Cryo-ET for investigating the role of respiratory chain organisation in health and disease

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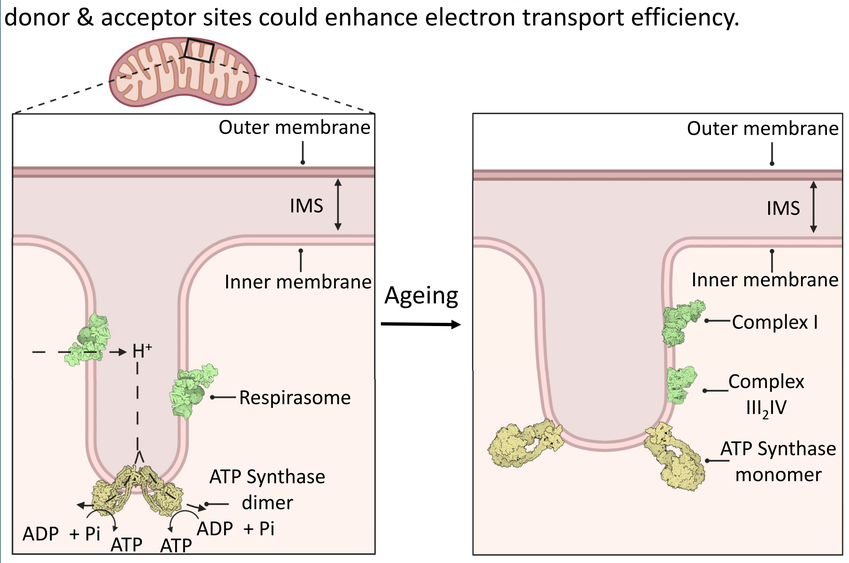
1. Background

The mitochondrial respiratory chain is the primary source of cellular energy - therefore defects in this system cause a range of severe pathologies. Electron transport through complexes I-IV facilitates pumping of protons into the inter-membrane space (IMS), which then flow back into the matrix through the ATP synthase to produce energy in the form of ATP. The complexes of this chain can be organised into **supercomplexes:**

(1) ATP synthase dimers: Dimer row formation induces crista membrane curvature, maintaining lamellar shaped cristae. Intriguingly, dimer architecture varies across species.

(2) Respirasomes: Comprising complexes I, III_2 and IV, it has been suggested that close proximity of electron donor & acceptor sites could enhance electron transport efficiency.



Ageing and disease…

Reduced stability of both ATP synthase dimers and respirasomes is associated with ageing and the latter with age-related diseases such as **Parkinson's**.

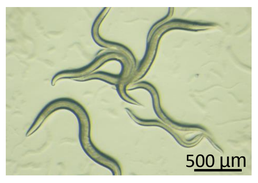
Increased respirasome stability has been observed in **cancer** cells.

Aims: Using *C. elegans* as a model system…

1. Investigate the relationship between ATP synthase dimer architecture & mitochondrial morphology
2. Determine the effect of reducing respirasome stability on mitochondrial morphology & respiratory chain organisation

2. Methods

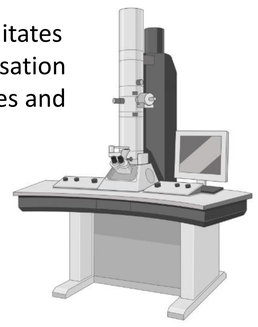
1 *C. elegans* as a model system



- High homology with human genome
- Widely used for ageing & mitochondrial studies
- Easily cultured, short life-cycle, numerous progeny
- Genetically tractable: genome fully sequenced
- Gap in the literature: ATP synthase organisation in invertebrates

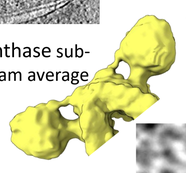
3 Cryo-ET to study the ETC *in situ*

Cryo-electron tomography facilitates study of the structure & organisation of complexes within membranes and whole organelles.

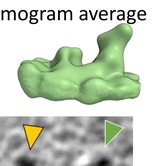


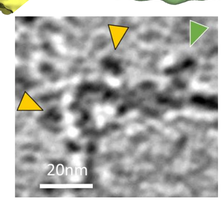
Tomogram of whole mitochondrion

ATP Synthase sub-tomogram average



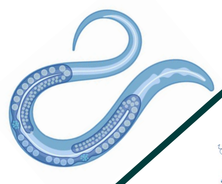
Respirasome sub-tomogram average

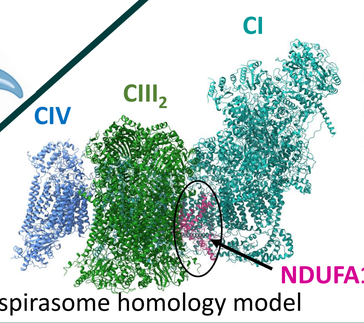




Crista membrane close-up

2 RNAi for reducing respirasome stability



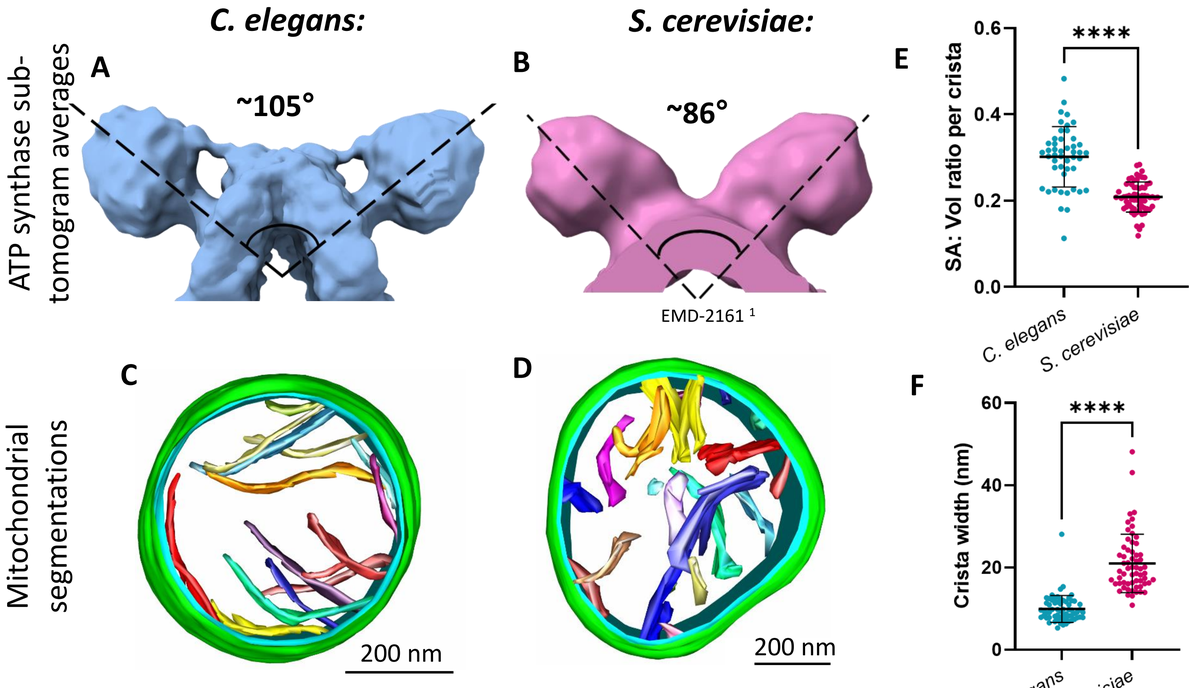


C. elegans respirasome homology model

Knocked down homologue of human accessory subunit of Complex I (NDUFA11) required for interaction with CIII

Induced knockdown through feeding the nematodes with RNAi engineered *E.coli*

3. Role of ATP synthase dimer organisation



- **Sub-tomogram averaging** revealed a novel angle (105°) between dimer heads in the *C. elegans* ATP synthase dimer **(A)** [2], unlike previously studied mammals and fungi which proffer an angle of 86° **(B)** [1].
- **Tomographic segmentation** of mitochondria isolated from *C. elegans* **(C)** and *S. cerevisiae* **(D)** revealed that a wider dimer angle in *C. elegans* was associated with increased crista surface area to volume ratio **(E)** & crista width **(F)**, indicative of flatter cristae [2].
- Flatter cristae may proffer *C. elegans* an energetic advantage in its soil-based habitat, where conditions range from near hypoxia to atmospheric.

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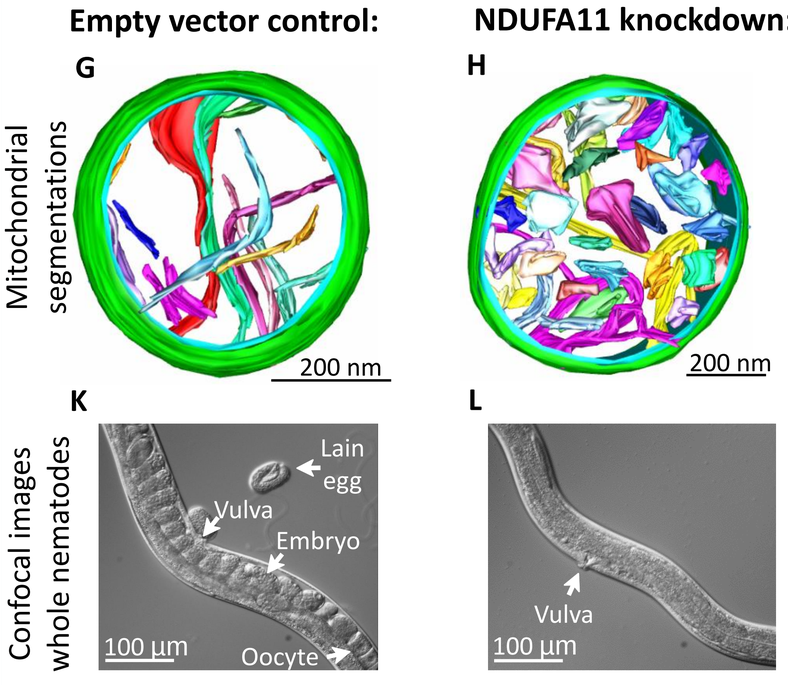
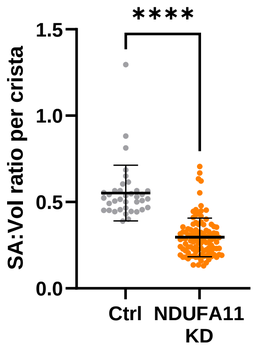
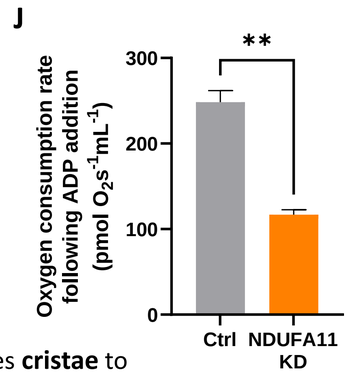
4. Role of respirasome stability







- **Reduced respirasome stability** induced by NDUFA11 knockdown causes **cristae** to **deform** from lamellar to sac-like **(G-I)**, losing curved ridges where ATPase dimer rows are localised [3].
- Some **cristae detach from junctions (G-H)**, which will reduce import of nuclear-encoded ETC subunits [3].
- This disruption in mitochondrial morphology is reflected by **impaired mitochondrial respiration (J)** and **severely inhibited reproduction** in the NDUFA11 knockdown nematodes **(K-L)** [3].

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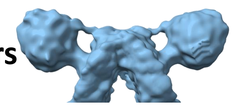
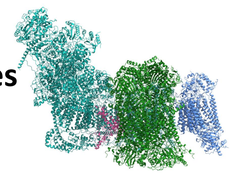
5. Conclusions & Future Work

ATP synthase dimers



- **Novel dimer interface** identified in *C. elegans*
- **Identified model system** for investigating the influence of dimer angle
- **Identified relationship** between **ATP synthase dimer angle** & **crista morphology**
- Energetic advantage likely proffered by flatter cristae: speculate that a **range of dimer angles evolved to suit bespoke energetic needs**
- Future studies investigating dimer angle & crista morphology in species inhabiting a range of environments will be key

Respirasomes



- **Reduced respirasome** stability induced by NDUFA11 knockdown severely **disrupts mitochondrial morphology & physiology**
- **Future sub-tomogram averaging** of CI and ATP synthase dimers will **confirm effect of NDUFA11 KD** on:
  - **Respiratory chain stoichiometry**
  - **ATP synthase dimer angle** (since curved ridges of crista membranes are lost in the KD)

6. Impact on Future Drug Development

- Frequency of age-related diseases is rising in an **ageing population**
- Despite an **established relationship between respiratory supercomplex association and age-related diseases**, it has been **impossible to exploit** this for drug development due to **lack of knowledge regarding the biological role** of respiratory supercomplexes.
- We have developed a basic understanding of the role that respiratory chain organisation plays in health & disease in the **model system *C. elegans***
- **Highly relevant to the human situation** due to high sequence identities in ETC subunits
- Potential for **pharmaceutical companies** to capitalise on these findings in the following ways in future…

1 Develop drugs to increase respirasome stability for treatment of age-related diseases e.g. Parkinson's

2 Develop drugs that reduce respirasome stability for chemotherapies: Potential to use a drug targeting NDUFA11

7. References

1. Davies *et al*., 2012. Structure of the yeast F_1F_o-ATP synthase dimer and its role in shaping the mitochondrial cristae. *Proc. Natl. Acad. Sci. USA.* **109**, 13602–13607.
2. Buzzard *et al*., 2023. Cryo-electron tomography of *C. elegans* mitochondria reveals how the ATP synthase dimer interface shapes crista membranes. *BioRxiv.* doi: 10.1101/2023.02.02.526626.
3. Knapp-Wilson *et al*., 2021. Maintenance of complex I and its supercomplexes by NDUF-11 is essential for mitochondrial structure, function and health. *J. Cell Sci.* **134**, jcs258399.