Non-Mendelian inheritance

Introduction

In this last lesson, we will consider situations where genes do not segregate in a Mendelian manner. This is true for genes that which are not located in the nucleus, such as mitochondrial genes, genes in plastids in plants or on other cytoplasmic structures, such as plasmids. Here, we will discuss the basics of mitochondrial inheritance and why yeast is well suited to study mitochondrial genetics. We will also look at how screens to identify mitochondrial genes can be set up in yeast.

A review of Mendel's laws

The two main genetic principles from Mendel are the Law of Segregation and the Law of Independent Assortment. According to the Law of Segregation, in a diploid organism that produces gametes by meiosis, each allele is segregated into a different gamete. The Law of Independent Assortment says that this segregation of alleles happens independently of other alleles. We now know that this is true for genetic loci that are not linked (not closely associated along a piece of DNA). When crossing two individuals with a known genotype, one can predict the probability that the offspring will have a particular genotype and phenotype using Mendel's two laws.

Non-Mendelian inheritance

Mendel's discoveries of segregation and independent assortment underlie the most common modes of genetic inheritance. However, there are inherited characteristics that do not obey Mendel's laws and are thus considered non-Mendelian. While genes located on the chromosomes in the nuclei of diploid cells underlie Mendelian inheritance, genes controlling traits inherited in a non-Mendelian fashion reside on cytoplasmic organelles and plasmids. We need to understand non-Mendelian modes of inheritance, because we need to rule out other patterns of inheritance to infer Mendelian inheritance. The key characteristic of non-Mendelian inheritance in eukaryotes is that the origin of the cytoplasm (and not the nuclear DNA) determines the phenotype. Cytoplasmic inheritance can be due to organelles or any other particles that can be passed on to the next cell generation and affect its phenotype.

Eukaryotic cells have organelles, which include mitochondria and chloroplasts. Most importantly for genetic analyses, mitochondria and chloroplasts have their own genomes and use their own transcriptional and translational machinery. A typical cell has many copies of the organellar genome, as there are many copies of mitochondria within the cell (and many copies of the chloroplast in plant cells and algae). In actively dividing cells, these organelles and their genomes must double, be partitioned at mitosis and passed on to the next generation. Thus, they will have special patterns of inheritance.

Mitochondrial inheritance

Let us concentrate here on the inheritance of mitochondria. In most higher eukaryotes (plants and animals), one parent will provide the organelles for the offspring. This parent is usually the female, as the oocyte contains the materials necessary in the cytoplasm for the development of the zygote. Thus, the egg cell contributes much more cytoplasm to the zygote than the male gamete, and in sperm cells, different processes ensure that sperm mitochondria do not compete with those from the egg. Thus, mitochondrial inheritance in animals and plants is uniparental and is passed from one generation to the next by maternal inheritance. There are diseases in humans that are caused by mutations in mitochondrial DNA. Typically, in these cases, the transmission of diseases occurs only via the mother.

This uniparental inheritance leaves little opportunity for genetic recombination between mitochondrial genes. Because all the mitochondrial DNA is inherited as a single unit (also called a haplotype, a group of genes that are inherited together from one single parent), mitochondrial DNA from different individuals can be used to infer the evolutionary history of populations. This has led to the proposition

of a "mitochondrial Eve", the most recent woman from whom all living humans descend.

In yeast, however, both haploid cells contribute equal amounts of mitochondria to the diploid zygote. Nevertheless, during the subsequent divisions, the "maternal" and "paternal" mitochondria become more or less randomly distributed to the daughter cells. After a few generations, the mitochondria of a given cell generally contains mitochondrial DNA only from one or the other parent cell, because only a small part of the mitochondrial DNA passes from the mothers to buds and to buds of buds (see figure 7-1).

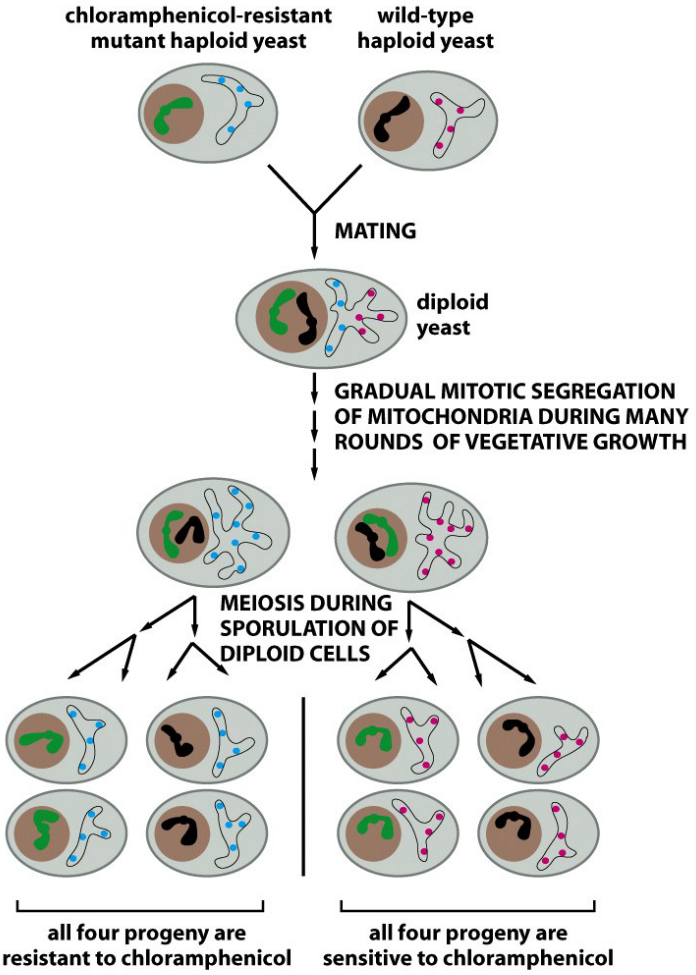

Figure 7-1 The difference in the patterns of inheritance between mitochondrial and nuclear genes of yeast cells. For nuclear genes (Mendelian inheritance), two of the four cells that result from meiosis inherit the gene from one of the original haploid parent cells (green chromosomes), and the remaining two cells inherit the gene from the other (black chromosome). By contrast, for mitochondrial genes (non-Mendelian inheritance), it is possible for all four of the cells that result from meiosis to inherit their mitochondrial genes from only one of the two original haploid cells. (adapted from figure 14-62, Molecular Biology of the Cell, Alberts, 5th edition, Garland Science)

When diploid cells in which the mitochondria have segregated, as described above, undergo meiosis to form four haploid daughter cells, each of the four haploid cells receives the same mitochondrial genes, and hence the segregation is 4:0 or 0:4 (mutant:wild type). Furthermore, in the vast generality of the cases exactly 50 % of the cells give a 4:0 pattern and the other 50 % give a 0:4 pattern. Note

that, as shown in figure 7-1, this is dramatically different from what happens to nuclear genes. If this type of cytoplasmic inheritance is observed for a certain gene, it shows that this gene must be present outside of the nucleus.

The use of yeast in studying mitochondrial genes

S. cerevisiae is an excellent model organism to genetically study mitochondrial function, because it is capable of satisfying its energy requirements with ATP generated by fermentation, i.e., without respiration and without needing a functional mitochondrion. Thus, oxidative phosphorylation and the presence of the mitochondrial genome are dispensable as long as fermentable carbon sources, such as glucose, are present in the growth medium. Therefore, yeast can survive mutations in mitochondrial genes that arrest oxidative phosphorylation (or even the total loss of mitochondrial DNA (mtDNA)) in the presence of fermentable carbon sources. These mutations would be lethal in many other eukaryotes. However, when yeast cells are grown on non-fermentable carbon sources, such as glycerol or ethanol, respiration and the presence of an intact mitochondrial genome become essential. This means that mutations that impair mitochondrial function can be isolated and identified by their conditional growth: they grow on fermentable carbon sources but not on non-fermentable ones. As mentioned above, in most higher eukaryotes, the transmission of mtDNA is difficult to study by crossing strains owing to uniparental inheritance. In yeast, however, mtDNA inheritance is biparental and thus, mtDNA transmission can be easily followed by crossing mutant and wild-type yeast strains.

The mitochondrial DNA of *S. cerevisiae* contains genes required for some mitochondrial functions. These genes encode tRNAs, rRNAs, cytochrome oxidase, and an ATPase. The remaining mitochondrial components are encoded in nuclear genes, including the genes encoding the subunits for mitochondrial RNA polymerase and mitochondrial ribosomes. Because both mitochondrial and nuclear genes are necessary for mitochondrial function in *S. cerevisiae*, mutations in either mtDNA or nuclear DNA can impair that function. Each class of mutation behaves genetically in a distinct fashion, indicating cytoplasmic or nuclear inheritance.

Mutants defective in oxidative phosphorylation form small colonies on medium containing limiting amounts of fermentable carbon sources. Respiratory-deficient strains carrying mutations in the nuclear genome are referred to as nuclear *petite* or *pet* mutants. Mutations in nuclear *PET* genes segregate 2:2 during meiosis, as expected for any single nuclear mutation.

Mutants with lesions in the mitochondrial genome have so-called rho mutations. These mutations segregate in a different fashion from nuclear pet mutations, indicating cytoplasmic inheritance. Rho mutations that are the result of the complete loss of mtDNA are called rho^0 mutations. Mutations where only part of the mtDNA is deleted are called rho-. If a rho^0 strain is crossed by a wild-type strain (rho^+), the cytoplasms of the two parents are mixed in the diploid. The resulting four spores in each tetrad all contain wild-type mitochondria. Therefore, the segregation is 4:0 rho^+:rho^0 (see figure 7-2).

When crossing a rho^- mutant strain with a rho^+ wild type strain, in most cases, 50 % of the tetrads will show a 4:0 and 50 % a 0:4 (rho^+:rho^-) segregation pattern. In some cases, some rho^- mutants show a bias towards the 4:0 (0:4>50 %), indicating that the mutated genome proliferates faster than the wild type one and takes over. These mutant mtDNAs are called hyper-suppressor. They generally corresponds to a mtDNA where the origin of replication of the mitochondrial genome is amplified through multiple tandem repetitions.

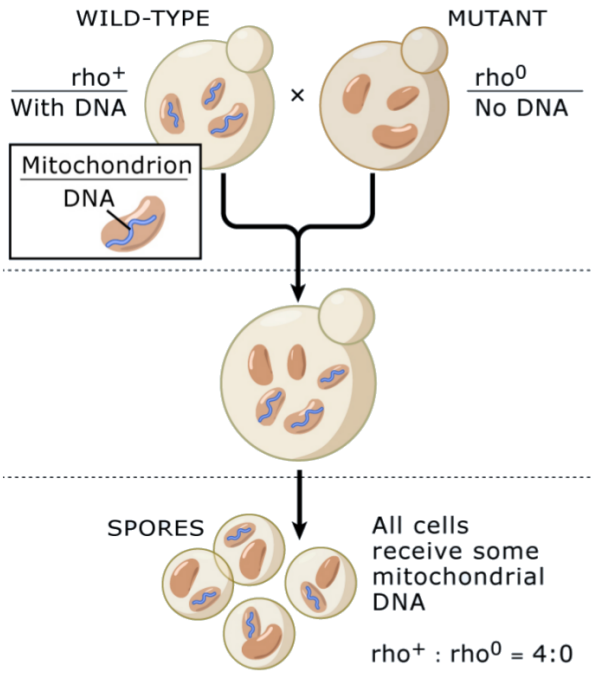

Figure 7-2 In this cross, one parent is wild type (*rho*$^+$) and the second parent lacks mitochondrial DNA (*rho0*). When the strains are crossed, all spores in a tetrad inherit mitochondria. Cytoplasmic inheritance is indicated by the 4:0 segregation pattern. (adapted from ergito.com)

In a screen for mutants defective in mitochondrial respiration, cells that cannot grow in the absence of fermentable carbon sources, such as glucose, can easily be selected for. For the identified mutants, it is straightforward to determine whether the affected gene is located on nuclear or mitochondrial DNA just by following the phenotypic segregation in tetrad analysis (see figure 7-2).

Rho$^-$ mutations can be used in complementation assays to detect whether a certain gene can complement and reverse the mutation, thus restoring mitochondrial function. Since mitochondria have their own genome, and are inherited by daughters independently of the nucleus, they are a suitable marker for the cytoplasm. *Rho*^{0} mutants are often used for this purpose.

Remember what you have learned about the behavior of cells mutant in the adenine biosynthesis pathway (*ade* mutants). We discussed that the shift in color from red to white (or vice versa) present in *ade1* or *ade2* mutants is often used as a non-selective phenotype in genetic screens. As mentioned before, *ade2* mutant cells are red because they accumulate an intermediate compound (AIR) that is oxidized to form a red pigment. Using this system, white colonies were identified as *petite* mutants that lack oxidative metabolism due to defective mitochondria. Due to this defect they cannot oxidize AIR and turn red. This exemplifies how a change in colony color can identify cells defective in mitochondrial function.

Summary

In this lesson, we have learned how yeast is used to study mitochondrial genes. Here, the great advantage is the ability of yeast to provide energy by respiration and by fermentation. Thus, genetic approaches are feasible to unravel mitochondrial biogenesis and function, and the regulatory cascades controlling mitochondrial gene function.