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Report

Normal Mitochondrial Dynamics Requires Rhomboid-7 and Affects *Drosophila* Lifespan and Neuronal Function

G. Angus McQuibban,[1,2,*] Jeffrey R. Lee,[2] Lei Zheng,[3,4] Mikko Juusola,[3,4] and Matthew Freeman[1,*]
[1] MRC Laboratory of Molecular Biology
Cambridge CB2 2QH
United Kingdom
[2] Department of Biochemistry
University of Toronto
Toronto M5S 1A8
Canada
[3] Physiological Laboratory
University of Cambridge
Cambridge CB2 3EG
United Kingdom

Summary

In addition to being energy generators, mitochondria control many cellular processes including apoptosis [1]. They are dynamic organelles, and the machinery of membrane fusion and fission is emerging as a key regulator of mitochondrial biology [2]. We have recently identified a novel and conserved mitochondrial rhomboid intramembrane protease that controls membrane fusion in *Saccharomyces cerevisiae* by processing the dynamin-like GTPase, Mgm1, thereby releasing it from the membrane [3]. The genetics of mitochondrial membrane dynamics has until now focused primarily on yeast [4]. Here we show that in *Drosophila*, the mitochondrial rhomboid (Rhomboid-7) is required for mitochondrial fusion during fly spermatogenesis and muscle maturation, both tissues with unusual mitochondrial dynamics. We also find that mutations in *Drosophila optic atrophy 1-like (Opa1-like),* the ortholog of yeast *mgm1*, display similar phenotypes, suggesting a shared role for Rhomboid-7 and Opa1-like, as with their yeast orthologs. Loss of human OPA1 leads to dominant optic atrophy, a mitochondrial disease leading to childhood onset blindness. *rhomboid-7* mutant flies have severe neurological defects, evidenced by compromised signaling across the first visual synapse, as well as light-induced neurodegeneration of photoreceptors that resembles the human disease. *rhomboid-7* mutant flies also have a greatly reduced lifespan.

Results and Discussion

Mitochondria form a tubular network in most eukaryotic cells, the result of a balanced set of opposing membrane fusion and fission reactions [5]. Disturbance of this balance disrupts mitochondrial architecture and function. Mitochondria are surrounded by double membranes and must maintain effective separation from the surrounding cytoplasm; there is considerable complexity in the mechanism underlying the membrane dynamics required for fusion and fission. In *Saccharomyces cerevisiae*, two dynamin-related large GTPases, Mgm1 [6, 7] and Fzo1 [8, 9], regulate the fusion reaction, although their precise functions remain unknown. It has recently become clear that Mgm1 is regulated by a two-stage proteolytic release from the inner mitochondrial membrane: first, the mitochondrial targeting sequence is removed by MPP (mitochondrial processing peptidase), a matrix-localized protease; second, the intramembrane serine protease activity of the mitochondrial rhomboid family member Rbd1/Pcp1 releases a soluble form of Mgm1 into the intermembrane space [3, 10–12]. Loss of Rbd1/Pcp1 leads to a phenotype similar to loss of Mgm1, implying that this intramembrane cleavage is necessary for full Mgm1 function.

Rbd1/Pcp1 and Mgm1 are conserved throughout eukaryotes, but much less is known about the genetic networks or mechanisms that control mitochondrial membrane dynamics in higher organisms. In humans, disruption of mitochondrial function is a major cause of inherited disease [13], including many neurological defects. Of particular relevance to our studies, mutation of OPA1, the human ortholog of Mgm1, leads to autosomal dominant optic atrophy, the most common familial, childhood-onset cause of blindness [14]. This implies that normal membrane dynamics are necessary for human mitochondrial function and provides a strong incentive for understanding the underlying biological control of this process.

Rhomboid-7 Regulates Mitochondrial Morphology

The only gene in *Drosophila* known to be needed specifically for mitochondrial fusion is *fuzzy onions* (*fzo*), which regulates mitochondrial fusion only in the male germline of flies [18]. In an attempt to extend the genetic analysis of mitochondrial membrane fusion in *Drosophila*, we have focused on Rhomboid-7, which is predicted to be mitochondrial by virtue of an N-terminal targeting sequence [15]. When Rhomboid-7 was expressed in mammalian cells (Figure 1A) or *Drosophila* cells (Figure 1B), the protein exclusively colocalized with Mitotracker and the F_1F_0 ATP synthase, respectively, both well-characterized markers for the mitochondrial network [16]. As an initial approach to revealing the function of Rhomboid-7, we used RNAi to disrupt gene expression in cultured *Drosophila* S2 cells [17] (Figures 1C–1E). Treatment with double-stranded (ds) RNA that targets *rhomboid-7* resulted in a majority of cells with a highly fragmented mitochondrial network (Figures 1C and 1D) and a quantitative reduction in rhomboid-7 expression (Figure 1E). As a control for specificity of this phenotype, cells were mock treated without dsRNA or treated with dsRNA that targets *fzo*, which is not expressed in S2 cells. Mitochondria were not affected in these controls

*Correspondence: angus.mcquibban@utoronto.ca (G.A.M.); mf1@mrc-lmb.cam.ac.uk (M.F.)
[4] Present address: Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN, United Kingdom.

(Figures 1C and 1D). By homology searching, we have identified the apparent *Drosophila* ortholog (CG8479) of *mgm1* and *OPA1*, which we here designate as *optic atrophy 1-like (opa1-like)* after the mammalian ortholog. Cells treated with dsRNA that targets *opa1-like* resulted in a fragmented mitochondrial network, a phenotype indistinguishable from *rhomboid-7* disruption, and a quantitative reduction in Opa1-like expression (Figures 1C–1E). These results suggest that Rhomboid-7 and Opa1-like participate in a similar process of maintaining mitochondrial morphology and that, as in other organisms, disruption of mitochondrial fusion leads to fragmentation. They also suggest that the machinery of membrane fusion control may be conserved between yeast and metazoans.

To complement the *Drosophila* RNAi analysis and to investigate whether a similar function was conserved in mammalian cells, mouse mitochondrial rhomboid (called PSARL, presenilins-associated rhomboid-like) [19] and mouse OPA1 were expressed in COS-7 cells. Cells that expressed high levels of either PSARL (Figure 1F) or OPA1 (Figure 1G) but not a catalytic mutant of PSARL (Figure 1H) displayed complete aggregation of the mitochondrial network, in a phenotypically identical fashion (for quantitation, see Figure S1 in the Supplemental Data available with this article online). This mitochondrial aggregation phenotype resembles the excess fusion caused by overexpression of the mitofusins [20], the mammalian homologs of Fzo. Cells that expressed either PSARL or OPA1 at low levels did not generally disrupt the normal tubular network seen in COS-7 cells (data not shown). In parallel, we examined the effects of overexpressing Rhomboid-7 in *Drosophila* S2 cells. As with mammalian cells, this led to mitochondrial aggregation (Figure S2). These data are consistent with the *Drosophila* RNAi experiments: together, they strongly suggest that, as in yeast, mitochondrial rhomboids and Opa1-like GTPases are involved in the control of mitochondrial membrane dynamics in both flies and mammals.

A *Drosophila rhomboid-7* Mutant

To investigate the requirements for a mitochondrial rhomboid in a multicellular organism, we have generated and characterized mutations in *Drosophila rhomboid-7*. The P element {RS3}CB-0229-3 is located in the 5′ UTR of *rhomboid-7* (Figure 2A), thereby potentially disrupting its expression but not the protein coding sequence. Flies homozygous for this insertion are viable and appear healthy but the males are sterile. Precise excision of this P element resulted in reversion to fully wild-type flies, demonstrating that the P element caused the phenotype. This precise excision line is used as a control for subsequent experiments. We have generated a predicted null allele of *rhomboid-7* by imprecise excision of {RS3}CB-0229-3. The resulting mutation, *rhomboid-7*$^{p\Delta 1}$, lacks the transcriptional start site and the first 18 codons of the protein (Figure 2A). In addition to removing the 5′ end of the gene, this deletion disrupts the mitochondrial targeting sequence, so if any residual protein were produced, it would not be targeted appropriately. Although some homozygous adults do emerge, 90% of *rhomboid-7*$^{p\Delta 1}$ flies die before pupariation. Death occurs during both embryonic and larval stages, but those that survive to pupariation develop to adults, although approximately 10% of these die during the process of eclosion, as they get stuck while crawling from the pupal case. Surviving flies appear morphologically normal but all die within 3 days. Males are sterile but the females are fertile. The progeny of homozygous females show exactly the same severity of phenotype as their parental generation and are not rescued by wild-type sperm, demonstrating that there is no maternal or paternal rescue of the homozygous zygotic phenotype. These results indicate that Rhomboid-7 is not essential for *Drosophila* development but that its absence nevertheless dramatically reduces viability.

Mitochondrial Fusion Defects in *rhomboid-7* Mutants

Since surviving males are sterile, we examined the testes of homozygous *rhomboid-7*$^{p\Delta 1}$ mutants and found that although there was no gross morphological disruption, the seminal vesicle was small and appeared empty (Figure 2B). This was confirmed by dissection: *rhomboid-7*$^{p\Delta 1}$ and P element {RS3}CB-0229-3 mutant testes contained no mature sperm (not shown). Significantly, there is an essential mitochondrial fusion process during *Drosophila* sperm maturation [21]. All the mitochondria in the spermatid coalesce adjacent to the nucleus, then undergo a process of massive membrane fusion. This results in the formation of the nebenkern, a mitochondrial derivative composed of two giant, intertwined mitochondria that eventually unfurl to fill the sperm tail, providing the energy for motility. Nebenkerns are easily seen by phase-contrast microscopy: they appear as phase-dark, round structures of equivalent size and are adjacent to the phase-light nucleus. In contrast to the uniform and regular shapes of control nebenkerns (Figure 2C, left), *rhomboid-7*$^{p\Delta 1}$ mutants (Figure 2C, middle) and P element {RS3}CB-0229-3 homozygous mutants (not shown) have irregularly shaped nebenkerns. This phenotype is strikingly similar to that caused by loss of *fuzzy onions* (*fzo*^{1}, an ethylmethane sulfonate-induced loss-of-function allele) [18, 21], the only other gene known to be required for mitochondrial membrane fusion in spermatids (Figure 2C, right). This strongly suggests that Rhomboid-7 may also participate in mitochondrial membrane fusion. We examined the structure of *rhomboid-7*$^{p\Delta 1}$ nebenkerns in more detail by transmission electron microscopy. Control nebenkerns show the typical onion-like structure of interleaved coils of membrane (Figure 2D, left). *rhomboid-7*$^{p\Delta 1}$ nebenkerns, however, were composed of many individual mitochondria that had coalesced beside the nucleus, but had failed to fuse (Figure 2D, right), again like *fzo*^{1} nebenkerns [18]. These data directly confirm that Rhomboid-7 is required for mitochondrial membrane fusion, at least in the formation of the nebenkern during spermatogenesis.

By analogy to yeast, we might expect Opa1-like to function in regulating mitochondrial membrane dynamics in *Drosophila*. We therefore examined the phenotype of *opa1-like* mutants. We have identified two independent P elements inserted into the first and second exons of the *opa1-like* gene (P{EPgy2}CG8479 and P{lacW}l(2)s3475, respectively). Both P element lines were early larval lethal. We therefore examined mitotic clones of *opa1*-like mutant cells in the male germline

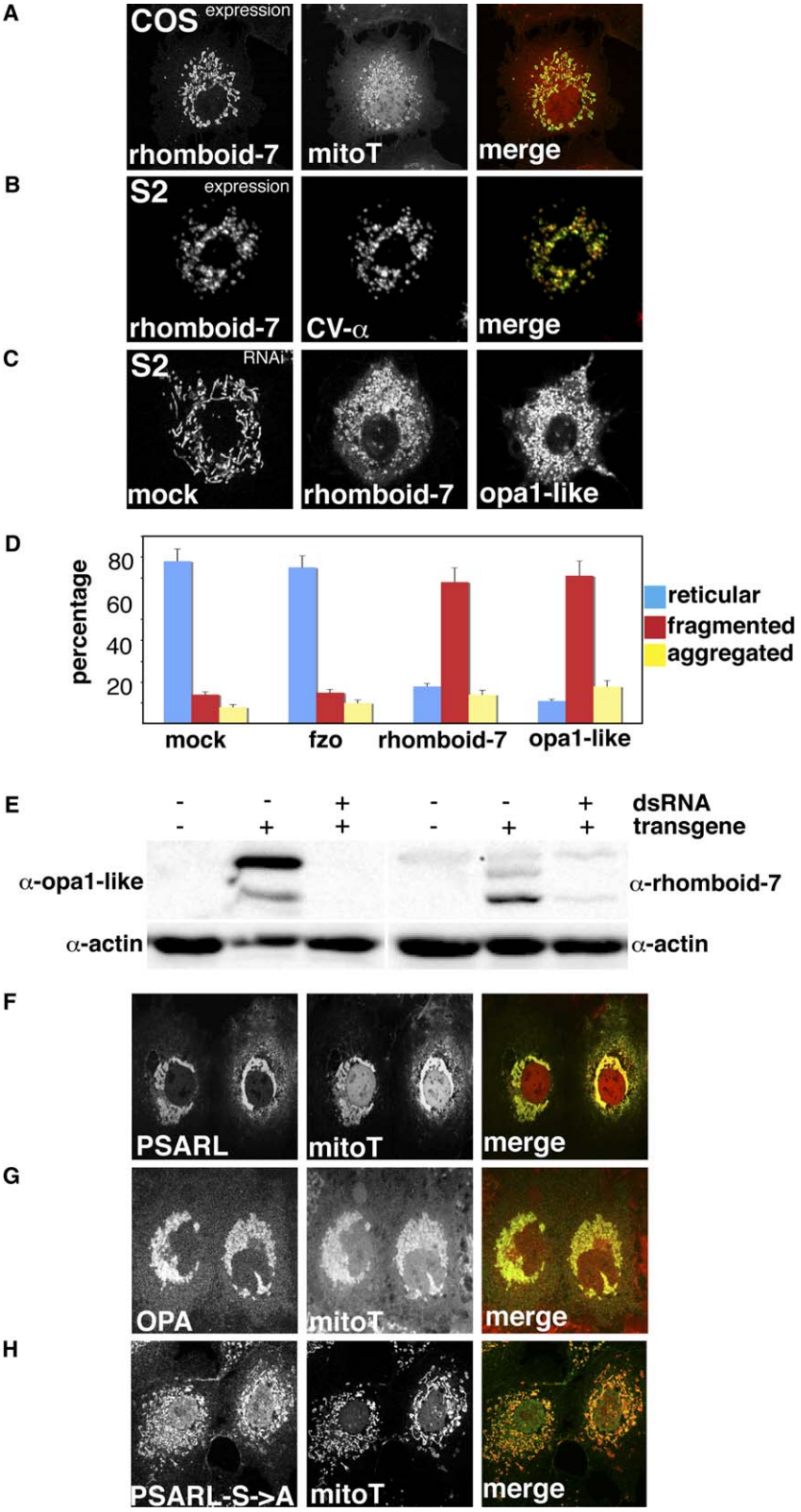

Figure 1. *Drosophila* Rhomboid-7 and Opa1-like Are Mitochondrial Proteins that Regulate Mitochondrial Morphology

(A) *Drosophila* Rhomboid-7-HA (left) expressed in COS-7 cells exclusively colocalizes with the mitochondrial dye Mitotracker, as shown in merged image.

(B) *Drosophila* Rhomboid-7-HA (left) expressed in *Drosophila* Scheider S2 cells colocalizes with the mitochondrial F_1F_0 ATP synthase component CV-α, as shown in merged image.

to determine whether Opa1-like, like Rhomboid-7 and Fzo, is required for nebenkern membrane fusion. *opa1-like*$^{P\{lacW\}l(2)s3475}$ (Figure 2E) and *opa1-like*$^{P\{EPgy2\}CG8479}$ (data not shown) mutant spermatids have nebenkerns with the same morphological defects as *rhomboid-7*$^{p\Delta 1}$ and *fzo*^{1} mutants. These data suggest that Rhomboid-7 and Opa1-like function to regulate the mitochondrial fusion that generates the nebenkern during spermatogenesis.

Surviving adult *rhomboid-7* mutants are morphologically normal, with the exception of a wing-position defect, in which the wings of about 60% of individuals hang down on either side of the abdomen, as opposed to control flies that have their wings tucked and positioned on top of the abdomen (Figure 3A). Such defects often indicate flight muscle abnormalities [22]. Examination of the indirect flight muscles of *rhomboid-7*$^{p\Delta 1}$ mutants by light microscopy showed a general disruption of the normal continuous pattern of the phase-dark banding (Figure 3B). Examination by electron microscopy showed many small mitochondria in the space between the myofibrils, as compared to larger mitochondria that completely filled the intramyofibril space in control flies (Figure 3C). As muscle maturation in *Drosophila* involves the fusion of many small mitochondria into larger ones over the first few days of life [23], these data indicate a role for Rhomboid-7 in mitochondrial fusion in flight muscles as well as spermatids—both tissues with unusual developmental requirements for high levels of fusion.

Neuronal Dysfunction in *rhomboid-7* Mutants

rhomboid-7$^{p\Delta 1}$ flies live for only 3 days whereas control flies live for an average of 60 days. In addition to this longevity defect, *rhomboid-7*$^{p\Delta 1}$ mutant flies are unable to fly, have extreme difficulty walking, and display erratic twitching in their legs and head. These motor defects could be caused by muscle or neurological disorder (or both), but in most tissues these are difficult to distinguish phenotypically. In order to test specifically whether neuronal activity was affected by *rhomboid-7* loss, we measured synaptic transmission in the retina, where muscle defects would be irrelevant, by recording electroretinograms (ERGs; this technique uses electrodes to record depolarisation of neurons in response to light [24]) of *rhomboid-7*$^{p\Delta 1}$ mutants and control flies. By recording extracellular potentials, an average synaptic transmission across the retina is measured. Additionally, intracellular recordings were made to record from single photoreceptors [25].

Throughout the stimulation, the responses of control flies showed prominent on- and off-transients (Figures 4A and 4B, lower traces); these spikes represent synaptic transmission from R1-6 photoreceptors to large monopolar cells, the first visual interneurons in the optic lobe. The amplitudes of these transients showed no obvious time or intensity dependency. In contrast, both on- and off-transients of *rhomboid-7*$^{p\Delta 1}$ mutants (Figures 4A and 4B, upper traces) were significantly smaller than those of control flies, with the off-transients showing strong time and intensity dependency. The off-transients to bright and dim pulses died out within 10–20 s and 20–40 s, respectively. Additionally, the graded receptor potential component of the *rhomboid-7*$^{p\Delta 1}$ ERGs was reduced in size during the experiments, much more than that observed with control flies. This was further confirmed by recording intracellular voltage responses of photoreceptors to a brief saturating light pulse. The responses of photoreceptors in *rhomboid-7*$^{p\Delta 1}$ mutants were less than half of those of control photoreceptors (Figure 4C), indicating reduced responsiveness to repetitive stimulation. These ERG recordings and intracellular voltage responses indicate that disruption of Rhomboid-7 prevents normal photoreceptor synaptic function, specifically constraining both the generation of light responses and the signal transfer across the first visual synapse.

Since all other known phenotypes associated with loss of rhomboid-7 or its yeast or mammalian homologs can be attributed to defects in mitochondrial fusion, we suspect that this is the cause of the abnormal synaptic transmission. There are many studies implicating mitochondria in synaptic function, although their precise role remains to be determined. Our data strongly suggest that fully efficient mitochondrial membrane fusion is necessary for normal photoreceptor activity. Consistent with this, it has recently been reported that loss of synaptic mitochondria prevents mobilization of the reserve pool of synaptic vesicles [26]. It will be interesting to determine whether impairment of mitochondrial fusion in synapses leads to the same specific defect. In fact, the basic need for mitochondrial fusion and fission in any cells is not fully understood; various theories have been proposed, but the precise reasons for mitochondrial networks being so dynamic remain mysterious.

As described above, mutations in the human *OPA1* gene cause dominant optic atrophy, a common form of childhood-onset blindness. We therefore tested for light-induced photoreceptor degeneration in *rhomboid-7* and control flies. Control flies show no defects

(C) RNA interference demonstrates the activity of *Drosophila* Rhomboid-7 and Opa1-like in regulating mitochondrial morphology. *Drosophila* Schneider S2 cells were mock treated (left) or treated with dsRNA that targets *rhomboid-7* (middle) or *opa1-like* (right) and stained with Mitotracker. Representative cells are shown.

(D) Quantification of three independent experiments (100 cells categorized per treatment) shows the significant shift from a reticular mitochondrial network (blue bars) in control samples (mock; fzo, Fuzzy onions, a protein that is not expressed in S2 cells) to a highly fragmented morphology (red bars) upon treatment with either *rhomboid-7* or *opa1-like* dsRNA. Error bars represent standard deviation.

(E) Treatment of S2 cells expressing Opa1-like (left) or Rhomboid-7 (right) with dsRNA targeted toward Opa1-like and Rhomboid-7, respectively, results in quantitative knockdown of protein expression. Specificity of knockdown and loading control is shown by equivalent expression of actin (bottom) in all treatments.

(F) Overexpression of mammalian mitochondrial rhomboid (PSARL-HA) in COS cells causes severe mitochondrial aggregation, a phenotype characteristic of overexpression of proteins that promote membrane fusion [20] (mitoT, mitotracker).

(G) Overexpression of mammalian OPA1-GFP in COS cells causes indentical severe mitochondrial aggregation (mitoT, mitotracker).

(H) Overexpression of a catalytically inactive mutant of PSARL-HA (catalytic serine changed to alanine) in COS cells does not disrupt normal mitochondrial morphology, indicating the requirement for protease activity to induce the membrane fusion phenotype (mitoT, mitotracker).

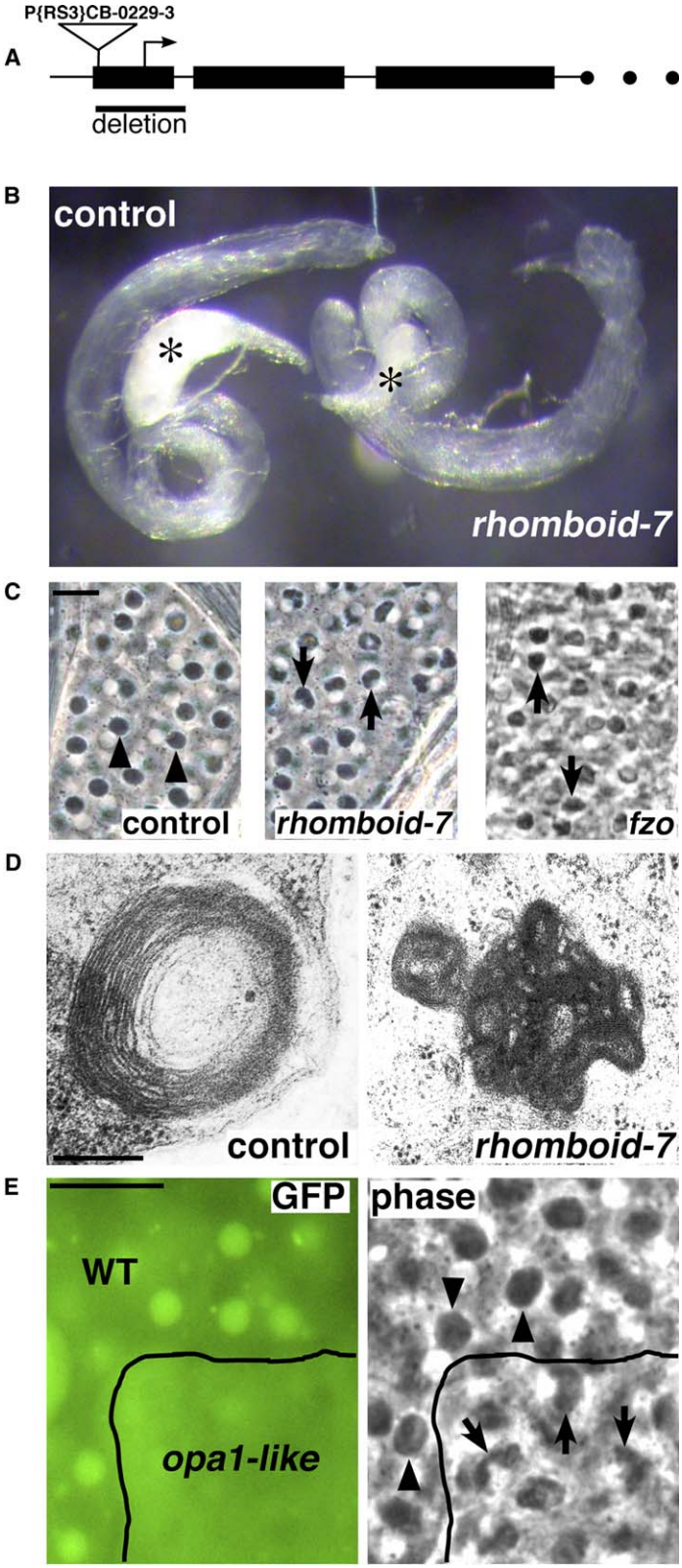

Figure 2. Mutations in *Drosophila rhomboid-7* and *opa1-like* Result in Nebenkern Formation Defects

(A) A schematic showing the genomic structure of the *Drosophila rhomboid-7* locus. The first three exons are displayed (the gene is composed of 6 exons). The first ATG is indicated by an arrow. The P element {RS3}CB-0229-3 is 9 nucleotides within the 5′ UTR of the *rhomboid-7* transcript. Imprecise excision of the P element resulted in a deletion of 135 nucleotides, removing sequences that encode for the first 18 amino acids of the Rhomboid-7 protein. The extent of the deletion, *rhomboid-7*$^{p\Delta 1}$, is indicated as a line beneath the genomic schematic.

(B) Analysis by light microscopy of live testes from control (left) and *rhomboid-7*$^{p\Delta 1}$ mutant (right) flies shows a complete absence of mature sperm in the mutant seminal vesicle (indicated by asterisks).

(C) Analysis by light microscopy by phase optics shows a nebenkern defect in *rhomboid-7*$^{p\Delta 1}$ and *fzo*^{1} mutants. Nebenkerns in control flies (left, phase-dark structures indicated by arrowheads) are nearly perfect circles of equivalent size to the nucleus (phase-light structures). Mutant *rhomboid-7*$^{p\Delta 1}$ testes (middle) have nebenkerns (indicated by arrows), but they are irregularly shaped and sized; they resemble *fzo*^{1} mutant nebenkerns (right, indicated by arrows). Scale bar equals 10 μm.

(D) Electron microscopy reveals that *rhomboid-7*$^{p\Delta 1}$ mutant nebenkerns undergo normal mitochondrial aggregation but fail to undergo membrane fusion. Nebenkerns from control flies (left) appear as interwoven layers of mitochondrial membranes indicative of the resulting two giant mitochondria that form the nebenkern. *rhomboid-7*$^{p\Delta 1}$ mutant nebenkerns (right) show multiple mitochondria that have aggregated but have failed to fuse. The irregular aggregation makes individual membranes difficult to resolve. Scale bar equals 2 μm.

(E) Clonal analysis reveals that Opa1-like participates in nebenkern formation. Cells mutant for *opa1-like* (P{lacW}l(2)s3475) were generated by making mitotic clones in the testes of otherwise wild-type flies. The tissue without green-stained nuclei (left, GFP fluorescence), outlined in both panels, indicates mutant tissue. Nebenkerns appear as near perfect circles in wild-type tissue (indicated by arrowheads), but in *opa1-like* mutant tissue, the nebenkerns have morphological defects indistinguishable from *rhomboid-7*$^{p\Delta 1}$ and *fzo*^{1} mutants (right, indicated by arrows, phase optics). Scale bar equals 10 μm.

after 3 days of continuous light (Figure 4D, left). In contrast, over the same period (the maximum length the mutants survive), *rhomboid-7*$^{p\Delta 1}$ mutant photoreceptors degenerated severely (Figure 4D, right), as shown by the almost complete loss of distinct rhabdomeres. This sensitivity to light implies that, in the absence of efficient mitochondrial membrane dynamics, photoreceptor neurons acquire activity-dependent damage at a much higher rate than normal, an interesting possible parallel with human dominant optic atrophy. Importantly, the

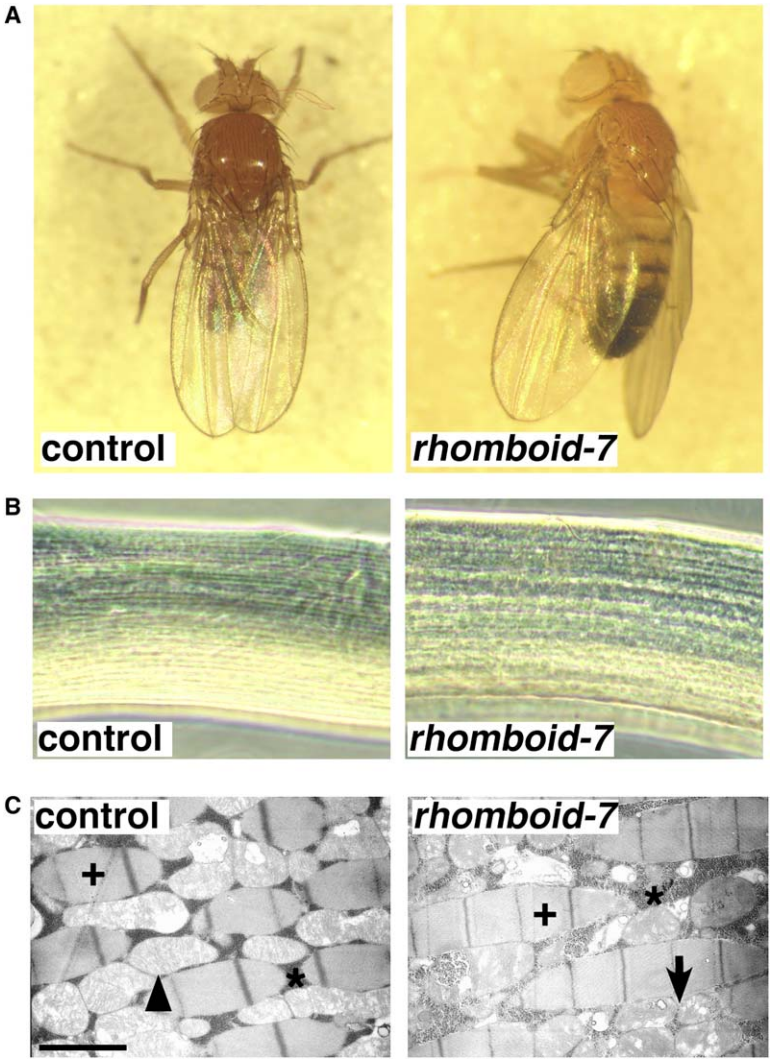

Figure 3. *rhomboid-7* Mutant Adults Have a Muscle Defect

(A) Surviving *rhomboid-7*$^{p\Delta 1}$ mutant adults (right) have a droopy wing phenotype as compared to the normal wing position in control flies (left).

(B) Light microscopy by phase-contrast optics reveals a significant disruption in the evenly spaced and horizontally oriented continuous phase-dark pattern of the myofilaments in the indirect flight muscles of *rhomboid-7*$^{p\Delta 1}$ mutants (right) as compared to control flies (left).

(C) Electron microscopy reveals a disruption in mitochondrial packing, size, and inner membrane organization of mitochondria in *rhomboid-7*$^{p\Delta 1}$ mutants. In wild-type tissue, mitochondria normally fill the space between the myofibrils (indicated with plus sign). *rhomboid-7*$^{p\Delta 1}$ mutant tissue (right, arrow indicates a cluster of four small, irregular mitochondria) has small mitochondria with significant loss of inner membrane cristae as compared to control flies (left, arrowhead indicates mitochondria). Asterisks indicate glycogen particles. Scale bar equals 1 μm.

rhomboid-7$^{p\Delta 1}$ mutant eyes showed no detectable degeneration under normal light or dark conditions (Figure S3), and it was these flies that showed the clear defect in signal transfer across the first visual synapse. This suggests that the synaptic defects are primary consequences of Rhomboid-7 loss, and not secondary to photoreceptor loss. Consistent with this conclusion that apoptosis may not be a primary phenotype of Rhomboid-7 loss, *Drosophila* S2 cells in which *rhomboid-7* was reduced by RNAi showed no extra sensitivity to cycloheximide-induced apoptosis (Figure S4).

Our results imply that not all mitochondrial membrane dynamics require functional Rhomboid-7 (otherwise the mutant would be fully lethal, as is the case for the mouse mitofusin genes MFN1 and 2 [27]). Instead, Rhomboid-7 is apparently required in places with a high requirement for mitochondrial fusion and/or energy demands. Loss of Opa1-like causes similar phenotypes to loss of Rhomboid-7 which, coupled with what has previously been shown in yeast, strongly suggests that they work in the same processes. It is worth noting, however, that *opa1*-like mutants are more severe than *rhomboid-7* mutants, indicating that Opa1-like has additional functions that are Rhomboid-7 independent. A similar conclusion has been reached in yeast, where loss of Mgm1 causes more severe failure of mitochondrial fusion than loss of Rbd1/Pcp1 [12]. One goal of this work was to investigate the consequences of disrupting mitochondrial fusion in metazoans, where the potential for complex phenotypes is greater than in yeast. Although we do not yet know the full mechanisms that underlie the synaptic transmission defects, our results lead us to suspect that they are caused by loss of full mitochondrial function at the synapse. The mechanism underlying the striking longevity defect is less obvious but could be related to neural malfunction in regions other than the retina.

Experimental Procedures

Protein Expression

rhomboid-7 cDNA (with the addition of HA at the C terminus) was cloned into the vector *pCMA* [28] that drives expression of transgenes in both *Drosophila* and mammalian cells. *PSARL* (with the addition of a HA-epitope tag at the C terminus) and *OPA1* (with the addition of GFP at the C terminus) cDNAs were cloned into the mammalian vector *pcDNA 3.1* (Invitrogen).

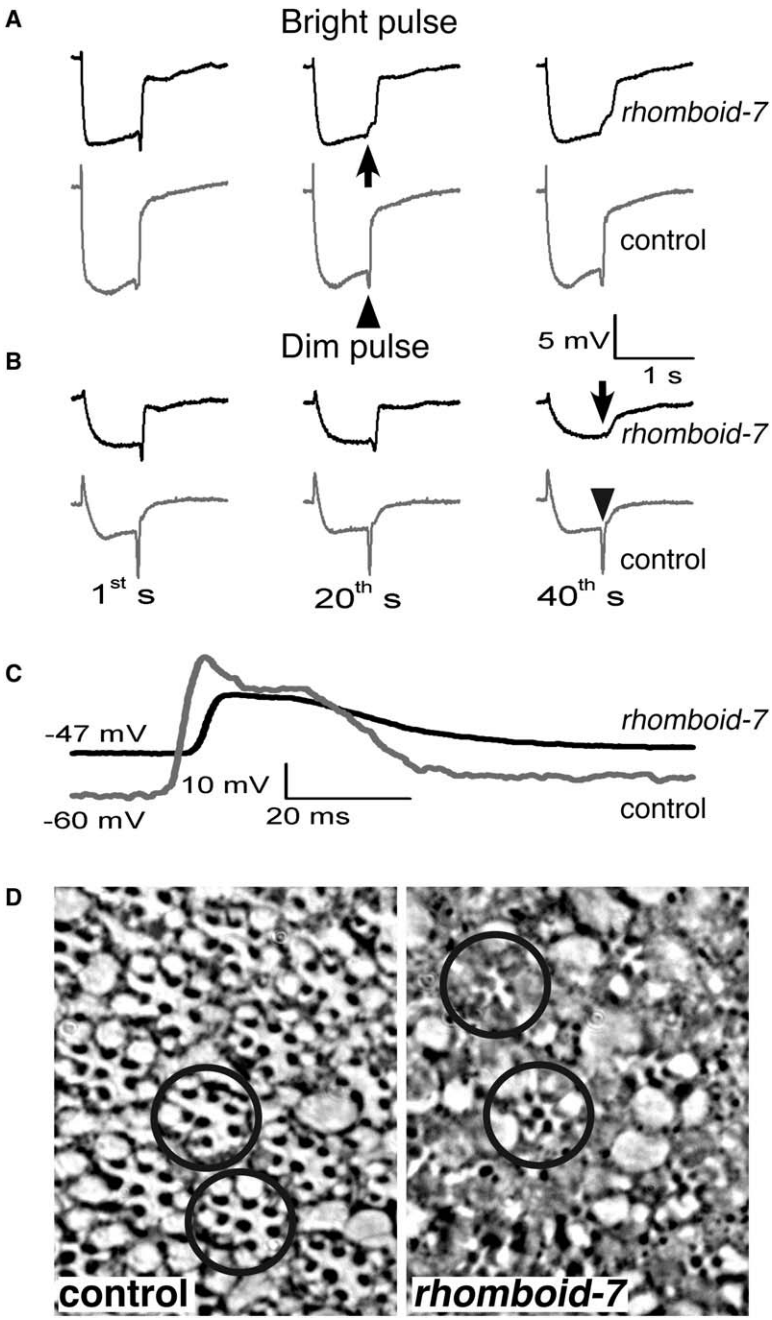

Figure 4. *rhomboid-7* Mutants Fail to Signal across the First Optical Synapse and Are Hypersensitive to Light-Induced Neurodegeneration

(A and B) Extracellular ERG recordings, made 2 days after eclosion, show that the on- and off-transients induced by light pulses are both time and intensity dependent in *rhomboid-7*$^{p\Delta 1}$ mutant eyes but not control eyes. Arrowheads indicate the off-transient in control tissue (wt, bottom traces in [A] and [B]) at the 20 s time point under bright pulse (A) and 40 s under dim pulse (B). In contrast, arrows indicate complete loss of off-transient in *rhomboid-7*$^{p\Delta 1}$ mutants (R7, upper traces in [A] and [B]) at the same time. Electrical potential and time scales are indicated.

(C) Intracellular recordings made 2 days after eclosion show that both the electrical potential and voltage response to light are significantly reduced in *rhomboid-7*$^{p\Delta 1}$ mutant eyes (black line) as compared to control flies (gray line). Electrical potential and time scales are indicated. The stimulus is a 10 ms long saturating light pulse.

(D) After 3 days of constant light exposure, sections through fixed adult eyes show that the normal pattern of seven phase-dark rhabdomeres in each ommatidium (indicated by circles) in control flies (left) are distorted or absent in *rhomboid-7*$^{p\Delta 1}$ mutants (right), demonstrating severe light-induced photoreceptor degeneration.

RNA Interference Assays

cDNA sequences (400 nt in length, sequences available upon request) for *fuzzy onions*, *rhomboid-7*, and *opa1-like* were PCR amplified from fly genomic DNA, and purified templates were used for in vitro transcription reactions to synthesize dsRNA, according to the manufacturer's instructions (Invitrogen). dsRNA was applied to S2 cells as described by Clemens et al. [29]. After 3 days, cells were stained with the vital dye Mitotracker (Molecular Probes) and observed by fluorescence microscopy.

Drosophila *Strains*

P element strains were obtained from public sources (P{RS3}CB-0229-3 from the Szeged stock center, P{EPgy2}CG8479 and P{lacW}l(2)s3475 from the Bloomington stock center). P element {RS3}CB-0229-3 was excised by P-transposase by standard genetic techniques. Control and *rhomboid-7*$^{p\Delta 1}$ mutant flies were grown under normal but constant light conditions for 3 days before adult heads were fixed, embedded, and sectioned as described by Freeman et al. [30].

EM Analysis

Drosophila testes were dissected in Ringers solution and fixed in 2% glutaraldehyde. EM was done as described in McQuibban et al. [3]. Indirect flight muscles were dissected in Ringers solution and imaged under phase optics by light microscopy and EM as described [3].

Electrophysiology

Extracellular ERGs were performed as described by Gengs et al. [24], and intracellular recordings were performed as described by Juusola and Hardie [25].

Immunostaining

Antibody staining was performed by standard techniques. Reagents used were as follows: antibodies against the antigens CV-α (Mitosciences), HA (Sigma), actin (Sigma), and the vital stain Mitotracker (Molecular Probes).

Supplemental Data

Four supplemental figures can be found with this article online at http://www.current-biology.com/cgi/content/full/16/10/982/DC1/.

Acknowledgments

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