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RESEARCH ARTICLE

Antioxidant proteins TSA and PAG interact synergistically with Presenilin to modulate Notch signaling in *Drosophila*

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ABSTRACT

Alzheimer's disease (AD) pathogenesis is characterized by senile plaques in the brain and evidence of oxidative damage. Oxidative stress may precede plaque formation in AD; however, the link between oxidative damage and plaque formation remains unknown. Presenilins are transmembrane proteins in which mutations lead to accelerated plaque formation and early-onset familial Alzheimer's disease. Presenilins physically interact with two antioxidant enzymes thiol-specific antioxidant (TSA) and proliferation-associated gene (PAG) of the peroxiredoxin family. The functional consequences of these interactions are unclear. In the current study we expressed a presenilin transgene in *Drosophila* wing and sensory organ precursors of the fly. This caused phenotypes typical of Notch signaling loss-of-function mutations. We found that while expression of TSA or PAG alone produced no phenotype, co-expression of TSA and PAG with presenilin led to an enhanced Notch loss-of-function phenotype. This phenotype was more severe and more penetrant than that caused by the expression of *Psn* alone. In order to determine whether these phenotypes were indeed affecting Notch signaling, this experiment was performed in a genetic background carrying an activated Notch (Abruptex) allele. The phenotypes were almost completely rescued by this activated Notch allele. These results link peroxiredoxins with the *in vivo* function of Presenilin, which ultimately connects two key pathogenetic mechanisms in AD, namely, antioxidant activity and plaque formation, and raises the possibility of a role for peroxiredoxin family members in Alzheimer's pathogenesis.

KEYWORDS Presenilin, Alzheimer's disease, peroxiredoxin, Notch

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease leading to dementia with a characteristic brain pathology marked by the accumulation of neurofibrillary tangles and senile plaques in the central nervous system (CNS) (Selkoe, 2001). The pathogenesis of AD is the subject of extensive study, but the molecular sequence of events ultimately leading to progressive neurodegeneration remains unclear (Smith et al., 2000; Van Gassen et al., 2000; Perry et al., 2002). Pathologic changes underlying the disease have been explored in post-mortem brain tissue (McLellan et al., 2003; Shan et al., 2003), neuroimaging (Fox and Schott, 2004; Schott et al., 2005), biochemical analyses of presenilins as part of the γ-secretase complex (Wolfe et al., 1999; Zhou et al., 2002), and functional analysis of implicated proteins in model organisms like *Drosophila* (Greeve et al., 2004; Iijima

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et al., 2004; Crowther et al., 2005). It has been suggested that there are two crucial neuropathological features of AD brains, the formation of amyloid plaques and the accumulation of oxidative stress (Lahiri and Greig, 2004). The strongest evidence for the crucial role of amyloidogenesis in AD pathogenesis comes from cases of familial AD (FAD). These early onset Mendelian cases result from mutations in the amyloid precursor protein (APP) (Goate et al., 1991) or mutations in one of the two presenilin genes, PSEN1 and PSEN2 (Levy-Lahad et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995). As part of the γ-secretase protein complex, presenilins are responsible for amyloid cleavage and FAD mutations in PSEN1 and PSEN2 lead to overproduction of the neurotoxic 42 amino acid form of β-amyloid (Martins et al., 1995; Borchelt et al., 1996; Duff et al., 1996; Scheuner et al., 1996; Citron et al., 1997; Xia et al., 1997; Mehta et al., 1998; Oyama et al., 1998). This human genetic evidence and the amyloid composition of the senile plaques have made the presenilin proteins a major focus of AD research (Haass and De Strooper, 1999).

In addition to aberrant amyloid processing, global oxidative damage plays a key role in the etiology of AD. Evidence for a role of oxidative damage in AD includes increased reactive carbonyl moieties in the brains of patients with AD (Smith et al., 1992), decreased antioxidant activity in mouse models and human AD brain (Gibson et al., 2000; Leutner et al., 2000; Schuessel et al., 2005), the accumulation of lipid peroxidation by-products in the brains and cerebrospinal fluid of patients with AD (Montine et al., 2002), the presence of oxidative damage to RNA in AD brains (Shan et al., 2003), abnormal oxidation of thiol proteases in the hippocampus in AD patients (Marcum et al., 2005), and mitochondrial abnormalities in AD brains (Smith et al., 2000). While both oxidation-reduction and amyloidogenesis are clearly fundamental to understanding AD, few studies have explored specific protein interactions using model organisms between the proteins involved in amyloidogenesis and those involved in oxidative stress.

There is clear evidence of a role for oxidative stress in mice with conditional knockout mutations in the Presenilin genes suggesting that these processes are linked (Gu et al., 2008). One possible explanation could involve the fact that presenilin proteins physically interact with a number of other proteins. Presenilins are eight transmembrane spanning proteins principally associated with the endoplasmic reticulum and the plasma membrane (Nowotny et al., 2000) and comprise the enzymatic part of the γ-secretase complex (Wolfe et al., 1999), responsible for regulated cleavage events that occur within the membrane. Known targets cleaved by γ-secretase include β-amyloid and the Notch receptor. In the case of Notch signaling, the γ-secretase activity of PSENs is activated by ligand binding to the Notch receptor. Intramembrane cleavage of Notch liberates the intracellular domain (ICD), which then translocates to the nucleus where it associates with the Suppressor of Hairless protein to activate transcription of target genes (De Strooper et al., 1999; Struhl and Greenwald, 1999). While presenilins are required for Notch signaling, overexpression of presenilin in *Drosophila* leads to a phenotype resembling Notch inactivation suggesting that expression of a single component of the complex acts in a dominant negative fashion (Ye et al., 1999). In addition to these γ-secretase functions, PSENs have been shown to physically interact with proteins involved in cell adhesion, cytoskeletal regulation, Wnt/wingless signaling pathway, intracellular calcium homeostasis, and apoptosis (Van Gassen et al., 2000; van de Hoef et al., 2009). However, in many cases the functional significance of these interactions is not known (Van Gassen et al., 2000). PSEN1 has also been found to physically associate with two antioxidant enzymes TSA and PAG, both of which are peroxiredoxin enzymes involved in the detoxification of hydrogen peroxide (Zhou et al., 2002; Patenaude et al., 2005).

Based on the evidence that both amyloidogenesis and oxidation-reduction are crucial components of AD pathogenesis and the lack of *in vivo* validation of protein-protein interactions with presenilin, we tested whether the antioxidant proteins TSA and PAG could modify the phenotypic effect of presenilin overexpression in *Drosophila*. *Drosophila* is well suited for such an analysis because of the relative ease in characterizing adult phenotypes, the ability to express transgenes in selected tissues using the GAL4-UAS system (Brand and Perrimon 1993), and the fact that *Drosophila* has already contributed to the study of AD (Greeve et al., 2004; Iijima et al., 2004; Crowther et al., 2005).

RESULTS

Peroxiredoxin enzymes TSA and PAG enhance the Notch inactivation phenotype caused by *Psn* expression in the wing

TSA and PAG physically interact with PSEN1 *in vitro* (Zhou et al., 2002); however the functional significance of this interaction *in vivo* remains elusive. We therefore turned to *Drosophila* as a model system to address this question. TSA and PAG share 76% amino acid homology, and both have been reported to catalyze the reduction of hydrogen peroxide using key cysteine residues and the protein thioredoxin as a cofactor (Fig. 1A). There is close homology between the four peroxiredoxin family members with two active cysteine residues in humans and the four two-cysteine family members in *Drosophila* (Fig. 1). We employed the *Drosophila* UAS-GAL4 transgenic expression system (Brand and Perrimon, 1993; Duffy, 2002) to determine whether TSA and PAG could interact with Presenilin *in vivo*. Interestingly, while *Drosophila Psn* is required for Notch cleavage and signaling, overexpression of *Psn* results in Notch loss-of-function phenotypes, possibly caused by a dominant negative effect

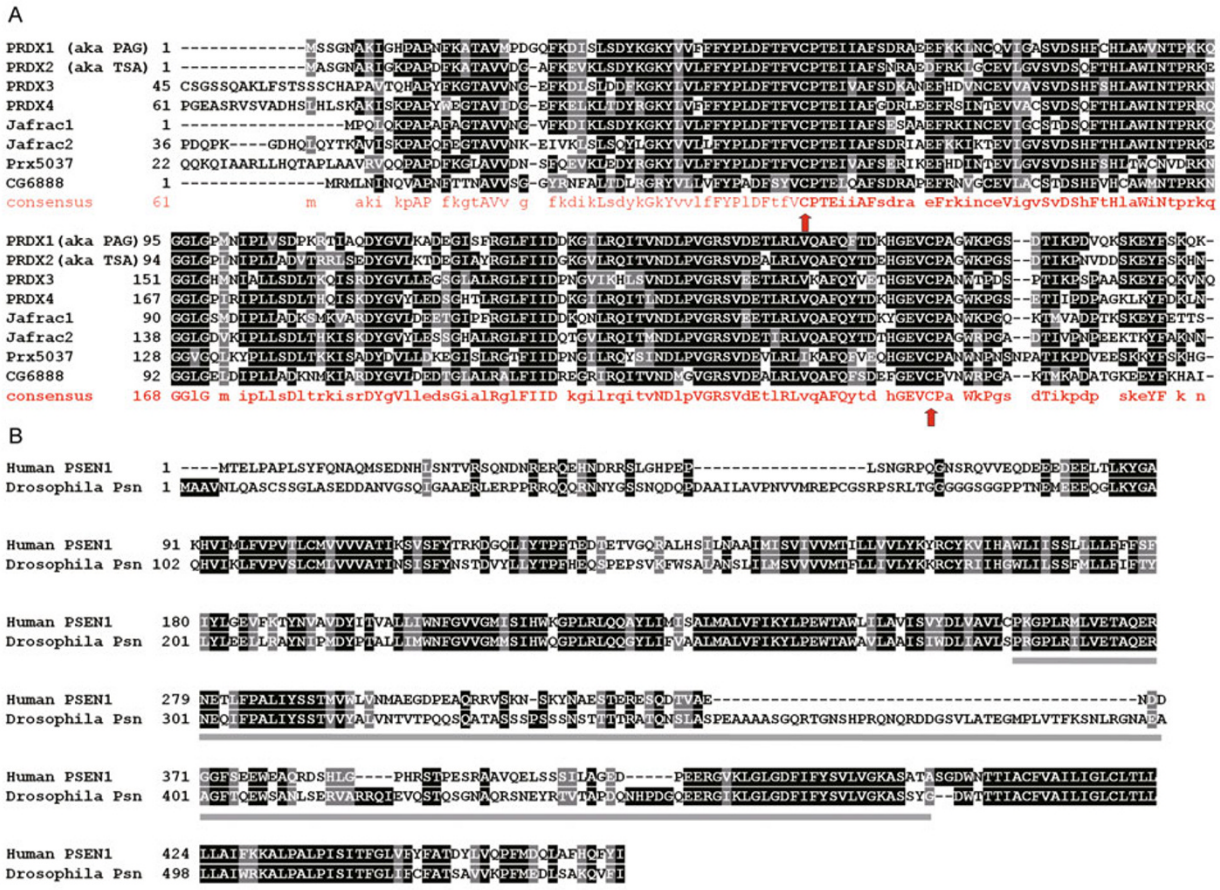

Figure 1. Amino acid sequence alignment of human and *Drosophila* peroxiredoxin enzymes and Human PSEN1 and *Drosophila* Psn. (A) Human and *Drosophila* two-cysteine peroxiredoxin family members are aligned. These proteins catalyze the reduction of hydrogen peroxide to water using a pair of cysteine residues (red arrows) and the thioredoxin protein as a co-factor. Human TSA and PAG share > 76% amino acid homology, and have been shown to interact with human PSEN1 protein. The four *Drosophila* two-cysteine family members show close homology to the human versions throughout the length of the proteins (consensus sequence is shown in red). (B) Human PSEN1 and *Drosophila Psn* are aligned. The *Psn* transgene shares areas of close homology with PSEN1. The protein-protein interaction with TSA and PAG has been shown to depend on the cytoplasmic loop of the PSEN1 protein between amino acids 265 and 407 (indicated by solid gray line), and this area shows conservation with *Psn* in distinct regions.

due to the overexpression of *Psn* (Ye and Fortini, 1999). We explored whether the addition of human TSA and PAG would modulate the phenotype due to overexpression of *Drosophila Psn*. The site of reported interaction with TSA and PAG, through the cytoplasmic loop region, is a portion of the protein which shows variable homology with distinct areas of strong homology (Fig. 1B, gray bar). We inserted human TSA and PAG coding sequences (Fig. 1) into the *Drosophila* pUAS expression vector and transformed these constructs into the *Drosophila* genome. We then expressed TSA and PAG in the developing wing using the *MS1096*-GAL4 driver (Guillen et al., 1995). The *Drosophila* wing is a good system for these studies because of its ordered structure with the L1–L5 wing veins (Fig. 2A) that have a well described development through developmental signaling pathways (Bier, 2005). The *MS1096*-GAL4 line drives strong GAL4 protein expression in the dorsal region of the wing pouch and weaker expression ventrally. Expression of TSA, PAG, or both with *MS1096*-GAL4 led to no abnormal phenotypes, indicating that strong expression of these peroxiredoxin enzymes alone does not interfere with normal wing development (Fig. 2A). When we expressed the *Psn* transgene with *MS1096*-GAL4, we observed a *Notch* loss-of-function phenotype with thickening of the L3 and L5 wing veins and a significant reduction in the size of the wing surface resulting in dorsal curling of the wings (Fig. 2B). However, when we co-expressed *Psn* with either TSA or PAG, or with both, we observed a significant increase in the severity of *Notch*-like phenotypes, most notably vein thickening (Fig. 2C). The wings were also reduced in size and exhibited stronger dorsal curling.

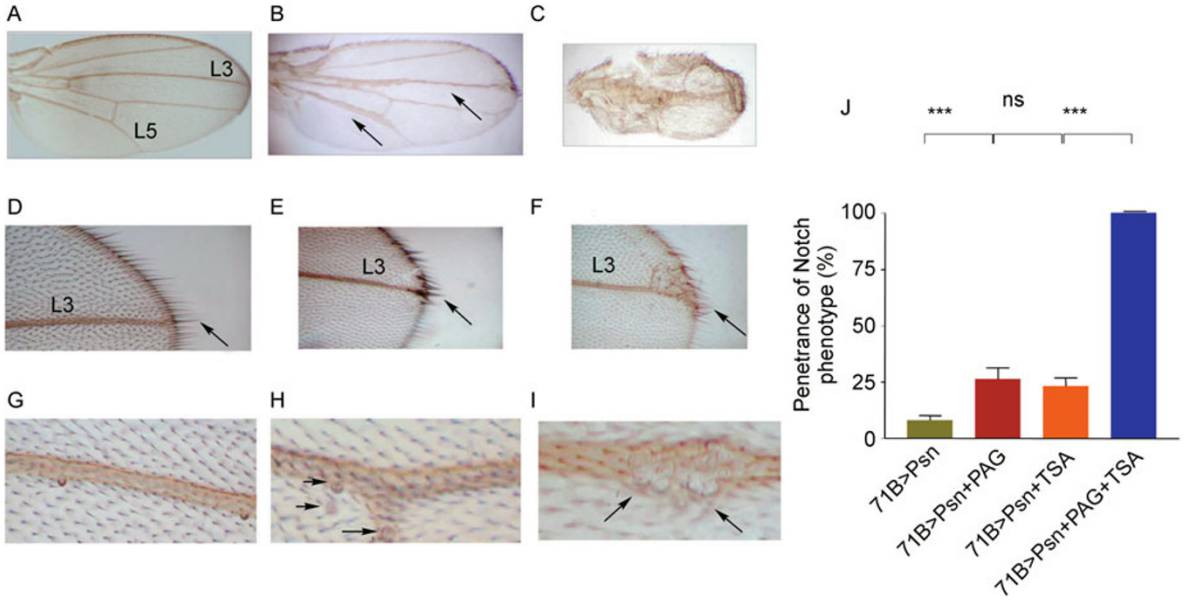

Figure 2. Wing phenotypes of *Psn* transgene, and *Psn* in combination with TSA and PAG. (A) *MS1096*-GAL4 > PAG-UAS; TSA-UAS progeny were indistinguishable from wild type. (B) *MS1096*-GAL4 > *Psn*-UAS progeny in contrast exhibited thickening of the L3, and L5 wing veins (arrows). (C) *MS1096*-GAL4 > *Psn*-UAS + PAG-UAS + TSA-UAS progeny exhibited severe broadening of the wing veins and reduction in wing size. (D) The *71B*- GAL4 > PAG-UAS + TSA-UAS progeny had the wild type pattern of double-row bristles on the anterior edge of the wing (arrow, wild type is shown). (E) *71B*-GAL4 > *Psn*-UAS progeny exhibited ectopic double-row bristles at the lateral end of the L3 wing vein (arrow). (F) *71B*-GAL4 > *Psn*-UAS + PAG-UAS + TSA-UAS progeny exhibited more pronounced clusters of ectopic double row bristles at the outer end of the L3 wing vein (arrow). (G) *71B*-GAL4 > PAG-UAS + TSA-UAS progeny had the wild type pattern of campaniform sensilla, a sensory organ precursor, with regular spacing along the L3 vein (wild type is shown). (H) *71B*-GAL4 > *Psn*-UAS progeny had ectopic campaniform sensilla along the L3 wing vein particularly near the cross-vein (arrows). (I) *71B*-GAL4 > *Psn*-UAS + PAG-UAS + TSA-UAS progeny had marked increase in severity of this phenotype with large clusters of ectopic companiform sensilla present along the L3 vein (arrows). (J) Penetrance of the Notch phenotypes in *71B*-GAL4 crosses grouped by genotype. While the *Psn* genotype had overall penetrance of 8%, the *Psn* + PAG had a penetrance of 26%, and *Psn* + TSA had a penetrance of 23%. Finally the *Psn* + PAG + TSA crosses had a penetrance of 100% which was significantly higher than the other genotypes.

Peroxiredoxin enzymes TSA and PAG enhance *Psn* sensory organ precursor phenotypes

In addition to the effect on the wing veins, we observed similar synergistic effects on the formation of sensory organs when co-expressing these proteins with *71B*-GAL4. The *71B*-GAL4 line drives moderate levels of GAL4 protein expression ubiquitously in regions of the wing anterior to the L5 vein (Guillen et al., 1995). Expression of the transgenes TSA or PAG with *71B*-GAL4 did not alter the wild type pattern of double row bristles (Fig. 2D) or campaniform sensilla (Fig. 2G) along the L3 wing vein. When we expressed UAS-*Psn* with the *71B*-GAL4 driver, we observed a modest number of ectopic sensory organs in a fraction of progeny, such as supernumerary double row bristles along the wing margin at the junction with the L3 wing vein (Fig. 2E) and extra campaniform sensilla along the L3 vein (Fig. 2H), both Notch loss-of-function phenotypes. Co-expression of TSA and PAG with *Psn* greatly increased the severity of ectopic clusters of both double-row bristles (Fig. 2F) and campaniform sensilla (Fig. 2I).

Another indicator of the genetic interaction between *Psn* and peroxiredoxins was an increase in the penetrance of Notch loss-of-function phenotypes defined as the presence of any ectopic campaniform sensilla or double-row bristle in the *71B*-GAL4 progeny. Expression of *Psn* alone resulted in only partial low-frequency penetrance, which was incrementally enhanced by co-expression with either TSA or PAG and was made fully penetrant if both TSA and PAG were co-expressed with *Psn* (Fig. 2J).

We also observed Notch loss-of-function phenotypes with ectopic sensory organs in other locations, other than the wing, when *Psn* was co-expressed with PAG or TSA. Ectopic bristles were observed anterior to the eye using the *A9*-GAL4 driver. *A9* is a low-expressing variant of the *MS1096*-GAL4 line (Guillen et al., 1995). Expression of TSA plus PAG resulted in no obvious phenotype (Fig. 3A, wild type shown), while expression of *Psn* alone produced a few ectopic bristles anterior to the eye (Fig. 3B, arrows). In contrast, when *Psn* was co-expressed with PAG plus TSA, the number of these

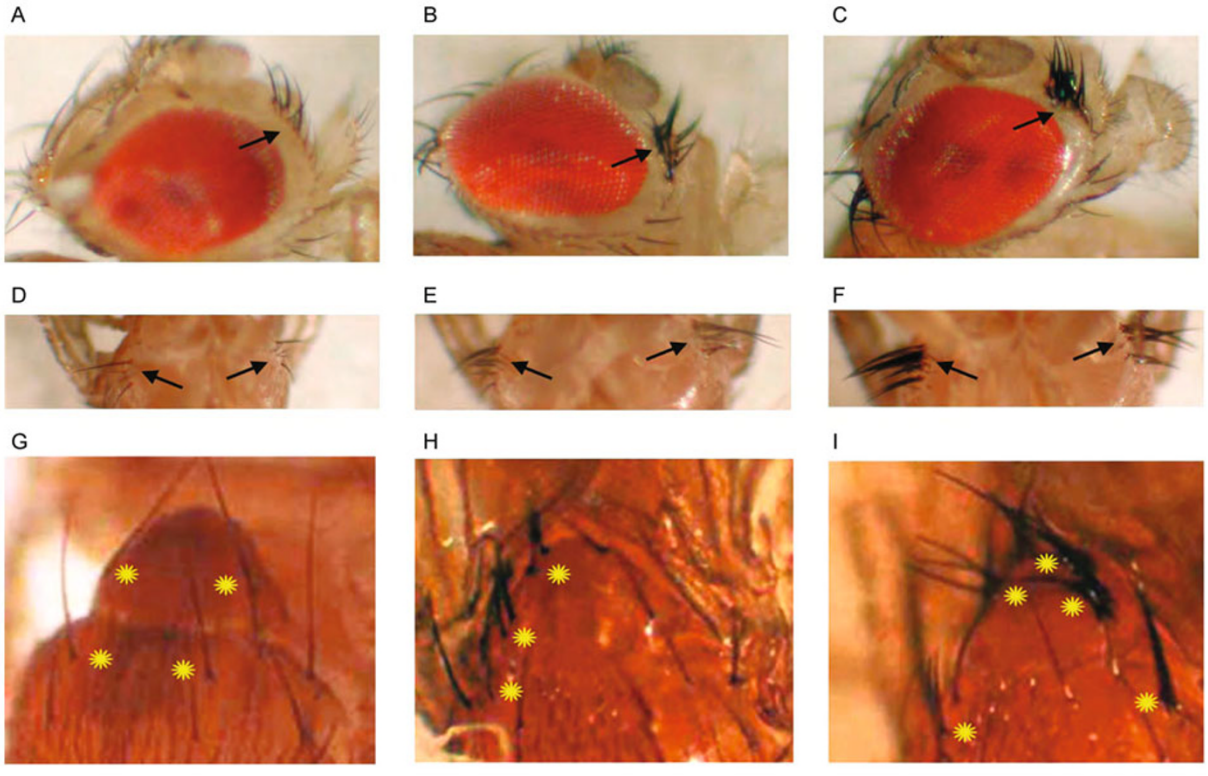

Figure 3. Bristle phenotypes of *Psn* transgene, and *Psn* in combination with TSA and PAG. (A) *A9*-GAL4 > TSA + PAG progeny demonstrated the wild type pattern of evenly spaced bristles anterior to the eye. (B) *A9*-GAL4 > *Psn* progeny displayed some ectopic clusters of bristles anterior to the eye (arrow). (C) *A9*-GAL4 > *Psn* + PAG + TSA progeny had a more severe phenotype with thicker clusters of ectopic bristles anterior to the eye. (D) *71B*-GAL4 > PAG + TSA progeny had wild type sternopleural bristles. (E) *71B*-GAL4 > *Psn* progeny displayed some ectopic sternopleural bristles. (F) *71B*-GAL4 > *Psn* + PAG + TSA progeny displayed a more severe phenotype with greater numbers of ectopic sternopleural bristles. (G) *MS1096*-GAL4 > TSA + PAG progeny were indistinguishable from wild type in terms of the macrochaetae (wild type shown). (H) *MS1096*-GAL4 > *Psn* progeny had ectopic macrochaetae that appeared to be in pairs next to the normal macrochaetae. (I) *MS1096*-GAL4 > *Psn* + PAG + TSA progeny had numerous macrochaetae clusters.

ectopic bristles increased substantially (Fig. 3C, arrow). A similar effect was observed using the *71B*-GAL4 driver. While expression of either TSA or PAG with *71B*-GAL4 had no phenotype in the sternopleural bristles (Fig. 3D, wild type shown), *Psn* expression produced a number of thick ectopic sternopleural bristles (Fig. 3E, arrow). This phenotype was markedly enhanced by co-expression of *Psn* with TSA and PAG (Fig. 3F, arrow). Finally, similar phenotypes were observed with regard to the scutellar macrochaetae with the *MS1096-GAL4* driver. While both wild type flies and those expressing TSA and PAG had four normal macrochaetae (Fig. 3G, yellow stars), expression of the *Psn* transgene led to an increase in the number of macrochaetae (Fig. 3H). This phenotype was enhanced by co-expression of *Psn* with TSA and PAG.

The synergism of TSA or PAG with *Psn* is blocked by activated Notch

The enhanced loss of Notch-like phenotypes resulting from co-expression of the peroxiredoxin enzymes TSA and PAG with *Psn* (e.g. thickened veins and increased formation of ectopic sensory organs) suggested that this synergy was mediated by reducing activity of Notch signaling since this pathway is known to be involved in restricting vein thickness, and in cell fate decisions of the sensory organ precursors to limit the number of cells assuming the sensory organ precursor cell fate through lateral inhibition (Artavanis-Tsakonas et al., 1999). The overexpression of *Psn* alone can produce Notch loss-of-function phenotypes, which presumably reflects a dominant negative effect caused by altering the stoichiometry of the multisubunit γ-secretase complex (Ye et al., 1999). We therefore hypothesized that the synergistic effect we observed with the addition of TSA and PAG was due to exacerbation of this dominant negative effect resulting in a further reduction in Notch signaling. We tested this hypothesis by co-expressing *Psn*, TSA and PAG with *MS1096*-GAL4 in flies carrying a dominant activated allele of Notch known as Abruptex (N^{Abx}) (Fig. 4). Since N^{Abx} can stimulate Notch signaling in the absence of γ-secretase, we

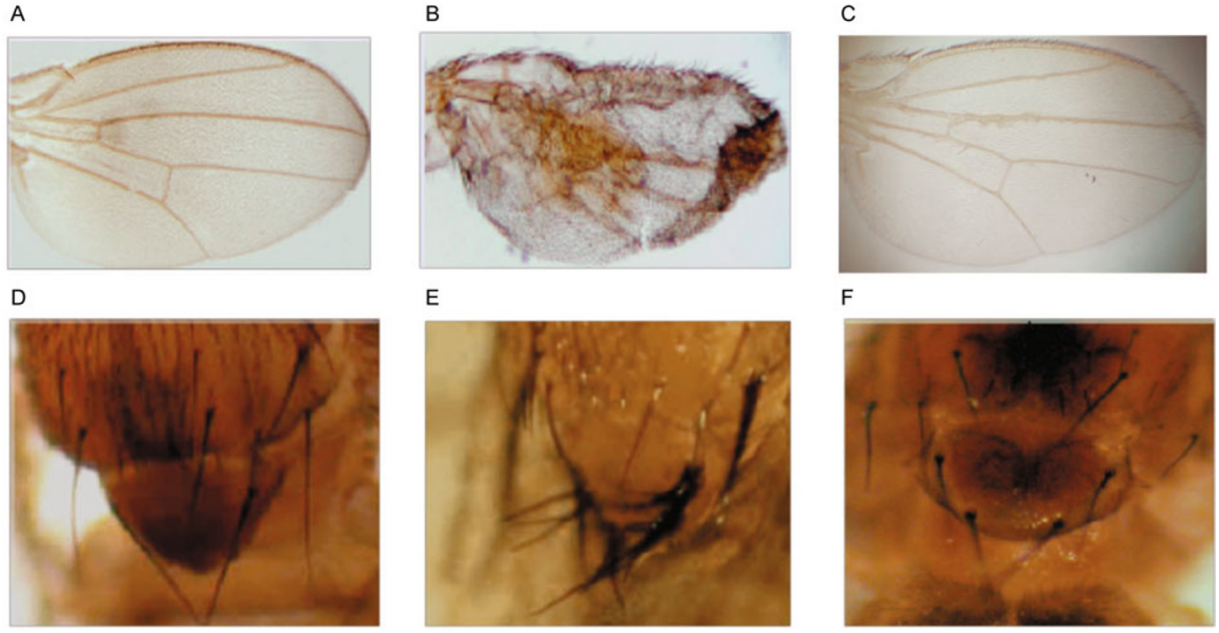

Figure 4. Rescue of *Psn* + TSA + PAG phenotypes with activated Notch abruptex (N^{Abx}). (A) The wild type wing. (B) *MS1096*-GAL4 > *Psn*-UAS + PAG-UAS + TSA-UAS phenotype with severe L3 and L5 wing-vein thickening. (C) N^{Abx} + *MS1096*-GAL4 > *Psn*-UAS + PAG-UAS + TSA-UAS shows partial rescue of the wing-phenotype with minimal L3 thickening and some L5 thickening. (D) The wild type pattern of macrochaetae on the scutellum. (E) Macrochaetae phenotype observed in the *MS1096*-GAL4 > *Psn*-UAS + PAG-UAS + TSA-UAS progeny. (F) Rescue of the phenotype with wild type pattern of macrochaetae in the N^{Abx} + *MS1096*-GAL4 > *Psn*-UAS + PAG-UAS + TSA-UAS progeny.

expected that this allele might rescue the vein and bristle pattern due to expression of *Psn* with PAG and TSA. Indeed, we found that flies expressing *Psn*, TSA and PAG in an N^{Abx} background had only residual thickening of the L3 and L5 wing veins (Fig. 4C, compared to wild type in 4A) which was significantly less severe than that observed in response to flies expressing *Psn* + TSA + PAG (Fig. 4B). In addition, the ectopic scutellar macrochaetae of flies co-expressing *Psn*, TSA and PAG (Fig. 4E) was nearly completely suppressed by N^{Abx} (Fig. 4F, compared to wild type in 4D). These data support the hypothesis that TSA and PAG act by aggravating the Notch inhibitory effect of *Psn* overexpression.

DISCUSSION

The underlying genetic heterogeneity of Alzheimer's disease has made understanding the pathogenesis of the disease difficult without the aid of model genetic organisms. It is clear that plaque formation and oxidative damage are key pathogenetic mechanisms in AD (Smith et al., 2000). The presence of early oxidative stress in mice with Presenilin mutations lends further support for the idea that plaque formation and oxidative damage are connected in AD (Gu et al., 2008). However, how these two pathogenetic processes are linked is not known. We have used *Drosophila* as a model system to explore a protein-protein interaction between presenilin and two peroxiredoxin family members TSA and PAG, which may provide a link between oxidative stress and plaque formation in AD.

There is genetic and biochemical evidence that peroxiredoxin family members play a role in the pathogenesis of AD. There are six peroxiredoxin family members in humans and the evidence for their involvement in AD is summarized in Table 1. Of these genes PRDX1 (PAG) was implicated in physical interaction with human Presenilin-1 and is primarily expressed in glia (Zhou et al., 2002; Hattori and Oikawa, 2007). PRDX2 (TSA) is globally expressed in the brain and has increased expression in the frontal cortex of patients with AD (Krapfenbauer et al., 2003) and is implicated in Parkinson's disease and amyotrophic lateral sclerosis (Boulos et al., 2007). Another family member PRDX6 has been more directly implicated in AD by linkage studies (Liu et al., 2007; Butler et al., 2009). The PRDX6 protein displays markedly increased expression in glia surrounding amyloid plaques in AD brain (Power et al., 2008). Overall these studies support a role for some of these family members in AD pathogenesis. Furthermore, they demonstrate an increase in the expression of PRDX family members in AD brain, an effect which our data suggest could impact the function of Presenilin. These observations suggest our study models a clinically relevant pathogenetic mechanism.

One advantage of the *Drosophila* wing as an assay system

Table 1 Genetic and biochemical evidence for a role for PRDX loci in Alzheimer's disease or other human neurodegenerative diseases

Gene name	Locus	Protein subcellular localization	CNS expression	Implication in neurodegenerative disease	Reference(s)
PRDX1	1p34.1	cytoplasm, melanosome, mitochondrion, nucleus	Glial not neuronal	Neuronal cell death in culture with high expression, rescued by Presenilin-1 co-expression	Zhou et al., 2002; Hattori et al., 2007
PRDX2	19p13.2	Cytoplasm, mitochondria	Global	Increased protein expression in the frontal cortex of patients with Alzheimer's and Parkinson's disease. Protects neurons in culture from ischemic and oxidative injury. Inactivation by S-nitrosylation promotes cell death in Parkinson's disease Loss of expression in motor neurons in ALS mouse models	Krapfenbauer et al., 2003; Boulos et al., 2007; Fang et al., 2007; Kato et al., 2005
PRDX3	10q26.1	Cytoplasm, early endosome, mitochondria	Neuronal	Reduced expression in the frontal cortex of patients with Parkinson's disease	Krapfenbauer et al., 2003
PRDX4	Xp22.11	Cytoplasm, mitochondria, extracellular space	Neuronal	Not implicated	N/A
PRDX5	11q13	Cytoplasm, mitochondria, peroxisome	Neuronal	Not implicated	N/A
PRDX6	1q25.1	Cytoplasm, lysosome	Glial not neuronal	Meta-analysis of linkage studies and individual linkage studies with significant LOD scores at 1q25, Marked increase in protein expression in glia surrounding neuritic plaques in AD brain	Butler et al., 2009; Liu et al., 2007; Power et al., 2008

is the ability to make use of a wealth of knowledge and an array of genetic tools for analyzing wing development. For example, in this study we observed phenotypes with overexpression of *Psn* which recapitulated Notch loss-of-function, and we observed a substantial increase in the penetrance and severity of these phenotypes when TSA and PAG were co-expressed with Psn. This led to the hypothesis that TSA and PAG enhance the dominant negative effect of overexpressed *Psn*. We were able to test whether this enhancement was mediated by the Notch pathway by suppressing this effect with an activated allele of Notch (Fig. 4). Since *Psn* acts in conjunction with three other components that comprise γ-secretase, it is likely that overexpression of *Psn* acts in a dominant negative fashion to inhibit Notch signaling by altering the normal stoichiometry of γ-secretase subunits (Struhl and Greenwald, 1999). Our results suggest that association of *Psn* with TSA or PAG aggravates this effect of *Psn* overexpression. Interestingly, this seemed to occur with the human peroxiredoxin isoforms in the presence of the *Drosophila Psn* transgene. While the portion of the PSEN1 protein which interacts with TSA and PAG is only partially conserved with *Psn*, our observations suggest a functionally significant effect across species. Furthermore, we saw an exacerbation of phenotype in response to overexpression of peroxiredoxin antioxidant enzymes, conditions that one might expect to provide oxidative protection. One possible explanation for these findings is that by associating with *Psn*, TSA and PAG further reduce the complete intact γ-secretase complex. Alternatively, Notch inactivation could be a result of either degradation or abnormal processing of the endogenous presenilin in response to the expression of *Psn* along with the mammalian transgenes. Either possibility could have obvious disease relevance, as it seems clear that increased expression of these proteins occurs in AD brain (Krapfenbauer et al., 2003; Hattori and Oikawa, 2007), and our data suggest that such an increase in expression could have an impact on Presenilin function and therefore indirectly could influence plaque formation.

While our results support a role for peroxiredoxins PAG and TSA in modifying the Notch inactivation phenotype caused by *Psn* overexpression, we cannot infer a direct role for these proteins in the processing of amyloid precursor protein, or in the pathogenesis of AD. Loss-of-function studies are needed to determine the mechanism of peroxiredoxin function and whether there is a clear role for endogenous peroxiredoxin in Notch signaling or amyloid cleavage. Further experiments in mammalian cells will be needed to determine whether peroxiredoxin family members are indeed involved in amyloid processing. Nonetheless, we have demonstrated that *Drosophila* provides an effective system for testing the *in vivo* relevance of AD related protein-protein interactions. As our wing assay for Psn activity has proven robust in validating a

suspected protein-protein interaction with Presenilin, it should be amenable to screening for new unknown functionally interacting partners of *Psn*.

MATERIALS AND METHODS

Drosophila stocks and transgenic constructs

The Presenilin transgene was provided by Mark Fortini (Ye and Fortini, 1999). Human TSA and PAG were subcloned into the pUAST vector and injected into w-embryos according to standard procedures (Rubin and Spradling, 1982). Injected larvae were grown and crossed to w- flies with w + selection in the next generation. PAG-UAS insertions were obtained on chromosomes 2 and X, while TSA-UAS insertions were obtained on chromosomes 3 and X. The following stocks were then generated using recombination (1) w-; +/+; *Psn*-UAS + TSA-UAS. (2) w-; PAG-UAS/CyO; *Psn*-UAS + TSA-UAS. (3) w-; PAG-UAS/CyO; TSA-UAS. (4) w-; PAG-UAS/CyO; *Psn*-UAS. (5) Notch Abruptex + *MS1096*-GAL4; +/+; +/+. (6) *MS1096*-GAL4 + TSA-UAS; +/+; +/+. (7) *MS1096*-GAL4 + PAG-UAS; +/+; +/+.

Fly mounting

Adult flies were anesthetized under carbon dioxide and selected for genotype if necessary and stored in isopropanol. Images of Macrochaetae, Sternopleural bristles, and anterior eye bristles were taken on fresh samples in isopropanol. Adult wings were dissected from the thorax and mounted on microscopic slides in Canada balsam. Fly wings were imaged with Nomarski optics.

Statistical analysis

The penetrance analysis was performed on a series of individual crosses using the *71B*-GAL 4 and the transgene stocks. The crosses were performed simultaneously under identical conditions at 29°C. Penetrance was defined based on phenotypic findings such as ectopic campaniform sensilla, ectopic double row bristles, and ectopic sternopleural bristles. Statistical analysis was performed using Graphpad Prism Software.

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ABBREVIATIONS

AD, Alzheimer's disease; PAG, proliferation associated gene (PRDX1); PRDX, Peroxiredoxin; PSEN1, Human Presenilin-1; *Psn*, *Drosophila* Presenilin; TSA, Thiol-specific antioxidant (PRDX2); UAS, upstream activator sequence

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