Generelt om mitokondrier og respirasjonskjede

Laurence Bindoff

UNIVERSITETET I BERGEN

European Reference Network

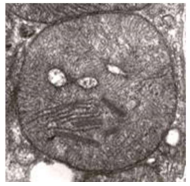
HELSE BERGEN
Haukeland universitetssjukehus

Oslo universitetssykehus

Learning objectives

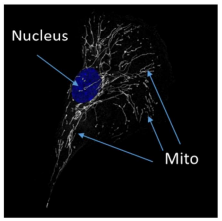
- Understand about
  - Mitochondria
    - What they are and what they do
    - Respiratory chain and mitochondrial energy metabolism
  - Mitochondrial genetics
    - Two genomes control the respiratory (mtDNA and nDNA)
  - Mitochondrial disease
    - Types and causes

What is a mitochondrion?



Old EM picture showing mitochondrion in cross section

Fibroblast cell stained for mitochondria (white) and nucleus (blue)



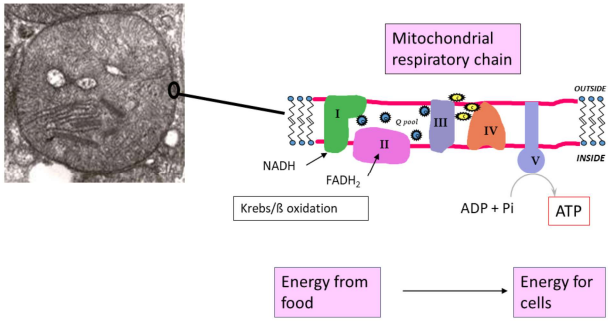
Bilder: LA Bindoff

Mitochondria are found in all cells except red blood cells and even these have mitochondria during their early development

Mitochondria in almost all cells are not seed-like organelles, but a reticulum or network

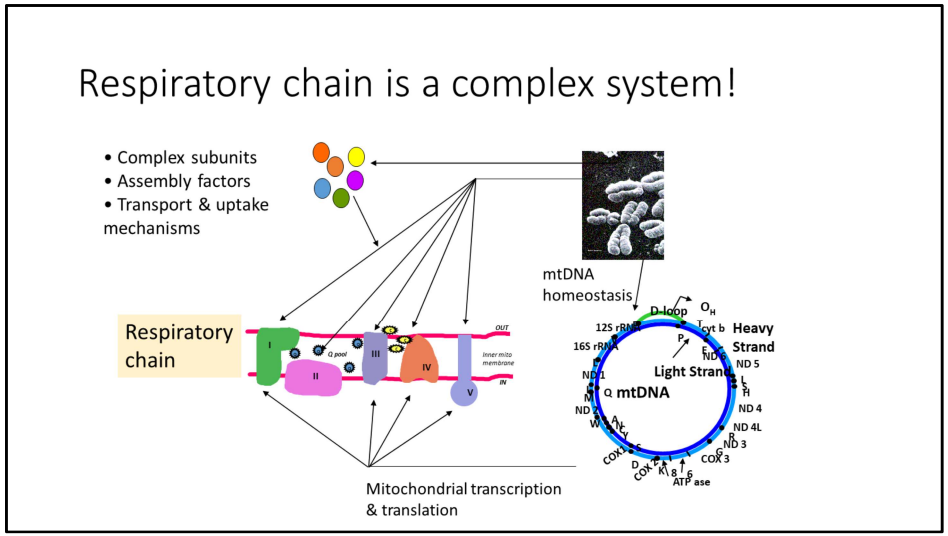
Mitochondria are constantly moving, fusing and splitting (fission). Fusion & Fission are important for mitochondrial homeostasis and defects in this process can give rise to disease (see later)

What do mitochondria do?



Bilder: LA Bindoff

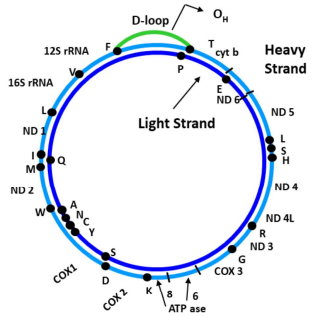
The mitochondrial respiratory chain is embedded in the inner mito membrane.
Its job is to use the energy derived from food to drive the phosphorylation of ADP to ATP. Since the reactions that release energy from food are oxidative the whole process is called “oxidative phosphorylation”



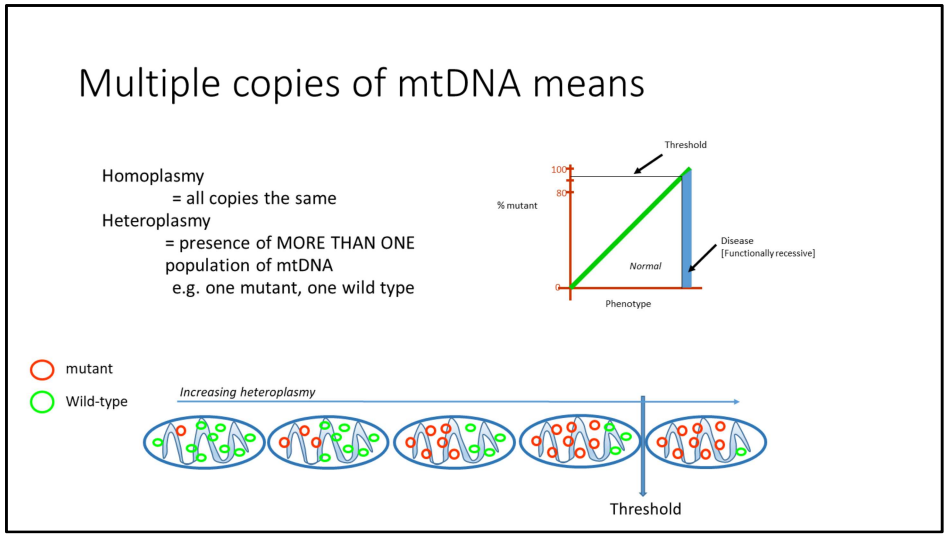
- 13 subunits/proteins are encoded by mtDNA: 7 go into complex I, one to complex III, 3 to complex IV and 2 to complex V. Complex II has no mtDNA encoded proteins
- Genes in the nucleus encode proteins that - 1. go into the complexes; 2. proteins involved with transport, uptake and assembly
- While mtDNA is separate from the nucleus, nuclear genes encode all the proteins required for mtDNA homeostasis including its polymerase (Polymerase gamma) and all of the proteins involved in transcription and translation.

Mitochondrial DNA

- Small (16,5kb), circular genome
- Economical
  - almost no non-coding (intronic) sequence
- Encodes 13 proteins, 22 tRNA and 2 rRNA
- Unprotected?
  - Exposed to damage?
- Multiple copies per cell

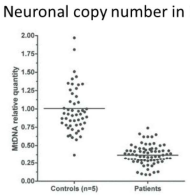


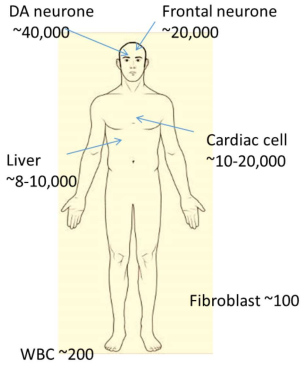
Mitochondrial DNA resembles a bacterial genome and is thought to be a remnant of a bacterial symbiont.
It is a compact genome with hardly any non-coding sequence. It encodes 22 transfer RNA (tRNA) and 2 ribosomal RNA (rRNA) needed for translating the 13 proteins encoded by mtDNA.
The two most important facts concerning this genome are: 1. it is (in humans) inherited almost exclusively from the mother and 2. it is found in multiple copies in each mitochondrion. Numbers of mtDNA vary between cell types from a few hundred in a skin fibroblast to tens of thousands in a neurone.



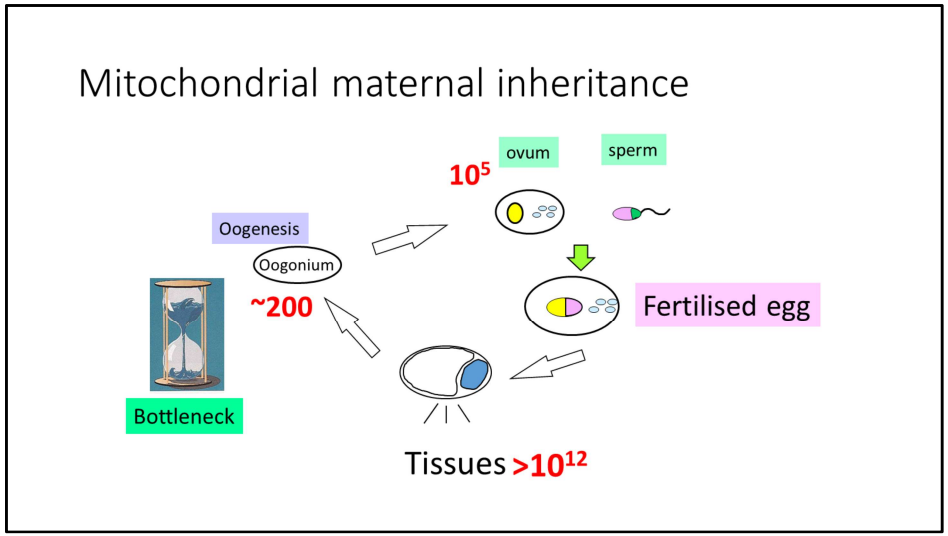
In view of the presence of multiple copies, a new genetic concept is needed when some copies (but not all) are mutated. It is assumed that in “normal” individuals, all mtDNA copies are the same: this is called homoplasmy. In those with mtDNA mutations affecting some but not all copies, the situation is called heteroplasmy. Heteroplasmy is an important concept since we see that it requires high levels of mutated mtDNA to cause a phenotype. In cases such as the m.3243A>G (the so-called MELAS mutation), this level may be as high as 90%. Once the level reaches this “threshold”, a cellular phenotype becomes manifest

Tissues copy number differences – depletion concept

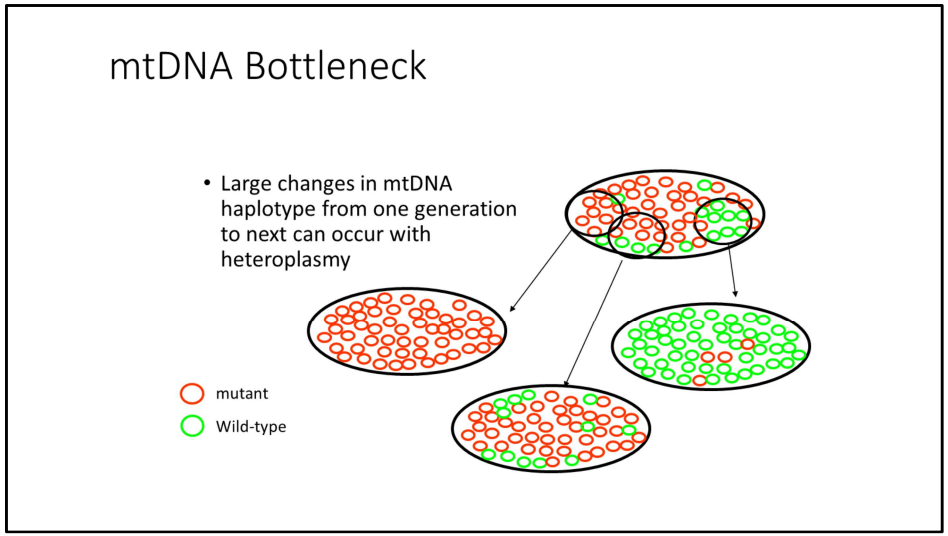




As noted earlier, numbers of mtDNA per cell vary according to tissue.
Depletion is the term we use when the number of copies falls below the normal (tissue) level.
Usually, we would not begin to suspect depletion before the level was under 50% since there is a great deal of biological variation. Mutations in genes involved with mtDNA homeostasis (e.g. polymerase gamma, TK2, DGUOK etc.) can give mtDNA depletion.



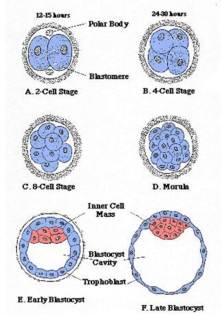
MtDNA is inherited from the mother.
The ovum has approx. 1-200,000 copies. However, prior to formation of the female germline (i.e. during oogenesis which is taking place early in embryogenesis) the number of mtDNA copies fall to low levels. Precursors of the ovum can have as few as 100-200 copies. This forms a "bottleneck" following which expansion of the mtDNA number can shift the proportion of mutant to wild-type mtDNA dramatically. See next slide.



Large changes in the balance (the degree of heteroplasmy) between normal and mutant mtDNA can occur when only a few copies are chosen to go into the next generation. See the 3 different examples.

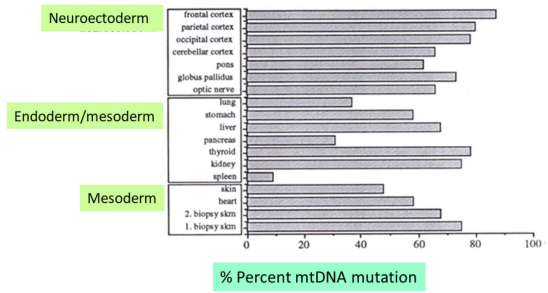
Mitochondrial segregation

- Mitochondrial division may start late
- ?at 64 cell stage
- Until then only segregation



Shifts in heteroplasmy from one generation to the next occur because of the bottleneck. There are, however, other ways the balance (the degree of heteroplasmy) can change. During early development of the foetus when cells are dividing and the primordial cell types are developing, mtDNA does not divide, but copies are “segregated” into daughter cells giving a continuous decline in mtDNA number. At some stage (perhaps 64 cell stage), mtDNA division starts again generating a similar situation to that described in the previous slide namely that there can be a shift in heteroplasmy.

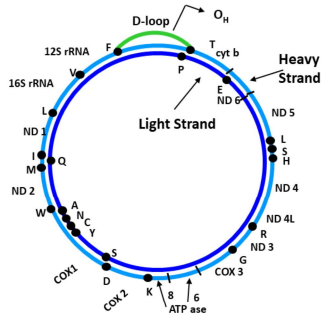
Tissue distribution of a mtDNA mutation



This slide shows the level of heteroplasmy in multiple different tissues taken post mortem from a patient with the m.3243A>G mtDNA mutation. Spleen represents the level in blood.

Mitochondrial DNA is highly polymorphic

- mtDNA acquires changes frequently
  - Mutations & polymorphisms!
- Uniparental inheritance
- Can be used to follow population movements



Some of you maybe aware that mtDNA is used on population studies. The reason is it is uniparentally inherited and (it is assumed) there is no exchange (equivalent to the crossing over seen in chromosomal DNA) of genetic material prior to inheritance by the next generation. Thus, the mtDNA we see in a population represents the maternal mtDNA that formed the population. mtDNA is used to plot maternal history while the Y chromosome is used for male lineages.

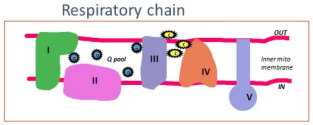
Summary

- Mitochondrial DNA
  - Small, circular, multi-copy, maternally inherited genome
  - 37 genes
    - That are all either respiratory chain components or required for their translation
- Respiratory chain
  - Most important pathway for ATP production
  - Complex control
    - 2 genomes
    - Construction must navigate
      - Dangerous environment
      - Two membranes

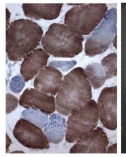
What do these facts mean for diagnosing mitochondrial disease

- Multiple mtDNA copies
  - *means you have to know about heteroplasmy & depletion*
- Variable tissue heteroplasmy
  - *means disease can be limited to one tissue*
  - *means you have to think about the tissue you use for diagnosis*
- Polymorphic mtDNA means
  - *means not every sequence changes is a mutation*
- Poor Phenotype – genotype correlation
  - *means you have to consider multiple possibilities*
- Two genomes required to build respiratory chain
  - *Means multiple sites/processes can be affected and all types of inheritance possible*

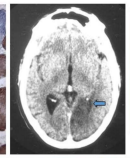
Respiratory chain diseases



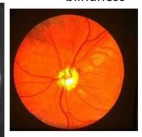
myopathy



acute brain lesions



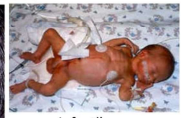
blindness



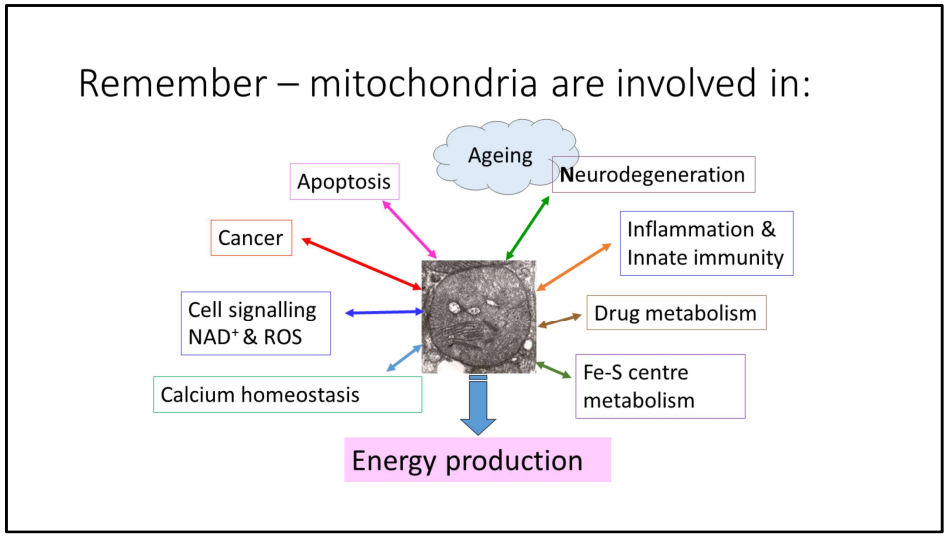
diabetes

Pixbay

deafness



Infantile encephalopathies



While this lecture focusses on energy metabolism and diseases associated with disruption of the respiratory chain, mitochondria perform multiple functions some of which (e.g. generating iron-sulphur centres) are equally important. Mitochondrial involvement in other diseases is therefore common. We define “mitochondrial” disease here as having to do with the respiratory chain and/or mtDNA. Mitochondria have also been (wrongly) been implicated in more diffuse processes such as ageing with the free radical theory being an excellent example. The theory goes: Mitochondria enclose a high energy system that generates dangerous intermediates (oxygen and hydroxyl radicals) that damage lipids, proteins and nuclei acids. Mitochondrial function declines with age. As function declines, the amount of free radicals rise and gradually damages tissues/organs leading to failure (i.e. ageing). While attractive, no evidence has been found that this is true!

Mitochondrial disease - general principles

- Affect any tissue
  - all tissues have mitochondria
- Present at any age
  - from birth to >80
- Demonstrate any pattern of inheritance
  - autosomal, X-linked, maternal

- **Particularly affect tissues that are:**
  - Terminally differentiated
    - High energy demand
- Muscle
- Retina
- CNS
- Pancreas
- Heart
- ALL & ANY!!

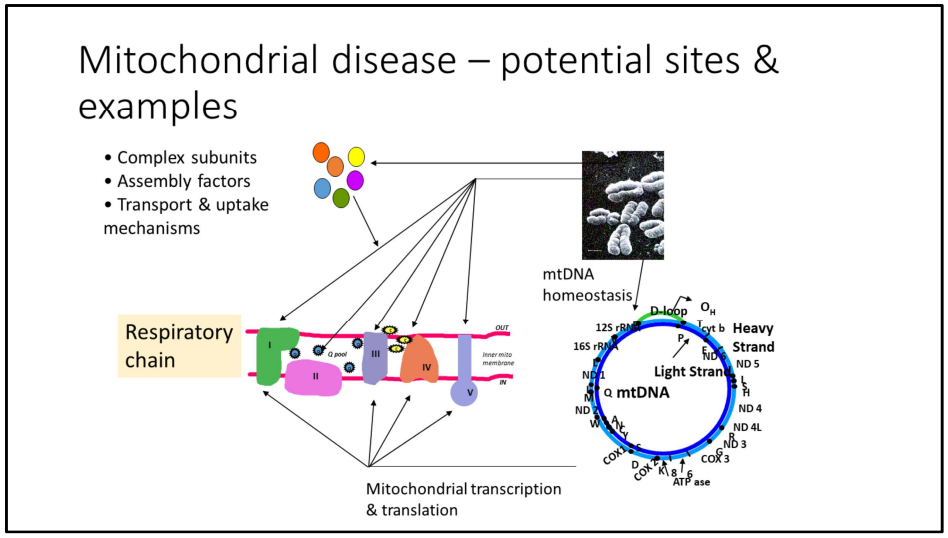
Adults and children have different disease profiles

- Adults
  - We see more mtDNA diseases than nDNA
  - However
    - mtDNA syndromes better characterised
    - Were the first to be recognised
- Infants/Children
  - We see more diseases due to nuclear gene defects than mtDNA
  - However, estimates suggest that ~20% are due to mtDNA mutation
  - Phenotypes are ofte non specific and thus much more difficult to diagnose
    - Encephalopathy, FTT

How common are mitochondrial diseases?

- Minimum prevalence
  - Gorman et al: *Ann Neurol* 2015; 77(5):753-9)
  - mtDNA mutations 1 in 5000
  - nuclear mutations 2.9 per 100,000
  - Combined 1:4300
- Maximum prevalence?
  - Elliot et al *Am J Hum Genet* 2008; 83(2):254-60.
  - "At least one in 200 healthy humans harbours a pathogenic mtDNA mutation that potentially causes disease in the offspring of female carriers"

- NOTE: Only screened for «known» genes
- Thus this is the tip of the iceberg
- Commonest inborn error of metabolism



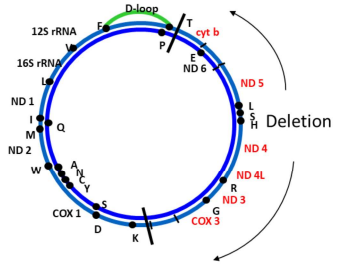
- 13 subunits/proteins are encoded by mtDNA: 7 go into complex I, one to complex III, 3 to complex IV and 2 to complex V. Complex II has no mtDNA encoded proteins
- Genes in the nucleus encode proteins that - 1. go into the complexes; 2. proteins involved with transport, uptake and assembly
- While mtDNA is separate from the nucleus, nuclear genes encode all the proteins required for mtDNA homeostasis including its polymerase (Polymerase gamma) and all of the proteins involved in transcription and translation.

Types of mitochondrial disease

- The next slides provide examples of mitochondrial diseases arising from defects in the various processes that make and keep mitochondria functioning
- More detailed descriptions of selected, common diseases will be given in later sections

Examples –
mtDNA mutation – deletion

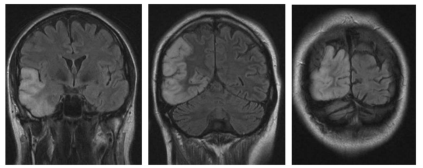
- KSS (onset <20)
  - ptosis/ophthalmoplegia
  - retinal pigment
  - cardiomyopathy
    - incl. conduction defect
  - myopathy
  - ataxia
  - etc.....
- CPEO (adult variant)

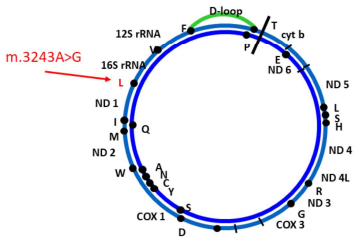


A single, large deletion of mtDNA was one of the first mtDNA mutations identified. The major phenotype associated with single deletions is progressive external ophthalmoplegia (PEO), but can include many other features. Disease onset defines the disorders with Kearns-Sayre syndrome (KSS) defined as having onset <18 years (and often retinal pigmentation, ataxia, cardiac conduction defects and high CSF protein) and chronic PEO (CPEO) being the adult variant. These diseases are a continuum sharing similar features although the adult form can just comprise PEO and mild proximal myopathy. Pearson's syndrome is also part of this spectrum, but differs in several ways not least in onset: Pearson's starts very early with refractory sideroblastic anaemia and exocrine pancreas dysfunction. The disease is caused by a single mtDNA deletion (which can be identified in multiple tissues including blood) and children who survive the initial haematological disease progress to develop KSS

mtDNA point mutation – m.3243A>G

- **M**itochondrial
- **E**ncephalopathy
- **L**actic
- **A**cidosis
- **S**troke-like episodes
  - Often associated with migraine prodrome
  - Common additional features
    - myopathy
    - diabetes*
    - recurrent vomiting
    - deafness *
    - etc





The 3243 point mutation in the tRNA for leucine (UUR) is the commonest known mtDNA mutation. When first described t was associated with a syndrome that bears the acronym MELAS and because of this, it is often called “the MELAS mutation”. We now know that this mutation causes many different phenotypes than just MELAS: the commonest is diabetes and deafness and since this is maternally inherited, it carries the acronym MIDD (maternally inherited diabetes and deafness). The mutation can also cause PEO, endrocrine disease incl. thyroid disease and cardiomyopathy. The important things to remember here are:

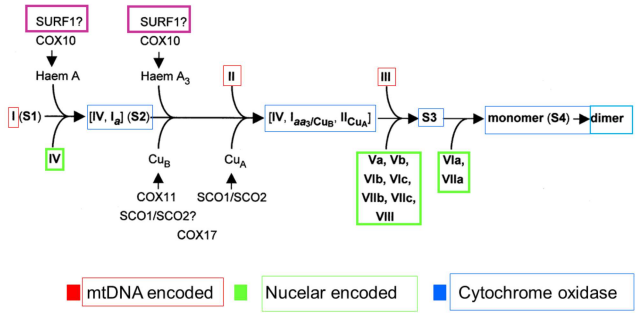
The level of heteroplasmy can differ from tissue to tissue meaning that phenotype depends in part on this. Biochemical analysis can show a predominant complex I defect or loss of complex I, III and IV activities. Risk of transmission varies but does relate to the level in the mother

Example – mutation affecting a respiratory chain complex subunit

- Leigh disease
  - Presents usually at or just after birth, but also later
    - Disease free interval
    - Failure to thrive
    - Vomiting/dysphagia
    - Elevated lactate (Blood &CSF)
  - Imaging
    - Lesions in brainstem/cord
  - Examples
    - Nuclear gene NDUFS1
    - mtDNA ATP6

The respiratory chain comprises over 85 protein subunits and complex I has the most with 45. The example of Leigh disease illustrates two things: first, that the same phenotype can arise from mutations affecting different proteins that are components of different complexes. Orphanet registers 28 nuclear genes that can cause Leigh disease (LD) including the example of NDUFS1 given here. Maternally inherited Leigh syndrome (MILS) is cause by mutations in mtDNA most commonly affecting the ATPase 6 gene.

Example – a nuclear encoded assembly protein (Surf1) –



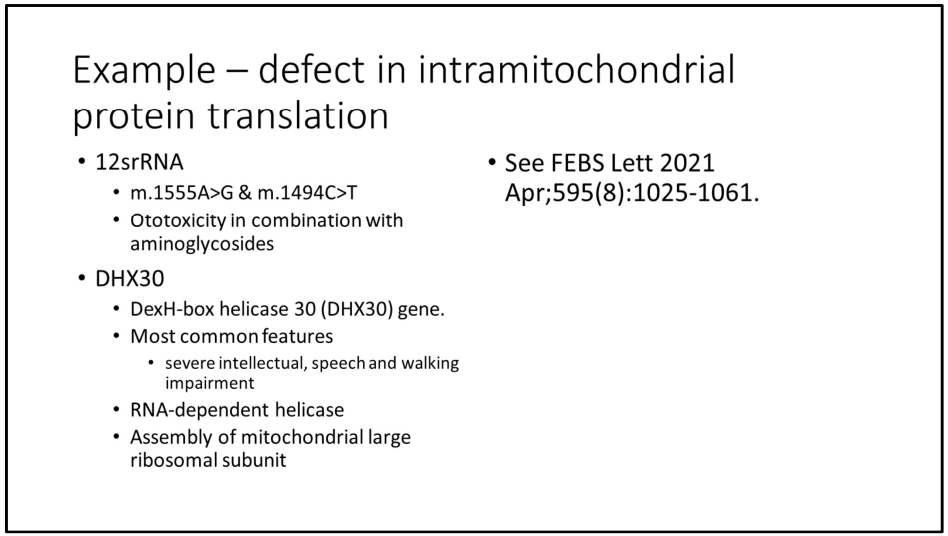
In addition to the multiple subunits that make up each of the complexes, assembly factors that place each subunit correctly or modify it (e.g. addition of copper or Fe-S centre) are essential. The example given here is of an assembly factor for complex IV (otherwise called cytochrome oxidase). SURF1 is required early in the assembly process and appears necessary for the correct incorporation of Haem a. Mutations in the gene encoding SURF1 cause Leigh disease

Protein uptake defect TIMM8A

- Mohr-Tranebjaerg syndrome
  - X-linked
    - Described in large Norwegian family (Mohr)
    - Deafness (onset 3-5)
    - Visual loss (ERG abn.)
    - Dystonia
    - Fractures
    - Mental retardation
  - Mutation in TIMM8A (DDP1)) gene
  - Small inner membrane space **chaperone**

- See FEBS Letters Volume 588, Issue 15, 1 August 2014, Pages 2484-2495

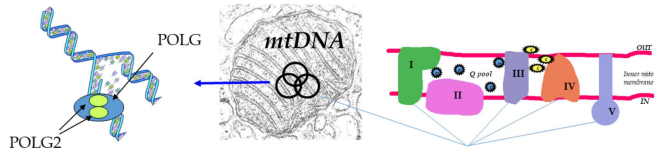
Nuclear gene products (i.e. proteins) need to be translated in the cytosol and protected (requirement for chaperones) and then taken up through first the outer and then the inner membrane. This requires multiple steps and many proteins of which TIMM8A is one. Loss of function in this protein causes Mohr-Tranebjærg syndrome

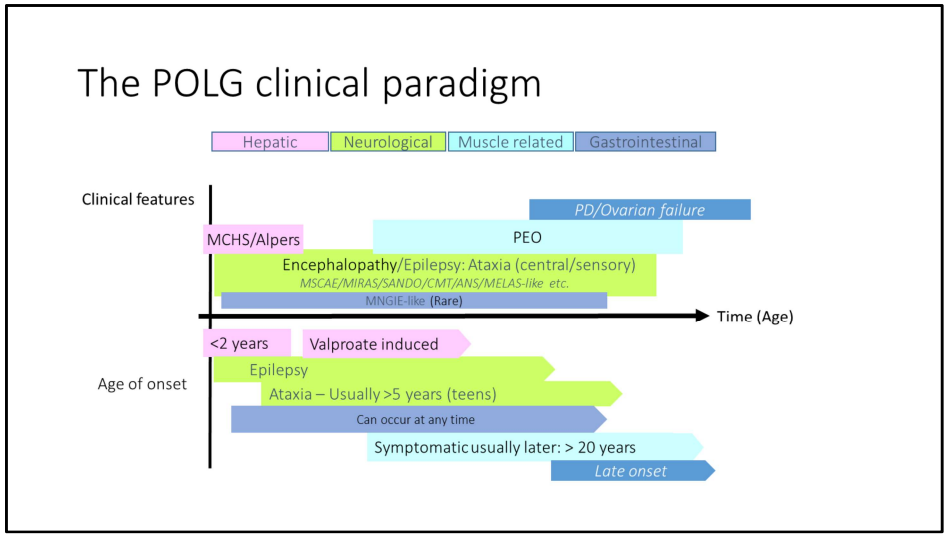


MtDNA genes encoding proteins must be translated and transcribed inside the mitochondrion. Only the tRNA and rRNA are encoded by mtDNA, the remaining proteins and cofactors must be transported to and taken up by mitochondria. The two examples given here show how the process of mitochondrial translation can be affected: mutation in the 12S rRNA gene cause hearing loss and were first discovered in those who developed deafness following the use of streptomycin an aminoglycoside. More recently, mutations in DHX30 were found to affect the assembly of the large ribosomal subunit and thus protein translation (Note: ribosomes are the structures on which protein translation takes place).

Example – mtDNA replication
Polymerase gamma: Polγ

- DNA dependant DNA polymerase gamma
  - Responsible for mtDNA replication & repair
- Heterotrimer
  - 1 catalytic (POLG) + 2 accessory (POLG2) subunits
- POLG mutations are common cause of mitochondrial disease

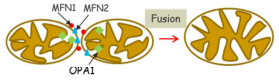


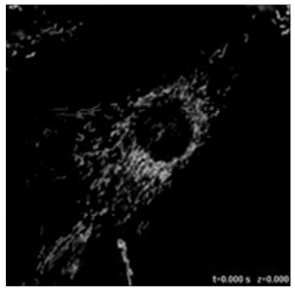


Mutations in POLG can affect all ages and give multiple phenotypes that particularly CNS but can also affect liver, peripheral nerve and bowel.
A plethora of names have been given to the different phenotypes including Alpers, myocerebrohepatic syndrome (MCHS), mitochondrial spinocerebellar ataxia and epilepsy (MSCAE), but these can be simplified by classifying by age of onset (see the section on POL gamma (dr Hikmat)

Example - disrupted mitochondrial fusion/fission

- Mitochondria are reticular
- Mitochondria constantly fuse & divide
- System needed for "quality control"
  - mitophagy





Mitochondria move! They do this for several reasons perhaps the simplest to understand is so that they get to places where energy is most needed. The other major reason for fusion and fission is to maintain mitochondrial "health". Being able to break open a reticulum (a long thin branched structure), remove a malfunctioning part and then fuse the ends together again is essential to maintain mitochondrial homeostasis. Multiple proteins are necessary since it is necessary to break and fuse both outer and inner membranes. Several og these proteins are associated with disease.

Proteins involved with fusion/fission

- Charcot Marie Tooth type 2a
  - AR (also AD)
  - Mutations in mitofusin 2 (MFN2)
  - Usu. presents early
  - Sensory/motor
  - Legs>arms
  - Other features incl.
    - Optic atrophy
    - Hearing loss
    - Tremor
- Dominant optic atrophy type 1
  - AD
  - Mutations in OPA1 gene
  - Can give
    - Pure OA
      - Visual problems <10
    - Syndromic OA (>60%)
      - Deafness
      - Ataxia
      - PEO
      - Myopathy
      - Axonal sensory neuropathy

Two examples of the diseases that result from mutations in genes that encode proteins having a role in fusion/fission