Pink1 Rescues Gal4-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. Pink1 (also identified as PARK6) 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 Gal4-induced phenotype. To further investigate the role of Pink1 in protection against toxic proteins, we have performed expression studies to determine the effects of increases and decreases in Pink1 on the Gal4-induced phenotype consisting of developmental defects in the Drosophila eye. Our results show that Pink1 is able to rescue the Gal4-induced phenotype, highlighting a protective role for Pink1 against toxic proteins. When expressing low levels of Gal4, reductions in Pink1 or parkin are not able to induce a phenotype. This suggests that Pink1 or parkin may counter Gal4 effects despite reductions, or that the effects of low level Gal4 may be alleviated by an alternative mechanism. Moreover, the Pink1 mechanism of action during differing types of cell stress, including degradation of toxic proteins, warrants further investigation.

Keywords

Drosophila melanogaster, Pink1, Parkin, Gal4, 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]. Pink1 (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, Pink1 is involved in mitochondrial protection, as loss of function of Pink1 results in substantial mitochondrial defects in sensitive tissues [6]-[10]. It is increasingly apparent that both Pink1 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 Pink1. In mitochondrial protection, the recruitment of parkin to the mitochondria by Pink1 results in the ubiquitination of various mitochondrial proteins, promoting mitophagy [13] [16]. In addition, Pink1 may have a protective role apart from the mitochondria, where an interaction with parkin could result in the tagging of cytosolic 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 Gal4 transcription factor [17]-[20]. We have shown the ability of parkin and Pink1 to rescue an α-synuclein-induced phenotype [17] [18] [21], and the ability of parkin overexpression to rescue a Gal4-induced phenotype [20]. The suppression of the effects of Gal4 is presumably through its targeting for proteosomal degradation by parkin. To further investigate the role of Pink1 in protection against toxic proteins, we have performed expression studies to determine the effects of Pink1 on the Gal4-induced phenotype of developmental defects in the Drosophila eye.

2. Materials and Methods
2.1. Fly Stocks and Culture
The UAS-Pink1 transgenic line was created from the GH20931 Drosophila melanogaster Pink1 clone [21]. The Pink1<sup>89</sup> mutant line [8] was provided by Dr. J. Chung (Seoul National University). The UAS-Pink1<sup>RNAi</sup> and UAS-parkin<sup>RNAi</sup> lines [9] [22] were provided by Dr. B. Lu (Stanford University). UAS-parkin was created previously in our laboratory [17]. The parkin<sup>45</sup> mutant line [23] was provided by Dr. L. Pallanck (Washington University). The GMR-Gal4 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. Pink1 Is Able to Rescue Gal4-Induced Rough Eye Phenotype
High levels of Gal4 expression in the developing Drosophila eye result in a characteristic rough eye phenotype [19]. At 25°C, GMR-Gal4 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 Pink1 transgene results in a significant reduction of the Gal4-induced phenotype, reducing the disruption to 5% (95% CI). Co-overexpression with two copies of the Pink1 transgene results in a further, significant reduction of the Gal4-induced phenotype, near control levels (0.5% disruption, 95% CI). These results suggest that an increase in Pink1, in a dose dependent manner, during eye development is able to alleviate the detrimental effects of Gal4 expression.

3.2. GMR-Gal4 Heterozygotes Show a Mild Rough Eye Phenotype at 29°C
Previous work in our laboratory indicates a mild Gal4-induced phenotype in GMR-Gal4 heterozygotes at 29°C, with intermediate levels of apoptosis [19]. Our results with GMR-Gal4 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-
Figure 1. Pink1 decreases the severity of the Gal4-induced phenotype. GMR-Gal4 homozygotes show a characteristic rough eye phenotype. Co-overexpression with one or two copies of the Pink1 transgene results in a significant reduction of the Gal4-induced phenotype. Genotypes shown include the control w^1118 (w^1118), GMR-Gal4/GMR-Gal4 (Gal4/Gal4), GMR-Gal4/GMR-Gal4; UAS-Pink1+/+ (Gal4/Gal4; Pink1), GMR-Gal4/GMR-Gal4; UAS-Pink1/UAS-Pink1 (Gal4/Gal4; Pink1/Pink1). Flies were raised at 25˚C. Error bars indicate standard error of the mean.

Figure 2. Reductions in Pink1 or parkin do not induce a rough eye phenotype in GMR-Gal4 heterozygotes. GMR-Gal4 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 Pink1, parkinRNAi, Pink1RNAi or when expressed in the Pink1^{B9} mutant background. Expression in a parkin mutant background resulted in apparent synthetic lethality (GMR-Gal4/+; park^{I5}/park^{I5}). Genotypes shown include w^1118 (w^1118), GMR-Gal4/+ (Gal4/+), GMR-Gal4/+; UAS-Pink1+/+ (Gal4/+; Pink1), GMR-Gal4/+; UAS-Pink1/UAS-Pink1 (Gal4/+; Pink1/Pink1), Pink1^{B9}/Y; GMR-Gal4/+ (Gal4/+; Pink1^{B9}), GMR-Gal4/+; UAS-Pink1RNAi+/+ (Gal4/+; Pink1RNAi), GMR-Gal4/+; UAS-parkinRNAi+/+ (Gal4/+; parkinRNAi). Flies were raised at 29˚C.
expression of either one or two copies of the Pink1 transgene has an effect. Therefore, possible protective effects of Pink1 overexpression on the GMR-Gal4 heterozygotes cannot be detected using this phenotype.

3.3. Reductions in Pink1 or Parkin Are Not Able to Induce a Rough Eye Phenotype in GMR-Gal4 Heterozygotes

It was of interest to determine if reductions in Pink1 or parkin have an effect on the subtle phenotype observed in the GMR-Gal4 heterozygotes at 29°C [19] (Figure 2). Our results show no appreciable change in eye morphology during co-overexpression of parkinRNAi, Pink1RNAi or when GMR-Gal4 heterozygotes are expressed in a Pink1 mutant background (Pink10/9) (Figure 2). These results suggest that reductions in Pink1 or parkin are not sufficient to induce the Gal4-induced phenotype. Expression in a parkin mutant background resulted in apparent synthetic lethality (GMR-Gal4+/; park4/park45). This implies that the broad protective functions of parkin are necessary to maintain a viable organism during this development.

4. Discussion

Increase in Pink1 expression during eye development is able to alleviate the detrimental effects of Gal4 expression in a dose dependent manner. The ability of Pink1 to counteract the effects of Gal4 is similar to previous results in our laboratory with parkin [20], supporting the theory that Pink1 is acting via parkin. Pink1 may interact with parkin to activate the ubiquitin-proteasomal system, resulting in the tagging of Gal4 for degradation. Alternatively, Pink1 may operate to protect the mitochondria from the effects of Gal4, recruiting parkin to the membrane to remove damage via mitophagy. Studies have shown that recruitment of parkin by Pink1 to de-polarized 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 Pink1, an accumulation of defective mitochondria results, leading to the neurodegeneration seen in Parkinson disease [14]. Pink1 may impart mitochondrial protection by interacting with other molecular chaperones at the mitochondrial membrane. For example, the phosphorylation of TRAP1 (Hsp75) by Pink1 has been shown to protect against oxidative stress and prevent cytochrome c release [25]. Identification of PINK1 targets is still in early stages. It is likely that PINK1 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-Gal4 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 Gal4-induced effect may be increased with reductions in Pink1 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 Gal4 are alleviated by an alternative mechanism, or that low levels of Pink1 or parkin may be sufficient to protect against low levels of Gal4. The lack of a phenotype observed using the Pink10/9 mutant may suggest that a functional kinase is not necessary for Pink1 to participate in protection against the effects of Gal4. Alternatively, the lack of a phenotype may be due to the residual activity of maternally-inherited Pink1. As many proteins have roles independent of their kinase function, investigations into other attributes of the Pink1 protein may shed light on unexplored roles or interactions.

In conclusion, Pink1 was shown to act against the toxic consequences of GMR-Gal4, in a manner similar to parkin, likely through the tagging of the Gal4 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 Pink1 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.

References


