Canadian Science Publishing | Genome

INSERTIONS OF MITOCHONDRIAL DNA INTO THE NUCLEUS. EFFECTS AND ROLE IN CELL EVOLUTION.

Journal:	*Genome*
Manuscript ID	gen-2019-0151.R3
Manuscript Type:	Review
Date Submitted by the Author:	09-Apr-2020
Complete List of Authors:	Puertas, Maria; Universidad Complutense, Departmento de Genetica Gonzalez-Sanchez, Monica; Universidad Complutense de Madrid, Departamento de Genética, Fisiología y Microbiología
Keyword:	mitochondrial DNA, NUMT, ageing, centromere, parasitic genetic element
Is the invited manuscript for consideration in a Special Issue? :	Not applicable (regular submission)

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1 **INSERTIONS OF MITOCHONDRIAL DNA INTO THE NUCLEUS. EFFECTS
2 AND ROLE IN CELL EVOLUTION.**

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13 ABSTRACT

14 We review the insertion of mitochondrial DNA (mtDNA) fragments into nuclear DNA
15 (NUMTS) as a general and ongoing process that has occurred many times during
16 genome evolution. Fragments of mtDNA are generated during the lifetime of organisms
17 in both somatic and germinal cells, by the production of reactive oxygen species in the
18 mitochondria The fragments are inserted into the nucleus during the double strand
19 breaks repair via the non-homologous end joining machinery, followed by genomic
20 instability, giving rise to the high variability observed in NUMT patterns among
21 species, populations or genotypes. Some de novo produced mtDNA insertions show
22 harmful effects, being involved in human diseases, carcinogenesis and ageing. NUMT
23 generation is a non-stop process overpassing the Mendelian transmission. This parasitic
24 property ensures their survival even against their harmful effects. The accumulation of
25 mtDNA fragments mainly at pericentromeric and subtelomeric regions is important to
26 understand the transmission and integration of NUMTs into the genomes. The possible
27 effect of female meiotic drive for mtDNA insertions at centromeres remains to be
28 studied. In spite of the harmful feature of NUMTs, they are important in cell evolution
29 representing a major source of genomic variation.

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31 Keywords: mitochondrial DNA, NUMT, ageing, centromere, parasitic genetic elements

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34 INTRODUCTION

35 The evolution of the DNA carrying cell organelles, mitochondria and chloroplasts, may
36 be considered under three related points of view: (i) the evolution from bacteria to
37 organelles, according to the endosymbiotic origin of the eukaryotic cell, (ii) the
38 evolution of organelle chromosomes producing a large variability in size and structure
39 in different species, resulting in the nearly-unidirectional loss of DNA from the former
40 bacterial chromosomes to the extant organelle chromosomes, including the insertion of
41 organelle coding genes in the main nuclear DNA, and (iii) the phylogenetic

42 relationships among species or populations that can be established to compare the DNA
43 sequences of their organelles, as well as the insertions of organelle DNA into nuclear
44 DNA

45 In the present work we review the insertion of mitochondrial DNA (mtDNA) fragments
46 into nuclear DNA (nDNA), considering that the mtDNA transfer to the nucleus seems
47 to be a general evolutionary trend that may have occurred many times along the genome
48 evolution and it is still an ongoing process, including the lifetime of many organisms.
49 This is a type of intracellular horizontal gene transfer whose effects on the individuals
50 undergoing the transfer and the long term evolutionary consequences are of general
51 biological interest

52 The comparison between mitochondrial and bacterial genomes has shown that
53 mitochondria share a common ancestor with the *Alphaproteobacteria*. The first
54 complete genome sequence of the obligate intracellular parasite *Rickettsia prowazekii*
55 showed similarities to mitochondrial genes (Andersson et al. 1998). More recently
56 Thrash et al. (2011) determined a common origin of mitochondria and SAR11 clade as a
57 sister group to the Rickettsiales. These results are accepted as a proof that the symbiotic
58 event that gave origin to mitochondria occurred only once in cell evolution.

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59 The sequencing of mtDNA in a number of animal, plant, fungi and protists has shown
60 that there is a large variation in structure and DNA amount among mitochondria of
61 different species. Remarkable is the case of Jakobid protists showing the most bacteria-
62 like mitochondrial genomes in size and genetic structure (Burger et al. 2013). All
63 mitochondrial genomes known are reductions of these protist mitochondrial genomes,
64 which indeed favors the idea of the uniqueness of the symbiotic event giving rise to
65 mitochondria. Despite their large diversity, a common feature shared by all known
66 mitochondria is that a few of their constitutive proteins are coded by the mitochondrial
67 genes, whereas most of the mitochondrial proteins are coded by nuclear genes.

68 Not all eukaryotes contain mitochondria. Anaerobic eukaryotes, such as some Ciliates,
69 Trichomonads, Amoeboflagellates and fungi do not contain mitochondria but their cell
70 energy is provided by organelles called *hydrogenosomes* which produce ATP and
71 hydrogen anaerobically. Other anaerobic or microaerophilic organisms, such as
72 Microsporidia, contain cell organelles called *mitosomes*. Although the evolutionary
73 origin of both hydrogenosomes and mitosomes is difficult to establish because both

74 have lost their DNA entirely, protein based phylogenies, particularly the Hps70 family,
75 show that both are evolutionarily related to mitochondria (Williams et al. 2002; Embley
76 et al. 2003).

77 All these facts may be interpreted as the evidence of a single symbiotic event giving rise
78 to mitochondria as a cell organelle, and a general evolutionary trend consisting in the
79 gradual loss of mitochondrial genes and their transfer to the nucleus that may end in the
80 total loss of the organelle chromosome. In this way, the initial single symbiotic event
81 may have resulted in a large variability of types of mitochondria and cell nuclei carrying
82 mitochondrial DNA fragments variable in number, size and location.

83

84 NUMTS, GENES AND PSEUDOGENES. IDENTIFICATION AND VARIABILITY

85 Mitochondrial DNA sequencing reveals that mitochondria carry an incomplete set of
86 genes for their own function, together to non-coding regions necessary for the
87 regulation of mtDNA replication and expression. One remarkable example is the
88 nuclear encoded DNA polymerase gamma (PolG) that replicates and repairs mtDNA
89 and is homologous to *E. coli* pol I (Ito and Braithwaite 1991). *Polg* nuclear mutations
90 affect the maintenance and proofreading function of mtDNA (Bailey et al. 2009). *Polg*
91 mice mutants suffer premature ageing and various deleterious effects (Trifunovic et al.
92 2004). This provides evidence that functional genes have been transferred from the
93 mtDNA to the nDNA. Most of the major protein complexes involved in oxidative
94 phosphorylation contains both nuclear and mitochondrial encoded subunits, which
95 promotes interesting questions on the co-evolution of mitochondrial and nuclear genetic
96 functional interactions within the same cell. Other mitochondrial genes transferred to
97 nDNA are unable to be expressed, maybe due, among other reasons, to the different
98 genetic codes used in the mitochondria and the cytosol. These genes reside in the nDNA
99 as pseudogenes.

100 Lopez et al. (1994) coined the term NUMT (**nu**clear **mit**ochondrial DNA segment) for
101 mtDNA sequences present in eukaryotic nuclei, which is generally applied to
102 pseudogenes only or, in broad sense, to mitochondrial sequences found in nDNA
103 ignoring if they are expressed or not. Singh et al. (2017) used *numtogenesis* to refer to
104 the transfer of mtDNA into the nuclear genome or, less specifically, the transfer of

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105 mitochondrial components into the nucleus. Similarly, in the case of plants, the term
106 NUPT refers to chloroplast DNA (cpDNA) inserted in nDNA.

107 NUMT identification is important both for the annotation and understanding of the
108 genomes and for evolutionary studies. The first works identifying NUMTs were carried
109 out by DNA hybridization, reporting the presence of sequences homologous to
110 mitochondrial genes in nDNA, mainly rRNA genes and cytochrome oxidase subunit I
111 gene. For example, Fukuda et al. (1985) isolated phage clones carrying DNAs
112 homologous to human mtDNA, estimating that human nDNA contains several hundred
113 copies of mtDNA-like fragments. The variety of organisms where homology of mtDNA
114 and cpDNA sequences was found in nDNA early made clear that this phenomenon was
115 of general occurrence (Zhang and Hewitt 1996; Bensasson et al. 2001). The advances of
116 whole genome sequencing projects allowed much more accurate studies, particularly in
117 the case of the human genome. Mourier et al. (2001) presented the first extensive
118 NUMT analysis; they found long NUMTs representing nearly all parts of the mtDNA.
119 Woischnik and Moraes (2002) found 612 independent integrations of mitochondrial
120 pseudogenes in the human genome evenly distributed among all nuclear chromosomes
121 as well as within each individual chromosome.

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122 The comparison of NUMTs among species shows a high variability. Richly and Leister
123 (2004) compared 13 species with sequenced mitochondrial and nuclear genomes
124 revealing large interspecific variation of NUMT copy number and size, although no
125 clear explanation exists for the interspecific diversity of NUMTs and NUPTs (Leister
126 2005). In certain protists the low NUMT and NUPT number can be due to the low
127 number of possible donor organelles per cell. Another possible explanation relates to
128 interspecific differences in the efficiency of integration of organelle DNA into the
129 nuclear genome.

130 Interestingly, a correlation between the abundance of NUMTs and the size of the
131 nuclear or the mitochondrial genomes, or of the nuclear gene density, is not evident.
132 However, in mosquitoes Ding et al. (2018) carried out a NUMT analysis of nineteen
133 mosquito species and concluded that the number, total length and density of NUMTs
134 are significantly correlated with genome size; moreover NUMTs are an important cause
135 of nuclear genome size expansion in mosquitoes. In fungi Krampis et al. (2006) and
136 Sacerdot et al. (2008) relied on a comparative analysis of the NUMT content. Results

137 revealed large differences in NUMT number and organization across the species. In
138 plants Ko and Kim (2016) found that NUMT patterns vary from species to species.

139 NUMT variability is found intraspecifically or even intraindividually as well. Lough et
140 al. (2008, 2015) studied NUMTs in a set of maize inbred lines, showing extensive
141 NUMT variation in size and location among lines, suggesting that mtDNA is being
142 incorporated or lost from the maize nuclear genome continuously. The same is true for
143 cpDNA insertions; Roak et al. (2010) studied the NUPTs into maize chromosomes of
144 the same lines. Like NUMTs, the positions of the NUPTs varied greatly among the
145 lines, suggesting that the transfers are recent as well as frequent. In *Aegilops speltoides*
146 the distribution of organellar-derived insertions differed among populations (Ruban et
147 al. 2014). Malik et al. (2016) found intraindividual variation of mtDNA levels which
148 differed significantly in mouse tissues. This variability is not functionally irrelevant.
149 Rand et al. (2006) compared the longevity in strains of *Drosophila simulans* carrying
150 mtDNAs with varying levels of divergence. The interspecific mtDNA strains showed a
151 very significant epistatic interaction effects depending of the nuclear and mtDNA
152 origins.

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153 The organellar DNA transferred to the nucleus can be deleted as demonstrated by
154 Sheppard and Timmis (2009) using a kanamycin resistance gene (neo) transferred from
155 cpDNA to the nucleus in tobacco. They found that the gene is highly unstable, with
156 deletion often occurring within a single generation, indicating that plastid DNA
157 insertion into and removal from the nuclear genome might be in dynamic equilibrium.
158 Schneider et al. (2014) found reversible accumulation of mtDNA in the mouse nucleus.
159 They studied the accumulation of mtDNA in embryonic and induced pluripotent stem
160 cells, reporting that upon differentiation, the level of mtDNA in these nuclei was
161 substantially reduced.

162 In spite of being essential for understanding the NUMT inheritance, there are few
163 studies comparing the mtDNA between somatic and germ lines. Sato et al. (2007)
164 compared the proportions of mitochondria carrying deleted mtDNA in various tissues at
165 various ages. Certain somatic tissues showed increases in the proportion of deleted
166 mtDNA with age, but the germ cells of females and their offspring showed a strong
167 decrease in deleted mtDNA with maternal age. It seems that female germ cells have
168 machinery that prevents the inheritance of defective mtDNA. de Paula et al. (2013b)

169 have shown that female gametes of *Aurelia aurita* do not transcribe mtDNA, lack
170 electron transport, and produce no free radicals. In contrast, male gametes actively
171 transcribe mitochondrial genes for respiratory chain components and produce reactive
172 oxygen species. The authors predict that quiescent oocyte mitochondria contain DNA as
173 an unexpressed template that avoids mutation accumulation by being transmitted
174 through the female germ line.

175

176 NUMT GENERATION AND INSERTION MECHANISMS

177 The existence of NUMTs implies that fragments of mtDNA must be produced in the
178 mitochondria and reach the nucleus despite of the physical barriers it must overcome. It
179 is generally accepted that mtDNA fragments are produced by the constant generation of
180 reactive oxygen species (ROS) in the mitochondria (Barja 2013). The mtDNA, devoid
181 of histones and located in the inner membrane of the mitochondria, very close to the
182 place of ROS production, is more vulnerable than nDNA to oxidative damage caused by
183 ROS.

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184 Several mechanisms have been proposed that would facilitate the exit of mtDNA from
185 the mitochondria and the entry into the nucleus. The most commonly accepted one is
186 that the fragments generated by ROS in the mitochondria, appear with a high 8-oxo-
187 deoxyguanosine content in the cytoplasm due to alterations of the organelle membrane,
188 either during division, fission/fusion events (Dimmer and Scorrano 2006), lysis (Mota
189 1963), mitophagy (Higgins and Coughlan 2014) or by the opening induction of a
190 permeability transition pore (Patrushev et al. 2004; Garcia and Chavez, 2007). Once in
191 the cytoplasm, mtDNA is protected from nucleases thanks to a mechanism mediated by
192 vacuoles which are degraded when they contact the nucleus (Campbell and Thorsness
193 1998). It has also been proposed (Kutsyi et al. 2005) that mtDNA could form a complex
194 with DNA-binding histone-like proteins avoiding degradation. Other pathways enabling
195 mtDNA to reach the nucleus are direct physical association between the mitochondrial
196 and nuclear membranes (Mota 1963) or encapsulation of the mitochondria inside the
197 nucleus (Jensen et al. 1976). It has been suggested that mtDNA could be transferred to
198 the nucleus in the form of mRNA with the help of a reverse transcriptase to complete
199 the process (Rodley et al. 2012). Nevertheless, some experimental studies do not
200 support this possibility. For example, Woischnik and Moraes 2002 found that large

201 NUMTs found in nDNA contain two or more mitochondrial genes with fragments of
202 non-coding regions. As well, Falkenberg et al. 2007 reported that the mitochondrial
203 transcripts are significantly shorter than the sequences of many NUMTs.

204 The mtDNA fragments that enter the nucleus must interact with the nDNA before their
205 integration for NUMTs formation. All regions of the mitochondrial genome are able to
206 interact with nDNA, as evidenced by the fact that recent human NUMTs contain
207 fragments originated from the entire mtDNA (Dayama et al. 2014). However, Doynova
208 et al. (2016), using chromosome conformation capture techniques to detect physical
209 interactions between mt- and nDNA in mammalian cells, showed that the D-loop region
210 exhibited a higher tendency to interact with the nuclear genome, probably because this
211 region is more prone to breakage than other mtDNA regions (Rothfuss et al. 2009).

212 MtDNA fragments are inserted into the nucleus during the process of double strand
213 breaks (DSBs) repair via the non-homologous end joining (NHEJ) machinery. This
214 hypothesis was first proposed by Blanchard and Schmidt (1996) and later confirmed by
215 Ricchetti et al. (1999) in a study on yeast in experimental conditions where homologous
216 recombination, the other DSBs repair mechanism, was avoided. Similar results
217 revealing the involvement of the NHEJ mechanism in the integration of mtDNA in the
218 nucleus were obtained in humans (Ricchetti et al. 2004). Hazkani-Covo and Covo
219 (2008) identified 35 and 55 lineage-specific NUMTs in the human and chimpanzee
220 genomes, respectively, showing that in 54% of the NUMT integration events examined
221 no deletions were detected. Considering that DSBs repair without NUMTs requires
222 nuclease processing of DNA ends that usually produce small deletions, these results led
223 the authors to propose that mtDNA fragments provide an alternative to nuclease activity
224 in DSBs repair via NHEJ using the mtDNA as a filler DNA.

225 Mitochondrial fragments are transferred to the nucleus in a single or several copies and,
226 in some cases, mtDNA suffers rearrangements prior or during the insertional event
227 (Ricchetti et al. 1999; Huang et al. 2003). These rearrangements include tandem
228 duplications or changes in gene order with respect to the organelle organization, as well
229 as the presence of fragments belonging to different regions of the mitochondrial
230 genome. Nevertheless, the evidence that older NUMTs tend to be shorter than recent
231 ones (Bensasson et al. 2003) and large recent insertions usually correspond to the whole
232 sequence of the mitochondrial genome (Huang et al. 2005) indicates that NUMTs can

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233 be fragmented after insertion. Matsuo et al. (2005) and Michalovova et al. (2013)
234 demonstrated that, once inside the nuclear genome, organellar DNA exhibits insertion
235 instability, with fragmentation and recombinational events in most cases mediated by
236 nuclear mobile elements.

237

238 mtDNA NUCLEAR INSERTIONS ARE INVOLVED IN DISEASE AND AGEING

239 Certain de novo mtDNA fragment insertion may produce harmful effects, being
240 involved in human disease. Willett-Brozick et al. (2001) described for the first time a
241 spontaneous recent germ line insertion of human mtDNA at the breakpoint junctions of
242 a familial constitutional reciprocal translocation. The 41-pb mitochondrial fragment was
243 captured during the repair of the DSBs involved in the translocation, revealing the
244 implication of this mechanism in the mtDNA transfer to the nucleus. A 251-bp mtDNA
245 insertion was found in a patient suffering from severe plasma factor VII deficiency, a
246 rare bleeding disorder. The mitochondrial fragment, containing the encoding DNA for
247 tRNA-Phe and part of the 12S subunit of rRNA, was integrated in the IVS 4 acceptor
248 splice site (Borensztajn et al. 2002). Turner et al. (2003) described a rare case of
249 Pallister-Hall syndrome caused by a transfer of mtDNA to the nuclear genome. The
250 fragment, 72-bp long, was found in exon 14 of the *GLI3* gene, generating a premature
251 stop codon and producing a truncated protein. Interestingly, this case was associated
252 with the radioactive contamination that followed the Chernobyl accident, revealing
253 again the link between NUMTs and DNA repair and instability. Cases of human
254 disease, type IV mucolipidosis (Goldin et al. 2004) and Usher syndrome type IC
255 (Ahmed et al. 2002), have also been related to mtDNA fragment nuclear insertions.
256 Millar et al (2010) reported an isolated case of lissencephaly caused by the insertion of a
257 mitochondrial genome-derived DNA sequence into the 50 untranslated region of the
258 PAFAH1B1 (LIS1) gene.

259 The mtDNA fragments are produced, transported and inserted from the mitochondria
260 towards the nucleus during the lifetime of the individuals in vital mitotic and post-
261 mitotic tissues. The consequences for the individual itself are important. Richter (1988)
262 first proposed that the oxidatively generated mtDNA fragments that escaped from
263 mitochondria and become integrated into the nuclear genome might transform cells to a
264 cancerous state.

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265 Several studies have concluded that both insertion and change in copy number of
266 mtDNA fragments are associated with carcinogenesis because mtDNA insertion may
267 disrupt tumor suppressor genes or activate oncogenes, contributing to cancer
268 development. Remarkably, surveys of thousands of human whole-cancer genomes have
269 shown that chromosomal rearrangements are frequently combined with mtDNA
270 fragments somatically transferred to the nucleus (Ju et al. 2015; Ju 2016). Mitochondrial
271 fragments have been identified in the *c-myc* oncogene of HeLa cells (Shay et al. 1991).
272 Srinivasainagendra et al. (2017) reported increased mtDNA insertions in the nuclear
273 genomes of colorectal adenocarcinomas. Mobile LINE elements with mitochondrial
274 inserts were found in the nuclear genome of mouse and rat tumors (Hadler et al. 1998).

275 The genomic instability caused by the insertions of mtDNA in the nuclear genome
276 during individual lifetime has also implications in ageing. The mitochondrial free
277 radical theory of ageing (MFRTA) (Harman 1972) proposes that ageing is caused by the
278 cumulative damage produced by the constant generation of ROS in the mitochondria
279 throughout the life of the individuals. It has been found that the mtDNA fragments have
280 a higher level of 8-oxo-deoxyguanosine, a marker of ROS-induced DNA oxidative
281 damage, than wild type non fragmented mtDNA (Suter and Richter 1999), supporting
282 ROS as causing agents of producing these fragments. The 8-oxodG levels are lower in
283 the heart and brain of long-than short-lived animal species in the case of mtDNA and
284 not in the nDNA (Barja and Herrero, 2000). MFRTA was updated and reviewed by
285 Barja (2013, 2017, 2019).

286 Studies in rat have shown that the amount of mtDNA in the nucleus increases with the
287 age of individuals in liver and brain (Caro et al. 2010). The mitochondrial regions
288 identified corresponded to cytochrome oxidase III and 16S rRNA. Interestingly, these
289 fragments contained the same SNPs found in the mitochondrial genome of the same
290 individuals, revealing their recent origin. Similar results showing an association
291 between increasing the copy number of mtDNA insertions and ageing were observed in
292 yeast, where the insertion of mtDNA fragments decreases the chronological life span,
293 measured as the time that non-proliferative cell populations can survive (Cheng and
294 Ivessa, 2010, 2012). Likewise, it has been proven in mice (Martínez-Cisuelo et al. 2016)
295 that age-related mtDNA fragment insertion is associated with the production of ROS in
296 the mitochondria throughout the life of individuals. The amount of mtDNA found in the
297 nuclei of hepatocytes and the rate of mitROS production of old mice decreased to levels

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298 similar to those found in young individuals (100% reversion) when the old ones were
299 treated during 7 weeks with rapamycin. This drug, that inhibits the TOR (Target Of
300 Rapamycin) protein, decreases the production of ROS in the mitochondrial complex I
301 and it is the only one described to date capable of increasing longevity in mammals in a
302 reproducible way (Harrison et al. 2009).

303

304 NUMTS ARE FREQUENTLY LOCATED AT PERICENTROMERIC AND/OR 305 SUBTELOMERIC REGIONS

306 In situ localization of NUMTs to chromosomes using mtDNA as probe provides data of
307 particular interest because NUMT position at specific chromosomes or chromosome
308 regions may reveal important features; however, not many works include these studies.
309 In situ localization in animal and plant species reveals frequent interactions between
310 mtDNA and nuclear heterochromatin such as the pericentromeric and/or subtelomeric
311 regions.

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312 Vaughan et al. (1999) localized mtDNA sequences to meiotic chromosomes of several
313 orthopteran species using in situ hybridization; mtDNA localization varied between
314 species being centromeric, telomeric or present throughout the chromosomes in
315 different species. Stupar et al. (2001) found an insertion of 270-kb mtDNA into the
316 pericentric region on the short arm of chromosome 2 of Arabidopsis. Michalovova et al.
317 (2013) found mtDNA and cpDNA insertions located at the pericentromeric regions of
318 Arabidopsis and rice. Lough et al. (2008, 2015) studied mtDNA insertions in a set of
319 maize inbred lines and found extensive variation in size and location among lines, but in
320 many lines the signals were located near the centromere or the telomere. Caro et al.
321 (2010) using FISH in bone marrow cells of young and old rats reported that 10 pairs of
322 chromosomes showed mtDNA signal located always at the pericentromeric region in
323 rats of both ages. Similar results were observed in the telocentric chromosomes of mice
324 where mtDNA fragments colocalize with pericentromeric satellite sequences (Martinez-
325 Cisuelo et al. 2016). Mustafa et al. (2018) obtained near-complete genomes of
326 mitochondria from wild sheep species or subspecies. In situ localization showed strong
327 hybridization to the centromeric regions of all autosomal sheep chromosomes, but not
328 the Y, with varying abundance of different mitochondrial regions. Recently, Koo et al.
329 (2018) have developed a technique of single-molecule mtFIBER FISH to study

330 numtogenesis in human, to aid in establishing a role for numtogenesis in cancer and
331 other human diseases.

332 B chromosomes (Bs) are supernumerary to the normal chromosome set (A
333 chromosomes or As) that are not required for the normal growth and development of the
334 B-carrier organism. In situ localization of mtDNA and cpDNA has been carried out in
335 the Bs of rye and *Aegilops speltoides* (Martis et al. 2012; Ruban et al. 2014). In both
336 species the B has accumulated large and significantly greater amounts of cp- and
337 mtDNA-derived sequences than the chromosomes of the normal set. Almost all parts of
338 the chloroplast and mitochondrial genomes were found on the Bs. In rye the organellar
339 DNA localizes in pericentromeric regions, whereas in *Ae. speltoides* insertions were
340 found along both arms of the Bs except at the pericentromere.

341 According to Michalovova et al. (2013), the frequent finding of mtDNA fragments
342 located at the pericentromeric regions is explained because the centromeres constitute a
343 stable genomic environment, being regions poor in genes, and because frequent DSBs
344 occur that are repaired using the NHEJ mechanism, thanks to which the fragments are
345 incorporated into the nuclear genome (Matsuo et al. 2005). This model predicts that in
346 species with great genome dynamism these blocks of pericentromeric mtDNA would be
347 fragmented by the insertion of transposable elements (TEs) taking them to other regions
348 of the genome, away from the centromeres. In addition, the ectopic recombination
349 mediated by TEs would be responsible for the remodeling of the regions where the
350 mtDNA fragments are found, producing their exit from the genome either when they are
351 forming a pericentromeric block or when they have been fragmented and are found in
352 other locations.

353 Centromeric function is highly conserved in all eukaryotes; however, centromeric DNA
354 shows remarkable sequence variability between species or even among different
355 chromosomes of the same species, as for example between A and B chromosomes
356 (Jones et al. 2008). This makes it remarkably difficult to define and identify the DNA
357 elements responsible for centromere activity. This large divergence is possible thanks to
358 the coevolution between the centromeric sequences and the kinetochore proteins. All
359 centromeres consist in repeated sequences, making difficult its sequencing. The
360 functional domain of the centromere consists in satellites, centromeric retrotransposons
361 or both. This functional region is surrounded by repeated sequences, possibly including

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362 mtDNA insertions in many cases, which are important for other centromeric functions
363 such as chromatid cohesion. The role of each of the regions is important in the
364 processes of cohesion and dissociation that occur in the centromeres during
365 chromosomal segregation in cell division (Guenatri et al. 2004).

366

367 mtDNA INSERTIONS MAY BE CONSIDERED AS PARASITIC GENETIC 368 ELEMENTS

369 Parasitic genetic elements (called selfish genetic elements as well) are those that
370 produce harmful effects to the host organisms, whereas they are maintained in
371 populations because they undergo a mechanism of drive resulting in higher than
372 Mendelian transmission. One of the best studied examples of parasitic genetic elements
373 are the B chromosomes (Bs), supernumerary to the normal A chromosome set, where
374 population dynamics of B carrying species depends on the strength of non-Mendelian
375 drive mechanisms counteracted by the harmful effect of Bs on the fitness of B-carrier
376 individuals (Jones et al. 2008).

377 Transposable elements (TEs) are considered parasitic elements as well, because they
378 may produce harmful effects when they move producing deleterious mutations and
379 possess mechanisms that promote their own higher than Mendelian transmission.
380 Besides their parasitic features, the TEs have been demonstrated to be a main
381 evolutionary force influencing or even driving the genomic and karyotypic evolution
382 (Ayarpadikannan and Kim 2014).

383 Interestingly, NUMTs and TEs share certain common features: they are “passengers” of
384 the nucleus that play various roles in processes affecting genome evolution and genetic
385 instability. They are instable *per se* and produce genomic instability. The effects of TE
386 and NUMTs in the genome vary from negligible to harmful producing various genetic
387 disorders and cancer. Recently, the possible adaptive phenotypic changes associated
388 with TEs were considered (Schrader and Schmitz 2019) indicating that the activity of
389 TEs might facilitate adaptive responses to environmental challenges.

390 Mitochondrial ROS production breaks mtDNA into mtDNA fragments which are
391 transferred to the nucleus, mainly at pericentromeric regions, during the lifetime of the
392 individual, promoting ageing at nuclear level. The mechanisms involved potentially

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393 include harmful effects such as the induction of major chromosomal rearrangements,
394 inhibition of cell division and transposon-mediated insertion and modification of
395 regulatory regions and structural coding genes (Barja, 2017). On the other hand, the
396 generation of NUMTs seems to be a non-stop process overpassing the Mendelian
397 transmission. These parasitic properties ensure their survival and spread in natural
398 populations, even against the harmful effects on the host. The accumulation of mtDNA
399 fragments mainly at centromeric and also subtelomeric regions is important to
400 understand the transmission and integration of NUMTs into the genomes which might
401 promote their own higher than Mendelian transmission.

402 The potential harmful effect of NUMTs is evidenced in the case of the parasitic B
403 chromosomes of rye and *Ae. speltoides.* The Bs show a much higher amount of
404 organelle derived DNA than the A chromosomes of the normal set. Insertions into A
405 chromosomes may disrupt gene function with lethal consequences. In contrast the Bs,
406 which are not required for growth and development, can tolerate more mutations
407 (Martis et al. 2012; Ruban et al. 2014).

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408 As in the case of TEs, the proposed parasitic features of NUMTs does not mean that
409 they are not important in cell evolution; contrarily, as stated by Leister (2005) NUMTs
410 and NUPTs are more than only “mutagens”. NUMTs and NUPTs can influence nuclear
411 processes such as replication or transcription, or they might even rebuild genes and their
412 products by providing new exons. If they can, this would constitute, in addition to the
413 ancient transfer of entire prokaryotic genes to the nucleus, a further contribution that
414 organelles make to the evolution of nuclear genomes. Popadin et al (2017) found that
415 the pseudogene Ps5, a large 9Kb-NUMT, was independently fixed in populations of
416 gorilla and the human/chip nascent populations, which implies that the spread of the
417 pseudogene within and across populations might have been driven by positive selection.
418 Besides, the rate of NUMT insertion is not constant and may correlate with critical
419 points in evolution. Gubin et al. (20017a, 2017b) estimated the times of incorporation of
420 18 selected NUMTs during the period of evolution of the human lineage after separating
421 from the chimpanzee and found a non-random rate of insertion, with one cluster situated
422 around 2.8 million years ago, corresponding to a period of major climate change and the
423 time of emerge of the genus Homo. Similarly, the reconstruction of the NUMT insertion
424 history in two bird species Geospiza fortis and Zonotrichia albicollis, and their closed
425 relatives, showed a remarkable acceleration of insertions in the ancestor of both species

426 followed by a slower accumulation in each lineage (Liang et al 2018). The results
427 indicate that mtDNA insertions represent a major source of nuclear chromosomal
428 variation. Whether or not mtDNA insertions might play an adaptive role in the
429 speciation processes is a matter of future research.

430 In the case of mtDNA fragment insertion into nDNA, the co-evolution between nuclear
431 and cytoplasmic genomes has to be considered differently in unicellular or asexually-
432 reproducing vs multicellular organisms with separated somatic and germ lines. In
433 asexually-reproducing organisms the NUMTs inserted into nDNA will be transmitted to
434 the progeny, unless a hypothetical detachment mechanism were present; whereas in
435 organisms with separated somatic and germ lines, only those NUMTS present in the
436 gamete DNA will be transmitted. The great majority of NUMT studies were carried out
437 in somatic tissues, thus lacking much information on this main point.

438 It should be also considered that the harmful effect of NUMT insertion during lifetime
439 of individuals (cancer, ageing) usually occurs after the reproduction of the individual
440 has occurred, and therefore it is reasonable to think that they will not affect significantly
441 the fertility either at the individual or at population level. It was also hypothesized
442 (Richly and Leister 2004) that factors, such as the number and/or stability of
443 mitochondria in the germline, or species-specific mechanisms controlling
444 accumulation/loss of nuclear DNA, might be responsible for the interspecific diversity
445 in NUMT accumulation, but as far as we know there are no population quantitative
446 studies relating the NUMT number and size polymorphism with effects on fitness.

447 Various studies propose a different role of female and male germ lines for NUMT
448 transmission. It has been hypothesized (Woischnik and Moraes 2002) that sperm
449 mtDNA, which is released from degenerating mitochondria after fertilization, could be
450 an important source of nuclear mtDNA pseudogenes transmitted to the progeny. In
451 contrast, Sato et al. (2007) proposed that female germ cells have machinery that
452 prevents the inheritance of defective mtDNA to the following generation because germ
453 cells are kept for a long time until they are ovulated. de Paula et al. (2013a, b) indicated
454 that suppressed mitochondrial metabolism in the female germ line may constitute a
455 mechanism for increasing the fidelity of mitochondrial DNA inheritance. They
456 proposed that quiescent oocyte mitochondria contain DNA as an unexpressed template
457 that avoids mutational accumulation by being transmitted through the female germ line.

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458 The avoidance of ROS-dependent mutation would be the evolutionary pressure
459 underlying maternal mitochondrial inheritance and the developmental origin of the
460 female germ line.

461 Female meiosis, and megagametogenesis in the case of plants, are moments of the vital
462 cycle where there is opportunity for a non-Mendelian drive mechanism to occur because
463 only one of the four meiotic products (and one of the nuclei of the megaspores) acts as
464 female gamete. When competition occurs for a meiotic product to be included in the
465 female gamete, the process is called "female meiotic drive", which is mainly associated
466 to repetitive sequences, such as those present in centromeres, subtelomeric
467 heterochromatin and other chromosome regions mainly composed by repetitive
468 sequences (Puertas and Villasante 2013, Lindholm et al. 2016). To explain centromere
469 drive, Iwata-Otsubo et al. (2017) proposed that amplified repetitive sequences act as
470 parasitic elements by promoting expansion of CENP-A chromatin and increased
471 transmission through the female germline. The study of female meiotic drive for
472 mtDNA insertions remains to be studied.

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