Bioenergetics of the Mitochondrion: Structure and Function of the ATPase
Then:  
Then:  

Now:  
Now:
Peter Mitchell (1920-1992)
CHEMIOSMOSIS

Photosynthesis
Respiration
Bacteriorhodopsin

Heat

Membrane Transport [ions, sugars, proteins]

ADP + Pi = ATP

Bacterial flagella

\[ \Delta \mu_H^+ = \Delta \psi - C \times \Delta pH \]
Metabolism in mitochondria
Charged ND residues in central membrane plane

49 kDa N-terminus
Transverse helix
ND2
ND3
ND4
ND4L
ND5
ND6
Discontinuous helices

FMN
51 kDa
75 kDa
30 kDa
24 kDa

TYKY
Ubiquinone-binding channel
PSST

L Sazanov

Subunit compositions of F-ATPases
What do we still need to know about the F-ATPase?

1. How is rotation generated during ATP hydrolysis?

2. How is rotation generated during ATP synthesis?

3. The role of cardiolipin

4. Is the permeability transition pore associated?

5. And others
1. How rotation is generated from ATP hydrolysis

- “Single molecule” rotational data mainly from bacteria
- Structural data almost entirely from mitochondria
- Reconciliation of these two data sets
Structures of inhibited F$_1$-ATPase did not explain the intermediate rotary steps
Bacterial

Mammalian

Catalytic Dwell

ATP Binding Dwell

40°

80°

Catalytic Dwell

Catalytic Dwell

Catalytic Dwell

ATP Binding Dwell

Phosphate Release Dwell

30°

65°

25°

Catalytic Dwell

Catalytic Dwell

Catalytic Dwell
Structural definition of phosphate release step

IF₁ stops the enzyme at the catalytic dwell

Thiophosphate stops F₁-ATPase at the phosphate release dwell
Active mammalian IF₁ is an antiparallel α-helical dimer
Thiophosphate inhibited bovine F$_1$-ATPase

Bason, Montgomery, Leslie & Walker 2015 PNAS 112, 6009-6014
2. How is rotation driven by the pmf?

- mechanical coupling mechanism
- the proton pathway
\[ \text{H}^+ / \text{ATP} = \text{ring symmetry/3} \]
3. Role of cardiolipin
Cardiolipin binding sites in mitochondrial proteins

- bound *permanently* to specific sites in structures of ADP/ATP translocase, and complexes III and IV

- in simulations, same sites occupied quickly and *permanently*

- binds *transiently* and *repeatedly* to specific sites in c-rings
### Residence times for cardiolipin

<table>
<thead>
<tr>
<th>c-ring</th>
<th>Inner</th>
<th>Outer</th>
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</thead>
<tbody>
<tr>
<td>c(_8)</td>
<td>520</td>
<td>230</td>
</tr>
<tr>
<td>deMe-c(_8)</td>
<td>500</td>
<td>280</td>
</tr>
<tr>
<td>c(_{10})</td>
<td>310</td>
<td>250</td>
</tr>
<tr>
<td>c(_{11})</td>
<td>620</td>
<td>350</td>
</tr>
</tbody>
</table>

In 500 ns, the active ring turns > 0.05°
Possible roles of cardiolipin in ATP synthase

• stabilises c-ring

• lubricates rotating ring

• facilitates proton dispersion from exit half-channel

• brings protons to entry half-channel
4. The mitochondrial permeability transition pore
Mitochondrial permeability transition pore

1976, Hunter et al

Allows molecules < 1.5 kDa to pass; diameter 2 nm

Consequences: mitochondrial depolarization, uncoupling of ox-phos, mitochondrial swelling

Proposed components: VDAC, ANT

Inducers: Ca$^{2+}$, adenine nucleotides, phosphate, oxidative stress

Inhibitor: Cyclosporin A
Pore opening and cyclophilin D

• peptidylprolyl *cis-trans* isomerase
• mitochondrial version of a larger family
• modulates PTP, but is not a component
• binding of cyclosporin A inhibits pore opening
Topic 1: The membrane rotors of ATP synthases in various species have a range of symmetries from 8-15. For example for humans it is 8; for Synechococcus it is 15. What are the bioenergetic consequences, and why do you think that the enzymes have evolved in this way?


In the past 20 years or so, bedaquiline is the only new drug introduced into clinical practice for treatment of tuberculosis. It inhibits the mycobacterial ATP synthase, but how? Given the urgent need for developing new drugs against multiply or even totally resistant bacterial pathogens, what features of the ATP synthase make it an attractive target for development of new drugs against bacterial pathogens, and what are the draw-backs? Remember, ideally the drug should not affect the human enzyme.

3. The permeability transition in human mitochondria refers to the opening of a non-specific membrane channel, known as the permeability transition pore (PTP), in the inner membrane. Opening is triggered by calcium ions, or reactive oxygen species, among other effectors, leading to swelling of the organelle, disruption of the inner membrane, loss of ATP synthesis and cell death. It is an uncharacterized part of cell death by necrosis and possibly apoptosis. Past proposals that the pore is provided by the voltage dependent ion channel in the outer membrane plus the adenine nucleotide translocase in the inner membrane have not withstood scrutiny. A recent proposal is that the pore is associated with the dimeric ATP synthase. How would you test this hypothesis? Remember, you will probably need to assay the functional PTP in a cellular context.

- Dimers of mitochondrial ATP synthase form the permeability transition pore. Georgio et al. (2013) PNAS 110, 5887-5892.
- An uncoupling channel within the c-subunit of the ATP synthase is the permeability transition pore. Alavian et al. (2014) PNAS 111, 10580-10585.
4. The electron transfer complexes in the inner mitochondrial membrane are organized in super-complexes. Describe the arrangement. What are the advantages of such an arrangement? Apart from ribosomes, where else in the mitochondrion are there indications of super-molecular organization? How would you improve the evidence? Think metabolons.


c-ring symmetries and H^+:ATP ratios

- 8-fold symmetry in metazoans
- 10-fold symmetry in yeast
- 10-15-fold in eubacteria
- 14-fold in chloroplasts
- Therefore, H^+/ATP = 2.7-5.0, depending on species
### P/O ratio in mammalian mitochondria

<table>
<thead>
<tr>
<th>e(^{-}) donor</th>
<th>P/O calc</th>
<th>P/O exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADH</td>
<td>10/3.7 = 2.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Succinate</td>
<td>6/3.7 = 1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Species</td>
<td>Sequence</td>
<td>Helix A Length</td>
</tr>
<tr>
<td>---------</td>
<td>----------</td>
<td>----------------</td>
</tr>
<tr>
<td>HOMSA</td>
<td>DIDTAAKFIGAGAATVGAGSGAGIGTVFGSLIIYARNPSLKQQLFSYAILGFALSEEAMGLFCLMVAFLILFAM-------</td>
<td>10</td>
</tr>
<tr>
<td>SACCE</td>
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<td>ILYTA</td>
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<tr>
<td>CLOPA</td>
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<td>11</td>
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<tr>
<td>SYNEL</td>
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<tr>
<td>CALTH</td>
<td>MGVLAAAIVGLAALGASFGVSNIVSRTIEGIAQRPESGVQLTTMFIGIGLVEAPIMAMVIAFIALGQ--------</td>
<td>13</td>
</tr>
<tr>
<td>SPIOL</td>
<td>MNPLIAASVIAAGLAVGLASIGPGVGQGTAAQGAVEGIAQRPEAEKGIRGTLLSSLAFMEALTITGYGLVVALALLFAANPFV----</td>
<td>14</td>
</tr>
<tr>
<td>SPIPL</td>
<td>MESNLTTAASVIAALAVGIGSIPGLGQGAAGQAVEGIAQRPEAEKGIRGTLLSSLAFMEALTITGYGLVVALVLFANPFV----</td>
<td>15</td>
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Mycobacterium tuberculosis

- No significant animal or environmental reservoir
- Humans: metastable equilibrium (HIV/AIDS and type II diabetes)

1.5 million people die/year from a curable disease (200,000 MDR-TB)

5-10% will develop TB

1.9 billion infected

Latent/persistence “inactive form?”

3.9 billion uninfected (for now...)

1.5 million dead

16.2 million sick

1.5 million people die/year from a curable disease (200,000 MDR-TB)
Most significant discovery in TB research – last 50 years

**A Diarylquinoline Drug Active on the ATP Synthase of *Mycobacterium tuberculosis***

Koen Andries,*, Peter Verhasselt, Jerome Guillemont, Hinrich W. H. Göhlmann, Jean-Marc Neefs, Hans Winkler, Jef Van Gestel, Philip Timmerman, Min Zhu, Ennis Lee, Peter Williams, Didier de Chaffoy, Emma Huitrí, Sven Hoffner, Emmanuelle Cambau, Chantal Truffot-Pernot, Nacer Lounis,† Vincent Jarlier

Shortens anti-tuberculosis treatment (8 weeks) and effective (bactericidal-sterilizing) in patients with drug-susceptible or drug-resistant TB

6-30 months → 2-4 months → 2 weeks
Subunit compositions of F-ATPases
Mycobacterial ATP synthase
Crystallisation

- Unit cell 103.2, 103.2, 615.9 Å
- Data collected to 4 Å
3. The permeability transition in human mitochondria refers to the opening of a non-specific membrane channel, known as the permeability transition pore (PTP), in the inner membrane. Opening is triggered by calcium ions, or reactive oxygen species, among other effectors, leading to swelling of the organelle, disruption of the inner membrane, loss of ATP synthesis and cell death. It is an uncharacterized part of cell death by necrosis and possibly apoptosis. Past proposals that the pore is provided by the voltage dependent ion channel in the outer membrane plus the adenine nucleotide translocase in the inner membrane have not withstood scrutiny. A recent proposal is that the pore is associated with the dimeric ATP synthase. How would you test this hypothesis? Remember, you will probably need to assay the functional PTP in a cellular context.

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