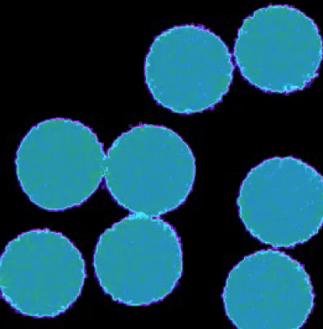
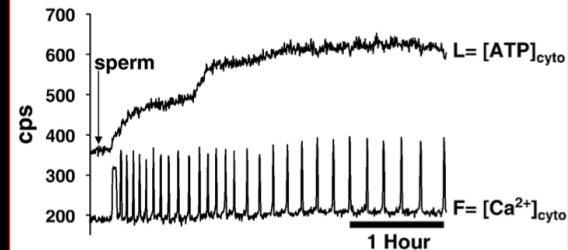


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In mouse oocytes injected with firefly luciferase the transfer of calcium to the mitochondria at fertilisation drives increased ATP generation

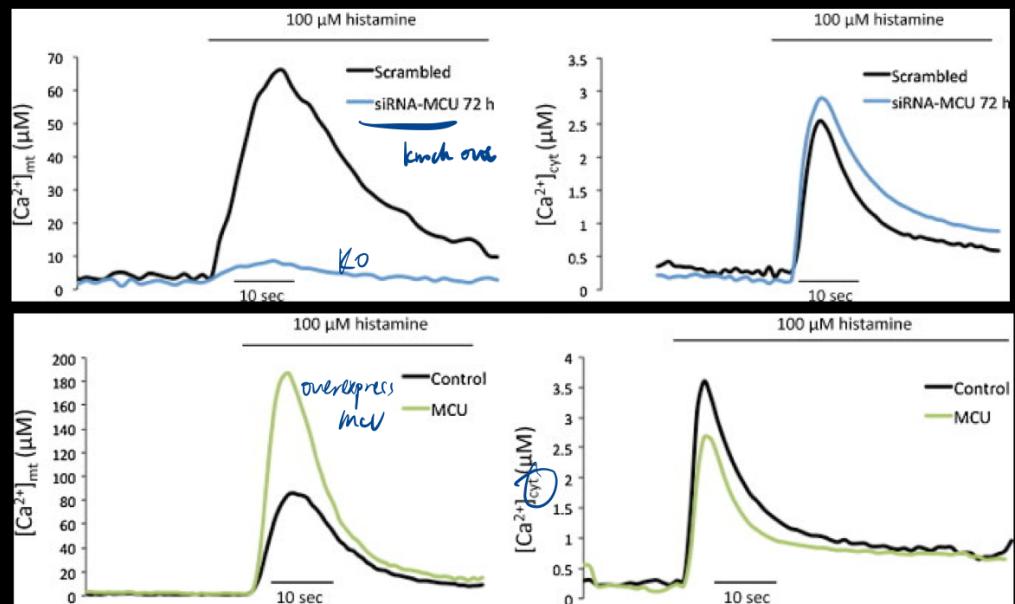


A) $[Ca^{2+}]_{cyto}$ and $[ATP]_{cyto}$



when fertilised, Ca^{2+} spikes,
2ATP↑

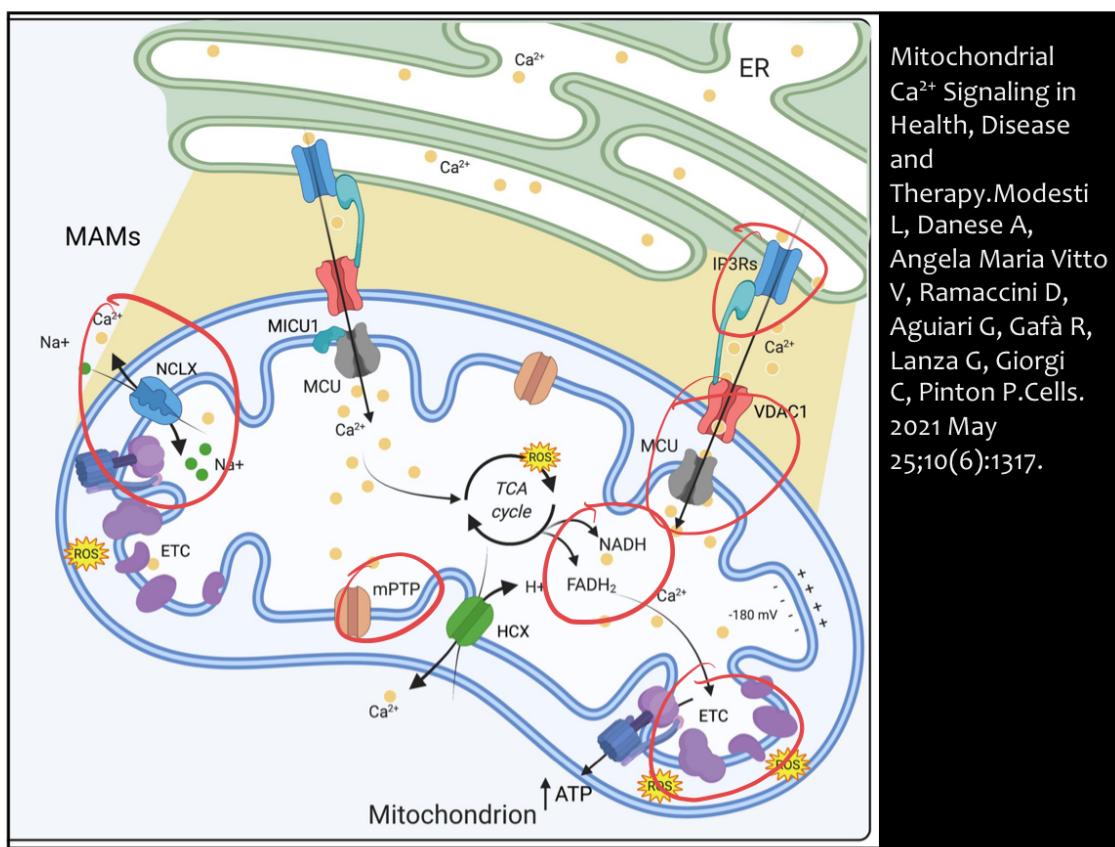
Molecular manipulation of MCU expression alters mitochondrial calcium uptake



D. De Stefani, A. Raffaello, E. Teardo, I. Szabo, R. Rizzuto A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter Nature, 476 (2011), pp. 336-340

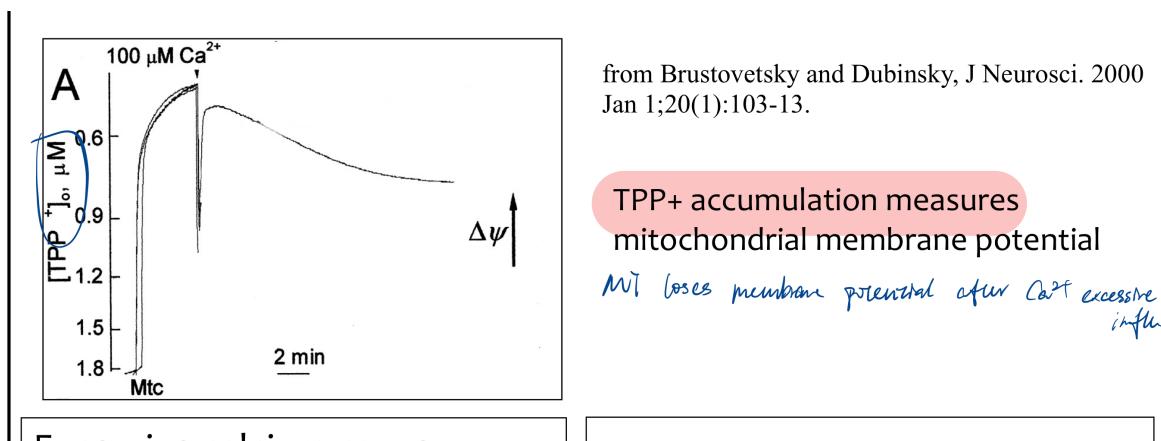
- MICU1 regulates the Ca²⁺ threshold in MT; which is higher than cytosol
- MT-ER contact site & Ca²⁺ signalling
 - ER: **IP3R** releases Ca²⁺;
 - MT: **VDAC1** — outer membrane Ca²⁺ channel: receive Ca²⁺ to MICU1 then MCU

MT & ER are usually very close to each other



Calcium overload and MT death

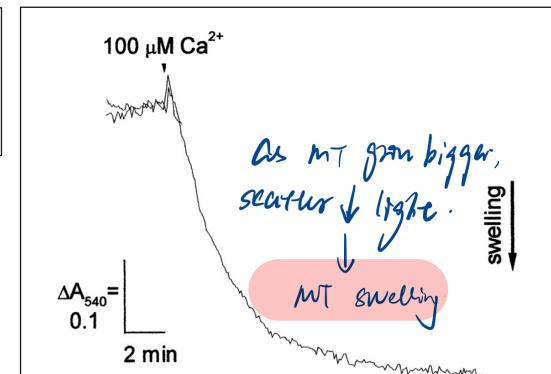
- Ca^{2+} accumulation can trigger opening to Permeability Transition Pore (mPTP) — its opening can lead to cell death
- Addition of excessive Ca^{2+} :
 - depolarise the MT membrane potential



- further leads to MT swelling
 - the larger the cell; the poorer scattering ability

Excessive calcium causes mitochondrial damage – loss of potential and swelling

in flow of H^+
 H_2O



- mPTP

- Modulate by:
 - **Cyclophilin D (CypD)** — mitochondrial matrix protein
 - CypD and Ca²⁺ bind to the OSCP and β subunits, respectively, inducing **conformational changes in ATP synthase** peripheral stalk subunits then modifying interactions of membrane embedded FO subunits
- Open by:
 - high Ca²⁺;
 - pro-oxidants (superoxides)
 - High ADP & Pi (**depletion** of ATP)
- Inhibited by:
 - high ATP
 - **cyclosporin A (CsA)**
 - **SfA - sanglifehrin**

Mitochondrial injury is caused by opening of a large transmembrane pore, the mitochondrial permeability transition pore (mPTP).

Opened by: High $[Ca^{2+}]_m$; ^{superoxide} Pro-oxidants; High Pi. <sup>depletion of ATP
↓ so high ADP
↑ Pi</sup>

Inhibited by: ATP; CsA (cyclosporin A); SfA (sanglifehrin)

Modulated by oxidation of SH- groups

Very large conductance – allows loss of intramitochondrial enzymes and Ca^{2+}

Formed from changed conformation of ATP

synthase/cyclophilin D?

regulates opening of the pore

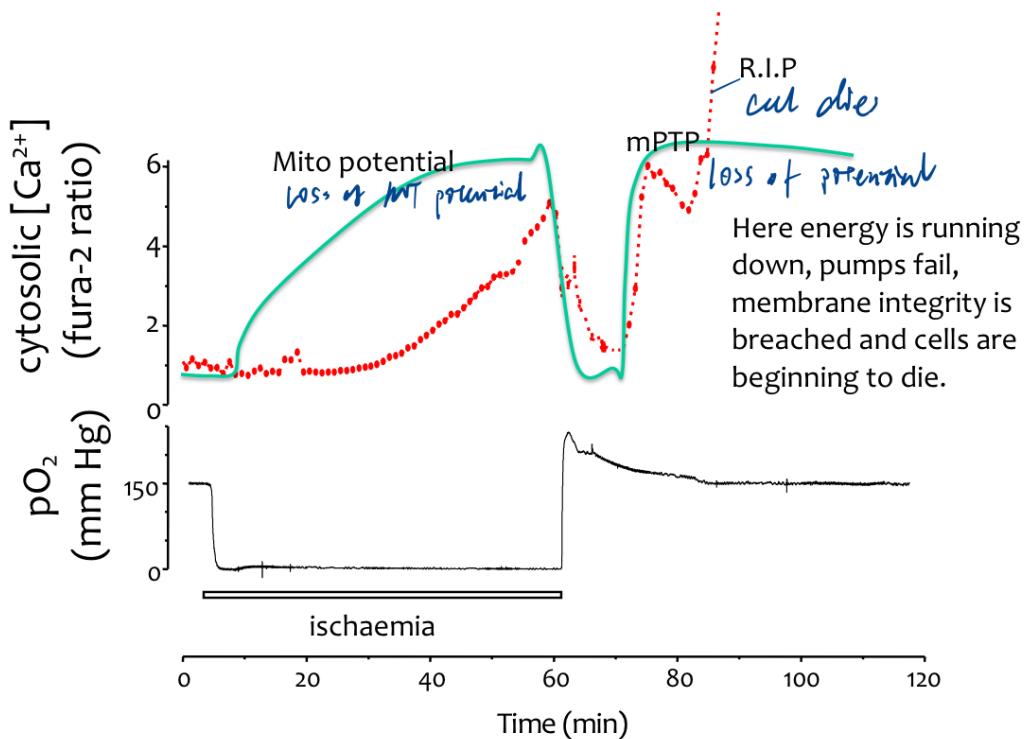
impt targets

▼ Study model: Cardiac Reperfusion Injury

- pathological change:
 - progressive fall in ATP — consumption outweighs production
 - rise in Ca^{2+} — why?
 - rise in Pi
 - collapsed MT membrane potential — no ETC no proton pump — **cannot uptake Ca^{2+}**
- Death happens during reperfusion
 - Now oxygen available: membrane potential reformed — H^+ pump — able to intake Ca^{2+} (MICU opening)
 - because there is high Pi (ADP) and low ATP in matrix — high rate of Oxidative Phosphorylation
 - — requires more Ca^{2+} to upregulate efficiency for TCA cycle and ETC

- Result: excessive Ca^{2+} influx and accumulation
- So, this induces mPTP opening

mitochondrial calcium ‘overload’ at reperfusion is a cause of cell death

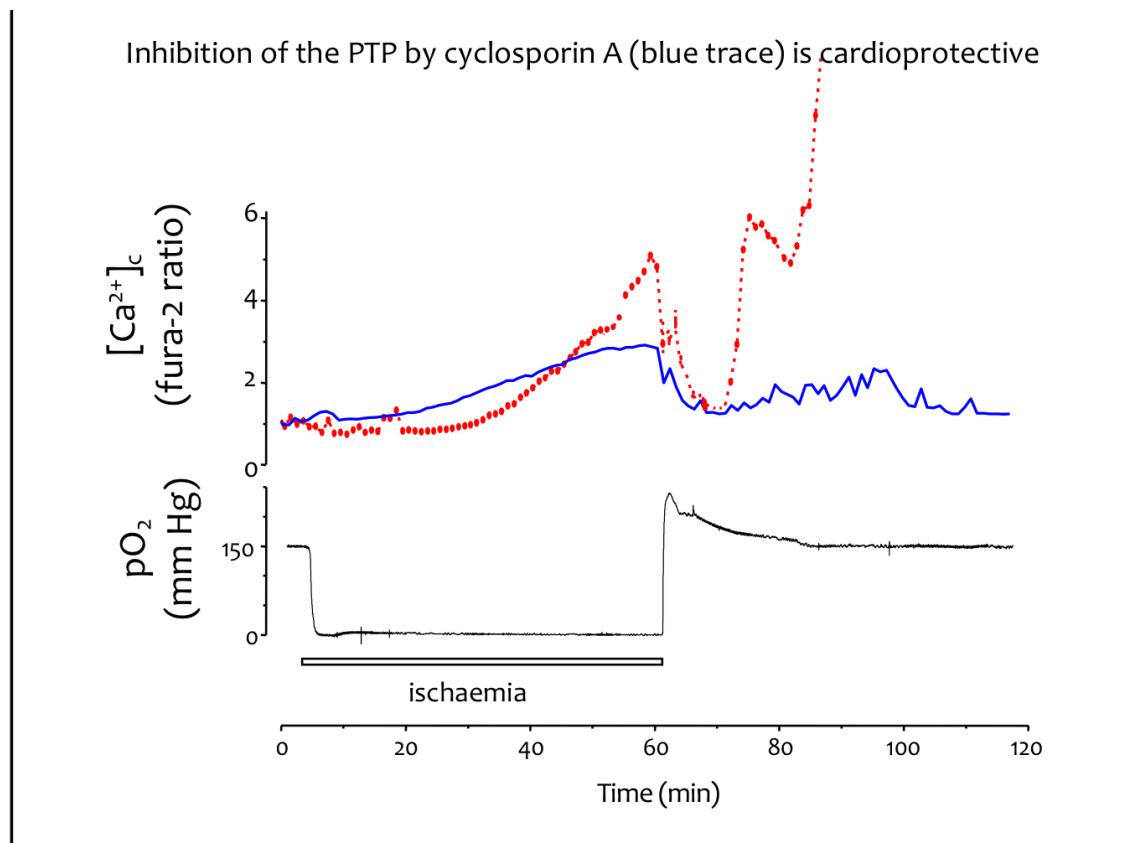


Rakhit et al., Circulation. 2001 May 29;103(21):2617-23.

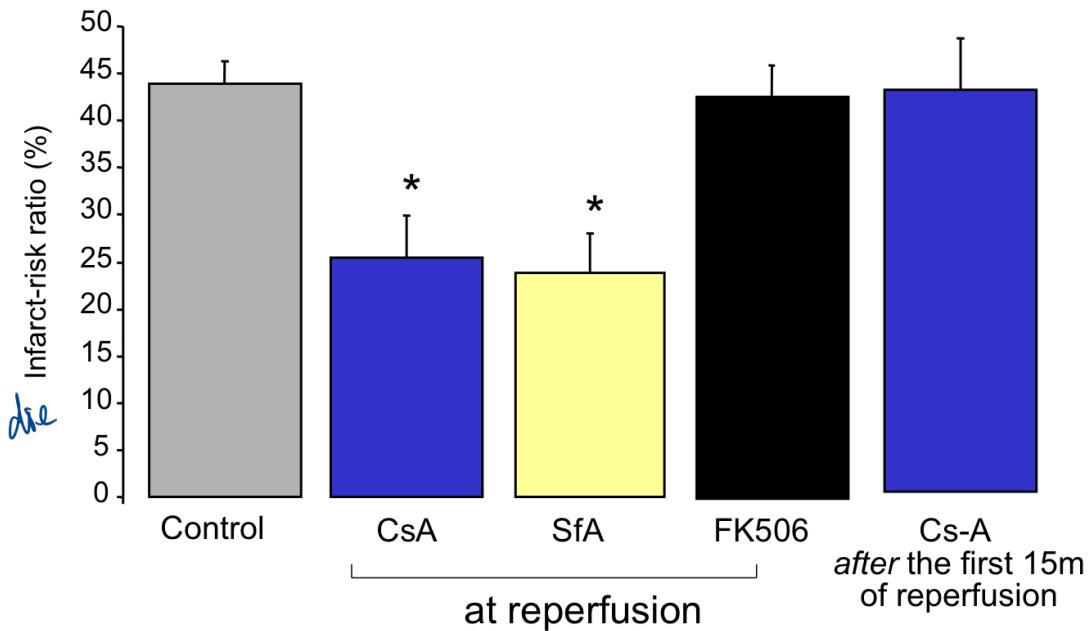
1. at ischaemia: MT loses membrane potential — fail to uptake Ca^{2+} → accumulation of cytosolic Ca^{2+}
2. at reperfusion: membrane potential restored → excessively uptake Ca^{2+} from cytosol → drop in cytosolic $[\text{Ca}^{2+}]$
3. accumulation of mt $[\text{Ca}^{2+}]$ leads to
 - a. opening of mPTP
 - b. damage the MT membrane potential
 - c. MT swelling

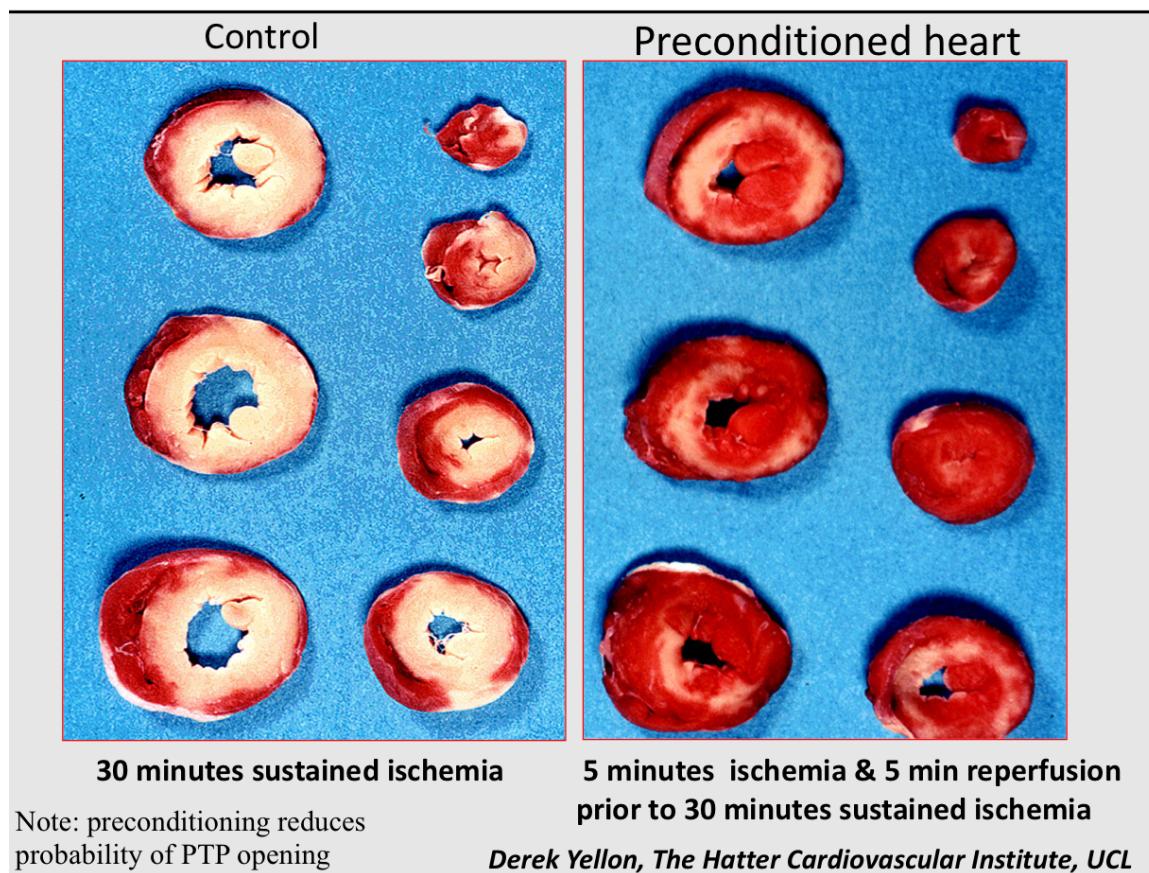
4. burst of MT — **drastic increase of cytosolic Ca²⁺ level & opening of mPTP** → cell death

- Inhibiting mPTP (using **cyclosporin A**) opening at reperfusion can suppress cell death



Inhibiting mPTP opening at reperfusion reduces infarct size following coronary artery occlusion in the intact heart

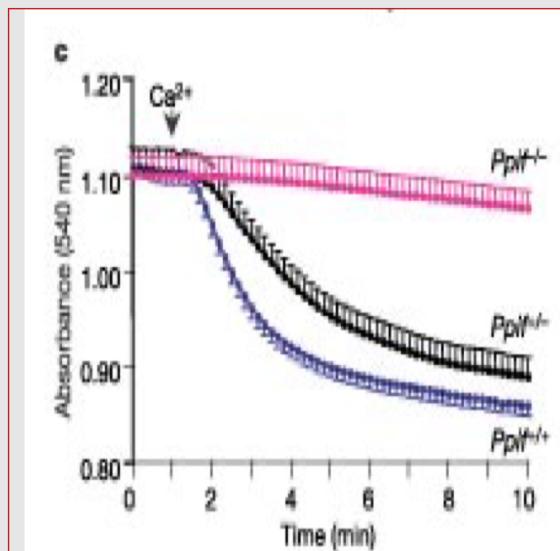




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- Inhibiting Cyclophilin D can also downregulate mPTP activity thus cell survival

Cyclophilin D modulates pore opening: following knockout of CypD the pore does not open



Mitochondrial swelling assay- absorbance of transmitted light changes as mitochondria swell after PTP opening (control, blue trace). The CypD (gene is called ppif) knockout is protected from pore opening.

Baines et al., Nature 2005 Mar 31;434(7033):658-62