J Plant Res (2010) 123:139–148
DOI 10.1007/s10265-009-0298-5



# Maternal inheritance of mitochondria: multipolarity, multiallelism and hierarchical transmission of mitochondrial DNA in the true slime mold *Physarum polycephalum*

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Received: 1 September 2009 / Accepted: 9 December 2009 / Published online: 16 January 2010


**Abstract** Direct evidence of digestion of paternal mitochondrial DNA (mtDNA) has been found in the true slime mold *Physarum polycephalum*. This is the first report on the selective digestion of mtDNA inside the zygote, and is striking evidence for the mechanism of maternal inheritance of mitochondria. Moreover, two mitochondrial nuclease activities were detected in this organism as candidates for the nucleases responsible for selective digestion of mtDNA. In the true slime mold, there is an additional feature of the uniparental inheritance of mitochondria. Although mitochondria are believed to be inherited from the maternal lineage in nearly all eukaryotes, the mating types of the true slime mold *P. polycephalum* is not restricted to two: there are three mating loci—*matA*, *matB*, and *matC*—and these loci have 16, 15, and 3 alleles, respectively. Interestingly, the transmission patterns of mtDNA are determined by the *matA* locus, in a hierarchical fashion (*matA* hierarchy) as follows: *matA7* > *matA2* > *matA11* > *matA12* > *matA15*/*matA16* > *matA1* > *matA6*. The strain possessing the higher status of *matA* would be the mtDNA donor in crosses. Furthermore, we have found that some crosses showed biparental inheritance of mitochondria. This review describes the phenomenon of hierarchical transmission of mtDNA in true slime molds, and discusses the presumed molecular mechanism of maternal and biparental inheritance.

**Keywords** *Didymium iridis* · Hierarchical transmission · Selective digestion · Maternal inheritance of mitochondria · mtDNA · *Physarum polycephalum*

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## Introduction

At present, the most widely accepted hypothesis on the maternal (or uniparental) inheritance of organelles is the active digestion hypothesis, which states that paternal organellar DNA is selectively digested either before fertilization in higher plants, or after fertilization in animals (Kuroiwa et al. 1982; Kuroiwa 1985).

In the case of mitochondria, we have obtained the first direct evidence of selective digestion of paternal mitochondrial DNA (mtDNA) in the zygote, using true slime mold *Physarum polycephalum* (Moriyama and Kawano 2003; Moriyama et al. 2005). The same result was obtained from the related species *Didymium iridis* (Moriyama et al. 2009; Fig. 1a). In that study, phase-contrast and epifluorescence observations using the DNA-specific fluorochrome DAPI revealed clearly that each elliptical mitochondrion in the myxamoeba of *D. iridis* has a rod-shaped mt-nucleoid (mtDNA protein complex; Fig. 1b). Each myxamoebal cell contained about 30 mitochondria before mating (Moriyama et al. 2009), and serial observations of mating showed that cell fusion occurred after mixing of two myxamoebal strains with different mating types. Soon after the fusion of two myxamoebal cells, a full complement of about 60 parental mitochondria mixed together. Each mitochondrion was characterized by the presence of a fluorescent mt-nucleoid. Through nuclear

fusion, the fused cells formed a uninucleate zygote about 3.5 h after mating. The fluorescent mt-nucleoid persisted in every mitochondrion until this stage (Fig. 1c), but mt-nucleoids began to disappear in about half of the mitochondria about 1 h after nuclear fusion (Fig. 1d) and were completely digested within about 5 h of mating (Fig. 1a, e). At this stage, their shapes were consistent with those of the myxamoebal stage.

The manner of selective digestion of mtDNA was almost identical in *P. polycephalum* (Moriyama and Kawano 2003). Polymerase chain reaction (PCR) analysis to detect parental mtDNA from a single cell confirmed that this selective digestion of mtDNA coincides with the elimination of paternal mtDNA in *P. polycephalum*. After the digestion of mtDNA, the number of paternal mitochondria that lost mtDNA remained unchanged until 36 h after mating, but maternal mitochondria increased greatly. After a further 24 h, however, the paternal mitochondrion was not observed. Electron microscopic observations revealed that paternal mitochondria, which had lost their mtDNA, had degraded during the 36–60 h after mating (Moriyama and Kawano 2003).

Recognition of the fact that the paternal mtDNA is selectively digested in the zygote could be a breakthrough in the study of the maternal inheritance of mitochondria. In *P. polycephalum*, an additional feature is the uniparental inheritance of mitochondria. *P. polycephalum* has more than 700 mating types, and the transmission pattern of mtDNA is determined by the multiallelic mating locus, *matA*. The present analysis on the relationship between transmission patterns and selective digestion of mtDNA provides new insights into the molecular mechanisms behind maternal inheritance.

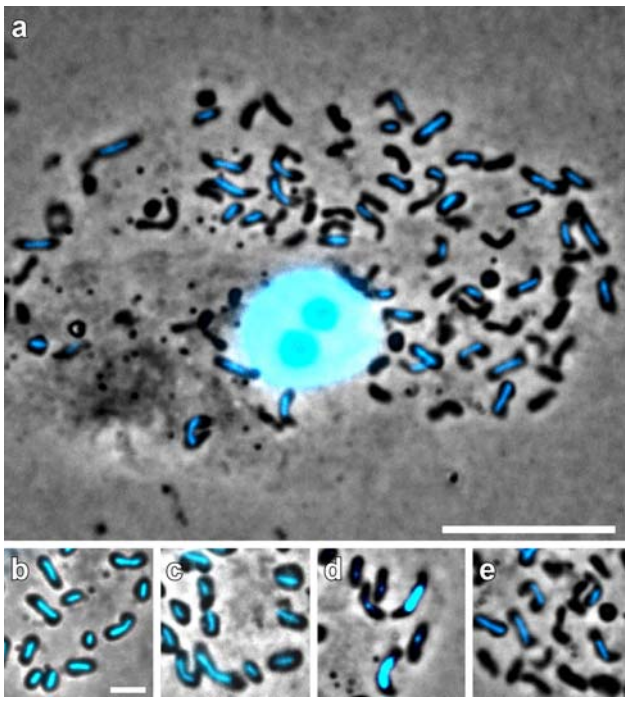


**Fig. 1** Selective digestion of mtDNA during zygote formation in the true slime mold *Didymium iridis*. Myxamoebal cell and zygotes were stained with 4′,6-diamidino-2-phenylindole (DAPI) and merged with phase-contrast images. **a** Uninucleate zygote at 5 h after mating. **b–e** The precise manner of mtDNA disappearance. **b** Myxmoeba. **c** Fused cell. **d** Uninucleate zygote 4.5 h after mating. **e** Uninucleate zygote 5 h after mating. The selective digestion of mtDNA was observed in the uninucleate zygote 5 h after mating (**a**). Each myxamoebal cell contained about 30 mitochondria before mating, and each mitochondrion contained a long rod-shaped mtDNA at its center (**b**). Fused cells had about 60 mitochondria, and their shapes were consistent with those of the myxamoebal stage (**c**). The selective digestion of mtDNA was observed in the uninucleate zygote 4.5 h after mating (**d**), and mtDNA was digested completely about 5 h after mating (**e**). *Bars* **a** 10 μm; **b–e** 2 μm (Modified from Moriyama et al. 2009)

## Maternal inheritance and the selective digestion hypothesis: a historical background

In 1909, Correns and Baur discovered that some genetic factors are not inherited in a Mendelian fashion (Baur 1909; Correns 1909). Baur realized that inheritable factors were present in the cytoplasm. Much later, mtDNA and chloroplast DNA (cpDNA) were discovered to be inheritable genomes located within the cytoplasm of eukaryotic cells. Multiple copies of these organellar genomes are organized into discrete DNA–protein clusters known as nucleoids within each mitochondrion and chloroplast (Kuroiwa et al. 1982; Kuroiwa 1991). The characteristic feature of cytoplasmic organelles is vegetative segregation. If more than two kinds of organelle DNA of different genetic background exist in a cell, they segregate over the course of repeated organelle and cell divisions (Birky 2001). Another characteristic feature of cytoplasmic genomes is their maternal (or uniparental) inheritance. The best-studied example is the uniparental inheritance of cpDNA in the green alga *Chlamydomonas reinhardtii*. The inheritance pattern of chloroplasts in *C. reinhardtii* is related closely to mating type, and the cpDNA of mating type-minus origin is eliminated in young zygotes within the first 6 h of mating (Kuroiwa et al. 1982).

Hutchison et al. (1974) found the first direct evidence for maternal inheritance of mtDNA in horse–donkey hybrids using restriction fragment length polymorphism (RFLP). Later studies reached the same conclusion in the rat (Hayashi et al. 1978; Kroon et al. 1978), the fruit fly *Drosophila melanogaster* (Reilly and Thomas 1980), humans (Giles et al. 1980), the cellular slime mold *Polysphondylium pallidum* (Mirfakhrai et al. 1990), and the malaria parasite *Plasmodium falciparum* (Creasey et al. 1993). These results indicate that maternal inheritance of mtDNA is common in almost all taxa.

The maternal inheritance of mitochondria was initially attributed to differences in cell volume and to differences in the number of mitochondria between male and female gametes. Fertilized eggs were thought to initially contain mitochondria from both parents, but the small number of mitochondria derived from the male gamete would then be lost during subsequent repeated cell divisions. As a result of vegetative segregation, most cells in a multicellular organism would contain mitochondria from the female parent (Dawid and Blackler 1972; Hutchison et al. 1974; Birky 1995; Ankel-Simons and Cummins 1996). However, PCR analysis has shown that paternal mtDNA was eliminated completely from the fertilized egg during the pronucleus stage (Kaneda et al. 1995). In contrast, paternal mtDNA can often be retained in interspecific hybrids, a phenomenon known as biparental inheritance or heteroplasmy. Biparental inheritance has called into question the strictness of maternal inheritance, leading to an energetic debate (Barr et al. 2005). Uniparental inheritance of mitochondria has been observed in the fungi *Microbotryum violaceum* (Wilch et al. 1992) and *Schizophyllum commune* (Specht et al. 1992), even though their gametes are almost identical in size (isogamy). Decreases have been observed in the mtDNA of flowering plants (Corriveau and Coleman 1991; Nagata et al. 1999). This decrease in mtDNA, which is organized into mt-nucleoids, occurs in the mature generative cells inside pollen grains before fertilization. These examples suggest that mtDNA inheritance is a more complex process than first thought.

The current central question is what are the molecular mechanisms that underlie the maternal inheritance of mtDNA. Understanding these mechanisms would help to explain cases where the unusual transmission of paternal mtDNA is observed.

As noted previously, a characteristic feature of the mitochondrial genome is its vegetative segregation during mitotic cell division. The maternal inheritance of mitochondria is a phenomenon that is observed during zygote formation, but even when a sexual cross generates heteroplasmic progeny, repeated cell division can produce homoplasmic cells (Birky 1995), and the mitochondrial genome seems to be inherited uniparentally. The life cycle of the true slime mold consists of two distinct vegetative forms: the uninucleate haploid myxamoeba and the multinucleate diploid plasmodium (Fig. 2a). During a sexual cross, the myxamoebae act as gametes; individuals of different mating types pair and fuse to form diploid zygotes that develop into macroscopic multinuclear plasmodia after repeated mitotic cycles without cell division (Bailey et al. 1990). Because no cell division takes place, mitochondrial segregation does not occur. However, uniparental inheritance of mitochondria has also been reported in the isogamous protists *P. polycephalum* (Kawano et al. 1987a, b; Kawano and Kuroiwa 1989; Meland et al. 1991) and *D. iridis* (Silliker and Collins 1988; Silliker et al. 2002). Loss of paternal mitochondria is presumed to happen at an early stage of zygote differentiation despite gametes of equal size being involved, and the zygote develops without

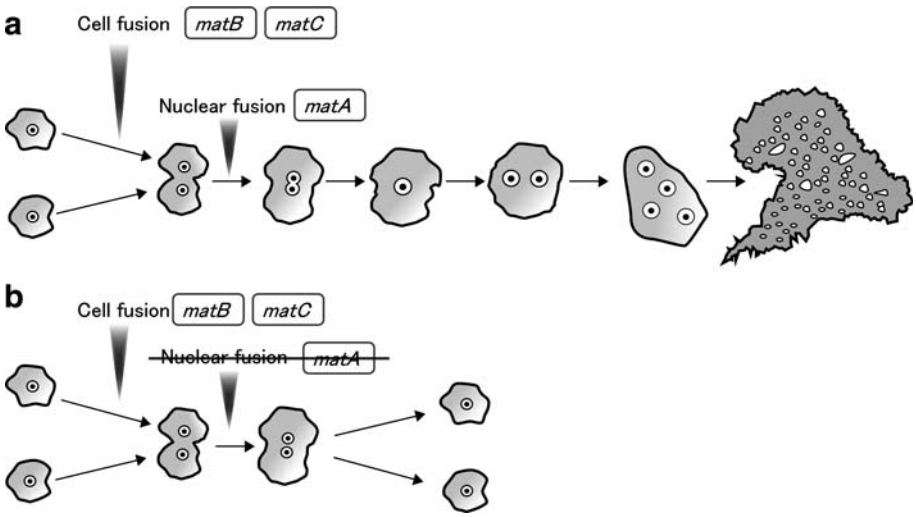


**Fig. 2** Genetic control of zygote formation in the mating of *Physarum polycephalum*. **a** *matA*–, *matB*–, and *matC*–heteroallelic cross. **b** *matA*–homoallelic cross. Zygote formation is under the control of three loci: *matA*, *matB*, and *matC*, with at least 16, 15, and 3 alleles, respectively. In the *matB*– or *matC*–homoallelic cross, the two amoebal cells cannot fuse. The *matA* locus controls nuclear fusion in the fused cell of the *matB*– and *matC*–heteroallelic cross. In the *matA*–, *matB*–, *matC*–heteroallelic cross, two amoebal cells can fuse and develop into a macroplasmodium followed by nuclear fusion and repeated mitosis without cell division (**a**). When the two myxamoebal cells have same *matA* allele, cells split apart after cell fusion and regenerate uninucleate amoebal cells (**b**) (Modified from Kawano et al. 1995)

cell division (Meland et al. 1991). Consequently, mitochondrial inheritance in this slime mold was thought to have an active mechanism during the sexual cross.

## Multipolar and multiallelic sexuality in eukaryotes

According to the consensus phylogeny of eukaryotes proposed by Baldauf (2003), the majority of eukaryotes can be assigned to one of eight major groups (Fig. 3). Organisms with stages of sexual reproduction can be found in all eight groups, and almost all organisms have two sexes or mating types (e.g., male and female, mating type-minus and -plus), but plasmodial slime molds and the fungal basidiomycetes have more than two sexes, multipolar mating types, and multiallelic mating loci (Kawano et al. 1995; Casselton 2008). This fact indicates that the multiple mating types evolved independently among diverse taxa.

The sex-determining genes of fungi reside in one or two specialized regions of the chromosome known as mating type (MAT) loci. These genes are sufficient to determine haploid cell identity, enable the attraction of a compatible mating partner, and prepare cells for sexual reproduction after fertilization. Basidiomycetes are thought to have diverged from ascomycetes, which have a bipolar mating types in one locus, like *MATa* and *MATα* in *Saccharomyces cerevisiae*. Basidiomycetes have a *matA* locus, which codes the homeobox domain, and have acquired another mating type locus, *matB*, which encodes pheromones and receptors. The mating type of *Coprinus cinereus* is determined by two unlinked *matA* and *matB* loci that have numerous alleles. To mate and develop efficiently, cells must have different alleles in both *matA* and *matB*. Several *matA* alleles are found in the natural population, resulting in an enormous number of combinations of fungus mating types (May and Matzke 1995). As a consequence, *C. cinereus* has thousands of mating types.

In the case of the true slime mold *P. polycephalum*, sexual crosses are controlled by three unlinked loci: *matA*, *matB*, and *matC* (Fig. 2; Dee 1966; Shinnick et al. 1978; Youngman et al. 1979; Kirouac-Brunet et al. 1981; Kawano et al. 1987a, b). Each *matA*, *matB*, and *matC* locus has several alleles, and to cross efficiently, myxamoebal cells must carry different alleles of at least *matA* and *matB* (Fig. 2a). The *matC* locus influences tolerance for elevated pH during cell fusion (Kawano et al. 1987a, b). When two myxamoebae are homoallelic for *matC*, pH limit for crossing is lower than that for *matC*–heteroallelic combinations. The *matB* locus regulates the efficiency of cell fusion during zygote formation (Pallotta et al. 1979; Youngman et al. 1981). When two myxamoebae are homoallelic for *matB*, cell fusion does not occur. The subsequent development of zygotes into plasmodia is regulated by the *matA* locus; *matA*–homoallelic fusion cells cannot complete nuclear fusion (Youngman et al. 1979; Holt et al. 1980: Fig. 2b). To date, at least 16, 15, and 3 alleles are known to exist in each *matA*, *matB*, and *matC* locus, respectively. Thus, at least 720 self-incompatible mating types exist in *P. polycephalum*.

## Hierarchical transmission of mtDNA in *P. polycephalum*

The mating type system of the true slime mold is particularly complex, and determining how mitochondrial inheritance is regulated in this organism is therefore of some interest. As noted previously, transmission of mtDNA is for

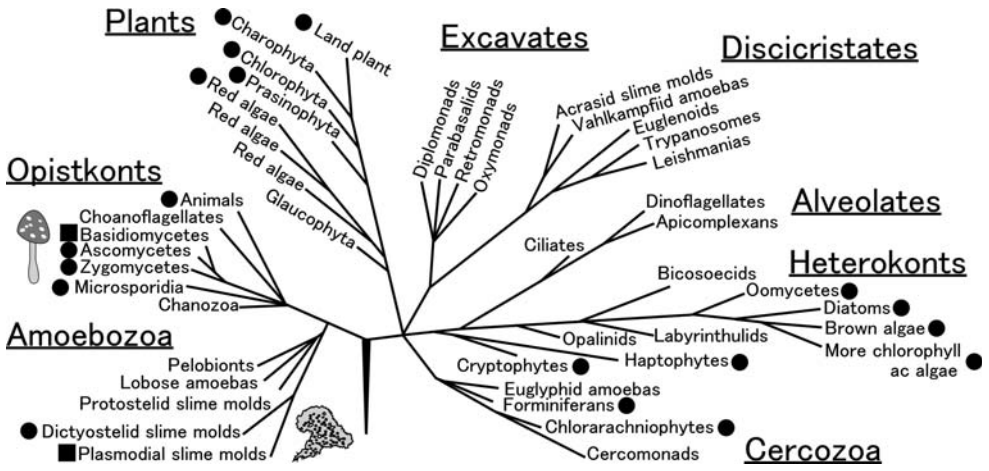


**Fig. 3** A consensus phylogeny of eukaryotes and the existence of sexual reproduction. The majority of characterized eukaryotes can be assigned to one of eight major groups (Baldauf 2003). *Closed circles* Sexual organisms with two sex or mating types, *closed squares* sexual organisms with multipolar and multiallelic mating types. Organisms with stages of sexual reproduction can be found in all eight groups. A multipolar and multiallelic mating system occurs only in basidiomycetes and plasmodial slime molds

the most part uniparental (Kawano et al. 1987a, b; Kawano and Kuroiwa 1989; Moriyama and Kawano 2003). The identity of the mtDNA donor in each pair is not determined by the loci of *matB* or *matC*, which regulate myxamoebal fusion. The results suggest that the combination of *matA* alleles present in the two myxamoebal cells determines which parent will be the mtDNA donor (i.e., maternal parent), and the paternal mtDNA is selectively digested in the zygote. Combinatorial analysis of the 13 *matA* alleles showed that particular pairs of *matA* alleles consistently lead to specific patterns of mtDNA inheritance (Kawano et al. 1987a, b, Kawano and Kuroiwa 1989). For example, in a sexual cross of *matA2* and *matA12* myxamoebal cells, the *matA2* cell always acts as the mtDNA donor, and the *matA12* cell is always the mtDNA recipient. In addition, a linear hierarchy exists among *matA* alleles concerning the loss of mtDNA. The mitochondrial donor is generally the myxamoeba cell that possesses the dominant *matA* allele, although this strain does not always act as the donor in other mating pairs. The *matA* hierarchy was first examined among four alleles and was found to function as follows: *matA2* > *matA11* > *matA12* > *matA1* (Kawano and Kuroiwa 1989). Similar results were confirmed independently by Meland et al. (1991). Existing research gave the tentative order of the *matA* hierarchy with regard to the inheritance of mtDNA as follows: *matA7* > *matA2* > *matA11* > *matA12* > *matA15*/*matA16* > *matA1* > *matA6* (note that *matA15* and *matA16* have not been tested against each other).

In the isogamous green alga *Chlamydomonas smithii*, selective digestion of mtDNA has also been reported, and the transmission pattern of mtDNA is determined by mating type. Meiotic progeny of sexual zygotes normally receive mtDNA from the mating type-plus parent (Aoyama et al. 2006). The mating type system of *P. polycephalum* is more complex than that of *C. smithii*, but the *matA* locus determines the transmission pattern of mtDNA.

## Selective digestion of mtDNA in *P. polycephalum*

To test whether strict uniparental inheritance occurs in *P. polycephalum*, the inheritance patterns of mtDNA were examined by PCR in all possible crosses between strains *matA1*, *matA2*, *matA11*, *matA12*, *matA15*, or *matA16* (Moriyama and Kawano 2003). Figure 4 shows an example of mtDNA inheritance patterns based on the *matA* hierarchy. In a cross of the haploid myxamoebal strains AI35 (*matA2*) and TU41 (*matA12*), the mtDNA of TU41 was lost in the diploid plasmodium (Fig. 4, upper left). In a cross involving AI16 (*matA1*) and TU41 (Fig. 4, lower left), however, the mtDNA of TU41 survived, and the mtDNA of AI16 was lost. Note that TU41 was the mitochondrial recipient (paternal) cell in the first cross but the donor (maternal) cell in the second. In most of the 60 crosses, the mtDNA was inherited strictly uniparentally as in AI35 × DP246 (Fig. 4, upper right), while some plasmodium showed biparental inheritance, as in DP246 × AI16 (Fig. 4, lower right). In 21 of 60 crosses, mtDNA of both parents was detected, and PCR estimation of parental mtDNAs showed that equal biparental inheritance occurred in 5 of the 21 crosses, but in other cross, the mtDNA was usually biased toward one or the other strain (Fig. 5). For example, the plasmodium of DP246 × AI16 contained mtDNA from both parents in equal amounts, but the plasmodium of DP246 × AI5 contained $10^{-3}$ as much mtDNA from AI5 compared to DP246 although *matA* type of AI16 is the same as that of AI5. These leakages of paternal mtDNA were observed only in crosses involving *matA15* and *matA16*, indicating that this is a genetically controlled phenomenon. The biparental inheritance of mtDNA was observed only in the combinations of *matA1* and *matA15*, *matA1* and *matA16*, and *matA2* and *matA15*, but 5 of 12 crosses of *matA1* and *matA15* (DP89 × AI5, DP89 × AI39, DP248 × AI2, DP248 × AI5, DP248 × AI39) showed uniparental inheritance of mtDNA, resulting

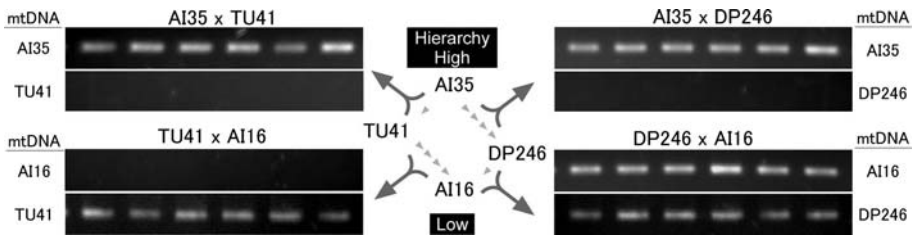


**Fig. 4** Transmission pattern of mtDNA in four representative crosses. The myxamoebal strains of AI35 (*matA2*), AI16 (*matA1*), TU41 (*matA12*), and DP246 (*matA16*) were used, and the dominance of mtDNA transmission was expected to be AI35 > TU41 > DP246 > AI16. Paternal mtDNA was detected by PCR from six plasmodia in the following crosses: AI35 × TU41, AI35 × DP246, TU41 × AI16, and DP246 × AI16. Except for DP246 × AI16, mtDNA was transmitted uniparentally in accordance with the *matA* hierarchy: the mtDNA of TU41 origin was eliminated in the plasmodia of AI35 × TU41. In TU41 × AI35; however, the mtDNA of TU41 was inherited uniparentally. In contrast, biparental inheritance of mtDNA was detected in AI16 × DP246. *Arrowheads* indicate the directions of the mtDNA transmission (modified from Moriyama and Kawano 2003)

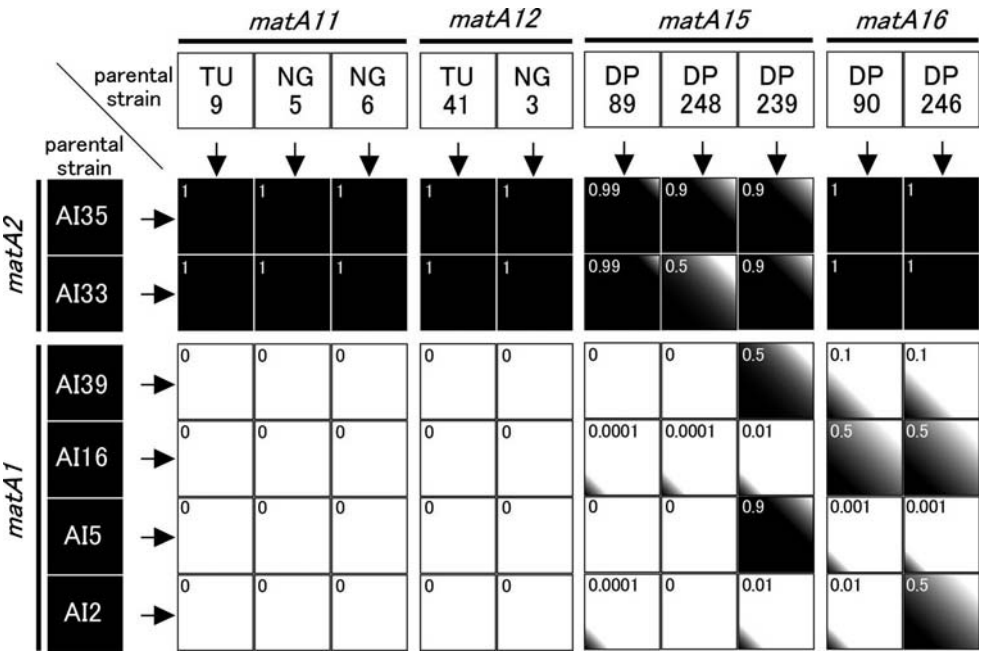


**Fig. 5** mtDNA transmission patterns investigated in 60 crosses of six *matA* alleles. *matA1* and *matA2* strains were crossed with *matA11*, *-12*, *-15*, and *-16* strains, and the ratios of maternal and paternal mtDNA were estimated using PCR. *Black* and *white boxes* indicate mtDNA genotypes. *Numbers* in the *boxes* denote the ratio of mtDNA from *matA1* or *matA2* origin (shown as *black*) in the plasmodium. Of the 60 crosses, 39 demonstrated strict uniparental inheritance of mtDNA, but some plasmodia showed leakage of paternal mtDNA, especially in the combinations of *matA1* and *matA15*, *matA1* and *matA16*, and *matA2* and *matA15* (modified from Moriyama and Kawano 2003)

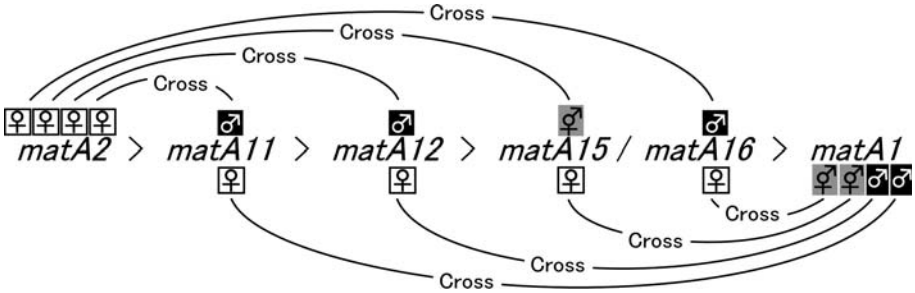


**Fig. 6** Tentative order of the *matA* hierarchy with regard to the inheritance of mtDNA. mtDNA is transmitted uniparentally in accordance with the *matA* hierarchy (*matA15* and *matA16* have not yet been tested against each other). The mitochondrial donor and the recipient are indicated in each cross as ♀ and ♂, respectively. Note that a mitochondrial recipient strain can be a donor when crossed with a strain lower on the hierarchy. The biparental inheritance of mtDNA is observed in some crosses and seems to be controlled genetically

in *matA*15 > *matA*1. Figure 6 shows the tentative order of the *matA* hierarchy with regard to the inheritance of mtDNA. The mitochondrial recipient strain can be a donor when crossed with a strain lower on the hierarchy.

In the crosses that showed biased biparental inheritance [e.g., DP246 (*matA*16) × AI5 (*matA*2)], the disappearance of mtDNA after mating was observed in about half the mitochondria (Moriyama and Kawano 2003). However, digestion of mtDNA in the recessive mitochondria from AI16 was not complete 24 h after mating; very faint small spots representing fluorescent mt-nucleoids persisted in some mitochondria (Fig. 7a). In addition, the persistent mt-nucleoids seemed to increase gradually in size for 24–36 h after mating, probably because of mtDNA replication (Fig. 7b). The value of their long axes (micrometers) and the relative amount of mtDNA as measured by fluorescence intensity were arranged in a scatterplot (Fig. 7c, d). At 24 h after mating, 18% of the mitochondria were vacant (0 μm) and 69% had well developed mt-nucleoids (0.5–1.8 μm; Fig. 7a,c), but some mitochondria contained small mt-nucleoids that emitted very faint fluorescence (0.1–0.25 μm). These very small mt-nucleoids may have been generated by incomplete mtDNA digestion, but such small mt-nucleoids were observed only rarely 36 h after mating (Fig. 7b, d). No degrading mitochondrion with small mt-nucleoid was observed, thus the incompletely digested mtDNA would replicate, and appeared to become larger.

In the plasmodia, the vacant mitochondria were completely eliminated, and the surviving mt-nucleoids and mitochondria were indistinguishable from each other. However, the biased biparental inheritance of mtDNA suggests that the incompletely digested mtDNA was of uniparental origin and that the normal copy number in each

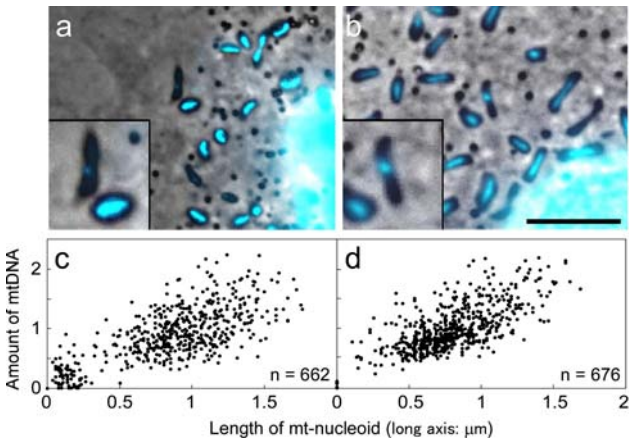


**Fig. 7** Incomplete digestion of mtDNA in a young zygote of DP246 (*matA16*) × AI5 (*matA1*). **a**, **b** Merged images of phase-contrast and DAPI fluorescence microscopy. **c**, **d** Relative amount of mtDNA versus mt-nucleoid length. A small amount of incompletely digested mtDNA was observed in the zygote of 24 h after mating (**a**). Some mt-nucleoids were short in length and small in amount (**a**, **c**, respectively). At 36 h after mating, those mitochondria seemed to restore the length of mt-nucleoids and amount of mtDNA in each mitochondrion during plasmodial development (**b**, **d**). *Bar* 5 μm (modified from Moriyama and Kawano 2003)

mitochondrion was restored during plasmodial development. These results suggest that complete digestion of paternal mtDNA is needed to destroy and eliminate mitochondria of paternal origin. Incomplete digestion of mtDNA and successive replications of mtDNA enable biased biparental inheritance.

## A hypothesis for the hierarchical transmission of mtDNA

A striking fact regarding the uniparental transmission of *P. polycephalum* is that all gametes can act as either donors or recipients of mtDNA. The dominance of mtDNA transmission depends entirely on the mating type locus *matA*. The uniparental inheritance of mtDNA has been studied to date mostly in systems of only two mating types or sexes. The situation is much more complex in *P. polycephalum*, in which more than two mating types and a linear dominance hierarchy of *matA* exist. Iwanaga and Sasaki (2004) proposed a model for the evolution and the dominance of *matA* alleles that entail a linear hierarchy as a

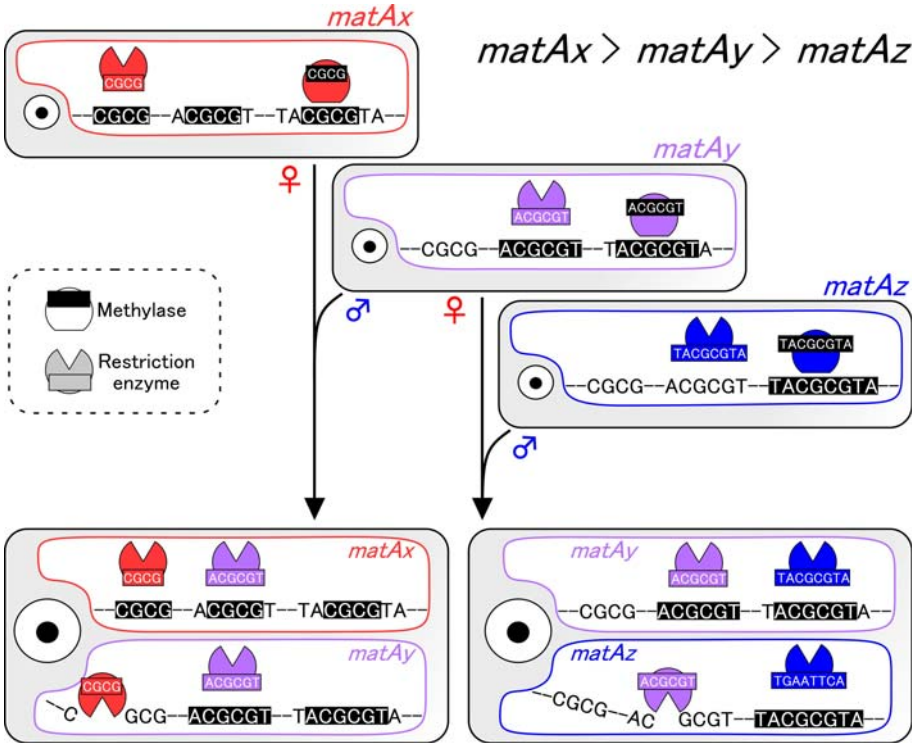


**Fig. 8** Theoretical model for the directional transmission of mtDNA. Each myxamoebal strain possesses a mitochondrial restriction enzyme and a mitochondrial methylase that recognize the same site. Recognition sites vary by *matA* alleles. Recognition sites are highly nested among the enzymes, and amoebal cells lower on the *matA* hierarchy have enzymes of longer recognition sites. The mtDNAs of both myxamoebal cells are methylated prior to cell fusion, but the number of methylated sites differs between the two cells. After cell fusion, the two restriction enzymes are imported into mitochondria, and the restriction enzyme higher on the *matA* hierarchy (shorter recognition site) cleaves the unmethylated recognition site of the mtDNA of lower *matA* origin ("Site-specific nuclease model": Iwanaga and Sasaki 2004)

"site-specific nuclease model". In this model, each myxamoebal strain is assumed to have a mitochondrial restriction enzyme and a mitochondrial methylase that recognize the same site (Fig. 8). Recognition sites vary by *matA* allele and are highly nested among enzymes, with myxamoebal cells lower on the *matA* hierarchy having the enzymes of longer recognition sites (e.g., CGCG ⊂ ACGCGT ⊂ TACGCGTA). The mtDNAs of both myxamoebal cells are methylated prior to cell fusion, but the number of methylated sites differs between the two cells. After cell fusion, the two restriction enzymes are imported into the mitochondria, and the restriction enzyme higher on the *matA* hierarchy (shorter recognition site) cleaves the unmethylated recognition site of the mtDNA of lower *matA* origin. Iwanaga and Sasaki (2004) predicted that to maintain the linear hierarchy of the nuclease methylase system, the length of the recognition sequence must be longer than the number of mating type alleles (hence must be longer than 16 in *P. polycephalum*). Although some nucleases are known to have exceptionally long recognition sites (e.g., the homing endonuclease *I-CeuI* recognizes 30 nucleotides), this model assumes that degenerated recognition sequences occur in the mtDNA and that repetition numbers of the recognition site exceed the number of mating type alleles. The assumption of highly nested recognition sequences is theoretically reasonable, and can account for the evolution of a directional transmission of mtDNA. However, degenerated repetitive sequences are not found in the mtDNA of *P. polycephalum* (Takano et al. 2001).

## Perspectives

We have identified two mitochondrial nuclease activities that are potentially involved in the active digestion of mtDNA (Moriyama et al. 2005). One is a $Ca^{2+}$-dependent high-molecular-weight nuclease complex, and its central activity was detected as a $Ca^{2+}$-dependent 50-kDa exonuclease band in SDS-PAGE/DNA zymography. This activity was observed in myxamoebal cells and zygotes but not in mature plasmodia. The other is a $Mn^{2+}$-dependent nuclease, and its activity was detected as a $Mn^{2+}$-dependent 13-kDa endonuclease band in SDS-PAGE/DNA zymography. This activity was observed from about 6 h after mixing the two myxamoebal strains. This timing corresponded precisely to that of selective mtDNA digestion. We have not yet identified which nucleases play the key role in the uniparental inheritance of mtDNA. Furthermore, the $Ca^{2+}$-dependent 50-kDa exonuclease cannot digest the mtDNA because the mtDNA is a circular molecule (Takano et al. 2001; Moriyama et al. 2005).

Given that both nucleases work in concert, however, we can provide the following explanation for the selective digestion and leakage of paternal mtDNA and for the hierarchy among *matA* alleles (Fig. 9). In myxamoebal cells, a 50-kDa exonuclease complex exists in the mitochondria prior to mating. There is a complicated hierarchy in the multiallelic *matA* locus, but we think the paternal and maternal cell are just determined during cell–cell recognition. After recognition of a myxamoebal cell of another *matA* allele, the maternal cell begins to express protective enzymes (e.g., methylase) against endonuclease activity that are imported into the mitochondria before mating (cell fusion). Shortly after cell fusion, the 13-kDa endonuclease is expressed in the zygote and is imported into each mitochondrion. The unprotected circular mtDNA of lower

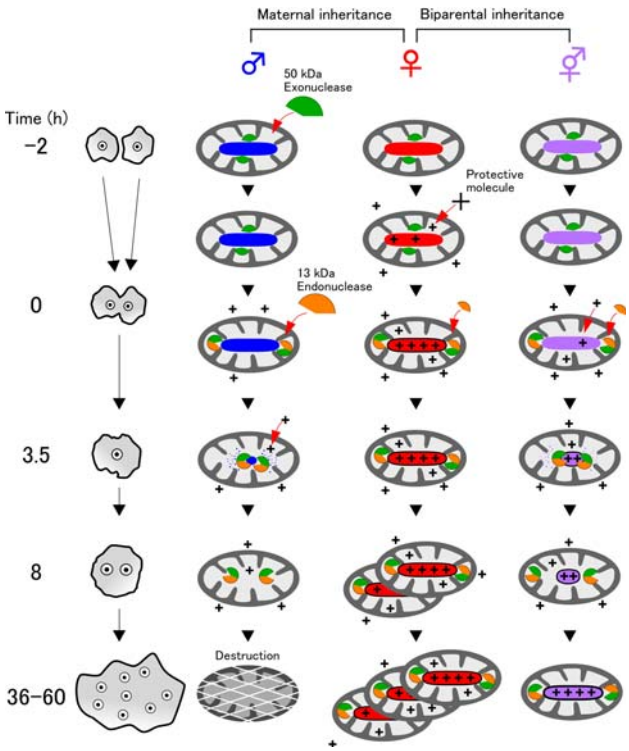


**Fig. 9** A model for the molecular mechanism of mitochondrial inheritance in *P. polycephalum*. A 50-kDa exonuclease exists in the mitochondria of myxamoebal cells prior to mating. There is a complicated hierarchy in the multiallelic *matA* locus, but we think the paternal and maternal cells (indicated as ♂ and ♀, respectively) are just determined during cell–cell recognition. After recognizing the amoebal cell of another *matA* allele, the maternal cell begins to express (or activates) protective enzymes against endonuclease activity (protective molecules are indicated by *plus*). Shortly after cell fusion, the 13-kDa endonuclease is expressed in the zygote and is imported into each mitochondrion. The unprotected circular mtDNA of lower *matA* origin is cleaved by this endonuclease, and the 50-kDa exonuclease complex then digests it completely. Because the maternal mtDNA is protected against nuclease, it survives. This concerted digestion of mtDNA by these two nuclease activities leads to the uniparental inheritance of mtDNA. The delayed expression of endonuclease or the accelerated protection of some mtDNA molecules of lower *matA* origin in the zygote would result in the biparental inheritance of mtDNA (indicated as ♀ and ♂)

*matA* origin is cleaved by this endonuclease, and the 50-kDa exonuclease complex then digests it completely. This concerted digestion of mtDNA by these two nuclease activities leads to the uniparental inheritance of mtDNA. In the case of biparental inheritance of mtDNA, the paternal mtDNA is not digested completely. This might be the result of delayed expression of nuclease or the rapid onset of mtDNA protection before completion of mtDNA digestion.

This model can be applied to the leakage of paternal mtDNA observed in interspecific hybrids in animals and plants. According to this explanation, *matA* alleles are assumed to work as determining factors for the expression of protective molecules against endonuclease. In ascomycetes, the MAT locus encodes *MATa* or *MAT*α, which regulate the expression of one pheromone–receptor pair (a-sex pheromone and α-sex pheromone receptor, or α-sex pheromone and a-sex pheromone receptor). In *P. polycephalum*, the phenomenon is quite complex, but the results of our study on selective digestion and the rare leakage of paternal mtDNA provided important clues for the universal mechanism of the maternal inheritance of mitochondria. The identification of the mitochondrial nuclease and protective molecule involved in mtDNA digestion would aid in understanding the molecular mechanism behind the uniparental inheritance of mtDNA and the dominance of *matA* alleles concerning mtDNA inheritance.

**Acknowledgments** The authors thank Dr. Tsuneyoshi Kuroiwa for his thoughtful suggestions, generous support and encouragement. We also would like to thank Dr. J. Mark Cock for checking our manuscript. This study was supported by grants for Scientific Research in Priority Areas (no. 13440246, no. 15440246, and 20440123 to S.K.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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