

## Deviations from Mendelian Genetics-Organelles

**Reading and problems for this and the next lecture:**

**Chapter 15**

**Problem set A below**

Mendel's laws do not apply to the genetic behavior of molecules that do not use mitotic and meiotic spindles to insure that daughter cells get equivalent sets of chromosomes. In the next two lectures, we will discuss exceptions to Mendel's laws.

**Mitochondria and chloroplasts are energy producing organelles of eukaryotic cells.**

Oxidative phosphorylation in mitochondria and photosynthesis in chloroplasts produce energy for animals and plants. Each organelle contains a double stranded circular DNA molecule that encodes components of the mitochondrion or chloroplast. For example, mammalian mitochondrial DNA (mtDNA) is a 16 kbp DNA that encodes thirteen proteins involved in oxidative phosphorylation, the large and small mitochondrial rRNAs, and a complete set of tRNAs required for mitochondrial protein synthesis. Since oxidative phosphorylation and mitochondrial gene expression as well as many other mitochondrial functions require more than these mitochondrial gene products, however, most mitochondrial proteins are encoded in the nucleus.

**Mitochondria and chloroplasts evolved from endosymbiotic bacteria**

The endosymbiont theory formulated by Lynn Margulis in the 1970s postulates that mitochondria and chloroplasts evolved from bacteria that invaded early eukaryotic cells. This theory is supported by similarities between prokaryotes and organelles. Some of these shared features are: (1) the circular genomes of prokaryotes and organelles are not complexed with histones; (2) the genomes of prokaryotes and organelles are organized with functionally related genes close to each other, and related genes are often expressed coordinately; (3) N-formyl methionine and tRNA<sup>fMet</sup> initiate translation in bacteria and organelles; (4) ribosomes are similar. This later feature was used to define which proteins were encoded in the nucleus and which in the organelle. For example, prokaryotic and

organelle ribosomes, but not eukaryotic cytoplasmic ribosomes, are sensitive to chloramphenicol, whereas eukaryotic cytoplasmic ribosomes, but not prokaryotic and organelle ribosomes, are sensitive to cycloheximide. In the presence of chloramphenicol radioactive *in vivo* labeling labels mitochondrial proteins encoded in the nucleus, whereas in the presence of cycloheximide, only mitochondrially encoded proteins are labeled.

### **Mitochondrial mutations in the yeast *Saccharomyces cerevisiae***

In each yeast cell there are 40-50 copies of the mtDNA. The number of mitochondria in yeast varies with growth conditions and involves cycles of fusion and fission. Yeast are particularly well suited to the study of mitochondrial genetics because mtDNA, but not the mitochondrion, is dispensable. In the absence of oxidative phosphorylation yeast can grow, albeit slowly, on fermentable carbon sources such as glucose.

The first mitochondrial mutants were isolated and studied by Boris Ephrussi in the 1940s. These mutant yeast cells grow slowly and thus give rise to small colonies. Because Boris was French, these mutants were called petites, named for the small size of the colonies they produced; strains wild-type for mitochondrial function are called grande. Petites generate ATP by glycolysis, a process that is much less efficient than oxidative phosphorylation, and thus the cells grow slowly. One diagnostic feature of petite mutants is that they do not grow at all if cultured in a nonfermentable carbon source such as glycerol or ethanol, which can only be metabolized by oxidative phosphorylation.

Mutations in either nuclear or mitochondrial genes can generate the petite phenotype. Nuclear mutations segregate like Mendelian traits in crosses, 2:2, and are called nuclear or segregational petites. Because most of the genes involved in mitochondrial function are encoded in the nucleus, there are many genes defined by nuclear petites.

Most mitochondrial mutations are deletions of the mitochondrial DNA that remove a significant proportion of the mitochondrial genome. Petite mutants can completely lack mitochondrial DNA, and these mutants are referred to as neutral or rho<sup>0</sup>; petite mutants that retain part of the mitochondrial genome are called rho<sup>-</sup>. Although lacking oxidative phosphorylation, rho<sup>0</sup>

and rho<sup>-</sup> yeast contain mitochondria. Mitochondria are essential for yeast viability, but those functions encoded by mtDNA are not. One interesting feature of rho<sup>-</sup> strains is the mtDNA of these and grande strains is the same size. The portion of the mtDNA that is not deleted is reiterated to generate a mtDNA of approximately the size of wild-type mtDNA.

### **The pattern of inheritance for the organelle genomes is not Mendelian**

Different yeast rho mutants vary in their inheritance patterns. For example, if a rho<sup>0</sup> petite mutant is crossed to a grande strain, all of the diploids are grande, and sporulation of these diploids results in all grande progeny. This is an example of uniparental inheritance. This is not maternal inheritance (see below) because both strains in the cross contribute equivalent amounts of cytoplasm to the diploid. This type of inheritance pattern is known as neutral. rho<sup>-</sup> strains behave differently in crosses. Depending on the type of deletion in the rho<sup>-</sup> strain, anywhere from 1-99% of the diploids generated from rho<sup>-</sup> petite to grande crosses are petites. If the diploids are grown mitotically many generations and sporulated, the progeny of the petite diploids are all petites and the progeny of the grande diploids are usually all grande. However, if a grande diploid from a rho<sup>-</sup> petite to grande cross is sporulated soon after being generated, the ratio of grande : petite spores can be 4:0, 3:1, 2:2 or 1:3. Often the mating of grande and rho<sup>-</sup> petites and the sporulation of the diploids result in all petite progeny. These types of petites are known as suppressive and reflect the replication advantage of the specific rho<sup>-</sup> mtDNA chromosome over that of the wild-type chromosome.

Why does the pattern of inheritance often change with the number of mitotic divisions that a rho<sup>-</sup>/grande diploid undergoes? The diploid originally starts out with mitochondria that contain a mixture of wild-type and mutant mtDNAs. Cells that contain such mixtures are called heteroplasmic, and cells containing a single mtDNA type, whether it is wild-type or mutant, are called homoplasmic. Because mtDNAs do not attach to the mitotic spindle during mitosis, they randomly segregate into the progeny cells. Eventually given enough cell divisions, cells are driven to be homoplasmic.

**In species with males and females, organelles are inherited from the mother. mRNA and proteins are also inherited from the mother.**

The female or male of a species is defined by the nature of the gametes it produces: females produce oocytes that contribute a large amount of cytoplasm, which includes mitochondria or chloroplasts, to the zygote, and males produce sperm that contribute essentially no cytoplasm to the zygote. Thus organelles are inherited from the mother in species with males and females. This inheritance can be easily demonstrated in any species that have been shown to contain mtDNA or chloroplast DNA (cpDNA) polymorphisms. Two individuals that are dimorphic for a mtDNA or cpDNA RFLP are crossed, and all the progeny can be shown to contain only the maternal mtDNA or cpDNA RFLP. This type of uniparental inheritance is called maternal inheritance or cytoplasmic inheritance.

A cautionary note on the terminology: the term "maternal inheritance" is used to describe organelle inheritance, while the term "maternal effect" is used to describe the products of nuclear genes of the mother that affect the phenotype of the offspring. The oocyte is packed with mRNAs and proteins derived from the maternal genome, and these molecules are essential for early development before transcription of the zygotes genome occurs. You will need to distinguish between maternal inheritance and maternal effect in problems showing inheritance patterns. We will discuss these distinctions in class.

### **MtDNA accumulates mutations rapidly**

In all species, mtDNA accumulates mutations approximately 10 times faster than nuclear DNA. Three differences between the nucleus and mitochondrion can explain the different mutation rates. First, repair mechanisms present in the nucleus are absent in mitochondria. Second, mitochondrial electron transport produces oxygen free radicals that can oxidize nucleotides, a mutagenic event. Third, mtDNA lacks histones, which are thought to protect DNA from damage. Any cell that contains a mutated mtDNA is heteroplasmic and subsequent divisions can cause progeny cells to contain a substantial proportion of mutant mtDNA.

## **Certain human genetic diseases are caused by mutations in mtDNA.**

Certain muscle and neurological diseases are caused by mutations in mtDNA; however, any mutation that severely disrupts mitochondrial function will not be tolerated unless wild-type mtDNA is also present. Human cells often contain approximately 100 mitochondria and 1000 mtDNA molecules. Any individual that is heteroplasmic for a mtDNA mutation will contain in different tissues various ratios of wild-type and mutant mtDNAs because of random segregation of mtDNA during somatic development.

The association between certain diseases and mtDNA lesions has only recently been made because of complex patterns of inheritance for these diseases. Because individuals bearing these mutations are almost invariably heteroplasmic (mutations that completely remove mitochondrial function will cause the cell to die if they become homoplasmic for the mutant DNA), individuals in a pedigree will have different amounts of mutant mtDNA. Thus, although maternally inherited sometimes only some of the progeny will exhibit the disease. Second, because different tissues can have different ratios of mutant to wild-type mtDNA, one individual in a pedigree can exhibit one organ pathology and another individual in the same pedigree will exhibit dysfunction in a different organ.

The variability of the phenotypes and patterns of inheritance of these diseases slowed their identification as being mitochondrial. Several features provide evidence for a mtDNA mutation causing the disease: (1) A pattern of inheritance is consistent with maternal inheritance. In other words, affected mothers will transmit the disease to children of both sexes, and affected fathers do not transmit their disease to their children. (2) Affected tissues will display a deficit in mitochondrial function. When samples from the tissue are taken, assays for mitochondrial oxidative function will show a defect. (3) Affected tissues should be heteroplasmic for wild-type and mutant mtDNAs.

One particularly well studied mitochondrial disease is myoclonic epilepsy and ragged-red fibre disease (MERRF). MERRF is maternally inherited, and severely affected individuals have myoclonic epilepsy (uncontrolled jerking), mitochondrial myopathy, and a defect in mitochondrial protein synthesis. The severity of the disease appears to be affected by two variables: percentage of mutant mtDNA and age. The amount of mutant DNA is caused by

variations in heteroplasmy discussed above. Presumably age is a factor because as cells get old, wild-type mtDNAs in a cell accumulate lesions that lowers the ability of the cell to produce energy. The lesion causing MERRF is a point mutation in the tRNA<sup>Lys</sup> gene. Severely affected tissues of individuals with MERRF contain higher ratios of mutant to wild-type mtDNA.

You might expect that it would be easy to guess at genetic diseases that are caused by deficits in mitochondrial function based on the phenotype of the affected individuals, but it really isn't. A mutation disrupting the mitochondrial tRNA<sup>Leu</sup> is associated with strokes and mitochondrial myopathy when present in a high percentage of the mtDNA (>85%), but is associated with maternally inherited diabetes mellitus and deafness when present in low percentage (5-30%). Thus, to conclude that a disease is due to a defect in the mitochondrial genome, the criteria outlined above must be fulfilled.

### **Uses of mtDNA**

Several features of human mtDNA make it a useful tool for studies in forensic pathology and human evolution. First, while a somatic cell has only two copies of a nuclear DNA sequence, the same cell will have hundreds of copies of a segment of mitochondrial DNA. The higher copy number of mtDNA increases the probability of being able to amplify from moribund tissue a specific DNA sequence by PCR. Second, mtDNA is inherited maternally and thus is not scrambled by recombination. These features of mtDNA inheritance make mtDNA one of the DNAs of choice for studying human lineages. The other chromosome that is useful for studying human lineages is the Y chromosome because like the mitochondrial chromosome, it is not altered by recombination and is only inherited from one parent. Finally, as mentioned above mtDNA mutates more rapidly than nuclear DNA. There is a highly variable noncoding region of 131 bps in human mtDNA that is particularly sensitive to change. The mutability of mtDNA ensures that other than maternally related individuals, most people will have distinct mtDNAs.

### **Forensic genetics**

Between 1976 and 1983, the military of Argentina kidnapped, jailed, and killed more than 10,000 dissidents that did not support the regime. Along with their parents, many infants and toddlers

disappeared. In 1977, four mothers and eventually many of the grandmothers of these children began to hold vigils in the main square of Buenos Aires to inform others that about the disappearance of their grandchildren with the goal of recovering their lost children and grandchildren. This group became known as the "Grandmothers of the Plaza de Mayo." They began to gather information that might lead them to the children, but realized that proof would require more. They contacted many organizations for help, including the American Association for the Advancement of Science (AAAS). They asked the AAAS for help with genetic testing that would stand up in the Argentinean courts after the military regime lost power. In 1983, the best way to confirm or exclude the familial relationship between two people was to compare the human lymphocyte antigens (HLAs) on white blood cells since these were highly polymorphic and allelic variants could be distinguished serologically. Even if a child's parents were missing or dead, the child should still have one allele shared by one of the paternal grandparents and the other shared by one of the maternal grandparents. The AAAS put the grandmothers in contact with Mary Claire King, then a geneticist at Cal. Professor King taught Argentine medical workers to analyze HLA markers on white blood cells, and the workers typed HLAs from family members of the missing children. The grandmothers set up a bank of the HLA information. The probability that a tested child belonged to the family claiming him or her on the basis of eye-witness accounts of abduction varied from 75-99%.

By the mid-1980s, the simpler cases had been solved and the limitations of the HLA approach became apparent. The advent of PCR and DNA sequencing now made it possible to analyze the DNA directly. Professor King turned to her former mentor and colleague Allan Wilson, a molecular biologist at Cal who was studying mtDNA. PCR amplification and sequencing of the highly variable region of mtDNA made it possible to match a child with his or her grandmother. The probability that two unrelated individuals would be identical for this 131 bp region is very small. In addition, the high copy number of the mtDNA aided in the identification of the remains of individuals killed by the military regime.

## **Human evolution**

In the 1980s, Allan Wilson pioneered the use of mtDNA to study human evolution. In two papers published in 1987 and 1991, he and his colleagues at Cal proposed that we all come from a population of humans that lived in Africa 200,000 years ago.

By comparing Restriction Fragment Length Polymorphisms (RFLPs) of mtDNAs from 143 individuals from around the globe and by sequence analysis of the highly variable region from 189 individuals, Wilson and his colleagues found greater sequence differences among native Africans than any other group, which included Europeans, Papua New Guineans, Asians, aboriginal Australians and individuals of Middle Eastern origin. In fact, the diversity of Africans is equivalent to the diversity of all groups combined. This led Wilson to propose that the African population has had the longest time to evolve variation and thus humans originated in Africa.

Having proposed that modern humans originated in Africa and populated the world by emigration from Africa, Wilson and colleagues calculated the date that the population of founder humans. They observed that 2.8% of the mitochondrial base pairs differed in the population of sub-Saharan Africans studied. They also know that chimpanzees and humans diverged about 5 million years ago and that human and chimpanzee mtDNAs differed at 15% of the base pairs of the mtDNA. Adjusting the data to account for multiple substitutions at the same base pair, they calculated that mtDNA has been diverging at a rate of 13.8% per million years. Assuming that this "molecular clock" is ticking at a constant rate over time, they determined that a "Mitochondrial Eve" from whom we are all derived lived  $2.8/13.8$  or 0.20 million years ago. Although there has been much argument about the assumptions and statistical methods used, most evolutionary geneticists accept that the women carrying our ancestral mtDNA lived in sub-Saharan Africa approximately 200,000 years ago.

Analysis of mtDNA sequences has also helped to support theories about how these early humans might have interacted with other Homo species as they migrated out of Africa. The replacement theory posits that as early *Homo sapiens* migrated out of Africa, they entered regions occupied by archaic Homo species and competed with them for resources. Eventually *H. sapiens* outcompeted these species leading to the extinction of these archaic species. A competing model, the regional continuity theory, posits that as *H. sapiens*



interbred with the other species. The Neanderthals were one of these archaic species, coexisting with modern humans for over 70,000 years until their extinction 28,000 years ago. In 1997 and then again in 2000 scientists were able to amplify by PCR nucleotides from the highly variable region of mtDNA sequences from two Neanderthals, one from western Germany and one from the Caucasus. When compared with human mtDNA sequences they found approximately three times as many differences between human and Neanderthal sequences than between pairs of humans. Moreover, the types and positions of the differences were distinct in the two groups. Using the types of calculations discussed above, the scientists estimated that the line that gave rise to Neanderthals and modern humans diverged between 365,000 and 853,000 years ago. The two Neanderthal sequences were much more closely related, and the scientists estimated that the ancestor to the two Neanderthals existed 150,000 to 350,000 years ago. The investigators argued that these differences support the replacement theory.

### **Problems set A**

1. You are given three haploid yeast strains, A, B and C, that are petite (no mitochondrial function). You cross A and B and obtain grande diploids. You grow the diploids for a few generations, sporulate them, and analyze 100 tetrads. All 100 tetrads produced two grande spores and two petite spores. You then cross B and C and obtain petite diploids. You grow the diploids for a few generations, sporulate them, and analyze 100 tetrads. All 100 tetrads produce four petite spores.

a) What kinds of petites were A, B and C? Be as specific as possible, and explain your reasoning

b) You cross A and C. Describe the types and quantities of the tetrads expected from this cross.

2. You are given a true breeding variant of squash that has yellow leaves (wild-type squash has green leaves). You cross this squash variant as the female parent with a wild-type squash as a male parent and find that all the progeny have yellow leaves. You

propose that yellow leaves are dominant to green leaves, that this is an example of maternal inheritance or that this is an example of a maternal effect gene. Describe a single genetic experiment to distinguish among these possibilities and the results for each scenario.

3. I removed this problem from the original set.

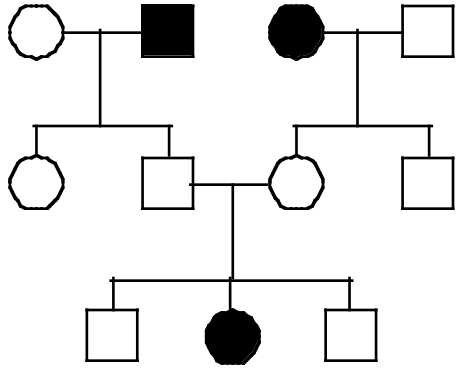
4. The cytoplasmically inherited trait *[PSI]* is thought to be a yeast prion form of the protein encoded by the *SUP35* gene. To test this you transform a *sup35* mutant with a plasmid containing a wild-type *SUP35* gene. You select for *[PSI]* "mutants" that still contain the wild-type *SUP35* gene. You now "kick out" the plasmid containing the wild-type *SUP35* gene from the *[PSI]* strain and propagate the strain lacking plasmid over many generations. Finally, you reintroduce the plasmid containing the wild-type *SUP35* and assay the transformants for the *[PSI]* phenotype. Remember: A prion is still susceptible to cellular proteases.

a) Why did you reintroduce the wild-type *SUP35* gene in the last step before scoring the cells for the *[PSI]* phenotype?

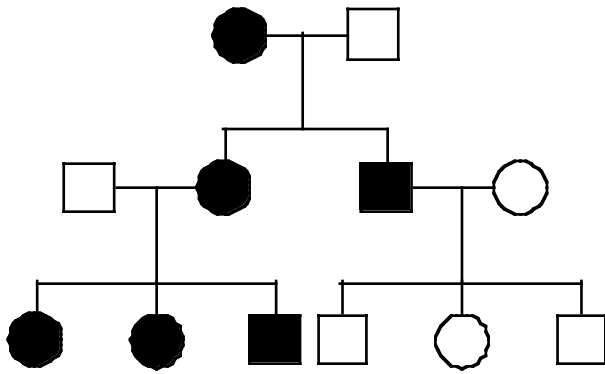
b) What result would you expect if *[PSI]* is not a prion form of the *Sup35* protein?

5. The affected individuals in the two pedigrees shown below display an optic nerve degeneration defects that leads to blindness.

Pedigree 1



Pedigree 2



for this defect.

a) What are the modes of inheritance for the diseases in the two pedigrees.

b) Describe a plausible explanation