Increased glutathione *S*-transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease

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Loss-of-function mutations of the *parkin* gene are a major cause of early-onset parkinsonism. To explore the mechanism by which loss of *parkin* function results in neurodegeneration, we are using a genetic approach in *Drosophila*. Here, we show that *Drosophila parkin* mutants display degeneration of a subset of dopaminergic (DA) neurons in the brain. The neurodegenerative phenotype of *parkin* mutants is enhanced by loss-of-function mutations of the *glutathione S-transferase S1* (*GstS1*) gene, which were identified in an unbiased genetic screen for genes that modify *parkin* phenotypes. Furthermore, overexpression of *GstS1* in DA neurons suppresses neurodegeneration in *parkin* mutants. Given the previous evidence for altered glutathione metabolism and oxidative stress in sporadic Parkinson's disease (PD), these data suggest that the mechanism of DA neuron loss in *Drosophila parkin* mutants is similar to the mechanisms underlying sporadic PD. Moreover, these findings identify a potential therapeutic approach in treating PD.

genetic modifier | neurodegeneration | parkin

Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the loss of dopaminergic (DA) neurons in the substantia nigra and the accumulation of proteinaceous intraneuronal inclusions known as Lewy bodies. The mechanisms responsible for neurodegeneration in PD are largely unknown, although previous work suggests that mitochondrial complex I dysfunction, oxidative stress, and aberrant proteolytic degradation may contribute to pathogenesis (1). The recent identification of genes responsible for rare inherited forms of parkinsonism presents an opportunity to establish neurodegenerative mechanisms that may be relevant to sporadic forms of PD.

Loss-of-function mutations of *parkin* are a common cause of autosomal recessive juvenile parkinsonism (ARJP), and parkin dysfunction may also contribute to late-onset sporadic PD (2–6). Patients with *parkin* mutations display many of the typical features of idiopathic PD, including locomotor dysfunction, reduced mitochondrial complex I activity, and degeneration of DA neurons in the substantia nigra. However, most ARJP cases have a significantly earlier age of onset and lack Lewy body pathology. Parkin has been shown to possess ubiquitin-protein ligase activity (7–9), which acts to confer substrate target specificity in the ubiquitin/proteasome protein degradation pathway. This finding has led to the model that toxic accumulation of parkin substrates may be responsible for DA neuron death. A number of putative substrates of parkin have been identified (10). Several of these parkin substrates, including the Lewy body component α-synuclein (11) and the putative G protein-coupled receptor Pael-R (12), have received considerable attention, in part because they implicate specific cellular pathways in *parkin*-mediated pathogenesis. However, the involvement of many of the identified parkin substrates in the etiology of ARJP remains controversial.

To identify pathways relevant to parkin pathogenesis, we are using a genetic approach to study a *Drosophila* ortholog of the *parkin* gene. Although the anatomy of the *Drosophila* brain differs from that of the vertebrate brain, many features of nervous system development and function are conserved in flies and humans, and this conservation has made *Drosophila* an excellent system in which to model neurodegeneration (13). In previous work, we showed that loss of *Drosophila parkin* function results in muscle degeneration and a late developmental defect in the formation of spermatids (14). Mitochondrial pathology is the earliest detectable phenotype associated with muscle degeneration and defective spermatids in *Drosophila parkin* mutants, suggesting that parkin is required for mitochondrial integrity.

In this study, we have examined DA neuron integrity in *Drosophila parkin* mutants. We show that *parkin* is required cell-autonomously to prevent degeneration of a subset of DA neurons in the brain. The neurodegenerative phenotype of *parkin* mutants is enhanced by a loss-of-function allele of the *glutathione S-transferase S1* (*GstS1*) gene that was identified in a screen for modifiers of a *parkin* partial lethal phenotype (15). Furthermore, we demonstrate that overexpression of *GstS1* prevented DA neuron degeneration in *parkin* mutants. These observations suggest that a variety of compounds known to induce glutathione *S*-transferase expression in mammals may provide a promising therapeutic strategy for PD.

Methods

Fly Strains. The *park*[25] null allele, hypomorphic *park*[Z472] allele, isogenic control *park*[rvA], and upstream activating sequence (UAS)-*park*[C2] transgene are described in ref. 14. The *park*[Z3678] hypomorph, which fails to complement other *parkin* alleles, has a noncoding point mutation. The tyrosine hydroxylase (TH)-GAL4 driver was a gift from S. Birman (Developmental Biology, Institute of Marseille, Marseille, France) (16). *GstS1*[K9303], *GstS1*[K8805], *GstS1*[4227], and *GstS1*[EP2223], 24B-GAL4, and Df(2R)ED1 were obtained from the Bloomington Stock Center (Bloomington, IN). *GstS1*[M26] was isolated by excision of *GstS1*[EP1227] (H.B., unpublished data). UAS-*GstS1* was constructed by cloning *GstS1* cDNA LD21131 (Berkeley *Drosophila* Genome Project, Berkeley, CA) into transformation vector pUASP. Germ-line transformants were generated by using

standard procedures. *Drosophila* cultures were kept under standard conditions, and all experiments were performed on flies raised at 25°C.

Analysis of Protein Carbonyl and Glutathione Content. Protein carbonyl content was calculated by using an established procedure (17). Glutathione levels were assayed by using a glutathione detection kit (Chemicon) according to the manufacturer's instructions.

Analysis of DA and Serotonergic Neurons. DA and serotonergic neurons were analyzed *in situ* by using anti-TH antiserum Ab152 (1:100, Chemicon) and anti-serotonin antiserum (1:100, Immunostar, Hudson, WI), respectively. Adult heads were dissected in cold PBS, and isolated brains were fixed in 4% paraformaldehyde/PBS for 30 min. Samples were washed in PBS/0.1% Triton X-100 and blocked for 1 h in 0.1 M Tris·Cl, pH 7.5/0.15 M NaCl/0.1% Triton X-100/10% heat-inactivated FBS. Anti-TH was incubated in blocking solution at 4°C overnight. After washing and incubating fluorescent secondary antiserum, samples were again washed and mounted between two glass coverslips by using ProLong antifade medium (Molecular Probes). This method of sample mounting allows visualization of the samples from both sides of the slide to optimize detection of all DA neuron clusters. Confocal microscopy was used to collect optical sections at 1-μm intervals. The number of TH-positive neurons within each of the major DA neuron clusters was determined by visual inspection of individual confocal Z-series images. Additionally, brains from adult flies segregating the *TH-GAL4* driver and a UAS-*GFP* transgene were dissected and fixed as described above. DA neurons were identified by using confocal microscopy to detect intrinsic GFP fluorescence, and the number of GFP-positive neurons was determined as described above. In all studies, DA neurons were counted with the investigator blinded to genotype. The same neuronal phenotypes were observed in completely independent experiments performed by three different investigators (A.J.W., D.A.T., and P.D.W.) at different institutions.

Behavior. Climbing assays were conducted as described in ref. 14. Recombinant chromosomes were generated between 24B-GAL4 and two *parkin* hypomorphic mutations, *park^{Z472}* and *park^{Z3678}*. These recombinants were tested with and without a UAS-*GstS1* transgene.
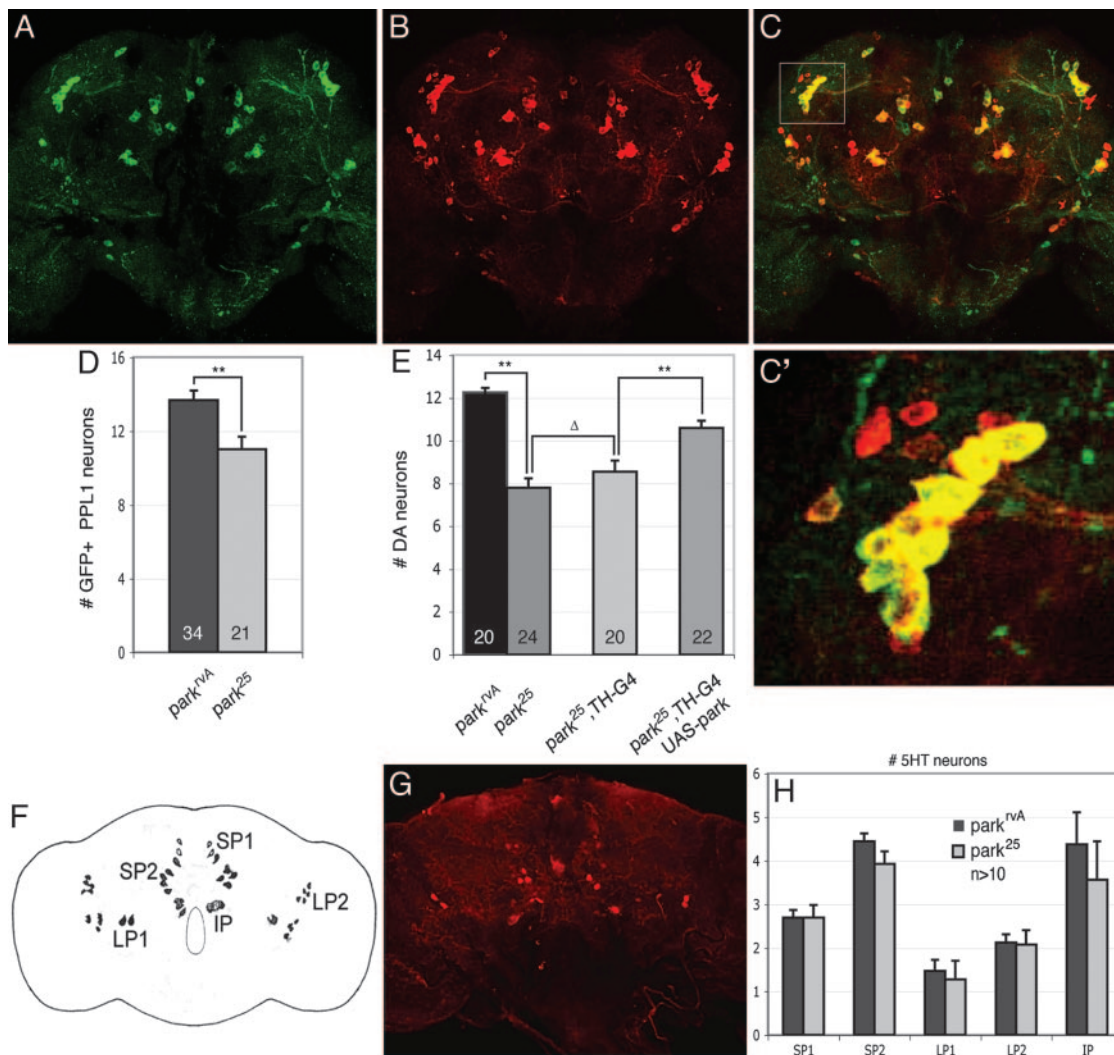
Results

In a previous study, we analyzed DA neuron viability in *parkin* mutants compared with controls by assessing the number of neurons positive for the DA neuron-specific marker TH in paraffin-imbedded head sections (14). Although no neuronal loss was detected, substantial variance in sample analysis might have obscured a subtle loss of DA neurons. Therefore, we repeated this analysis by using confocal microscopy of whole-mount adult brains stained with antiserum to TH (Fig. 1). This method allowed us to visualize individual DA neurons in the adult *Drosophila* brain at high resolution and to reproducibly identify all of the previously reported DA neurons (18), thus providing a sensitive approach for a quantitative analysis of DA neurons in *parkin* mutants.

Using this method, we compared the number of neurons in each of the DA neuron clusters in *parkin* mutants and isogenic control animals. Importantly, to control for investigator bias, all experiments were carried out with the experimenter blinded to the sample genotypes throughout the analysis. In agreement with previous work (14, 19), no gross anatomical defects were observed in the cortical or neuropil regions of the adult *Drosophila* brain, and TUNEL staining experiments did not reveal detectable differences between brains of *parkin* mutants and controls
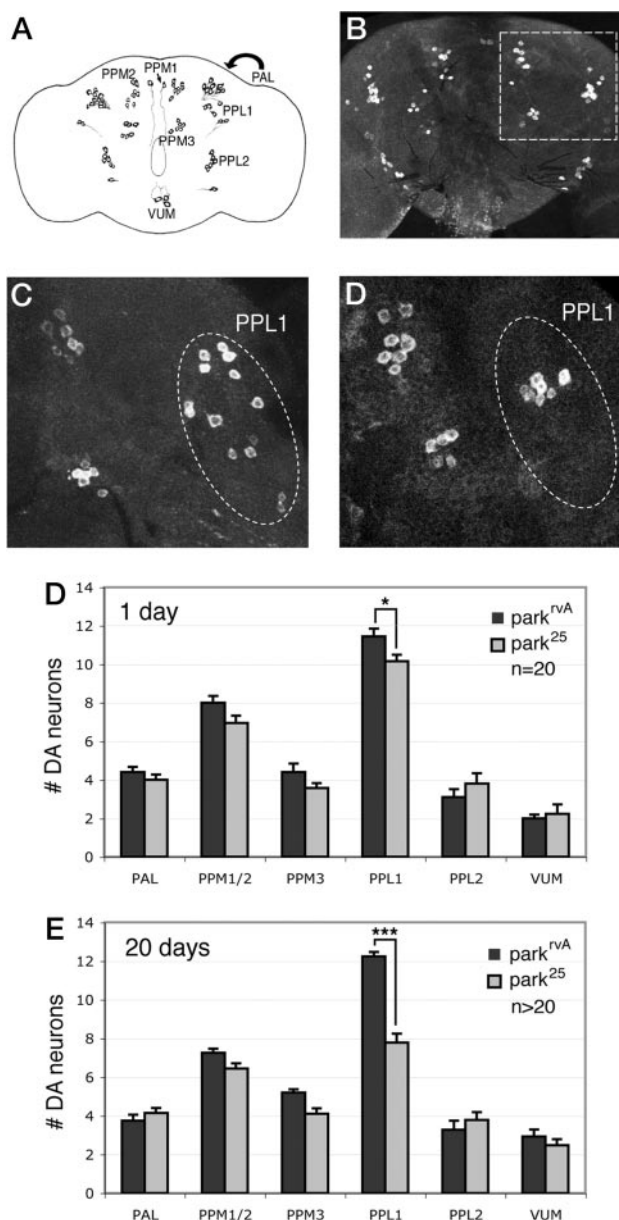
Fig. 1. A subset of DA neurons degenerates in *parkin* mutants. (*A*) Diagram shows the locations of the major DA neuron clusters in the adult *Drosophila* brain. PAL, protocerebral anterior lateral; PPM, protocerebral posterior medial; VUM, ventral unpaired medial. The PAL cluster is located in the same position but anterior to the PPL1 cluster, as designated by the arrow. (*B*) A projected Z-series confocal image from a WT adult brain stained with anti-TH to label DA neurons. DA neurons within the boxed section are shown at higher magnification in *C* and *D*. (*C* and *D*) Representative images from WT (*C*) and *parkin* mutant (*D*) brains aged 20 days. Fewer DA neurons are detected in the PPL1 cluster in *parkin* mutants relative to WT. (*E* and *F*) The number of DA neurons in each major DA neuron cluster in WT (black bars) and *parkin* mutants (gray bars) in 1-day-old (*E*) and 20-day-old (*F*) adult flies. *parkin* mutants display a significant decrease in the number of PPL1 neurons at 1 day of age (*, $P < 0.05$). Neuron loss in *parkin* mutants is more extensive in the PPL1 cluster at 20 days of age (***, $P < 0.0001$). Statistical significance was calculated by using Student's *t* test. n refers to the number of brains used for neuron counts. Animals homozygous for the *parkin* null allele *park^{25}* or bearing the isogenic *park^{rvA}* chromosome, which has a WT allele of *parkin*, were used in these analyses.

(data not shown), indicating that the vast majority of neurons are intact and healthy in *parkin* mutants. Furthermore, no dramatic differences were observed between DA neurons of *parkin* mu-

Fig. 2. Neuron loss in *parkin* mutants is specific to DA neurons and results primarily from loss of a cell-autonomous requirement of *parkin*. (*A–C*) Expression of GFP driven by TH-GAL4 (*A*) and anti-TH (*B*) staining reveals a high degree of colocalization of signals (*C*). The boxed area in *C* marks the PPL1 cluster and is magnified in *C′*. (*D*) Analysis of GFP-positive neurons in 20-day-old adult *parkin* mutants and age-matched WT controls bearing the TH-GAL4 driver and a UAS-GFP transgene confirms the cell loss in the PPL1 DA neuron cluster of *parkin* mutants (∗∗, $P < 0.005$, Student's *t* test). (*E*) DA neuron loss is significantly reduced by expression of a *parkin* transgene using the TH-GAL4 driver compared with *parkin* mutant or *parkin* mutants bearing the TH-GAL4 driver alone. Expression of GAL4 from the TH-GAL4 driver did not significantly affect DA neuron viability. (∗∗, $P < 0.001$; Δ, $P = 0.3$) (*F*) Diagram shows all major serotonergic neurons in the adult brain. SP, subaesophageal; LP, lateral protocerebral; IP, inferior medial protocerebral. (*G*) Representative confocal micrograph of a WT adult brain stained with anti-5-hydroxytyrosine to reveal serotonergic neurons. (*H*) No significant difference in the number of serotonergic neurons is detectable in 20-day-old adult *parkin* mutants relative to age-matched controls, indicating that neuron loss is specific to a subset of DA neurons. Statistical significance was calculated by using ANOVA and Bonferroni's post hoc test for planned comparisons. Numbers shown in histograms refer to the number of brains used for neuron counts. *park25* or *parkrvA* homozygotes were used in all analyses of neuronal viability.

tants and control animals (Fig. 1). However, quantification of the different DA neuron clusters of 1-day-old *parkin* adults relative to age-matched controls revealed a significant reduction in the number of DA neurons in one of the clusters, the protocerebral posterior lateral (PPL) 1 (Fig. 1). In 20-day-old *parkin* adults, the TH-positive PPL1 neurons showed a further decrease in numbers (Fig. 1). Again, no significant difference was detected in any other DA neuron cluster in 20-day-old *parkin* mutants. By contrast, the number of TH-positive neurons in the PPL1 cluster during the late pupal stage, in which the adult CNS is already fully formed, was indistinguishable between *parkin* mutants and controls (data not shown). These data indicate that loss of parkin function in *Drosophila* results in the progressive degeneration of DA neurons in the PPL1 cluster in the adult brain.

Additional experiments were performed to further characterize the neurodegenerative phenotype of *parkin* mutants. To confirm that loss of TH-positive neurons in *parkin* mutants was not an artifact of anti-TH immunostaining, we used an alternative method to detect DA neurons. The DA neuron-specific driver *TH-GAL4* was used to drive GFP expression in *parkin* mutants, and GFP fluorescence was used as a marker of DA neurons. Although this GAL4 driver does not express GFP in all of the TH-positive neurons in the PPL1 cluster (ref. 16 and Fig. 2), experiments with this driver revealed significantly decreased numbers of DA neurons in the PPL1 cluster of 20-day-old *parkin* mutants relative to age-matched control animals (Fig. 2). To verify that the loss of PPL1 neurons derives from loss of parkin function, the *TH-GAL4* driver was used to induce expression of a *parkin* transgene (*UAS-park*) in an effort to rescue the observed degeneration. Results of this analysis revealed that transgenic expression of *parkin* in DA neurons significantly attenuated DA neuron loss in the PPL1 cluster (Fig. 2). These results confirm

the neurodegenerative phenotype of *parkin* mutants and demonstrate that parkin is required cell-autonomously for DA neuron integrity. The incomplete rescue of DA neuron loss by transgenic expression of *parkin* may reflect lack of expression of the *TH-GAL4* driver in some of the PPL1 neurons, inefficient expression of transgenic *parkin*, and/or an additional nonautonomous role of parkin in DA neuron integrity.

Neurodegeneration in PD is largely restricted to a subset of DA neurons in the brain. To evaluate the specificity of neuron loss in *Drosophila parkin* mutants, the integrity of other catecholaminergic neurons was assessed. Specifically, antiserum against serotonin (5-hydroxytyrosine) was used to analyze serotonergic neurons in 20-day-old *parkin* mutants and age-matched isogenic controls (20). In contrast to our analyses of DA neurons, no significant neuronal loss was observed in any of the serotonergic clusters in *parkin* mutants (Fig. 2). These results, in conjunction with our previous work and the observation that overall brain volume was not altered in *parkin* mutants, indicate that neurodegeneration in *parkin* mutants is selective for a subset of DA neurons in the central nervous system.

In an effort to identify pathways that influence the *Drosophila parkin* phenotypes, we performed a genetic screen for dominant modifiers of a *parkin* partial pupal lethal phenotype, which appears to result from muscle dysfunction (15). A loss-of-function allele of *GstS1* was the strongest enhancer recovered from this screen. To further assess the involvement of *GstS1* in *parkin* pathogenesis, we explored the effects of altered *GstS1* function on other *parkin* phenotypes. In previous work, we showed that parkin function is required in the musculature for normal geotactic climbing behavior. Thus, we tested whether *GstS1* mutations influence the climbing phenotype of *parkin* mutants. All of the *GstS1* alleles tested, including several that were not used in our genetic screen, were also found to enhance a climbing defect of *parkin* mutants when heterozygous with a WT allele of *GstS1* (Fig. 3). By contrast, *GstS1* mutations alone had no effect on climbing ability in a background of WT *parkin* or in *parkin* heterozygotes (Fig. 3 and data not shown). Moreover, overexpression of a UAS-*GstS1* transgene in the musculature, using the mesoderm GAL4 driver, *24B-GAL4*, was sufficient to restore the climbing ability of two hypomorphic *parkin* mutants to the WT (Fig. 3). Considering the extensive muscle degeneration observed in *parkin* null mutants, hypomorphic mutants were used in this assay to potentiate the detection of small differences in climbing ability.

To extend these findings to the neurodegenerative phenotype of *parkin* mutants, we analyzed the effect of altered *GstS1* activity on DA neuron integrity in *parkin* mutants. Although GstS1 appears to be predominantly expressed in flight muscle, GstS1 expression is also detected in the adult head (21). To explore the effects of reduced GstS1 dosage on the *parkin* DA neuron loss phenotype, *parkin* mutants were crossed to a stock bearing a viable null allele of GstS1 designated *GstS1^{M26}*. *parkin* mutants bearing the *GstS1^{M26}* mutation in trans to a deletion chromosome that removes the *GstS1* gene [*Df(2R)ED1*] were recovered at low frequency and displayed dramatically shortened lifespan (data not shown). Analysis of DA neuron integrity in 1-day-old *parkin* mutants bearing this combination of *GstS1* alleles revealed significantly enhanced DA neuron loss relative to *parkin* mutants alone (Fig. 4). Loss of *GstS1* function alone had no affect on DA neuron viability in 1-day-old adults (Fig. 4). Conversely, transgenic overexpression of *GstS1* only in DA neurons, using the *TH-GAL4* driver in conjunction with a *UAS-GstS1* transgene, was able to significantly attenuate the loss of DA neurons in 20-day-old *parkin* mutants (Fig. 4). Moreover, the degree of rescue conferred by *GstS1* expression was comparable to that observed with transgenic *parkin* expression (Fig. 4). These results
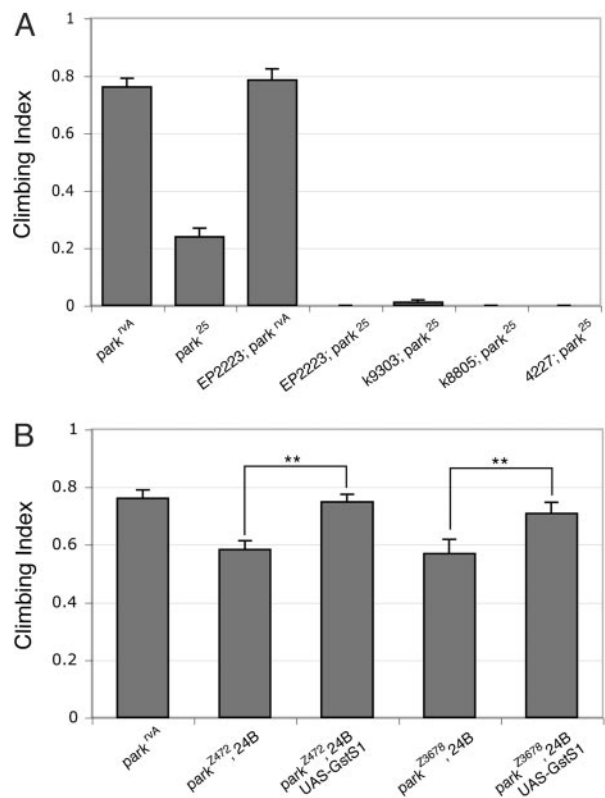

Fig. 3. GstS1 activity modifies locomotor deficits in *parkin* mutants. (*A*) Loss-of-function alleles of *GstS1* enhance the climbing defect of *parkin* mutants. Each loss-of-function *GstS1* allele, *EP2223*, *k09303*, *k08805*, and *04227*, used in this analysis was heterozygous with a WT allele of *GstS1*. *park^{25}* or *park^{rvA}* homozygotes were used in all climbing assays. Enhancement of the *parkin* climbing defect by each of the *GstS1* alleles was significant ($P < 0.005$, Student's *t* test). (*B*) Directed expression of a GstS1 transgene (UAS-GstS1) by a muscle GAL4 transgene (24B) alleviates the partial climbing deficits seen in two *parkin* hypomorphic mutants (*park^{Z472}* and *park^{Z3678}*). Suppression of climbing deficits from GstS1 overexpression was significant (**, $P < 0.001$) by Student's *t* test.

indicate that *GstS1* potently modulates DA neuron viability in *parkin* mutants.

Glutathione *S*-transferases catalyze the conjugation of reduced glutathione to a variety of substrates, including the products of reactive oxygen species (22), and substantial evidence suggests that oxidative stress is a major contributing factor in sporadic PD (23). Our results demonstrating *GstS1* modification of the *parkin* loss-of-function phenotypes suggest that parkin may act to limit oxidative stress-induced cellular damage. To begin to investigate this hypothesis, we tested whether *parkin* mutants display other evidence of oxidative stress. One consequence of oxidative stress is the production of protein carbonyls. Examination of the level of protein carbonyl content revealed significantly elevated levels of protein carbonyl content in 1-day-old adult *parkin* mutants (10.4 nmol of carbonyls per mg of protein) relative to age-matched isogenic controls (2.6 nmol of carbonyls per mg of protein). These results suggest that parkin confers protection from damage by reactive oxygen species. However, we did not detect significant alterations in the levels of glutathione in 1-day-old *parkin* mutants, indicating that oxidative damage in *parkin* mutants is not simply a consequence of decreased glutathione levels.

Discussion

Substantial evidence suggests that oxidative stress contributes to DA neuron loss in sporadic PD (24). In particular, increased
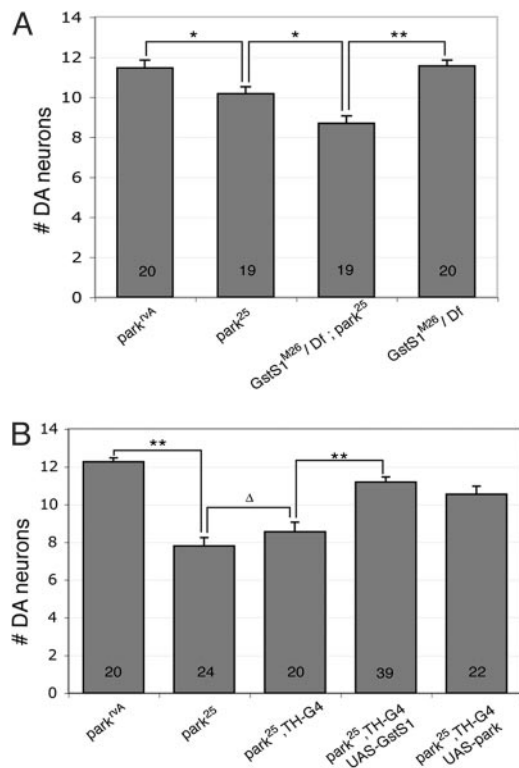
Fig. 4. GstS1 activity influences DA neuron viability in *parkin* mutants. (*A*) A *GstS1* null allele (*GstS1*[M26]) in trans to a deletion (Df) of the *GstS1* region [*Df(2R)ED1*] enhances DA neuron loss detected in the PPL1 cluster of 1-day-old *parkin* mutants. *GstS1* null mutants (*GstS1*[M26]/*Df*) alone manifest no DA neuron loss in a WT *parkin* background. (*B*) Transgenic overexpression of GstS1 in DA neurons significantly suppresses DA neuron loss in 20-day-old *parkin* mutants (park[25],TH-G4; UAS-GstS1). The degree of rescue conferred by GstS1 is comparable to that achieved by transgenic expression of *parkin* in DA neurons (park[25],TH-G4; UAS-park). Statistical significance was calculated by using ANOVA and Bonferroni's post hoc test for planned comparisons. (∗, $P < 0.05$; ∗∗, $P < 0.001$; Δ, $P = 0.3$). Numbers shown in bars refer to the number of brains analyzed for neuron counts. park[25] or park[rvA] homozygotes were used in all analyses of neuronal viability.

levels of lipid peroxidation, oxidatively damaged proteins and DNA, and decreased levels of glutathione have been documented in the substantia nigra of PD patients (23). One potential source of this oxidative stress is increased free radical formation resulting from partial inhibition of mitochondrial complex I. Many PD patients display decreased complex I activity in the brain and peripheral tissues, and several complex I inhibitors cause a PD-like syndrome in humans and animal models (25). Our studies of *Drosophila parkin* mutants reveal many features in common with sporadic PD. We have previously reported that mutations in *Drosophila parkin* result in profound mitochondrial defects in multiple tissue types. In this report, we demonstrate that fly *parkin* mutants also display progressive degeneration of a subset of DA neurons and that this degeneration can be rescued by overexpression of a glutathione *S*-transferase, a factor that has been implicated in the cellular response to oxidative stress (26). These observations demonstrate that fly *parkin* mutants recapitulate key features of ARJP and suggest that the mechanisms responsible for DA neuron loss in ARJP are similar to the mechanisms responsible for sporadic PD.

Three previous analyses of neuronal integrity in *Drosophila parkin* mutants, including a study from our laboratory, failed to detect DA neuron loss (14, 19, 27). However, the specific DA neuron cluster that degenerates in *parkin* mutants (PPL1) was not analyzed in two of these studies (19, 27). Although the PPL1

neuron cluster was assessed in our previous study of *Drosophila parkin* mutants, the substantial variation in DA neuron counts observed in this study would not have allowed detection of the reduction of DA neurons reported in our current study. A major motivation of the present study was to apply unbiased quantitative methods to analyze all of the DA neuron clusters in the adult *Drosophila* brain in an effort to detect subtle alterations in neuron structure and integrity. Results described here indicate that confocal microscopy is a powerful method to facilitate detection of subtle neuron loss in *Drosophila*.

To understand how mutations in *parkin* result in DA neuron death in the human disease, substantial effort has been invested in the identification of targets of the parkin ubiquitin-protein ligase activity. A number of parkin substrates have been identified (10). Two of these substrates, α-synuclein and Pael-R, have received considerable attention, in part because *in vitro* studies have shown that these components are cytotoxic when overexpressed, and both of these proteins appear to accumulate in the brains of ARJP individuals (11, 12). Furthermore, overexpression of parkin has been shown to attenuate the toxicity of these proteins *in vitro* and *in vivo* (11, 12, 27–31). These findings have led to a model whereby accumulation of these proteins causes DA neuron death in ARJP individuals. Although most of the previously identified parkin substrates appear to have *Drosophila* orthologs, BLAST searches of the *Drosophila* genome sequence have failed to detect orthologs of *α-synuclein* or *Pael-R*. Thus, our results demonstrating DA neuron degeneration in *Drosophila parkin* mutants suggest that α-synuclein and Pael-R are not obligate in DA neurodegeneration. Although our work challenges the absolute requirement of α-synuclein and Pael-R in the etiology of ARJP, these factors may contribute to the severity and/or magnitude of cell loss in ARJP individuals. However, the fact that DA neuron degeneration can occur in the absence of α-synuclein and Pael-R indicates that additional factors are clearly involved in ARJP pathogenesis. The identification of these factors is paramount to our understanding of the molecular etiology of this disorder.

Previous work on GstS1 function in *Drosophila* suggests that this factor may play a role in detoxifying products of oxidative damage (26). Our finding that altered *GstS1* activity influences the *parkin* mutant phenotypes raises the possibility that parkin may also offer protection from the effects of oxidative stress. Further support for this hypothesis comes from the findings that oxidative damage in flies, mice, and cell lines and sensitivity to oxidative stress agents in flies and cell lines correlates inversely with parkin activity (19, 32–34). Our recent results from transcriptional profiling of *parkin* mutants and a genetic screen for *parkin* modifiers also demonstrate that oxidative stress response elements are up-regulated and that mutations in oxidative stress response components enhance the *parkin* mutant phenotypes (15).

There are potentially many ways that parkin might confer protection from the effects of oxidative stress. For example, parkin may recognize substrates bearing a specific oxidative modification and target these damaged proteins for degradation. Support for this model is provided by the finding that the HOIL-1 ubiquitin-protein ligase, a protein with a similar domain structure to parkin, has recently been shown to recognize and ubiquitinate an oxidatively modified form of its substrate IRP2 (35). Alternatively, the findings that altered parkin function results in mitochondrial defects in cell lines, flies, mice, and humans raises the possibility that parkin may directly influence mitochondrial integrity (14, 32, 36, 37). Studies in yeast have identified a ubiquitin ligase involved in mitochondria fission (38), and ubiquitination is also important for the insertion of proteins into the outer mitochondrial membrane (39, 40). An intriguing possibility is that parkin may label oxidatively damaged mitochondrial proteins and target the entire mitochondrion for destruction by autophagy. Indeed, the ultrastructural mor-

phology of mitochondria in *parkin* mutant flies is strikingly similar to the appearance of mitochondria that are engulfed during autophagy (41). Furthermore, previous work has shown that ubiquitination of mitochondrial proteins and the formation of other ubiquitin-like conjugates can target mitochondria for autophagic degradation (42, 43).

Our findings suggest a potential therapeutic approach for ARJP and possibly sporadic PD. There are a number of compounds that are known to induce glutathione *S*-transferase activity in vertebrates (44). Although these compounds have been studied primarily because of their protective effects against cancer, our current results suggest that these compounds might be useful in the treatment of ARJP. There are several reasons to believe that this potential treatment strategy could extend beyond ARJP. First, as mentioned above, significant evidence suggests that oxidative stress is involved in the etiology of sporadic PD (24). Furthermore, alleles of the *Gst omega-1* gene have been found to influence the age of onset of PD in humans (45), and loss-of-function mutations of the yeast *GstS1* homolog, *gtt1*, enhance the toxicity of α-synuclein in this organism (46). Because mutations that increase α-synuclein expression result in heritable forms of PD and α-synuclein is a component of the Lewy body inclusions associated with sporadic PD, the finding that reduced *gtt1* activity in yeast enhances α-synuclein toxicity suggests that altered glutathione *S*-transferase activity may be relevant to sporadic PD pathogenesis. A number of excellent models of

α-synucleinopathy currently exist (46–49) that can be used to test this hypothesis.

In summary, our findings that DA neuron loss occurs in *Drosophila parkin* mutants and that DA neuron loss can be modified by a putative oxidative damage detoxification factor suggest that the mechanisms responsible for DA neuron death in *Drosophila parkin* mutants and sporadic PD are conserved. Furthermore, the observation that a modifier of the reduced viability and climbing phenotypes of *Drosophila parkin* mutants also modifies the DA neuron loss phenotype suggests an underlying similarity in the etiology of the muscle and neuron phenotypes in *Drosophila parkin* mutants. The ease of detecting modifiers of the *Drosophila parkin* viability and behavioral phenotypes and our current demonstration of the relevance of one of these factors to DA neuron integrity illustrate the potential of this system to identify genetic pathways and therapeutic factors relevant to an understanding of PD pathogenesis and treatment.

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