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Interactions between *Rbf* and *Drp1* in Drosophila dopaminergic cells enhance aging

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ABSTRACT. Known for tumor-suppression transcriptional regulation activities, the Retinoblastoma (Rb or Rbf in flies) protein plays a crucial role in cell-cycle regulation and apoptosis. More recently, an established a cytosolic role and mitochondrial localization, the Rb protein seems to cooperate with Drp1 to regulate mitochondrial quality in a transcription-independent manner. Control of mitochondrial quality is vital for the assurance of cell survival, and the failure of this function may result in catastrophic mitochondrial dysfunction and cell death. Here we exploited the *UAS-Gal4* expression system to direct expression in neurons via the *Ddc-Gal4* directing transgene. The directed expression of *Drp1* in neuronal populations that include the dopaminergic (DA) neurons under the control of the *Ddc-Gal4* transgene can produce a robust Drosophila model of Parkinson Disease (PD) with a severely reduced median lifespan and premature locomotor dysfunction. These are significantly suppressed when *Rbf* is also overexpressed. Intriguingly, the co-expression of *Drp1-RNAi* and *Rbf-RNAi* can restore aging defects associated with *Rbf* loss of function. A delicate balance between *Rbf* and *Drp* activities, to benefit the health of the mitochondrial network, seems to be essential during aging.

Key words: *Drosophila melanogaster*; aging; climbing ability; mitochondria; Drosophila model of Parkinson disease; *Rbf*; *Drp1*

INTRODUCTION

The retinoblastoma (*Rb/Rbf*) gene encodes a tumor suppressor protein that acts as a crucial transcriptional regulator of cell proliferation and apoptosis. The *Rb* gene was first identified as tumor suppressor protein-encoding gene and was named so as mutant forms of the gene cause formation of a retinal cancer: retinoblastoma (Wen-Hwa et al., 1987). Since the initial discovery, the loss of function of *Rb*/*Rbf* has been linked to numerous types of human cancers (Du et al., 2012). In humans, the Rb protein can bind to the E2F transcription factor and then act to suppress the transcription of the *cyclin* and *CDK* genes. The *Rb/Rbf* activity predominantly depends on its phosphorylation status. Once, the inactivation of the Rb/Rbf protein was thought to be due to the inactivation of its catalytic site, but recent evidence found the movement of Rb from the nucleus to the cytoplasm can suppress its activity (Jiao et al., 2008). Further studies centered upon the cellular localization of Rb protein and found a fraction localizes in mitochondria regardless of the cell types, abnormal or not (Ferecatu et al., 2009). The Rb protein detected in mitochondria has been determined to suppress apoptosis in a manner that is independent of transcriptional regulator activity (Ferecatu et al., 2009; Hilgendorf et al., 2013). The Rb protein interacts with the pro-apoptotic Bcl-2 family protein, Bax, *in vivo* and can activate Bax to promote apoptosis. Apart from nuclear activity, mitochondrial localization suggests the existence of a cytosolic or mitochondrial function for the Rbf protein in eukaryotes.

The regulation of mitochondrial-mediated apoptosis with pro-apoptotic Bcl-2 proteins and Dynamin-related protein (Drp1) seems to be well-conserved between Drosophila and humans. The Rb protein is found to interact with the Drp1 in mediation of mitochondrial-dependent cell death as induced by cadmium in hepatocytes (Zhang et al., 2019). The inhibition of Drp1 acts to counteract cell death induced by the localization of the Rb protein to the mitochondrial surface induced by $CdCl_2$ (Zhang et al., 2019). Drp1 protein plays a major role in mitochondrial quality control and mitochondrial apoptosis (Sebastián et al., 2017; Favaro et al., 2019). In *Drosophila melanogaster*, the overexpression of *Rbf* gene triggers apoptosis through the activation of the JNK pathway (Milet et al. 2014). This *Rbf*-induced apoptosis requires the presence of the pro-apoptotic Bcl-2 family protein, Debcl, and the mitochondrial fission protein, Drp1, and is dependent upon mitochondrial fragmentation (Clavier et al., 2015). *Rbf* can act to suppress the transcription of *Buffy*, the anti-apoptotic *Bcl-2* family gene (Clavier et al., 2014) which promotes the interaction between Drp1 and Debcl (the sole pro-apoptotic Bcl-2 protein in flies) to induce apoptosis (Clavier et al., 2015). In contrast to the consequences of the overexpression of *Rbf*, flies deficient for *Rbf* are sensitive to apoptosis due to the upregulation of the apoptotic gene *hid* (Ariss et al., 2018). The localization of the Rbf protein to, or near, the mitochondria suggest that an influence upon the function of the Drp1 protein is of particular interest.

The processes that contribute to mitochondrial quality control are not isolated signaling pathways, but rather seem to be a network of interconnected activities that share a collection of common intermediate components at several levels. The mechanisms that contribute to mitochondrial dynamics maintain the homeostasis of the cell and assist in the function of the mitochondrial network. The UPR^{mt} transcription factor can induce the transcription of *Drp1* during periods of stress to promote mitophagy or the controlled degradation of select mitochondria (Nargund et al., 2015). The Drp1 protein can promote apoptosis in *C. elegans*, *D. melanogaster* and cell culture (Jagasia et al., 2005; Goyal et al., 2007). The Drp1 protein can promote apoptosis in Bcl-2 protein-dependent and independent manners (Cassidy-Stone et al., 2008; Oettinghaus et al., 2016). The activities related to transcription of the Rb/Rbf protein are well conserved and include the regulation of metabolic

pathways, control of oxidative phosphorylation and mediation of mitochondrial functions through the control of the very important E2F transcription factor (Dyson 2016). Many mitochondrial activities are vital for the survival of cells and the assurance of the quality of mitochondria, and the failure of these processes results in phenotypes characterized by the dysfunction of mitochondria and subsequent cell death.

Although recently, we have demonstrated that loss of *parkin* function more than supresses the reduced median lifespan and impaired locomotion over time that results from the overexpression of *Rbf* in dopaminergic neurons by *Ddc-Gal4*, little is known about the contribution of *Rbf* towards mitochondrial health and neurodegeneration (Hasan and Staveley, 2024). As we have demonstrated, both the overexpression and inhibition of *Drp1* act to phenocopy PD-like phenotype in Drosophila, the anticipated role of the mitochondria in PD pathogenesis suggest that the *Drp1*-induced model of PD an attractive model for investigation of the role of *Rbf*. We utilized the *Drosophila melanogaster* as a model organism to study the phenotypic effects of *Rbf* and *Drp1* gene interaction. We examined the effects of over expression and inhibition of the expression of transcription regulator *Rbf* in selected neurons along with modified expression of *Drp1* upon aging and locomotion over time to reveal a balance of complex interactions that can improve aging.

MATERIALS AND METHODS

Drosophila melanogaster stocks and media

All stocks were maintained on a standard media prepared from cornmeal/molasses/yeast/agar medium treated with propionic acid and methylparaben to resist fungal growth. Aliquots of media were poured into plastic vials, allowed to solidify, and refrigerated at 4°C until used. Stocks are kept at room temperature while crosses and experiments were carried out at 25°C. The *UAS-Rbf RNAi1*^{HMS03004} (y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.HMS03004}attP2/TM3, Sb[1]); the *UAS-Rbf RNAi2*^{GL01293} *(y[1] sc[*] v[1] sev[21]; P{y[+t7.7] v[+t1.8]=TRiP.GL01293}attP40); UAS-Rbf (w[*]; P{w[+mC]=UAS-Rbf.D}III); UAS-Drp1 (y[1] w[*]; P{w[+mC]=FLAG-FlAsH-HA-Drp1}3, Ki[1]);* the *UAS-Drp1-RNAi1*^{JF02762} *(y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.JF02762} attP2}); UAS-Drp1-RNAi2*^{HMC03230} *(y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.HMC03230}attP40); Ddc-Gal4*$^{4.36}$*(w[1118]; P{w[+mC]=Ddc-Gal4.L}Lmpt[4.36];* and *UAS-lacZ* stocks were obtained from Bloomington Drosophila Stock Center at Indiana University, Bloomington, Indiana, USA. The *Ddc-Gal4/CyO; UAS-Drp1/TM3, Ddc-Gal4/CyO; UAS-Drp1-RNAi /TM3*; *Ddc-Gal4/CyO; UAS-Rbf-RNAi /TM3* derivative lines were generated through the use of standard recombination methods and used to overexpress and inhibit *Drp1* and inhibit *Rbf* in the select dopaminergic (DA) neurons by use of the *Ddc-Gal4*$^{4.3D}$ transgene.

Survival assay

Several crosses of virgin females and males were made, and a cohort of critical class males collected upon eclosion. At least 250 flies were aged per genotype in the cohorts of 25 or less per vial on fresh media, replenished every two-five days to avoid crowding. Flies were observed and scored every second day for the presence of deceased adults. As a rule, flies were considered dead when movement was not observed upon agitation (Todd et al., 2012). Longevity data were analyzed with

GraphPad Prism version 8 statistical software, and the Mantel-Cox test compared survival curves. Significance was determined at a 95% confidence level ($P \leq 0.05$) with Bonferroni correction.

Locomotor analysis

The 70 male flies of the critical class were collected within 24 hours and maintained as ten flies in each vial. The food was changed twice every week. Every week 50 males of each genotype were assayed, in groups of 10, for their ability to climb a glass tube divided into five levels of 2 cm each according to the established protocol (Todd et al., 2012). The climbing index was calculated for each week using GraphPad prism version 8 statistical software. The climbing curve was fitted using non-linear regression and determined at a 95% confidence interval ($P \leq 0.05$).

RESULTS

Alteration of the expression of *Rbf* along with *Drp1* directed by the *Ddc-Gal4*$^{4.3D}$ transgene

The overexpression of *Drp1* leads to a compromised lifespan and diminished climbing ability over time. In these experiments, the control *Ddc-Gal4*$^{4.3D}$ *UAS-Drp1 UAS-lacZ* critical males were determined to have a median lifespan of 58 days (n=282). The overexpression of *Rbf* in the *Ddc-Gal4 UAS-Drp1* critical class flies results in much-increased median life span of 74 days (n=294) compared to control with a P-value at <0.0001 as determined by log-rank (Mantel-Cox) test. The two *UAS-Rbf-RNAi* transgenes, *UAS-Rbf-RNAi1* ^{HMS03004} and *UAS-Rbf-RNAi2*^{GL01293}, when expressed along with *Ddc-Gal4 UAS-Drp1*, results in a median life span of 56 (n=253) and 60 days (n=280) (Figure 1A) similar to control with a P-value at 0.0012 and 0.3854, respectively, as determined by log-rank (Mantel-Cox) test. The non-linear fit of the climbing curve shows overexpression of *Rbf* by *Ddc-Gal4 UAS-Drp1* rescue the decline in climbing ability compared to control at 95% CI with P-value < 0.0001. The non-linear fitting of the climbing curve shows inhibition of *Rbf* by *Ddc-Gal4 UAS-Drp1; UAS-Rbf-RNAi1* ^{HMS03004} and *Ddc-Gal4 UAS-Drp1; UAS-Rbf-RNAi* ^{GL01293} is very similar to the control flies at 95% CI with P-value at 0.3012 and 1.762 respectively (Figure 1B) (n=50).

Altered expression of *Rbf* along with *Drp1-RNAi* directed by *Ddc-Gal4*$^{4.3D}$ has little effect

The *Ddc-Gal4*$^{4.3D}$ *UAS-Drp1-RNAi UAS-lacZ* critical males were determined to have a median lifespan of 70 days in 323 flies. Overexpression of *Rbf* in the *Ddc-Gal4 UAS-Drp1-RNAi* expressing flies results in the increased median life span of 74 days (n=275) compared to the control with a P-value of <0.0001 as determined by log-rank (Mantel-Cox) test. The two *UAS-Rbf-RNAi* transgenes, *UAS-Rbf-RNAi* ^{HMS03004} and *UAS-Rbf-RNAi2*^{GL01293}, when expressed along with *Ddc-Gal4 UAS-Drp1-RNAi*, results in a median life span of 72 (n=253 flies) and 70 days (n=280 flies), respectively (Figure 2A) similar to control with P-value of 0.3679 and 1.1737 respectively, as determined by log-rank (Mantel-Cox) test. The non-linear fit of the climbing curve shows overexpression of *Rbf* by *Ddc-Gal4 UAS-Drp1-RNAi* further contributes to the loss of climbing ability throughout the life of critical class flies compared to control at 95% CI (P<0.0001). The non-linear fit of the climbing curve shows inhibition of *Rbf* by *Ddc-Gal4 UAS-Drp1; UAS-Rbf-RNAi1* ^{HMS03004} and *Ddc-Gal4 UAS-Drp1; UAS-Rbf-RNAi2* ^{GL01293} is very similar to the climbing ability of control flies at 95% CI with P-value at 0.0452 and 0.1229 respectively (Figure 2B) (n=50).

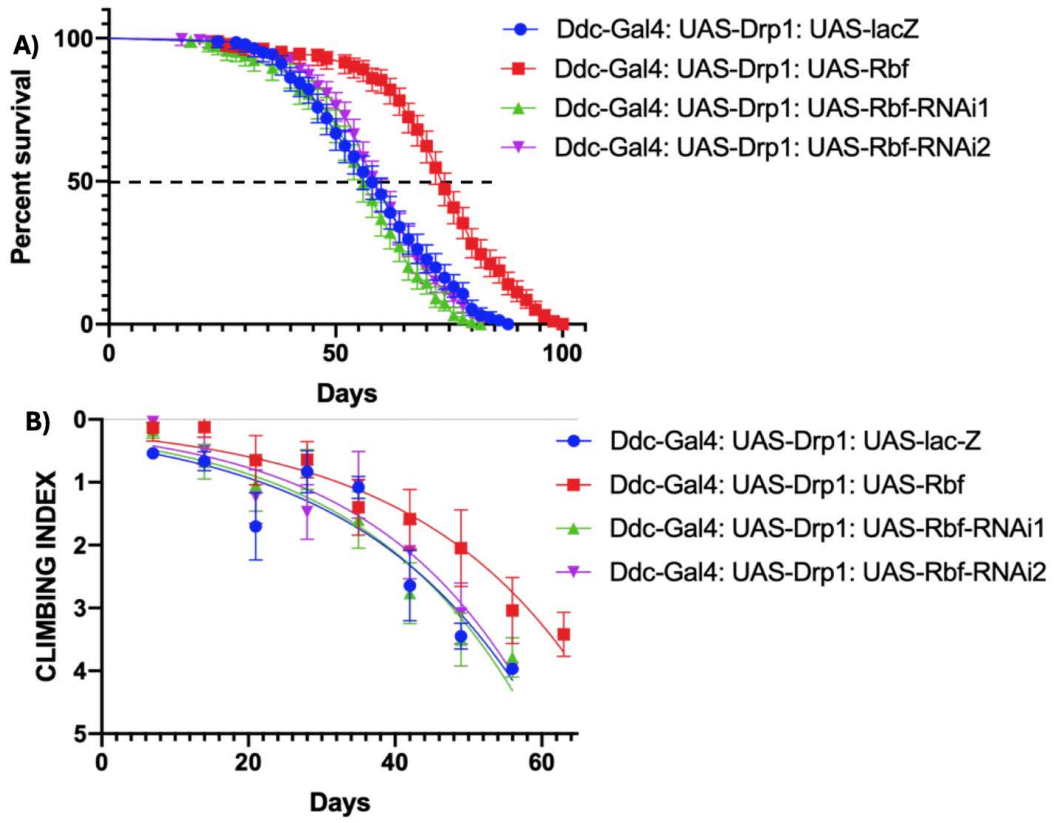

Figure 1. Altered expression of *Rbf* in the *Ddc-Gal4$^{4.3D}$ UAS-Drp1* model of PD.

Figure 1A. In the control, *Ddc-Gal4$^{4.3D}$ UAS-Drp1 UAS-lacZ* critical class males were determined to have a median life span of 58 days (n=282). The overexpression of *Rbf* along with *Drp1*, resulted in a median lifespan of 74 days (n=294), much higher than 58 days of control determined by the Log-rank Mantel-Cox test P-value of <0.0001, with Bonferroni correction. The inhibition of *Rbf* by either of two RNAi transgenes, *UAS-Rbf-RNAi1 ^{HMS03004}* and *UAS-Rbf-RNAi ^{GL01293}*, directed by the *Ddc-Gal4$^{4.3D}$* along with the *UAS-Drp1* transgene resulted in the median lifespans of 56 (n=266) and 60 days (n=263) similar to 58 days of control, determined by Log-rank Mantel-Cox test at P-value 0.0012 and 0.3854 respectively, with Bonferroni correction. The graph of longevity assay was generated by GraphPad prism8. **Figure 1B.** The GraphPad prism8 generated a graph of the climbing abilities of *Ddc-Gal4 UAS-Drp1* flies that express *Rbf, Rbf-RNAi* and control. The abilities of flies that overexpress *Rbf* has improved compared to control as determined in the non-linear fitting of the climbing curve by a 95% confidence interval (p<0.0001). The abilities of flies expressing *UAS-Rbf-RNAi's* are very similar to control as determined in the non-linear fitting of the climbing curve by a 95% confidence interval with a p-value of 0.3012 and 1.762 (n=50). The graph of longevity assay was generated by GraphPad prism8 non-linear regression curve.

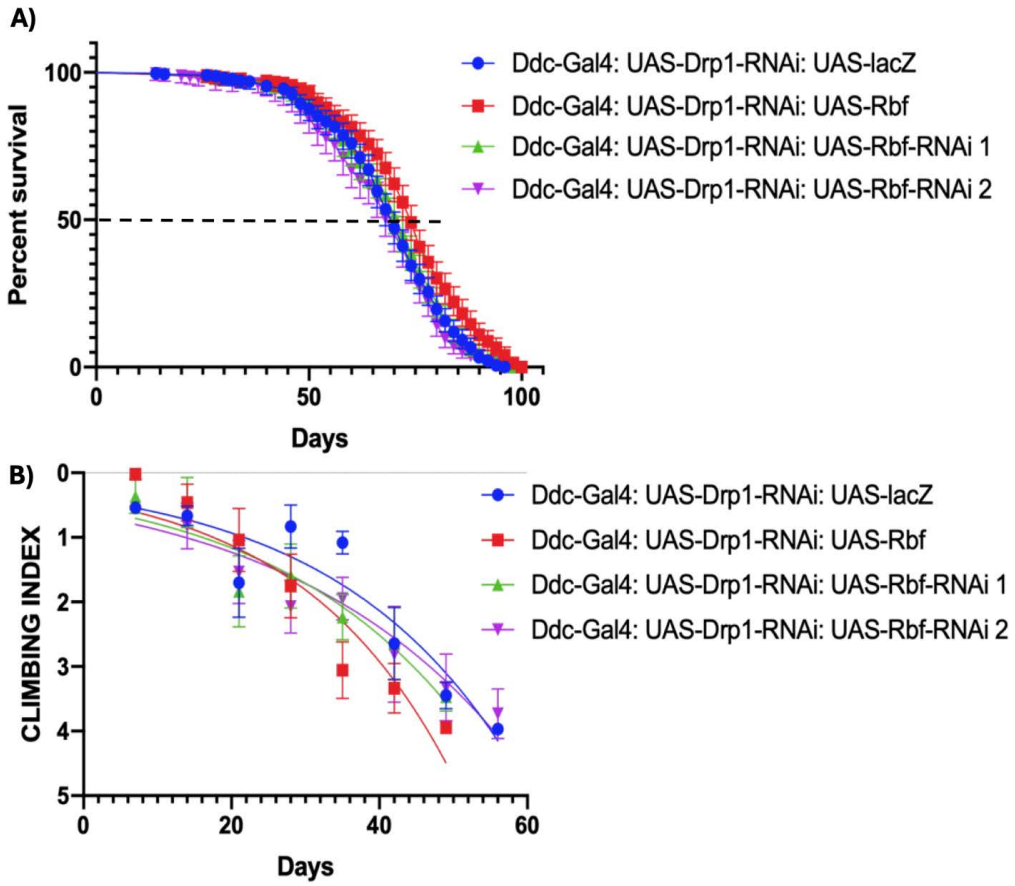

Figure 2. Altered expression of *Rbf* in the *Ddc-Gal4$^{4.3D}$ UAS-Drp1-RNAi* flies.

Figure 2A. In the control, *Ddc-Gal4$^{4.3D}$ UAS-Drp1-RNAi UAS-lacZ* critical class males were determined to have a median life span of 70 days (n=323). The overexpression of *Rbf* , along with *UAS-Drp1-RNAi* , results in a median lifespan of 74 days (n=275), slightly higher when compared to 70 days of control determined by the Log-rank Mantel-Cox test at P-value of <0.0001, with Bonferroni correction. The inhibition of *Rbf* by two RNAi lines, *UAS-Rbf-RNAi1 ^{HMS03004}* and *UAS-Rbf-RNAi ^{GL01293}*, directed by the *Ddc-Gal4$^{4.36}$* along with the *UAS-Drp1-RNAi* transgene results in the median lifespan of 72 (n=266) and 70 days (n=263) similar to 70 days of control, determined by Log-rank Mantel-Cox test at P-value 0.3679 and 0.1737 respectively (n~250), with Bonferroni correction. The graph of longevity assay was generated by GraphPad prism8. **Figure 2B.** The GraphPad prism8 generated a graph of the climbing abilities of *Ddc-Gal4 UAS-Drp1-RNAi* flies when expressing *Rbf, Rbf-RNAi's* and the control *lacZ* transgenes. The climbing abilities of flies overexpressing *Rbf* has further compromised compared to control as determined in the non-linear fitting of the climbing curve by a 95% confidence interval (p<0.0001). The climbing abilities of flies expressing *UAS-Rbf-RNAi's* are very similar to control as determined in the non-linear fitting of the climbing curve by a 95% confidence interval with a p-value of 0.0452 and 0.1229 (n=50). The graph of longevity assay was generated by GraphPad prism8 non-linear regression curve.

Ddc-Gal4$^{4.3D}$ directed expression of *Drp1-RNAi* can rescue *Rbf-RNAi* aging defects

In this experiment, the control *Ddc-Gal4*$^{4.3D}$ *UAS-Rbf-RNAi; UAS-lacZ* critical class males were determined to have a median lifespan of 58 days (n=351). The overexpression of *Drp1* along with the directed *RNAi* inhibition of *Rbf via Ddc-Gal4* results in flies with a similar median lifespan of 56 days with a sample size of 350 flies (P-value=0.0006). The inhibition of *Drp1* by either of two RNAi transgenes, *UAS-Drp1-RNAi1* and *UAS-Drp1-RNAi2*, results in greater median lifespans of 66 days (n =313) and 76 days (n=322), both increased compared to the control (Figure 3A) as determined by log-rank (Mantel-Cox) test at a P value at <0.0001. The overexpression of *Drp1* in *Ddc-Gal4 Rbf-RNAi* does not seem to influence the locomotor abilities overtime. Notably, the inhibition of *Drp1* by expression of *Drp1-RNAi* acts to rescue the climbing ability lost by the RNA-interference of *Rbf* directed by the *Ddc-Gal4* transgene as determined in the non-linear fitting of the climbing curve by 95% confidence interval at a P-value=0.0001 (**Figure 3B**).

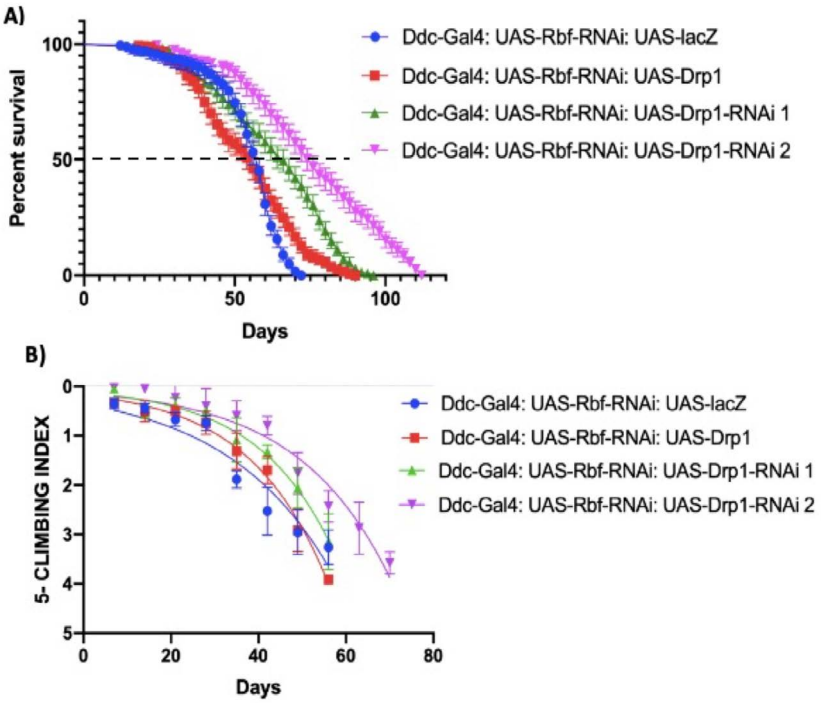

Figure 3. Expression of *Drp1-RNAi* coupled with *Ddc-Gal4*$^{4.3D}$ *Rbf-RNAi*^{HMS03004}.

Figure 3A. The graph of the longevity assay generated by GraphPad prism8 with altered *Drp1* expression in *Ddc-Gal4 Rbf-RNAi*^{HMS03004} expressing flies. The overexpression of *Drp1* results in the median lifespan of 56 days (n=351), similar to 58 days of control (*lacZ/Rbf-RNAi*^{HMS03004}) determined by Log-rank Mantel-Cox test, with Bonferroni correction. The inhibition of *Drp1* in neurons using *Ddc-Gal4* transgene along with *Rbf-RNAi*^{HMS03004} results in an increased lifespan of 66 days (n=313) with UAS-*Drp1-RNAi1*^{JF02762} and lifespan of 76 days (n=322) with *UAS-Drp1-RNAi2*^{HMC03230} compares to 58 days of control done by Log-rank Mantel-Cox test, with Bonferroni correction. **Figure 3B.** The GraphPad prism8 generated a graph of the climbing abilities of *Ddc-Gal4 Rbf-RNAi*^{HMS03004} flies when expressing *Drp1, Drp1-RNAi* and control. The climbing abilities of *Ddc-Gal4 Rbf-RNAi Drp1-RNAi* flies are significantly increased compared to control as determined in the non-linear fitting of the climbing curve by a 95% confidence interval (n=50).

DISCUSSION

The cytosolic and nuclear role of Rb/Rbf protein is crucial in cell differentiation and cell survival. The activation of *Rb* can lead to increased cell survival and cell death dependent upon the molecular cues and cellular environment. The mutant or inactivated *Rb* gene is prevalent in some types of cancer, conversely increased expression of *Rb* is quite common in cancerous cells (Shi et al., 2000; Patel et al., 2020). The overexpression and inhibition of *Rbf* in selected neurons of flies, have adverse effects and decrease median lifespan and climbing ability (Hasan et al., 2024). Altered expression of Rb/*Rbf* homologues in mice has catastrophic effects, to include lethality (Vooijs et al., 1999; Lipinski et al., 1999). The overall function of *Rb/Rbf* is well conserved throughout evolution in worms, flies, mice and mammals (van den Heuvel et al., 2008). The crucial role of *Rbf* affects cellular health adversely when expression changes under normal conditions.

The directed expression of *Rbf* with *Drp1* resulted in the suppression of the *Drp1* overexpression phenotype of decreased longevity and age-dependent loss in climbing ability. The mechanistic basis is not clear, the rescue of *Drp1* overexpression phenotype may suggest the activation of the *Rbf*-mediated cell proliferation pathway or inhibition of Drp1 induced apoptosis. The inhibition of *Rbf* in selected neurons that express *Drp1* did not enhance the phenotypes of decreased lifespan and age-dependent loss of climbing ability. A plausible explanation is that the toxic effects of excessive apoptosis due to *Drp1* overexpression is sufficient to generate the observed phenotypes (Willems et al., 2015; Nagdas et al., 2017), and inhibition of *Rbf* activity does not confer an additional disadvantage. Alternatively, loss-of-*Rbf*-induced toxicity precede the effects of *Drp1*-induced toxicity, but the effect is not additive, as such, additional phenotypes may not be observable. The lifespan and climbing abilities of the flies that overexpress *Drp1*, or inhibit *Rbf*, individually are very close to each other. The phenotypic effect of *Drp1* overexpression and *Rbf* inhibition together is not additive.

The role of *Rbf/Rb* in apoptosis is influenced by its interaction with multiple proteins. The mitochondrial fission protein Drp1 functions with Bcl-2 family proteins to promote mitochondrial fragmentation during apoptosis (Clerc et al., 2014; Wang et al., 2015; Zhang et al., 2016). In Drosophila, the Rbf protein regulates mitochondrial fragmentation hence apoptosis by promotion of excessive ROS production. The pro-apoptotic Debcl and Drp1 co-localize at the mitochondria, and their interactions can be disrupted by the overexpression of the anti-apoptotic *Buffy* (Clavier et al., 2015). The inhibition of *Drp1* in *Rbf-RNAi* background acted to rescue the phenotypes associated *Rbf* loss of function. This suggests that the inhibition of *Drp1*, may impede the molecular process which was responsible for *Rbf* inhibition PD phenotype. The inhibition of *Rbf* in *Drp1-RNAi* background has a phenotype that is very similar to *Drp1* inhibition alone. It is likely that Drp1 and Rbf function on the same biological process and, hence, show a similar outcome. Plausibly cytosolic Rbf functions downstream of Drp1 and is recruited by Drp1 on mitochondrial to promote cell death (Zhang et al., 2019). With the transcriptional activity of Rbf in mind, the Drp1 protein may function downstream of Rbf and has an epistatic effect over the *Rbf*-associated phenotypes.

The *Rbf* overexpression with *Drp1* inhibition slightly improved the lifespan but caused early onset in the climbing ability defect. The molecular mechanisms by which Rb/Rbf protein affects apoptosis and cell proliferation is convoluted. It is important to understand that as a transcription regulator, *Rbf* can promote contrary things like cell proliferation and cell death. Additionally, Rbf can localize in cytoplasm or mitochondria to maintain mitochondrial dynamics or apoptosis (Hilgendorf et al., 2013; Zhang et al., 2019). The total rescue of *Drp1* overexpression and partial

recovery of Drp1 inhibition phenotype by *Rbf* overexpression is very interesting. In my experiments, the inhibition of *Rbf* is beneficial when combined with the inhibition of the mitochondrial fission protein *Drp1.* The numerous biological functions dependent upon the *Rbf* transcription regulator and the dynamic structure of mitochondria are obscure. The elucidation of the link to mitochondrial dysfunction remains challenging. The activity of Rb at the mitochondria may be an essential way to control for extremes of Rb expression leading to mitophagy, autophagy, cell death, organismal impaired or enhanced survival. The Rbf protein does play major role in maintaining mitochondrial health transcriptionally. The Drp1 localizes in mitochondria and a fraction of Rbf localize in mitochondria to facilitate cell death. The decrease in lifespan and age-dependent loss in climbing ability observed in *Drp1* overexpression flies is rescued by *Rbf* overexpression. The inhibition of Rbf gene activity by the directed expression of an *RNAi* transgene in the selected neurons does not affect PD-like symptoms induced by *Drp1* overexpression or inhibition in Drosophila. Interaction studies are required to chart out the overall effect of cytosolic and nuclear role of Rbf in interaction with Drp1. Additionally, it is important to elucidate the molecular changes associated with the loss of function of these protein in development and function of dopaminergic neurons of Drosophila.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Ariss MM, Islam ABMMK, Critcher M, Zappia MP, et al. (2018). Single cell RNA-sequencing identifies a metabolic aspect of apoptosis in *Rbf* mutant. *Nat. Commun.* 9: 5024.

Cassidy-Stone A, Chipuk JE, Ingerman E, Song C, et al. (2008). Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. *Dev. Cell* 14: 193–204.

Clavier A, Baillet A, Rincheval-Arnold A, Coléno-Costes A, et al. (2014). The pro-apoptotic activity of *Drosophila* Rbf1 involves dE2F2-dependent downregulation of *diap1* and *buffy* mRNA. *Cell Death Dis.* 5: e1405.

Clavier A, Ruby V, Rincheval-Arnold A, Mignotte B, et al. (2015). The Drosophila retinoblastoma protein, Rbf1, induces a Debcl- and Drp1-dependent mitochondrial apoptosis. *J. Cell Sci.* 128: 3239-3249.

Clerc P, Ge SX, Hwang H, Waddell J, et al. (2014). Drp1 is dispensable for apoptotic cytochrome c release in primed MCF10A and fibroblast cells but affects Bcl-2 antagonist-induced respiratory changes. *Br. J. Pharmacol.* 171: 1988–1999.

Du W, Searle J. (2012). The Rb pathway and cancer therapeutics. *Curr. Drug Targets*. 10: 581–589.

Dyson NJ. (2016). RB1: A prototype tumor suppressor and an enigma. *Genes Dev.* 30: 1492-1502.

Favaro G, Romanello V, Varanita T, Desbats MA, et al. (2019). DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nat. Commun.* 10: 2576.

Ferecatu I, Le Floch N, Bergeaud M, Rodríguez-Enfedaque A, et al. (2009). Evidence for a mitochondrial localization of the retinoblastoma protein. *BMC Cell Biol.* 10: 50.

Goyal G, Fell B, Sarin A, Youle RJ, et al. (2007). Role of mitochondrial remodeling in programmed cell death in *Drosophila melanogaster*. *Dev. Cell.* 12: 807–816.

Hasan A, Staveley BE. (2024). *Rbf* and healthy ageing in Drosophila: interactions with *park*in, *Buffy* and *Debcl*. *Genet. Mol. Res.* 23: gmr19282.

Hilgendorf KI, Leshchiner ES, Nedelcu S, Maynard MA, et al. (2013). The retinoblastoma protein induces apoptosis directly at the mitochondria. *Genes Dev.* 27: 1003-1015.

Jagasia R, Grote P, Westermann B, Conradt B. (2005). DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in *C. elegans*. *Nature.* 433: 754–760.

Jiao W, Lin HM, Datta J, Braunschweig T, et al. (2008). Aberrant nucleocytoplasmic localization of the retinoblastoma tumor suppressor protein in human cancer correlates with moderate/poor tumor differentiation. *Oncogene* 27: 3156–3164.

Lipinski MM, Jacks T. (1999). The retinoblastoma gene family in differentiation and development. *Oncogene* 18: 7873-7882.

Milet C, Rincheval-Arnold A, Moriéras A, Clavier A, et al. (2014). Mutating RBF can enhance its pro-apoptotic activity and uncovers a new role in tissue homeostasis. *PLoS One.* 9: e102902.

Nagdas S, Kashatus DF. (2017). The interplay between oncogenic signaling networks and mitochondrial dynamics. *Antioxidants.* 6: 33.

Nargund AM, Fiorese CJ, Pellegrino MW, Deng P, et al. (2015). Mitochondrial and nuclear accumulation of the transcription factor ATFS-1 promotes OXPHOS recovery during the UPRmt. *Mol. Cell.* 58: 123–133.

Oettinghaus B, D'Alonzo D, Barbieri E, Restelli LM, et al. (2016). DRP1-dependent apoptotic mitochondrial fission occurs independently of BAX, BAK and APAF1 to amplify cell death by BID and oxidative stress. *Biochim Biophys Acta.* 1857: 1267–1276.

Patel JM, Goss A, Garber JE, Torous V, et al. (2020). Retinoblastoma protein expression and its predictors in triple-negative breast cancer. *npj Breast Cancer.* 6: 19.

Sebastián D, Palacín M, Zorzano A. (2017). Mitochondrial dynamics: coupling mitochondrial fitness with healthy ageing. *Trends Mol. Med.* 23: 201-215.

Shi YZ, Hui AM, Li X, Takayama T, et al. (2000). Overexpression of retinoblastoma protein predicts decreased survival and correlates with loss of p16INK4 protein in gallbladder carcinomas. *Clin. Cancer Res.* 6: 4096-4100.

Todd AM, Staveley BE. (2012). Expression of Pink1 with α-synuclein in the dopaminergic neurons of Drosophila leads to increases in both lifespan and healthspan. *Genet. Mol. Res.* 11: 1497-1502.

van den Heuvel S, Dyson NJ. (2008). Conserved functions of the pRB and E2F families. *Nat Rev Mol Cell Biol.* 9: 713-724.

Vooijs M, Berns A. (1999). Developmental defects and tumor predisposition in Rb mutant mice. *Oncogene.* 18(38): 5293-5303.

Wang P, Wang P, Liu B, Zhao J, et al. (2015). Dynamin-related protein Drp1 is required for Bax translocation to mitochondria in response to irradiation-induced apoptosis. *Oncotarget.* 6: 22598–22612.

Wen-Hwa L, Bookstein R, Hong F, Young LJ, et al. (1987). Human retinoblastoma susceptibility gene: Cloning, identification, and sequence. *Science.* 235: 1394–1399.

Willems PHGM, Rossignol R, Dieteren CEJ, Murphy MP, et al. (2015). Redox homeostasis and mitochondrial dynamics. *Cell Metab.* 22: 207-218.

Zhang S, Che L, He C, Huang J, et al. (2019). Drp1 and RB interaction to mediate mitochondria-dependent necroptosis induced by cadmium in hepatocytes. *Cell Death Dis.* 10: 1–17.

Zhang Z, Liu L, Wu S, Xing D. (2016). Drp1, Mff, Fis1, and MiD51 are coordinated to mediate mitochondrial fission during UV irradiation-induced apoptosis. *FASEB J.* 30: 466–476.