Mitochondrial protein Prel-like is required for development of dendritic arbors and prevents their regression in the *Drosophila* sensory nervous system

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Dynamic morphological changes in mitochondria depend on the balance of fusion and fission in various eukaryotes, and are crucial for mitochondrial activity. Mitochondrial dysfunction has emerged as a common theme that underlies numerous neurological disorders, including neurodegeneration. However, how this abnormal mitochondrial activity leads to neurodegenerative disorders is still largely unknown. Here, we show that the *Drosophila* mitochondrial protein Prel-like (Prel), a member of the conserved PRELI/MSF1 family, contributes to the integrity of mitochondrial structures, the activity of respiratory chain complex IV and the cellular ATP level. When Prel function was impaired in neurons in vivo, the cellular ATP level decreased and mitochondria became fragmented and sparsely distributed in dendrites and axons. Notably, the dendritic arbors were simplified and downsized, probably as a result of breakage of proximal dendrites and progressive retraction of terminal branches. By contrast, abrogation of the mitochondria transport machinery per se had a much less profound effect on the arbor morphogenesis. Interestingly, overexpression of Drobl-1 (Debcl), a *Drosophila* Bax-like Bcl-2 family protein, in the wild-type background produced dendrite phenotypes that were reminiscent of the *prel* phenotype. Moreover, expression of the Drobl-1 antagonist Buffy in *prel* mutant neurons substantially restored the dendritic phenotype. Our observations suggest that Prel-dependent regulation of mitochondrial activity is important for both growth and prevention of breakage of dendritic branches.

**KEY WORDS:** *Drosophila*, Prel, Dendrite, Mitochondria, Neurodegeneration

**INTRODUCTION**

Mitochondria are important for multiple cellular events such as ATP production, Ca²⁺ regulation, axonal and dendritic transport of organelles, and the release and re-uptake of neurotransmitters at synapses (Detmer and Chan, 2007). Mitochondria in most healthy cells exist as tubules of variable size and undergo dynamic morphological changes that depend on the balance of fusion and fission. This fusion-fission cycle ensures mixing of metabolites and mitochondrial DNA and influences organelle shape and bioenergetics functionality (Chan, 2006b; Okamoto and Shaw, 2005). The dynamin-related GTPases have been shown to have central roles in the fusion-fission dynamics of mammalian mitochondria. The mitofusins (MFNs) are proteins localized at the mitochondrial outer membrane that are required for the fusion of mitochondria (Santel, 2006), whereas OPA1 in the inner membrane mediates the fusion (Olichon et al., 2006). The key component of the fusion machinery is dynamin-related protein 1 (Drp1) (Labrousse et al., 1999; Smirnova et al., 2001).

Dysfunction of mitochondria is highly connected to neurodegenerative diseases, and abrogation of the fusion machinery is an early and causal event in neurodegeneration (Chan, 2006a; Knott et al., 2008; Lin and Beal, 2006). Mutations in MFN2 cause the autosomal dominant disease Charcot-Marie-Tooth (CMT) type 2A, a peripheral neuropathy of long motor and sensory neurons; and Purkinje neurons in the mouse model have aberrant mitochondrial distribution, ultrastructure and electron transport activity (Chen et al., 2007; Santel, 2006; Zuchner et al., 2004). Mutations in OPA1 cause autosomal dominant optic atrophy (ADOA), the most commonly inherited form of optic nerve degeneration (Alexander et al., 2000; Delettre et al., 2000; Olichon et al., 2006). However, it is still largely unknown as to how the abnormal mitochondrial morphology leads to neurodegenerative disorders.

Other consequences of impaired mitochondrial fusion-fission dynamics in the nervous system model have also been studied. In *Drosophila*, observation of *opa1* and *drp1* mutants has revealed impaired mitochondrial fusion-fission dynamics in the nervous system. An eye-specific homozygous mutation of *opa1* causes rough and glossy eye phenotypes in adult flies, suggesting that an increase in apoptosis is occurring (Yarosh et al., 2008). Mutations in *drp1* result in elongated mitochondria that are mostly absent from the presynapses (Verstreken et al., 2005). It has been reported that dendritic mitochondria are more metabolically active than axonal mitochondria (Overly et al., 1996) and that the dendritic distribution of mitochondria and their activity are essential and limiting for the development and morphological plasticity of dendritic spines in cultured hippocampal neurons (Li et al., 2004). In *Drosophila* loss of mitochondrial complex II activity causes degeneration of photoreceptors and disruption of mitochondrial protein translation severely affects the maintenance of terminal arborization of dendrites (Chihara et al., 2007; Mast et al., 2008). Nevertheless, it is not yet well understood how proper mitochondrial morphology, distribution and activity contribute to the formation and maintenance of dendritic arbors.
Here, we addressed this question by using Drosophila dendritic arborization (da) neurons. Individually identified da neurons are classified into classes I-IV in order of increasing field size and arbor complexity, and they produce dendritic arbors of stereotypic patterns in a two-dimensional manner between the epidermis and muscles (Grueber et al., 2002; Orgozo and Grueber, 2005; Sugimura et al., 2003).

Here, we show that Prel (protein of relevant evolutionary and lymphoid interest)-like (Prel), a Drosophila mitochondrial protein of the conserved PRELI/MSF1 family (Dee and Moffat, 2005), contributes to the integrity of mitochondrial structure and activity, and to the morphogenesis of dendritic arbors. Mutant prel class IV neurons simplified and downsized their dendritic arbors, and showed breakages of their major branches without detectable signs of apoptosis. Furthermore, we observed genetic interactions between Prel and the Drosophila Bax-like Bel-2 family proteins Drob-1 (also known as Debel) and Buffy (Colussi et al., 2000; Igaki et al., 2000; Quinn et al., 2003). All of these observations suggest that Prel-dependent control of mitochondrial activity has a pivotal role in the development and maintenance of dendritic arbors.

MATERIALS AND METHODS

Drosophila strains

We used the Gal4-UAS system (Brand and Perrimon, 1993) to express transgenes and to visualize da neurons. Gal4 lines used were Gal4UBI75 (Hayashi et al., 2002), Gal4∈ (Ainsley et al., 2003), Gal4109(2)80 (Gao et al., 1999), Gal80-2-21 (Grueber et al., 2003) and Gal80-in(c155). UAS marker fly stocks were provided from the Bloomington Stock Center or the Drosophila Genetic Resource Center at the Kyoto Institute of Technology. UAS-drob-1/debdcl/dbrg-1/dBok (Senou-Matsuda et al., 2005), UAS-drone [DN] (Quinn et al., 2000), UAS-p35 (Yoo et al., 2002) and UAS-Buffy/Drob2 (Quinn et al., 2003) were gifts from M. Miura (University of Tokyo, Japan). The milt mutant strain was milt82/CyO (Stowers et al., 2002).

GS screening and isolation of prel mutant

To identify genes causing abnormal dendritic patterns, we adopted the P-element-based gene search (GS) system (Toba et al., 1999). For the screening, female flies that expressed Gal4∈ UAS-mCD8::GFP were crossed to the male flies of individual GS lines to induce overexpression of GS-vector flanking sequences in F1 progeny. Out of 3000 GS lines screened, we found that 47 lines showed abnormal dendrite phenotypes; analysis of GS-vector flanking sequences in F1 progeny. Out of 3000 GS lines screened, we focused on one of the GS lines, prel3758 (Quinn et al., 2003) were gifts from M. Miura (University of Tokyo, Japan).

Molecular cloning

To identify prel mutations, we isolated genomic DNA from the wild-type flies (+/+) or heterozygous flies of 56 jump-out stocks that were homozygous lethal. Then, we identified the 1452 bp deletion including the prel ORF region by using genomic PCR and sequencing (Fig. 3A). Both prel and opa1 cDNA were synthesized from embryos (5-20 hours AEL) by using Ready-To-Go RT-PCR Beads (Amerham Pharmacia) and primers that were designed on the basis of the wild-type gene sequences in FlyBase (http://flybase.bio.indiana.edu). To construct pUAST-mito::tdTomato, we assembled the cDNA fragment of human cytochrome c oxidase subunit 8A gene of pCAG-mito::VENUS (Okita et al., 2004), tdTomato of pRTSET-tdTomato (Shaner et al., 2004) and pUAST (Brand and Perrimon, 1993). For the construction of prel-GAL4, the 2-kbp upstream sequence of the prel coding region was inserted into pPTGAL4 (Sharma et al., 2002), pUAST-HA::Drob1 (Senou-Matsuda et al., 2005) was a gift from M. Miura, and pUAST-TMyc::Buffy/Drob2 was donated by T. Igaki (Kobe University, Japan). dsRNAs for the dynamin-related domain of opa1, full-length of prel and EGFP were synthesized by using a MEGAscript kit (Ambion).

MARCM analysis, image acquisition and quantification of dendritic trees

MARCM analysis and time-lapse recordings were performed basically as described previously (Lee and Luo, 1999; Satoh et al., 2008). To acquire images of da neurons in adults, we removed the heads, wings and legs of adult flies and mounted the abdomens in 90% glycerol. All of the images of da neurons, except for those of immunostaining of Fig. S2 and Fig. S3E-S3G in the supplementary material, were acquired from whole-mounted live larva or from the adult abdomens by using a Zeiss LSM 510 META laser-scanning confocal microscopy system. For quantification of dendritic patterns of da neurons, we used Neurobyte software (Kurabo). MetaMorph software (Molecular Devices) was used to estimate the amount of fluorescent mito::GFP signals (Satoh et al., 2008). Mitochondrial signal index is indicated as mitochondrial signal (pixel) divided by the total length of neuronal processes (μm).

Antibodies, RNAi, subcellular fractionation, immunoblotting and assays of ATP levels and enzymatic activities

GST fusion proteins of Drosophila Prel and Opa1 were expressed and used for generating rat antibodies. For RNAi experiments S2 cells were cultured with 20 μg/ml dsRNA of the full-length prel or GFP-coding sequence. For DNA transfection we used Nucleofector R and its reagent (Amauxo). The mitochondrial fraction of S2 cells was prepared by using a Qproteome Mitochondria Isolation Kit (Qiagen). For immunoblotting, we used anti-HA antibody 16B12 (Covance), anti-α-tubulin antibody DM1A Ab (Sigma) and anti-Complex-V α-subunit antibody 15H4C4 (Invitrogen). The basic methods for assays of ATP levels and enzymatic activities were described previously (Senoo-Matsuda et al., 2005; Trounce et al., 1996).

Light and electron microscopy

S2 cells were seeded into concanavalin-A-coated glass-bottomed culture dishes, incubated with Mitotracker Orange (Invitrogen), and then fixed for immunostaining. Mitochondrial membrane potential was analyzed by using tetramethylrhodamine methyl ester (TMRM; Invitrogen). For EM, fixed S2 cells were embedded in Epon812 (Nacalai Tesque) to prepare ultrathin sections. For immuno-EM of S2 cells, prepared ultrathin sections were exposed to mouse anti-HA antibody 16B12 (Covance). After washing with PBS, the grids were incubated with anti-IgG antibody that had been conjugated with 18 nm gold particles (Jackson ImmunoResearch) and stained with uranyl acetate and lead citrate. All of the EM specimens were examined with an H-7650 transmission electron microscope (Hitachi) operating at 80 kV. Detailed information of all of the methods will be promptly provided upon request.

RESULTS

Prel is a Drosophila mitochondrial protein of the Prel/MSF1 family and required for shaping filamentous mitochondria in S2 cells

We conducted a screening by using the Gene Search (GS) system to hunt for genes that control complex morphology of dendritic arbors of the class IV neuron at larval stages. One of the aims of this system is to drive overexpression or misexpression of genes neighboring the GS-vector insertion site (Toba et al., 1999). Out of 3000 GS lines screened, we focused on one of the GS lines, G59160, and its candidate gene, prel-like (prel), which is conserved throughout eukaryotes. The predicted product of prel is 236 amino acids in length and a member of the PRELI/MSF1 family in Drosophila (Dee and Moffat, 2005). The closest human homolog is PRELI; and its amino acid sequence shows 46% identity to that of Preli-like through the entire length. In yeast, Ups1p, a member of this family, is localized in mitochondria and regulates their shape (Wennberg et al., 2006). To confirm whether the Prel protein is localized to mitochondria in Drosophila cells, we fractionated lysates of Drosophila Schneider 2 (S2) cells and showed that both endogenous and exogenously expressed Prel proteins were predominantly...
Mitochondrial protein Prel suppresses degeneration

Fig. 1. Prel is a mitochondrial protein and its knockdown affects mitochondrial morphology of S2 cells. (A) Western blot analysis of Prel in S2 cells. Individual proteins were detected by the antibodies listed on the right. α-tubulin and CV-α, a component of mitochondrial F1F0 ATP synthase, are loading controls for cytoplasmic and mitochondrial proteins, respectively. Endogenous Prel and exogenously expressed Prel::3HA are indicated by the arrow and arrowhead, respectively. Cytoplasmic fraction: mito, mitochondrion-enriched fraction. (B–N) Light micrographs of S2 cells (B–D,F–I,L) and ultrastructure of mitochondria in S2 cells (E,G,H,M,N). In the light micrographs (except for C and J), mitochondria were visualized by using Mitotracker Orange. (F–H) EGFP dsRNA-treated S2 cells as a control. (B–D,I–K) Prel::3HA expressing S2 cells were stained with Mitotracker Orange (B,J) and for HA (C,J). The expression level was lower in B–D than in I–K. Signal intensity of Prel::3HA in I–K was five times higher than in B–D. (E) Localization of Prel::3HA in mitochondria is shown by immunoelectron microscopy (yellow arrows). (I–N) Prel-knockdown cells. Scale bars: 10 μm in B–D,F–I,L; 200 nm in E,G,H,M,N. (O,P) Quantification of mitochondrial morphology in EGFP dsRNA-treated cells as a control, in prel- or opa1-knockdown cells, and in Prel::3HA-expressing cells. Evaluation was made on the basis of light micrographs of S2 cells (O) and ultrastructure of mitochondria in S2 cells (P). (P) Mitochondria that have an abnormally expanded matrix (M) and/or only a few thin cristae (N) were defined as disorganized mitochondria. Data are presented as mean ± s.d. of three independent sets of experiments. **P<0.001 (one-way analysis of variance between groups (ANOVA) with Tukey’s HSD post-hoc analysis).

Both prel knockdown and its exogenous expression reduce mitochondrial activity in S2 cells

It is well known that the mitochondrial oxidative phosphorylation activity in cultured mammalian cells reflects mitochondrial shape and generates an electrochemical proton gradient across the mitochondrial inner membrane (Yaffe, 1999). To study whether prel knockdown affected mitochondrial activity in live S2 cells, we used tetramethylrosamine methyl ester (TMRM) to detect the mitochondrial membrane potential. Quantitative comparisons of the mitochondrial membrane potential showed that knockdown significantly reduced this potential when compared with the potential of control healthy cells (Fig. 2A). We also analyzed the ATP level as an indicator of mitochondrial metabolism and showed that knockdown of either prel or opa1 and overexpression of prel reduced the ATP level (Fig. 2B), although the effects were less severe than the acute one caused by treating the cells with hydrogen peroxide or the protonophore carbonyl cyanide m-chlorophenylhydrazone (CCCP) (Fig. 2A,B). We also measured mitochondrial respiratory chain enzyme activities in prel-knockdown cells. The enzymatic activity of neither rotenone-sensitive NADH-cytochrome c oxidoreductase (an indicator of complex I and II) nor antimycin-A-sensitive succinate-cytochrome c oxidoreductase (complex II and III) was affected, whereas KCN-sensitive cytochrome c oxidase activity (complex IV) was decreased in prel-knockdown cells (Fig. 2C–E). All of these results suggest that prel knockdown or its expression beyond the physiological level not only had deleterious effects on the morphology but also on the activity of the mitochondria.

detected in the mitochondria-enriched fraction but hardly found in the cytoplasm (Fig. 1A). Under the light microscope, the expressed Prel was mostly colocalized with the mitochondrial marker Mitotracker Orange (Fig. 1B–D), and it appeared to be associated with the cristae when observed by immunoelectron microscopy (Fig. 1E).

By both light and transmission electron microscopy, we examined the role of Prel in shaping mitochondria in S2 cells. More than 80% of the control S2 cells had a filamentous mitochondrial network (Fig. 1F,O), and parallel, accordion-like folds of cristae structures were observed in each mitochondrion (Fig. 1G,H,P). Both knockdown of prel and its exogenous expression caused significant fragmentation of mitochondria (Fig. 1I–L,O). At the ultrastructural level, prel-knockdown cells had many mitochondria with lower electron density. Those mitochondria took on a round shape, having an abnormally expanded matrix (Fig. 1M) and only a few thin cristae (Fig. 1N). It is known that OPA1, one of the mitochondrial dynamin-related GTPases, is important for both fusion of inner membranes and maintenance of the structure of cristae (Chan, 2006b; Cipolat et al., 2006; Frezza et al., 2006; Meeusen et al., 2006; Okamoto and Shaw, 2005). We showed that knockdown of opa1 in S2 cells affected the mitochondrial structure in a very similar manner to that found with knockdown of prel (Fig. 1O,P; see Fig. S1 in the supplementary material).
showed that mito::GFP signals were more numerous in axons than in control class IV neurons (Fig. 3B,C,F,G); and our quantification S4E-G in the supplementary material, were acquired from whole-da neurons, except for those of immunostaining of Fig. S2 and Fig. membrane-bound fluorescent proteins. All of the images of larval mitochondria in da neurons by expressing fluorescent proteins that (Lee and Luo, 1999). Throughout this study, we visualized larvae by using a mosaic analysis in this and subsequent experiments.

Both prel loss-of-function and its overexpression cause mislocalization and fragmentation of mitochondria in class IV neurons

prel mRNA was found to be ubiquitously distributed in the embryo till stage 17 (data not shown). We observed that prel was expressed in class IV da neurons by using two Gal4 lines that were expected to trap an enhancer region of prel (see Fig. S2A-F in the supplementary material). To study phenotypes of the loss of prel function, we remobilized the GS vector of GS14515 and isolated a deletion (designated prel1 or simply prel hereafter) that almost totally lacked the open reading frame (Fig. 3A). prel1 homozygous mutants were lethal at early larval stages before their dendritic patterns became mature, and this lethality was recovered by broad deletion mutant. White boxes represent untranslated regions (UTRs), and cross-hatched boxes are protein-coding regions. NP0738, GS9160 and GS14515 strains have P-element insertions in the 5' UTR. Genomic organization of the prel allele was characterized by PCR using a pair of primers (arrows). Amplified fragments of yw (+/+) and heterozygous prel1/+ of adult flies are shown. (B-I) Distribution of mitochondria in ddaC neurons. Mitochondria were visualized with mito::GFP (B,D,F,H; green in C,E,G,I) and neuronal plasma membranes were labeled with myr::mRFP (magenta in C,E,G,I). Insets in B,D,F,H). High-magnification images of cell bodies. The yellow arrow in H indicates a ddaC neuron. (B-E) Clones of the wild-type (B,C) and prel (D,E). (F-I) Control ddaC (F,G) and ddaC-overexpressing Prel::3HA (H,I). Tracings of axons (blue) and dendritic arbors (magenta) of representative wild-type (C) and prel (E) clones are shown to the right of the merged images. All images were taken at the third-instar larval stage; dorsal is to the top and anterior is to the left. Detailed genotypes of the animals and clones imaged in all figures can be provided upon request. Scale bars: 100 μm in B,D,F,H; 10 μm in insets of B,D,F,H. (J-M) Quantification of mitochondrial signals in both dendrites (J,L) and axons (K,M). (J,K) Clones of the wild-type (WT) and prel (prel). (L,M) Control ddaC (control) and ddaC-overexpressing Prel::3HA (UAS-prel). In this and all subsequent graphs, numbers of neurons analyzed are indicated above individual bars, unless described otherwise. Data are presented as mean ± s.d. ***P<0.001 for each value compared with control neurons by Student’s t-test.

**Fig. 2. Prel affects mitochondrial activity in S2 cells.**

(A) Quantitative comparisons of mitochondrial membrane potential. The membrane potential is indicated by the TMRE intensity. The fluorescence intensity of 100 cells was measured under each condition. (B) Quantitative comparisons of the cellular ATP content. Cells were treated for 5 days with dsRNA of either EGFP, prel or opa1, for 30 minutes with hydrogen peroxide or for 4 hours with carbonyl cyanide m-chlorophenylhydrazone (CCCP), a protonophore. (C-E) Mitochondrial respiratory chain enzymatic activities. (C) Complex I+III activity (rotenone-sensitive NADH-cytochrome c oxidoreductase). (D) Complex II+III activity (antimycin A-sensitive succinate-cytochrome c oxidoreductase). (E) Complex IV activity (KCN-sensitive cytochrome c oxidase). The data are presented as mean ± s.d. of three independent experiments; ***P<0.001 (ANOVA with Tukey’s HSD post-hoc analysis).

**Fig. 3. Both prel loss-of-function and overexpression causes mislocalization and fragmentation of mitochondria in class IV neurons.** (A) The exon-intron structure of prel, P-element insertion sites and the prel deletion mutant. White boxes represent untranslated regions (UTRs), and cross-hatched boxes are protein-coding regions. NP0738, GS9160 and GS14515 strains have P-element insertions in the 5' UTR. Genomic organization of the prel allele was characterized by PCR using a pair of primers (arrows). Amplified fragments of yw (+/+) and heterozygous prel1/+ of adult flies are shown. (B-I) Distribution of mitochondria in ddaC neurons. Mitochondria were visualized with mito::GFP (B,D,F,H; green in C,E,G,I) and neuronal plasma membranes were labeled with myr::mRFP (magenta in C,E,G,I). Insets in B,D,F,H). High-magnification images of cell bodies. The yellow arrow in H indicates a ddaC neuron. (B-E) Clones of the wild-type (B,C) and prel (D,E). (F-I) Control ddaC (F,G) and ddaC-overexpressing Prel::3HA (H,I). Tracings of axons (blue) and dendritic arbors (magenta) of representative wild-type (C) and prel (E) clones are shown to the right of the merged images. All images were taken at the third-instar larval stage; dorsal is to the top and anterior is to the left. Detailed genotypes of the animals and clones imaged in all figures can be provided upon request. Scale bars: 100 μm in B,D,F,H; 10 μm in insets of B,D,F,H. (J-M) Quantification of mitochondrial signals in both dendrites (J,L) and axons (K,M). (J,K) Clones of the wild-type (WT) and prel (prel). (L,M) Control ddaC (control) and ddaC-overexpressing Prel::3HA (UAS-prel). In this and all subsequent graphs, numbers of neurons analyzed are indicated above individual bars, unless described otherwise. Data are presented as mean ± s.d. ***P<0.001 for each value compared with control neurons by Student’s t-test.
in dendrites by an order of magnitude (WT and control in Fig. 3J-M). The distribution of mitochondria in dendrites was not necessarily restricted to branching points. The density of the mitochondrial signals was significantly reduced in both axons and dendrites either in prel mutant neurons or in the wild-type background neurons overexpressing prel (Fig. 3D,E,H,I; prel and UAS-prel in Fig. 3J-M). We found a filamentous mitochondrial network in cell bodies of the control neurons (insets of Fig. 3B,F); by contrast, both prel loss-of-function and prel overexpression appeared to cause fragmentation of mitochondria (insets of Fig. 3D,H). All of these results suggest that prel was necessary for proper mitochondrial distribution in neuronal processes and the tubular morphology in class IV da neurons.

**Dysfunction of prel causes simplification, downsizing and local breakage of dendritic arbors of class IV neurons**

Loss of prel function and its overexpression affected not only the subcellular distribution and shape of mitochondria in da neurons, but also the complex dendrite morphogenesis of class IV neurons. In contrast to the complicated and expansive dendritic arbors of the control neurons (Fig. 4A), those of the prel mutant neurons exhibited shorter, smaller, much less-branched dendritic arbors (Fig. 4B,C). A closer look at the distal regions of the mutant dendritic arbors showed that the terminal branches tended to be shorter than those of the wild-type neurons, making the mutant distal dendrites appear fuzzy. Strikingly, arbors of 9 out of 21 prel neurons had been locally destroyed, as judged by the presence of detached branches nearby (Fig. 4C and 4C′; see also Fig. S3A-B′ in the supplementary material). To examine whether there was a hot spot for this destruction, we plotted the spatial distribution of breakpoints in the mutant arbors (Fig. S3C in the supplementary material). The wild-type class IV neuron ddaC develops its receptive field with a radius of about 400 μm, whereas the radius of the mutant neuron was reduced to about 300 μm. Of the 55 breakage points examined, 22 were located within a 100 μm radius; and 51 out of 55 were within a 200 μm radius (see Fig. S3C in the supplementary material). This quantification showed that prel loss-of-function caused branch breakages more in the proximal area of the arbor than in the distal one. In contrast to this arbor destruction, we observed no axonal breakage, at least not close to the cell body, in any of the 21 mutant neurons observed.

These phenotypes of the mutant neurons were indeed due to loss of prel function, as shown by the fact that they were rescued to normal when a prel transgene was expressed (Fig. 4D). Overexpression of prel in the wild-type neurons also simplified and downsized their dendritic arbors (Fig. 4E). All of the above phenotypes were shown to be statistically significant by our quantification (Fig. 4F,G). It should be noted that we found no apoptotic signs in the prel mutant class IV neurons at the stage examined (see Fig. S4 in the supplementary material), making the possibility that the dendritic phenotype was a secondary consequence in dying cells less likely. In contrast to the effects on dendritic arbors of class IV neurons, those of class I-III appeared to be hardly affected by loss of prel function or prel overexpression (see Fig. S5 in the supplementary material).

**The dendritic phenotype of prel mutant neurons is much more severe than that of milt mutant neurons**

As described above, the prel mutant class IV neurons showed two classes of mitochondrial phenotypes: a reduction in mitochondrial signals in both dendrites and axons; and fragmentation in the cell body, which presumably decreased mitochondrial activity. We attempted to clarify cause-and-effect relationships between each of these mitochondrial phenotypes and the malformation of the dendritic arbor. To understand effects of the localization on dendrite morphogenesis, we examined how dendritic morphology was affected when the transport of mitochondria was blocked by using a loss-of-function mutation of milton (milt). Milt is an adaptor protein between mitochondria and kinesin and is required for mitochondrial transport in photoreceptor axons (Glater et al., 2006; Stowers et al., 2002) and in oocytes (Cox and Spradling, 2006). Milt was expressed...
in da neurons in wild-type larvae (see Fig. S2G-I in the supplementary material). In milt mutant class IV neurons, mitochondria were hardly distributed in dendrites or axons, as we had expected (Fig. 5A,C,E,F). In contrast to this strong mislocalization phenotype, abnormalities of dendritic arbors of the milt mutant neurons were substantially milder than those of the prel neurons (Fig. 5B,D,G,H). These results imply that a reduction in mitochondrial signals in dendrites and axons might per se not lead to severe morphological abnormalities of dendritic arbors.

Dendritic phenotypes caused by overexpression of Drob-1, a Drosophila Bcl-2 family protein, are reminiscent of those of prel mutant neurons

We then explored the relationship between alterations of mitochondrial activity and dendrite morphogenesis. For this purpose, we investigated previously isolated genes that control mitochondrial membrane structure and activity, including a Drosophila protein of the Bcl-2 family, Drob-1 (also known as Debcl) (Colussi et al., 2000; Igaki et al., 2000). It is known that members of the mammalian Bcl-2 family control permeabilization of mitochondrial outer membrane in apoptotic cells and also are involved in mitochondrial respiration (Danial et al., 2003; Karbowski et al., 2006; Youle and Strasser, 2008). As described later, we found that overexpression of prel or drob-1 decreased ATP levels in neurons, suggesting impaired mitochondrial function. This overexpression of Drob-1 caused simplification and downsizing of dendritic arbors of class IV neurons and, in addition, breakage of proximal branches (Fig. 6A,E,F). Of the 20 neurons examined, arbors of four neurons had such breakages. The overexpression also resulted in a decrease in mitochondrial density in neuronal processes (Fig. 6C,D,G), all of which were similar to those of prel mutant neurons (Fig. 3D,E,H,I; Fig. 4B,C).

Expression of Drob-1 antagonist Buffy in prel mutant neurons restores their dendritic morphology

The above results suggest that prel dysfunction or drob-1 overexpression resulted in the malformation of dendritic arbors, primarily by way of reducing mitochondrial activity. It has been shown that Buffy, another fly Bcl-2 family protein, acts as a Drob-1 antagonist in photoreceptor cells (Quinn et al., 2003). Consistent with the antagonistic molecular function of Buffy, the drob-1-overexpression phenotype was suppressed by co-overexpressing buffy. Overexpression of buffy alone hardly affected dendrite morphogenesis or mitochondrial distribution (Fig. 6E-G; see Fig. S6 in the supplementary material). Based on these observations, we addressed whether expression of Buffy might suppress the assumed reduction in the mitochondrial activity of the prel mutant class IV neurons and consequently restore its dendrite phenotype. We found that the abnormal morphology of dendritic arbors of the prel neurons was remarkably recovered to normal when the buffy transgene was expressed (Fig. 6B), and there were no significant differences between dendritic arbors of the wild-type neurons and those of prel mutant neurons expressing buffy on the basis of either of the two parameters examined (Fig. 6E,F). All of these results suggest that the aberrant dendritic arbors of the prel neurons are mostly due to mitochondrial dysfunction.

Progressive regression of dendritic arbors when prel function is impaired in larval and adult neurons

Loss-of-function mutations in the genes controlling mitochondrial fusion, such as MFN2 and OPA1, are associated with neurodegenerative diseases (Chan, 2006a; Knott et al., 2008); however, it has not been reported that mutations of the human prel gene are linked to any of these diseases. To investigate whether dysfunction of prel in mitochondria elicits late-onset neurodegeneration, we observed class IV neurons in larvae and adults.

We performed time-lapse recordings of dendritic arbors of prel mutant class IV neurons from an early second-instar stage until the mature late third-instar stage (see Figs S7 and S8 in the supplementary material). In the wild-type neuron, the dendritic arbor elaborated terminal branches (see Fig. S7 in the supplementary material). By contrast, terminal branches of the mutant neurons were eliminated, and major branches became almost bald over this time period (see Fig. S8 in the supplementary material). This time-lapse analysis, together with the ‘local destruction’ phenotype, indicates that intact mitochondrial function prevented both of the two modes of dendrite regression: the breakage of proximal branches and the retraction of terminal branches.

Dramatic remodeling of dendritic arbors occurs in da neurons undergoing metamorphosis (Kuo et al., 2005; Williams and Truman, 2005). Two out of the three class IV neurons in each abdominal
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**DISCUSSION**

The molecular function of Preli

We showed that both *prel* loss-of-function and its overexpression abrogated mitochondrial structures and activity in S2 cells and also in *Drosophila* neurons. Apparently an appropriate expression level of Preli is required for controlling mitochondrial shape, structure of the cristae, the activity of respiratory chain complex IV and the cellular ATP level. What then is the exact molecular function of Preli?

The molecular function of the Preli family is largely unknown in multicellular organisms. It has been recently shown that Ups1p, the yeast homologue of Preli, regulates the level of cardiolipin (CL), a phospholipid of mitochondrial membranes (Osman et al., 2009; Tamura et al., 2009). CL is known to be located predominantly in the mitochondria and has diverse mitochondrial functions including stabilization of the respiratory chain supercomplex (Joshi et al., 2009). These reports imply that the reduction in the complex IV activity and the ATP level in the *prel*-knockdown cells could be attributed to the altered phospholipid composition. Further biochemical study is required to measure the complex IV activity and phospholipid composition in various genetic backgrounds including Buffy or Drob1 overexpression, which might help to find evidence for a molecular pathway that includes Preli and Buffy.

However, it has been also proposed that loss of Ups1p affects the function of the yeast OPA1 homolog Mgm1p, which is important for both fusion of inner membranes and maintenance of the structure of the cristae (Chan, 2006; Cipolat et al., 2006; Frezza et al., 2006; Meeusen et al., 2006; Okamoto and Shaw, 2005). Mgm1p is imported into mitochondria, and its function is regulated by proteolytic cleavage (Griparic et al., 2007; McQuibban et al., 2003; Song et al., 2007; Tamura et al., 2009). This import and cleavage pattern is altered, and the mitochondria fragmented, in the yeast *ups1p* mutant (Sesaki et al., 2006; Tamura et al., 2009). We showed that the knockdown of *Drosophila opa1* in S2 cells affected the structure and activity of mitochondria similarly to *prel* knockdown, suggesting that *Drosophila* Preli is also required for organizing the mitochondrial inner membrane structure in concert with *Drosophila* Opa1. However, we could not provide positive evidence for any functional linkage between these two molecules. Neither *prel* knockdown nor its overexpression strongly affected the cleavage pattern of endogenous Opa1 in S2 cells (data not shown). A better

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**Fig. 6. Expression of Drob-1, a *Drosophila* Bcl-2 family member, affects dendritic pattern formation of class IV neurons.**

(A–D) Dendritic arbors and mitochondrial distribution of representative class IV ddaC neurons of the wild-type overexpressing drob-1 (A, C, D) and the *prel* mutant clone overexpressing buffy (B). (A) The primary branch was severed in the proximal region of the arbor of the drob-1-overexpressed neuron shown (yellow arrow in A). The *prel* mutant phenotype could be rescued to normal by overexpression of buffy (B). The mitochondria were visualized with mito::GFP (C; green in D), and the neuronal plasma membrane was labeled with myr::mRFP (magenta in D). The yellow arrow in C indicates a ddaC neuron. Tracings of the axon (blue) and the dendritic arbor (magenta) of the drob-1-overexpressed neuron (D) are shown at the right of the merged image. Scale bars: 100 μm. (E, F) Quantification of total length of dendritic branches (E) and the number of terminal branches (F) of wild-type ddaC clones (WT), *prel* mutant clones (prel), *prel* mutant clones that overexpressed buffy (*prel + UAS-buffy*), *prel* overexpressing neurons (UAS-*prel*), neurons that coexpressed *prel* and buffy (UAS-*prel* + UAS-*buffy*), drob-1 overexpressing neurons (UAS-drob-1), neurons that coexpressed drob-1 and buffy (UAS-drob-1 + UAS-*buffy*), and buffy overexpressing neurons (UAS-*buffy*). (G) Quantification of mitochondrial signals in ddaC neurons of control ddaC neurons (Gal4pUAS-my::mRFP UAS-mito::GFP) (WT), and those of ddaC overexpressing either prel (UAS-prel), prel and buffy (UAS-prel + UAS-*buffy*), drob-1 (UAS-drob-1), drob-1 and Buffy (UAS-drob-1 + UAS-*buffy*) or buffy (UAS-*buffy*). Data are presented as mean ± s.d. **P<0.01 and ***P<0.001 (ANOVA with Tukey’s HSD post-hoc analysis).
Effects of mitochondrial dysfunction on different classes of neurons

Mitochondria are abundant in regions of intense energy consumption, such as muscles, sperm and neurons; and OPA1 expression is high in the retina, brain, testis, heart and skeletal muscle (Alexander et al., 2000). The CMT type 2A phenotype due to MFN2 mutations might reflect the extreme cell geometry, as shown by the fact that long peripheral nerves are particularly sensitive to perturbations produced by MFN2-mediated mitochondrial dysfunction (Santel, 2006). Class IV neurons, which develop expansive and complicated dendritic arbors, probably consume the highest amount of ATP; thus they are very vulnerable to a loss or reduction in the prel-dependent mitochondrial function. The differential Gal4 expression of the trap line and the cis-element fusion line suggests that Prel is expressed most strongly in class IV neurons among the four subclasses (see Fig. S2 in the supplementary material).

Abnormal mitochondrial distribution is not necessarily linked to the severe morphological defect of the dendritic arbor

We had speculated that the severe dendritic phenotype of the prel class IV neurons could be primarily due to the misdistribution of mitochondria. However, our attempts to correlate the mitochondrial localization and the dendritic phenotype suggested that such a view might be naive. Loss of function of milt dramatically reduced the mitochondrial density in neuronal processes; nevertheless, the dendritic phenotype of the milt mutant neuron was much less profound than that of the prel neurons. Similarly, milt mutant eyes are indistinguishable from the wild-type eyes in their external and photoreceptor morphology in spite of the paucity of mitochondria in photoreceptor axon terminals (Stowers et al., 2002). These observations imply the possibility that the visible misdistribution of mitochondria per se might not necessarily lead to the severe morphological abnormality of dendritic arbors or axons.

How can we interpret these observations? Overexpression of prel diminished the ATP level in vivo, where the local ATP concentration within the cell might fall below a threshold that is necessary for dendritic growth and maintenance. However, mitochondria that remain in the cell body of the milt neuron might maintain their ATP-producing activity, and at least a subpopulation of synthesized ATP molecules might diffuse a long distance to reach the distal region in the dendritic arbor. These hypotheses would be testable if the ATP level in the cell body and its diffusion inside dendritic branches could be visualized and measured quantitatively in various genetic backgrounds (Imamura et al., 2009).

Prel-dependent control of mitochondrial activity prevents regression of dendritic branches

Dysfunction of mitochondria correlates with neurodegenerative diseases and is an early and causal event in neurodegeneration (Chan, 2006a; Knott et al., 2008; Lin and Beal, 2006). The results of this study imply that the evolutionarily conserved Prel might prevent
neurodegeneration in other animal species, as discussed below. When Prel function was impaired, branches in the proximal region of the arbor were degraded, and terminal branches were eliminated at the mature larval stage; and in adult flies, overall arbor retracted. These phenotypes are reminiscent of degradation of neurite branches within and near amyloid deposits in the brain of a transgenic mouse model of Alzheimer disease, which is speculated to occur through mitochondrial dysfunction, oxidative stress and calcium deregulation (Tsai et al., 2004). The branch destruction of the prel mutant neuron could also be due to a ‘physical’ reason. Transport of various cargos might be impaired when ATP is limited, leading to a defect in mechanical strength of the membrane. Such fragile branches could be ruptured during larval locomotion.

It has been intensively studied whether the mutant proteins that are associated with hereditary neurodegenerative diseases affect mitochondrial function. Our study has provided cellular and genetic evidence that Prel is a novel target of such research. Future studies should be directed towards further characterization of the Prel protein by using both fly and vertebrate systems to clarify its function in mitochondria and its involvement in mechanisms that prevent the regression of dendritic arbors.

Acknowledgements

The antibodies and fly stocks were provided by the Developmental Studies Hybridoma Bank at the University of Iowa, the Bloomington Stock Center and the Drosophila Genetic Resource Center at Kyoto Institute of Technology. We thank Yuh-Nung Jan for communicating data before publication. We are also grateful to M. Miura, T. Igaki, L. Luo, Y. N. Jan, T. Schwarz, T. Schroeder, Y. Hiromi and A. Kadota for other fly strains and materials. We thank M. Miura, H. Chihara, M. Yamamoto, Y. Kozutsumi and A. Kawaguchi for their technical advice and/or discussion; S. Yonehara for use of the DNA sequencer and Multilabel Counter; and K. Shimizu and M. Futamata for their technical assistance. This work was supported by grants from the programs Grants-in-Aid for Scientific Research on Priority Areas Molecular Brain Science (17024025 to T.U.) and Systems Genomics (17017032 to T.A.). A.T. and T.T. are recipients of a fellowship of the Japan Society for the Promotion of Science.

Supplementary material

Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/136/22/3757/DC1

References


Mitochondrial protein Prel suppresses degeneration


