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*Pink*1 Rescues *Gal*4-Induced Developmental Defects in the *Drosophila* Eye

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Abstract

Parkinson disease pathology often includes the presence of ubiquitin-positive, *α*-synuclein-enriched inclusions in the remaining neurons. *Pink*1 (also identified as *PARK*6) encodes a serine-threonine kinase involved in mitochondrial protection that works with *parkin* to ubiquitinate various proteins, promoting mitophagy. The *parkin* protein works to tag cystolic proteins for degradation, and previous work in our laboratory has shown the ability of *parkin* to rescue a *Gal*4-induced phenotype. To further investigate the role of *Pink*1 in protection against toxic proteins, we have performed expression studies to determine the effects of increases and decreases in *Pink*1 on the *Gal*4-induced phenotype consisting of developmental defects in the *Drosophila* eye. Our results show that *Pink*1 is able to rescue the *Gal*4-induced phenotype, highlighting a protective role for *Pink*1 against toxic proteins. When expressing low levels of *Gal*4, reductions in *Pink*1 or *parkin* are not able to induce a phenotype. This suggests that *Pink*1 or *parkin* may counter *Gal*4 effects despite reductions, or that the effects of low level *Gal*4 may be alleviated by an alternative mechanism. Moreover, the *Pink*1 mechanism of action during differing types of cell stress, including degradation of toxic proteins, warrants further investigation.

Keywords

***Drosophila melanogaster*, *Pink*1, *Parkin*, *Gal*4, Toxic Protein, Parkinson Disease**

1. Introduction

Parkinson disease (PD) is the most prevalent neurodegenerative movement disorder [1]. Characterized by a progressive loss of dopaminergic neurons, PD pathology often includes the presence of Lewy bodies, ubiquitin-positive and *α*-synuclein-enriched inclusions, in the remaining neurons. Although sporadic forms of PD are believed to be more common, many familial forms share features with sporadic PD, including protein aggregation

and mitochondrial dysfunction [2]. *Pink*1 (*PTEN induced putative kinase* 1) encodes a serine-threonine kinase that has been linked to autosomal recessive and some sporadic forms of Parkinson disease [3]-[5]. Targeted to the mitochondria, *Pink*1 is involved in mitochondrial protection, as loss of function of *Pink*1 results in substantial mitochondrial defects in sensitive tissues [6]-[10]. It is increasingly apparent that both *Pink*1 and *parkin*, acting in the same pathway, are necessary for proper mitochondrial integrity and function [11] [12]. The *parkin* E3 ubiquitin ligase acts downstream of *Pink*1. In mitochondrial protection, the recruitment of *parkin* to the mitochondria by *Pink*1 results in the ubiquitination of various mitochondrial proteins, promoting mitophagy [13]-[16]. In addition, *Pink*1 may have a protective role apart from the mitochondria, where an interaction with *parkin* could result in the tagging of cystolic proteins for degradation. This may be an important, but largely overlooked role, as neurodegenerative diseases are often characterized by the accumulation of toxic proteins.

Our laboratory has examined the adverse effects of expressing proteins that can produce toxicity, including *α*-synuclein and the *Gal*4 transcription factor [17]-[20]. We have shown the ability of *parkin* and *Pink*1 to rescue an *α-synuclein*-induced phenotype [17] [18] [21], and the ability of *parkin* overexpression to rescue a *Gal*4-induced phenotype [20]. The suppression of the effects of *Gal*4 is presumably through its targeting for proteosomal degradation by parkin. To further investigate the role of *Pink*1 in protection against toxic proteins, we have performed expression studies to determine the effects of *Pink*1 on the *Gal*4-induced phenotype of developmental defects in the *Drosophila* eye.

2. Materials and Methods

2.1. Fly Stocks and Culture

The *UAS-Pink*1 transgenic line was created from the GH20931 *Drosophila melanogaster Pink*1 clone [21]. The $Pink1^{B9}$ mutant line [8] was provided by Dr. J. Chung (Seoul National University). The $UAS\text{-}Pink1^{RNAi}$ and $UAS\text{-}parkin^{RNAi}$ lines [9] [22] were provided by Dr. B. Lu (Stanford University). *UAS-parkin* was created previously in our laboratory [17]. The $parkin^{45}$ mutant line [23] was provided by Dr. L. Pallanck (Washington University). The *GMR-Gal*4 flies [24] were obtained from the Bloomington *Drosophila* Stock Center at Indiana University. All crosses were performed using standard techniques. All flies were cultured on standard cornmeal/yeast/molasses/agar media.

2.2. Scanning Electron Microscopy of the *Drosophila* Eye

Flies were aged three days past eclosion on standard cornmeal/yeast/molasses/agar media at either 25˚C or 29˚C. Flies were then frozen at −80˚C and examined under dissecting microscope. Flies were mounted, desiccated overnight and coated in gold before photography at 170 times magnification with a Hitachi S-570 SEM. Area of disruption was determined by the presence of fused or enlarged (>150%) ommatidia. Areas of ommatidial disruption were compared using GraphPad Prism 5. Error bars represent standard error of the mean.

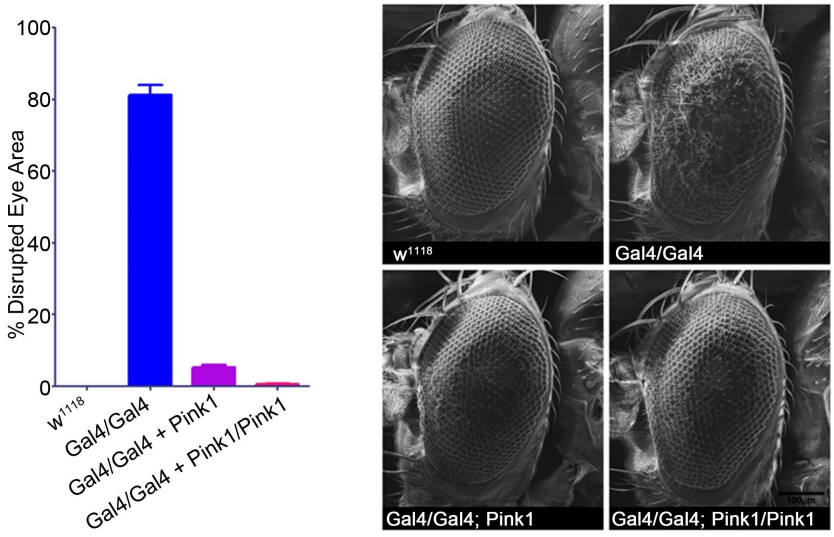
3. Results

3.1. *Pink*1 Is Able to Rescue *Gal*4-Induced Rough Eye Phenotype

High levels of *Gal*4 expression in the developing *Drosophila* eye result in a characteristic rough eye phenotype [19]. At 25˚C, *GMR-Gal*4 homozygotes have a rough eye phenotype characterized by an 81% ommatidial disruption of the eye area (**Figure 1**). Co-overexpression with one copy of the *Pink*1 transgene results in a significant reduction of the *Gal*4-induced phenotype, reducing the disruption to 5% (95% CI). Co-overexpression with two copies of the *Pink*1 transgene results in a further, significant reduction of the *Gal*4-induced phenotype, near control levels (0.5% disruption, 95% CI). These results suggest that an increase in *Pink*1, in a dose dependent manner, during eye development is able to alleviate the detrimental effects of *Gal*4 expression.

3.2. *GMR-Gal*4 Heterozygotes Show a Mild Rough Eye Phenotype at 29˚C

Previous work in our laboratory indicates a mild *Gal*4-induced phenotype in *GMR-Gal*4 heterozygotes at 29˚C, with intermediate levels of apoptosis [19]. Our results with *GMR-Gal*4 heterozygotes at 29˚C show this mild phenotype, with an unevenness of the ommatidial surface, and no visible fusing of ommatidia or enlargement greater than 150% (**Figure 2**). Due to the subtle nature of the phenotype, it is difficult to determine if co-over-



(a) (b)

Figure 1. *Pink*1 decreases the severity of the *Gal*4-induced phenotype. *GMR-Gal*4 homozygotes show a characteristic rough eye phenotype. Co-overexpression with one or two copies of the *Pink*1 transgene results in a significant reduction of the *Gal*4-induced phenotype. Genotypes shown include the control w^{1118} (w^{1118}), *GMR-Gal*4/*GMR-Gal*4 (*Gal*4/*Gal*4), *GMR-Gal*4/*GMR-Gal*4; *UAS-Pink*1/+ (*Gal*4/*Gal*4; *Pink*1), *GMR-Gal*4/*GMR-Gal*4; *UAS-Pink*1/*UAS-Pink*1 (*Gal*4/*Gal*4; *Pink*1/*Pink*1). Flies were raised at 25˚C. Error bars indicate standard error of the mean.

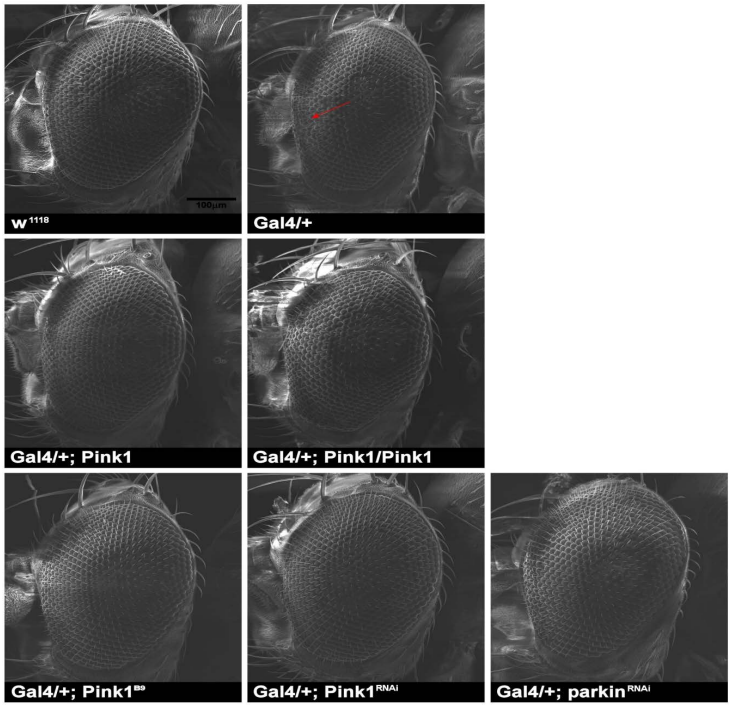


Figure 2. Reductions in *Pink*1 or *parkin* do not induce a rough eye phenotype in *GMR-Gal*4 heterozygotes. *GMR-Gal*4 heterozygotes show a mild phenotype of unevenness in the ommatidial surface, with no visible fusing of ommatidia or enlargement over 150% (red arrow). No changes were observed with co-overexpression of *Pink*1, $parkin^{RNAi}$, $Pink1^{RNAi}$ or when expressed in the $Pink1^{B9}$ mutant background. Expression in a *parkin* mutant background resulted in apparent synthetic lethality (*GMR-Gal*4/+; $park^{45}/park^{45}$). Genotypes shown include w^{1118} (w^{1118}), *GMR-Gal*4/+ (*Gal*4/+), *GMR-Gal*4/+; *UAS-Pink*1/+ (*Gal*4/+; *Pink*1), *GMR-Gal*4/+; *UAS-Pink*1/*UAS-Pink*1 (*Gal*4/+; *Pink*1/*Pink*1), $Pink1^{B9}/Y$; *GMR-Gal*4/+ (*Gal*4/+; $Pink1^{B9}$), *GMR-Gal*4/+; *UAS-*$Pink1^{RNAi}$/+ (*Gal*4/+; $Pink^{RNAi}$), GMR-*Gal*4/+; *UAS-*$parkin^{RNAi}$/+ (*Gal*4/+; $parkin^{RNAi}$). Flies were raised at 29˚C.

expression of either one or two copies of the *Pink*1 transgene has an effect. Therefore, possible protective effects of *Pink*1 overexpression on the *GMR-Gal*4 heterozygotes cannot be detected using this phenotype.

3.3. Reductions in *Pink*1 or *Parkin* Are Not Able to Induce a Rough Eye Phenotype in *GMR-Gal*4 Heterozygotes

It was of interest to determine if reductions in *Pink*1 or *parkin* have an effect on the subtle phenotype observed in the *GMR-Gal*4 heterozygotes at 29˚C [19] (**Figure 2**). Our results show no appreciable change in eye morphology during co-overexpression of $parkin^{RNAi}$, $Pink1^{RNAi}$ or when *GMR-Gal*4 heterozygotes are expressed in a *Pink*1 mutant background ($Pink1^{B9}$) (**Figure 2**). These results suggest that reductions in *Pink*1 or *parkin* are not sufficient to induce the *Gal*4-induced phenotype. Expression in a *parkin* mutant background resulted in apparent synthetic lethality (*GMR-Gal*4/+; $park^{45}/park^{45}$). This implies that the broad protective functions of *parkin* are necessary to maintain a viable organism during this development.

4. Discussion

Increase in *Pink*1 expression during eye development is able to alleviate the detrimental effects of *Gal*4 expression in a dose dependent manner. The ability of *Pink*1 to counteract the effects of *GMR-Gal*4 is similar to previous results in our laboratory with *parkin* [20], supporting the theory that *Pink*1 is acting via *parkin*. *Pink*1 may interact with *parkin* to activate the ubiquitin-proteasomal system, resulting in the tagging of *Gal*4 for degradation. Alternatively, *Pink*1 may operate to protect the mitochondria from the effects of *Gal*4, recruiting *parkin* to the membrane to remove damage via mitophagy. Studies have shown that recruitment of *parkin* by *Pink*1 to depolarized mitochondria results in the ubiquitination of mitochondrial proteins VDAC1 [15] and mitofusin [13] [16], leading to recruitment of autophagic proteins or decreases in mitochondrial fusion. It is hypothesized that when this process is impaired, by mutations in either *parkin* or *Pink*1, an accumulation of defective mitochondria results, leading to the neurodegeneration seen in Parkinson disease [14]. *Pink*1 may impart mitochondrial protection by interacting with other molecular chaperones at the mitochondrial membrane. For example, the phosphorylation of TRAP1 (Hsp75) by *Pink*1 has been shown to protect against oxidative stress and prevent cytochrome c release [25]. Identification of *PINK*1 targets is still in early stages. It is likely that *PINK*1 will be identified to interact with various proteins, and serves to protect against multiple stressors such as toxic proteins, oxidative stress and mitochondrial dysfunction.

The *GMR-Gal*4 heterozygotes exhibit a mild phenotype at 29˚C, with intermediate levels of apoptosis in the eye imaginal discs [19]. It was thought that this mild *Gal*4-induced effect may be increased with reductions in *Pink*1 or *parkin*, inducing a measurable rough eye phenotype. Our results show that these reductions are not sufficient to induce a rough eye phenotype, implying that either the effects of *Gal*4 are alleviated by an alternative mechanism, or that low levels of *Pink*1 or *parkin* may be sufficient to protect against low levels of *Gal*4. The lack of a phenotype observed using the $Pink1^{B9}$ mutant may suggest that a functional kinase is not necessary for *Pink*1 to participate in protection against the effects of *Gal*4. Alternatively, the lack of a phenotype may be due to the residual activity of maternally-inherited *Pink*1. As many proteins have roles independent of their kinase function, investigations into other attributes of the *Pink*1 protein may shed light on unexplored roles or interactions.

In conclusion, *Pink*1 was shown to act against the toxic consequences of *GMR-Gal*4, in a manner similar to *parkin*, likely through the tagging of the *Gal*4 protein for degradation by the ubiquitin-proteasomal system, or through protection of the mitochondria via mitophagy or, perhaps, both. The understanding of the ability of *Pink*1 to protect against protein toxicity, even an introduced protein, may provide extremely valuable clues on the way to the development therapeutics strategies to treat or prevent Parkinson disease.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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