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1 **Title**

2 Is mitochondrial morphology important?

3

4 **Authorship**

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10

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12 mitochondrial morphology; fission and fusion; mitochondrial dysfunction; genetic disease,
13 mitochondrial dynamics

14

15 **Abstract**

16 Mitochondria are double membrane-bound organelles whose network morphology in cells is
17 shaped by opposing events of fusion and fission executed by dynamin-like GTPases.
18 Mutations in these genes can perturb the form and functions of mitochondria in cell and animal
19 models of mitochondrial diseases. An expanding array of chemical, mechanical, and genetic
20 stressors can converge on mitochondrial shaping proteins and disrupt mitochondrial
21 morphology. In recent years, studies aimed at disentangling the multiple roles mitochondrial
22 shaping proteins play beyond fission or fusion have provided insights into the homeostatic
23 relevance of mitochondrial morphology. Here, I will review the pleiotropy of mitochondrial
24 fusion and fission proteins with the aim of understanding whether mitochondrial morphology
25 is important for cell and tissue physiology.

26

27

28 Mitochondrial form and function

29 Mitochondria are double membrane-bound organelles that perform an armada of
30 metabolic, biosynthetic, and signaling functions to control the life and death of the cell [1,2].
31 Over 125 years ago, Karl Benda coined the term *mitochondria* (an amalgamation of the Greek
32 words for *thread* and *granules*), which still today aptly describes the morphology this essential
33 organelle adopts in most cell types. Landmark electron microscopy studies by Palade and
34 Sjöstrand in the 1950s provided insights into the ultrastructure of mitochondrial membranes
35 and depicted mitochondria as isolated and independent organelles within cells[3,4]. Recent
36 convergent advances in cell biology have allowed us to image cells with unprecedented
37 spatiotemporal resolution[5–7], allowing us to appreciate that mitochondria can modulate their
38 morphology and establish interconnected networks that span across the cell, contacting and
39 communicating with other organelles in a dynamic fashion and in ways that vary across cell
40 types, tissues, and organisms[8,9]. The opposing events of mitochondrial membrane fission
41 and fusion that are executed by dynamin-like GTPases: DRP1 performs fission at the outer
42 mitochondrial membrane (OMM) upon recruitment from the cytosol to specific OMM receptors
43 while Mitofusins (MFN1/2) perform OMM fusion, and OPA1 catalyzes inner mitochondrial
44 membrane (IMM) fusion[10]. The discovery of the first mitochondrial shaping proteins in yeast
45 in the 1990s[11] provided a molecular framework to understand how the genetic, chemical,
46 and mechanical lesions that disrupt their mammalian counterparts impact diverse
47 mitochondrial and cellular functions [2,10].

48 The spatial compartmentalization of mitochondrial functions, the maintenance of
49 cellular homeostasis over time, and the integration of adequate responses to stress relies on
50 the organization of the IMM and OMM. The OMM interacts with other subcellular organelles
51 such as the endoplasmic reticulum (ER), trans-Golgi network (TGN), lipid droplets (LD),
52 lysosomes, and cytoskeleton to regulate complex homeostatic and signaling processes[8,9].
53 The IMM delimits the matrix, where mitochondrial DNA (mtDNA) is replicated and expressed,
54 and extends internally to form cristae, which are convoluted invaginations that harbor essential
55 macromolecular complexes such as the machinery of oxidative phosphorylation (OXPHOS)
56 that synthesizes the bulk of cellular ATP. As such, cristae structure and density direct the
57 bioenergetic capacity of mitochondria[12] and insults that perturb their ultrastructure can lead
58 to reduced OXPHOS activity[13]. Similarly, chemical and genetic perturbations that tip the
59 balance of fission and fusion (Figure 1) often correlate with altered OXPHOS output leading
60 to the notion that hyperfused mitochondria increase bioenergetic capacity while shorter,
61 fragmented mitochondria are bioenergetically impaired[14–16]. More recently, self-supervised
62 deep learning studies were used to revisit the mitochondrial morphology changes triggered by
63 well-known mitochondrial poisons, which revealed intercellular and intracellular diversity of

64 mitochondrial shapes well-beyond what has been classified with simpler, supervised
65 approaches [17].

66 Beyond ATP synthesis, mitochondria also act as signaling organelles by orchestrating
67 multi-layered homeostatic cellular responses to a wide array of stressors in ways that have
68 been proposed to depend on mitochondrial morphology, membrane integrity and
69 composition[1,9]. An ample of body of literature has led to the inference that elongation of the
70 mitochondrial network confers protection against cell death signaling and mitophagy while
71 fragmented mitochondria facilitate these processes[10,18,19]. Here, we will revisit the
72 conventional wisdom that the overall network morphology of mitochondria is paramount for
73 metabolism and signaling at the level of the organelle, cell, and tissue.

74

75 **The relationship between mitochondrial form and function – cause or consequence?**

76 The long-standing notion that mitochondrial function is inextricably linked to the overall
77 network morphology in ways that are relevant for cellular and tissue homeostasis originated
78 from the interpretation of two separate groups of observations. The first involves situations
79 where targeted disruption the mitochondrial shaping proteins (such as OPA1, MFN1/2, DRP1
80 and its receptors) correlate with impairment of one or more mitochondrial functions, often with
81 negative repercussions at the level of the cell, tissue or organism[2,10,18]. The second arises
82 in cell or animal models in which genetic, chemical, mechanical, or infectious lesions trigger a
83 perturbation of mitochondrial morphology that can be ameliorated or fully corrected through
84 additional genetic or chemical interventions targeting mitochondrial dynamics[13,20–29]. For
85 the latter, improved mitochondrial form *and* function often correlates with rescued cell and
86 tissue functions, but this does not *per se* constitute formal proof of a causal role of imbalanced
87 mitochondrial dynamics in disease. In both situations, it is becoming increasingly evident that
88 the pleiotropic function of mitochondrial shaping proteins and the questionable specificity of
89 the chemical inhibitors designed to modulate their individual activities make it challenging to
90 draw definitive conclusions regarding the functional relevance of mitochondrial network
91 morphology.

92

93 **Pleiotropic functions of mitochondrial fusion factors**

94 **OPA1**

95 *OPA1* encodes a dynamin-like GTPase that localizes to the IMM where it executes
96 mitochondrial fusion[30,31]. *OPA1* ablation or mutation prevents IMM fusion, leading to a
97 fragmentation of the mitochondrial network due to unabated division[32–34]. Pathogenic
98 variants in *OPA1* are primarily associated with Dominant Optic Atrophy (DOA) and DOA “plus”,
99 which is a more severe, syndromic version of DOA involving extra-ocular symptoms, but also
100 Behr syndrome, familial forms of Parkinson’s Disease (PD) and rare forms of infantile

101 encephalomyopathy[35–40]. Hypomorphic *OPA1* patient-derived primary fibroblasts
102 generally show impaired mitochondrial fusion rates and a fragmented mitochondrial
103 network[13,34]. In mice, whole-body genetic deletion of *Opa1* is incompatible with embryonic
104 development and deletion in the skeletal muscle, nervous system, retina, heart, and immune
105 cell compartments triggers deleterious organ sequelae, highlighting the physiological
106 relevance of OPA1[41–48]. However, not all tissues appear to depend as heavily on OPA1
107 for function, as evidenced by hepatocyte-specific deletion studies that yield mice in seemingly
108 perfect health despite an absence of OPA1, mitochondrial fragmentation, and cristae loss
109 [49,50]. Indeed, physiological profiling of *Opa1* mutant mice carrying mutations akin to those
110 causing human diseases show peculiar tissue-specific defects, rather than organism-wide
111 dysfunction[47,51–55].

112 Disrupting OPA1 function triggers fragmentation of the mitochondrial network and
113 deleterious effects on cell and tissue health, which has been used as an argument for the
114 central role of OPA1 in mitochondrial fusion in safeguarding normal cell and animal
115 physiology[19]. However, OPA1 exercises pleiotropic functions within mitochondria that
116 extend beyond mitochondrial fusion, which include the shaping of the IMM into cristae[13,56–
117 58], the maintenance of mtDNA[39,59,60], maintenance of mitochondrial phospholipids such
118 as cardiolipin[13], assembly of OXPHOS complexes[13,61–63], ROS signaling[41,64–67], the
119 restriction of mitochondrial outer membrane permeabilization (MOMP)[68,69], and the release
120 of mtDNA to the cytoplasm that drives overactive innate immune signaling and
121 inflammation[41,42,70].

122 The role of OPA1 in mitochondrial fusion and network morphology regulation can be
123 separated from its other functions under certain experimental conditions[49,65,71]. For
124 example, synthetically suppressing mitochondrial division in OPA1-deficient cells through the
125 genetic ablation of DRP1 leads to a complete re-establishment of tubular mitochondrial
126 morphology in cultured cells without correcting IMM fusion defects[13,23,72–74]. This
127 correlates with an improvement OXPHOS activity, and mitochondrial membrane potential and
128 tumor cell growth in vitro (but not in vivo)[71,75]. However, repressing DRP1 in OPA1
129 deficiency does not fully rescue normal mitochondrial morphology in hepatocytes of mice[23]
130 nor in muscle of nematodes[76], highlighting a more complex in vivo situation. In neurons,
131 OPA1-dependent mitochondrial morphology and distribution defects can be restored via DRP1
132 ablation, but this does not rescue neuronal viability and function[74]. It is important to consider
133 that DRP1 ablation alone impairs mitochondrial and peroxisomal fission[77] and is associated
134 with altered bioenergetic efficiency[78,79], fatty acid oxidation[80], calcium buffering[79,81],
135 mitophagy[23,82,83], mitosis[27,84], impaired tumor growth[85], initiation of the integrated
136 stress response (ISR)[86] and cell death sensitivity[87,88], making it challenging to interpret
137 the phenotypic outcome of cells and tissues deficient for both OPA1 and DRP1. Inhibition of

138 mitochondrial fission in OPA1-deficient cells can also be achieved through the suppression of
139 PGS1, which encodes a phosphatidyl glycerophosphate (PGP) synthase in the IMM that
140 catalyzes the synthesis of PGP, a phospholipid precursor of cardiolipin (CL)[13]. In OPA1
141 mutant human and mouse fibroblasts, PGS1 ablation slows down mitochondrial fission to
142 rescue tubular mitochondrial morphology, yet this does not rescue OPA1-dependent cristae
143 disruption and these double mutant cells still manifest apoptotic sensitivity, mtDNA depletion,
144 and bioenergetic defects[13], consistent with the early observations that deletion of *Opa1*
145 disrupts apoptosis-induced cristae remodeling independently of mitochondrial fusion[56]. On
146 the other hand, in OPA1 knockout mouse fibroblasts, re-expression of wild type and GTPase-
147 dead OPA1 variants can ameliorate cristae structure, cell death sensitivity, and bioenergetic
148 efficiency without rescuing mitochondrial fusion and tubular network morphology[57]. In lung
149 adenocarcinomas, the ablation of either OPA1 or Mitofusins drives mitochondrial
150 fragmentation through impaired fusion, yet only OPA1 ablation is sufficient to alter tumor
151 growth[71], pointing to a role of OPA1 in tumorigenesis that goes beyond mitochondrial fusion.
152 In line, human fibroblasts derived from patients carrying pathogenic variants in *OPA1* that
153 compromise GTPase activity do not necessarily lead to mitochondrial fragmentation or cristae
154 dysfunction[13,34] but still manifest other mitochondrial and cellular dysfunctions[34,38].
155 Collectively, these studies reveal that it is possible to functionally uncouple the fusion roles of
156 OPA1 from other mitochondrial functions and reveals that counteracting mitochondrial
157 fragmentation is not necessarily sufficient to rescue organellar and cellular dysfunctions
158 caused by OPA1 loss. Therefore, given our current knowledge regarding its many
159 mitochondrial functions, it appears imprudent to attribute aberrant cellular dysfunction caused
160 by OPA1 deficiency to an impairment of mitochondrial network morphology per se.

161

162 **Mitofusins**

163 The Mitofusin *fzo*, whose *fuzzy onion* namesake reflects mitochondrial dysmorphology
164 observed in mutant drosophila spermatids, was the first mitochondrial fusion protein identified
165 in metazoans[89]. In mammals, OMM fusion is executed by two paralogs, Mitofusin 1 (MFN1)
166 and Mitofusin 2 (MFN2), which are dynamin-like GTPases anchored into the OMM that
167 coordinate membrane tethering and subsequent fusion[90,91]. OMM fusion activity, which is
168 crucial for maintaining an interconnected mitochondrial network in cells[90,92], occurs through
169 both homo- and heterotypic interactions and requires GTP hydrolysis. Deletion of either *Mfn1*
170 or *Mfn2* or inhibition of GTPase activity inhibits OMM fusion in cells, resulting in the
171 fragmentation of the mitochondrial network due to unopposed mitochondrial fission. In vivo,
172 homozygous deletion of either Mitofusin leads to embryonic lethality[92] and tissue-specific
173 ablation or expression of pathogenic variants in the central nervous system, heart, muscle,

174 liver, and immune cells impair organ function, underscoring physiological significance of
175 Mitofusins[93–98].

176 In vivo, studies comparing the phenotypic consequences of tissue-specific ablation of
177 MFN1 versus MFN2 have revealed markedly different phenotypic outcomes, which could
178 reflect the tissue-specific expression patterns of MFN1 and MFN2 and/or the distinct and
179 specialized roles they have evolved to play in organelle biology[95,97–107]. In humans,
180 pathogenic variants in *MFN2* (but not *MFN1*) cause Charcot-Marie-Tooth type 2A (CMT2A)
181 disease, a peripheral neuropathy characterized by axonal degeneration and distal muscular
182 atrophy[108]. Beyond OMM fusion, MFN2 interacts with OMM adapter proteins MIRO1 and
183 MIRO2 (encoded by *RHOT1* and *RHO2*, respectively) [10], which are required for the
184 trafficking and distribution of mitochondria along microtubules. It is therefore not surprising
185 that peripheral nerve axons are particularly sensitive to MFN2 deficiency. More recently,
186 pathogenic *MFN2* variants were discovered in patients suffering from multiple symmetric
187 lipomatosis (MSL) with or without peripheral neuropathy (a hallmark of CMT)[109,110], thus
188 expanding the clinical heterogeneity of Mitofusin dysfunction. No pathogenic variants in *MFN1*
189 have yet been attributed to human genetic diseases, but studies correlating decreased *MFN1*
190 expression with heart failure in patients manifesting idiopathic dilated cardiomyopathy suggest
191 that MFN2 expression alone may be insufficient to ensure tissue homeostasis in certain
192 contexts[111]. In a surprisingly large proportion of CMT2A and MSL patient-derived fibroblasts
193 deficient for MFN2, mitochondrial network morphology and fusion rates were not found to be
194 impaired[109,112–115], which points to either cell type-specific effects of MFN2-dependent
195 mitochondrial fusion and/or fusion-independent dysfunctions of mutant MFN2 contributing to
196 pathology[116].

197 Indeed, MFN2, but not MFN1, has been found to localize both to the endoplasmic
198 reticulum (ER) surface as well as the OMM[117,118]. The dual localization of *MFN2* gene
199 products facilitates the process of ER-mito tethering and the formation of mitochondrial ER
200 contact sites (MERCs), which are critical for inter-organelle metabolite and lipid exchange,
201 autophagy, mitochondrial fission, and cell death signaling[9]. Genetic deletion of *MFN2*
202 disrupts MERCs and also triggers the activation of an ER stress response[95,119,120]. In
203 addition to these defects organelle defects, ablation of MFN2, but not MFN1, impairs
204 ubiquinol synthesis[121], reduces bioenergetic efficiency [121,122], and energy
205 expenditure[102]. In macrophages MFN2, but not MFN1, is required for the reactive oxygen
206 species (ROS) signaling and cytokine production independently of mitochondrial fusion[123].
207 In Purkinje cells, MFN2, but not MFN1, is required for dendritic outgrowth, spine formation,
208 and cerebellar function[124], although whether this is due impaired OMM fusion and/or altered
209 mitochondrial trafficking is unclear. While both Mitofusins are required to maintain tubular
210 mitochondria under basal conditions in mouse embryonic fibroblasts (MEF) cells[92,125],

211 MFN2, and not MFN1, is dispensable for stress-induced mitochondrial hyperfusion (SiMH),
212 which is characterized by an elongation of the mitochondrial network that can be triggered by
213 nutrient starvation, amino acid deprivation, and translation inhibition [14,15,126]. The notion
214 that mitochondrial elongation protects against cell death is attributed in part to the fact that
215 genetic ablation of critical proteins required for SiMH (e.g. SLP2 and MFN1) sensitize cells in
216 culture to programmed and regulated cell death[14,15,126] which mirrors the observation the
217 ablation of DRP1, which promotes mitochondrial elongation, protects against apoptosis[87].
218 However, it there appears to be far less known regarding SiMH and cell death protection in
219 vivo.

220 Given their partially overlapping roles in mitochondrial fusion, genetic deletion of both
221 *Mfn1* and *Mfn2* was initially conceived to unequivocally block OMM fusion to drive
222 mitochondrial fragmentation. Double knockout (DKO) cells show more dramatic disruption of
223 mitochondrial morphology than single Mitofusin mutants [92], highlighting cooperativity in
224 OMM fusion. In mice, tissues devoid of both MFNs generally exhibit more severe
225 mitochondrial, cellular, and physiological defects[28,120,122,124,127,128], although there is
226 no evidence this is a direct result of impaired OMM fusion. Double MFN1/MFN2 knockout
227 (DKO) in skeletal muscle, but not cardiac muscle[128], drives the accumulation of mtDNA
228 mutations and deletions[127], sustaining the prevailing theory that mitochondrial fusion is
229 required to ensure matrix content mixing, maintain mtDNA copy number, and prevent the
230 accumulation of pathogenic mtDNA variants[19]. This theory is predicated on the observation
231 that promoting mitochondrial elongation through the inhibition of DRP1 in heteroplasmic cells
232 carrying supra-threshold levels of pathogenic mtDNA can modulate mtDNA
233 heteroplasmy[129]. As previously mentioned, DRP1 ablation alone induces pleiotropic cellular
234 defects, including disruption of nucleoid dynamics[130,131], cristae structure[71,78,131],
235 mitophagy[132], and mitosis[133] (Figure 4), making it difficult to ascribe heteroplasmic shifts
236 exclusively to changes in mitochondrial morphology. Moreover, OPA1 ablation, which also
237 impairs mitochondrial fusion, does not trigger the same defects in mtDNA nucleoid dynamics
238 as the ablation of Mitofusins[128]. Therefore, given the multiple roles of Mitofusin beyond
239 fusion, particularly those linked to mitophagy, transport, and bioenergetic efficiency, there is
240 insufficient evidence to support the hypothesis that defective mitochondrial membrane fusion
241 is responsible for defects in mtDNA stability.

242 The chemical toolkit to manipulate Mitofusin function has expanded in the last years.
243 First, medium-throughput chemical screening identified Leflunomide as an MFN1/MFN2-
244 dependent inducer of mitochondrial fusion, which acts through the mitochondrial de novo
245 pyrimidine synthesis pathway to boost *MFN1* and *MFN2* transcription and, consequently,
246 mitochondrial fusion[134]. Leflunomide, a clinically-approved drug used in the management
247 and treatment of rheumatoid arthritis, inhibits dihydroorotate dehydrogenase (DHODH), which

248 is the IMM enzyme that catalyzes oxidation of dihydroorotate into orotate using ubiquinone as
249 co-substrate electron acceptor. Leflunomide can dampen doxorubicin-induced cell death in
250 wild type but not DKO MEFs, which is reminiscent of the increased cell death resistance
251 conferred upon induction of SiMH[14]. Yet, whether cell death resistance by Leflunomide is
252 caused by mitochondrial elongation or is related to the inhibition of pyrimidine synthesis and/or
253 the transfer of electrons to ubiquinone (whose biosynthesis is also under the control of
254 MFN2[121]) has not been determined. An independent chemical screening campaign
255 identified mitochondrial fusion inducer hydrazone M1[135] as an inducer of mitochondrial
256 elongation acting in an MNF1/MFN2 and OPA1-dependent manner. In cells, M1-induced
257 mitochondrial elongation correlates with reduced cytochrome *c* release and increased
258 apoptotic resistance, yet this drug also affects the levels of Complex V, which is critical for
259 cristae remodeling and cell death signaling via OPA1[64] and mitochondrial motility[135]. M1
260 provides remarkable benefits against insults that damage mitochondria both in vitro and
261 vivo[29,136], but until its mechanism of action and target(s) have been clarified, this molecule
262 provides limited additional insights into the relevance of OMM fusion. Finally, the most
263 promising new tools to dissect the relevance of OMM fusion for cellular health emerged from
264 the creation of rationally-designed HR1 mini-peptide therapies, which aimed to rescue
265 mitochondrial dysfunction caused by CMT2A mutations. These peptides were able to restore
266 inter-mitochondrial tethering and fusion activities that are disrupted by pathogenic MFN2
267 variants, effectively rescuing mitochondrial fragmentation, trafficking, and depolarization and
268 significantly improves physiological outcomes in MFN2 cell and mouse models[137,138].
269 Altogether, the cellular and organismal consequences that derive from the chemical and
270 genetic manipulation of Mitofusins has highlighted their biological relevance and their
271 importance in organellar dynamism.

272 Recently, the discovery that ER and mitochondrial functions of MFN2 can be
273 functionally and physically uncoupled provided new ground to explore the relevance of OMM
274 fusion. Alternative splicing of the *MFN2* gene yields distinct ER resident proteins ERMIT2 and
275 ERMIN2, which resemble truncated versions of MFN2 yet lack a fully competent GTPase
276 domain[139]. ERMIT2 and ERMIN2 are expressed in various mouse and human tissues, with
277 only the former being required to mediate ER-mito tethering by anchoring into the
278 mitochondrial associated membranes (MAM) of the ER[139]. ERMIT2 and ERMIN2
279 expression in MFN2-deficient MEFs was unable to rescue mitochondrial morphology however
280 MFN2 or ERMIN2 expression could ameliorate aberrant ER morphology[139]. Synthetic
281 rescue of calcium homeostasis and lipid transfer defects by ERMIT2 in MFN2-deficient cells
282 and liver-specific knockout mice highlights the functional relevance of the ER-mito tethering
283 function of Mitofusins[9], which can be uncoupled from their OMM fusion activities.
284 Determining whether this paradigm holds true in other tissues and whether other signaling

285 and biosynthetic defects caused by MFN2 ablation (Figure 3) rely primarily on ER-related
286 functions of the *MFN2* gene will provide significant insights into the functional importance of
287 organellar contacts and OMM fusion alike.

288

289 **Pleiotropic functions of mitochondrial fission factors**

290 **DRP1**

291 Dynamamin-related protein 1 (DRP1) is a highly conserved protein of the Dynamamin family
292 that is required for mitochondrial fission in yeast, nematodes, and mammals[10]. DRP1,
293 encoded by the *DNM1L* gene, is a cytosolic protein that dynamically localizes to both
294 mitochondrial and peroxisomal membranes, where it oligomerizes and drives membrane
295 constriction in a GTP-dependent manner[10]. Drp1-mediated mitochondrial fission occurs at
296 membrane contact sites with the endoplasmic reticulum (ER)[140], the trans-Golgi network
297 (TGN)[141], and the actin cytoskeleton[142] and is regulated by post-translational
298 modifications on DRP1 at the OMM[10] and is by replication of mtDNA at the IMM[143] (Figure
299 4). Membrane recruitment of DRP1 in mammals is mediated by membrane-anchored OMM
300 proteins that act as receptors mitochondrial fission factor (MFF)[144], mitochondrial dynamics
301 protein of 49 kDa (MiD49, encoded by *MIEF2*)[145], MiD51 (encoded by *MIEF1*) and
302 mitochondrial fission 1 protein (FIS1)[146]. The relative contribution of these four DRP1
303 receptors to mitochondrial division has been judged by the impact their individual and
304 combinatorial ablation have on overall mitochondrial network elongation, DRP1 recruitment to
305 mitochondria, and protection against stress-induced mitochondrial fission (SiMF)[147]. In
306 MEFs, equivalent and dramatic alterations of mitochondrial morphology were observed in both
307 MFF/MiD49/MiD51 triple KO (TKO) and quadruple MEFs lacking all 4 proteins, which
308 suggested that MFF, MiD49, and MiD51, but not FIS1[144,147], execute cooperative roles in
309 mammalian mitochondrial division. Despite their namesakes, both MFF and FIS1 are also
310 involved in peroxisomal division and their ablation leads to peroxisomal and mitochondrial
311 elongation[148,149] while MiDs are exclusively mitochondrial. MiDs contain a
312 nucleotidyltransferase domain, and MiD51 necessitates ADP as a co-factor to stimulate both
313 DRP1 oligomerization and GTPase activity. Although these receptors co-localize with DRP1
314 at ER-constriction sites in distinct foci[73,150], MFF and MiD49/51 can independently
315 influence DRP1 recruitment and activity[147]. This has led to the notion that MFF and MiDs
316 have distinct yet complementary roles in mitochondrial fission: MiDs recruit the GTP-bound
317 state of DRP1 to facilitate oligomerization, whereas MFF selectively recruits oligomeric and
318 active forms of DRP1 [151]. Intriguingly, genetic disruption of MFF[152] or MiD49[153] in
319 humans triggers mitochondrial elongation that can be observed in patient-derived fibroblasts
320 and is associated with a severe neurodevelopmental disorder in the case of MFF[152,154]
321 and a pure myopathy in the case of MiD49 (encoded by *MIEF2*)[153].

322 Recently, time-lapse imaging studies performed with unprecedented spatiotemporal
323 resolution allowed for the tracking individual membrane scission events along the
324 mitochondrial network, which revealed that under physiological conditions, DRP1-dependent
325 mitochondrial division occurs either in the mid-zone, requiring MFF, or at the periphery, where
326 FIS1 is required[155]. Resulting daughter mitochondria borne of mid-zone fission had on
327 average, higher metabolic activity and quality and were more likely to engage in subsequent
328 mitochondrial fusion events compared to daughter mitochondria generated from peripheral
329 fission, which typically yielded smaller, more fragmented mitochondria harboring higher
330 damage and lower mitochondrial membrane potential. Hence, the molecular composition of
331 the fission machinery on the OMM impacts where, when and how mitochondria divide,
332 although it remains to be determined whether such dichotomous mitochondrial division
333 decisions occur in vivo. Beyond organelle division, DRP1 was found to be required for the
334 formation of mitochondrial derived vesicles (MDVs, reviewed elsewhere[156]); an intercellular
335 vesicular transport system of specific cargo originating at mitochondria implicated in cell
336 signaling and quality control[157]. While both MDV formation and peripheral mitochondrial
337 fission depend on DRP1, the vesicle composition, size and molecular adapters involved
338 appear to be different[155,157]. DRP1 and its receptors are subject to various layers of post-
339 translational regulation that can modulate mitochondrial division rates under physiology and
340 pathological conditions and perturbation of these upstream regulators can negatively impact
341 the proliferation, identity, and fate of cells[133,158–162] (Figure 4).

342 DRP1 ablation in various cellular contexts and animal models results in notable
343 elongation of both mitochondria and peroxisomes[163,164] and leads to a wide range of
344 pleiotropic defects in cells and animal models. In humans, patients harboring pathogenic
345 variants in *DNM1L* typically present with a devastating encephalopathy due to defective
346 mitochondrial and peroxisomal fission (EMPF1) [77,78,165] with a minority of patients
347 manifesting more minor neurological symptoms[166,167]. In mice, homozygous deletion of
348 *Dnm1l* is embryonically lethal[168] and heterozygous mutant mice carrying loss-of-function
349 variants exhibit peroxisomal and mitochondrial dysfunction, reduced lifespan, and
350 multisystemic defects including cardiac failure[169]. Surprisingly, short-term ablation of DRP1
351 in the heart appears benign under basal conditions. In fact, the genetic deletion in post-natal
352 cardiomyocytes[170] or chemical inhibition using Drpitor1a (a Drp1 GTPase inhibitor), mdivi-
353 1 (an inhibitor of yeast Dnm1)[171], P110 (an inhibitor of DRP1-FIS1 interaction)[172], or
354 hydralazine (an anti-hypertensive heart failure medication purported to inhibit GTPase
355 activity)[173] all promote mitochondrial elongation and cardioprotective advantages against
356 surgically-induced ischemia-reperfusion (IR) or pressure-overload injury[170–173].
357 Dampening DRP1 activity is associated with improved injury outcome and effectively curtails
358 the deleterious effects on oxidative damage, MOMP, calcium retention capacity, and cell

359 death. On the other hand, long-term DRP1 ablation in cardiomyocytes causes irreparable
360 damage to cardiac function[28,174], promoting MOMP, mitophagy, and cardiomyocyte cell
361 death. Despite a key role of DRP1 in peroxisomal fission, the form and function of this
362 organelle were not evaluated in any of these studies, and so it remains to be determined how
363 DRP1 influences cardiac function.

364 Inhibition of DRP1 in the brain is associated with adverse neurological and behavioral
365 effects, with heterogeneous effects depending on the region or cell type of the brain that is
366 targeted [163,175,176] and some of these effects appear to be independent of mitochondrial
367 fission[177]. DRP1 ablation has deleterious effects on the axonal trafficking of mitochondria
368 (but not other organelles) and synaptic function, yet but whether these defects are caused by
369 alterations in mitochondrial morphology or due to defects in OXPHOS and/or calcium buffering
370 remain elusive [175,178,179] . On the other hand, in vivo chemical inhibition of DRP1 has
371 been proposed to alleviate neurotoxicity in mouse models of neurodegeneration[180–182],
372 although the inadequate target specificity (e.g., mdivi1)[183,184], limited efficacy (e.g.
373 P110)[185], and limited solubility (e.g., Drpitor1a)[186] have complicated the interpretation of
374 in vivo studies, however spectacular the reported phenotypic improvements of drug-treated
375 animals may be.

376 In the liver, genetic deletion of *Dnm1l* in hepatocytes causes liver damage, impaired
377 fatty acid metabolism, reduced weight gain, and protection against high fat diet (HFD)-induced
378 dysregulation of glucose homeostasis. DRP1 ablation promoted mitochondrial largening, ER
379 stress, mitophagy, and activation of the integrated stress response (ISR)[23,187]. Silencing
380 of *Dnm1l* in the liver of mice using N-acetyl galactosamine-siRNA conjugates had similar
381 negative effects and promoted Metabolic Dysfunction-Associated Steatohepatitis (MASH,
382 formerly NASH)[86]. Notably, inhibition of OPA1 in DRP1-deficient hepatocytes was sufficient
383 to improve mitochondrial shape and rescue mice from liver damage and defective liver
384 mitophagy. However, these hepatocytes exhibited exacerbated cristae loss (relative to
385 individual OPA1 or DRP1 knockouts) without any observable physiological dysfunctions,
386 similarly to the knockout of Complex I of the ETC[188]. Unfortunately, none of these studies
387 explored the impact of DRP1 ablation on the form nor function of peroxisomes, which are
388 established as regulators of hepatic metabolism in health and disease[189].

389

390 **OMA1**

391 OMA1, so called for its overlapping activity with the *m*-AAA protease, is a zinc-
392 dependent mitochondrial protease anchored in the IMM that is responsible for the proteolytic
393 cleavage and degradation of substrates in the IMM and IMS[190,191]. The most well-studied
394 substrate of OMA1 is OPA1, whose cleavage by at the S1 site by OMA1 converts L-OPA1 to
395 S-OPA1 (Figures 2 and 5). Under basal conditions, the modest proteolytic activities of OMA1

396 and the *i*-AAA protease (encoded by *YME1L1*), which cleaves at S2 to yield similar isoforms
397 of S-OPA1, strikes a balance between L-OPA1 and S-OPA1 to maintain tubular mitochondrial
398 morphology in cells[33,192]. An armada of stress conditions can trigger the hyperactivation
399 of OMA1 in vitro and in vivo, tipping the balance of S-OPA1 at the expense of L-OPA1, driving
400 the mitochondrial network to fragment due to a combination of accelerated fission (S-OPA1)
401 and reduced IMM fusion (L-OPA1)[18]. Ablation of OMA1 alone does not alter mitochondrial
402 morphology yet protects against SiMF in a manner similar to the ablation of DRP1 or
403 PGS1[13,193]. Unlike other mitochondrial shaping proteins, the whole-body ablation of OMA1
404 yields healthy knockout mice in which the only phenotypes reported thus far are mild
405 alterations in response to cold challenge or high fat diet feeding when knockout mice are bred
406 on a mixed genetic background[20,21,192]. In vivo, whole-body or tissue specific ablation of
407 OMA1 confers substantial protection against genetic, pharmacological, and surgical lesions
408 that trigger mitochondrial fragmentation, cellular dysfunction, and organ failure, pointing to the
409 restorative and therapeutic potential of rebalancing mitochondrial morphology in disease[20–
410 22,24,194]. Yet, other studies have demonstrated that OMA1 ablation is not the panacea for
411 all disorders characterized by imbalanced OPA1 processing. For example, in knockin mice
412 carrying the pathogenic G58R mutation in the IMS protein CHCHD10, which causes a rare
413 autosomal-dominant mitochondrial myopathy and cardiomyopathy characterized by
414 imbalanced OPA1 processing and mitochondrial fragmentation, the additional ablation of
415 OMA1 accelerated mortality and dysfunction of *Chchd10*^{G58R} mice[195]. In the case of
416 *COX10*, whose disruption in humans is responsible for congenital cardiomyopathy [196],
417 cardiomyocyte-specific *Cox10* knockout causes an early-onset cardiomyopathy that is
418 accelerated, not diminished, by the additional ablation of OMA1[197]. Finally, deletion of
419 *YME1L1* in the central nervous system leads to the hyperactivation of OMA1 and
420 fragmentation of axonal mitochondria, promoting neurodegeneration, paralysis, and
421 death[198]. Inhibiting OMA1 in the CNS of these mice restores wild type mitochondrial
422 morphology yet worsens the progression of the disease. While ablation of *YME1L* triggers
423 OMA1-dependent fragmentation of the mitochondrial network in both the CNS and heart[20],
424 the beneficial effects of OMA1 inhibition exhibit tissue specificity that is independent of
425 mitochondrial morphology, which could be explained either by the action of other OMA1
426 substrates whose proteolytic cleavage is required for this activation (Figure 5). Recently, a
427 newly identified OMA1 substrate DELE1 (DAP3 Binding Cell Death Enhancer 1), emerged
428 from CRISPR screens deployed to identify regulators of the integrated stress response
429 (ISR)[199,200]. DELE1 is a nuclear-encoded IMM-associated protein whose OMA1-
430 dependent cleavage enables its export from mitochondria to the cytosol, where it triggers the
431 engagement of the eIF2alpha and ATF4 signaling pathway (Figure 5)[201]. Ablation of either
432 OMA1 or the cleavage of DELE1 blunts the ISR, which has shown to be detrimental or

433 advantageous in various disease contexts, including in models of mitochondrial disease[202–
434 204]. Unfortunately, the multifaceted nature OMA1 and the incomplete cartography of its many
435 substrates cast considerable doubt over the specific functional relevance of the OMA1-OPA1
436 axis. Fortunately, new genetic tools that allow for the inactivation of proteolytic cleavage sites
437 in OPA1 or ablate DELE1 altogether, while keeping OMA1 activity intact, hold the potential to
438 help explain how these OMA1 substrates contribute to cellular homeostasis[205].

439

440 **Mitochondrial transport and distribution**

441 Mitochondrial dynamics is often synonymously employed to refer to mitochondrial
442 membrane fission and fusion events, but it also encapsulates the dynamic movement and
443 motility of mitochondria. In mitotic cells, mitochondrial fragmentation and fission at metaphase
444 is a pre-requisite for the appropriate segregation of individual mitochondria to daughter cells
445 following cytokinesis. Interfering with the mitochondrial fission disrupts cell cycle progression
446 and instigates mitotic spindle defects, chromosomal instability, replication stress and
447 G2/M arrest (reviewed elsewhere[206]).

448 In post-mitotic cells, the intracellular trafficking and movement of mitochondria has
449 been best studied in polarized cells such as neurons[207]. Neurons exhibit significant
450 heterogeneity in mitochondrial morphology: axonal mitochondria are round, fragmented, and
451 sparse, dendritic mitochondria are longer and more densely packed whereas the soma
452 contains an interconnected network of mitochondria reminiscent of what is observed in
453 epithelial cells. In neurons, transport is accomplished by latching mitochondria onto
454 microtubules tracks through motor adaptor complexes that can walk mitochondria in the plus-
455 end (anterograde) or minus-end direction. Anterograde movement of mitochondria is driven
456 by kinesin-1 motor Kif5B while retrograde transport is directed by Dynein/Dynactin, both of
457 which tether to the OMM through the motor-binding protein Milton (encoded by TRAK1/2) and
458 the OMM-anchored adaptor MIRO1 and MIRO2, calcium-binding GTPases that physically and
459 functionally interact with Mitofusins[207] and the neuron-specific mitochondrial protein
460 syntaphilin (SNPH), which control mitochondrial motility and quality[208–210]. Anterograde
461 trafficking of mitochondria from the soma throughout the axon serves to meet the metabolic
462 and biosynthetic demands of the entire neuron while retrograde motility was initially proposed
463 to facilitate recycling and degradation at the soma[211]. However, there are conflicting reports
464 regarding the differential statuses and functionalities of mitochondria that move (or not) in
465 different directions [212–214] as well as the true contribution of axonal organelle quality control
466 that must be performed at the soma[207,215]. Nevertheless, the dynamic regulation of
467 mitochondrial morphology appears to be molecularly and functionally connected to motility
468 and transport. In healthy neurons, timelapse imaging of anterograde and retrograde trafficking
469 show that mitochondria occur with organelles of a specific size range, suggesting that cargo

470 size may be both rate-limiting and influenced by mitochondrial shape[216–219]. Indeed,
471 fission and fragmentation are required to dissociate mitochondria from an extensively
472 interconnected network in the soma and permit entry into the axon, suggesting that reducing
473 mitochondria to a defined size is a prerequisite for axonal trafficking. In cortical axons,
474 mitochondria enter with a size of ~0.1 to 1µm and maintain these short, uniform lengths along
475 the axon through continuous events of fusion and fission[219,220]. The conditional ablation of
476 DRP1 in mouse dopaminergic neurons promotes mitochondrial elongation and compromises
477 both anterograde and retrograde trafficking of mitochondria, leading to depletion of axonal
478 mitochondria, neuronal cell death, and the manifestation of levodopa-responsive PD[178]. In
479 flies, *drp1* was identified in a forward genetic screen for genes required for the synaptic
480 localization of mitochondria but not for their function in the soma, further underscoring the that
481 elongated mitochondria disrupts their proper trafficking[179]. However, impaired the
482 mitochondrial motility in DRP1-deficient mouse neurons can be partially rescued by
483 exogenous addition of ATP, which suggests that bioenergetic and cristae defects resulting
484 from DRP1 deficiency may also underlie improper axonal distribution of mitochondria [175].

485 So, when it comes to mitochondrial trafficking does size really matter? In the neurons
486 of flies, the different lengths of individual mitochondria followed by timelapse imaging, ranging
487 of ~0.1 to 1 µm, do not have an appreciable impact on run velocities[220–222]. Yet,
488 suppression of MFF expression in mouse cortical neurons slows down mitochondrial fission
489 rates, promotes mitochondrial elongation in axons (but not dendrites), and effectively hampers
490 the axonal entry of mitochondria without impairing mitochondrial motility along the axon nor
491 mitochondrial membrane potential, matrix ATP levels, or redox potential[219]. Hence, there
492 appears to be compartment-specific dependencies of mitochondrial morphology for axonal
493 entry but not necessarily for axonal transport. Intriguingly, fragmentation of the mitochondrial
494 network observed in primary retinal ganglion cell (RGC) neurites from an *Opa1*^{Q285STOP} mouse
495 model for DOA was associated with increased mitochondrial motility[223]. While OXPHOS
496 capacity was also compromised in *Opa1*^{Q285STOP} neurites, it is unlikely that this is responsible
497 for the increased mitochondrial motility given that treatment of wild type mouse cortical
498 neurons with mitochondrial poisons is sufficient to attenuate mitochondrial motility without
499 altering mitochondrial morphology[218]. However, mitochondrial fragmentation is not
500 necessarily associated with more rapid axonal trafficking. Expression of pathogenic variants
501 in MFN2 associated with CMT2A in of dorsal root ganglion cultures also trigger mitochondrial
502 fragmentation yet reduce both anterograde and retrograde motility of mitochondria [224]. As
503 MIRO2 ablation phenocopies the motility and distribution defects of MFN2-deficient
504 mitochondria[74] but does not cause mitochondrial fragmentation, it is likely that mitochondrial
505 trafficking defects in CMT2A reflect the key role of MFN2 as part of the adaptor complex
506 between mitochondria, kinesin motors, and microtubules, rather than a consequence of

507 impaired OMM fusion. In line, inhibition of mitochondrial fission via the suppression of Drp1
508 can rescue motility and distribution of Opa1-deficient but not Mitofusin-deficient mitochondria
509 in neurons[74]. However, restoring normal motility and distribution in Opa1/Drp1-deficient
510 neurons did not rescue cell viability, cristae disruption, nor OXPHOS dysfunction, highlighting
511 once again the separable bioenergetic and signaling functions of mitochondrial shaping
512 proteins[13,74]. Altogether, these data argue that mitochondrial fragmentation observed in a
513 variety of inborn and acquired neurological disorders may not be the primary cause of
514 pathology.

515

516

517

518 **Concluding remarks**

519 The field of mitochondrial dynamics has grown in leaps and bounds since the earliest
520 pictorial hand-drawn representations of mitochondrial fission, fusion, and motility by Margaret
521 Lewis[225]. The last years have seen significant advances in our understanding of the
522 molecular machinery that is required to fuse, divide, and traffic mitochondria within mammalian
523 cells[10]. High-throughput chemical and genetic screening are continuing to reveal complex
524 networks and layers of molecular regulation that fine-tune these dynamic processes while
525 advances in microscopy have helped mature our understanding of the function of membrane
526 contact sites between mitochondria and other organelles including the endoplasmic reticulum,
527 Golgi network, and lysosomes, each of which heralds different fates for the future sites of
528 mitochondrial membrane remodeling and cell signaling[9]. These studies also have provided
529 a foundation for our understanding of how mitochondria receive signals to modify their form
530 and functions while also providing us with a finer appreciation for the tremendous
531 heterogeneity in mitochondrial morphology that exists within single cells, which is a
532 phenomenon whose biological significance we have yet to fully comprehend[1,2]. Moving
533 forward, technological advances in super-resolution and event-driven microscopy, chemical
534 probe development, artificial intelligence, and image analyses will be invaluable tools for the
535 functional exploration of the intracellular heterogeneity of mitochondrial morphology, which, in
536 turn, will help expand our *visual vocabulary* of mitochondrial structures and behaviors beyond
537 the simplified classification schemes that have populated earlier reviews on the topic ([Figure](#)
538 [1](#)). In so doing, we can expect these explorations to yield exciting discoveries regarding the
539 role of mitochondrial shaping proteins and provide a holistic understanding of how
540 mitochondria respond to stress and broadcast signals to the rest of the cell to determine its
541 fate and functions.

542

543 **Glossary**

544 **Autosomal dominant optic atrophy (ADOA):** inherited bilateral degeneration of the optic
545 nerve caused by pathogenic variants in *OPA1* and the most common autosomally inherited
546 optic atrophy in ~80% of patients. Approximately 20% of *OPA1* patients manifest ADOA or
547 DOA “plus”, a more severe, syndromic version of DOA involving extra-ocular symptoms.

548

549 **Behr syndrome:** an autosomal recessive, early-onset optic atrophy accompanied by
550 spinocerebellar degeneration resulting in ataxia, peripheral neuropathy, intellectual disability,
551 and developmental delay associated with pathogenic variants in *OPA1*.

552

553 **Cardiolipin (CL):** a non-bilayer-forming phospholipid unique to mitochondria required for the
554 optimal function of various mitochondrial enzymes and macromolecular complex formation,
555 mitochondrial fusion, and cristae formation.

556

557 **Charcot-Marie-Tooth type 2A (CMT2A):** a hereditary motor and sensory neuropathy
558 belonging to the most common class of inherited neurological disorders. Type 2A is caused
559 by mutations in *MFN2*.

560

561 **Drpitor1a:** a small molecule DRP1 inhibitor that was identified by in silico screening and
562 validated by its ability to prevent mitochondrial fragmentation in cultured cells. Drpitor1a
563 exhibits greater potency than mdivi-1 and confers resistance against cardiac ischemia-
564 reperfusion injury and tumor growth in mouse models.

565

566 **Encephalopathy due to defective mitochondrial and peroxisomal fission-1 (EMPF1):** a
567 neurological disorder characterized by delayed psychomotor development, hypotonia and, in
568 many cases, childhood mortality. EMPF1 is caused by pathogenic variants in *DNM1L*,
569 inherited in autosomal dominant or recessive fashion.

570

571 **Integrated Stress Response (ISR):** is a stress-induced signaling pathway that can be
572 triggered by mitochondrial and metabolic dysfunctions, proteotoxic stress, and pathogen
573 infection. Four distinct cytosolic kinases, PERK, PKR, GCN2, and HRI, can converge to
574 phosphorylate the alpha subunit of translation initiation factor 2 (eIF2 α) in response to distinct
575 stimuli. ISR signaling can be initiated by DELE1, whose cleavage by OMA1 inside
576 mitochondria promotes DELE1 translocation to the cytosol where it activates HRI.

577

578 **M1 Hydrazone:** a cell-permeable hydrazone compound that enhances mitochondrial fusion
579 in a Mitofusin and *OPA1*-dependent manner. M1 does not alter ER nor lysosomal
580 morphologies. Its mechanism of action is unknown.

581

582 **Metabolic Dysfunction-Associated Steatohepatitis (MASH):** previously known as NASH
583 (Non-alcoholic Steatohepatitis) is a stage of fatty liver disease that is characterized by
584 inflammation (hepatitis) and liver damage that can lead to cirrhosis, liver failure and
585 hepatocellular carcinoma.

586

587 **Mdivi-1:** first-in-class inhibitor of DRP1 identified in phenotypic synthetic rescue screen of
588 yeast mutants defective for mitochondrial fusion (*fzo-1*) whose survival could be rescued by
589 inhibiting mitochondrial fission. Mdivi-1 attenuates mitochondrial fission in yeast and human
590 cells but also interferes with Complex I activity in mammalian cells.

591

592 **Mitophagy:** the selective, autophagic removal of defective or dysfunctional mitochondria.
593 Several pathways exist, including the PINK1-PARKIN pathway in which dissipation of the
594 mitochondrial membrane potential causes PINK1 to translocate to the OM, where it is
595 recognized by the E3 ubiquitin ligase PARKIN that marks it for lysosomal degradation.
596 Mitophagy receptors including NIX1, BNIP3, and FUNDC1 contain LIR domains that bind LC3
597 proteins to promote mitochondrial degradation.

598

599 **Mitochondrial-Endoplasmic Reticulum Contact Sites (MERCs) :** sites at which the OMM
600 and the ER surfaces are juxtaposed at constant distances (usually <30 nm.). MERCs serve to
601 facilitate the inter-organellar transfer of phospholipids and Ca²⁺ and act as nodes governing
602 mitochondrial fission, cell death signaling, and autophagy.

603

604 **Mitochondrial Outer Membrane Permeabilization (MOMP):** Regulated increase in OMM
605 permeability through the oligomerization of Bcl-2 family proteins BAX and BAK to form pores
606 that allow molecules typically sequestered inside mitochondria to escape to the cytosol to
607 trigger apoptosis and/or innate immune signaling. MOMP can be initiated by a variety of
608 chemical, genetic, and infectious triggers.

609

610 **Oxidative phosphorylation (OXPHOS):** electron transfer along the respiratory chain
611 complexes (I–IV) in the IMM driving the accumulation of a proton gradient that is harnessed
612 by ATP synthase (complex V) to produce ATP from ADP and inorganic phosphate

613

614 **P110:** a seven amino acid peptide that is designed to inhibit pathological mitochondrial
615 fragmentation by inhibiting the physical interaction between DRP1 and FIS1. P110 peptide
616 targets amino acids 49 through 55 in region 110 of DRP1 and does not affect interactions with
617 other adaptors MiD51, MiD49, and MFF.

618

619 **Parkinson's Disease (PD):** neuromuscular disorder characterized by dopamine deficiency
620 and basal ganglia degeneration. Pathogenic variants in *PARK2* (which encodes PARKIN) and
621 *PINK1* are frequent causes of early-onset PD. Mutations in *OPA1* have also been associated
622 with PD.

623

624 **Peroxisomes:** single membrane-bound organelles responsible for fatty acid oxidation, ether
625 phospholipid biosynthesis, and ROS metabolism. Mitochondria contribute to the de novo
626 biogenesis of peroxisomes, which can also grow and divide from pre-existing peroxisomes.

627

628 **Purkinje cells:** a type of neuron exclusive to the cerebellar cortex characterized by extensive,
629 flat dendritic trees replete with intricate branching. Purkinje cells control and coordinate motor
630 activity and their degeneration are associated with a variety of mitochondrial and
631 neurodegenerative disorders.

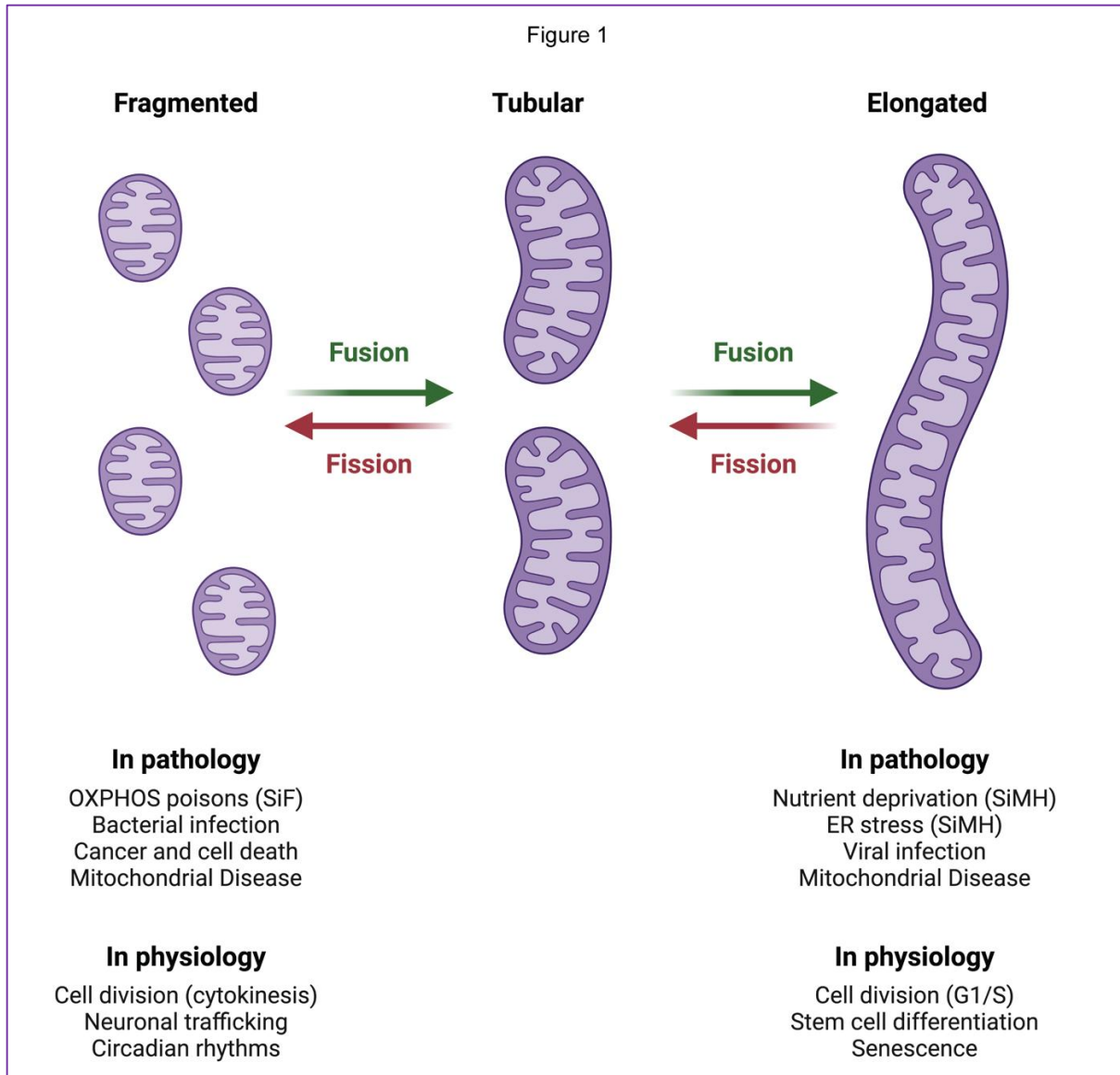
632

633 **Stress-induced mitochondrial hyperfusion (SiMH):** a protective response that constitutes
634 the elongation of mitochondrial tubules in response to stress conditions including starvation,
635 amino acid deprivation, UV irradiation, cytosolic translation inhibition, that would otherwise
636 induce cell death. SiMH depends upon mitochondrial fusion and MFN2 (but not MFN1), SLP2,
637 and L-OPA1.

638

639 **Stress-induced fission (SiF):** mitochondrial network fragmentation triggered by various
640 genetic, chemical, or infectious lesions that promote the excessive fission of mitochondria.
641 SiF is characterized by the cleavage of L-OPA1 by the mitochondrial metalloprotease OMA1,
642 causing a loss of profusion L- OPA1 and accumulation of the pro-fission S-OPA1, recruitment
643 of DRP1 to the OMM, and mitochondrial fragmentation.

644



645

646

647 **Figure 1 –Membrane fission and fusion control the morphology of mitochondria**

648 Balanced mitochondrial membrane fusion events shape the morphology of mitochondria.

649 Mitochondrial morphology exists on a multi-dimensional spectrum but can be arbitrarily

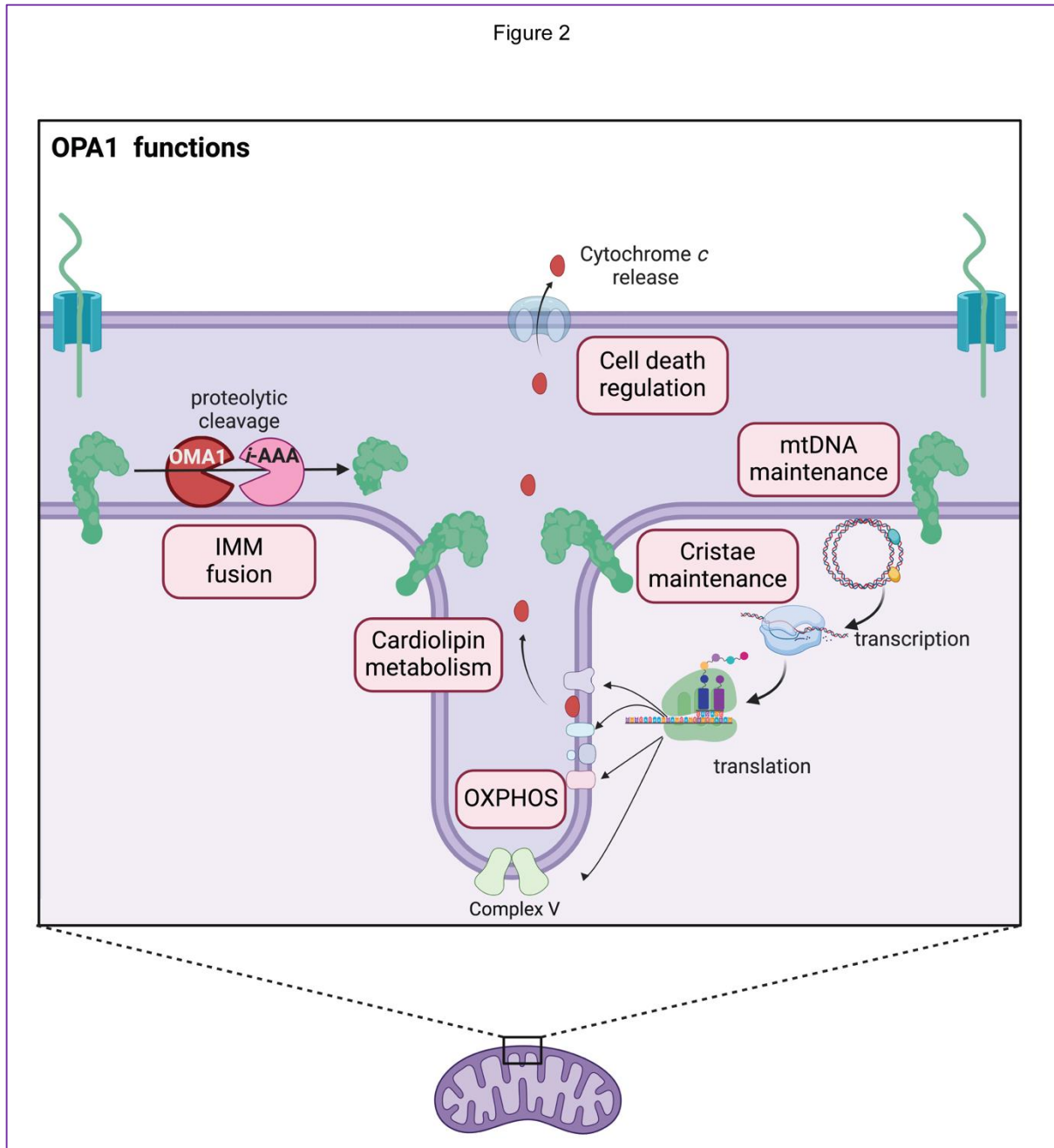
650 classified into network morphologies corresponding to fragmented, tubular, and elongated

651 classes that reflect the balance of mitochondrial membrane fusion and fission.

652

653

Figure 2



654

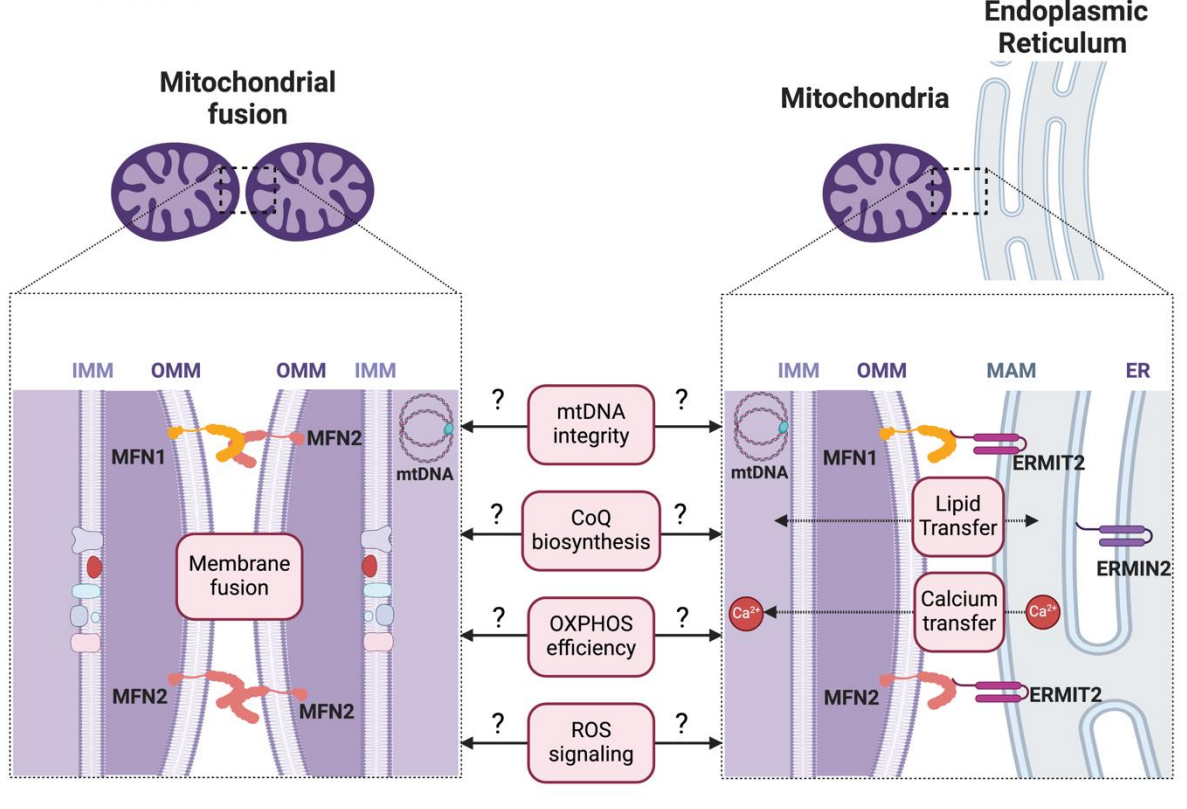
655 **Figure 2 – The multifaceted roles of OPA1 in mitochondrial biology**

656 Optic Atrophy 1 (OPA1) encodes a dynamin-like GTPase (green) translated on cytosolic
657 ribosomes and imported into the inner mitochondrial membrane (IMM), where it is essential
658 for IMM fusion. OPA1 is proteolytically cleaved a membrane-anchored long (L-OPA1) form to
659 a soluble, short (S-OPA1) form by the mitochondrial metallopeptidase OMA1 and the *i*-AAA
660 protease YME1L. In addition to the execution of IMM fusion, OPA1 is required for the
661 formation of cristae, protection against the release of pro-apoptotic factors (e.g. cytochrome
662 *c*), biogenesis of cardiophilin, maintenance of mtDNA levels, and oxidative phosphorylation
663 (OXPHOS).

664

Figure 3

MFN functions



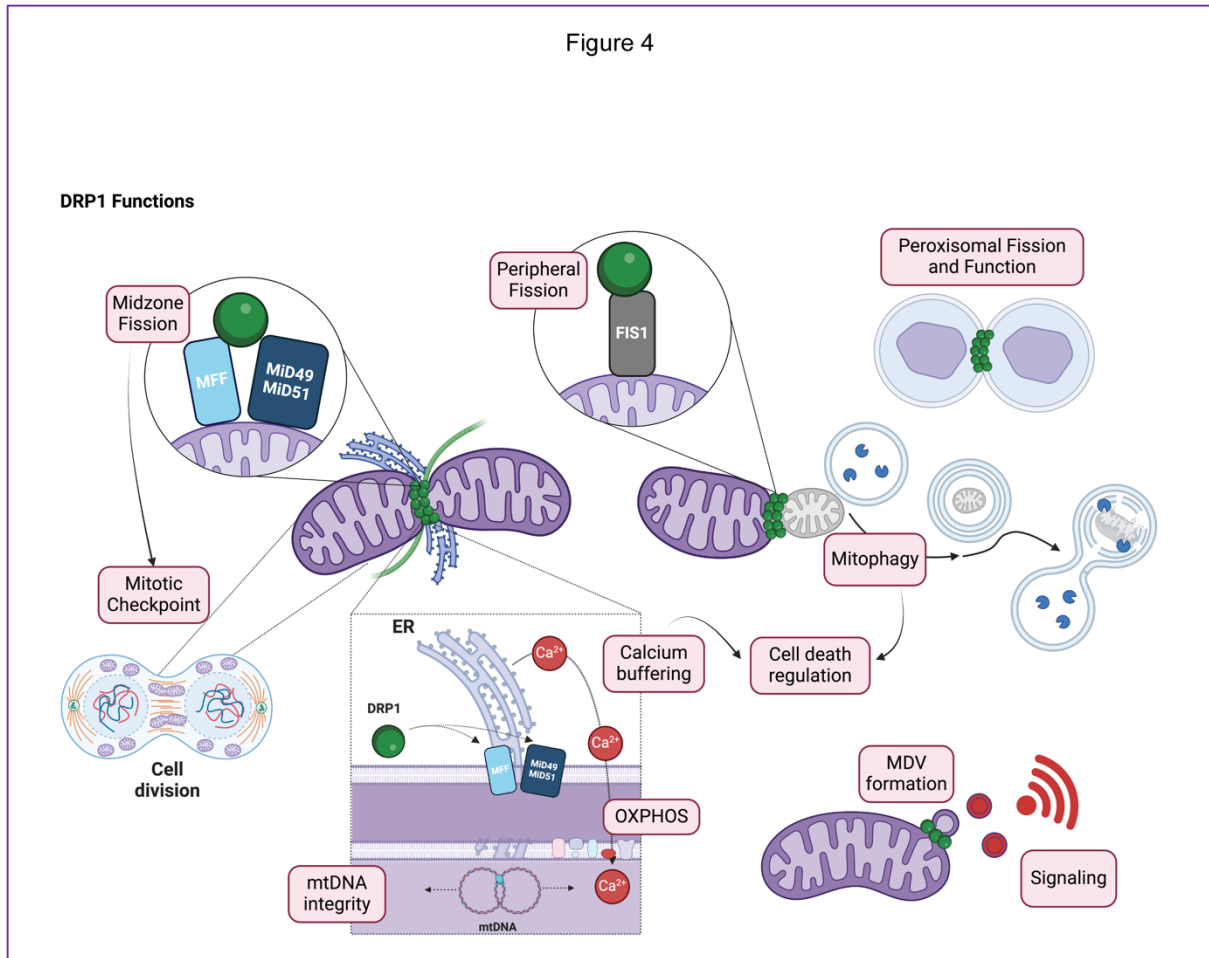
665

666 Figure 3 –Intra and intraorganellar functions of Mitofusins

667 Mitofusin 1 (MFN1) and Mitofusin 2 (MFN2) are paralogous genes encoding dynamin-like
 668 GTPases anchored into the outer mitochondrial membrane (OMM), where they execute
 669 partially overlapping functions. MFN1 and MFN2 tether membranes through homo- and
 670 heterotypic interactions, executing OMM fusion through GTP hydrolysis. Alternative splicing
 671 of the *MFN2* gene yields ERMIN2 and ERMIT2, protein isoforms that localize to endoplasmic
 672 reticulum (ER) and Mitochondrial-ER contact sites (MERCs) at Mitochondrial Associated
 673 Membranes (MAMs), respectively. Disruption of mitofusin-dependent ER-mitochondrial
 674 tethering (but not OMM fusion) is associated with impaired lipid and calcium trafficking.
 675 Genetic ablation or mutation of mitofusins is associated with defective mtDNA nucleoid
 676 dynamics and integrity, ubiquinol (CoQ) biosynthesis, OXPHOS function, reactive oxygen
 677 species (ROS) and ER stress signaling. Whether these defects arise from defects in OMM
 678 fusion and/or ER-mitochondrial tethering has not been determined.

679

Figure 4



680

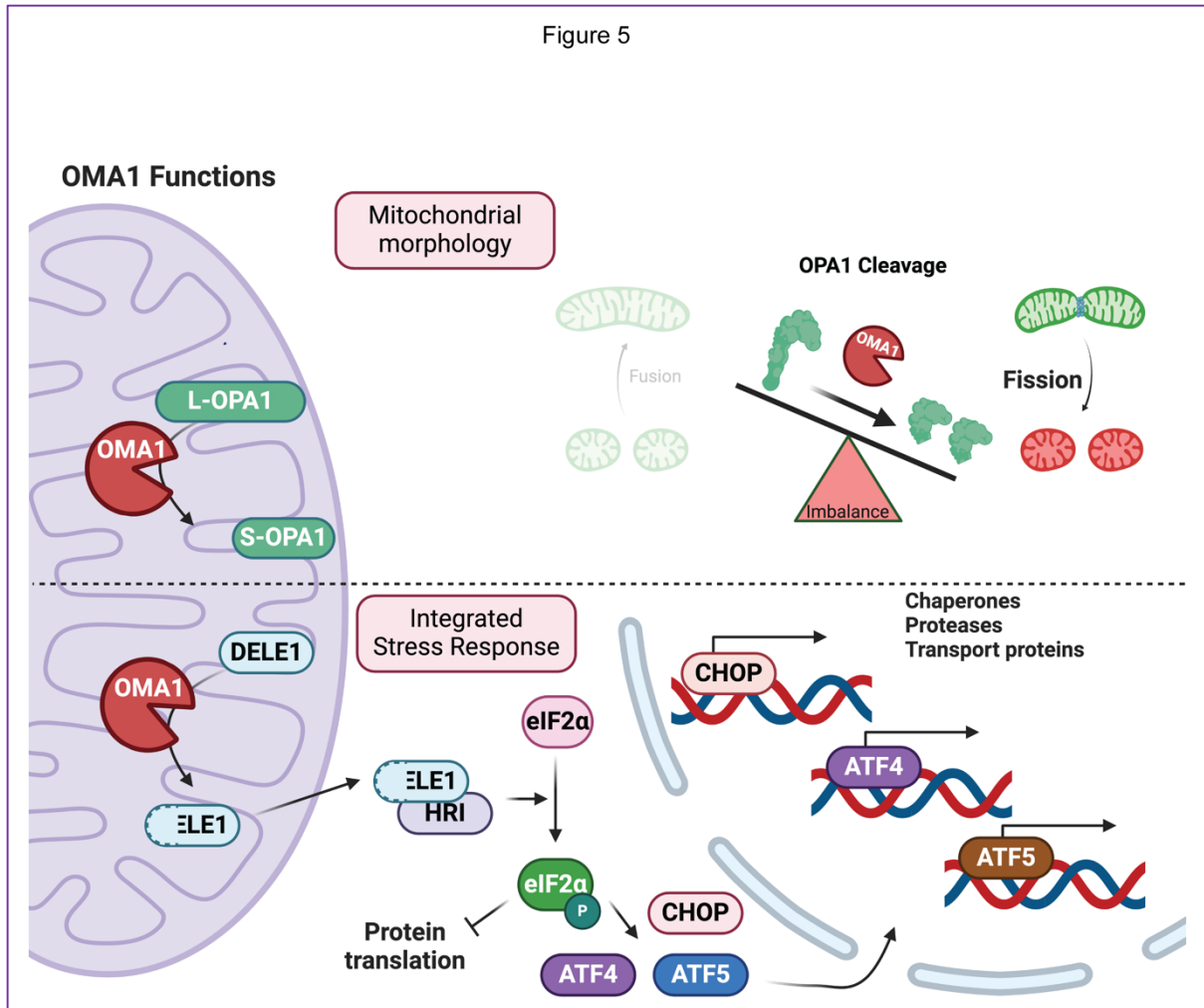
681 **Figure 4 – The role of DRP1 in organelle membrane dynamics**

682 DNM1L encodes the cytosolic GTPase Dynamin Related Protein 1 (DRP1, green), which
683 controls mitochondrial division, peroxisomal division, and formation of mitochondrial derived
684 vesicles (MDVs, red) and downstream signaling through recruitment to membrane receptors
685 MFF, MiD49, MiD51, and FIS1. Mitochondrial division occurs at contact sites with the
686 endoplasmic reticulum (ER, blue) and actin cytoskeleton (green) and is triggered by the
687 replication of mitochondrial DNA (mtDNA). DRP1 deficiency can impair mitochondrial fission
688 (midzone and peripheral), MDV formation, mitophagy, calcium (Ca^{2+}) buffering, peroxisomal
689 fission, oxidative phosphorylation (OXPHOS), mtDNA integrity, and mitotic checkpoint
690 clearance.

691

692

Figure 5



693

694

Figure 5 – The dual role of OMA1 in cellular homeostasis

695

The inner mitochondrial membrane (IMM) metalloprotease OMA1 controls mitochondrial

696

morphology and integrated stress response (ISR) signaling through substrate proteolysis.

697

OMA1 cleaves the pro-fusion long OPA1 (L-OPA1) to soluble short OPA1 (S-OPA1), whose

698

accumulation under stress conditions promotes mitochondrial fission and fragmentation.

699

OMA1 also controls the ISR by cleavage at the IMM of DELE1 into a mature, short form that

700

is exported to cytosol where it interacts with HRI to promote the phosphorylation of eIF2α

701

kinase leading to a global reduction in cytosolic translation and upregulation of chaperones,

702

proteases, and transport proteins through the action of stress response transcription factors

703

CHOP, ATF4, and ATF5.

704

705

706 **Declaration of interests**

707 The author declares no competing interests.

708

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712

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Outstanding Questions

- Can we individually study the mitochondrial shaping functions of pro-fission and pro-fusion proteins separately from their other non-mitochondrial shaping functions?
- What are the best tools to use to specifically modulate mitochondrial network morphology without modulating mitochondrial cristae and mtDNA integrity?
- What is the pertinence of peripheral fission versus mid-zone fission in vivo?
- What is the contribution of impaired mitochondrial fission versus impaired peroxisomal fission in cell and animal models in which DRP1 or its receptors are absent?
- How do primary alterations in mitochondrial morphology alterations impact organelle contact site biology?