Mitochondrial structure-function relationships

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Mitochondrial structure is diverse
Mitochondrial structure is dynamic

DYNAMICS: Motility – Structure – Position – Matrix
Concept of research

Regulation

Filamentous

Fusion

Fission

Fragmented

Consequences
Mitochondrial dynamics is regulated by multifunctional fusion and fission proteins

- Apoptosis (Drp1, OPA1, hFis1, Mfn2)
- ER morphology (Mfn2)
- ER mitochondria communication (Mfn2)
- Peroxisome fission (hFis1, Drp1)
- OXPHOS expression (Mfn2)

• Cell survival
• Ca^{2+} and ATP handling
• Metabolism
Mitochondrial and cellular metabolism is sustained by the respiratory chain
Complex I: structure and function

Willems et al., Cell Calcium, 2008
http://www.scripps.edu/mem/biochem/CI/
Complex I assembly requires multiple assembly chaperones

- CIA30/NDUFAF1
- Mimitin/B17.2L/NDUF12L
- NDUFAF12L
- C6orf66
- C8orf38
- C20orf7

Dieteren et al., JBC, 2008
http://www.scripps.edu/mem/biochem/CI/
Complex I expression and activity: reduced in patient fibroblasts

**SO:** Mutations reduce The expression/activity of fully-assembled complex I

Ugalde et al., Hum. Mol. Gen. 2004
Verkaart et al., BBA, 2007
Human complex I deficiency: \( \Delta \psi \) is depolarized

Distelmaier et al. (Submitted)
Human complex I deficiency: NAD(P)H levels are elevated

Verkaart et al., BBA, 2007
Measurement of cellular ROS using CM-H₂DCFDA

CM-H₂DCFDA $\xrightarrow{\text{hydrolysis}}$ CM-H₂DCF

ROS $\xrightarrow{\text{oxidation}}$ CM-DCF

Koopman et al., 2006
Koopman et al., 2007, AJP Cell Phys., 2007
Human complex I deficiency: Reactive oxygen species levels are elevated

Verkaart et al., BBA, 2007
Koopman et al., AJP, 2007
Mitochondria-ER Ca\textsuperscript{2+} handling

Tethers: (A) \textit{InsP}_3R \rightarrow \textit{mtHSP70} \rightarrow \textit{VDAC/Porin}
(B) \textit{Mfn2}_{ER} \rightarrow \textit{Mfn2}_{mito}

Valsecchi et al. (Submitted)
Cytosolic calcium handling in human skin fibroblasts: Fura-2

- Peak calcium level
- Basal calcium level
- Calcium removal rate
- 1μM bradykinin

Graph showing Fura-2 ratio (340/380 nm) over time (s)
Human complex I deficiency: Ca\textsuperscript{2+} and ATP handling are disturbed

Visch et al., JBC, 2004; Visch et al., BBA, 2005
Visch et al., AJP, 2006; Willems et al., Cell Calcium, 2008
Valsecchi et al. (Submitted)
Human complex I deficiency: Key cellular consequences

(I) Less fully-assembled and active complex I protein

(II) Depolarized mitochondrial membrane potential

(III) Increased NAD(P)H and ROS levels

(IV) Altered cellular and mitochondrial ATP/Ca\(^{2+}\) handling

Q1: How does this relate to mitochondrial morphology?
Q2: Can complex I deficiency be mitigated?
Visualizing mitochondrial structure with fluorescent proteins and cations

Koopman et al., Methods., 2008

\[ \Delta \psi (\text{mV}) = \frac{2.303 \cdot RT}{z F} \log \left( \frac{[\text{Cation}]_{\text{in}}}{[\text{Cation}]_{\text{out}}} \right) \]
Complex I deficient patient cell lines: Different mitochondrial morphologies

Koopman et al., AJP, 2005; Koopman, Cytometry, 2006; Koopman et al., Methods, 2008; Willems et al. (Submitted)
Mitochondrial morphology correlates with residual complex I activity

Koopman et al., 2007, AJP Cell Phys. (in press)
A ‘good’ mitochondrial structure relates to better mitochondrial function and less ROS.
The number of mitochondria per cell relates to mitochondrial Ca\(^{2+}/\text{ATP}\) handling.

Willems et al. (Submitted)
Cell biological consequences of human complex I deficiency

Valsecchi et al. (Submitted)
Mitochondria become more branched in Parkinson’s disease and HIBM

Fibroblast from patients with Parkinson’s disease

Myoblasts from patients with Hereditary Inclusion Body Myopathy

Eisenberg et al., Hum. Mol. Genet., 2008