

## NON-MENDELIAN INHERITANCE

**Yoshiki Nishimura and Tsuneyoshi Kuroiwa**

*University of Tokyo, Japan*

**Keywords:** non-Mendelian inheritance, chloroplast DNA, mitochondrial DNA, DAPI (4' 6-diamidino-2- phenylindole), optical tweezer

### Contents

#### 1. Chloroplast Inheritance

##### 1.1. Early Cytological Observations of Chloroplast Inheritance

##### 1.2. Chloroplast Inheritance in Isogamous Organisms

###### 1.2.1. Unicellular Green Algae: *Chlamydomonas reinhardtii*

###### 1.2.2. Discovery of Chloroplast DNA

###### 1.2.3. Molecular Mechanism of Non-Mendelian Inheritance of cpDNA

###### 1.2.4. Transformation of cpDNA

###### 1.2.5. Active Digestion of mt- cpDNA Revealed in a Single Zygote by Using the Optical Tweezer in *C.reinhardtii*

###### 1.2.6. Chloroplast Inheritance in Other Isogamous Algae

##### 1.3. Chloroplast Inheritance in Anisogamous and Oogamous Organisms

###### 1.3.1. Anisogamous and Oogamous Algae, and Ferns

###### 1.3.2. Malarial Parasites: *Plasmodium falciparum*

##### 1.4. Chloroplast Inheritance in Higher Plants

###### 1.4.1. Angiosperms

###### 1.4.2. Gymnosperms

#### 2. Mitochondrial Inheritance

##### 2.1. Early Genetic Analysis of Mitochondrial Inheritance

##### 2.2. Discovery of Mitochondrial DNA

##### 2.3 Mitochondrial Inheritance in Isogamous Organisms

###### 2.3.1. Yeast-*Sccharomyces cerevisiae*

###### 2.3.2. Unicellular Green Algae: *Chlamydomonas reinhardtii*

###### 2.3.3. True Slime Mold: *Physarum polycephalum*

##### 2.4. Mitochondrial Inheritance in Oogamous Organisms

###### 2.4.1. Mammals

###### 2.4.2. Humans

###### 2.4.3. Higher Plants

#### Glossary

#### Bibliography

#### Biographical Sketches

### Summary

Mitochondria and chloroplasts carry their own DNA. These cytoplasmic DNAs are inherited in a non-Mendelian fashion, different from nuclear encoded genes. There are three patterns in non-Mendelian inheritance; maternal, biparental and paternal; in nearly all eukaryotes, individuals inherit mitochondrial and chloroplast genes from only the maternal parent.

Eukaryotic organisms have been classified by differences in the sizes of female and male gametes into at least three different groups: isogamous, anisogamous, and oogamous species. When gametes are alike in appearance they are called isogametes. Gametes having only a slight difference in size are said to be anisogametes and such species exhibit anisogamy. When gametes differ in size and activity, as they do in animals, higher plants, ferns, mosses, and algae such as *Volvox*, *Fucus* and *Polysiphonia*, the organisms are said to exhibit oogamy.

Until recently, maternal inheritance of organelle DNA was postulated to occur by simple dilution of male mt and cp DNA in zygotes, since in multicellular organisms gametes are anisogamous or oogamous, and the male contributes very little cytoplasm (mitochondria or chloroplasts) to zygotes. But this cannot explain the finding that non-Mendelian inheritance occurs in isogamous algae *C.reinhardtii*, even though female ( $mt^+$ ) and male ( $mt^-$ ) gametes are isogamous and contribute equal amounts of cytoplasm. This suggests the presence of developmentally programmed processes that preferentially digest paternal organelle DNA and thus lead to the maternal inheritance of organelle DNA. In this article, various patterns of non-Mendelian inheritance in isogamous, anisogamous, and oogamous organisms are summarized and research into the mechanisms that underlie non-Mendelian inheritance are presented.

Investigation of the molecular mechanism of non-Mendelian inheritance is important in many fields. For example, malarial parasites—*Plasmodium falciparum*—contain mitochondrial and chloroplast like genomes in their cytoplasm, and these are inherited maternally. Since targets for certain anti-malaria drugs are likely to be in cytoplasmic genomes, a study of cytoplasmic inheritance would be important in understanding the emergence and spread of resistance to these compounds in populations of parasites.

## 1. Chloroplast Inheritance

### 1.1. Early Cytological Observations of Chloroplast Inheritance

Nine years after the rediscovery of Mendel's principles, plant geneticists, Correns and Baur described a very unusual phenomenon that could not be explained by means of Mendel's laws. A 1909 paper illustrated the results of reciprocal crosses in which the inheritance of green, variegated, and white leaves in four-o'clocks (*Mirabilis jalapa*) was investigated. The fertilization of eggs from a plant with variegated leaves by pollen from a green-leaved plant produced uniformly variegated offspring. Surprisingly, the reciprocal cross, in which the leaves of the mother plant were green and those of the father were variegated, did not lead to the same outcome. Instead, all of the progeny from this cross displayed green leaves. These results seemed to indicate that the offspring inherited their form of variegation from their mother only. At the same time, Baur found that the green and variegated patterns of leaves of *Pelargonium zonale* were inherited in a biparental fashion.

Between 1910 and 1950, the first observations of non-Mendelian inheritance in higher plants were followed by extensive cytological and genetic observations. In variegated plants, mutant chloroplasts were observed with electron-microscopes. One of the central questions at issue was whether the mutant chloroplasts themselves were the culprit of

the leaf variegation rather than effects of different cytoplasm on plastid development: for example, somatic mutation in a nuclear gene that could affect the cellular environment or the chloroplasts themselves. This idea was supported by the observation of mixed cells containing both green and white chloroplast types. Until the discovery of cpDNA in plastids, this observation provided the best evidence for the presence of a genetic element in chloroplasts.

## 1.2. Chloroplast Inheritance in Isogamous Organisms

### 1.2.1. Unicellular Green Algae: *Chlamydomonas reinhardtii*

In higher plants, genetic analyses of mechanisms of maternal inheritance have proved possible, but biochemical analyses have been difficult since double fertilization must be performed in cells embedded in various tissues. Many workers prefer to use microorganisms with a short lifecycle in which genetic and biochemical analyses are relatively easy. In 1954 Sager showed for the first time that uniparental inheritance of cytoplasmic genes for resistance to streptomycin occurred in *C.reinhardtii*. Since then, many studies of the mechanisms of plastid inheritance at the genetic and biochemical level have been performed using *C.reinhardtii*.

*C.reinhardtii* is a unicellular green algae that has various favorable traits for genetic and biochemical researches, such as a fast sexual cycle, biochemically distinct mutant phenotypes, and the fact that it contains only a single chloroplast. Vegetative cells are converted into gametes by nitrogen starvation. On mixing, gametes of opposite mating types fuse to form diploid zygotes. Not only nuclei but also chloroplasts fuse during the first three hours after mating. As zygotes are germinated, meiosis occurs to yield four haploid meiotic products. The nuclear mating type segregate in a normal Mendelian ratio 2:2. The cp genes are transmitted in a uniparental manner from only *mt*<sup>+</sup> parent.

### 1.2.2. Discovery of Chloroplast DNA

Between 1960 and 1970, the existence of unique species of DNA in chloroplasts was demonstrated. In 1962, Ris and Plaut first reported electron-microscope observations of DNA-like fibers within the chloroplasts of *C.reinhardtii*. These fine fibrils appeared to become clumped in an electron-transparent area, the nucleoid, in the matrix of chloroplasts. Such DNA-containing regions have also been observed in the chloroplasts of many other plants, such as red, brown, and green algae. In 1963, Sager and Ishida observed that a unique species of DNA could be enriched to 40% of the total in a chloroplast preparation from *C.reinhardtii*. On the basis of these data, the presence of DNA in chloroplasts was first accepted.

By combining observations on the molecular weights of individual organelle DNA molecules with data that yielded estimates of the total amount of DNA per cell, it has been shown that there are several or many DNA molecules per organelle and per cell; for example, a chloroplast of *C.reinhardtii* contains 80 copies of cpDNA molecules. Multiple copies of DNAs in chloroplasts are not naked *in vivo* but are organized by proteins into DNA-protein complexes called “chloroplast (cp) nucleoids.” Application of DNA-specific fluorochrome, DAPI (4',6-diamidino-2-phenylindole) further refined

the observation of cp nucleoids. In 1978, Kuroiwa and colleagues examined the distribution and behavior of cpDNA by staining with DAPI and revealed that the number, distribution, and shape of the chloroplast nucleoids change actively during the development and division of chloroplasts, and these parameters differ among various plants.

### 1.2.3. Molecular Mechanism of Non-Mendelian Inheritance of cpDNA

In 1972, one biochemical study showed that  $mt^-$  cpDNA is digested in the period 6–24 hours after mating. This observation, together with the finding of DNAs in chloroplasts, led to an important hypothesis that the mechanisms of non-Mendelian inheritance were based on an enzymatic reaction that eliminates most cpDNA molecules from the  $mt^-$  parent after mating.

To explain the preferential digestion of  $mt^-$  cpDNA, it has been proposed that the maternal transmission of cpDNA is governed by a methylation-restriction system analogous to that found in bacteria: after gametic fusion, the  $mt^-$  cpDNA is digested by a restriction enzyme while the modified  $mt^+$  cpDNA remains unaffected. Modification is assumed to occur as a result of methylation. Studies using CsCl density gradient, high-pressure liquid chromatography, and restriction endonucleases have shown that  $mt^+$  cpDNAs are methylated but  $mt^-$  cpDNA are not. Furthermore, the isolation of DNA methyltransferases from *C.reinhardtii* with molecular weights of 60 000 and 200 000 Da has been reported.

But arguments against this hypothesis were subsequently put forward, based on the observation that normal uniparental inheritance patterns occurred even in a nuclear mutant *me1* in which cpDNAs were constitutively methylated to high levels in both  $mt^+$  and  $mt^-$  vegetative cells. Sager and Grabowy countered this conclusion with their observation that additional methylation occurred during gametogenesis of  $mt^+$ , but not  $mt^-$  cells carrying the *me1* mutation. And additional incorporation of methyl groups into cpDNA occurred at the 7 h zygote stage in *C.reinhardtii*, which might be responsible for the protection. Furthermore, a report indicated that treatment of cells with the methylation inhibitors (L-Ethionine and 5-azacytidine) that resulted in a hypomethylation of gamete cpDNA had no effect on the subsequent uniparental inheritance of  $mt^+$  cpDNA.

In 1982, the results of a fluorescent microscopy study were published by Kuroiwa *et al.*. The cpDNA in *C.reinhardtii* is localized into 8–10 nucleoids, easily distinguished from the much larger cell nucleus when stained with the DNA specific fluorochrome DAPI. Within 50 minutes after mating, each cp nucleoid contributed by the  $mt^-$  parent begins to dissolve from its periphery until the DAPI fluorescence disappears completely, while the cp nucleoids from the  $mt^+$  parent remain intact. Based on the morphological characteristics of the  $mt^-$  cp nucleoid disappearance, an involvement of DNase I-like nucleases was suggested. But since the preferential disappearance of  $mt^-$  cp nucleoids occurs well before the DNA digestion is detected by biochemical techniques six hours after zygote formation, the changes in cpDNA molecules that cause the preferential disappearance remained controversial. Further research required a development of technique to transform chloroplast genomes, which is described later in this paper.

To identify the factors involved in the preferential disappearance of  $mt^-$  cp nucleoids, young zygotes of *C.reinhardtii* were treated with specific inhibitors of translation in chloroplasts (chloramphenicol, erythromycin) and in the cytoplasm (cycloheximide), with inhibitors of transcription in chloroplasts (rifampicin) and in the cytoplasm (actinomycin D and  $\alpha$ -amanitin), at various temperatures, with chelating agents (EDTA, EGTA), and with UV light. These experiments indicated that *de novo*  $mt^+$  nuclear gene expression that is sensitive to UV irradiation would be responsible for the preferential disappearance.

It was in 1985 that identification of nucleases specifically required for the uniparental inheritance of cpDNA was attempted. Consequently, a set of  $Ca^{2+}$  dependent nucleases was found that displayed both endo- and exo-nucleolytic activity. The extracts were composed of six nucleases and this preparation was collectively referred to as nuclease C. Previous reports that EGTA inhibited the specific disappearance of  $mt^-$  cp nucleoids and the fact that the  $mt^+$  cp nucleoids became resistant against the nuclease C treatment suggested a possible involvement of the nuclease C in  $mt^-$  cpDNA destruction. But the hypothesis was rendered unlikely by the observation that the nuclease C activity was found to be present throughout the entire life cycle—in zygotes,  $mt^+$  and  $mt^-$  vegetative cells, and gametes. Until now, the actual enzymatic mechanism responsible for the  $mt^-$  cpDNA destruction is still unknown.

In 1988, an examination was made of the polypeptides that are synthesized in the cytoplasm and related to the preferential disappearance of  $mt^-$  cp nucleoids by incorporation of [ $S^{35}$ ]methionine and two-dimensional gel electrophoresis. About 200 polypeptides were synthesized within 30 minutes after mating and six of these polypeptides appeared to be essential for the preferential disappearance of  $mt^-$  cp nucleoids. These polypeptides are probably involved in the activation of nuclease C, perhaps via alterations in biochemical or biophysical parameters such as the concentration of intracellular  $Ca^{2+}$ , permeability of chloroplast membrane, the structure of lysosomes, and so on.

In addition, interesting data was obtained about the protection of  $mt^+$  cp nucleoids, using a permeabilized cell model. When the cell model was incubated with the nuclease C fraction, cp nucleoids in  $mt^+$  and  $mt^-$  vegetative cells and  $mt^-$  gamete disappeared completely, but cp nucleoids in  $mt^+$  gametes remained unaffected. These results suggest the presence of an unidentified mechanism to protect  $mt^+$  cpDNA from nuclease C activated during the gametogenesis.

In parallel with these works, in 1993, an acidic polypeptide in zygotes was found that binds to chloroplast nucleoids (Ezy1). This polypeptide is expressed almost immediately upon zygote formation. Expression of *ezy-1* is also selectively inhibited when  $mt^+$ , but not  $mt^-$ , gametes are UV irradiated. Similar behavior was reported for the gene *zys1A, B*. These gene products might play a key role in the preferential digestion of  $mt^-$  cpDNA.

-  
-  
-

TO ACCESS ALL THE 17 PAGES OF THIS CHAPTER,  
Visit: <http://www.eolss.net/Eolss-sampleAllChapter.aspx>

### **Bibliography**

Gillham N.W. (1994). *Organelle Genes and Genomes*. New York: Oxford University Press. 424 pp. [A comprehensive book that reviewed almost all fields of research concerning mitochondria and chloroplast. Their structure, evolution, genetics, and biogenesis are lucidly summarized.]

Kuroiwa T. (1991). The replication, differentiation, and inheritance of plastids with emphasis on concept of organelle nuclei, *International Journal of Cytology* **128**, 1–62. [This paper extensively presents historical aspects and recent discoveries in chloroplast inheritance, chiefly emphasizing the concept of organelle nuclei.]

### **Biographical Sketches**

**Yoshiki Nishimura** graduated from the University of Tokyo in 1996, and the Graduate-School of Science, University of Tokyo in 2001.

**Tsuneyoshi Kuroiwa** attended the Graduate School of University of Tokyo at 1971 and received research training at the Tokyo Metropolitan Isotope Research Center in 1971/1972. He was Associate Professor of Department of Biology, Okayama University from 1972 to 1977, Associate Professor and Professor of National Institute for Basic Biology from 1977 to 1987, and became Professor at the University of Tokyo at 1987. He served as Director of Biological Science, Consultant of School of Science, Director of the Botanical Garden, and Vice dean of the School of Science of the University of Tokyo in 1987–2000. At present, he is Professor at the University of Tokyo, and President of the Japanese Society of Botanical Sciences. He was awarded The Society Prizes by the Japan Society of Electron Microscopy and Japanese Society of Plant Morphology in 1998, in connection with the discovery of plastid and mitochondria dividing apparatuses.