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Inner-membrane proteins PMI/TMEM11 regulate mitochondrial morphogenesis independently of the DRP1/MFN fission/fusion pathways

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Mitochondria are highly dynamic organelles that can change in number and morphology during cell cycle, development or in response to extracellular stimuli. These morphological dynamics are controlled by a tight balance between two antagonistic pathways that promote fusion and fission. Genetic approaches have identified a cohort of conserved proteins that form the core of mitochondrial remodelling machineries. Mitofusins (MFNs) and OPA1 proteins are dynamin-related GTPases that are required for outer- and inner-mitochondrial membrane fusion respectively whereas dynamin-related protein 1 (DRP1) is the master regulator of mitochondrial fission. We demonstrate here that the *Drosophila PMI* gene and its human orthologue *TMEM11* encode mitochondrial inner-membrane proteins that regulate mitochondrial morphogenesis. PMI-mutant cells contain a highly condensed mitochondrial network, suggesting that PMI has either a pro-fission or an anti-fusion function. Surprisingly, however, epistatic experiments indicate that PMI shapes the mitochondria through a mechanism that is independent of *drp1* and *mfn*. This shows that mitochondrial networks can be shaped in higher eukaryotes by at least two separate pathways: one PMI-dependent and one DRP1/MFN-dependent.

Keywords: mitochondrial morphogenesis; PMI; TMEM11; tubulation

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INTRODUCTION

Mitochondria are highly dynamic and undergo remodelling through cycles of fission and fusion. Fission and fusion are essential for maintenance of mitochondrial function and to adapt mitochondria to cellular needs (Detmer & Chan, 2007). Components of the mitochondrial fission and fusion machineries were first identified in fly and yeast and are also conserved in humans (Okamoto & Shaw, 2005; Westermann, 2008). Mitofusins (MFNs) 1 and 2 are outer-membrane dynamin-like GTPases that bridge neighbouring mitochondria to promote their tethering and fusion (Hales & Fuller, 1997; Chen *et al*, 2003). Another dynamin-like GTPase, OPA1, is associated with the mitochondrial inner membrane and cooperates with MFN1 to promote mitochondrial fusion (Olichon *et al*, 2002; Cipolat *et al*, 2004). Mitochondrial fission is also regulated by a conserved GTPase, dynamin-related protein 1 (DRP1; Bleazard *et al*, 1999; Smirnova *et al*, 2001). The shape of the mitochondrial network results from a balance between fission and fusion, which antagonize each other in a dose-dependent manner (Sesaki & Jensen, 1999).

The original purpose of this work was to characterize PGRP-LD, a putative member of the peptidoglycan-recognition protein family. Peptidoglycan-recognition protein are eukaryotic peptidoglycan-binding proteins that are essential for both invertebrate and vertebrate immune responses (Royet & Dziarski, 2007; Charroux *et al*, 2009). However, as in the case of about 20 loci in the *Drosophila* genome, *PGRP-LD* is transcribed as a bi-cistronic mRNA, which also encodes an uncharacterized protein called PMI (supplementary Fig S1A online; http//www.flybase.org). Unexpectedly, phenotypic analyses of *PMI_PGRP-LD*-null mutants led us to conclude that the *Drosophila* PMI protein is a novel determinant of mitochondrial morphogenesis, the function of which is conserved in its human orthologue TMEM11.

RESULTS

Loss of PMI function leads to defects in mitochondrial fission

PMI_PGRP-LD-mutant flies hatched as morphologically normal adults but displayed paralysis under stress conditions (supplementary

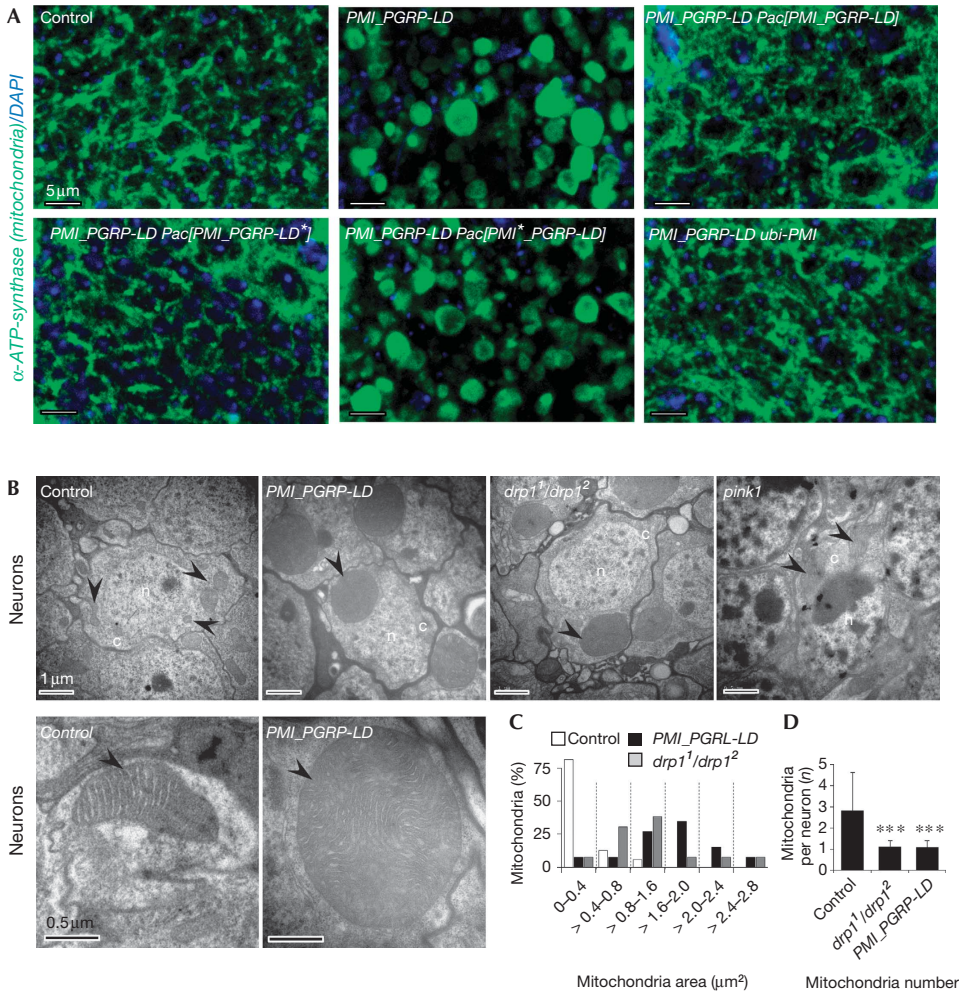

Fig 1 | *PMI* function is required to shape the mitochondrial network. (**A**) Fly adult brains labelled with ATP synthase antibodies. Mitochondria of *PMI_PGRP-LD* mutant neurons (*PMI_PGRP-LD*) are round and well individualized. This is in contrast to the dense and thin filamentous network observed in controls. *PMI_PGRP-LD* genomic construct restores mitochondrial shape (*PMI_PGRP-LD Pac[PMI_PGRP-LD]*), as does a construct in which *PGRP-LD* has frameshift mutations (*PMI_PGRP-LD Pac[PMI_PGRP-LD*]*) or a transgene allowing the expression of *PMI* only (*PMI_PGRP-LD ubi-PMI*). Genomic constructs containing PMI frameshift mutations have no rescuing activity (*PMI_PGRP-LD Pac[PMI*_PGRP-LD]*). (**B**) Transmission electron microscopy (TEM) pictures of adult neurons. *PMI_PGRP-LD* mitochondria are abnormally enlarged and have an internal ultrastructure similar to that of *drp1* mutant, with no sign of cristae alterations (lower panels), in contrast to *pink1* swollen mitochondria. Arrows, mitochondria; n, nucleus; c, cytoplasm. (**C**) Size distribution of adult neuron mitochondria. The mitochondrial section area was measured on TEM pictures. In controls, more than 75% of mitochondrial sections are smaller than 0.4 µm², whereas in *PMI* and *drp1* mutants, 85% and 61% of the mitochondria have a section area of more than 0.8 µm², respectively. Student's *t*-test: control compared with *PMI*, $P<0.001$; control compared with *drp1*, $P<0.001$. Control, $n=101$ mitochondria, *PMI_PGRP-LD*, $n=85$. Three independent brains. (**D**) Number of mitochondria per neuron. Consistent with impaired fission, *PMI* and *drp1* mutants have significantly fewer mitochondria per cell. Student's *t*-test results: *** $P<0.001$. Control, $n=46$ cells; *PMI_PGRP-LD*, $n=84$. Three independent brains.

Fig S1A,B online and data not shown). As this behaviour, known as 'bang sensitivity', has been associated with invalidation of mitochondrial proteins (Verstreken *et al*, 2005; Fergestad *et al*, 2006), we analysed the consequences of *PMI_PGRP-LD* inactivation on mitochondrial structure. Whereas mitochondria of wild-type cells formed a dense network of thin tubules that completely fill the soma, *PMI_PGRP-LD* mitochondria were large, round and often as big as nuclei (Fig 1A; supplementary Fig S2 online). By using genomic rescue constructs, we showed that the mitochondrial phenotype was due to *PMI* but not due to *PGRP-LD* inactivation. Although of abnormal size, *PMI_PGRP-LD* mitochondria had a normal ultrastructure with an intact outer membrane and well-formed cristae similar to that of *drp1*-mutant mitochondria, but different from *pink1* mitochondria (Fig 1B,C, supplementary Fig S3A,B online). As a result of impaired fission, *drp1*- and *pink1*-mutant cells had fewer mitochondria per cell (Fig 1B,D; supplementary Fig S3A,C online). Similar observations were made for PMI-mutant neurons and myocytes (Fig 1B,D;

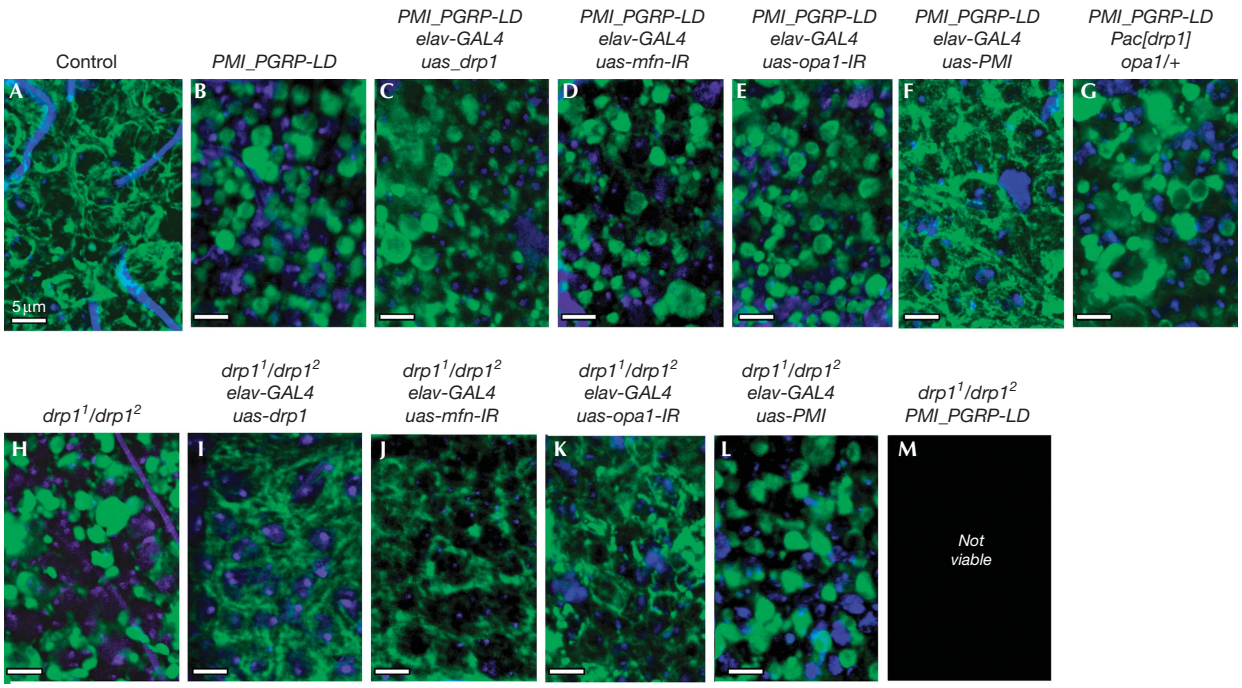

Fig 2 | *PMI* does not genetically interact with components of the fission/fusion machinery. Antennal lobe cortex from adult fly brain labelled with ATP synthase antibodies (green) and 4,6-diamidino-2-phenylindole (DAPI; blue). Wild-type control is shown in (**A**). *PMI_PGRP-LD* mutant phenotype (**B**) is not rescued by overexpression of *drp1* (**C**) or by downregulation of *mitofusin/marf* (**D**) or *opa1* (**E**). Adding one copy of *drp1* and removing one copy of *opa1* has no effect on *PMI_PGRP-LD* mitochondrial shape (**G**). $drp1^1/drp1^2$ mitochondrial morphology (**H**) is rescued by overexpression of *drp1* (**I**) or RNAi-mediated downregulation of *mfn/marf* (**J**) or *opa1* (**K**). Neuronal overexpression of PMI rescues *PMI_PGRP-LD* (**F**) but not *drp1* phenotypes (**L**). *PMI_PGRP-LD* or $drp1^1/drp1^2$ mutants are viable, but $drp1^1/drp1^2$;*PMI_PGRP-LD* double mutants are lethal (**M**). RNAi, RNA interference.

supplementary Fig S3A,C online). Consistent with this, three-dimensional reconstruction data revealed a marked condensation of the mitochondrial network in *PMI* cells, with the overall volume occupied by mitochondria unchanged (supplementary Fig S4A,B online). These results suggest that the function of PMI is to promote mitochondrial fission or prevent fusion, and that its inactivation leads to excessively fused and less numerous mitochondria.

PMI shapes mitochondria independently of *drp1*/*mfn*

Mitochondrial shape is controlled by a balance between two antagonistic pathways that promote fusion and fission, respectively. To determine the genetic place of *PMI* with respect to fission/fusion regulators, we conducted epistasis experiments. Mitochondrial enlargement found in fission mutants is due to unbalanced fusion, and can therefore be rescued by reducing levels of the pro-fusion proteins MFN or OPA1 (Bleazard *et al*, 1999; Sesaki & Jensen, 1999; Fekkes *et al*, 2000; Mozdy *et al*, 2000; Cerveny *et al*, 2001; Griffin *et al*, 2005; Deng *et al*, 2008; Poole *et al*, 2008; Yang *et al*, 2008; Park *et al*, 2009). Consistently, inactivation of *mfn/marf* and *opa1* was sufficient to restore normal filamentous mitochondrial network in *drp1* neurons (Fig 2J,K). By contrast, reducing either *mfn/marf* or *opa1* transcript levels did not modify the *PMI_PGRP-LD* phenotype (Fig 2D,E). Defaults in mitochondrial morphogenesis observed in fission mutants are dependent on the fission protein DRP1 (James *et al*, 2003; Tondera *et al*, 2005; Deng *et al*, 2008; Poole *et al*, 2008; Yang *et al*, 2008; Park *et al*, 2009; Zhao *et al*, 2009). However, although *drp1* overexpression rescued *drp1* mutants (Fig 2I), it had no effect on *PMI_PGRP-LD* mitochondria (Fig 2C). In addition, whereas adding one copy of *drp1* or removing one copy of the *opa1* gene rescued *pink1* phenotype (supplementary Fig S5I,K,L online), *PMI_PGRP-LD* mutants in which *drp1* and *opa1* were respectively increased and decreased were not rescued (supplementary Fig S5H online, Fig 2G). Finally, *drp1*- and *pink1*-mutant phenotypes were not affected by providing ectopic PMI (Fig 2L, supplementary Fig S5J online). These results indicate that PMI regulates mitochondrial shape independently of the canonical pathways, implicating *mfn/marf*, *opa1* and *drp1*. This was supported by the additive effect of *drp1* and *PMI* mutations. Indeed, *drp1* and *PMI* mutants hatched into viable adults (supplementary Fig S5N,O online), but the *drp1;PMI* double mutant was embryonically lethal (supplementary Fig S5M online).

Fly and human PMI are inner-membrane proteins

To study PMI localization, we generated flies carrying a CFP::PMI amino-terminal fusion expressed under *PMI* endogenous regulatory sequences. The CFP signal was associated with mitochondria in all cells, but it was not uniformly distributed on mitochondria, and instead accumulated as dots (Fig 3A,B). This was confirmed using a PMI::mCherry carboxy-terminal fusion that similarly targets mitochondria and accumulates along them in neurons (Fig 3C). PMI orthologues are present in many genomes including those of bilaterians, lower metazoans and choanoflagellates (supplementary Fig S6A,B online). Proteomic analyses suggested that the human PMI orthologue TMEM11 is part of the mitochondriome (Pagliarini *et al*, 2008). This was confirmed by

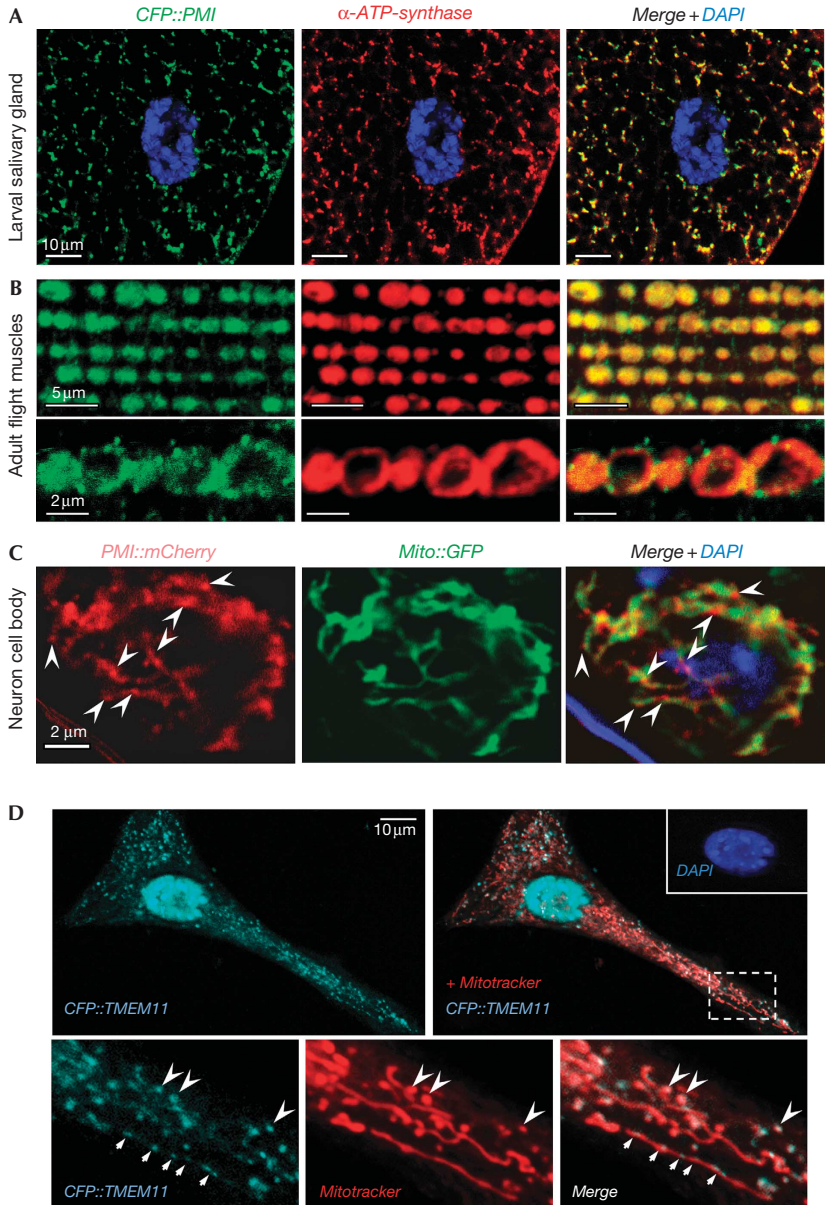

Fig 3 | *Drosophila* and human PMI are mitochondrial proteins. (**A**) Salivary gland cells from amino-terminal CFP::PMI larvae stained for ATP synthase (red). CFP::PMI (green) is targeted to mitochondria. (**B**) In adult myocytes, CFP::PMI (green) colocalizes with mitochondrial ATP synthase (red) and accumulates as dots. (**C**) *actin-GAL4 UAS-PMI::mCherry*, mito::GFP larval neuron. PMI::mCherry accumulated as dots along or at the tip of mitochondrial tubules (arrows). (**D**) C2C12 cells transfected with CFP::TMEM11 (blue) and stained with mitotracker (red) and 4,6-diamidino-2-phenylindole (DAPI; nucleus). CFP signal is shown as dots scattered throughout the cytoplasm. Bottom, CFP::TMEM11 (blue) colocalized with mitochondria (red, arrows), and often accumulated as dots along mitochondrial tubules (small arrowheads).

the detection of endogenous TMEM11 in the mitochondrial fraction of human cells (Fig 4C) and by using CFP-tagged TMEM11 that colocalized with mitotracker (Fig 3D). As for PMI, TMEM11 expression pattern was not uniform but discrete, forming a beaded necklace along mitochondrial tubules (Fig 3D). The submitochondrial localization of PMI was determined by electron microscopy. In N-terminal CFP::PMI- or C-terminal PMI::CFP-expressing myocytes, the majority of gold beads were present in areas filled with cristae (Fig 4A,B). A similar pattern was found for the inner-membrane protein ATP synthase, whereas the outer-membrane protein VDAC was mostly detected at the mitochondrial periphery (Fig 4A,B). The presence of three putative transmembrane domains in PMI sequence (supplementary Fig S6B online) suggests that PMI is an inner-membrane-associated protein.

We then determined TMEM11 submitochondrial localization by carrying out proteolysis assays. Although protease treatment of isolated mitochondria degraded outer-membrane protein (VDAC), it did not affect inter-membrane space (Smac/DIABLO), inner membrane (COX4), or matrix-associated proteins (HSP60) (Fig 4D). In this assay, TMEM11 functioned similarly to HSP60,

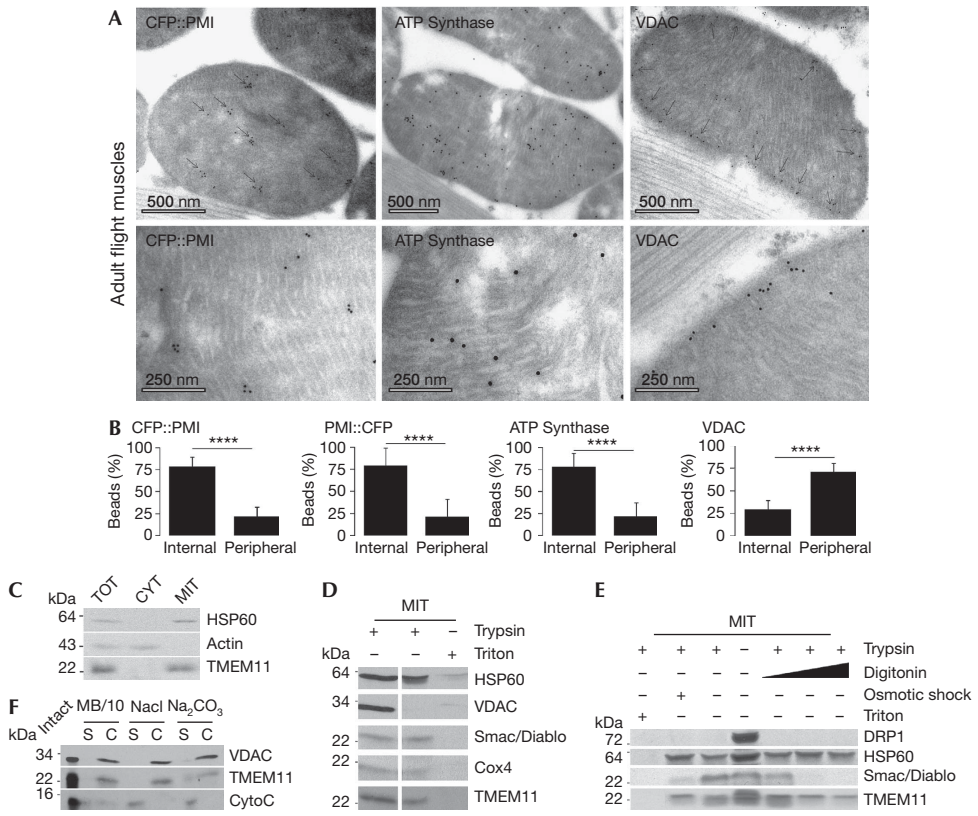

Fig 4 | *Drosophila* and human PMI localized at the mitochondrial inner membrane. (**A**) Transmission electron microscopy (TEM) images of CFP::PMI adult flight muscle labelled with GFP (CFP::PMI), ATP synthase or VDAC antibodies. Secondary antibody is conjugated to 10 (CFP::PMI and VDAC) or 15 nm (ATP synthase) gold beads. Similarly to inner-membrane protein ATP synthase, CFP::PMI staining is inside the mitochondria (arrows) in areas in which cristae are visible (higher magnification). The outer-membrane protein VDAC is detected all around the mitochondria (arrows). (**B**) Gold bead distribution for CFP::PMI (amino-terminal), PMI::CFP (carboxy-terminal), ATP synthase and VDAC immunolabelling. Mitochondria analysed: CFP-tagged PMI ($n=59$), ATP synthase ($n=28$), VDAC ($n=28$). Beads counted: CFP-tagged PMI ($n=736$), ATP synthase ($n=900$), VDAC ($n=1034$). Student's *t*-test: **** $P<0.0001$; three independent stainings. (**C**) Identical cell-equivalent amounts of HeLa cells post-nuclear supernatant (TOT, 100 μg of protein) separated into cytosol plus light membranes (CYT) and mitochondrial (MIT) fractions were analysed by western blot using TMEM11, mitochondrial HSP60 and actin antibodies. Results demonstrate that TMEM11 is a mitochondrial protein. (**D**) Mitochondria were incubated in the absence or presence of trypsin and triton. Samples were analysed by western blot using HSP60 (matrix), VDAC (outer membrane), Smac/DIABLO (intermembrane space), COX4 (inner membrane) and TMEM11 antibodies. Results demonstrate that TMEM11 is localized inside the mitochondria. (**E**) Mitochondria incubated or not in hypo-osmotic buffer (osmotic shock) were incubated in the absence or presence of trypsin, triton and increasing concentrations of Digitonin. Samples were analysed by western blot using HSP60 (matrix), DRP1 (outer membrane), Smac/DIABLO (intermembrane space) and TMEM11 antibodies. These results show that TMEM11 is a matricial protein. (**F**) Mitochondria (intact or sonicated) were incubated in hypo-osmotic buffer (MB/10) alone or supplemented by 1-M NaCl or 0.1-M Na_2CO_3, pH 11, and centrifuged to separate membrane pellets (P) from soluble protein supernatants (S). Samples were analysed by western blot using VDAC, TMEM11 and cytochrome C antibodies. With both treatments, TMEM11 remained in membrane pellets as did the integral membrane protein VDAC, whereas cytochrome C, peripherally associated to the inner membrane, was readily extracted by either treatment. This demonstrates that TMEM11 is a transmembrane protein.

Smac/DIABLO and COX4, indicating that it is located inside the mitochondria. Consistently, TMEM11 was degraded when mitochondrial membranes were solubilized before protease incubation (Fig 4D). However, unlike the inter-membrane space protein Smac/DIABLO, TMEM11 was resistant to proteolysis after outer-membrane disruption by osmotic shock or digitonin treatment (Fig 4E). Finally, we showed that TMEM11 is an integral membrane protein as it remained in the membrane pellet after salt or alkali extraction, as VDAC did (Fig 4F). These results demonstrate that TMEM11 is a mitochondrial inner-membrane protein facing the matrix.

TMEM11 regulates mitochondrial shape in human cells

We tested whether TMEM11 is also implicated in controlling mitochondrial morphology. In human cells treated with non-relevant short-interfering RNA (siRNA), nearly 90% of the cells have tubular mitochondria filling the cytoplasm (Fig 5A,E). In TMEM11 siRNA-treated cells, up to 45% of cells no longer show mitochondrial tubules, instead they have spherical and enlarged mitochondria (balloon phenotype; Fig 5A,D,E). DRP1 knockdown resulted in a less pronounced condensation of the mitochondrial network, with some mitochondria forming dense spherical entities on typical hyper-elongated tubules

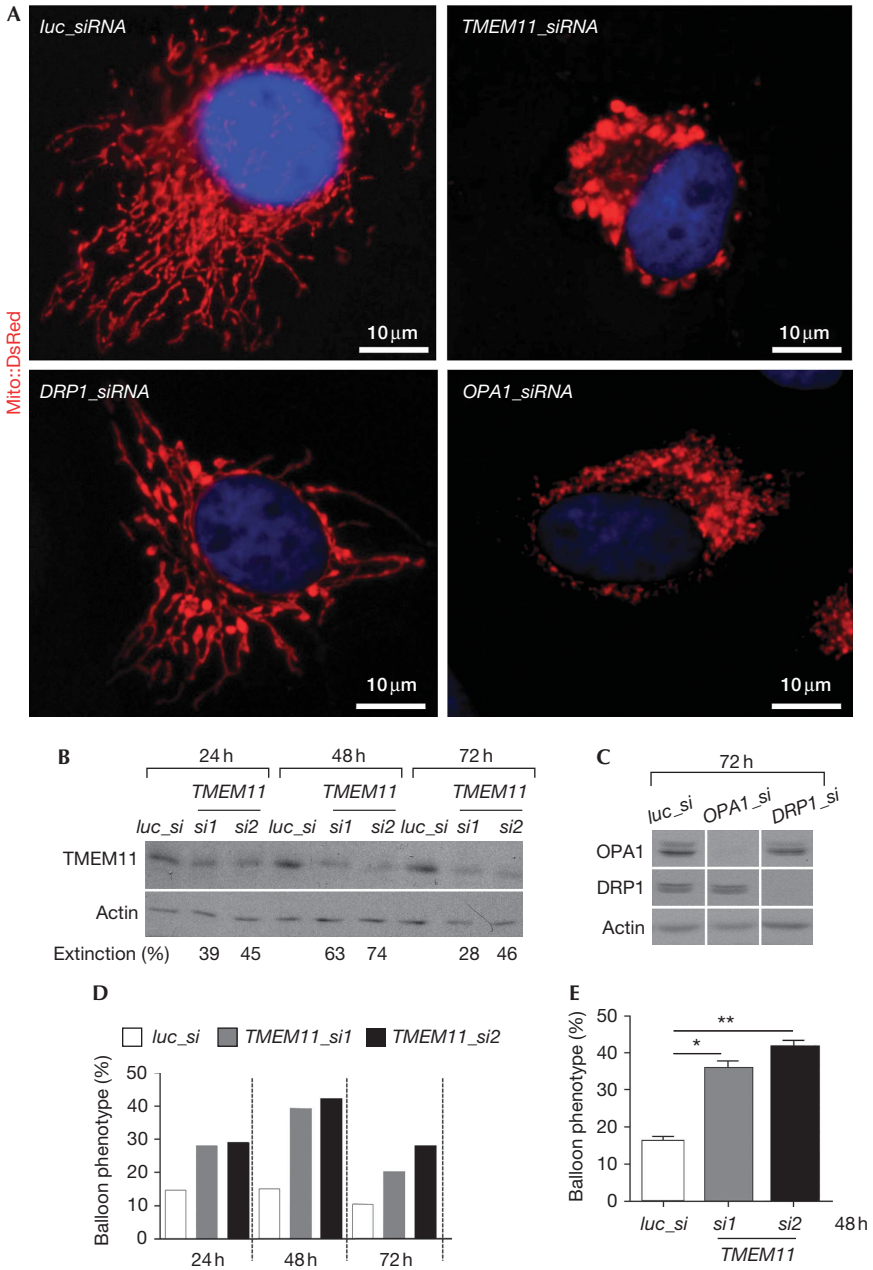

Fig 5 | TMEM11 is required to shape mitochondria in human cells. (**A**) Human cells co-transfected with either *luciferase* siRNA or siRNA targeting *TMEM11*, dynamin-related protein 1 (*DRP1*) or *OPA1* and a *mito::DsRed* reporter construct. In control cells (*luc_si*), mitochondria are organized into a dense network of tubules. In cells treated with *TMEM11_siRNA* (two different siRNAs tested), mitochondria condense into large spherical entities that aggregate around the nucleus. This is referred to as 'balloon phenotype'. Cells treated with *DRP1_siRNA* have dense, spherical mitochondria and hyperfilamented mitochondrial tubules. Mitochondria in *OPA1_siRNA*-treated cells were more numerous and smaller. (**B**) Time-course western blot experiments showing the extinction efficiency of two siRNAs (si1, si2) on TMEM11 protein level, compared with control siRNA against *luciferase* (*luc_si*). (**C**) Western blot experiments showing protein levels of OPA1 and DRP1 in cells treated with specific siRNA (*DRP1_si* or *OPA1_si*) or a non-relevant siRNA (*luc_si*). (**D**) Data show quantification of mitochondrial balloon phenotype 24, 48 or 72 h after transfection. $n = 300$ cells in each condition. (**E**) Mean of three independent experiments showing the percentage of cells that exhibit a balloon phenotype at 48 h when treated with TMEM11 siRNA (si1, si2) or *luciferase siRNA*. Differences as compared with *luc_si* were statistically significant: $^{*}P < 0.05$, $^{**}P < 0.01$. $n = 900$ cells in each condition. siRNA, short-interfering RNA.

(Fig 5A,C). Similar to TMEM11 siRNA-treated cells, OPA1-depleted cells had spherical mitochondria, but they were smaller and more numerous, consistent with mitochondrial fragmentation (Fig 5A,C). In conclusion, reduction of TMEM11 levels in human cells results in a condensation of the mitochondrial network with a loss of tubular shape.

DISCUSSION

PMI-depleted cells contain enlarged but less numerous mitochondria. As the overall mitochondrial volume within a given cell is identical in wild-type and *PMI*-mutant cells, the *PMI*-mutant phenotype is not a consequence of increased mitochondrial biogenesis (supplementary Fig S4B online). PMI/TMEM11 mitochondrial defects instead evoke the phenotype reported for mutants that affect mitochondrial fission (Bleazard *et al*, 1999; Fekkes *et al*, 2000; Mozdy *et al*, 2000; Cerveny *et al*, 2001; Smirnova *et al*, 2001; James *et al*, 2003; Tondera *et al*, 2005; Deng *et al*, 2008; Poole *et al*, 2008; Yang *et al*, 2008; Park *et al*, 2009; Zhao *et al*, 2009). Although *PMI*- and *drp1*-mutant mitochondria have similar morphological features, our results indicate that *drp1* and *PMI* regulate mitochondrial shape by independent mechanisms. This is unexpected as the fission proteins characterized so far are components of this pathway (James *et al*, 2003; Tondera *et al*, 2005; Deng *et al*, 2008; Poole *et al*, 2008; Park *et al*, 2009; Zhao *et al*, 2009). The mitochondrial-fission mutant phenotype is the result of unbalanced fusion leading to condensation of the mitochondrial network (Sesaki & Jensen, 1999; Fekkes *et al*, 2000; Mozdy *et al*, 2000; Cerveny *et al*, 2001; Chen *et al*, 2003; Deng *et al*, 2008; Poole *et al*, 2008; Park *et al*, 2009). However, we were unable to revert *PMI*-mutant phenotypes by reducing levels of the fusion-promoting genes *mfn/marf* or *opa1*. This demonstrates that the mitochondrial phenotype observed in flies lacking PMI does not result from *mfn*- or *opa1*-dependent hyperfusion. In contrast to core components of the fission/fusion machineries (*drp1*, *mfn/marf*, *opa1*), mutations of which induce developmental lethality in fly, PMI function is mostly dispensable for normal development. Consistently, we were unable to identify a yeast PMI orthologue, although such proteins exist for mitochondrial fission/fusion core proteins. To our knowledge, the only other described mutations that are epistatic to both fission (*drp1*) and fusion (*mfn*) genes are those affecting the yeast *mdm31* and *mdm32* genes. Although the mode of action of these proteins remains unknown, they control 'tubulation', a process that gives the mitochondria its default tubular shape (Dimmer *et al*, 2005). It has been proposed that these inner-membrane proteins might physically bridge both mitochondrial membranes in a manner that maintains the integrity of the organelle (Okamoto & Shaw, 2005). Although animals have tubular mitochondria, no tubulation gene orthologues have been found in higher eukaryote genomes, suggesting that tubulation might involve a different set of molecules. This could be a possible function for the inner-membrane proteins PMI/TMEM11. However, alternatively, PMI/TMEM11 could regulate fission/fusion processes at the level of the inner membrane, which are so far uncharacterized.

As mentioned above, PMI is transcribed from a bi-cistronic locus that also encodes PGRP-LD, a microbial-recognition protein. As all *Drosophila* bi-cistronic mRNAs analysed so far encode proteins that are implicated in the same biological process (Ben-Shahar *et al*, 2007), PGRP-LD might function together with PMI to regulate mitochondrial shape and function in response to infection. Further work will be required to address this issue. In mammals, the only TMEM11-identified interactor is BNIP3 (http://www.string-db.org), a Bcl2-like stress sensor that affects mitochondrial morphology and regulates mitophagy, cell death or survival in response to hypoxia and infection (Zhang *et al*, 2008; Carneiro *et al*, 2009; Chiche *et al*, 2009; Landes *et al*, 2010). Therefore, TMEM11 and PMI might be molecular switches that, on activation, adapt the mitochondria to different physiological needs.

METHODS

PM1_PGRP-LD-mutant locus was obtained by ends-out gene targeting. For *Pac[PMI_PGRP-LD]* rescue constructs, the *PMI_PGRP-LD* locus (fragment from 494 bp before *PMI* ATG to 282 bp after *PGRP-LD* stop) was recovered from BAC29P05 (BACPAC Resource Center) into *P[acman]* vector. In derived *Pac[PMI*_PGRP-LD]* and *Pac[PMI_PGRP-LD*]* constructs, frameshift mutations were added. The *PMI_PGRP-LD* locus was modified by recombineering to create the *Pac[CFP::PMI]* and *Pac[PMI::CFP]* reporters.

Drosophila stocks were maintained at 25 °C on a cornmeal-agar diet. *UAS-PM1, UAS-PM1::mCherry, Pac[PMI_PGRP-LD], Pac[CFP::PMI]* and *Pac[PMI::CFP]* transgenic lines were generated by *phiC31*-mediated transgenesis. $drp1^{1}$ and $drp1^{2}$ alleles and *Pac[drp1]* were obtained from Dr P. Verstreken; *UAS-mfn-IR, UAS-opa1-IR, UAS-drp1* from Dr M. Guo; *opa1, pink1* from Dr J. Chung and *UAS-mito::GFP* line, *elav-GAL4* from Bloomington stock center.

For *in situ* labelling of mitochondria, we used ATP synthase antibody (1/300; MitoSciences) on fixed tissues permeabilized in 0.5% Triton. MitoTracker CMXROS (Molecular Probe) and pDsRed2-Mito plasmid (Clontech) were used for staining on cell culture.

For western blot, we used TMEM11 antibody (1/500; Proteintech). siRNA for *luciferase* and *TMEM11* (si1: J-00540-09 and si2: J-005440-10) were from Dharmacon Research. For detailed experimental procedures see supplementary material online.

Supplementary information is available at EMBO *reports* online (http://www.emboreports.org).

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Author contribution: T.R., M.M. F.M. carried out the experiments and performed data analysis. L.A.-P. and P.B. designed and carried out the experiments on TMEM11. M.P. designed cloning approaches and realized all constructs involving recombineering technology. F.R. performed immunogold labelling. A.F. realized classical cloning and fly transgenesis. J.R. and T.R. together designed the research and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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