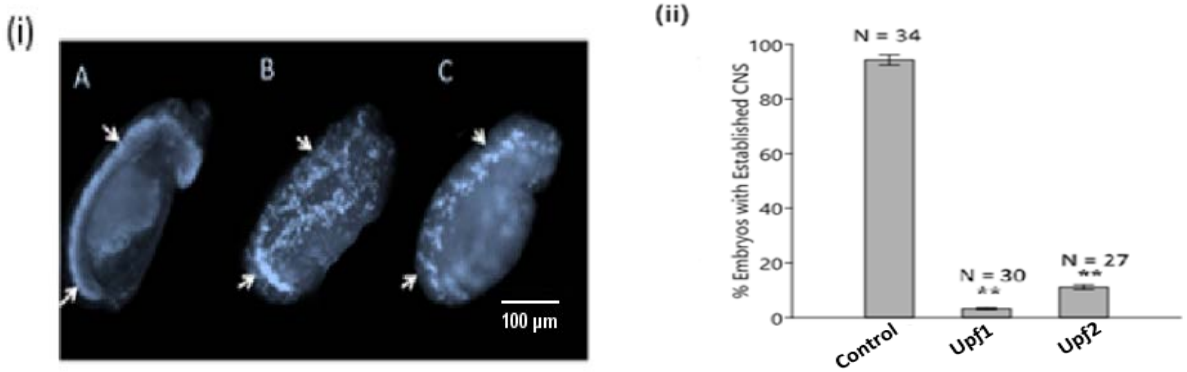

Figure 1. Central nervous system establishment in *Upf*1 and *Upf*2 mutant embryos**. (i)** Lateral confocal images of the central nervous system in embryos. Anti-repo staining (a glial marker) showing the ventral nerve cord of stage 12 to 14 in **(A)** Control, (**B)** and **(C)** maternal mutant embryos of *Upf*1 and *Upf*2 respectively displaying unestablished CNS. Arrows point to the position of the nerve cord. Anterior end on the right. **(ii)** Percentage of embryos with established CNS in FRT19A (control embryos), $Upf1^{13D}$ (maternal homozygous *Upf*1 mutant embryos), and $Upf2^{29AA}$ (maternal *Upf*2 mutant embryos). * Indicates significant difference compared to control ($p<0.0001$, ANOVA, and Tukey's pairwise). Data are presented as means ± SEM

Subsequently, temperature-sensitive SimGal4>UAS-*RNAi* was used to drive the localized depletion of Upf1, Upf2, and Upf3 in the CNS at an early and late stages of embryogenesis (stages 5 and 13 respectively). The result showed a significant reduction of hatching in both the early and late stage depleted embryos of Upf1 and Upf2 but not Upf3. However, there was slight improvement of hatching in the embryos of late expression of RNAi. The embryos that hatched displayed high chances of reaching adulthood as indicated by the percentage of larva adult escapers (Table 2).

Table 2. Hatching analysis of the embryos with Upf loss of functions localized in the CNS

Average hatching and survival of the genotypes: *Sim-Gal4>UAS* (control), *Sim-Gal4>UASUpf1i (Upf1-RNAi), Sim-Gal4>UASUpf2i (Upf2-RNAi)* and *Sim-Gal4>UASUpf3i (Upf3-RNAi).* *Indicate significant difference compared to the control ($p<0.001$), † Indicate significant difference when compares with the Upf3-RNAi ($p<0.05$). The proportion of survival is calculated as P = f/n; n is the total number of larvae collected for rearing; f = number of larvae reaching the adult stage

Time of expression of Gal4	Genotype (no of female flies, N= 25)	Average No. of eggs laid	Percentage hatching	Percentage of larva adult escapers
Early Embryogenesis	*Sim-Gal4>UAS*	131.2±5.5	95.12±5.1	96.31±5.4
	Sim-Gal4>UAS-Upf1i	122.8±3.9†	12.05±2.1*†	78.38±2.5*
	Sim-Gal4>UAS-Upf2i	105.6±5.0	21.97.0±4.5*†	84.48±3.2
	Sim-Gal4>UAS-Upf3i	159.6±8.4	65.04±3.9	94.22±4.8
Late Embryogenesis	*Sim-Gal4>UAS*	252.2±6.9	83.98±0.8	93.31±3.0
	Sim-Gal4>UAS-Upf1i	116.1±4.4†	37.51±4.3*†	81.18±1.9
	Sim-Gal4>UAS-Upf2i	124.6±5.0	32.74±1.7*†	87.25±6.8
	Sim-Gal4>UAS-Upf3i	207.7±3.2	65.05±4.2	91.70±2.0

Furthermore, the lateral view of the Upf1 and Upf2 embryos showed a significant reduction in the area of VNC with respect to the total area of the embryo in the early depleted embryos of Upf1 and Upf2 (Figure 2). However, no significant difference is noticed when the depletion was delayed till the late stage of embryogenesis (Figure 3)

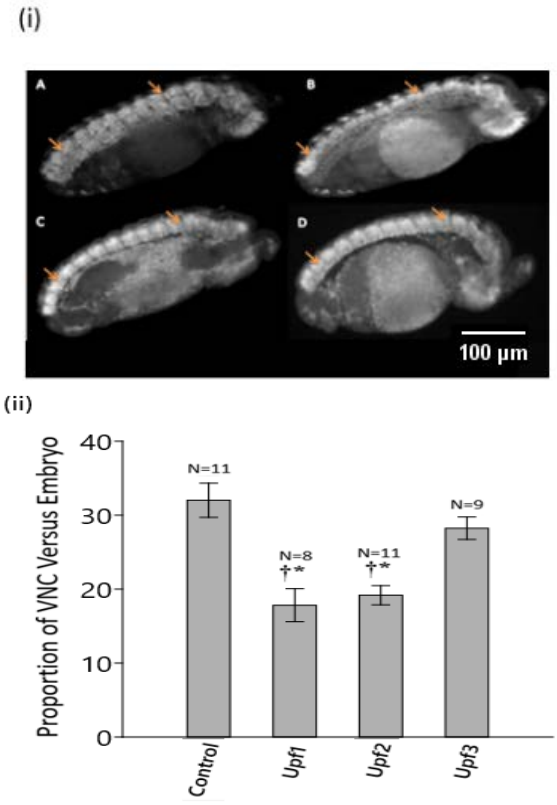

Figure 2. Central nervous system establishment in embryos with early *Upf* loss of functions localized in the CNS. (i) Lateral view of CNS of embryos. (A) Control, (B) *Upf1*i, (C) *Upf2*i, *and* (D) *Upf3*i. Arrows point to the ventral nerve cord. (ii) The area of ventral nerve cord compared to the total area of the embryos in the genotypes: Control (*Sim-Gal4>UAS*), *Upf1i* (*SimGal4UASUpf1i*), *Upf2i* (*Sim-Gal4>UASUpf2i*) and *Upf3i* (*Sim-Gal4>UASUpf3i*); *indicate significant difference when compared with the control, $p<0.001$. †Indicate significant difference compared to the *Upf3*i, $p<0.05$. (ANOVA and then Turkey's pairwise were used for the statistical analysis).

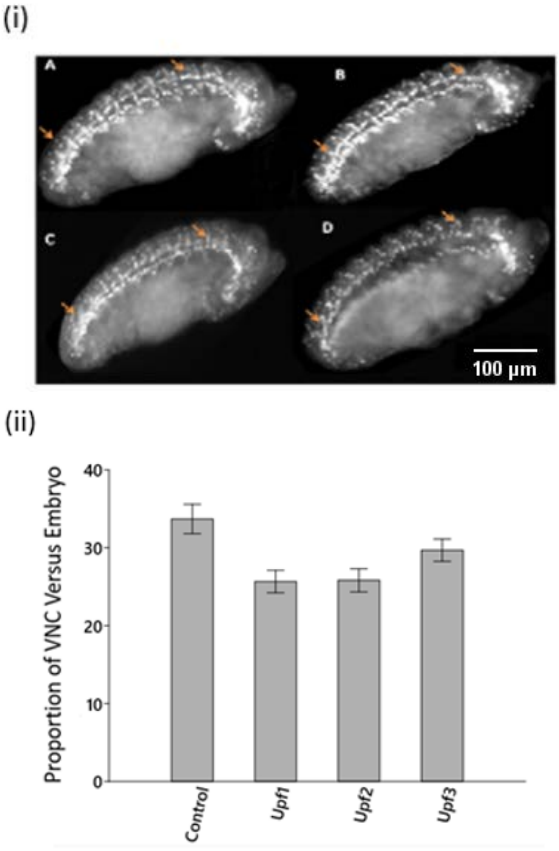

Figure 3. Central nervous system establishment in embryos with late Upf loss of functions localized in the CNS. (i) Lateral view of CNS of embryos. (A) Control (Sim-Gal4>UAS), (B) Upf1i (SimGal4UASUpf1i), (C) Upf2i (Sim-Gal4>UASUpf2i), and (D) Upf3i (Sim-Gal4>UASUpf3i). Arrows point to the ventral nerve cord (ii) The area of ventral nerve cord compared to the total area of the embryos in the genotypes: Control (Sim-Gal4>UAS), Upf1i (SimGal4UASUpf1i), Upf2i (Sim-Gal4>UASUpf2i) and Upf3i (Sim-Gal4>UASUpf3i);

Due to the observed improvement in hatching and survival of the embryos with delayed depletion of Upf1 and Upf2, the resultant third instar larva brains of these embryos were analyzed. The result showed normal VNC development but a significant reduction in the area of optic lobes of Upf1 and Upf2 mutant larval brains, but there was no significant reduction of any parameter noticed in the Upf3 mutants' larval brain (Figures 4 and 5).

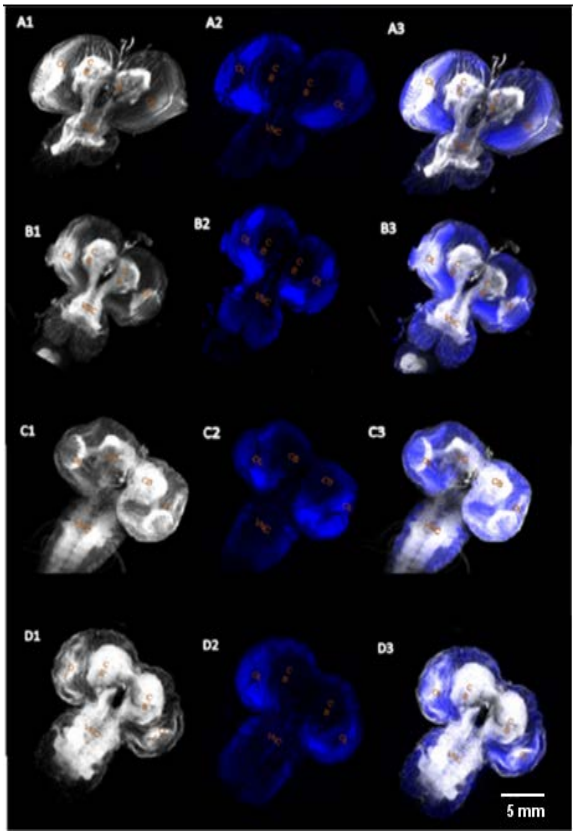

Figure 4. The central nervous system of mutant larvae. Confocal images of third instar larvae brains stained with a dachshund (gray) labeling the CNS tissues and Hoechst (blue) for nuclei. (A1 – A3) brain from the control (Sim-Gal4>UAS-GFP). (B1 – B3) Brain from the *Upf1*i (SimGal4>UAS-*Upf1*i). (C1 – C3) Brain from the *Upf2*i (SimGal4>UAS-*Upf2*i). (D1 – D3) Brain from the *Upf3*i (SimGal4>UAS-*Upf3*i). A1, B1, and C1: Dachshund; A2, B2, C2: Hoechst; A3, B3, C3: composite images; OL: optic lobe; CB: central brain; VNC: ventral nerve cord.

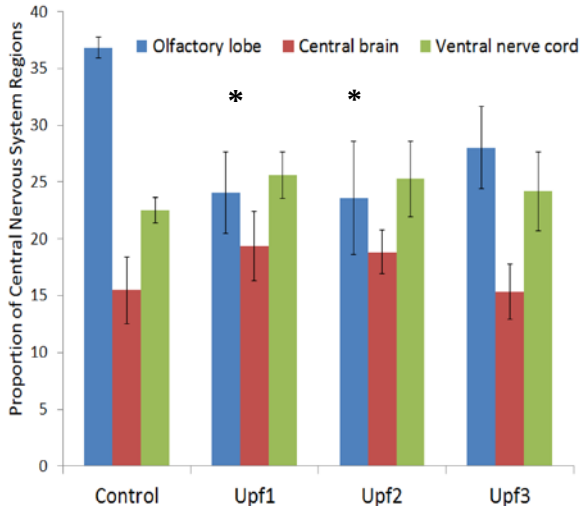

Figure 5. The proportion of central nervous system regions in larvae with loss of function of NMD factors in late NS development. The proportion of the area of OL (optic lobes), CB (central brains) and VNC (ventral nerve cords) to the total area of the larval brain, for the genotypes: Control (*Sim-Gal4>UAS*), *Upf1i* (*Sim-Gal4>UAS-Upf1i*), *Upf2i* (*Sim-Gal4>UAS-Upf2i*) and *Upf3i* (*SimGal4UASUpf3i*). *Indicate significant difference when compared with the control ($p<0.001$; ANOVA and Tukey's pairwise).

4. Discussion

NMD machinery ensures the production of functional proteins by eliminating mRNAs with PTC. Studying the developmental involvement of this pathway has proven important in pursuit of potential therapies in cancer and developmental biology. Similarly, NMD factors show promising roles in mammalian neuronal differentiation and survival of neural progenitors *in vitro* (Lou *et al.*, 2014) and in synaptic architecture and synaptic vesicle cycle efficacy (Long *et al.*, 2009), but their *in vivo* role in nervous system development and function has been hard to test due to the requirement for viability of most core NMD factors. In this work, we focused on the development of CNS during early embryonic and larval stages of *Drosophila*, in the absence of core NMD factors *Upf1*, *Upf2*, and *Upf3*, Owing to the remarkable similarities of insects with vertebrate in brain and nerve cord development, especially regarding the expression and function of homologous genes (Holley *et al.*, 1995; Arendt and Nübler-Jung, 1999; Denes *et al.*, 2007). Thus, we checked the ventral nerve cord (VNC) development in *Upf1* and *Upf2* maternally mutant embryos and found that VNC failed to be established in most of the embryos (Figure 1). The embryos were incompatible with life, very few survived to the second instar larvae stage, and none survived to adulthood (Table 1)

To investigate the localization effect of NMD absence in the CNS development, we used a temperature-sensitive GAL4/UAS system to derive the expression of *RNAi* and depleted the *Upf1*, *Upf2* and *Upf3* at early stage of embryogenesis specifically during the midline cells development. We found that there was a slight improvement in the survival and establishment of VNC compared to the maternally mutant embryos, but lethality was still near 80 percent. Also, significant reduction in the area size of the VNC of *Upf1* and *Upf2* depleted embryos was also observed in those embryos (Table 2). Although the restriction of the repression of the mutant core NMD factors to the midline reduced the burden of early cellular damage as noticed in the maternally mutant embryos, high mortality and reduced VNC were still noticed (Figure 3). This indicates that the expression of NMD factors in the nervous system at this stage of development is necessary for organismal viability. Nevertheless, delaying the depletion of Upf1 and Upf2 till the late stage of embryogenesis slightly improved the hatching approximately from 12% to 38% and 22% to 33% for Upf1 and Upf2 respectively (Figure 2). Still there was significant difference when compared with the control or Upf3 depleted embryos. These findings suggest that NMD plays a role in both early and late embryogenesis.

Dpp protein is implicated in the determination of the initial dorsal-ventral pattern of *Drosophila* embryo, by providing the signal for the border establishment along the domains of the dorsal-ventral axis, which happen before gastrulation at the cellular blastoderm stage (stage 5-6) 2-3hours after egg laying (Raz and Shilo, 1993). Previous work showed that the elimination of the *Drosophila* zygotic Dpp pathway resulted in embryos with no ventral structures including CNS (Raz and Shilo, 1993). The work of Shum *et al.*, (2016) in hESCs that established the role of NMD in regulating TGF-β and BMP signaling

(mammalian orthologue pathways of *Drosophila* Dpp*) in* ectoderm and mesoderm differentiation suggests that activation of Dpp (TGF-β homolog) may have been disturbed due to the absence of NMD and, consequently, the establishment of CNS (VNC in this case) was interfered with. In support of this hypothesis, our analysis of GSCs showed that Dpp signaling was disrupted in *Drosophila* germline stem cells (Unpublished). Avery *et al* (2011) showed that the localization of Gurken-Torpedo signaling pathway (TGF-alpha – EGF in vertebrates) that targets Broad-Complex (BR-C) was adequate in NMD mutant eggs. Since the restriction of BR-C expression depends on the interaction between EGF (Gurken) and BPM (Deng and Bownes, 1997), then the integrity of DPP signaling in this system might have been disrupted in the CNS phenotype described in this work.

The loss of NMD at midline cells developmental stage in Sim>Gal4 embryos might have affected the characteristic cell division of the neuroectoderm. Consequently, the patterning of the *Upf1* and *Upf2* depleted embryos during embryogenesis is compromised (Chang *et al.*, 2000), resulting in the reduced area of the VNC (Figure 4 and 5). Another possibility could be the compromised establishment and maintenance of the ventral epidermal and neuronal cell lineage (Chang *et al.*, 2000) due to the loss of NMD. This may have caused molecular deficit and defects in the establishment of the VNC, which results in the observed low survival rate.

Full establishment of cellular identities within ventral ectoderm and the differentiation of the ventral epidermis are achieved at the end of stages 9 and 12 of embryonic development respectively (Raz and Shilo, 1993). At stage 13, the ganglion mother cells will start differentiating into neurons. When expression of the RNAi was delayed to the beginning of stage 13, a dramatic improvement of viability and hatching of the embryos was noticed, indicating that the requirement of NMD for viability is due to its role in early embryogenesis. Moreover, analysis of the third instar larval brain from those embryos showed no reduction in the size of the central brain and the ventral nerve cords of the mutant larvae. However, we found a significant reduction in the optic lobes of the *Upf1* and *Upf2* larva but not in *Upf3* mutants (Figure 4). Interestingly, Lou *et al* shows that the deficit of *Upf1* and *Upf3B* in mouse neuronal stem cells led to reduced proliferation and increased differentiation into neural progenitors which expressed some markers of mature neurons (Lou *et al*., 2014). A similar mechanism could be at play in *Drosophila* optic lobes since the formation of vesicles of the optic lobes from the OL precursors begins at stage 13 (Hartenstein, 1993; Spindler and Hartenstein, 2010). However, NMD is a strictly regulated process in guiding the neuronal development. The *Upf1* and *Upf2* also have other non NMD functions that makes it difficult to tell whether the phenotypic expression is a result of NMD malfunction or other non NMD functions of the factors. Furthermore, the staining method employed was a proxy to determining the overall structure of the CNS using glial cells. The distribution, organization and complexity in relationship between the glial cells and neural formation are yet to be fully encompassed (Bahrampour *et al*., 2017). As such, the interference of glial cells factors in the observed defects may not be ruled out. Thus, an unbiased analysis of the house keeping genes in relation to these phenotypes may provide better explanation for NMD regulation and also the level of NMD participation in the phenotypes.

5. Conclusion

Our work has demonstrated neurodevelopmental and neurophysiology defects in flies mutants for core NMD factors. We showed the failure to establish CNS in *Upf1* and *Upf2* maternally mutant embryos. When the loss of *Upf1* and *Upf2* was localized in the nervous system, the result is nearly lethal with death occurring at the embryonic stage. We also revealed that those flies with loss of function of *Upf1* and *Upf2* localized to the NS exhibited a reduction of the areas of the ventral nerve cord in embryos and the optic lobes in the larvae.

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