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

Now
Peter Mitchell (1920-1992)
CHEMIOOSMOSIS

Photosynthesis

Respiration

Heat

Bacteriorhodopsin

Membrane Transport [ions, sugars, proteins]

ADP + Pi = ATP

Bacterial flagella

$\Delta \mu_{H^+} = \Delta \psi - C \times \Delta p\text{H}$
Metabolism in mitochondria
ATP synthase from bovine mitochondria at 12 Å resolution

T Spikes, V Kumar, M G Montgomery & J E Walker, unpublished
Subunit compositions of F-ATPases

Mitochondria

Eubacteria and chloroplasts
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 cardiolpin
4. How is the enzyme assembled
5. Is the permeability transition pore associated?
I. 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
Kinosita, Yoshida, Noji & colleagues
Structures of inhibited F$_1$-ATPase did not explain the intermediate rotary steps

- **Bearing:** polyphenols
- **NBS:** azide, efrapeptin, nucleotide analogs
- **C-t domain:** aurovertin, IF$_1$
- **c-ring-a:** oligomycin
Structural definition of phosphate release step.

\[ \text{IF}_1 \text{ stops the enzyme at the catalytic dwell} \]

\[ \text{Thiophosphate stops } F_1-\text{ATPase at the phosphate release dwell} \]
Active mammalian IF₁ is an antiparallel α-helical dimer in crystals
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
W. Junge
$\text{H}^+/\text{ATP} = \text{ring symmetry}/ 3$
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
A Duncan, A Robinson, & J E Walker (2016) PNAS 113, 8587-8692
## Residence times for cardiolipin

<table>
<thead>
<tr>
<th>c-ring</th>
<th>Time (ns)</th>
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<tr>
<td></td>
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<tr>
<td>c₈</td>
<td>520</td>
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<td>deMe-c₈</td>
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<tr>
<td>c₁₁</td>
<td>620</td>
<td>350</td>
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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. Assembly of human ATP synthase
Δb
$\Delta b$

$\rho^0$

$\Delta c$
Peripheral stalk destabilised
Peripheral stalk destabilised

Δe (or g)

Δf
Δ6.8kD

partial loss of ATP6, ATP8, DAPIT
Δ6.8kD

ΔDAPIT

partial loss of ATP6, ATP8, DAPIT
Glucose

Partial loss of ATP6, ATP8, DAPIT

Galactose
4. The mitochondrial permeability transition pore
Mitochondrial permeability transition pore

1976, Hunter et al

Allows molecules $< 1.5$ kDa to pass; diameter $2 \text{ nm}$

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?


2. 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.** Georgia 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

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<tr>
<th>e(^-) donor</th>
<th>P/O calc</th>
<th>P/O exp</th>
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<tr>
<td>NADH</td>
<td>10/3.7 = 2.7</td>
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<tr>
<td>Succinate</td>
<td>6/3.7 = 1.6</td>
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<tr>
<td>Species</td>
<td>Helix A</td>
<td>Helix B</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

3.9 billion uninfected (for now...)

Latent/persistence "inactive form?"

1.5 million dead

16.2 million sick
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 Huintric, 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

Eubacteria and chloroplasts

Mitochondria
Mycobacterial ATP synthase
Crystallisation

- Unit cell 103.2, 103.2, 615.9 Å
- Data collected to 4 Å
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Human HAPI cells

- near haploid human cell line used with CRISPR-cas9
- derived from myeloid leukaemia
- fragment of chr15 in chr19, and reciprocal translocation chr 9 and 22
- ATP5G1-G3 on chrs 17, 12 and 2
Expression of subunit c in human HAPII cells
Identification of a HAPI clone devoid of subunit c
Mitochondrial F-ATPase and the PTP: conclusions

- subunit c is not involved in forming the PTP
- nor are subunits ATP6 and ATP8
The PTP: association with other ATPase subunits

- Have made HAPI deletion strains for subunits b, oscp, e, f, g, DAPIT and 6.8PL
- PTP assays to follow
- Characterisation of vestigial complexes tells us about assembly
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.


