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1 TITLE

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3 **Mitophagy during development and stress in *C. elegans***

4

5 KEYWORDS

6

7 mitochondria, mitochondria homeostasis, mitophagy, *Caenorhabditis elegans*, paternal

8 mitochondria elimination, aging, stress, heteroplasmy

9

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17

18

19 ABSTRACT

20

21 Mitochondria is a key cellular organelle, which is tightly supervised by multiple oversight

22 cellular mechanisms regulating mitochondrial biogenesis and mitochondria maintenance

23 and/or elimination. Selective autophagy of mitochondria, *id est* mitophagy, is one of the

24 cellular mechanisms controlling mitochondria homeostasis. The nematode *Caenorhabditis*

25 *elegans* has recently emerged as a powerful model organism to study the roles and functions

26 of mitophagy. We present here the current knowledge on cellular and molecular mechanisms

27 underlying the selective elimination of mitochondria by autophagy in *C. elegans* in the

28 context of developmental processes, aging and adaptive responses to various stresses

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36 **1) Mitochondria homeostasis is regulated through multiple supervision systems.**

37 **1.1 Mitochondrial functions**

38 Mitochondria are double-membrane organelles originating from an endosymbiotic
39 event whereby an alpha protobacteria has been domesticated by eukaryotic cell (Sagan, 1967).
40 This organelle possesses its own genome (mtDNA) (Nass and Nass, 1963) and integrates
41 multiple catabolic pathways through oxidative phosphorylation to produce adenosine
42 triphosphate (ATP) (Hall et al., 2012). This organelle shelters essential metabolic pathways
43 such as the Krebs cycle and fatty acid β -oxidation, as well as the biosynthesis of heme and
44 lipids and part of the urea cycle. Mitochondria is the major site for reactive oxygen species
45 (ROS) production, which could act either as signaling molecule when produced at low doses
46 or be detrimental for the cells when produced at higher dose (Shadel and Horvath, 2015).
47 Mitochondria are involved in the early step of the signaling pathway of apoptosis and
48 participate to cell calcium homeostasis. Mitochondria are dynamic organelles that consistently
49 undergo fission and fusion events and move over the cell cytoskeleton tracks (Summerhayes
50 et al., 1983). Such a dynamic is essential for the quantitative and qualitative adaptation of
51 mitochondria to the cell physiology variations, and the participation to calcium regulation and
52 spatial segregation of mtDNA and matrix components. This dynamic allows the specific
53 localization of mitochondria to subcellular places where energy is required (Sun et al., 2013),
54 and eventually modulate the way mitochondria establish contact sites with other organelles.
55 Large mechanochemical GTPases from the Dynamin family are responsible for the fluidity of
56 the mitochondria network reticulum by controlling fusion and fission of mitochondria
57 (Praefcke and McMahon, 2004; van der Bliet et al., 2013). Because mitochondria are
58 essential to the cell, its dysfunction could jeopardize cell homeostasis and trigger apoptosis,
59 inflammation or senescence (Bock and Tait, 2019). Indeed, numerous human diseases are
60 associated with mitochondria dysfunction (Alston et al., 2017).

61

62 **1.2 Mitochondrial maintenance**

63 Mitochondrial homeostasis is the result of a constant equilibrium between biogenesis,
64 reparation and degradation processes.

65 On the one hand, a series of mechanisms allow the building of new mitochondrion,
66 increasing the mitochondrial mass. These processes include the replication of mtDNA, the
67 expression of mitochondrial genes, the import of nuclear-encoded proteins and lipids, and
68 mitochondrial dynamic, which encompasses transport, fusion and fission. Overall, these

69 mechanisms constitute the mitochondrial biogenesis, which requires a tight crosstalk between
70 two different genomes that is called the nucleo-mitochondrial interaction (Grivell, 1995;
71 Melber and Haynes, 2018).

72 On the other hand, there is a set of mechanisms involved in maintenance, reparation
73 and eventually the removal of dysfunctional and damaged mitochondria. A first level of
74 action is the regulation of protein degradation via mitochondrial proteases and the ubiquitin-
75 and proteasome-dependent OMMAD (outer mitochondrial membrane associated degradation)
76 (Xu et al., 2016). A second mechanism is the mitochondrial unfolded protein response
77 (UPR^{mt}), an evolutionary conserved adaptive transcriptional stress response, which promotes
78 normal mitochondrial function recovery. UPR^{mt} modifies nuclear genes transcription to foster
79 protein folding and to prevent aggregate formation, to induce proteases and to limit ROS
80 accumulation. Noticeably, UPR^{mt} can also promotes mitochondrial biogenesis by stimulating
81 the mtDNA replication and the mitochondrial import of lipids and proteins (Naresh and
82 Haynes, 2019). An additional maintenance mechanism relies on the formation of
83 mitochondrial-derived vesicles that allows the selective elimination of mitochondrial damaged
84 proteins and lipids in response to an oxidative stress (Sugiura et al., 2014). It depends on the
85 formation of buds, containing the jeopardized mitochondrial material, which are addressed to
86 late endosomes for further degradation within the lysosomes (Soubannier et al., 2012).
87 Finally, the massive elimination of dysfunctional mitochondria could occur through jettisoned
88 exosphere containing mitochondria (Melentijevic et al., 2017), endosomal microautophagy
89 (Hammerling, 2016) or mitochondrial-selective autophagy. Overall, these multiple
90 mechanisms involved in quality control of mitochondria are modulated by intrinsic and
91 environmental contexts.

92 This review focusses on the functions and the molecular mechanisms of selective
93 degradation of mitochondria by autophagy, *id est* mitophagy, in the model organism
94 *Caenorhabditis elegans*.

95

96 **2) Overview of the autophagy mechanisms**

97 Autophagy allows the addressing of intracellular components to lysosome for
98 degradation and recycling. It was first termed by de Duve (De Duve, 1963) to describe the
99 cell degradation process taking place in the double-membrane organelle containing parts of
100 cytosol and pieces of organelle structures. Indeed, three different types of autophagy co-exist
101 within the cell depending on the mechanisms of delivery of cytoplasmic components to the
102 lysosome: microautophagy, chaperone-mediated autophagy and macroautophagy (Boya et al.,

103 2013). Microautophagy (MI) is defined as the engulfment of cytosolic cargoes directly via
104 invagination of the lysosomal/vacuolar membrane. It can degrade soluble proteins or even
105 bigger cargoes such as portion of the nucleus, lipid droplets, mitochondria and endosomes
106 (Kissová et al., 2007; Tuttle et al., 1993; Tuttle and Dunn, 1995; Veenhuis et al., 1983;
107 Yokota et al., 1993). MI depends on the activity of the Endosomal Sorting Complexes
108 Required for Transport (ESCRT) machinery (Oku et al., 2017). Interestingly a
109 microautophagy related mechanism, named endosomal microautophagy (eMI), shares with
110 MI many features but involves endosome (Mukherjee et al., 2016; Sahu et al., 2011;
111 Uytterhoeven et al., 2015). For example, in mouse embryonic fibroblasts, organelle such as
112 mitochondria could be guided to the Rab5 positive early endosome and are engulfed in a
113 ESCRT-0, -I and -II-dependent mechanism (Hammerling et al., 2017).

114 Chaperon-mediated autophagy (CMA) is another type of autophagy that directly imports
115 substrates to lysosome for degradation (Majeski and Dice, 2004; Massey et al., 2004). In a
116 similar way to eMI, CMA degrades protein, which interact with HSPA8 through a KFERQ
117 like motif, and nearly 30% of cytosolic components could be degraded via CMA (Dice,
118 1990). Neither microautophagy nor CMA has been yet scrutinized in *C. elegans*, but while the
119 former is evolutionary well conserved the latter is presumably not conserved in *C. elegans*,
120 which is devoid of LAMP2A (Eskelinen et al., 2005; Kaushik and Cuervo, 2018).

121 Macroautophagy (hereafter referred to as autophagy) is present in all eukaryotes and
122 characterized by the formation of a double-membrane vesicle, the autophagosome, which is
123 dedicated to cytoplasmic materials sequestration and delivery to the endo-lysosomal system.
124 Initially, autophagy has been studied in starvation condition and was considered a non-
125 selective degradation process, during which autophagosomes engulfed indistinctly bulk part
126 of the cytoplasm (Figure 1). However, a growing number of experimental results
127 demonstrated that selective autophagy could target specific cargoes using a series of dedicated
128 receptors. Autophagy receptors can specifically recognize the cargoes to be degraded and
129 bridge them to autophagosomes by interacting with Atg8/LC3 (Komatsu and Ichimura, 2010).
130 Based on the engulfed cargo, selective autophagy was further classified into mitophagy
131 (mitochondria), pexophagy (peroxisome), nucleophagy (nucleus), ER-phagy (ER), lipophagy
132 (lipid droplets), aggrephagy (protein aggregates), xenophagy (pathogens) and so forth
133 (Galluzzi et al., 2017).

134 Molecular machinery is well conserved between eukaryotes and Atg genes have been initially
135 characterized in yeast (Tsukada and Ohsumi, 1993). However, Atg genes have been
136 duplicated during evolution resulting in a more complex situation in mammals.

137 In the last 15 years, *C. elegans* has emerged as a powerful model to study autophagy (Chen et
138 al., 2017) and genetic screens identified new autophagy genes some of which are absent in
139 yeast (Tian et al., 2010).

140

141 **3) Mitophagy is a selective degradative process that contributes to mitochondria quality** 142 **control**

143 **3.1 Generalities**

144 Mitophagy is the process that selectively engulfs pieces or whole mitochondrion into
145 autophagosomes, to form mitoautophagosome that subsequently fused with lysosomes for
146 degradation. The term mitophagy was first coined by Lemaster (Lemasters, 2005) based on
147 the work performed in yeast by Camougrand and colleagues (Kissová et al., 2004). Since this
148 initial molecular description of mitophagy in yeast, multiple *in vivo* evidences for the
149 physiological importance of mitophagy have been put forward in other organisms. While in
150 yeast, mitophagy is mediated by the receptor ATG32, *C. elegans* and mammals present
151 several receptors and signalling pathways regulating mitophagy (Table1). Mitophagy is
152 involved in multiple physiological processes, including programmed red blood cells
153 maturation, apoptotic cell death, stress-responses (hypoxia, heat shock, etc...), and
154 neurodegenerative diseases that challenge mitochondria functioning. The discovery of
155 mitophagy and the detailed description of its various molecular mechanisms and underlying
156 functions have been recently reviewed (Montava-Garriga and Ganley, 2019; Palikaras et al.,
157 2018; Pickles et al., 2018). Therefore, we briefly state a general overview of the principles
158 that lead to selective elimination of mitochondria via autophagy.

159 Mitophagy events covers three main categories: the basal, the stress-induced and the
160 developmentally programmed mitophagy. The mitophagy flux can be divided into two major
161 steps, the tagging of mitochondria to be eliminated and the subsequent formation of the
162 mitoautophagosome. Usually, mitophagy is triggered by modifications of the cell
163 environment or stress conditions or developmental signalling that lead to mitochondria
164 activity/parameters alteration and specific mitochondria tagging. It means that environmental
165 or cellular stresses or other mitophagy stimuli are translated into a specific molecular
166 modification of the outer mitochondrial membrane that primes mitochondria for degradation.
167 Thereafter, primed mitochondria recruits the autophagy machinery leading to the formation of
168 an isolation membrane (IM), also called phagophore, nearby mitochondria. Finally, IM
169 enwraps the targeted mitochondria and closes to form a mitoautophagosome, which
170 subsequently fuses with the lysosome for degradation.

171

172 **3.2 Tagging unwanted mitochondria**

173 Overall, the selective elimination of mitochondria via autophagy requires the specific
174 localization to the mitochondrial surface of a set of molecules recognized as cargoes for
175 autophagosome (Table 1). These evolutionary conserved pathways include ubiquitin and
176 autophagy receptors, both potentially harbouring post-translational modification such as
177 polyubiquitination and/or phosphorylation. The best understood mechanism involves PTEN-
178 induced putative kinase protein 1 (PINK1) and the E3 ubiquitin ligase Parkin (Figure 2).
179 However, alternative E3-ubiquitin ligases could be involved such as Mul1 (Rojansky et al.,
180 2016). When mitochondria are damaged and loss their membrane potential, PINK1 is
181 stabilized on the OMM and thus recruits Parkin, which subsequently ubiquitylates proteins at
182 the OMM. Then, these multiple ubiquitin motifs are recognized by a set of autophagy receptor
183 such as P62, NBR1, NDP52, Optineurin, TAXBP1 and ALLO-1 (Table 1). These receptors
184 harbour both an ubiquitin binding site that interacts with ubiquitylated OMM proteins and a
185 LC3 interacting region (LIR) motif that connects onto the IM, thus enabling the tethering
186 between both organelles (Figure 2). Alternatively, a series of ubiquitin-independent
187 mitophagy employ specific autophagy receptors directly localized to the mitochondrial
188 membranes, such as Atg32, BCL2L13, DCT-1/NIX/BNIP3L, BNIP3, FUNDC1, FKBP8,
189 PHB2 and AMBRA1 (Figure 2 and Table 1). In this case, the mitophagy stimulus triggers the
190 increase of autophagy receptor density at the OMM by both transcriptional and post-
191 translational regulations to promote and strengthen direct interaction with Atg8/LC3
192 autophagy proteins. Additionally, alternative mechanisms allow the exposure of inner
193 membrane of the mitochondria (IMM) autophagy receptor, PHB-2 and cardiolipin. This set of
194 mitophagy receptors is differently employed depending on the organism, cellular context,
195 developmental stage and environment. For example, when yeast, are submitted to nitrogen
196 starvation the OMM protein Atg32, is upregulated and acts as an autophagy receptor, through
197 its Atg8 interacting motif, leading to the specific recognition of mitochondria by forming
198 autophagosome. Similarly, during the maturation of mammalian red blood cell, mitochondria
199 are removed by a mechanism involving mitophagy via increased expression of the OMM
200 protein NIX, which recruits isolation membranes to mitochondria through its LIR (Table 1).

201

202 **3.3 Formation and maturation of the mitoautophagosome**

203 The mitophagy receptors, mediate the interactions with the autophagy machinery to
204 trigger autophagosome formation around mitochondria. Damaged mitochondria represent a

205 platform for the multiple formation of mitophagosomes (Dalle Pezze et al., 2020; Zachari et
206 al., 2019). Different receptors interact with the autophagosome nucleation UNC-51-Like
207 Kinase 1 (ULK1) complex or the ATG8/LC3/GABARAP autophagosomal proteins. For
208 example, ULK1 can interact directly with FUNDC1 and BCL2L13 whereas NDP52 contact
209 FIP200 which is a ULK1 protein complex member to trigger mitochondria degradation
210 (Vargas et al., 2019). Atg8/LC3/GABARAP are ubiquitin-like proteins, which localize to
211 autophagosomal membrane through conjugation to phosphatidylethanolamine and mediate
212 interactions with a number of mitophagy receptors possessing a LIR motif. Moreover, it has
213 been shown that ATG8 homologues use a LIR motif to interact with ULK1 and could regulate
214 autophagy (Alemu et al., 2012; Kraft et al., 2012). However, Atg protein including ULK1,
215 WIPI-1, Atg14 and Atg16L1 can directly associate with depolarized mitochondria in the
216 absence of LC3 (Itakura et al., 2012). In particular conditions, LC3/GABARAP homologs are
217 dispensable for autophagosomes formation during PINK1/Parkin-dependent mitophagy but
218 essential for autophagosomes-lysosome fusion (Nguyen et al., 2016). Additionally, studies in
219 erythrocytes, suggest that the ubiquitin like conjugating system made of Atg5 and Atg7 is
220 dispensable for mitophagy (Nishida et al., 2009). Overall, these studies question the
221 importance of interactions between mitophagy receptors and Atg8/LC3 protein.

222 Importantly, recent publications showed that the extension of the autophagosomal membrane
223 around the mitochondria is regulated by the Rab family of small GTPases and their canonical
224 regulators Rab-GEF (GTP exchange factor) and Rab-GAP (GTPase activating protein)(Tan
225 and Tang, 2019). During mitophagy, Rab7, whose activity is regulated by two Rab GAPs
226 TBC1D15 and TBC1D17, is also recruited to the OMM where it mobilizes vesicles to build
227 up autophagosomes. Both proteins are also recruited to the outer mitochondrial membrane via
228 the OMM protein FIS and could interact via their LIR motifs with the nascent
229 autophagosomal membrane containing LC3/GABARAP proteins. Inactivation of these GAPs
230 lead to the accumulation around mitochondria of autophagosomal like membranes. It seems
231 that these GAPs help to guide the extension of the autophagosomal membrane around
232 mitochondria and spatially control the Rab7 activity which itself controls vesicle recruitment
233 to the autophagosomal membrane (Yamano et al., 2014). Finally, once mitoautophagosome is
234 formed it ultimately fuses with the lysosome for degradation. Before this ultimate step of
235 mitophagy, mitoautophagosomes acquire different protein complexes to gain any capacity to
236 fuse. These proteins complexes are tethering factors allowing the establishment of contact
237 zones with lysosome and SNARE proteins to trigger membrane fusion between tethered
238 organelles.

239

240 **4) Tools to study mitophagy in *C. elegans***

241 In recent years, the model organism *C. elegans* has been a leading animal to study the
242 physiological role of mitophagy (Figure 3). This organism allows addressing key biological
243 questions regarding mitophagy functions during the ageing process and in the selective
244 clearance of paternal mitochondria. *C. elegans* has multiple experimental advantages amongst
245 them its genetic toolkit and its ease for cellular biology approaches. Specifically, strategies
246 used to observe and analyse mitophagy in *C. elegans* are mainly based on imaging techniques
247 employing combinations of photonic and electron microscopies. Immunostaining approach on
248 fixed tissue to visualize both autophagosome and mitochondria has been developed.
249 Antibodies against LGG-1 or LGG-2, the two homologues of the ubiquitin-like protein ATG8
250 that label the autophagosome membrane could be combined with marker of mitochondria. A
251 colocalization analysis is indicative of possible mitophagy events or close contacts between
252 both organelles. Hence, in *C. elegans*, live imaging of mitophagy events is often monitored by
253 colocalization between fluorescent tagging of mitochondrial and autophagosomal markers
254 (Figure 4). Multiple ways are available to visualize mitochondria, which have been recently
255 reviewed (Rolland, 2014). Briefly, multiple transgenic lines expressing different
256 mitochondrial protein (either addressed to the OMM, IMM or the matrix) tagged with
257 fluorophore are available. These strains include known *C. elegans* mitochondria autophagy
258 receptors such as DCT-1/NIX or ALLO-1 (Palikaras et al., 2015; Sato et al., 2018).

259 Alternatively, mitochondria could be labelled with fluorescent dyes (red and deep red
260 Mitotracker, tetramethylrhodamine ethyl ester (TMRE) and rhodamine 6G) (Rolland, 2014).

261 *C. elegans* autophagosome are generally monitored in transgenic lines expressing either GFP
262 or mCherry tagged LGG-1 and LGG-2 proteins. Interestingly, transgenic lines expressing
263 autophagy proteins, LGG-1 and SQST-1, tagged with a novel fluorophore, CemOrange2 that
264 is separable from GFP and far-red fluorescent proteins should facilitate multiplex imaging in
265 the future (Thomas et al., 2019). Several combination of organelle tags have been developed
266 by N. Tavernarakis and his colleagues. They created strains with a dual fluorescent reporter
267 system that combines a mitochondria marker, the mitochondrial-targeted GFP, expressed
268 along with an ubiquitously expressed LGG-1 tagged with DsRed. Additionally, they adapted
269 the Rosella tool (Rosado et al., 2008) by constructing a *C. elegans* strain expressing the
270 mitoRosella synthetic gene, to study mitophagy in *C. elegans* (Palikaras et al., 2015). The
271 mitoRosella is a mitochondria-targeted pH sensitive GFP fused to the pH insensitive DsRed.
272 In the acidic autolysosome, the GFP part of the biosensor is quenched while the DsRed

273 fluorophore is resistant. Thus, healthy/normal mitochondria could be observed thanks to both
274 green and red signals they emitted. Subsequently, once mitophagy events occur,
275 autophagosome engulfs mitochondria and then fuses with lysosome, which results in the
276 quenching of the GFP signal and the reduction of the GFP/DsRED ratio indicative of the
277 mitophagy flux. This tool has been originally adapted to study mitophagy in the large body
278 wall muscle cells of *C. elegans* but also adapted to the nervous system (Fang et al., 2019). In
279 addition, the overall mitochondrial homeostasis could be analysed through its mass
280 modification. These observations could be assessed in *C. elegans* by quantifying the overall
281 fluorescence signal from mitochondrial targeted GFP specifically expressed in intestinal cells
282 under the control of the *ges-1* promoter (Palikaras et al., 2015). Additionally, addressing the
283 Timer protein to the mitochondria (MitoTimer), allows to monitor mitochondrial biogenesis
284 and mitophagy (Laker et al., 2014). The dual emission fluorescent “Timer” probe initially
285 fluoresces in green then shifts irreversibly to red fluorescence when it mature following a
286 form of oxidation (dehydrogenation) of the Tyrosine 67 residue (Terskikh et al., 2000). The
287 MitoTimer Red/Green fluorescence ratio does not depend on the overall expression of
288 MitoTimer nor in changes in mitochondria pH. On one hand, the green fluorescence level
289 indicates newly synthesized MitoTimer protein addressed to the mitochondrial matrix and
290 reflects the increase of MitoTimer production or import and mitochondrial biogenesis. On the
291 other hand, the level of red fluorescence, which indicates older mitochondria, mainly depends
292 on degradative pathways such as autophagy, mitochondrial proteases or the ubiquitin
293 proteasome system. Overall, measurements of red fluorescence over the green signal emitted
294 by MitoTimer allows collection of quantitative data that mirrors multiple parameters
295 controlling the mitochondrial homeostasis and therefore should be interpreted cautiously for
296 monitoring mitophagy. In *C. elegans*, MitoTimer has been so far only expressed in
297 mechanosensory neuron but it is a promising complementary tool to monitor mitochondria
298 biogenesis in other cells and tissues. Alternatively, a dual Fluorescent Protein tag fused to the
299 mitochondrial protein TOMM-7 has been proposed to monitor the mitophagy flux by western
300 blot (Chapin et al., 2015). Finally, direct observation of mitoautophagosome could be
301 performed in *C. elegans* with electron microscopy (EM) (Al Rawi et al., 2011; Meléndez et
302 al., 2003). This technic allows visualization of autophagosome, possibly at different
303 maturation steps (Manil-Ségalen et al., 2014). Additionally, EM analysis of autophagosomes
304 from wild type and mutant strains could provide insights into the mechanism defects that are
305 associated with genome alteration (Wang et al., 2016). It helps to monitor and distinguish
306 normal mitochondria from modified ones, targeted by autophagosome, with altered structure

307 such as matrix aggregates or cristae disappearance (Wang et al., 2016; Zhou et al., 2016).
308 Interestingly, EM and direct observation of fluorescent reporter construct labelling either
309 distinct autophagosomal or mitochondrial proteins could be combined in *C. elegans* by
310 applying the Correlative Light and Electron Microscopy (CLEM) technique. This technique
311 has been used to analyse the mechanism underlying apoptotic corpse elimination through
312 consecutive recruitment of LGG-1 and LGG-2 during *C. elegans* development (Largeau and
313 Legouis, 2019) and could be adapted to further study mitophagy in *C. elegans*.

314

315 **5) Physiological and developmental mitophagy in *C.elegans***

316 The model organism *C. elegans*, due to its intrinsic multiple experimental advantages
317 and specific autophagy tools developed by the research community, has lead the way to the
318 discovery of key developmental and physiological roles for mitophagy. Hereafter, we will
319 present and discuss mitophagy mechanisms involved in *C. elegans* development and stress
320 response.

321

322 **5.1 Elimination of paternal mitochondria**

323 In humans, as in most animals, the mtDNA is maternally inherited (Birky, 2001), with
324 exceptional pathological cases in which paternal mtDNA could be transmitted to the offspring
325 (Luo et al., 2018, 2013). Multiple experimental evidences using *C. elegans* and other model
326 organisms have demonstrated that paternal mitochondria are not simply diluted upon
327 fertilization but actively eliminated (see Box 1). Albeit some variations between species, the
328 process of paternal mitochondria elimination (PME) is essential and relies on a selective
329 mitophagy mechanism named allophagy. Allophagy designs the allogeneic (non-self)
330 organelle autophagy in which paternal mitochondria, and membranous organelles (MO) that
331 enter the oocyte are eliminated by autophagy (Al Rawi et al., 2011; Sato and Sato, 2011; Zhou
332 et al., 2011) (Figure 5). MO are sperm specific organelles that derived from the Golgi
333 apparatus and probably play a role similar to the vertebrate acrosome (Nishimura and
334 L'Hernault, 2017). Electron microscopy analyses of the fertilized oocyte revealed that
335 paternal mitochondria are small and rounded whereas maternal ones are thin and tubular.
336 Paternal mitochondria lose their membrane potential, have no cristae but present thicker
337 membranes containing holes and dark speckles within the matrix. In the meantime,
338 ubiquitination events take place on paternal mitochondria and MO (Sato et al., 2018) and the
339 depletion of the E1 ubiquitin activating E1 enzyme UBA-1 or the E2 ubiquitin conjugating
340 enzymes UBC-16 and UBC-18 alters the PME (Molina et al., 2019). This ubiquitination is at

341 least partially Parkin-independent since *pink-1* and *pdr-1* mutants display no defect in PME
342 (Lim et al., 2019). Probably, two parallel but independent mechanisms contribute to PME.
343 One is dependent on ubiquitination of the OMM proteins and involves both proteasome and
344 mitophagy, the other is ubiquitination-independent and involved FNDC-1, the orthologue of
345 FUNDC1 (FUN14 domain containing 1)(Lim et al., 2019). The paternal mitochondria
346 ubiquitination triggers the recruitment of the mitophagy receptor ALLO-1, which upon
347 phosphorylation by the IKKE-1 kinase interacts with LGG-1, via a LIR domain, and
348 participates to the formation of autophagosome around the paternal mitochondria (Sato et al.,
349 2018). An additional mitophagy receptor, the prohibitin PHB-2, is localized to the IMM and
350 becomes accessible for allophagy following alteration of the OMM (Wei et al., 2017) (Figure
351 5). The initial alteration of the OMM could be due to the process OMMAD because depletion
352 of the two proteasomal subunits RPN-10 and RAD-23 affects PME (Molina et al., 2019; Zhou
353 et al., 2011).

354 Several studies reported differential functions for LGG-1 and LGG-2, the homologues of the
355 mammalian GABARAP and LC3, respectively, during allophagy. After fertilization, they
356 both localize in clusters containing paternal mitochondria and MOs, which degradation is
357 completed at the 8-cell stage (Al Rawi et al., 2011; Sato and Sato, 2011; Zhou et al., 2011).
358 Genetic studies revealed that the allophagic clusters are completely abolished in *lgg-1* null
359 mutant but persist for longer time in *lgg-2* mutants, due to a delay in the formation of
360 autolysosome. These data illustrate that *lgg-1* is essential for autophagosome formation,
361 whereas *lgg-2* has a role in autophagosome fusion with lysosome. Moreover, LGG-2 interacts
362 with VPS-39, an Homotypic fusion and vacuole Protein Sorting (HOPS) complex subunit, to
363 mediate the tethering and acidification of autophagosomes with lysosomes downstream of
364 Rab5-Rab7 (Djeddi et al., 2015; Manil-Ségalen et al., 2014).

365

366 **5.2 Mitophagy and iron metabolism**

367 Several proteins of the mitochondrial electron transport chain need iron as a key
368 element to accomplish their functions, and iron deregulation is often detrimental for
369 mitochondria functions. Recently, a study on *C. elegans* identifies that the induction of
370 mitophagy is part of an adaptive iron starvation response induced as a protective mechanism
371 against mitochondrial stress (Schiavi et al., 2015). Mitochondrial respiration is dependent of
372 iron-sulfur (Fe-S) clusters, which biosynthesis is stimulated by the mitochondrial protein
373 Frataxin (FXN) (Gervason et al., 2019). Mutations of *fxn* result in dysfunctions of the
374 respiratory chain and lead to multiple symptoms observed in Friedreich's ataxia. In *C.*

375 *C. elegans*, the moderate depletion using RNAi, of FXN homologue FRH-1, induces lifespan
376 extension (Ventura and Rea, 2007). Interestingly, mitophagosome formation and mitophagy
377 are induced in *frh-1* RNAi worms, and lifespan extension depends on PDR-1, PINK-1, DCT-
378 1, as well as on the autophagy receptor p62/SQST-1. EM analysis of *frh-1* worms revealed the
379 accumulation of electron-dense iron deposits within mitochondrial matrix, which is consistent
380 with data in mouse (Puccio et al., 2001). *Frh-1* (RNAi) mimics a hypoxia state through
381 cytosolic iron deprivation, which activates the hypoxia-inducible factors HIF-1 and AHA-1,
382 to modulate longevity (Zhang et al., 2009). Moreover, *frh-1* RNAi can also upregulate the
383 expression of *dct-1* and a series of globin genes, while it downregulates the expression of
384 *sqst-1/p62*. Globin genes encode iron-containing proteins that regulate the cellular redox
385 status in response to hypoxia (Hoogewijs et al., 2007). Upon iron deprivation, using the iron
386 chelator 2,2'-dipyridyl, the worms present a mitophagy-dependent increased longevity, which
387 overlaps the FRH-1 depletion.

388 Interestingly, *C. elegans* infection by *Pseudomonas aeruginosa* also induces an iron
389 related mitophagy. Amongst the virulence factors produced by *P. aeruginosa*, siderophores,
390 such as pyoverdine, retrieve the iron from its host. Consequently, *C. elegans* infected by *P.*
391 *aeruginosa* experiments an acute iron sequestration similar to the slight decrease of *frh-1*
392 mRNA level. This iron sequestration induced a mitochondrial turnover through a protective
393 mitophagy process. For example, *pink-1* and *pdr-1* depletion sensitize animals to *P.*
394 *aeruginosa* (Kirienko et al., 2015) indicating that mitophagy is necessary for *C. elegans*
395 resistance to the infection, which probably impairs mitochondrial function through iron
396 chelation and damages mitochondria. Therefore, iron chelation-induced mitophagy eliminates
397 damaged mitochondria to preserve energy homeostasis during this pathogen infection.

398

399 **5.3 Dual effect of mitophagy on heteroplasmy maintenance.**

400 In most animals, mtDNA is maternally inherited and typically, individuals harbour a
401 unique mtDNA genotype defining the homoplasmic state. However, germline and somatic
402 mutations can lead to the coexistence of a mixture of mutant and normal mtDNA within the
403 same individual, qualified as heteroplasmy and responsible of numerous human
404 mitochondrial diseases (Wallace and Chalkia, 2013). Several *C. elegans* heteroplasmic strains
405 have been isolated (Konrad et al., 2017; Tsang and Lemire, 2002). These strains combined
406 with powerful novel technical possibilities such as purification of mitochondria from specific
407 tissues (muscle, neuron, intestine, epidermis, gonad) even from a single worm make *C.*
408 *elegans* a powerful model for studying mechanisms regulating heteroplasmy state

409 maintenance (Ahier et al., 2018). The *C. elegans* heteroplasmic strain, harbouring a 3.1-
410 kilobase deletion (*uaDf5*) has twice mtDNA molecules compared to the wild type. Dissection
411 of worms shows that *uaDf5* content varies within tissues of individual worms (Liau et al.,
412 2007). Interestingly, PDR-1/Parkin is required for maintaining low levels of *uaDF5* mtDNA
413 in *C. elegans* suggesting that this key protein involved in triggering mitophagy could have a
414 beneficial role (Valenci et al., 2015). However, the mechanism underlying heteroplasmic
415 status maintenance seems much more complicated. Two recent reports have shown that the
416 heteroplasmic strain constitutively induces UPR^{mt}, which promotes proliferation of the
417 *uaDF5*. Interestingly, the loss of the UPR^{mt} transcription factor ATFS-1 facilitates mitophagy
418 activity and induces a decrease of mtDNA copy-number (Gitschlag et al., 2016; Lin et al.,
419 2016). The expression of an error prone mtDNA polymerase in *C. elegans* generates multiple
420 mtDNA deletions with a variable heteroplasmic state, and displays multiple phenotype defects
421 such as neuromuscular dysfunction. Surprisingly, a genetic screen to identify modulators of
422 this mutator phenotype reveals the paradox that a reduced mitophagy may be beneficial for
423 this worm (Haroon et al., 2018). Altogether, these reports point out a very complex
424 implication of mitophagy in the regulation of the heteroplasmic state in *C. elegans*.

425

426 **6) Mitophagy during aging and neurodegeneration in *C. elegans***

427 **6.1 Mitophagy in aging and stress conditions**

428 A recent work revealed a novel physiological role for mitophagy, which allows
429 mitochondria clearance all along the aging process in *C. elegans* (Figure 6). The progressive
430 accumulation of the mitochondrial mass, observed during aging of the worm, could induced
431 some persistent cell malfunctions and be responsible of the decline of health associated with
432 aging (Palikaras et al., 2015). Interestingly, this phenotype is further enhanced when key
433 autophagy gene or the mitophagy Pink/parkin pathway are depleted, demonstrating that the
434 mitochondria homeostasis is partly modulated via mitophagy during aging. The mitophagy
435 receptor DCT-1 (DAF-16/FOXO Controlled, germline Tumour affecting-1), homologue of
436 NIX/BNIP3L and BNIP3, is crucial during this process and its depletion results in the
437 accumulation of abnormal mitochondria, similarly to *pdr-1* and *pink-1* animals. DCT-1 is
438 localized at the OMM, and contains a LIR motif, which mediates its interaction with LGG-1
439 (Palikaras et al., 2015). The overexpression of DCT-1 allows worm to better adapt to various
440 stressful condition such as heat shock, UV exposure and pharmacological treatment
441 challenging mitochondria (FCCP, paraquat). The ubiquitination of the lysine 26 of DCT-1
442 increases upon mitophagy induction, in a PDR-1 and PINK-1-dependent manner and the three

443 mutants are more sensitive to mitochondrial stress. DCT-1 participates with PINK-1 and
444 PDR-1 to the extension of lifespan dependent on both the insulin pathway (*daf-2* mutant) and
445 mitochondrial proteins (*clk-1* and *isp-1* mutants).

446 This study also showed that *dct-1* gene expression and mitophagy are both regulated
447 by the transcription factor SKN-1, the homologue of the mammalian NRF2/NFE2L2. SKN-1
448 is activated under oxidative stress conditions (Ghose et al., 2013; Ploumi et al., 2017) and
449 promotes mitochondrial biogenesis by up-regulating several mitochondria genes. SKN-1
450 depletion leads to the modification of mitochondrial morphology, an increase of cytoplasmic
451 Ca²⁺ and a mitochondrial membrane depolarization and blocks DCT-1 mediated mitophagy.
452 By coupling mitophagy and mitochondrial biogenesis, SKN-1 is a key player of a loop which
453 regulation is important during the aging of animals (Figure 6). Interestingly, an additional
454 mechanism of control of mitophagy during aging in *C. elegans* relies on the specific
455 interaction of two mRNAs, from *puf-8* and *mff-1* genes. MFF-1 is an OMM protein that
456 recruits the key mitochondrial fission factor DRP-1 to mitochondria. In old adult worms, *puf-*
457 *8* mRNA inhibits the translation of *mff-1* mRNA, decreasing the mitochondrial fission and
458 also the mitophagy, which, ultimately results in the accumulation of altered mitochondria
459 (D'Amico et al., 2019).

460 Finally, a recent report studied the involvement of mitophagy in *mev-1* mutants that
461 are highly sensitive to environmental stresses. This study also demonstrates that the
462 mitophagy genes *dct-1* and *pdr-1* but *pink-1* are required for *mev-1* to survive to heat shock
463 (Civelek et al., 2019). This conclusion is further supported by results indicating that
464 mitophagy deficient animals are more sensitive to the ultraviolet C radiation (Hartman et al.,
465 2019).

466 Overall, these results indicate that mitophagy is a key cellular process that helps *C.*
467 *elegans* to cope with stressful environmental context.

468

469 **6.2 Pharmacological modulation of mitophagy protects from age-induced decline**

470 Three molecules that specifically modulate mitophagy in *C. elegans* have been
471 identified: Urolithin A (UA), tomatidine and nicotinamide adenine dinucleotide (NAD⁺). UA,
472 a part of the organic compounds known as benzo-coumarins, is the first natural compound
473 shown to induces mitophagy in worm as well as rat and mammalian cells (Ryu et al., 2016).
474 The UA precursor, ellagitannin, is found in multiple fruits and is processed in UA by the
475 microbiota. UA can extend lifespan and improve worm fitness by inducing mitophagy. In
476 young adult, UA treatment induces mitochondria fragmentation and leads to the reduction of

477 mitochondrial content. The latter phenotype depends on the mitophagy genes *pink-1*, *dct-1*,
478 *skn-1* or the autophagy genes *bec-1*, *sqst-1*, *vps-34*, which depletion inhibits the lifespan
479 extension following UA exposure. Indeed, UA treatment induces autophagy, which is
480 detected by the increased LGG-1 positive autophagosomes. In older worms, UA exposure
481 leads to mitochondrial content increase and to a higher transcription rate of respiratory chain
482 subunit genes, compared to control animals. During the UA long-term treatment, the
483 mitochondrial biogenesis is facilitated by the initial mitophagy induction observed in young
484 adult. The enhanced mitochondrial biogenesis improves mitochondrial function through
485 stabilizing ATP content and increasing the respiration capacity, and therefore leads to a
486 prolonged locomotion and pharyngeal pumping activities of treated worms. Interestingly, this
487 result suggest that UA is capable to uncouple mitophagy and mitochondrial biogenesis during
488 aging; stimulating first mitophagy then mitochondrial biogenesis. Interestingly, most of the
489 effects of UA on *C. elegans* are also observed in mammalian muscle and intestinal cells and,
490 mice and rat aging models treated with UA show improvement of their muscle activity. This
491 molecule shows a high potential to be used as a nutritional supplement to improve
492 mitochondria function and muscle quality in human elderly people and clinical trials aiming
493 to test the effect of UA on human health have been undertaken (Andreux et al., 2019).

494 Tomatidine is a steroid like compound enriched in unripe tomatoes. This molecule
495 have beneficial effects in mouse cells for anti-inflammatory (Chiu and Lin, 2008) and anti-
496 tumorigenic effects (Fujiwara et al., 2012). In mouse skeletal muscle cells, tomatidine
497 promotes muscle fitness partially through increasing mitochondrial mass (Dyle et al., 2014).
498 Interestingly, tomatidine treatment extends worm lifespan, as well as health span (Fang et al.,
499 2017), as manifested by the improvement of swimming and pharyngeal pumping in aged
500 animals. Interestingly, tomatidine induces moderate ROS production, which in turn activates
501 SKN-1/Nrf-2 pathway. SKN-1, in turn, promotes PINK-1/DCT-1-dependent mitophagy and
502 mitochondrial biogenesis to keep mitochondria healthy. Additionally, tomatidine action seems
503 to be dependent on the key UPR^{mt} gene *atfs-1* and its beneficial effect has been further
504 extended to primary rat cortical neurons and human HEK-203 cells (Fang et al., 2017). This
505 study further provides experimental evidences indicating that somehow pharmacological
506 treatment, activating mitophagy through SKN-1 pathway, may provide new approaches to
507 improve healthy aging. It is highly possible that UA induced mitophagy and lifespan
508 extension shares the same pathway triggered by tomatidine treatment.

509 NAD⁺ is an important coenzyme linked to the cell energy homeostasis. It is part of the
510 redox couple NAD⁺/NADH, which provides the mitochondria respiratory chain with electrons

511 and protons. NAD⁺ is also a coenzyme for the nuclear deacetylase sirtuin 1 that modulates
512 chromatin structure and regulates multiple cellular pathways including aging. In *C. elegans*,
513 NAD⁺ depletion inhibits mitophagy while its replenishment stimulates mitophagy by
514 upregulating DCT-1 through the sirtuin homolog SIR-2.1 and DAF-16 dependent mechanism
515 (Fang et al., 2017). Interestingly, it has been also shown that NAD⁺ depletion is a major driver
516 of the metabolic defects observed in the premature aging autosomal recessive disease Werner
517 syndrome (WS). Both in WS model worm, *wrn-1* mutant, and in patient cells, a depletion of
518 NAD⁺ is associated with mitochondria dysfunction and defective mitophagy. The NAD⁺
519 replenishment improves the mitochondrial function by restoring mitophagy in a DCT-1- and
520 UNC-51 dependent mechanism (Fang et al., 2019), which opens future therapeutic strategy
521 for several diseases (Connell et al., 2019).

522 Overall, *C. elegans* has been a leading model organism to put forward drug that
523 selectively modulate mitophagy and contribute to set up numerous pre-clinical and clinical
524 trials aiming to analyse putative translation of these mitophagy inducers drug beneficial effect
525 to human.

526

527 **6.3 Mitophagy and neurodegenerative processes**

528 Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis
529 associated with frontotemporal dementia (ALS/FTD) are the major diseases that affect the
530 functioning of the brain in the human population. These pathologies have currently no
531 effective treatment and result in a progressive destruction of the nervous system leading to a
532 loss of: memory, cognitive functions, sensory abilities, speech and locomotion.

533 AD, PD and ALS/FTD share similar cellular dysfunctions regarding calcium
534 homeostasis, lipid metabolism, energy production and recycling of cellular waste products,
535 related to mitochondria and autophagy, and which are mostly linked to mitochondrial
536 associated membranes function (Area-Gomez and Schon, 2017; Paillusson et al., 2016).

537 Several *C. elegans* models of neurodegenerative diseases have been used to challenge the
538 possible causal link between these disease-associated gene mutations and nervous system
539 dysfunctions (Alexander et al., 2014). The exploration of the link between mitophagy and
540 neurodegeneration, in animal models of AD, indicates that conserved mitochondrial stress
541 response signature emerges comprising UPR^{mt} and mitophagy pathways (Sorrentino et al.,
542 2017). Mitophagy induction by NAD⁺ boosting compound attenuates A β toxicity in *C.*
543 *elegans*. The stimulation of the UPR^{mt} and mitophagy can decrease protein aggregation,
544 increase worm fitness and delays progression of symptoms. By adapting for neural expression

545 the genetic tools for studying mitophagy (see section 4), a recent report provided additional
546 evidences for mitophagy involvement in neurodegenerative diseases in *C. elegans* (Fang et
547 al., 2019). The treatment of worms with UA (see section 6) triggers a *dct-1*, *pink-1* and *pdr-1*
548 dependant mitophagy flux in nerve cells without inducing bulk autophagy. The authors also
549 tested the beneficial effect of mitophagy in two *C. elegans* models of AD, the pan-neuronal
550 expression of the human A β 1-42 protein (Voglis and Tavernarakis, 2008) and the neuronal
551 expression of the pro-aggregant F3 Δ K280 tau fragment (Fatouros et al., 2012). The
552 pharmacological stimulation of mitophagy is beneficial for both models and restore the
553 cognitive defects observed on learning and memory. A similar effect has been reported in
554 mice model of AD, suggesting a conserved role of mitophagy in neurodegenerative diseases
555 (Fang et al., 2019).

556 Multiple *C. elegans* models of PD, including genetic and pharmacological induced models
557 have been reported in the literature (Cooper and Van Raamsdonk, 2018). Mutations in parkin
558 and Pink1 cause autosomal recessive form of PD and the depletion of both genes in *C.*
559 *elegans* lead to accumulation of defective mitochondria. *pink-1* null mutant presents
560 mitochondrial cristae defect both in muscle and neuron, as well as increased sensitivity to
561 oxidative stresses and neuron dysfunction (Sämann et al., 2009). Surprisingly, the defects in
562 *pink-1* mutant could be suppressed by depleting *lrk-1*, the homologue of LRRK2 which is
563 associated with PD in human (Biskup et al., 2006). LRK-1 functions in axonal-dendritic
564 polarity of synaptic vesicles in neurons of *C. elegans* (Sakaguchi-Nakashima et al., 2007).
565 LRK-1 and PINK-1 act antagonistically in neurite outgrowth, suggesting that the mitophagy
566 defect in *pink-1* mutant could affect neuron outgrowth defect. Finally, patients with ALS/FTD
567 accumulate TDP-43 (Tar DNA-binding protein 43) in degenerating neuron (Neumann et al.,
568 2006). Interestingly, knockdown of TDP-43 enhances autophagosome and lysosome
569 biogenesis indicating somehow a link between ALS/FTD and autophagy. (Xia et al., 2016).
570 When overexpressed in the *C. elegans* nervous system, the human TDP-43 protein
571 accumulates in the nucleus and results in an uncoordinated locomotion associated with
572 abnormal synapse structure (Ash et al., 2010). The full exploitation of these worm models
573 regarding any defect in mitophagy and the implication on ALS/FTD is still pending.

574

575 **7) CONCLUSION**

576 Recent researches performed with the nematode *C. elegans* have clearly contributed to
577 the better understanding of the molecular mechanisms of mitophagy and its physiological
578 roles at the scale of a whole animal. The most representative inputs from these studies

579 concerns the fields of developmental biology, aging, adaptation to stress and
580 neurodegenerative pathologies.

581 Studies in *C. elegans* revealed that the maternal restricted transmission of mtDNA
582 requires the selective elimination of paternal mitochondria by mitophagy and led to the
583 identification of an original mechanism that is independent of Pink/parkin and relies on the
584 mitophagy receptor ALLO-1. These data, supported by studies in the model *Drosophila*
585 *melanogaster* paved the way for understanding this essential question in mammals (see Box
586 1). Additionally, the worm *C. elegans* provided the first clue on a regulatory mechanism that
587 coordinates mitochondrial biogenesis and mitophagy during aging and involved the master
588 regulator SKN-1. It also contributed to demonstrate that an inner membrane protein,
589 Prohibitin 2, could act as a mitophagy receptor (Wei et al., 2017), and to identify the links
590 between iron deficiency and mitophagy (Schiavi et al., 2015). Finally, *C. elegans* studies have
591 been important to better understand the relationships between neurodegenerative diseases and
592 mitophagy and allowed to test and validate novel molecules that could be further developed as
593 therapeutics to enhance mitophagy and improve healthy aging.

594 Several technological improvements for studying mitophagy and mitochondrial
595 biogenesis have been possible using *C. elegans*, taking advantage of its transparency to
596 developed the fluorescent biomarkers Rosella (Palikaras et al., 2015) and MitoTimer (Laker et
597 al., 2014; Terskikh et al., 2000). The power of genetic approaches and engineering of
598 transgenic nematodes using molecular biology approaches strongly suggests that additional
599 live-imaging tools will appear soon in *C. elegans*. Nevertheless, the mitochondrial
600 degradation *per se* often lacks clear experimental demonstration in *C. elegans*, and would
601 strongly benefit of the development of biochemical tools for the quantification of the delivery/
602 degradation of mitochondrial cargos into the lysosome (Chapin et al., 2015).

603 One can expect that in the near future, studies in *C. elegans* will bring new insights on
604 molecular mechanism controlling mitophagy, and identify the roles for mitophagy in many
605 biological processes. The important question of the basal mitophagy is particularly difficult to
606 address, which explains why most of the studies that identified the mitophagy mechanisms are
607 generally done in the context of a massive and non-physiological stress of the mitochondria.
608 *C. elegans* could be useful to identify whether the signalling events, the mitophagy receptors
609 and the regulation of the basal mitophagy are different from the developmental or
610 pathological processes. By the simplicity of its anatomy and the presence of multiple tissues,
611 *C. elegans* is also a powerful system to decipher the variations of autophagy/mitophagy
612 functions between organs and during the whole life cycle. Neurology and aging pathologies

613 are one obvious target but the differentiation and physiology of other cell types could be
614 studied as well as trans-differentiation processes that have been described in the nematode. *C.*
615 *elegans* could also open the study of autophagy/mitophagy in very complex biological
616 processes such as circadian rhythm, the sleep-like state lethargus or very long starvation
617 survival period.

618 Finally, another key point in the field of mitophagy is to understand the mechanisms
619 of cooperation and coordination between the various mitochondria quality control pathways.
620 How UPR^{mt}, mitophagy, proteasome and other processes like the exophers jettison
621 (Melentijevic et al., 2017) are globally regulated to cope with a dysfunctional mitochondria
622 network remains unclear and *C. elegans* could be useful to address such a question.

623

624

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631

632

633 **Box 1. Convergence of struggles to eliminate paternal mitochondria**

634 Multiple mechanisms, depending on the animal, act before or just after fertilization
635 during the early embryogenesis, to eliminate paternal mitochondria. Early work from
636 Sutovsky and colleagues showed that paternal mitochondria are ubiquitinated for an efficient
637 degradation mediated by proteasome in fertilized cow and monkey eggs (Sutovsky et al.,
638 2000). Nishimura and colleagues then showed, in the fish medaka (*Oryzias latipes*), that the
639 paternal mtDNA number is progressively reduced at the end of the spermatogenesis and then
640 is specifically and progressively digested just after fertilization (Nishimura et al., 2006). In
641 *Drosophila melanogaster*, sperm mtDNA is eliminated during spermatogenesis, by two
642 mechanisms. The first one occurs at the elongation stage of postmeiotic sperm development,
643 during which, mitochondrial nuclease, Endonuclease G (EndoG), eliminates only mtDNA
644 rather than the complete mitochondria. Later on during spermatogenesis, the residual mtDNA
645 is eliminated during spermatid individualization stage, through a cellular remodelling process
646 that modify spermatid tail (DeLuca and O’Farrell, 2012). This mechanism is similar to the

647 one observed in *C. elegans* whereby the endonuclease CPS-6 originally localized to the
648 intermembrane space has access to the mtDNA, after rupture of the IMM by an unknown
649 mechanism. This step seems to be important for the subsequent PME steps (Zhou et al.,
650 2016). Moreover, an additional active mechanism exists also in drosophila eggs to get rid of
651 paternal mitochondria (Politi et al., 2014). This mechanism uses non conventional autophagy
652 and endocytic pathways to eliminate sperm mitochondria within the eggs even if these sperm
653 mitochondria is devoid of DNA. Paternal mitochondria destruction (PMD) initiates with
654 vesicles resembling MVB that put mitochondria into small fragments that are subsequently
655 sequestered in autophagosomes that finally fuse with lysosomes. P62 and ubiquitination are
656 involved but this mechanism is not Parkin dependent (Politi et al., 2014). In mice, the
657 situation is debated. One report indicates that most motile sperm eliminate their mtDNA and
658 leave only vacuolar mitochondria before fertilization. Then the sperm mitochondria persist
659 until morula stage, even they are observed to colocalize with autophagy proteins p62 and LC3
660 just after fertilization. Nonetheless, their clearance is independent of autophagy pathway (Luo
661 et al., 2013). However, this conclusion is challenged by a functional analysis showing that
662 paternal mitochondria are maintained in 60% of the embryos depleted for Parkin and Mul1.
663 Therefore, the authors concluded that in mice, PMD required a Parkin and Mul1 dependent
664 autophagy (Rojansky et al., 2016). Sutovsky and colleagues addressed the PME question in
665 another vertebrate, the porcine zygote, and provided evidences for another type of active
666 paternal mitochondria destruction mechanism. This mechanism is based on a P62/SQSTM1-
667 dependent autophagy, VCP-mediated dislocation of ubiquitinated mitochondria protein and
668 presentation to the 26S proteasome (Song et al., 2016). Overall, multiple different systems
669 have been selected during the evolution to specifically eliminate paternal mitochondria in
670 most animals suggesting that PME step is critical for the subsequent animal development.

671

672

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674

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1197 **Legends of figures**

1198

1199 **Figure 1. The three types of autophagy in most eukaryotic cells**

1200 (A) Macroautophagy, which is commonly named autophagy, is characterized by the
1201 sequestration of cargos within a double membrane structure, called the autophagosome. In
1202 mammals, the autophagy starts by the formation of a flat membranous structure, the
1203 phagophore, which elongates and close to generate a double membrane vesicle. Finally, the
1204 external membrane of the sealed autophagosome fused with the lysosome to generate an
1205 acidic and degradative compartment named the autolysosome, allowing recycling of
1206 autophagic cargoes. The core autophagy machinery forms various complexes that acts in four
1207 major steps. the ULK1/2 (unc-51 like autophagy activating kinase 1/2) complex is involved in
1208 the initiation step of the process. The class III phosphatidylinositol 3-kinase (PI3K) complex
1209 induces locally PI3P (phosphatidylinositol 3 phosphate) synthesis and allows the nucleation
1210 of the phagophore by recruiting the PI3P binding complex. The Atg12 and the Atg8
1211 conjugation systems are controlling the elongation of the phagophore to form the
1212 autophagosome through lipidation of Atg8s to phosphatidylethanolamine (PE).

1213 (B) Membrane invagination of either the endosome or the lysosome allows the direct
1214 sequestration of cargoes within degradative compartments by endosomal microautophagy or
1215 microautophagy, respectively.

1216 (C) Chaperone-mediated autophagy (CMA) targets proteins containing the KFERQ motif that
1217 is recognized by several chaperones, including Hsp70 (Heat shock protein 70kDa). During
1218 CMA, LAMP2 (Lysosomal-Associated Membrane Protein) mediates the transfer of Hsp70-
1219 protein aggregates within the lysosome.

1220

1221 **Figure 2. Ubiquitin-dependent or independent mitophagy pathways involve multiple**
1222 **receptors.**

1223 Two main mitophagy pathways allow the selective degradation of mitochondria by autophagy
1224 in physiological and stress induced conditions. **(A)** On one hand, mitochondrial receptors
1225 mediate mitophagy through the direct interaction with Atg8/LC3 proteins. Such mitochondrial
1226 receptors are outer mitochondrial membrane proteins NIX NBIP3 (NIX- NIP-3-Like Protein
1227 X / BNIP3L- BNIP3 like), FUNDC1 (FUN14 Domain Containing 1), BCL2L13 (Bcl2-like
1228 protein 13), and inner membrane protein prohibitin or the phospholipid cardiolipin. **(B)** On the
1229 other hand, the Pink1 (PTEN-induced putative kinase 1)-Parkin dependent mitophagy
1230 pathway requires cytosolic protein adapters. Upon mitochondrial damage, PINK1, that is
1231 usually cleaved and processed, is stabilized at the outer mitochondrial membrane where it
1232 phosphorylates both ubiquitin residues on mitochondrial protein and the E3 ubiquitin ligase
1233 Parkin. Activation of Parkin results in the high density of phospho-ubiquitin chains linked to
1234 substrates, which triggers the mitochondrial recruitment of the receptors OPTN (Optineurin),
1235 NDP52(Nuclear dot protein 52 kDa), SQSTM1(sequestosome 1), NBR1 (Neighbor of
1236 BRAC1) and TAX1BP1 (Tax1-binding protein) and their interaction with the autophagy
1237 machinery. In both pathways, Atg8/LC3s can interact with ULK1 (unc-51 like autophagy
1238 activating kinase 1) to induce autophagosome formation.

1239

1240 **Figure 3. Schematic representation of *C. elegans* life cycle indicating autophagy and**
1241 **mitophagy characterized processes.**

1242 The development cycle of *C. elegans* is rapid and stereotyped (3.5 days at 20 °C), and
1243 consists of an embryonic stage and four larval stages before producing an adult animal that
1244 can live several weeks. In response to environmental stress an alternative developmental
1245 pathway results in the formation of a diapause stage called dauer larva. The figure only shows
1246 hermaphrodite animals, which are fertile for several days in absence of cross-fertilization by
1247 males. The various types of autophagy and the associated cargo are indicated in orange.

1248

1249 **Figure 4. Detection of mitophagy by microscopy in *C. elegans***

1250 **(A)** Live imaging confocal microscopy of mitophagy events (yellow arrows) monitored by the
1251 colocalization between mitochondria stained with the fluorescent Mitotracker (red) and
1252 autophagosomes labelled with GFP::LGG-1 (green), the *C. elegans* homolog of GABARAP.
1253 **(B)** Transmission electron microscopy image of muscle cells showing regular mitochondria

1254 (m) and a stressed-mitochondria containing dark inclusions, engulfed by an autophagosome
1255 (yellow arrow).

1256

1257 **Figure 5. Degradation of spermatozoid-inherited mitochondria by autophagy in *C.***
1258 ***elegans***

1259 Three processes are involved in the degradation of paternal mitochondria during fertilization
1260 (see also Box 1). **(Left)** The internal membrane (IMM) breakdown occurs shortly after
1261 fertilization and causes the relocation of the endonuclease CPS-6 from the intermembrane
1262 space to the matrix of paternal mitochondria. CPS-6 catalytic activity triggers the degradation
1263 of paternal mitochondrial DNA (mtDNA) and promotes paternal mitochondrial elimination
1264 (PME) through autophagy. **(Right)** As soon as fertilization occurs, paternal mitochondria are
1265 further ubiquitinated, allowing the recognition and binding by the autophagy receptor ALLO-
1266 1. ALLO-1 interacts with the IKKE-1 kinase, which phosphorylates ALLO-1 and probably
1267 other substrates that could be present on mitochondria. Then ALLO-1 interacts with LGG-1
1268 through its LIR domain to allow the formation of autophagosomes around paternal
1269 mitochondria. **(Middle)** Paternal mitochondria are also tagged by two specific mitophagy
1270 receptors, the outer mitochondrial membrane (OMM) protein FNDC-1 and the inner
1271 mitochondrial membrane protein, prohibitin PHB-2. After the depolarization and rupture of
1272 the OMM, which is dependent of the proteasome, PHB-2 is exposed to the cytosol and
1273 interacts with LGG-1.

1274

1275 **Figure 6. SKN-1 coordinates mitochondrial biogenesis and mitophagy during *C. elegans***
1276 **aging.**

1277 The mitochondrial homeostasis depends on the balance between mitochondrial biogenesis and
1278 degradation. In physiological condition, a basal mitophagy flux regulates the mitochondrial
1279 content. Mitochondrial damages result in the increase of cytosolic Ca²⁺ and ROS causing the
1280 releasing of several transcription factors, including SKN-1 (SKiNhead-1). The mitochondrial
1281 quality control is first ensured by UPR^{mt} (Unfolded Protein Response in mitochondria), an
1282 adaptive transcriptional response that promotes protein refolding and mitochondrial recovery.
1283 When dysfunctions are persistent and the UPR^{mt} overwhelmed, elimination of the defective
1284 mitochondria occurs through mitophagy. The transcriptional factor SKN-1 promotes the
1285 activation of nuclear genes coding for mitochondrial proteins and mitophagy components,
1286 such as DCT-1 (Daf-16/ FOXO controlled germline tumor affecting-1) which is localized at
1287 the outer mitochondrial membrane and can interact with LGG-1 to mediate mitophagy.

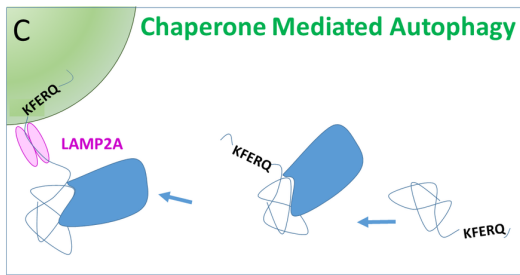
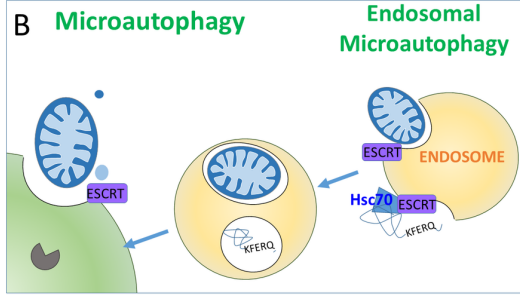
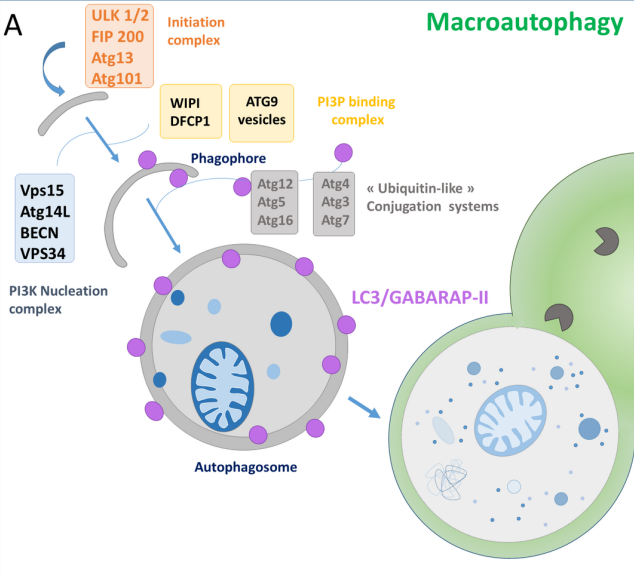
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Table 1 : Key mammalian proteins involved in mitophagy and their homologues in *C. elegans*
 Autophagy receptors as well as cargos are engulfed by autophagosomes for degradation
 References are key publications linked to the mitophagy functions of the proteins. n.d : not determined.

Mitophagy actors in mammals	<i>C.elegans</i> homologues	Function	Localisation	References
AMBRA1 - Activating molecule in Beclin-1-regulated autophagy	n.d	Mitophagy inducer Parkin and LC3 interactor	cytosolic	(Strappazon et al., 2015; Strappazon and Cecconi, 2015; Van Humbeeck et al., 2011)
BCL2-L-13 – Bcl2-like protein 13 -Homologue of yeast Atg32	n.d	Mitophagy receptor	OMM	(Murakawa et al., 2015; Sakakibara et al., 2015)
BNIP3 - BCL2/adenovirus E1B 19kDa protein interacting 3	DCT-1 - Daf-16/ FOXO controlled germline tumor affecting-1	Mitophagy receptor	OMM	(Hanna et al., 2012; Quinsay et al., 2010; Schiavi et al., 2015)
BNIP3L - BNIP3 like / NIX - NIP-3-Like Protein X	DCT-1 - Daf-16/ FOXO controlled germline tumor affecting-1	Mitophagy receptor	OMM	(Palikaras et al., 2015; Schweers et al., 2007)
Cardiolipin is a major membrane phospholipid localized in mitochondria	Cardiolipin	Lipid mitophagy receptor	Translocates from IMM to OMM	(Chu et al., 2013; Sakamoto et al., 2012)
FKBP8 - FK506-binding protein (FKBP) family	n.d	ATG8 interacting protein- Adaptor	OMM	(Bhujabal et al., 2017)
FUNDC1 – FUN14 Domain Containing 1	FNDC-1	Mitophagy receptor	OMM	(Lim et al., 2019; Liu et al., 2012)
LC3 - light chain 3 / GABARAP - GABA(A) receptor-associated protein -Homologue of yeast Atg8	LGG-2 / LGG-1	Initiation, elongation and closure of autophagosome/ recognize specific cargos to degrade	Cytosolic protein conjugated to autophagosome	(Alemu et al., 2012; Manil-Ségalen et al., 2014)

NDP52 - Nuclear dot protein 52 kDa	n.d	Ubiquitin-binding receptor protein	Cytosolic protein	(Vargas et al., 2019)
NBR1 – Neighbor of BRAC1 gene	n.d	Ubiquitin-binding receptor protein	Cytosolic protein	(Hollville et al., 2014)
NRF2/NFE2L2 - Nuclear factor (erythroid-derived 2)-like 2	SKN-1 -SKiNhead 1	Transcription factor	Cytosolic and nucleus	(Fang et al., 2017; Ghose et al., 2013)
OPTN - Optineurin	n.d	Ubiquitin-binding receptor protein	Cytosolic protein	(Padman et al., 2019)
PHB2 – Prohibitin 2	PHB-2	Mitophagy receptor	IMM	(Wei et al., 2017a)
PINK1 -PTEN-induced putative kinase 1	PINK-1	Serine/threonine kinase	OMM	(Kawajiri et al., 2010; Narendra et al., 2010)
PRKN – Parkin	PDR-1 Parkinson's Disease Related 1	E3 ubiquitin ligase	Cytosolic protein	(Narendra et al., 2008; Schiavi et al., 2015)
SQSTM1/p62 –sequestosome 1	SQST-1	Ubiquitin-binding receptor protein	Cytosolic protein	(Geisler et al., 2010; Zhang et al., 2017)
TAX1BP1 – Tax 1 binding protein 1	n.d	Ubiquitin-binding receptor protein	Cytosolic protein	(Whang et al., 2017)

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- Atg8 family
- Chaperones
- ESCRT Endosomal Sorting Complexes Required for Transport

Figure 1 Leboutet et al.

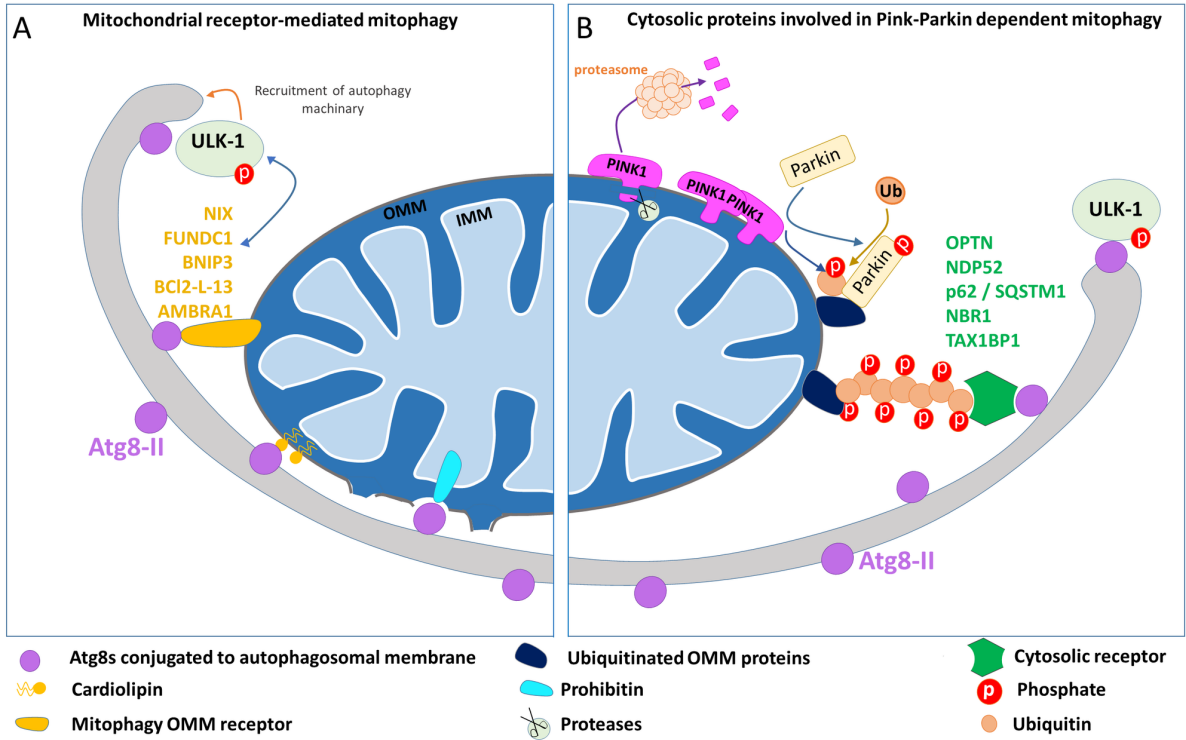


Figure 2 Leboutet et al.

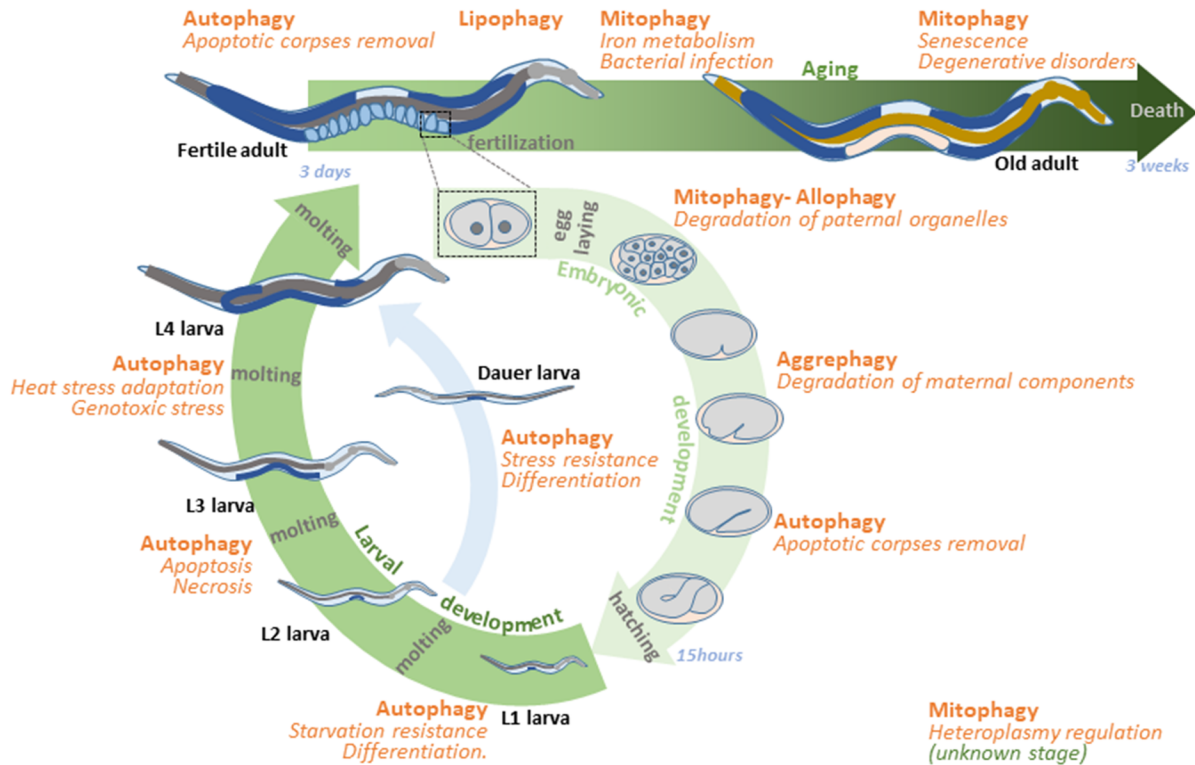


Figure 3 Leboutet et al.

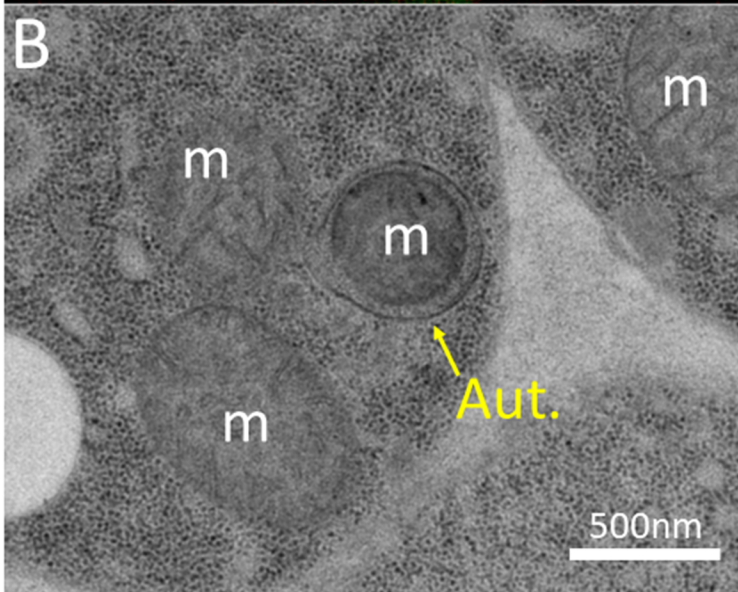
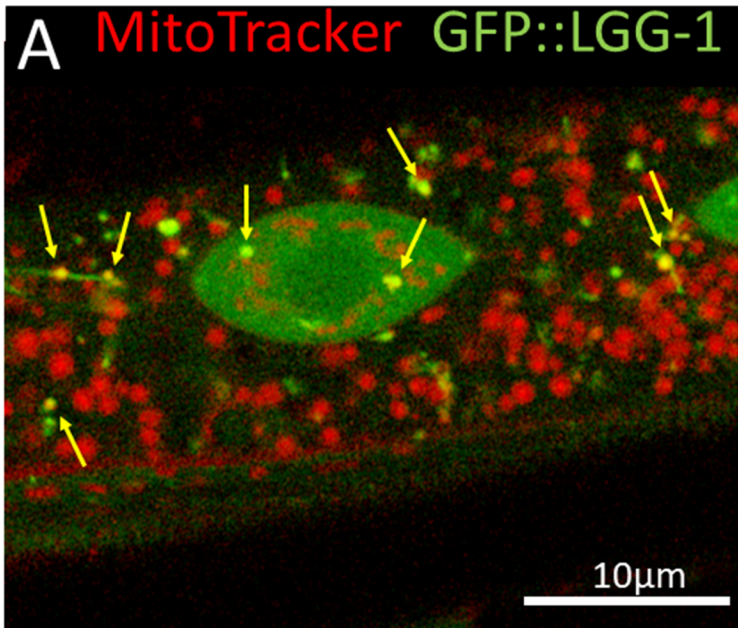
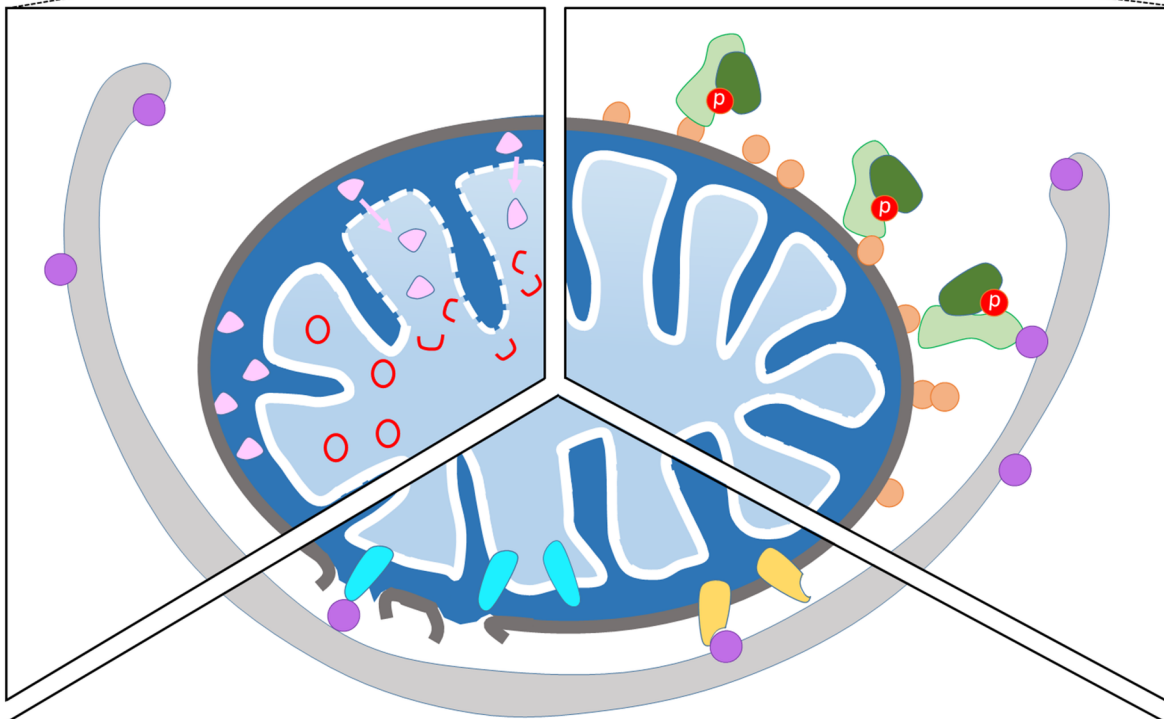
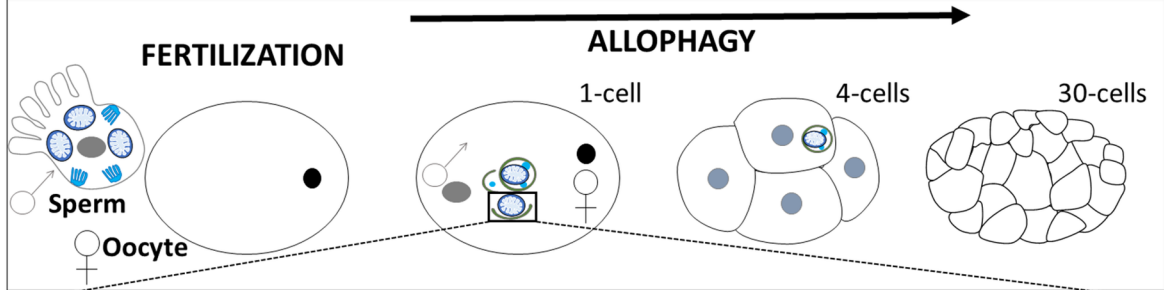


Figure 4 Leboutet et al.



Paternal mitochondria
 Membranous Organelle
 Gamete pronuclei

CPS-6
 mitochondrial DNA
 LGG-1 PE-conjugated

Prohibitin
 FNDC-1

Phosphate
 Ubiquitin
 IKKE-1
 ALLO-1

Figure 5 Leboutet et al.

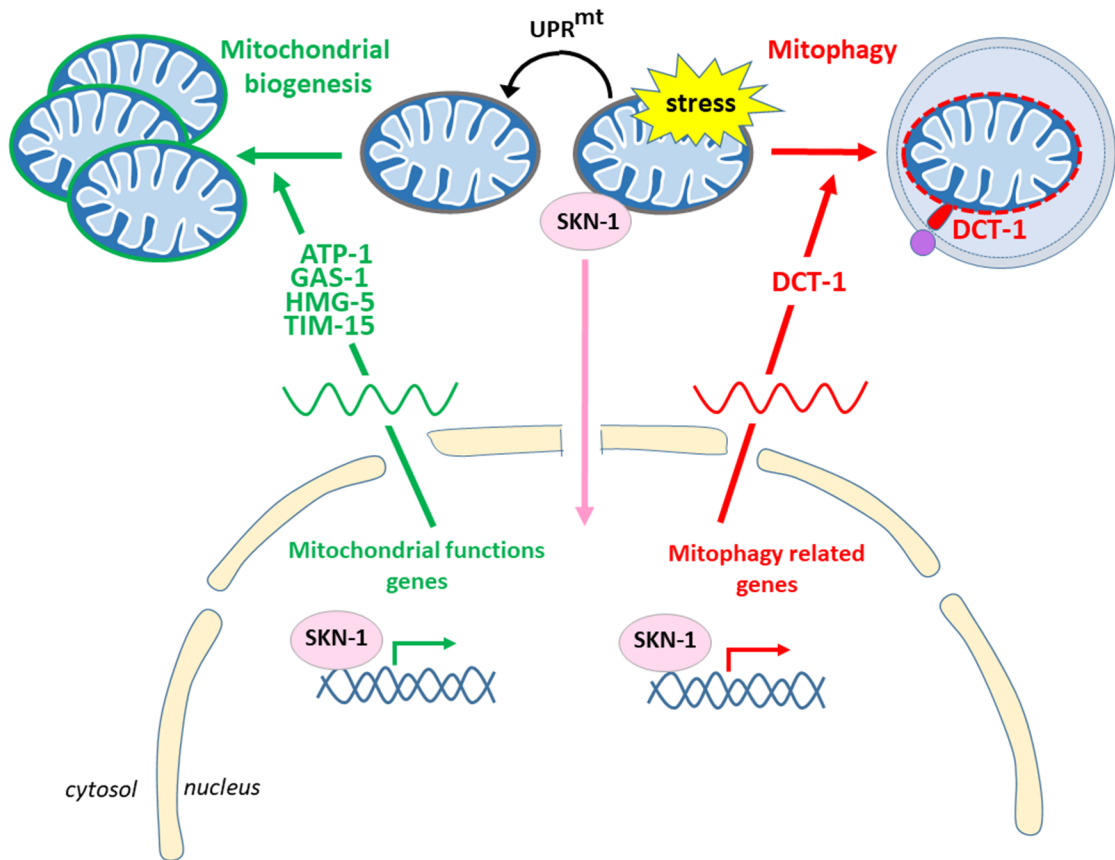


Figure 6 Leboutet et al.