Identifying potential *PARIS* homologs in *D. melanogaster*

E.M. Merzetti and B.E. Staveley

Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada

Corresponding author: B.E. Staveley
E-mail: bestave@mun.ca

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ABSTRACT. Mitochondrial destruction leads to the formation of reactive oxygen species, increases cellular stress, causes apoptotic cell death, and involves a cascade of proteins including PARKIN, PINK1, and Mitofusin2. Mitochondrial biogenesis pathways depend upon the activity of the protein PGC-1 α . These two processes are coordinated by the activity of a transcriptional repressor, Parkin interacting substrate (PARIS). The PARIS protein is degraded through the activity of the PARKIN protein, which in turn eliminates the transcriptional repression that PARIS imposes upon a downstream target, PGC-1 α . Genes in this pathway have been implicated in Parkinson's disease, and there is a strong relationship between mitochondrial dysfunction and pre-mature neuron death. The identification of a PARIS homolog in *Drosophila melanogaster* would increase our understanding of the roles that PARIS and interacting genes play in higher organisms. We identified three potential PARIS homologs in *D. melanogaster*, one of which encodes a protein with similar domains to the *Homo sapiens* PARIS protein, CG15436. The *Drosophila* eye is formed from neuronal

precursors, making it an ideal system to assay the effects of altered gene expression on neuronal tissue formation. The eye-specific expression of RNAi constructs for these genes revealed that both *CG15269* and *Crol* caused neurodegenerative phenotypes, whereas *CG15436* produced a phenotype similar to *srl-EY*. *Crol-RNAi* expression reduced mean lifespan when expressed in dopaminergic neurons, whereas *CG15436-RNAi* significantly increased lifespan. *CG15436* was PARIS-like in both structure and function, and we characterized the effects of decreased gene expression in both the neuron-rich *D. melanogaster* eye and in dopaminergic neurons.

Key words: *PGC-1 α* ; *Spargel*; *PARIS*; *CG15436*; *CG15269*; *Crol*

INTRODUCTION

Parkinson's disease (PD) is the second-most-common neurodegenerative disorder, and affects over 1.6% of individuals over 65 years of age, with an associated annual healthcare cost of an estimated \$25 billion dollars (Beitz, 2014). PD patients present with symptoms related to impaired motor control, including bradykinesia, postural rigidity, and resting tremor, and, in the later stages, diminished cognitive function, including dementia and memory loss (Klockgether, 2004). The former symptoms are well-known to occur as a direct result of lost neurons in the *substantia nigra pars compacta* region of the brain, leading to a decrease in the neurotransmitter, dopamine. Efforts to combat PD have focused on symptom alleviation, and have failed to prevent progression or stem the initial onset of the disease. PD is caused by inherited and environmental factors, and in many cases, a combination of genetic risk factors and exposure to harsh environmental conditions, such as toxins, leads to disease symptoms (Mitsui, 2013). Many genes that are implicated in PD are either related to or directly linked to the proper maintenance and upkeep of mitochondria. Mitochondria are sites of oxidative phosphorylation, and produce much of the energy of a given cell; the impaired function of these organelles in neurons with high energy requirements may be the origin of the pre-mature mortality and disease onset found in PD (Ryan et al., 2015). The intracellular balance of mitochondria is an essential part of proper cell function, and changes to this balance can result in a number of negative consequences. In the event of mitochondrial damage, impaired organelles are repaired, or removed and recycled into usable components. If removal is necessary, a separate process initiates the formation of new mitochondria, in order to restore the energetic capacity of the cell (Palikaras and Tavernarakis, 2014). Mutations of genes involved in either of these processes can cause a loss of cellular energy and eventual cell mortality, and have been directly linked to the onset of PD (Guo, 2012). Identifying and characterizing the genes responsible for mitochondrial impairment in PD may lead to new therapeutic targets and novel options in disease management.

Parkin and *PTEN-induced putative kinase 1 (PINK1)* are two genes that function in the proper turnover of damaged or dysfunctional mitochondria, and prevent the accumulation of potentially toxic-impaired organelles (Eiyama and Okamoto, 2015). *Parkin* encodes an E3 ubiquitin ligase that leads to the ubiquitination and subsequent destruction of cellular proteins (Narendra et al., 2008). Mutations to *Parkin* result in the degeneration of dopaminergic neurons, probably by allowing the aggregation of multiple dysfunctional mitochondria that

eventually leads to overall cell death (Greene et al., 2003). *PINK1* is a serine/threonine-protein kinase that acts by recruiting *Parkin* to damaged mitochondria (Koh and Chung, 2010). Similarly, mutations to *PINK1* lead to the degeneration and dysfunction of dopaminergic neurons (Yang et al., 2006). Under normal physiological conditions, mitochondria prevent the outer-membrane accumulation of the PINK1 protein. If the mitochondria become depolarized this no longer occurs, allowing a build-up of PINK1 in the mitochondria, which then recruits the *Parkin* protein (PARKIN) and begins ubiquitin-mediated organelle destruction (Eiyama and Okamoto, 2015). Mutations in *Parkin* and *PINK1* have been linked to dopaminergic neurodegeneration and early-onset forms of human PD (Greene et al., 2005; Whitworth et al., 2008). The direct link between mitochondrial impairment and neurodegeneration indicates an important role of both *Parkin* and *PINK1* in overall organism viability.

The *Parkin*-mediated removal of impaired mitochondria leads to an overall decrease in the number of cell organelles; this mitochondrial deficit is corrected by a parallel pathway involving the peroxisome proliferator-activated receptor-gamma co-activator (*PGC*) family of genes (Scarpulla, 2011). The three members of this gene family [*PGC-1 α* , *PGC-1 β* , and *PGC-1 related cofactor (PRC)*] all share similar and partially redundant functions in mitochondrial biogenesis. *PRC* is mainly present in proliferating cells, while *PGC-1 α* and *PGC-1 β* are both found in tissues with high energetic requirements, such as skeletal muscle (Russell et al., 2005; Gleyzer and Scarpulla, 2011; Rowe et al., 2012). Although *PGC-1 α* and *PGC-1 β* are expressed in the same tissues and have been linked to the regulation of exercise endurance, *PGC-1 α* has specifically been found to be induced by stress, exercise, and fasting (Watson et al., 2007). Under normal physiological conditions, *PGC-1 α* is repressed by the negative regulatory protein, PARIS (Parkin interacting substrate). The PARIS protein in *Homo sapiens* is coded for by *ZNF746* (herein referred to simply as *PARIS*) and is localized to the neurons, including those in the *substantia nigra pars compacta* (Shin et al., 2011). Overexpression of *PARIS* in a mouse model resulted in the destruction of dopaminergic neurons, which could be rescued with the overexpression of either *Parkin* or *PGC-1 α* (Shin et al., 2011). *Parkin* appears to act as a master regulator by maintaining the balance between mitochondrial removal and biogenesis, and ensures that the cell receives an appropriate amount of energy for normal function. Therefore, the altered expression of downstream components, including *PARIS* and *PGC-1 α* , results in disease phenotypes in otherwise healthy organisms.

Drosophila melanogaster has been successfully used as a model organism for human disease research, and similarities between the nervous system of *Drosophila* and *H. sapiens* allow for the complex study of neurodegenerative disorders (West et al., 2015). The neuron-rich *Drosophila* eye is an ideal system with which to study the broad effect of genetic mutation on neuronal tissue, and contains a highly sensitive array of ommatidia and bristle tissues that respond to, and are altered by, any negative change to neuron cell architecture (Mishra and Knust, 2013). This makes the eye an excellent tissue with which to assess the effect of gene disruption on neuronal viability and altered gene activity, and provides a relatively rapid and quantifiable model of degeneration (Lee et al., 2014).

Many genes (including *Parkin* and *PINK1*) that are implicated in PD pathology have functional *D. melanogaster* homologs, and exhibit severe neuronal dysfunction when their expression is altered (Clark et al., 2006). Unlike the three *PGC* family members present in humans, *D. melanogaster* only has a single *PGC-1 α* homolog, *spargel (srl)* (Tiefenböck et al., 2010). Our previous research has indicated that altered *srl* expression leads to Parkinsonian phenotypes in *D. melanogaster*, providing a powerful model with which to study the complex

interactions that underlie disease progression (Merzetti and Staveley, 2015). The activities of *Parkin*, *PGC-1 α* , and *PINK1* homologs indicate that mitochondrial biogenesis and maintenance pathways are highly conserved across organisms. However, the only *H. sapiens* gene that is involved in the *Parkin/PINK1* pathway without a putative *D. melanogaster* homolog is *PARIS*. In this study, we aimed to identify potential *PARIS* candidates in *D. melanogaster*, and to characterize these candidates in neuron tissue in order to determine their potential roles in disease pathways.

MATERIAL AND METHODS

Drosophila culture

D. melanogaster stock lines were obtained from various sources; the *Ddc-Gal4*^{HL4.3D} (*Ddc-Gal4*) line was a gift from J. Hirsh (University of Virginia, USA) (Lin et al., 2000); *Glass Multiple Reporter-Gal4*¹² (*GMR-Gal4*), *UAS-lacZ*⁴⁻²⁻¹ (*UAS-lacZ*), *srl: y w; P{EPgy2^{srlEY05931}}* (*srl-EY*), and *srl: y sc v; P{TRiP.HMS00858}attP2* (*UAS-srl*^{HMS00858}) (*UAS-srl-RNAi*) were obtained from the Bloomington *Drosophila* Stock Center, Indiana University, Bloomington, IN, USA; and RNAi transgenes were obtained from the Vienna *Drosophila* Resource Center, Austria [stock Nos. 7779 (*UAS-Crol-RNAi*), 43655 (*UAS-CG15269-RNAi*), and 39986 (*UAS-CG15436-RNAi*)]. The flies were raised on a cornmeal-yeast-molasses-agar medium; a standard recipe was used that contained 65 g/L cornmeal, 10 g/L nutritional yeast, 5.5 g/L agar, 50 mL/L fancy-grade molasses, 5 mL 0.1 g/mL methyl 4-hydroxybenzoate in 95% ethanol, and 2.5 mL propionic acid. The medium was stored at 4°C and warmed to room temperature before use.

Scanning electron microscopy of the *D. melanogaster* eye

GMR-Gal4 virgin female flies were mated with *UAS-lacZ*, *UAS-Crol-RNAi*, *UAS-CG15269-RNAi*, *UAS-CG15436-RNAi*, *UAS-srl-EY*, and *UAS-srl-RNAi* males. The resulting male progeny from each individual cross were collected in groups of no more than 20 at three days of age and frozen at -80°C. Using forceps, the flies were mounted on metal stubs and desiccated for 48 h before imaging. Scanning electron micrographs were taken of the left eye of each mounted male fly at 130X magnification on a Mineral Liberation Analyzer 650F. The number of bristles and ommatidia was obtained using ImageJ.

Ageing analysis

Ddc-Gal4 virgin female flies were mated with *UAS-lacZ*, *UAS-Crol-RNAi*, *UAS-CG15269-RNAi*, and *UAS-CG15436-RNAi* males. Male offspring were collected in groups of up to 20 and stored in vials. The vials were scored for survival every 48 h, and the media were changed every 72 h or whenever a death event was scored. The resulting survival curves were compared using the GraphPad Prism software (log-rank, Mantel Cox test).

Bioinformatic analysis

Protein domains were identified using ScanProsite (De Castro et al., 2006), and Pfam

(Finn et al., 2014) alignment was generated by ClustalW2 (Larkin et al., 2007). Sequence identity and similarity scores were calculated using EMBOSS Needle (emboss.sourceforge.net). Protein sequences were obtained from UniProt [GenBank accession Nos. NP_001156946.1 (PARIS), NP_477243.1 (CROL), NP_609739.2 (CG15269), and NP_608840.1 (CG15436)].

RESULTS

Genes *crol*, *CG15436*, and *CG15269* share a high degree of protein conservation with *PARIS*

The *H. sapiens* *PARIS* gene encodes a protein that is predicted to contain 644 amino acids, and has homologs in the chimpanzee, rhesus monkey, cow, mouse, and rat. A detailed bioinformatic analysis of *PARIS* revealed the presence of an N-terminal Kruppel-associated box (KRAB) domain and two C-terminal zinc-finger double domains (Figure 1A). KRAB domains are transcriptional repressors found in C2H2 zinc-finger proteins. These functional modules comprise the largest group of transcriptional regulators in mammals, and are limited to the tetrapod superclass of organisms (Urrutia, 2003). Despite the significance of this gene in the regulation of mitochondrial biogenesis and cellular energy production, no known *PARIS* homologs have been identified in non-mammalian lineages.

Figure 1. *Homo sapiens* *PARIS* protein shared conserved protein domains with the putative *Drosophila melanogaster* homologs *Crol*, *CG15269*, and *CG15436*. **A.** Aligned protein sequences show the position of protein domains in three putative *D. melanogaster* homologs of *PARIS*. Orange indicates a KRAB domain, gold indicates a ZAD domain, and green indicates zinc-finger double-binding domains. **B.** Alignment of the zinc-finger double-binding domain in the *Homo sapiens* *PARIS* and *D. melanogaster* *CG15436* proteins revealed a high degree of amino acid (aa) conservation, with 51 of 74 aa either fully conserved or replaced by an aa of similar activity. Dark gray indicates the same aa, light gray indicates aa with similar functions.

The *PARIS* protein was used as a query to identify potential *D. melanogaster* homologs using the National Center for Biotechnology Information BLAST® tool (Altschul et al., 1990). The top three candidates were *CG15269* with 15.4% identity (23.8% similarity), *CG15436* with 12.8% identity (20.5% similarity), and crooked legs (*CROL*) with 10.1% identity (14.1% similarity) to the *PARIS* *H. sapiens* protein. The overall protein structures of these putative homologs were compared to the *PARIS* protein in order to identify potential conserved domains. *CG15436*, *CG15269*, and *CROL* all contained C-terminal zinc-finger double domains similar to those found in the *PARIS* protein (Figure 1A). *CROL* is a protein that is composed of 962 amino acids and is a negative regulatory factor that is involved in

proper limb development, while CG15269 encodes a predicted protein of 587 amino acids with no identified function. The activity of CROL and the presence of zinc-finger motifs in CG15269 suggest that it may also function as a negative transcriptional repressor. In addition to the zinc-finger motifs found in all three putative homologs, CG15436 contained a specialized N-terminal zinc-finger domain known as a zinc-associated domain (ZAD). ZADs may act in a similar fashion to KRAB domains, and are thought to be the *D. melanogaster* equivalent of this domain (Jauch et al., 2003). Alignment of the N-terminal KRAB domain of PARIS and the ZAD of CG15436 revealed a high degree of protein sequence similarity, with 51 amino acids either identical or of a similar functional domain over a 75-amino-acid span (Figure 1B). CG15436 is a 34-amino-acid-long protein that has not yet been characterized. Due to the presence of multiple zinc-finger domains, it is believed that the activity of this protein is related to transcriptional regulation, and a single homolog has been identified in *Xenopus tropicalis*. Based on the presence of both N-terminal C2H2 zinc-finger domains and the C-terminal ZAD, CG15436 appears to be the most PARIS-like gene identified by our bioinformatic analysis.

Crol-RNAi and CG15436-RNAi cause degeneration when expressed in the *D. melanogaster* eye

The adult *D. melanogaster* eye is composed of two separate yet equally important tissues: ommatidia and bristles. These tissues stem from neuronal precursors, and can be easily imaged and quantified using a scanning electron microscope, making the eye a highly sensitive system for determining the effects of altered gene expression in neurons. We expressed RNAi transgene lines of the three putative PARIS candidates driven by GMR-Gal4 to determine the consequences of reduced gene function (Figure 2A). Eye-specific expression of the CG15269-RNAi construct resulted in a slight yet significant loss of ommatidia at 25°C. This effect was exacerbated at 29°C, a temperature used to increase the amount of transgene expression, as higher temperatures increase the efficiency of the Gal4-UAS system, indicating that further expression of CG15269-RNAi caused further tissue destruction (Figure 2B). Expression of the *Crol-RNAi* construct caused a substantial decrease in both ommatidia and bristle formation at 25°C compared to in a *UAS-lacZ* control, and a severe loss of both bristles and ommatidia at 29°C (Figure 2C). This effect was more severe than that found with the *CG15269-RNAi* construct.

CG15436-RNAi has a non-degenerative effect similar to *srl-EY* expression in the *D. melanogaster* eye

To determine if one of the four candidate PARIS genes was acting in pathways related to *srl* function, we investigated the effect of altered *srl* expression in the *D. melanogaster* eye (Figure 3A). The driven expression of a *srl-EY* transgene construct caused a slight decrease in ommatidia number at both 25° and 29°C, and a slight decrease in bristle count at 29° compared to a *UAS-lacZ* control (Figure 3B and C). There was no decrease in bristle number at 25°C. Expression of a *srl-RNAi* transgene caused a sharp and substantial decrease in both ommatidia and bristle number at both 25° and 29°C. In comparison, expression of a CG15436-RNAi transgene caused no change in ommatidia and bristle number at 25°C, and only a slight decrease in both tissues at 29°C. Expression of both *srl-EY* and CG15436-RNAi transgenes seem to cause similar effects when driven by a GMR-Gal4 driver in the *D. melanogaster* eye.

Figure 2. Expression of *Crol-RNAi* and *CG15269-RNAi* transgenes caused a decrease in both ommatidia and bristle count in the *Drosophila melanogaster* eye. **A.** Scanning electron micrographs of *D. melanogaster* eyes taken at a horizontal field width of 500 μ m. The genotypes are as follows: I) *GMR-Gal4/UAS-lacZ* (25°C); II) *GMR-Gal4/Crol-RNAi* (25°C); III) *GMR-Gal4/CG15269-RNAi* (25°C); IV) *GMR-Gal4/CG15436-RNAi* (25°C); V) *GMR-Gal4/UAS-lacZ* (29°C); VI) *GMR-Gal4/Crol-RNAi* (29°C); VII) *GMR-Gal4/CG15269-RNAi* (29°C); and VIII) *GMR-Gal4/CG15436-RNAi* (29°C). The flies exhibited a decrease in the number of ommatidia (**B**) and bristles (**C**) when *Crol-RNAi* and *CG15269-RNAi* transgenes were expressed at 25°C. This effect intensified at 29°C, causing a further decrease in tissue numbers for both transgenes, with *Crol-RNAi* being more severe in both cases. Expression of *CG15436-RNAi* had no effect on ommatidia and bristle number at 25°C, and exhibited only a slight decrease in tissue number at 29°C. All of the counts were compared with those in a *UAS-lacZ* control; comparisons were made using one-way analysis of variance, and significance was tested using a Tukey post-hoc test, N = 10. Images were taken using a FEI MLA 650 scanning electron microscope. **P < 0.01, ***P < 0.001. Images were taken at an identical magnification, scale bar = 100 μ m.

Figure 3. Expression of *srl-EY* and *CG15436* transgenes show a similar phenotype, while expression of a *srl-RNAi* line caused a decrease in ommatidia and bristle number in the *Drosophila melanogaster* eye. **A.** Scanning electron micrographs of *D. melanogaster* eyes taken at a horizontal field width of 500 μ m. The genotypes are as follows: I) *GMR-Gal4/UAS-lacZ* (25°C); II) *GMR-Gal4/Crol-RNAi* (25°C); III) *GMR-Gal4/CG15269-RNAi* (25°C); IV) *GMR-Gal4/CG15436-RNAi* (25°C); V) *GMR-Gal4/UAS-lacZ* (29°C); VI) *GMR-Gal4/Crol-RNAi* (29°C); VII) *GMR-Gal4/CG15269-RNAi* (29°C); and VIII) *GMR-Gal4/CG15436-RNAi* (29°C). The flies exhibited a decrease in both ommatidia (**B**) and bristle (**C**) count when a *srl-RNAi* transgene was expressed at 25° and 29°C. Expression of *srl-EY* had no effect on ommatidia count and caused a slight decrease in bristle count at 25°C, and a slight decrease in both ommatidia and bristle count at 29°C. Similarly, there was no effect on the eye when a *CG15436* transgene was expressed at 25°C, and there was a slight decrease in both ommatidia and bristle count at 29°C. All of the counts were compared with those in a *UAS-lacZ* control; comparisons were made using one-way analysis of variance, and significance was tested using a Tukey post-hoc test, N = 10. Images were taken using a FEI MLA 650 scanning electron microscope. *P < 0.05, **P < 0.01, ***P < 0.001. Images were taken at an identical magnification, scale bar = 100 μ m.

Ddc-Gal4-directed expression of *Crol*-RNAi reduces mean lifespan, while *CG15436*-RNAi increases mean lifespan in *D. melanogaster*

Dopaminergic neurons are essential producers of dopamine, and the altered expression of genes implicated in neurodegenerative disease in these neurons led to a decrease in mean lifespan compared to controls. This results in an ideal system with which to identify potential causative disease genes by assaying the tissue-specific consequences of altered gene expression in dopaminergic neurons. To determine the effect of altered putative *PARIS* homolog expression in dopaminergic neurons, the *Ddc-Gal4* transgene was used to direct the inhibition of the three candidate genes via *RNAi* (Figure 4). Tissue-specific expression of *Crol*-*RNAi* in dopaminergic neurons resulted in a significant decrease in mean lifespan (56 days) compared to in *UAS-lacZ* controls (60 days), while the directed expression of a *CG15436*-*RNAi* transgene resulted in a significant increase in mean lifespan (64 days). Expression of a *CG15269* transgene did not result in a significant difference in mean lifespan (62 days) compared to the *UAS-lacZ* controls.

Figure 4. Dopaminergic expression of a *CG15436*-*RNAi* transgene increased while expression of a *Crol*-*RNAi* transgene decreased mean lifespan in *Drosophila melanogaster*. Altered expression of *Crol* in the dopaminergic neurons by expression of a *Crol*-*RNAi* transgene caused a significant decrease in mean longevity compared to that in *UAS-lacZ* controls. *CG15436* seemed to have an opposite effect, as expression of a *CG15436*-*RNAi* transgene caused a significant increase in lifespan compared to that in *UAS-lacZ* controls. *CG15269*-*RNAi* expression had no significant effect on mean lifespan when expressed in dopaminergic neurons. All of the transgene lines were driven by the *Ddc(II)*-*Gal4* driver, and longevity is shown as percent survival ($P < 0.05$ as determined by the Mantel-Cox log-rank test), $N > 150$.

DISCUSSION

Beginning with the sequence of the *H. sapiens* *PARIS* protein, bioinformatic analysis led to the identification of three similar genes with the potential to be *D. melanogaster* ortholog(s) and/or homolog(s). Two of these genes, *CG15269* and *CG15436*, have not been investigated to any great extent, except that they have been characterized as encoding zinc-finger-containing proteins. The third gene, *crooked-legs* (*Crol*), has been identified as a transcription factor that is involved in leg morphogenesis, and is a mediator of cell cycle progression that has been studied as a potential interacting factor in models of Huntington's disease (D'Avino and Thummel, 1998; Mitchell et al., 2008). Although *Crol* has been implicated in models of human disease, little or no analysis of its effects upon a range of neuronal tissues has been performed. Interestingly, the KRAB motif, as found in the *H. sapiens* *PARIS* protein product, is a highly conserved motif found in over one third of all mammalian zinc-finger transcription factors (Urrutia, 2003). The KRAB domain is not well conserved in

D. melanogaster zinc-finger transcription factors, although a domain with a similar function and composition exists: ZAD (Chung et al., 2002). The presence of this domain in only one of the three putative *D. melanogaster* homologs suggests that *CG15436* may be the most likely *PARIS* candidate. In addition to this N-terminal ZAD, *CG15436* contains two C-terminal zinc-finger double domains, similar to those found in the human *PARIS* gene product. This domain is important in protein-protein binding interactions, and although some KRAB domain proteins exist without the C-terminal zinc-finger domains, these proteins have been found to function in the sequestration of KRAB-activating proteins and not in the activity of the domain (Chung et al., 2007). ZAD-containing proteins may require the C-terminal zinc-finger double domains for proper co-factor binding (Jauch et al., 2003). The activity of KRAB domain proteins is dependent upon the presence of a co-activator identified as Kruppel associate protein-1 (KAP-1) (Friedman et al., 1996). KAP-1 is a member of the multi-component transcriptional intermediary factor 1 (TIF1) family, which in humans contains four structurally and functionally similar proteins (Le Douarin et al., 1995; Khetchoumian et al., 2004). Although no ZAD direct-binding factor has been identified, a single TIF1 protein family member has been identified in *D. melanogaster*, *Bonus* (*Bon*) (Beckstead et al., 2001). The presence of conserved activation and binding domains in the *H. sapiens* *PARIS* and *D. melanogaster* *CG15436* proteins provides a large base of evidence for homologous function across species.

PARIS is a directly binding inhibitor of the PGC-1 α protein in *H. sapiens*; lowered expression would be predicted to result in a reduction of inhibition, and thus an increase in mitochondrial biogenesis through elevated *PGC-1 α* activity. We expressed RNAi transgene constructs for our three putative *PARIS* homologs to determine the consequences they have on the formation of eye tissues. *D. melanogaster* eye-specific expression of a *Crol*-RNAi transgene caused a severe disruption in the formation of ommatidia and bristles in the *D. melanogaster* eye. *Crol* is expressed in the imaginal discs of *D. melanogaster* eyes, suggesting that it may play a similar role in eye development (D'Avino and Thummel, 1998). This is the first time altered *Crol* expression has been assessed and characterized in the *D. melanogaster* eye. *D. melanogaster* eye-specific expression of a *CG15436*-RNAi transgene resulted in no significant change in ommatidia number at 25°C, and an increase in bristle number at the same temperature. At 29°C, the numbers of both ommatidia and bristles were slightly lower than those in *UAS-lacZ* controls. The role of the *CG15436* gene has not yet been identified; however, it may act as a DNA-binding transcription factor, based on the presence of zinc-finger motifs. The third putative homolog, *CG15269*, exhibited a slight yet significant decrease in ommatidia and bristle number at 25°C, and a more substantial decrease in both tissues at 29°C compared to *UAS-lacZ* controls. This gene currently has no identified function, but, like *PARIS*, contains the zinc-finger motifs that are associated with transcriptional repression. We present the first evidence that *CG15269* may play a role in the development of *D. melanogaster* eye tissue, and that the altered expression of this gene may lead to developmental defects in these tissues. These eye-specific developmental phenotypes that are associated with the loss of function of the three putative *PARIS* homologs *CG15436*, *CG15269*, and *Crol* have not been previously identified, and could indicate the involvement of all three genes in neuronal development.

Using the *Ddc-Gal4* transgene, the *PARIS* candidate genes were inhibited in the dopaminergic neurons in order to determine the consequences of improper gene function and potential links to disease. Altered *CG15269* expression had no effect on mean lifespan in dopaminergic neurons. This result differs from the data obtained from the *D. melanogaster* eye; however, the eye is a more sensitive system, and allows for the detection of subtler changes than

do aging assays. Therefore, it is possible that *CG15269* does play a role in neuronal formation, but does not have a strong enough effect when altered to cause pre-mature organism mortality. The expression of a *Crol-RNAi* construct caused a significant decrease in mean longevity compared to control flies. This supports the evidence found in the *D. melanogaster* eye that *Crol* is involved in neuronal tissues, and the altered expression of this gene leads to tissue disruption, and in this case, reduced mortality. Finally, altered *CG15436* expression caused a significant increase in mean lifespan compared to controls. This result would be expected from a *PARIS* homolog, because RNAi inhibition of this gene would cause an increase in mitochondrial biogenesis through the *PGC-1 α* pathway. Of the three putative *PARIS* homologs we identified and characterized in this study, *CG15436* was the best candidate, and may be the *D. melanogaster* homolog of *PARIS*.

In this study, we identified and evaluated potential *D. melanogaster* functional homologs of the recently discovered human gene, *PARIS*. Finding a functional homolog of *PARIS* would provide a better *D. melanogaster* model of the *srl*-mediated pathway of mitochondrial biogenesis that is initiated by the PD gene, *Parkin*. Through this process, we found that three genes previously uncharacterized to have a neuronal function: *Crol*, *CG15436*, and *CG15269*, are important in the proper functioning of both dopaminergic and precursor eye tissue neurons. Of these three genes, *CG15436* was the most *PARIS*-like in both structure and function. Fully characterizing the *D. melanogaster* pathway of mitochondrial biogenesis will generate a more precise model with which to study human disease, and potential preventative treatments for neurodegenerative disease.

Conflicts of interest

The authors declare no conflict of interest.

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