Mitochondria

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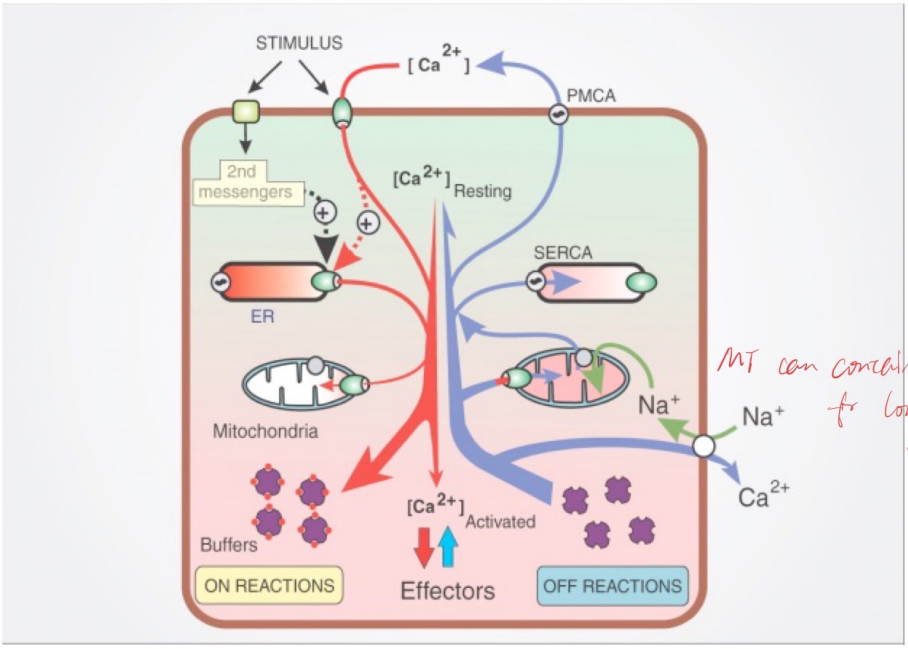
Mitochondria and Physiology

- They are the only reason we need to breathe **oxygen — the major energy providers in cells**, the process of which requires oxygen
- they are sensitive to cellular state — **house proteins that can initiate apoptotic cell death**
- **accumulate calcium** and help shape spatial and temporal calcium signal patterning



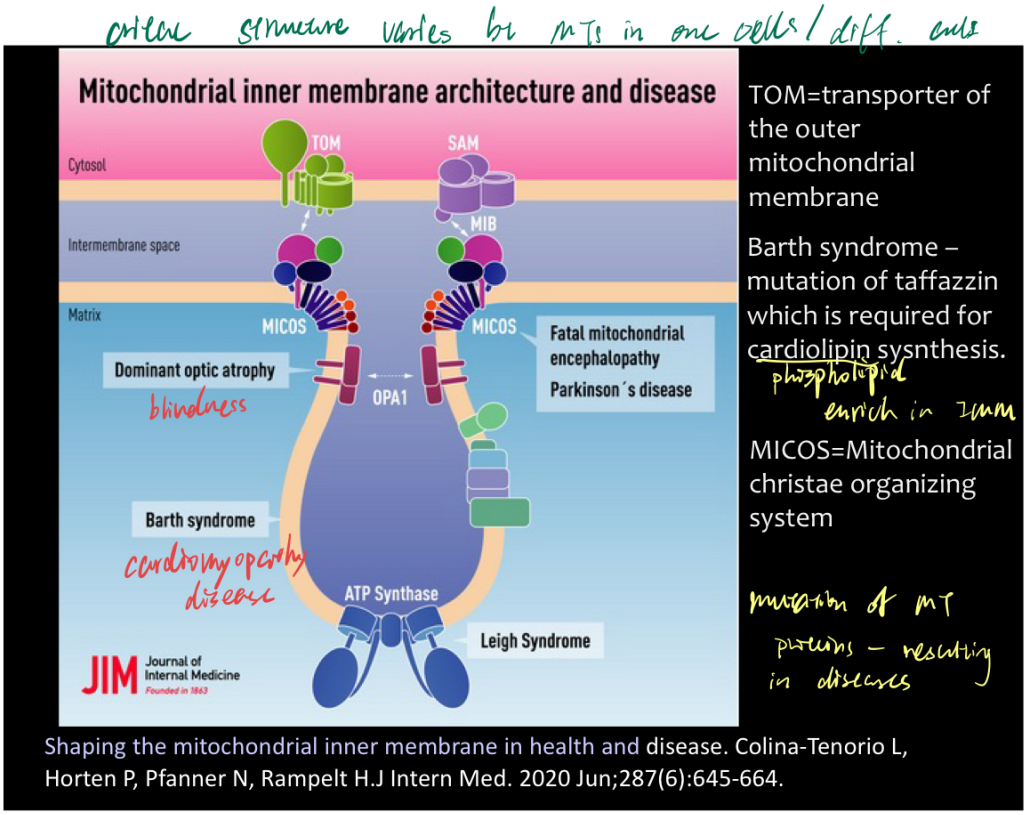
Cell Signalling Biology - Michael J. Berridge - www.cellsignallingbiology.org - 2008

- human mitochondrial DNA (mtDNA) is a double-stranded, circular molecule of 16 569 bp and contains 37 genes coding for two rRNAs, 22 tRNAs and **13**

polypeptides. The mtDNA-encoded polypeptides are all subunits of **enzyme complexes of the oxidative phosphorylation system.**

- house a number of synthetic enzymes involved in processes: manufacture of haem, biosynthesis of steroids

Cristae structure



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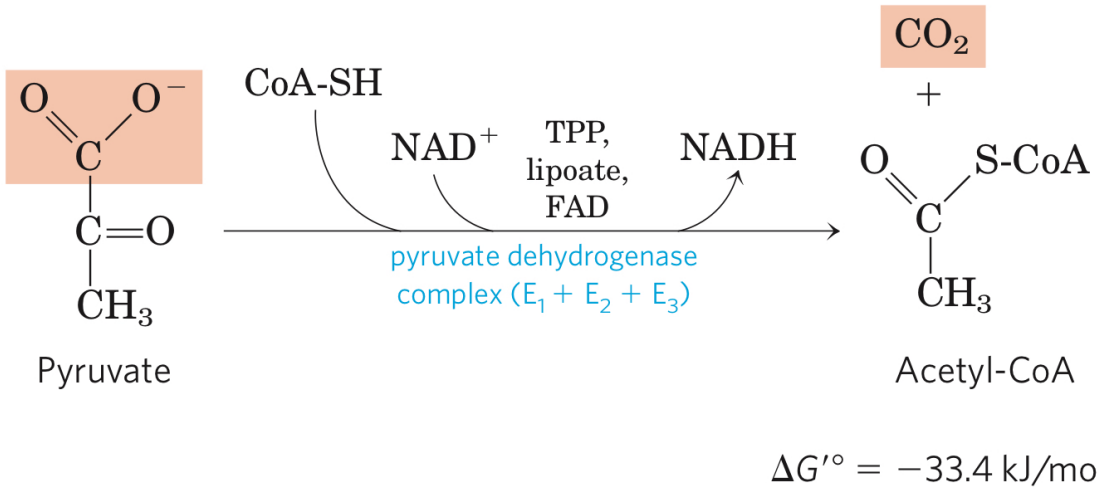
- TOM: transporter of outer membrane of mitochondria
- MICOS: mitochondrial cristae organising system
- SAM
- Barth syndrome: mutation of tafazzin — protein required for synthesis of cardiolipin (phospholipid essential for cristae structure) — lead to Cardiomyopathy — problems of heart chamber muscles

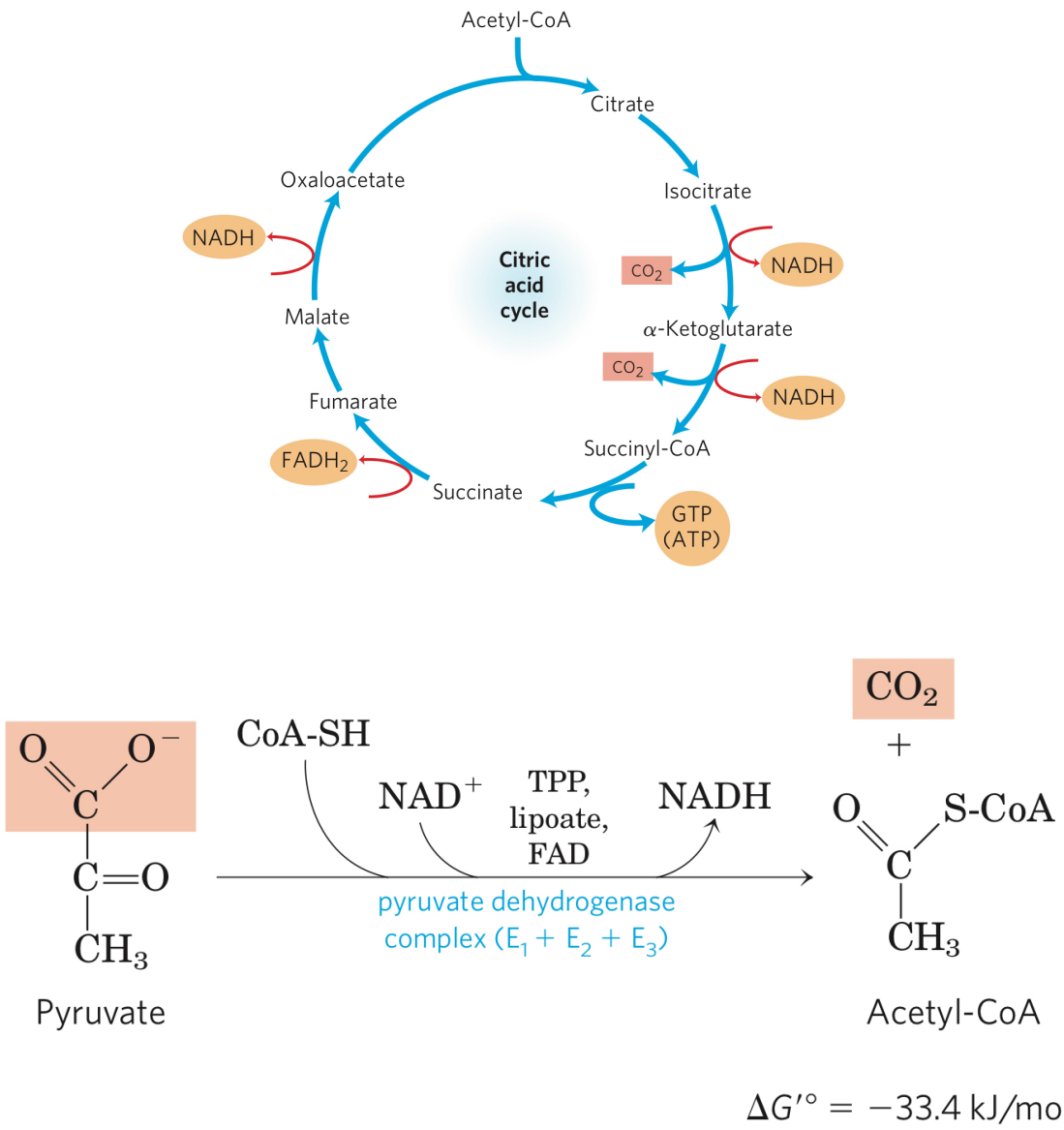
- OPA1: regulate MT fission and fusion — mutation leads to blindness

TCA + ETC



$\Delta G'^{\circ} = -33.4$ kJ/mol

FIGURE 16–2 Overall reaction catalyzed by the pyruvate dehydrogenase complex. The five coenzymes participating in this reaction, and the three enzymes that make up the enzyme complex, are discussed in the text.



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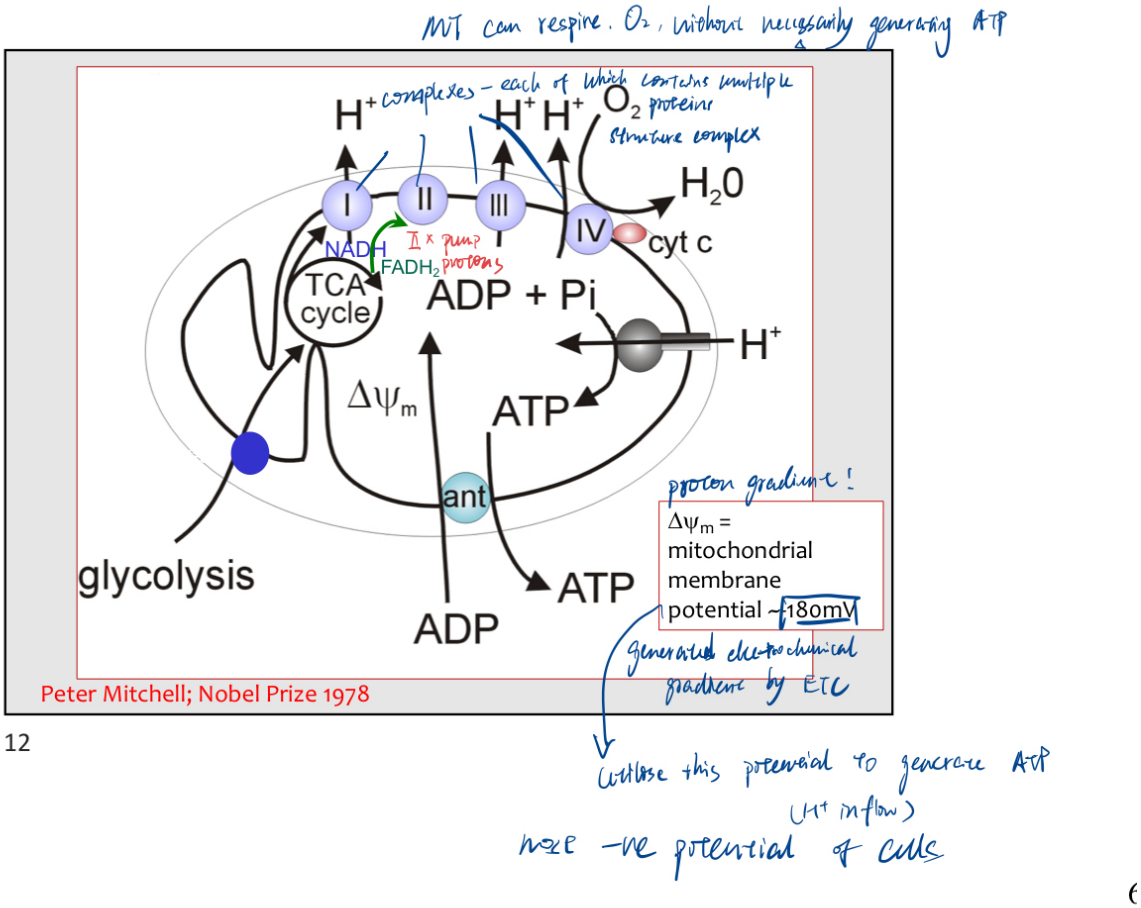
- MT take up pyruvate via an **active transporter —** link reaction (participation of pyruvate dehydrogenase) — Enter The Citric Acid Cycle as **Acetyl-CoA**

- Results: production of Reduced e- carriers (NADH & FADH2) — offer energy required for ETC
- ETC: complexes contain more than one proteins (polypeptide chains)
  - Complex I composed of 43 polypeptide chains
    - oxidise NADH & reduce coenzyme Q (ubiquinone) — pass to complex III (gathering point of electrons)
    - produce proton flux — produce electrochemical potential across the inner mitochondrial membrane
  - Complex II
    - conversion of succinate to fumarate
    - At the same time oxidise succinate-bound FADH2 to FAD and reduce coenzyme Q
    - do not directly contribute to the proton gradient used to produce ATP
  - Complex III
    - oxidse coenzymeQ — reduce cytochrome C
    - Proton pumping — gathering point of electrons
    - Complex III is a major site of reactive oxygen species **(ROS)** production within the mitochondria. The transfer of electrons through Complex III can result in the partial reduction of oxygen to form superoxide radicals (O2•−), which can subsequently give rise to other ROS. While ROS have physiological roles in cell signaling, excessive ROS production can lead to oxidative stress and damage to cellular components.
  - Complex IV
    - reduced cytochrome C is oxidised
    - Then the O2 is reduced to form H2O with H+
      - 4 cytochrome c^2+ + 4 H^+ + O2 → 4 cytochrome c^3+ + 2 H2O
    - Pump H+

- Up to this point: MT can respire without synthesising ATP (but building up electrochemical gradient)

- The proton gradient is expressed as the mitochondrial membrane potential (between the MT intermembrane space & matrix
- Then the H+ utilises this gradient to enter the matrix via ATP synthase
  - ATP synthase contains H+ channels
  - Motor that converts ADP into ATP
- ATP is then exported out of MT via anti-porter: exchanging ADP inside



How can we know about how MT work in cells? How they dysfunction when diseases?

▼ Key Technique & measurement

▼ measurement of O2 consumption

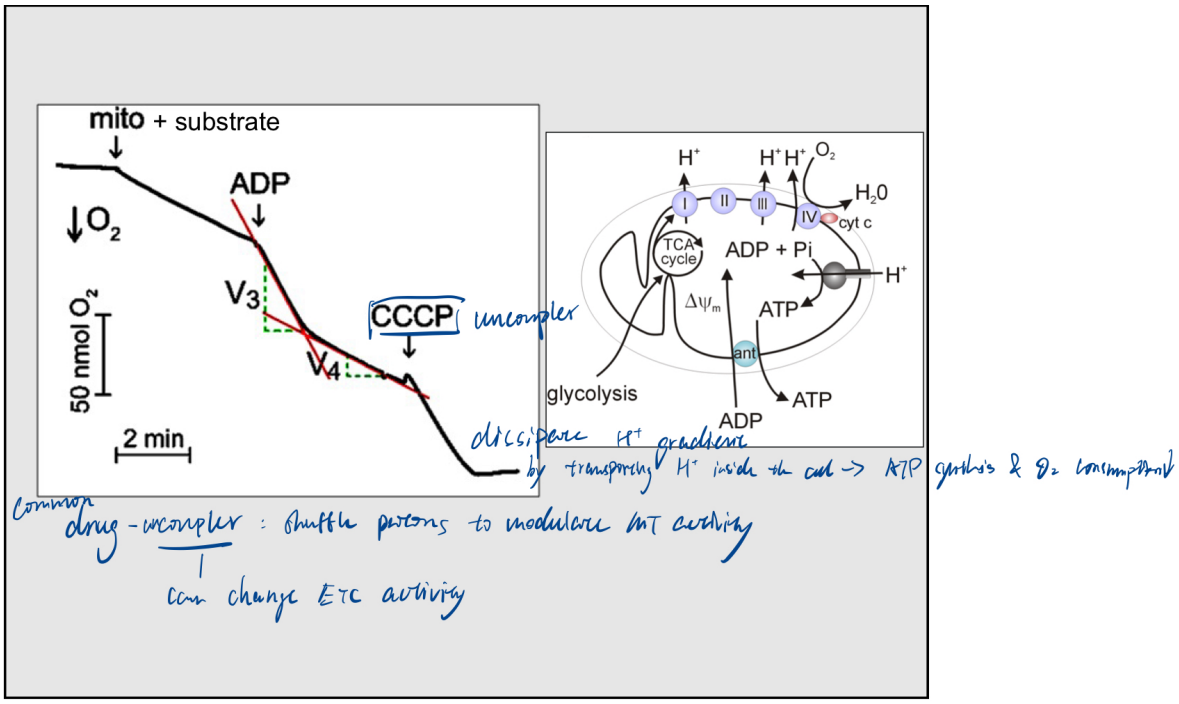
- Clark Electrode

  The **Clark electrode** is an electrode that measures **ambient oxygen partial pressure** in a liquid using a **catalytic platinum** surface according to the net reaction:

  O2 + 4 e– + 4 H+ → 2 H2O

  It improves on a bare platinum electrode by use of a membrane to reduce fouling and metal plating onto the platinum.

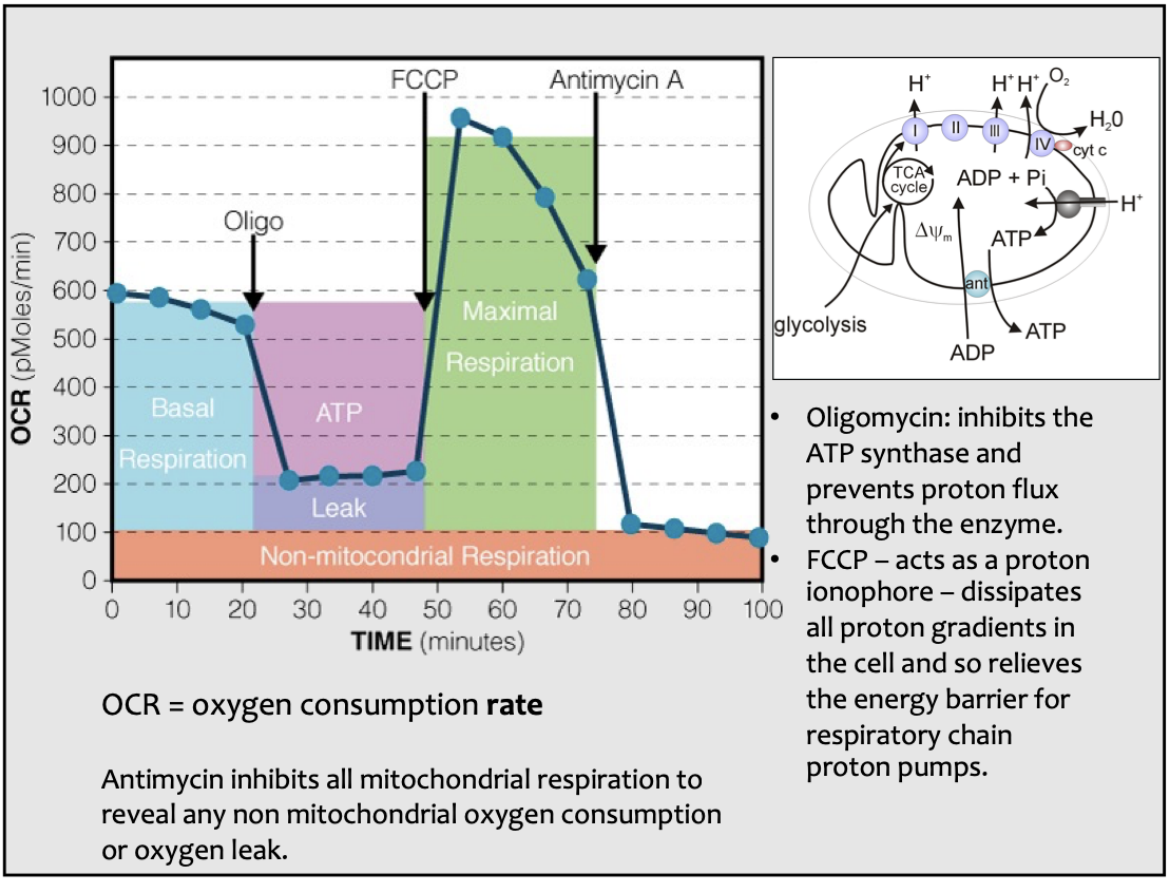
  - The current you get is proportional to the amount of O2 in cells
- For accuracy, important to generate an really airtight system that does not allow O2 to leak in
- **Seahorse Assay**
  - measure **oxygen consumption rate (OCR) and proton efflux rate (PER) or extracellular acidification rate (ECAR)** in real time, at intervals of approximately 5-8 minutes
  - can culture cells in a plate and add drugs — no need for a cuvette



- The Clark Electrode: plot a graph of O2 consumption against time:
  - The slope measures the rate of O2 consumption
  - Addition of ADP can accelerate the consumption
  - **Uncoupler**
    - CCCP: allows **protons** to freely flow back into the mitochondrial matrix through the inner mitochondrial membrane without passing through ATP synthase, thus dissipating the proton gradient and preventing ATP synthesis
    - With the uncoupler present, electron transport continues, pumping protons across the inner mitochondrial membrane, but ATP synthesis is uncoupled from proton movement. Therefore, **to maintain the proton gradient and continue electron transport, the rate of oxygen consumption increases — to maintain the MT membrane potential!!**
    - The increase in oxygen consumption occurs because the electron transport chain works harder to pump protons to maintain the proton gradient, even though ATP synthesis is not occurring. This increased oxygen consumption is not coupled to ATP production

but rather reflects the demand for more oxygen to sustain the increased activity of the electron transport chain

- **Inhibitor of ATP synthase (oligomyosin)**
  - the proton gradient will continue building up
  - still Oxygen consumption goes on for a while — then stop ETC
  - until a point when no more proton can be pumped out against the gradient



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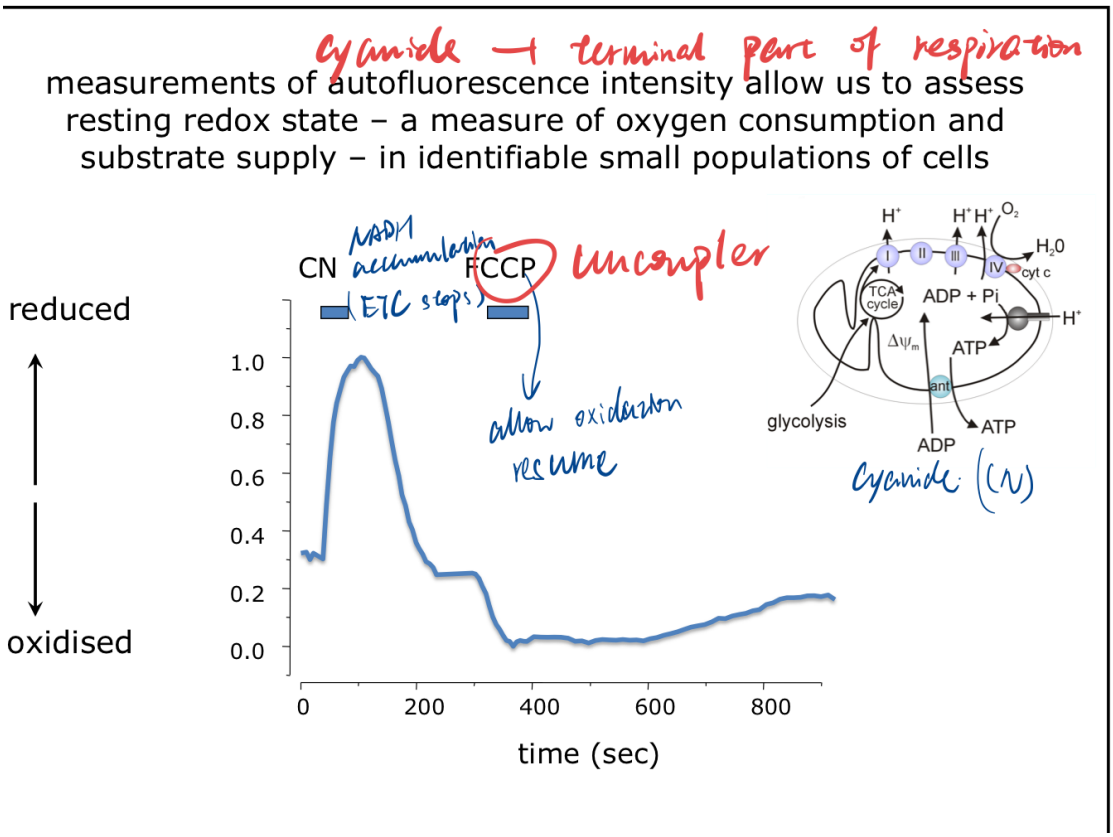
MT have distinct organisation & structures in different cell types

- In skeletal muscle: MT aligned in pairs along Z lines (interfibrillar and subsarcolemmal MT)

- Cardiac muscle: account for 40% cell volume (normally in other cell types : 20%)

Fluorescent probes for monitoring MT activity

- sharing characteristic of the probes:
  - lipophilic: can pass freely across MT membranes
  - cationic: likely to accumulate in -ve compartment (healthy MT has a strong -ve membrane potential)
- TMRM
  - accumulates in the negatively charged mitochondrial matrix in a manner proportional to the mitochondrial membrane potential
  - brighter when MT is respiring
  - When TMRM accumulates within the mitochondria, its fluorescence intensity increases due to the reduced quenching effect compared to when it's in the cytoplasm
  - The brighter the signals, the greater the membrane potential
  - After addition of uncoupler — depolarise membrane potential — signal gets dimmed — then leave the MT to the nucleus & cytoplasm
- Measuring NADH — redox state



MT genome & Heteroplasmy

- maternal inheritance
- mt mutations can primarily affect CNS
- severity seems to have correlation of how many mutant MT in the cell
- **Heteroplasmy** describes the situation in which two or more mtDNA variants exist within the same cell (e.g. wildtype and mutant). Heteroplasmies are often caused by de novo mutations occurring either in the germline or in the somatic tissues. In fact, heteroplasmy levels often vary even between the cells or somatic tissues of the same individual, leading to situations where only specific cells, tissues, or organs are affected by mitochondrial dysfunction. The accumulation of these mutations in somatic tissues over time may be a central factor in aging.

Mitochondria Quality Control — MT homeostasis

▼ Biogenesis

- PGC-1 alpha: master regulator of MT biogenesis: transcriptional **coactivator**
  - promote expression of regulatory TF
  - can be activated via phosphorylation
  - downstream of Ca2+ - AMPK pathway
- NRF (nuclear respiratory factor)
  - turn on expression of nuclear encoded ETC proteins, which will be further transported to MT
- TFAM
  - translocate to MT
  - turn on transcription translation MT genome