Alteration of Drp1 Expression in Drosophila Models of Parkinson Disease

Azra Hasan  
Memorial University of Newfoundland

Brian Ernest Staveley (✉ bestave@mun.ca)  
Memorial University of Newfoundland  https://orcid.org/0000-0002-4892-9287

Research article

Keywords: Ddc (dopa decarboxylase), Drosophila, Drp1, GMR (glass multiple reporter), Mitochondrial dynamics, park, Parkinson Disease, RNAi (ribonucleic acid interference)

DOI: https://doi.org/10.21203/rs.3.rs-39652/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background: Parkinson Disease (PD) and other neurodegenerative diseases have a significant relationship with mitochondrial dysfunction. The substantial effect of mitochondrial dynamics in PD has led us to study the role of the gene encoding the mitochondrial fission protein \textit{Drp1} in Drosophila models. \textit{Drp1} is a member of the highly conserved, dynamin family of protein encoding genes. \textit{Drp1} plays an essential role in the maintenance of the mitochondrial, peroxisomal, and endoplasmic reticulum (ER) dynamics, and has been found to regulate processes during homeostasis and cell survival.

Results: The directed expression of \textit{Drp1} in \textit{Drosophila melanogaster} neurons under the control of the \textit{Ddc-Gal4} transgene decreases the lifespan and compromises climbing ability over time. The directed inhibition of \textit{Drp1} produces a novel model of Parkinson Disease, as this causes little change in mean lifespan but a significant decrease in locomotor or climbing abilities. Interestingly, the loss of \textit{park} dependent Drosophila model of PD is rescued by the directed inhibition of \textit{Drp1}.

Conclusion: The compromised climbing abilities in flies with directed inhibition of \textit{Drp1} has produced a new model for Parkinson Disease and can be used to further investigate the mechanism(s) underlying PD and other neurodegenerative diseases. Interestingly, the combined inhibition of both \textit{Drp1} and \textit{park} suppresses Parkinson Disease phenotypes and promotes survival.

Background

Mitochondria are critical organelles in the process of survival at the cellular level. Mitochondria accumulate damage over time to become dysfunctional and contribute to the process of ageing and eventually to the death of an organism [1]. Mitochondria are known to be the powerhouse of the cell, but its role goes beyond the production of ATP (oxidative phosphorylation); it is responsible for various aspects of energy homeostasis, oxidative stress, calcium handling, cell signalling, and cell survival [2]. The central role of mitochondria in energy regulation and signalling implies that its dysfunction would have devastating effects upon cellular functions. Such is especially true for the nervous system because subtle changes in signalling can have catastrophic consequences leading to neurodegenerative disease.

The shape and size of mitochondria are not fixed properties; mitochondrial morphology depends upon a number of factors, including stage of the cell cycle and cell type, and can change quite quickly in response to external stimuli or metabolic cues. Changes to the mitochondrial network seem to differentially influence a number of signalling pathways [3]. Therefore, mitochondria undergo fission and fusion frequently in order to change their structure in response to the specific requirements of the cell, under a wide range of circumstances. Mitochondrial fission allows segregation of damaged mitochondria components while the process of fusion facilitates the exchange of mitochondrial material vital to maintain homeostasis within the mitochondrial network. Mitochondrial fusion helps compromised mitochondria, with highly damaged DNA and protein, to actively exchange components with other more healthy mitochondria to decrease the severity of heteroplasmy, and help with functional
complementation [4, 5]. Mitochondrial fission allows for the segregation of irreversibly damaged portions of the mitochondrial network and subsequent degradation. Mitochondrial fission necessarily requires dynamin-related protein 1 (Drp1) and FIS1 [6]. Nevertheless, an explicit understanding of the factors promoting fission and fusion remains limited.

The Drp1 gene encodes a dynamin family GTPase protein comprised of a characteristic Dynamin like protein family domain (Dynamin and Mx protein domains), a dynamin central domain, and a dynamin GTPase effector domain. While predominantly located in the cytoplasm, a small fraction is located upon the cytoplasmic surface of the mitochondrial tubules. Overexpression of Drp1 causes mitochondrial fragmentation, whereas inhibition results in the elongation of the mitochondrial network. Drp1 protein function is regulated by post-translational modification via phosphorylation, where a well-documented phosphorylation site, S616, promotes fission through an increase in activity. In contrast, phosphorylation of another site, S637, acts to lessen fission through reduced activity [7, 8, 9]. Drp1 polymerizes to form a spiral structure around the mitochondrial tubule, and then utilizes its GTPase activity to constrict the tubule and eventually cause fragmentation of the mitochondria [10, 11, 12, 13]. Presumably, a low membrane potential promotes fission, while a high membrane potential obstructs fission [14]. Through its role in mitochondrial fission, Drp1 controls mitochondrial morphology and function.

The role of Drp1 protein is not limited to mitochondrial fission. In addition, it participates in peroxisomal fragmentation [15] and maintains the morphology and function of the Endoplasmic Reticulum (ER) [16, 17]. Also, Drp1 is required for a standard rate of cytochrome c release and caspase activation during programmed cell death [18]. The role of Drp1 in apoptosis is not clear, but the product of the Bax gene, a pro-apoptotic Bcl-2 family protein, has been found to co-localize with Drp1 at the site of mitochondrial fission [19]. Drp1 plays role in Bcl-2 regulated apoptosis, peroxisomal and ER fragmentation.

The Drp1 protein interacts with other proteins involved in a number of mitochondrial processes, such as the products of Pink1 (PTEN-induced putative kinase 1) and park [20]. Mutation of the Pink1 and park genes are among the most prominent causes of early onset PD. The roles of Pink1 and park are vital to ubiquitin-dependent mitophagy. The protein product of park is involved in the process of ubiquitination that plays a crucial role in the proteasomal directed degradation of proteins, such as Drp1 [21]. Activated Akt can increase Drp1 phosphorylation and its localization to the mitochondria, to promote “mitofission” with an accompanying increase in the generation of reactive oxygen species [22]. Drp1 knockouts increase nuclear translocation of transcription factor foxo and enhance the expression of its downstream targets [23]. Akt protein inhibits the nuclear translocation of foxo [24, 25] and regulates Pink1/park dependent mitophagy [26]. Foxo transcriptionally upregulates the Pink1/park pathway in mammals and Pink1 appears to function upstream of foxo in D. melanogaster [27, 28]. Such a feedback mechanism implies a complex relationship between Drp1 and park, Akt1 and foxo.

Here, we propose that gain-of-function of Drp1 results in a Parkinsonian-like phenotype. We use drosophila to model Parkinson disease because it is an excellent model system in which to study interactions between genes, including PD genes [29, 30]. The inhibition of Drp1 in the dopaminergic
neurons of a mouse MPTP model of PD gives protection against mitochondrial translocation of p53 and the loss of dopaminergic neurons [31]. In our experiment, we used the UAS-Gal4 system to direct and to inhibit the expression of the Drp1 gene in Drosophila. The GMR-GAL4 transgene directs expression in the developing eye [32] and the Ddc-Gal4 transgene directs expression in dopaminergic and serotonergic neurons [33, 34]. Overexpression of Drp1 has toxic effects; although, its inhibition slightly improves the lifespan, the climbing ability over time is compromised. In an established park-RNAi model of PD [35], we directed and inhibited the expression of the Drp1 gene. The PD-like phenotypes of Ddc-Gal4 park RNAi were rescued by the expression of Drp1-RNAi transgenes. The strategy is to identify the basic mechanism in simple model organism and then further validate the finding in mammalian model organism.

**Results**

Drp1 is highly conserved between Homo sapiens and Drosophila melanogaster

The *D. melanogaster* Drp1 protein sequence was sourced from NCBI protein and the conserved sequences were identified using NCBI CDD. NCBI protein Blast of Drp1 protein of *D. melanogaster* (NP_608694.2) with the *H. sapiens*, identified dynamin-1-like protein (isoform 4) (NP_001265392.1), it is 65% identical with a bit score of 957. The multiple sequence alignment of the two proteins derived by Clustal Omega (Fig. 1A) shows a highly conserved dynamin-like protein family domain, a dynamin central domain, and a dynamin GTPase effector domain. Two well-documented phosphorylation sites are identified; S606 and S627 in dynamin-1-like protein isoform 4 of *H. sapiens*; and S616 and T637 in Drp1 of *D. melanogaster*. A template-based modeling of *D. melanogaster* Drp1 protein by use of a combination of empirically derived energy functions and physics-based simulated folding was produced using Phyre2. The modeled *D. melanogaster* Drp1 protein (i) and the *H. sapiens* Dynamin-1 like protein (ii) from the NCBI database share an identical structure (Fig. 1B). The amino-terminus region of the Drp1 protein is highly conserved and has a consensus LC3-interacting region (LIR) sequence for binding to the ATG8/LC3 protein as determined by the Eukaryotic Linear Motif (ELM) resource. As this protein is so highly conserved, it seems very likely that the functions are highly conserved.

The overexpression and inhibition of Drp1 with Ddc-Gal4

The overexpression of Drp1 by the Ddc-Gal4 transgene results in a decreased lifespan of 56 days compared to the control of 68 days shown in Fig. 2A. Inhibition of Drp1 by two distinct RNAi transgenes, via the UAS-Drp1-RNAi1 and UAS-Drp1-RNAi2 directed by the Ddc-Gal4 transgene, results in lifespans of 70 and 72 days, respectively; very similar to the 68 days observed in the control, as determined by log-rank at a P < 0.0001 (Fig. 2A). While, the overexpression of Drp1 by Ddc-Gal4 does little to alter locomotion over time, the climbing abilities of flies expressing Drp1-RNAi's are severely compromised as determined by 95% confidence interval in a nonlinear fitting of the climbing curve (Fig. 2B).

The inhibition of Drp1 in the Ddc-Gal4 UAS-park-RNAi model of PD
The loss of function of park has led to the establishment of a number of Drosophila models of PD. The Ddc-Gal4 park-RNAi UAS-lacZ critical males have a median lifespan of 58 days. Overexpression of Drp1 in the Ddc-Gal4 park-RNAi expressing flies results in a similar life span of 58 days which is not significantly different compared to the control. The two UAS-Drp1-RNAi transgenes, UAS-Drp1-RNAi1 and UAS-Drp1-RNAi2, when expressed along with Ddc-Gal4 park-RNAi, results in a much-increased median life span of 84 and 76 days, respectively; determined by log-rank at a P < 0.0001 (Fig. 3A). The overexpression of Drp1 by Ddc-Gal4 along with park-RNAi slightly increases the climbing ability over time. However, the locomotor activity of the critical classes with the directed expression of the Drp1-RNAi transgenes are significantly increased (Fig. 3B) as determined by 95% confidence interval in a nonlinear fitting of the climbing curve.

**Overexpression of Drp1 during development of the eye decreases ommatidia and bristle number**

The inhibition and overexpression of Drp1, directed by the GMR-Gal4 transgene in the developing eye of flies affects development. The expression of UAS-Drp1 and UAS-Drp1-RNAi1 in developing eye directed by GMR-Gal4 transgene results in significantly higher mean number of ommatidia, 716.9 and 718.8, respectively compared to 703 for the lacZ control flies (Fig. 4B) as determined by unpaired T test with a P value of 0.0483 and 0.0484. The mean of interommatidial bristle produced through inhibition by the UAS-Drp-RNAi1 and UAS-Drp1-RNAi2 transgene was significantly higher at and 556.7 (P value = 0.0406) and 578.7 (P value = 0.0023) compare to 536 of control flies as determined by an unpaired T test. The mean number of interommatidial bristle for UAS-Drp1 flies was 541.6 compared to 536 of control, which is not significant as determined by an unpaired T test (P value = 0.6128). The ommatidia area, compared to the control of 200 um 2 per ommatidium, of the overexpression critical class was 213.7 um 2 (P value = 0.0303) and the ommatidium area produced by the UAS-Drp1-RNAi1 and UAS-Drp1-RNAi2 transgenes were 225 um 2 (0.0490) and 217 um 2 (P value = 0.0011), which are all significantly different as determined by unpaired T test(Fig. 4D).

**Discussion**

The critical role played by the structure of the mitochondria in the function of the organelle suggests that the product of the Drp1 gene acts as an essential component in the regulation of a number of sub-cellular processes. Excessive mitochondrial fragmentation is associated with dysfunctional metabolic diseases and a “hyper-fused” mitochondrial network serves to protect from metabolic insult and autophagy [36]. In the skeletal muscle of mice, Drp1 overexpression causes the severe impairment of post-natal muscle growth as it results in attenuated protein syntheses and the downregulation of growth hormone pathways [37]. The high fat and high glucose conditions cause excessive oxidative stress and mitochondrial fragmentation mediated by the Drp1 protein [38, 39]. These phenotypes are similar to the overexpression of DLP1/Drp1 and inhibition of the MFN2 gene. In contrast, the levels of MFN2 mRNA are increased by acute weight loss [40]. The effect of Drp1 overexpression upon physiological processes is toxic.
The balance between mitochondrial fission and fusion is very delicate. For example, a newborn girl with microcephaly, along with other developmental defects, was shown to have defective mitochondrial and peroxisomal fission likely due to a dominant negative mutation in the Drp1 homologue [41]. Drp1 is essential for embryonic development in mice such that homozygous mutants or knockout Drp1 mice die during embryogenesis [42]. The Drp1 protein assists in caspase-independent mitochondrial fission to amplify apoptosis [43]. The acute overexpression and inhibition of the Drp1 gene has shown similar phenotypes [23], most likely due to an extreme disruption in mitochondrial morphology.

Increasing the expression of Drp1 in the neurons of Drosophila under the Ddc-Gal4 transgene seems to decrease longevity, and Drp1 has been found to increase the ROS levels in mitochondria [44]. In our experiments, the lifespan of flies that overexpress Drp1 was significantly decreased when compared to controls, which may be due to elevated oxidative stress beyond a threshold of a normal lifespan. The inhibition of Drp1 in dopaminergic neurons through the directed expression of the Drp1-RNAi’s results in a slight increase in lifespan. Drp1 overexpression and inhibition does significantly influence the number of ommatidia or interommatidial bristles, and significantly increase ommatidia area when expressed under the control of the GMR-Gal4 transgene.

The well-documented function of Drp1 is the promotion of mitochondrial fission and the inhibition of mitochondrial fusion[45]. Mitochondria host a number of cellular processes, especially oxidative phosphorylation, and are thus under continuous cellular stress and require repair and replacement [46, 47, 48]. The lower tolerance of mitochondria to fission, compared to fusion, suggests that a continuous network of mitochondria can survive greater injury due to a lower rate of mitophagy and a slower rate of mitochondrial biogenesis, up-to an optimum level. The overexpression of Drp1 results in the excessive fragmentation of the mitochondria such that its efficiency can be diminished to the point that functional complementation by fellow mitochondria is negligible. Mitochondria can become a distinct burden for the cell to upkeep instead of being an efficient “powerhouse of the cell”. Clearly, the level of Drp1 expression plays a key role in cell survival.

The park gene is crucial to the function of Pink1-dependent mitochondrial mitophagy. The loss of the park protein is a cause of great cellular stress as a major mitophagy mechanism is compromised to potentially result in the accumulation of non-functional mitochondria. Under normal circumstances, the park ubiquitin ligase recruits the Drp1 protein to mediate mitochondrial fragmentation during mitophagy [20, 49]. In mouse embryonic fibroblasts, loss of park does not have any effect upon the number of mitochondria [50]. Furthermore, the loss of park along with the loss of Drp1 increases the number of mitochondria by threefold, which can be interpreted that park controls mitochondria fragmentation in a Drp1 knockout background. Therefore, park can negatively regulate Drp1-independent mitochondrial division. The impact of the loss of park can be observed only when the majority of mitochondrial fission activity, mediated by Drp1, is absent.

In our experiments, the critical class flies that have the directed co-expression of Drp1-RNAi and park-RNAi inhibitory transgenes live longer than those that express park-RNAi and over-express Drp1 under the Ddc-
Gal4 transgene. The inhibition of Drp1 and park though directed RNA interference promotes survival. This could be due to the establishment of an altered mitochondrial network to enhance homeostasis and benefit cellular health.

Conclusion

The Drp1 is not a major Parkinson disease gene, but it has been associated with cell death pathways in neurons. The overexpression of Drp1 gene in neurons results in the reduced survival and an age-dependent decline in locomotor ability. The knockdown of Drp1 in the Ddc-Gal4-transgenes of Drosophila results in an age-dependent loss in climbing ability, phenotypes that are strongly associated with neuronal degeneration and Parkinson disease. Thus, the compromised climbing abilities in flies with directed inhibition of Drp1 have produced a new model of Parkinson Disease and can be used to investigate further the mechanisms underlying PD and other neurodegenerative diseases. The co-inhibition of the parkin with Drp1 results in the rescue of the phenotypes observed, it is possible that Drp1 and parkin participate in cellular pathways that promote cell death. Further studies are required to better understand the interaction between parkin and Drp1 in these neurons. Overall our experiments are allowing us to contribute to our understanding of mitochondrial health and enhanced conditions of homeostasis.

Materials And Methods

Bioinformatic analysis

Protein sequences were obtained from the National Center of Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/protein/). The conserved domains were identified using NCBI Conserved Domain Database (https://www.ncbi.nlm.nih.gov/cdd/), and Eukaryotic Linear Motif (http://elm.eu.org/). Multiple sequence alignment was done using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) to reveal the conservation of domains. The Homo sapiens Dynamin-1 like protein (DLP-1/Drp1) structure (PDB ID 4BEJ) was obtained from NCBI structure database (https://www.ncbi.nlm.nih.gov/structure/) and Drosophila melanogaster Drp1 protein structure was developed using Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) modeling tool. The final models were edited with the PyMOL software (https://pymol.org/2/) to highlight the N-terminus, C-terminus and LIR regions.

Drosophila stocks and media

The Ddc-Gal4 line was received from Dr. J. Hirsch of University of Virginia. The GMR-Gal4; UAS-lacZ4-1, UAS-Drp1 (y[1] w[+]; P[w+ mC] = FLAG-FlAsH-HA-Drp1)3, Ki[1]); the UAS-Drp1-RNAi1 (y[1] v[1]; P[y+[ t.7.7] v+[ t.1.8] = TriP.JF02762 attP2}); and UAS-Drp1-RNAi2 (y[1] v[1]; P[y+[ t.7.7] v+[ t.1.8] = TriP.HMCO3230 attP40) stocks were obtained from Bloomington Drosophila Stock Center at Indiana University, Bloomington, Indiana, USA. The UAS-park-RNAi line was obtained from Dr. B. Lu [51, 52]. The
Ddc-Gal4 park-RNAi line was produced through standard methods [53]. All flies were maintained on standard cornmeal/molasses/yeast/agar media treated with propionic acid and methylparaben to resist fungal growth. Stocks were maintained at room temperature (22° ± 3° C), whereas crosses and experiments were kept at 25°C.

Survival assay

Female virgins of the Ddc-Gal4 genotype were collected every 8 to 12 hours for several days. The confirmed virgin flies were then crossed with UAS-lacZ, UAS-Drp1, UAS-Drp1-RNAi1 and UAS-Drp1-RNAi2 males. Critical class male progeny were collected from mating's until approximately 250 flies of each genotype were obtained. To avoid over-crowding, the flies were maintained in cohorts of 25 or less per vial on standard media. Flies were scored every second day for viability and were transferred to new food every two to five days. Scoring continued until all flies had died [55]). Longevity data were analyzed using GraphPad Prism version 8 statistical software (graphpad.com), and survival curves were compared by Mantel-Cox test. Significance were determined at 95% confidence level (P ≤ 0.05) with Bonferroni correction.

Locomotor analysis

Approximately 70 male flies of the critical class were collected within a 24-hour period and maintained as cohorts of 10 flies in each vial. Media was replenished twice a week. The climbing assay was performed as previously described according to a standard protocol [54, 55]. Briefly, every week 50 males were assayed, in groups of 10, for their ability to climb a glass tube divided into 5 levels of 2 cm each. 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 ≤ 0.05).

Biometric analysis of the Drosophila melanogaster eye

Female virgins of the GMR-Gal4 genotype were collected every 8 to 12 hours for several days. The confirmed virgins were then crossed with the males of UAS-lacZ, UAS-Drp1, UAS-Drp1-RNAi1 and UAS-Drp1-RNAi2 genotypes. Critical class male progeny were collected for each genotype. The collected flies were kept as cohorts of 10 flies or less upon fresh media and allowed to age for 3 to 4 days. The flies were prepared for scanning electron microscopy following the standard protocol [35]. Ommatidia and interommatidial bristle counts were performed on 10 or more flies of each genotype using National Institute of Health (NIH) ImageJ software. The ommatidium area was calculated by measuring the area of 5 distinct ommatidial “rosettes” per fly eye and then dividing by 7 to determine the mean area of each ommatidium; done on 10 eyes of each genotype. The Biometric analysis was performed using GraphPad Prism version 8 statistical software. Significance were determined at 95% confidence level (P ≤ 0.05).

Abbreviations
Ddc
dopa decarboxylase
Drp1
dynamin related protein 1
GMR
glass multiple reporter
PD
Parkinson disease
Pink1
PTEN-induced putative kinase 1
RNAi
ribonucleic acid interference

**Declarations**

*Ethics approval and consent to participate*

The study was approved by the Animal Care Committee of the Memorial University of Newfoundland as a Category of Invasiveness Level A protocol. Consent was not applicable for the study.

*Consent for publication*

Not Applicable

*Availability of data and materials*

All data generated or analyzed during this study are included in the article.

*Competing interests*

The authors declare no competing interests.

*Funding*

The study was funded by School of Graduate studies, Memorial University and by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant. Funding organizations were not involved in the design of the study.

*Author's contributions*

Azra Hasan performed the statistical analysis of bioinformatic, survival, climbing and biometric data. Brian E Staveley devised and participated in the experimental design, supervision of the study and revision to the draft of manuscript.
Acknowledgements

Not Applicable

Author’s Information

Department of Biology, Memorial University of Newfoundland, St. John’s, NL, A1B 3X9, Canada

Azra Hasan & Brian E. Staveley

References

1. Harman D. The aging process: Major risk factor for disease and death. Med Sci. 1991;88:5360–3. http://www.pnas.org/content/88/12/5360.full.pdf. Accessed 22 Dec 2017.

2. Galluzzi L, Kepp O, Kroemer G. Mitochondria. Master regulators of danger signalling. Nat Rev Mol Cell Biol. 2012;13:780–8.

3. Hoitzing H, Johnston IG, Jones NS. What is the function of mitochondrial networks? A theoretical assessment of hypotheses and proposal for future research. BioEssays. 2015;37:687–700. doi:10.1002/bies.201400188.

4. Chan DC. Fusion and Fission: Interlinked Processes Critical for Mitochondrial Health. Annu Rev Genet. 2012;46:265–87. doi:10.1146/annurev-genet-110410-132529.

5. Nakada K, Sato A, Hayashi JI. Mitochondrial functional complementation in mitochondrial DNA-based diseases. International Journal of Biochemistry Cell Biology. 2009;41:1907–13.

6. Sebastián D, Palacín M, Zorzano A. Mitochondrial Dynamics: Coupling Mitochondrial Fitness with Healthy Aging. Trends Mol Med. 2017;23:201–15. doi:10.1016/J.MOLMED.2017.01.003.

7. Chang C-R, Blackstone C. Drp1 phosphorylation and mitochondrial regulation. EMBO Rep. 2007;8:1088–9. doi:10.1038/sj.embor.7401118. author reply 1089-90.

8. Cribbs JT, Strack S. Reversible phosphorylation of Drp1 by cyclic AMP-dependent protein kinase and calcineurin regulates mitochondrial fission and cell death. EMBO Rep. 2007;8:939–44. doi:10.1038/sj.embor.7401062.

9. Chang C-R, Blackstone C. Cyclic AMP-dependent Protein Kinase Phosphorylation of Drp1 Regulates Its GTPase Activity and Mitochondrial Morphology. J Biol Chem. 2007;282:21583–7. doi:10.1074/jbc.C700083200.

10. Mears JA, Lackner LL, Fang S, Ingerman E, Nunnari J, Hinshaw JE. Conformational changes in Dnm1 support a contractile mechanism for mitochondrial fission. Nat Struct Mol Biol. 2011;18:20–6. doi:10.1038/nsmb.1949.

11. Lackner LL, Horner JS, Nunnari J. Mechanistic analysis of a dynamin effector. Science. 2009;325:874–7. doi:10.1126/science.1176921.

12. Ingerman E, Perkins EM, Marino M, Mears JA, McCaffery JM, Hinshaw JE, et al. Dnm1 forms spirals that are structurally tailored to fit mitochondria. J Cell Biol. 2005;170:1021–7.
13. Legesse-Miller A, Massol RH, Kirchhausen T. Constriction and Dnm1p Recruitment Are Distinct Processes in Mitochondrial Fission. Mol Biol Cell. 2003;14:1953–63. doi:10.1091/mbc.e02-10-0657.

14. Twig G, Elorza A, Molina AJA, Mohamed H, Wikstrom JD, Walzer G, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J. 2008;27:433–46. doi:10.1038/sj.emboj.7601963.

15. Koch A, Thiemann M, Grabenbauer M, Yoon Y, McNiven MA, Schrader M. Dynamin-like Protein 1 Is Involved in Peroxisomal Fission. J Biol Chem. 2003;278:8597–605. doi:10.1074/jbc.M211761200.

16. Wikstrom JD, Israeli T, Bachar-Wikstrom E, Swisa A, Ariav Y, Waiss M, et al. AMPK Regulates ER Morphology and Function in Stressed Pancreatic β-Cells via Phosphorylation of DRP1. Mol Endocrinol. 2013;27:1706–23. doi:10.1210/me.2013-1109.

17. Pitts KR, Yoon Y, Krueger EW, McNiven MA. The Dynamin-like Protein DLP1 Is Essential for Normal Distribution and Morphology of the Endoplasmic Reticulum and Mitochondria in Mammalian Cells. Mol Biol Cell. 1999;10:4403–17. doi:10.1091/mbc.10.12.4403.

18. Breckenridge DG, Kang B-H, Kokel D, Mitani S, Staehelin LA, Xue D. Caenorhabditis elegans drp-1 and fis-2 regulate distinct cell-death execution pathways downstream of ced-3 and independent of ced-9. Mol Cell. 2008;31:586–97. doi:10.1016/j.molcel.2008.07.015.

19. Karbowski M, Lee Y-J, Gaume B, Jeong S-Y, Frank S, Nechushtan A, et al. Spatial and temporal association of Bax with mitochondrial fission sites, Drp1, and Mfn2 during apoptosis. J Cell Biol. 2002;159:931–8. doi:10.1083/jcb.200209124.

20. Buhlman L, Damiano M, Bertolin G, Ferrando-Miguel R, Lombès A, Brice A, et al. Functional interplay between Parkin and Drp1 in mitochondrial fission and clearance. Biochim Biophys Acta - Mol Cell Res. 2014;1843:2012–26. doi:10.1016/j.bbamacr.2014.05.012.

21. Wang H, Song P, Du L, Tian W, Yue W, Liu M, et al. Parkin Ubiquitinates Drp1 for Proteasome-dependent Degradation. J Biol Chem. 2011;286:11649–58. doi:10.1074/jbc.M110.144238.

22. Kim DI, Lee KH, Gabr AA, Choi GE, Kim JS, Ko SH, et al. Aβ-Induced Drp1 phosphorylation through Akt activation promotes excessive mitochondrial fission leading to neuronal apoptosis. Biochim Biophys Acta - Mol Cell Res. 2016;1863:2820–34. doi:10.1016/j.bbamcr.2016.09.003.

23. Favaro G, Romanello V, Varanita T, Andrea Desbats M, Morbidoni V, Tezze C, et al. DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. Nat Commun. 2019;10:2576. doi:10.1038/s41467-019-10226-9.

24. Boccitto M, Kalb RG. Regulation of Foxo-dependent transcription by post-translational modifications. Curr Drug Targets. 2011;12:1303–10. http://www.ncbi.nlm.nih.gov/pubmed/21443461. Accessed 6 Nov 2017.

25. Burgering BMT, Medema RH. Decisions on life and death: FOXO Forkhead transcription factors are in command when PKB/Akt is off duty. J Leukoc Biol. 2003;73:689–701. doi:10.1189/jlb.1202629.

26. McCoy MK, Kaganovich A, Rudenko IN, Ding J, Cookson MR. Hexokinase activity is required for recruitment of parkin to depolarized mitochondria. Hum Mol Genet. 2014;23:145–56.
27. Li W, Du M, Wang Q, Ma X, Wu L, Guo F, et al. FoxO1 Promotes Mitophagy in the Podocytes of Diabetic Male Mice via the PINK1/Parkin Pathway. Endocrinology. 2017;158:2155–67. doi:10.1210/en.2016-1970.

28. Webb AE, Brunet A. FOXO transcription factors: key regulators of cellular quality control. Trends Biochem Sci. 2014;39:159–69. doi:10.1016/j.tibs.2014.02.003.

29. Staveley BE. Successes of Modelling Parkinson Disease in Drosophila. In: Mechanisms in Parkinson's Disease - Models and Treatments. InTech; 2012. p. 233–50.

30. Xiong Y, Yu J. Modeling Parkinson's disease in Drosophila: What have we learned for dominant traits? Frontiers in Neurology. 2018;9 APR:228.

31. Filichia E, Hoffer B, Qi X, Luo Y. Inhibition of Drp1 mitochondrial translocation provides neural protection in dopaminergic system in a Parkinson's disease model induced by MPTP. Sci Rep. 2016;6:32656.

32. Freeman M. Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. Cell. 1996;87:651–60.

33. Riemensperger T, Issa AR, Pech U, Coulom H, Nguyễn MV, Cassar M, et al. A Single Dopamine Pathway Underlies Progressive Locomotor Deficits in a Drosophila Model of Parkinson Disease. Cell Rep. 2013;5:952–60.

34. Li H, Chaney S, Forte M, Hirsh J. Ectopic g-protein expression in dopamine and serotonin neurons blocks cocaine sensitization in Drosophila melanogaster. Curr Biol. 2000;10:211–4.

35. M'Angale PG, Staveley BE. Overexpression of Buffy enhances the loss of parkin and suppresses the loss of Pink1 phenotypes in Drosophila. Genome. 2017;60:241–7. doi:10.1139/gen-2016-0165.

36. Wai T, Langer T. Mitochondrial Dynamics and Metabolic Regulation. Trends Endocrinol Metab. 2016;27:105–17. doi:10.1016/J.TEM.2015.12.001.

37. Touvier T, De Palma C, Rigamonti E, Scagliola A, Incerti E, Mazelin L, et al. Muscle-specific Drp1 overexpression impairs skeletal muscle growth via translational attenuation. Cell Death Dis. 2015;6:e1663. doi:10.1038/cddis.2014.595.

38. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. Proc Natl Acad Sci U S A. 2006;103:2653–8. doi:10.1073/pnas.0511154103.

39. Schneeberger M, Dietrich MO, Sebastián D, Imbernón M, Castaño C, García A, et al. Mitofusin 2 in POMC Neurons Connects ER Stress with Leptin Resistance and Energy Imbalance. Cell. 2013;155:172–87. doi:10.1016/J.CELL.2013.09.003.

40. Bach D, Naon D, Pich S, Soriano FX, Vega N, Rieusset J, et al. Expression of Mfn2, the Charcot-Marie-Tooth Neuropathy Type 2A Gene, in Human Skeletal Muscle. Diabetes. 2005;54:2685–93. doi:10.2337/DIABETES.54.9.2685.
41. Waterham HR, Koster J, van Roermund CWT, Mooyer PAW, Wanders RJA, Leonard JV. A Lethal Defect of Mitochondrial and Peroxisomal Fission. N Engl J Med. 2007;356:1736–41. doi:10.1056/NEJMoA064436.

42. Ishihara N, Nomura M, Jofuku A, Kato H, Suzuki SO, Masuda K, et al. Mitochondrial fission factor Drp1 is essential for embryonic development and synapse formation in mice. Nat Cell Biol. 2009;11:958–66. doi:10.1038/ncb1907.

43. Oettinghaus B, D’Alonzo D, Barbieri E, Restelli LM, Savoia C, Licci M, et al. DRP1-dependent apoptotic mitochondrial fission occurs independently of BAX, BAK and APAF1 to amplify cell death by BID and oxidative stress. Biochim Biophys Acta - Bioenerg. 2016;1857:1267–76. doi:10.1016/j.bbabio.2016.03.016.

44. Nagdas S, Kashatus DF. The Interplay between Oncogenic Signaling Networks and Mitochondrial Dynamics. Antioxidants. 2017;6:33. doi:10.3390/antiox6020033.

45. Chang CR, Blackstone C. Dynamic regulation of mitochondrial fission through modification of the dynamin-related protein Drp1. In: Annals of the New York Academy of Sciences. Blackwell Publishing Inc.; 2010. p. 34–9.

46. Dan Dunn J, Alvarez LA, Zhang X, Soldati T. Reactive oxygen species and mitochondria: A nexus of cellular homeostasis. Redox Biol. 2015;6:472–85. doi:10.1016/J.REDOX.2015.09.005.

47. Lambert AJ, Brand MD. Reactive Oxygen Species Production by Mitochondria. In: Methods in molecular biology (Clifton NJ). 2009. 165–81. doi:10.1007/978-1-59745-521-3_11.

48. Murphy MP. How mitochondria produce reactive oxygen species. Biochem J. 2009;417:1–13. doi:10.1042/BJ20081386.

49. Wang Y, Tang C, Cai J, Chen G, Zhang D, Zhang Z, et al. PINK1/Parkin-mediated mitophagy is activated in cisplatin nephrotoxicity to protect against kidney injury. Cell Death Dis. 2018;9:1113. doi:10.1038/s41419-018-1152-2.

50. Roy M, Itoh K, Iijima M, Sesaki H. Parkin suppresses Drp1-independent mitochondrial division. Biochem Biophys Res Commun. 2016;475:283–8. doi:10.1016/j.bbrc.2016.05.038.

51. Yang Y, Nishimura I, Imai Y, Takahashi R, Lu B. Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila. Neuron. 2003;37:911–24.

52. Yang Y, Gehrke S, Imai Y, Huang Z, Ouyang Y, Wang JW, et al. Mitochondrial pathology and muscle and dopaminergic neuron degeneration caused by inactivation of Drosophila Pink1 is rescued by Parkin. Proc Natl Acad Sci U S A. 2006;103:10793–8.

53. Githure M, Staveley P. BE. Bcl-2 homologue Debcl enhances α-synuclein-induced phenotypes in Drosophila. doi:10.7717/peerj.2461.

54. Todd AM, Staveley BE. Novel assay and analysis for measuring climbing ability in Drosophila. Drosoph Inf Serv. 2004;87:101–8. http://www.ou.edu/journals/dis/DIS87/4 - Techniques.pdf. Accessed 6 Oct 2019.

55. Todd A, Staveley B. Expression of Pink1 with α-synuclein in the dopaminergic neurons of Drosophila leads to increases in both lifespan and healthspan. Genet Mol Res. 2012;11:1497–502.
A. Drp1 is evolutionarily conserved in Drosophila. Clustal Omega multiple sequence alignment of D. melanogaster Drp1 (NP_608694.2) protein with the H. sapiens (NP_001265392.1) shows evolutionarily conserved domains identified using the NCBI Conserved Domain Database (CDD) and further confirmed by the Eukaryotic Linear Motif (ELM) resource. The two well documented phosphorylation sites are identified, S606 and S627 in dynamin-1-like protein (DLP-1) isoform 4 of H. sapiens; and S616 and T637 in Drp1 of D. melanogaster. The asterisks indicate the residues that are identical; the colons indicate the conserved substitutions; and the dots indicates the semi-conserved substitutions. Colour differences indicate the chemical nature of amino acids: red indicates small hydrophobic (includes aromatic) residues; blue indicates acidic; magenta indicates basic; and green indicates basic with hydroxyl or amine groups.
Figure 2

B (i). The original Dynamin-1 like protein (DLP-1) structure of H. sapiens (NP_001265392.1) from the NCBI structure database. B (ii). The Phyre2 web portal for protein modelling, prediction and analysis mediated the development of a model of the Drp1 protein of D. melanogaster (NP_608694.2) from a 76% identical protein with a confidence of 100%. The N terminus is coloured in Magenta; C terminus is coloured in Red and a consensus ATG8 binding region at N terminus is coloured in orange.
Figure 3

Altered Drp1 expression under the control of Ddc-Gal4 influences the survival and climbing ability of flies. A. The GraphPad prism8 generated graph of the longevity assay for the expression of Drp1, Drp1 RNAi’s under the control of Ddc-Gal4 transgene. The directed expression results in decreased median lifespan of 56 days compare to 68 days of control calculated by Log-rank Mantel Cox test, with Bonferroni correction. The inhibition of Drp1 under the control of Ddc-Gal4 transgene results in increased lifespan of 70 days with UAS-Drp1-RNAi1 and 72 days with UAS-Drp1-RNAi2 compare to 68 days of control done by Log-rank Mantel Cox test, with Bonferroni correction. B. The GraphPad prism8 generated graph of the climbing abilities of flies with overexpression of Drp1, Drp1 RNAi’s and control. The climbing ability of Drp1 overexpression and Drp1 RNAi’s flies is significantly compromised compared to control as determined in nonlinear fitting of the climbing curve by 95% confidence interval.
Altered Drp1 expression when coupled with Ddc-Gal4 park-RNAi. A. The graph of longevity assay generated by GraphPad prism8 with altered Drp1 expression in Ddc-Gal4 park-RNAi expressing flies. The overexpression results in median lifespan of 58 days similar to 58 days of control (lacZ/Park-RNAi) determined by Log-rank Mantel-Cox test, with Bonferroni correction. The inhibition of Drp1 in neurons using Ddc-Gal4 transgene along with Park-RNAi results in increased lifespan of 84 days with UAS-Drp1-RNAi1 and lifespan of 76 days with UAS-Drp1-RNAi2 compare to 58 days of control done by Log-rank Mantel Cox test, with Bonferroni correction. B. The GraphPad prism8 generated graph of the climbing abilities of flies with overexpression of Drp1, Drp1 RNAi and control. The climbing abilities of and Drp1 RNAi flies has significantly increased compared to control as determined in nonlinear fitting of the climbing curve by 95% confidence interval. 

Figure 4
Figure 5

The phenotypic effects of altered Drp1 expression in D. melanogaster eye. A. Scanning electron micrograph of the altered Drp1 expression under the control of GMR-Gal4 transgene. The genotypes are (a) GMR-Gal4/UAS-lacZ (Control); (b) GMR-Gal4/UAS-Drp1; (c) GMR-Gal4/UAS-Drp1-RNAi1 (d) GMR-Gal4/UAS-Drp1-RNAi2. B. The ommatidia number for control is 703, the inhibition and expression of Drp1 results in slight increase ommatidial count compared to control. C. The interommatidial bristle count for the control is 536 and for the expression and inhibition lines are slightly significant more for the two RNAi transgenes mean value of 556.7 and 578. D. The ommatidium area of the Drp1 expression transgene GMR-Gal4 UAS-Drp1 and the inhibition transgenes GMR-Gal4 UAS-Drp1-RNAi1 and GMR-Gal4 UAS-Drp1-RNAi2 are significantly higher than control.

Supplementary Files
This is a list of supplementary files associated with this preprint. Click to download.

- HasanStaveleyDrp1NC3RsARRIVEGuidelinesChecklist.docx