
ROS and MITOCHONDRIA
Jakob Troppmair

About me

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- **MOLECULAR TRANSPLANTATION BIOLOGY**
- **MOLECULAR ONCOLOGY**

The paper

LETTER

doi:10.1038/nature13909

Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS

Edward T. Chouchani[1,2]*, Victoria R. Pell[2]*, Edoardo Gaude[3], Dunja Aksentijević[4], Stephanie Y. Sundier[5], Ellen L. Robb[1], Angela Logan[1], Sergiy M. Nadtochiy[6], Emily N. J. Ord[7], Anthony C. Smith[1], Filmon Eyassu[1], Rachel Shirley[7], Chou-Hui Hu[2], Anna J. Dare[1], Andrew M. James[1], Sebastian Rogatti[1], Richard C. Hartley[8], Simon Eaton[9], Ana S. H. Costa[3], Paul S. Brookes[6], Sean M. Davidson[10], Michael R. Duchen[5], Kourosh Saeb-Parsy[11], Michael J. Shattock[4], Alan J. Robinson[1], Lorraine M. Work[7], Christian Frezza[3], Thomas Krieg[2] & Michael P. Murphy[1]

https://www.nature.com/articles/nature13909#s1

ET Chouchani *et al. Nature* **000**, 1-5 (2014) doi:10.1038/nature13909

nature

The background

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Ischemia-Reperfusion-Injury (IRI)

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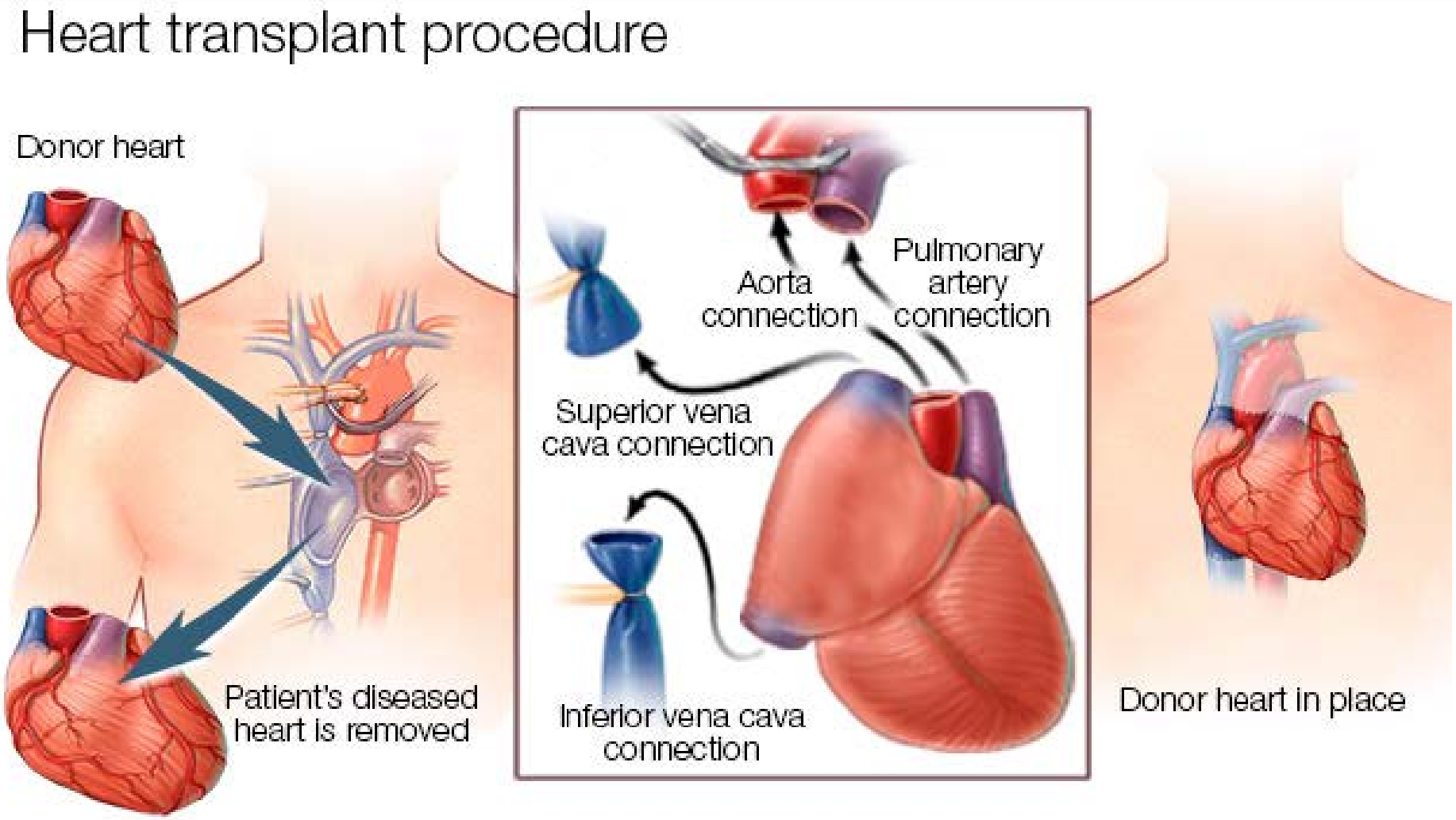
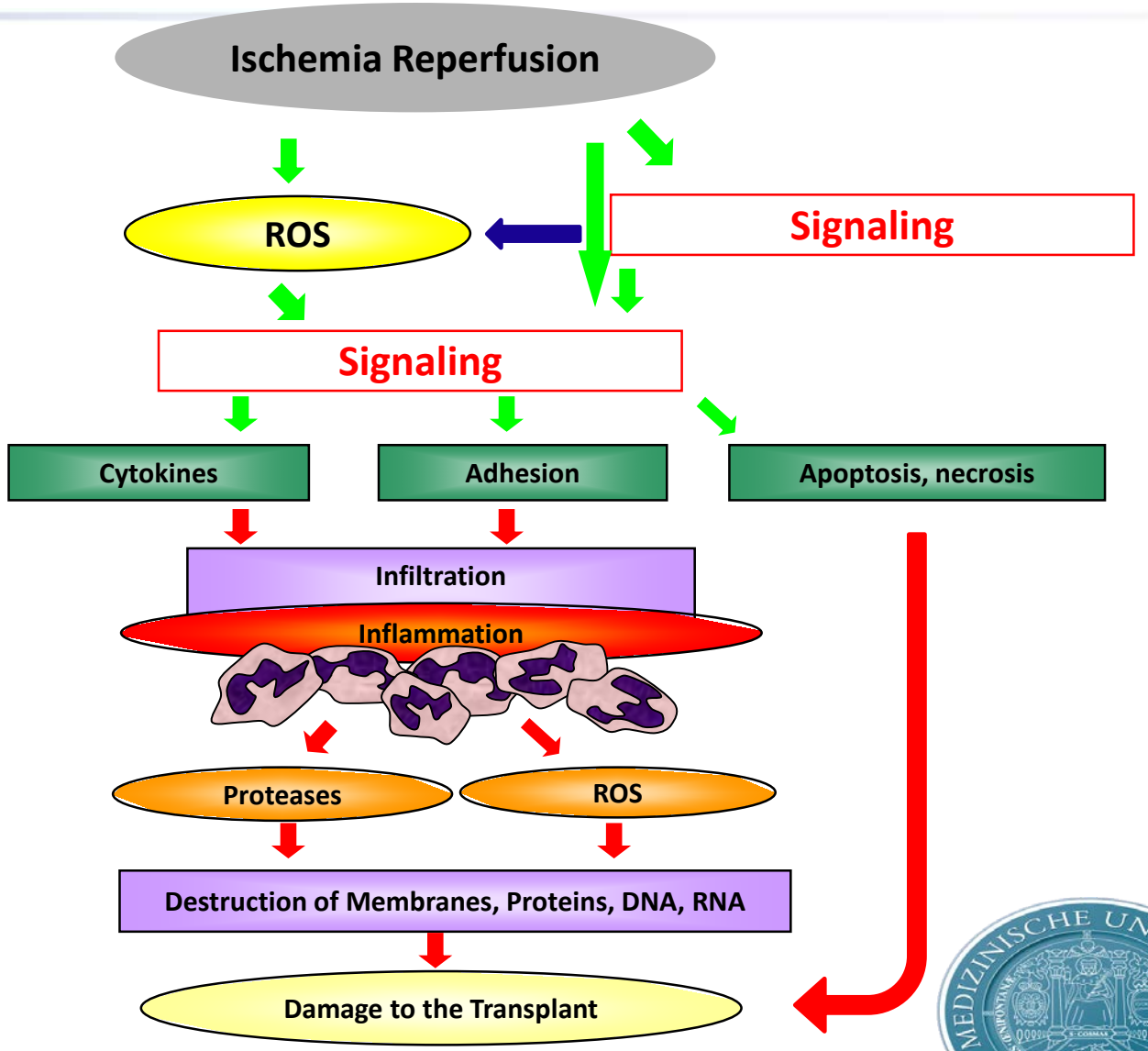
nature

Ischemia-reperfusion injury (IRI)



Ischemia/reperfusion injury (IRI)

Ischaemia-reperfusion injury occurs when the blood supply to an organ is disrupted (ischemia) and then restored (reperfusion), and underlies many disorders, notably heart attack and stroke. While reperfusion of ischaemic tissue is essential for survival, it also initiates oxidative damage, cell death and aberrant immune responses through the generation of mitochondrial reactive oxygen species (ROS).



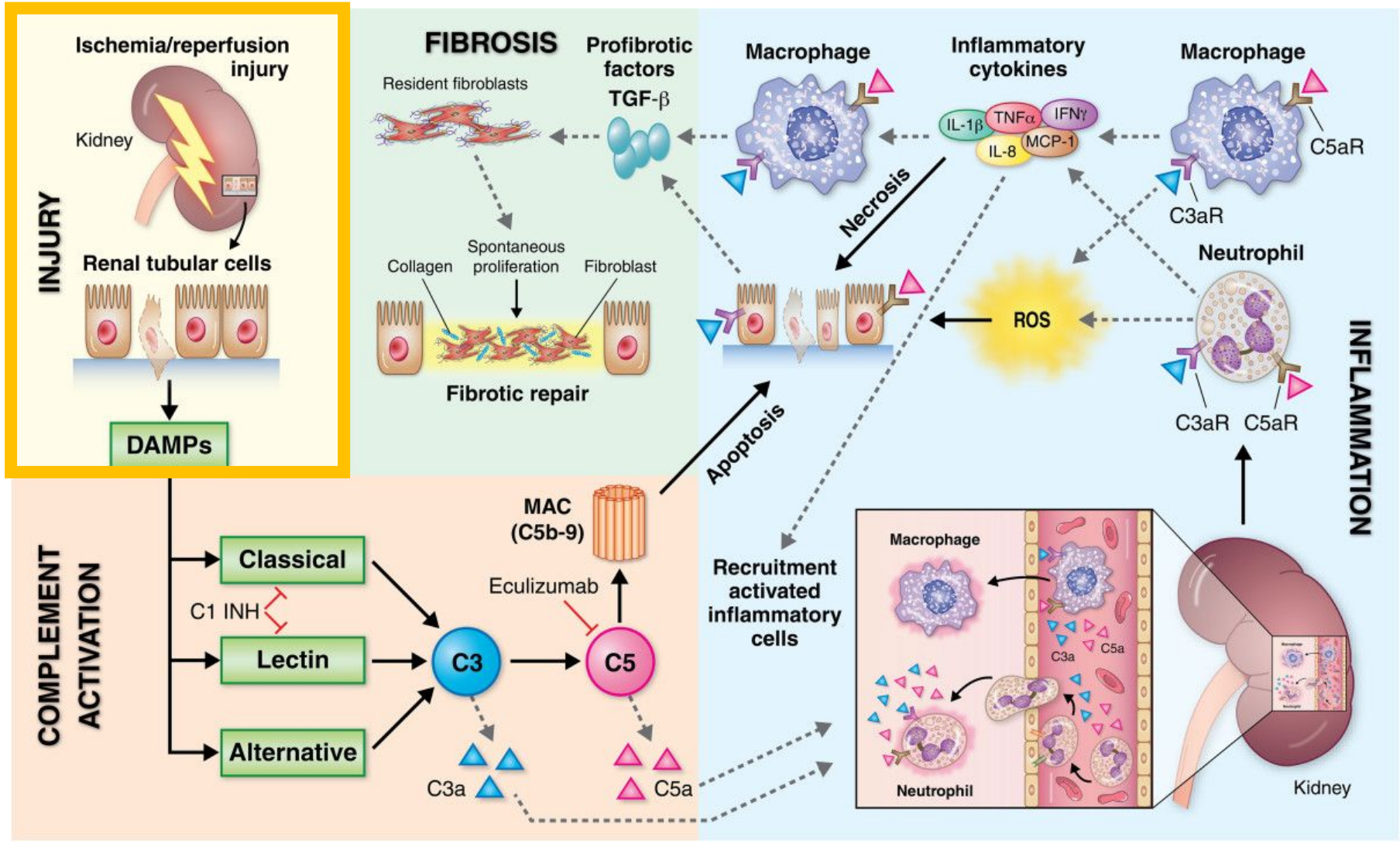
IRI: not only in transplantation

Table 1 Examples of ischemia and reperfusion injury

Affected organ	Example of clinical manifestation
Single-organ ischemia and reperfusion	
Heart	Acute coronary syndrome
Kidney	Acute kidney injury
Intestine	Intestinal ischemia and reperfusion; multiorgan failure
Brain	Stroke
Multiple-organ ischemia and reperfusion	
Trauma and resuscitation	Multiple organ failure; acute kidney injury; intestinal injury
Circulatory arrest	Hypoxic brain injury; multiple organ failure; acute kidney injury
Sickle cell disease	Acute chest syndrome; pulmonary hypertension, priapism, acute kidney injury
Sleep apnea	Hypertension; diabetes
Ischemia and reperfusion during major surgery	
Cardiac surgery	Acute heart failure after cardiopulmonary bypass
Thoracic surgery	Acute lung injury
Peripheral vascular surgery	Compartment syndrome of extremity
Major vascular surgery	Acute kidney injury
Solid organ transplantation	Acute graft failure; early graft rejection

Nat Med. 2011 Nov 7;17(11):1391-401.

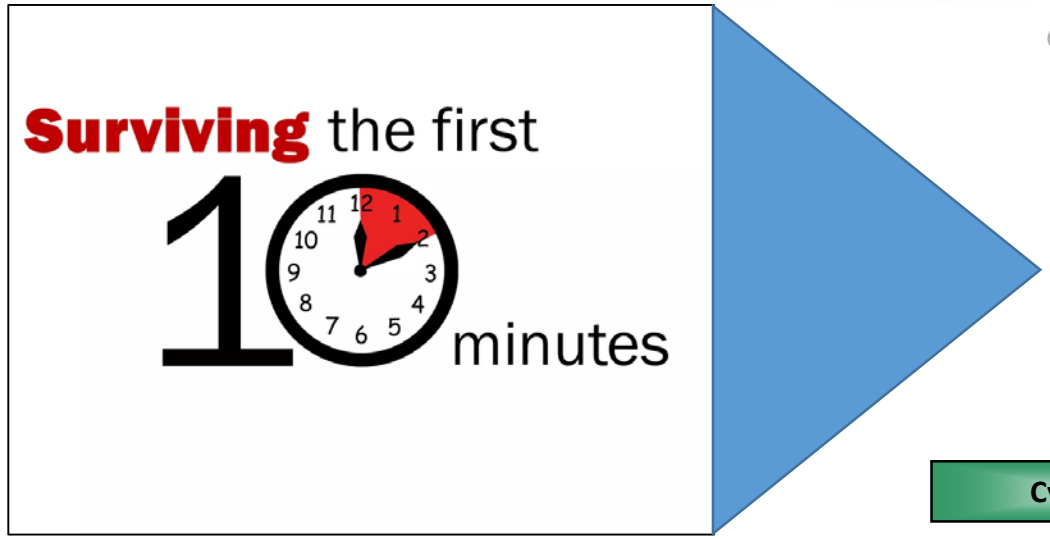
Ischemia/reperfusion injury (IRI)

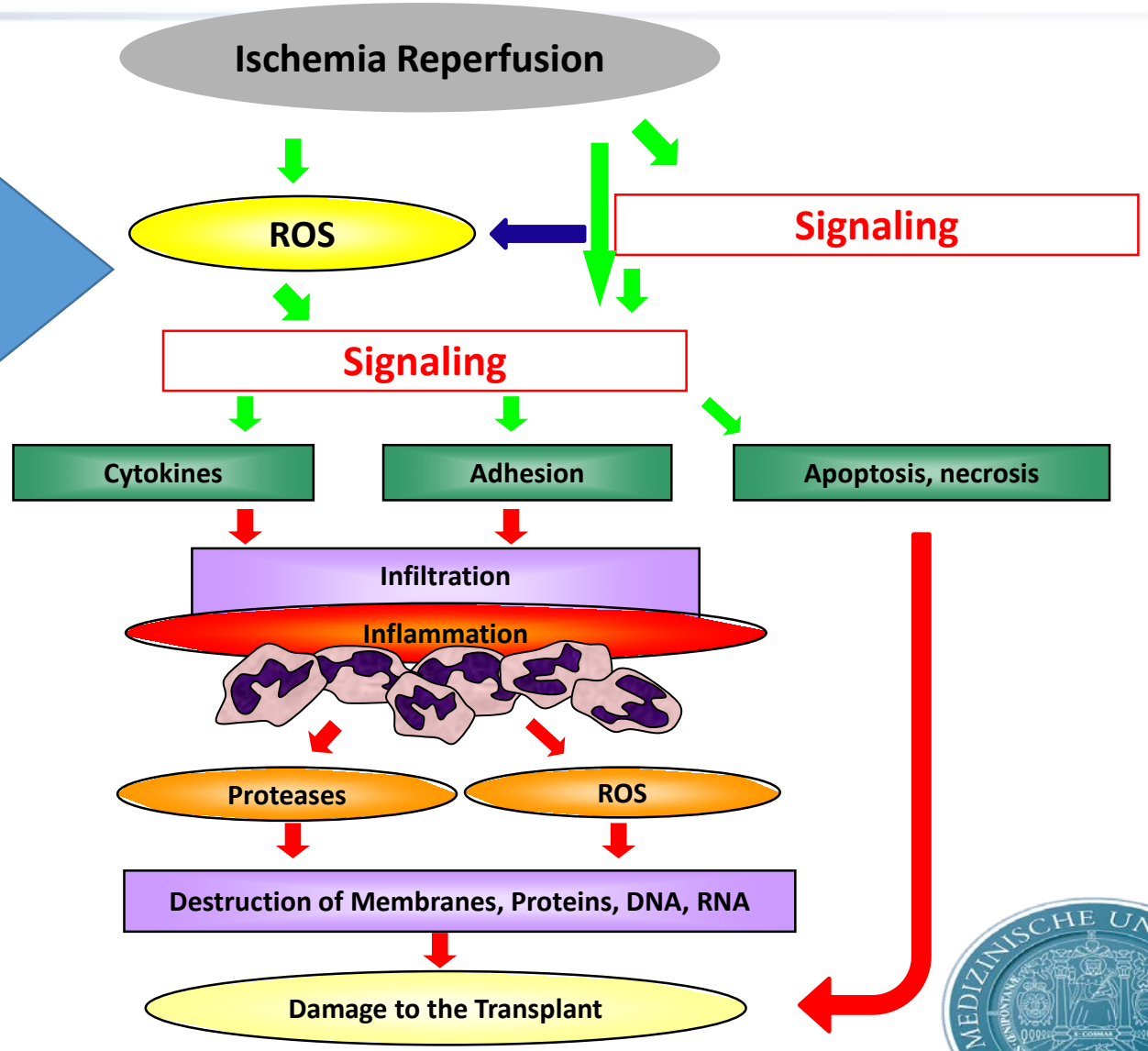


Ischemia/reperfusion injury (IRI)

Processes occur over weeks, months, years.

IRI: Key decisions occur early





The paper

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Mitochondria

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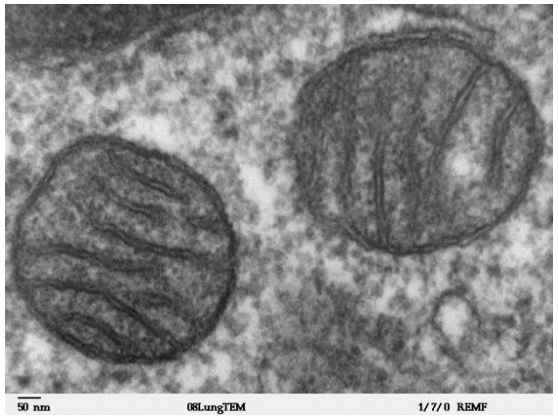
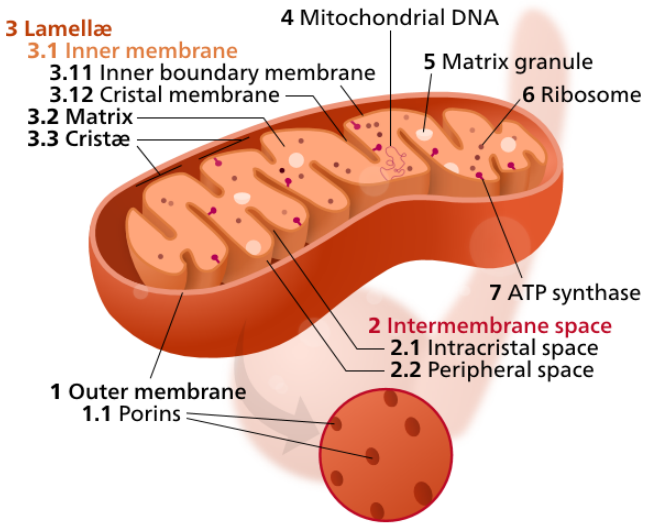
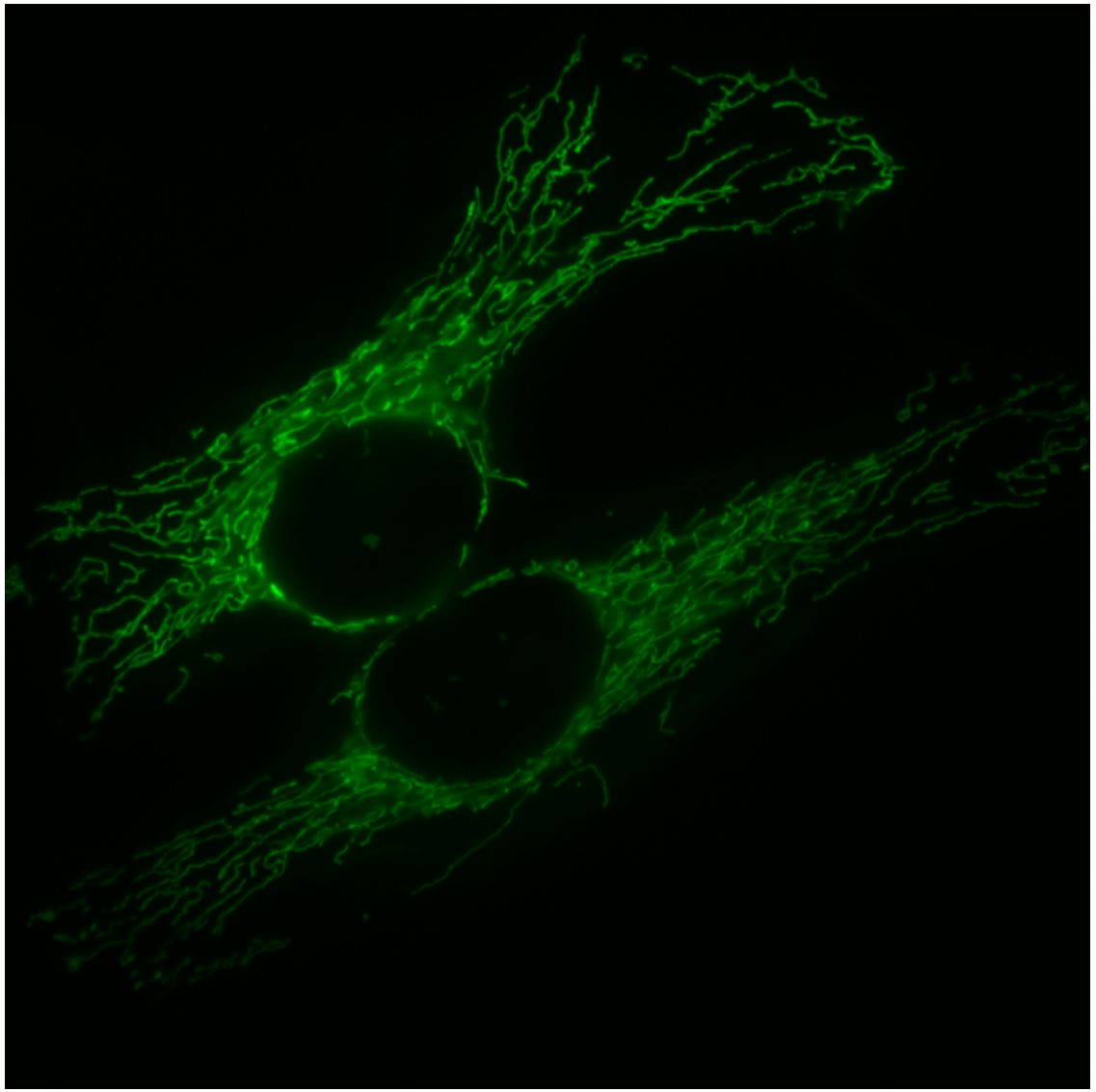
nature

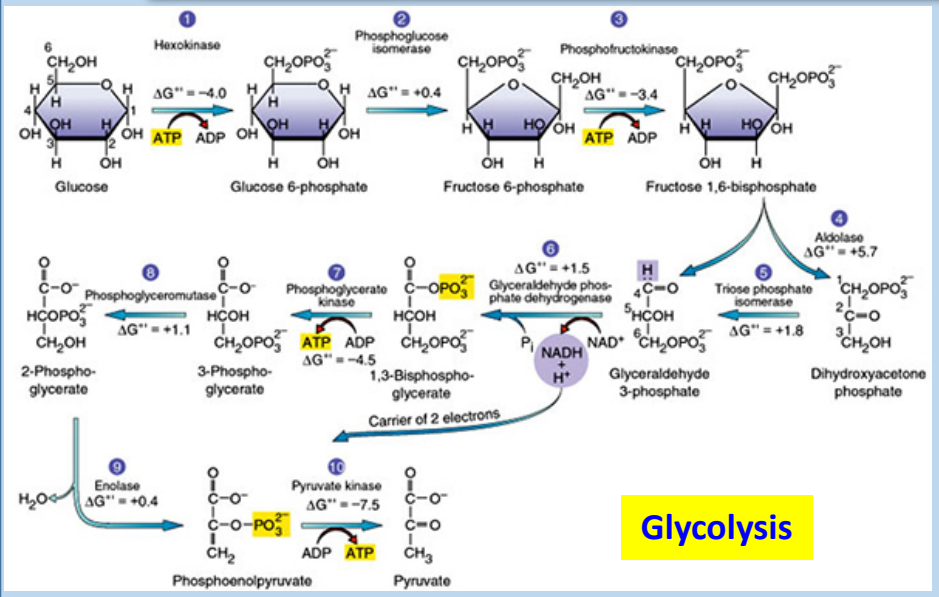
Mitochondria



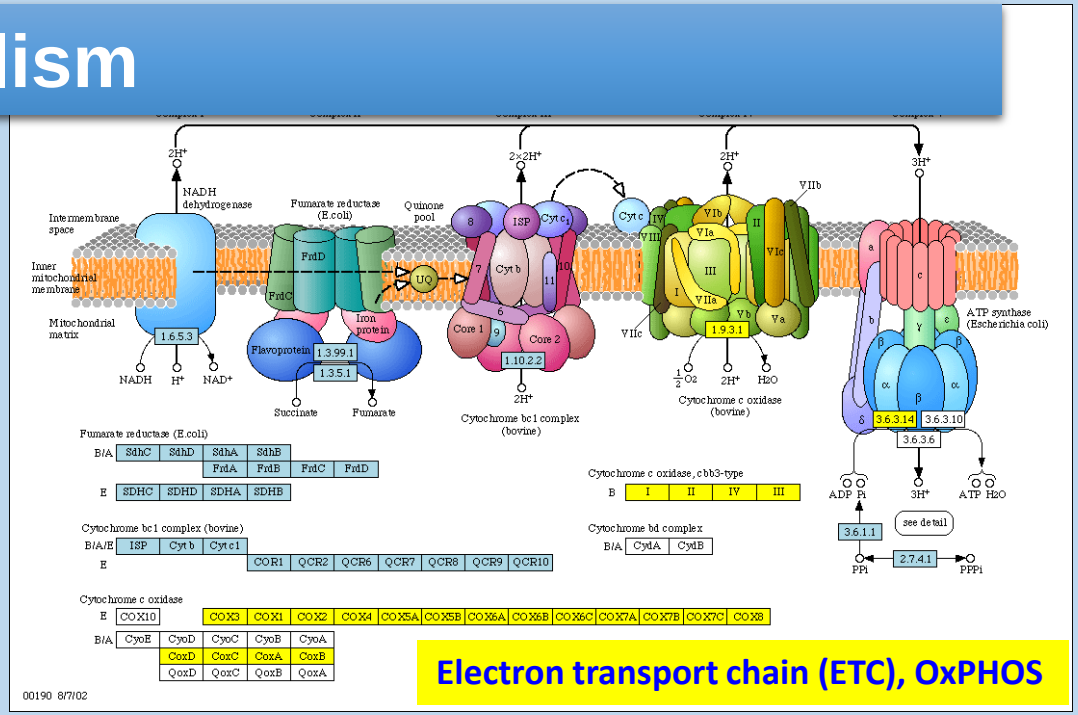




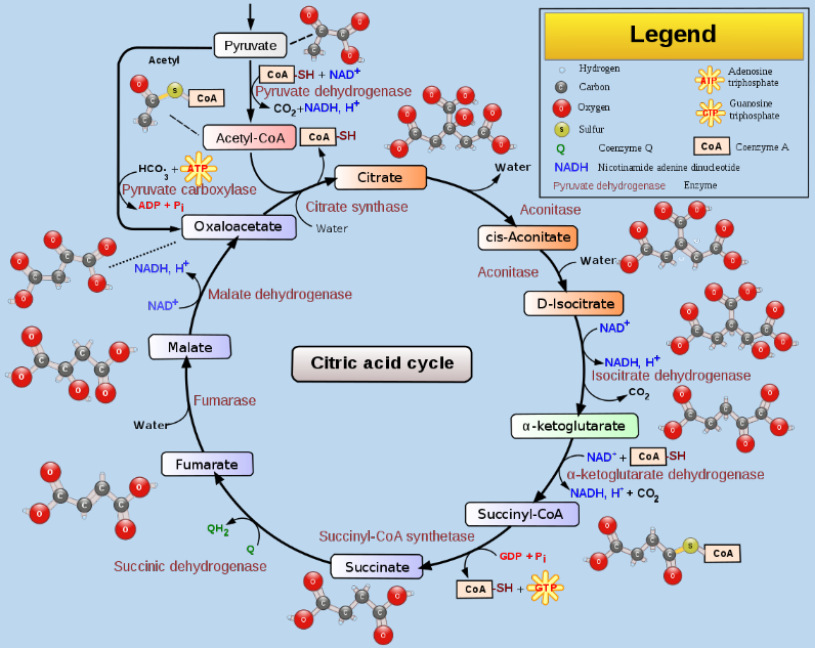
Metabollism



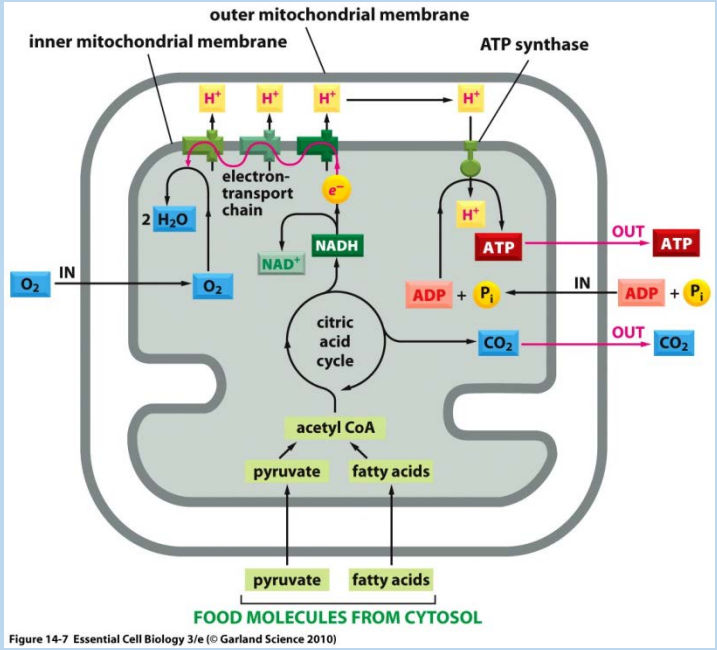
Glycolysis



Electron transport chain (ETC), OxPHOS



citric acid cycle (CAC), tricarboxylic acid (TCA) cycle, Krebs cycle



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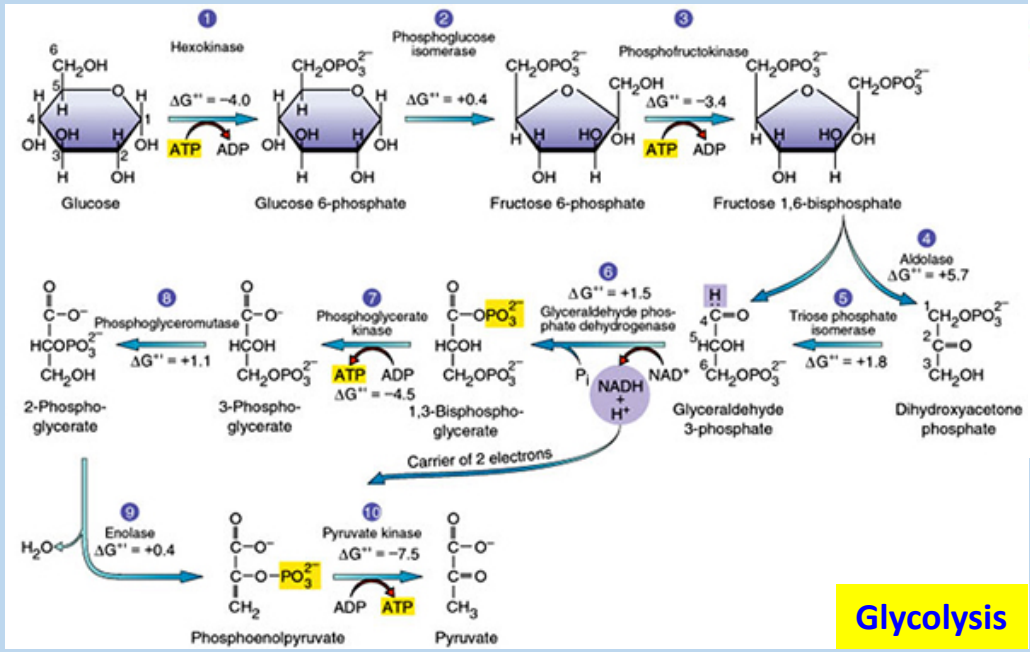

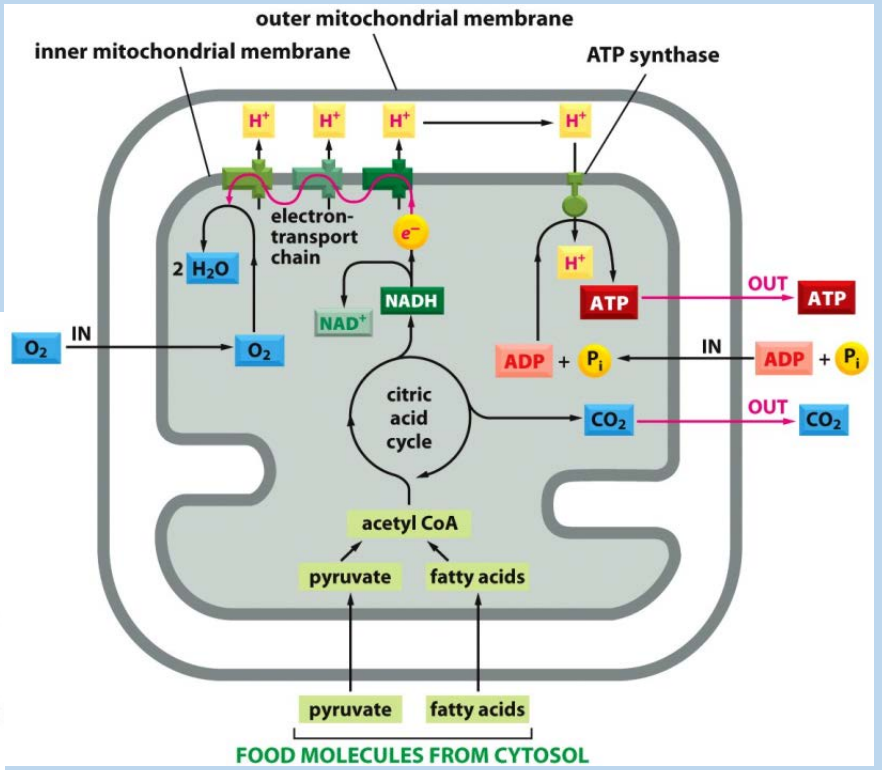

Glycolysis

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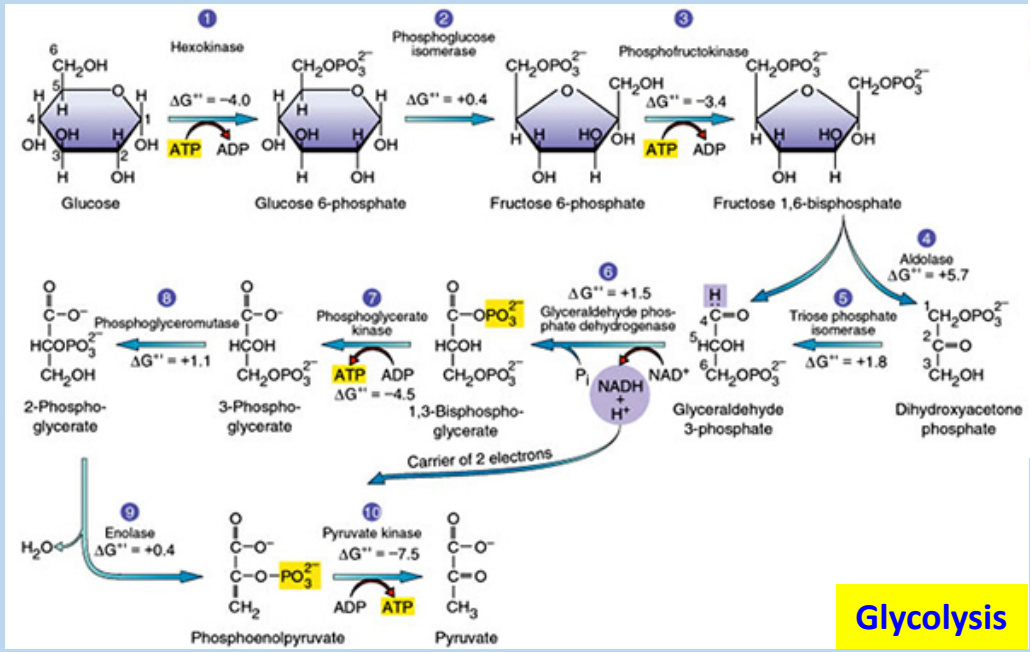
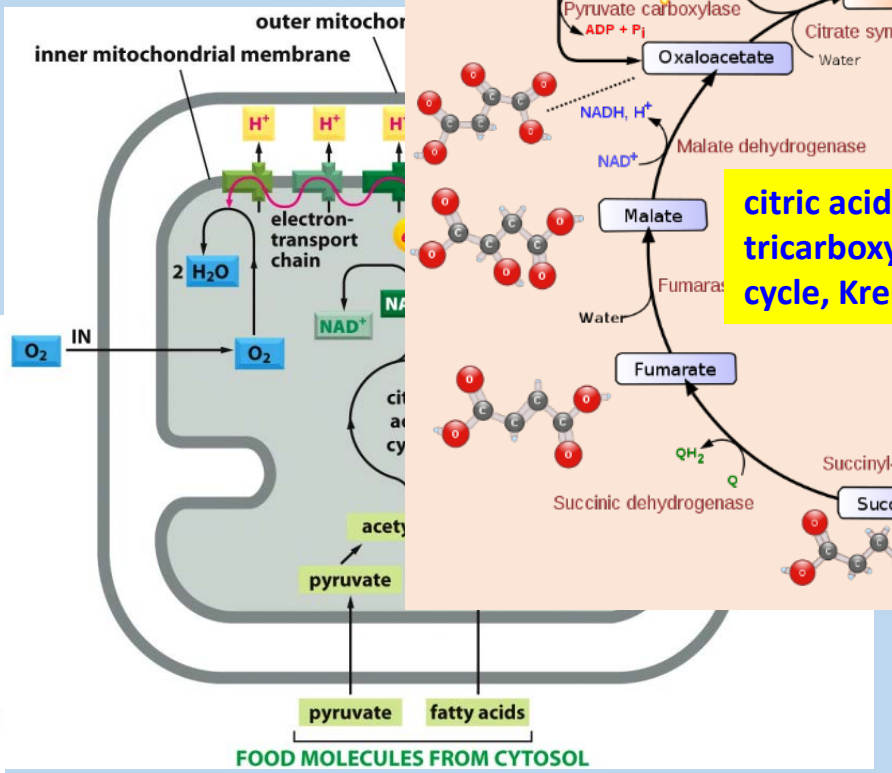
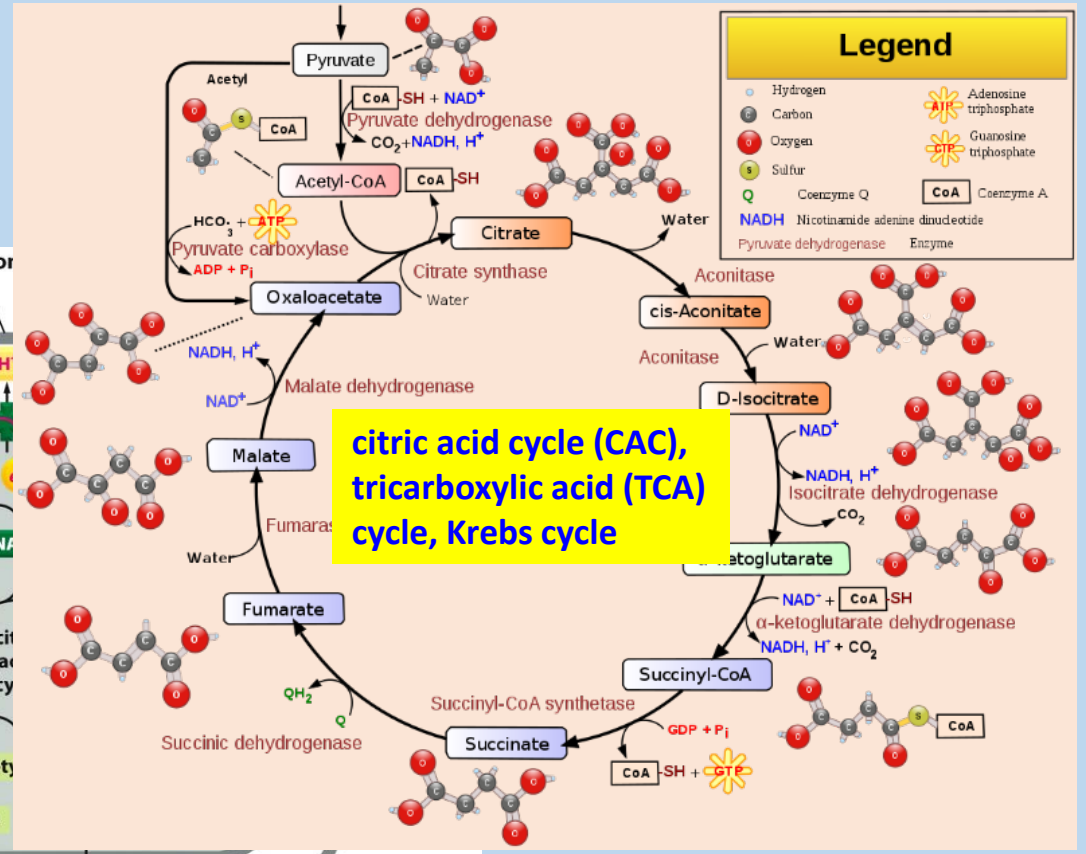


Glycolysis





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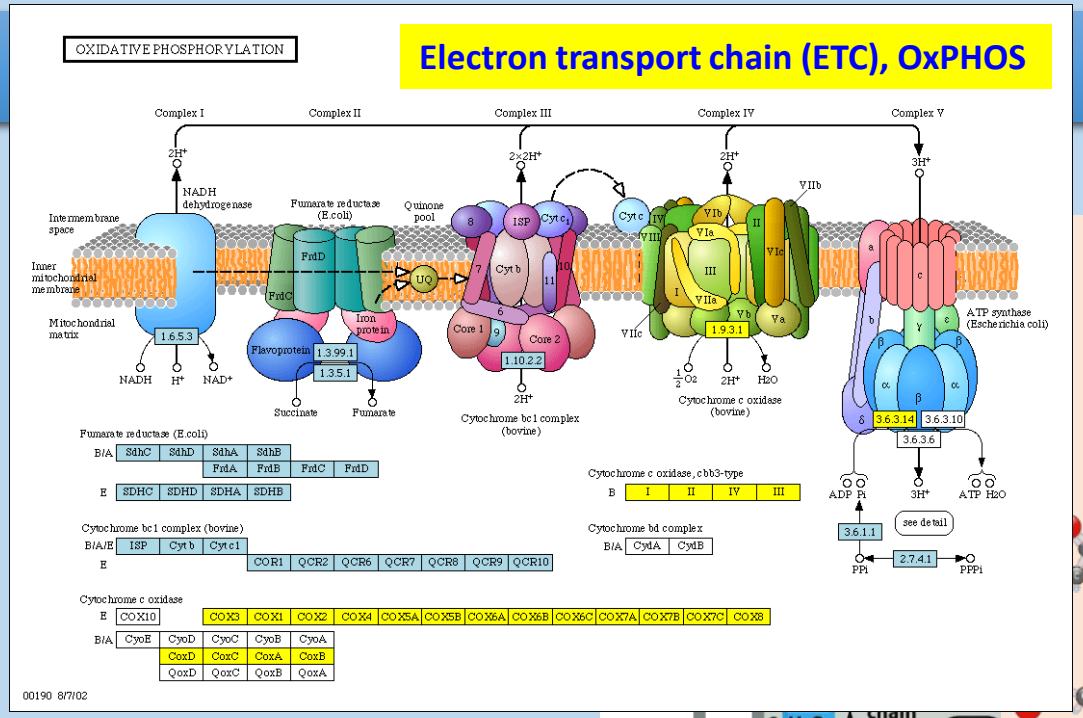
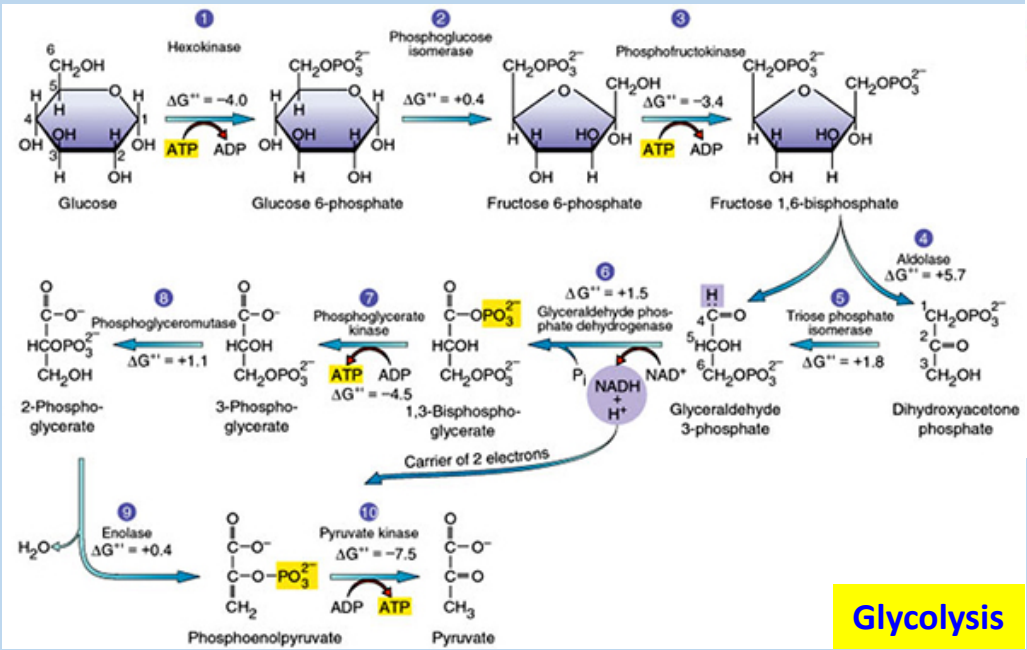
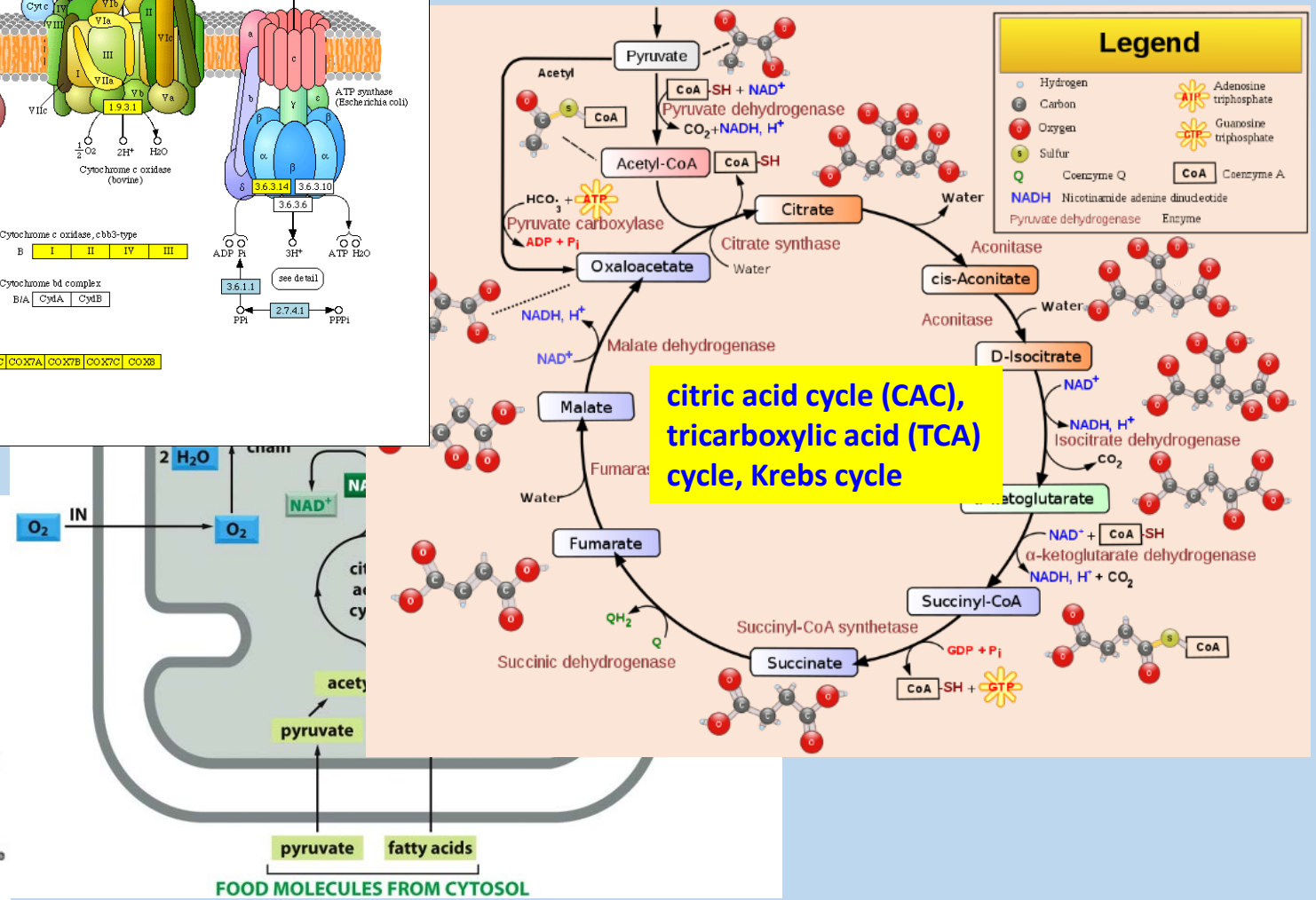

OXIDATIVE PHOSPHORYLATION
Electron transport chain (ETC), OxPHOS
Complex I
Complex II
Complex III
Complex IV
Complex V
NADH dehydrogenase
Fumarate reductase (E.coli)
Quinone pool
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
Succinate
Fumarate
Cytochrome bc1 complex (bovine)
Cytochrome c oxidase (bovine)
ATP synthase (Escherichia coli)
Fumarate reductase (E.coli)
Cytochrome c oxidase, cbb3-type
Cytochrome bc1 complex (bovine)
Cytochrome bd complex
Cytochrome c oxidase
00190 8/7/02


Hexokinase
Phosphoglucose isomerase
Phosphofructokinase
Glucose
Glucose 6-phosphate
Fructose 6-phosphate
Fructose 1,6-bisphosphate
Aldolase
Triose phosphate isomerase
Glyceraldehyde phosphate dehydrogenase
Phosphoglyceromutase
Phosphoglycerate kinase
2-Phospho-glycerate
3-Phospho-glycerate
1,3-Bisphospho-glycerate
Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate
Carrier of 2 electrons
Enolase
Pyruvate kinase
Phosphoenolpyruvate
Pyruvate
Glycolysis


Legend
Hydrogen
Carbon
Oxygen
Sulfur
Coenzyme Q
Nicotinamide adenine dinucleotide
Adenosine triphosphate
Guanosine triphosphate
Coenzyme A
Enzyme
Pyruvate
Acetyl-CoA
Pyruvate dehydrogenase
Pyruvate carboxylase
Citrate
Citrate synthase
Water
Oxaloacetate
Aconitase
cis-Aconitate
D-Isocitrate
Isocitrate dehydrogenase
α-ketoglutarate dehydrogenase
Succinyl-CoA
Succinyl-CoA synthetase
Succinate
Succinic dehydrogenase
Fumarate
Malate
Malate dehydrogenase
citric acid cycle (CAC), tricarboxylic acid (TCA) cycle, Krebs cycle
pyruvate
fatty acids
FOOD MOLECULES FROM CYTOSOL

Reactive oxygen species (ROS)

LETTER

doi:10.1038/nature13909

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nature

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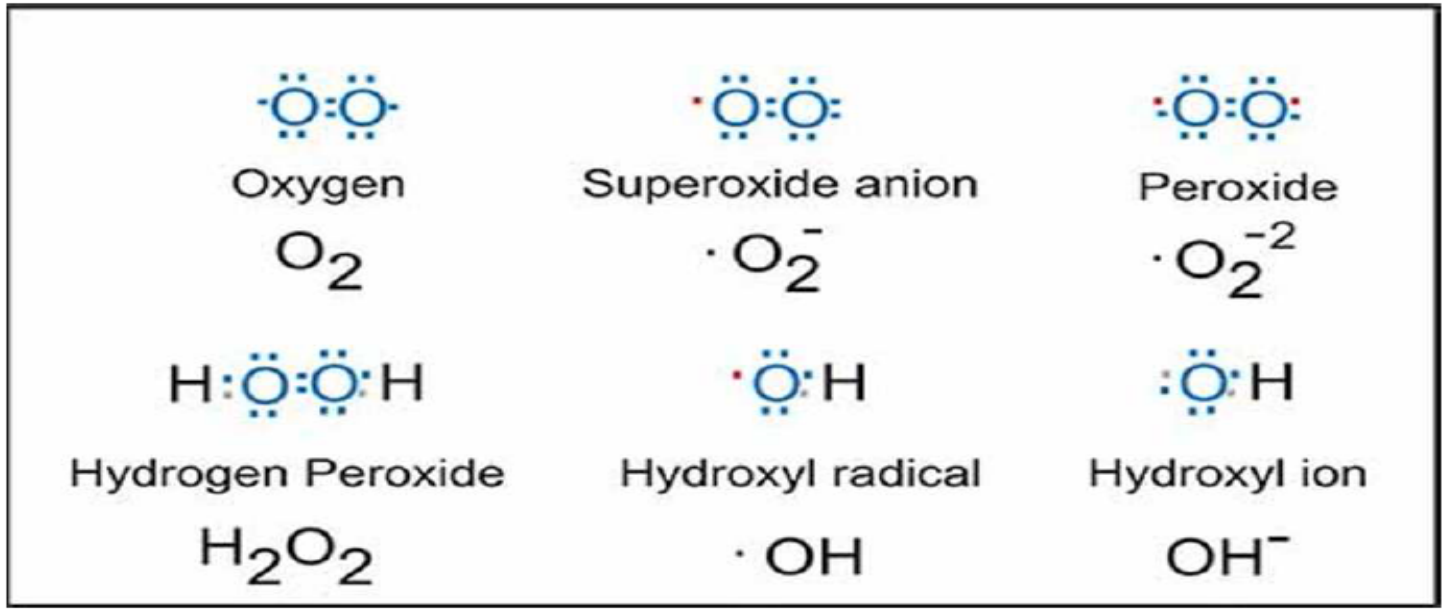
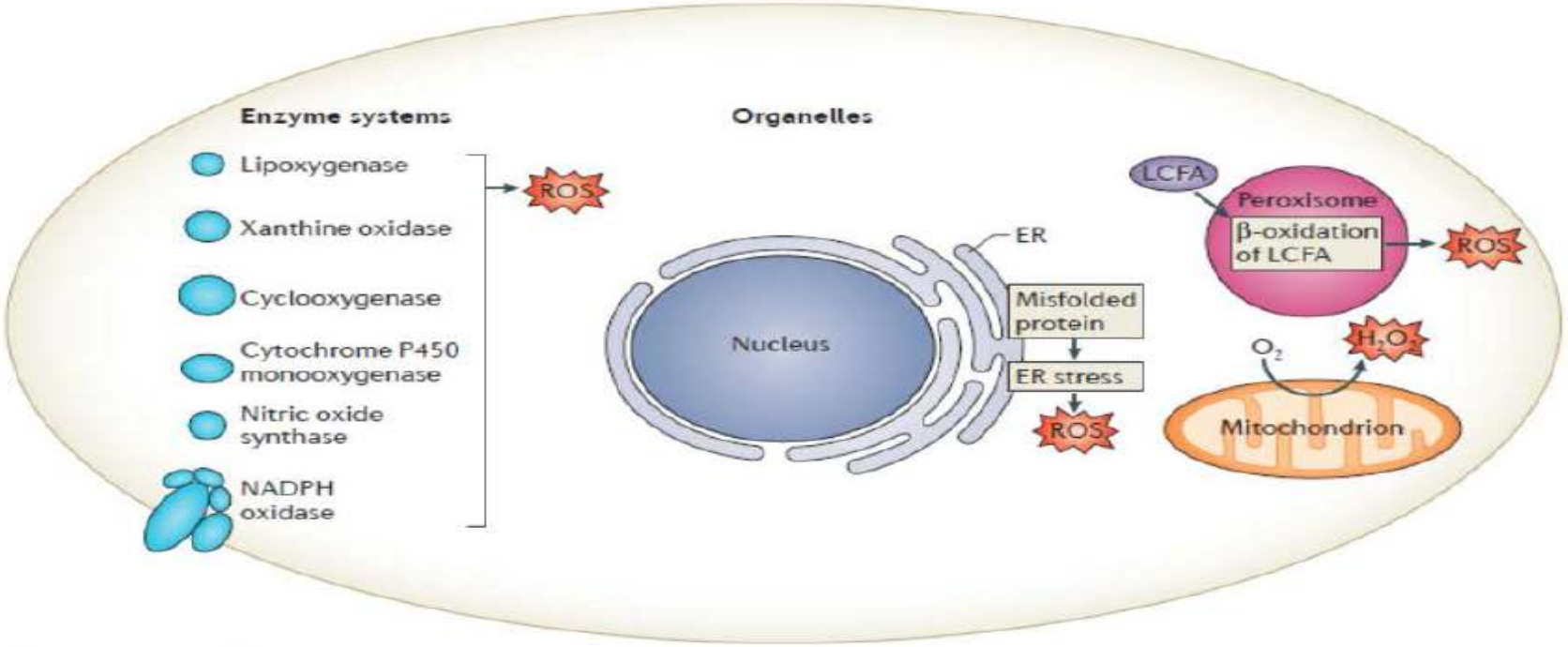


Reactive oxygen species (ROS): Electron structures of common reactive oxygen species. Each structure is provided with its name and chemical formula. The • designates an unpaired electron.

ROS, reactive oxygen species: chemically highly reactive, the reduction of molecular oxygen (O_2) produces superoxide, the precursor of most other reactive oxygen species.

Reactive oxygen species (ROS)



Intracellular sources of ROS

Nature Reviews Molecular Cell Biology 15, 411–421 (2014)

Reactive oxygen species (ROS)

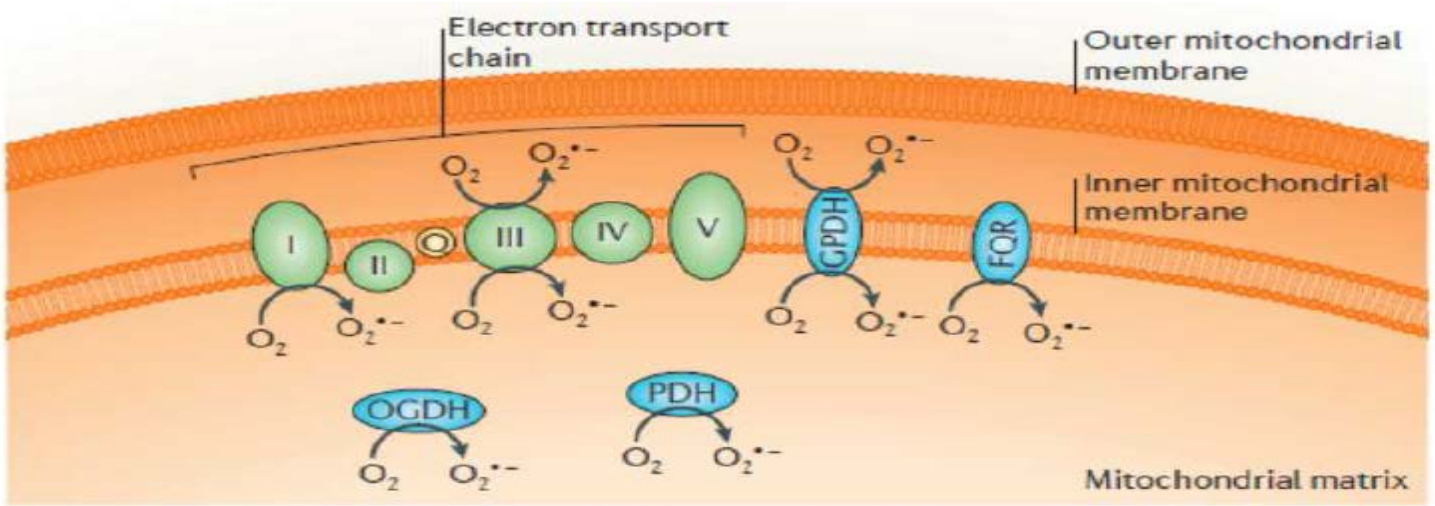
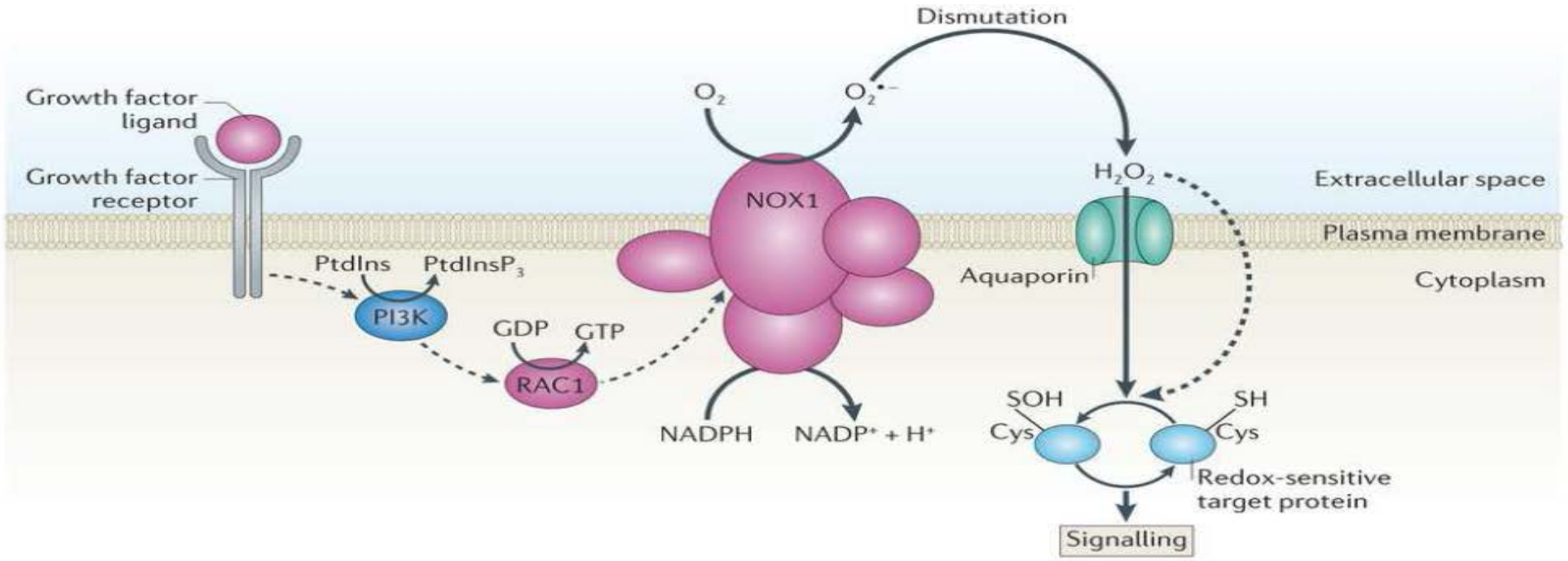


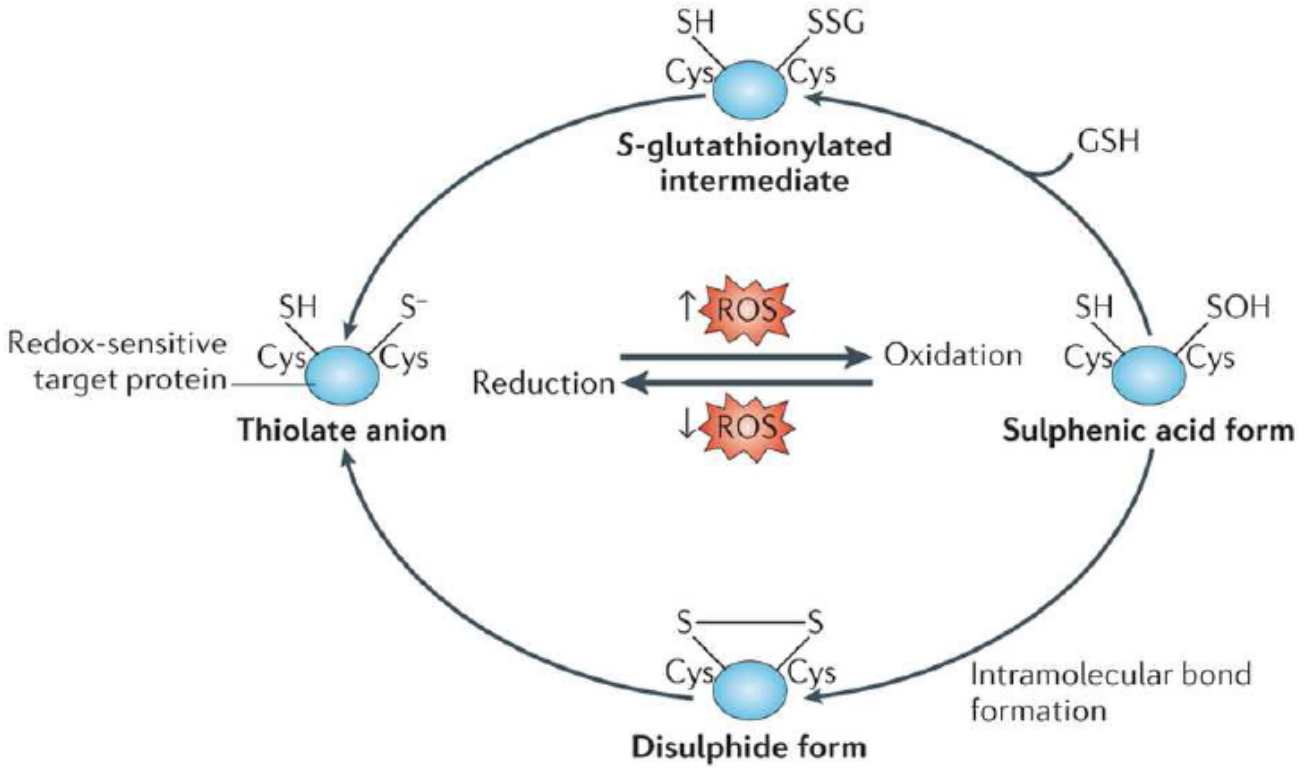
Figure 1 | **Sites for the generation of reactive oxygen species in mitochondria.** Multiple sites of mitochondrial superoxide anion ($O_2^{\bullet-}$) production have been mapped. Quantitatively, complex I and complex III of the electron transport chain are the major sites of oxidant production, with the generation of superoxide anions occurring both on the matrix side of the mitochondrion and in the inner mitochondrial membrane space. Other contributors include metabolic enzymes in the mitochondrial matrix, such as OGDH (2-oxoglutarate dehydrogenase) and PDH (pyruvate dehydrogenase), and the mitochondrial membrane forms of GPDH (glycerol 3-phosphate dehydrogenase; also known as GPDM) and the FQR (electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial) system (see REFS 5,7 for more details). Q, coenzyme Q10.

Reactive oxygen species (ROS)



ROS: root of all evil

Reduction–oxidation (redox)-sensitive target proteins have reactive Cys residues that can form thiolate anions (S−) at a physiological pH. Oxidation of this residue results in a sulphenic acid (SOH) moiety that often leads to a change in function of the target protein. This sulphenic acid form can be further oxidized (not shown) or reversed either by direct reduction or through other processes, including forming an intramolecular disulphide bond or conjugating with glutathione (GSH) to form an S-glutathionylated (SSG) intermediate. ROS, reactive oxygen species; SH, thiol.



Nature Reviews | Molecular Cell Biology

ROS as signaling molecules

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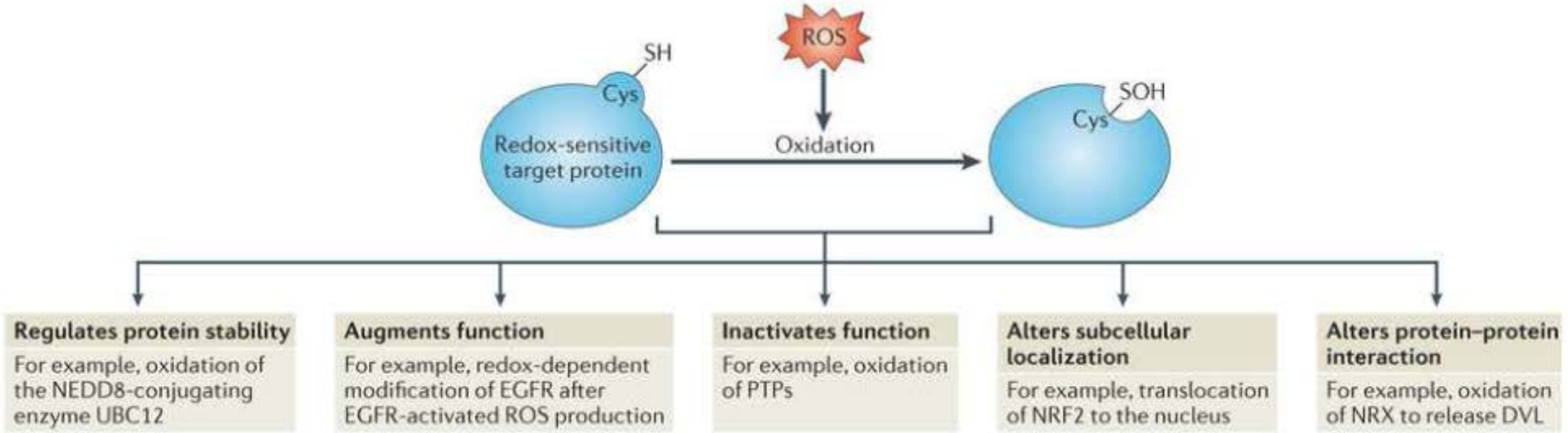

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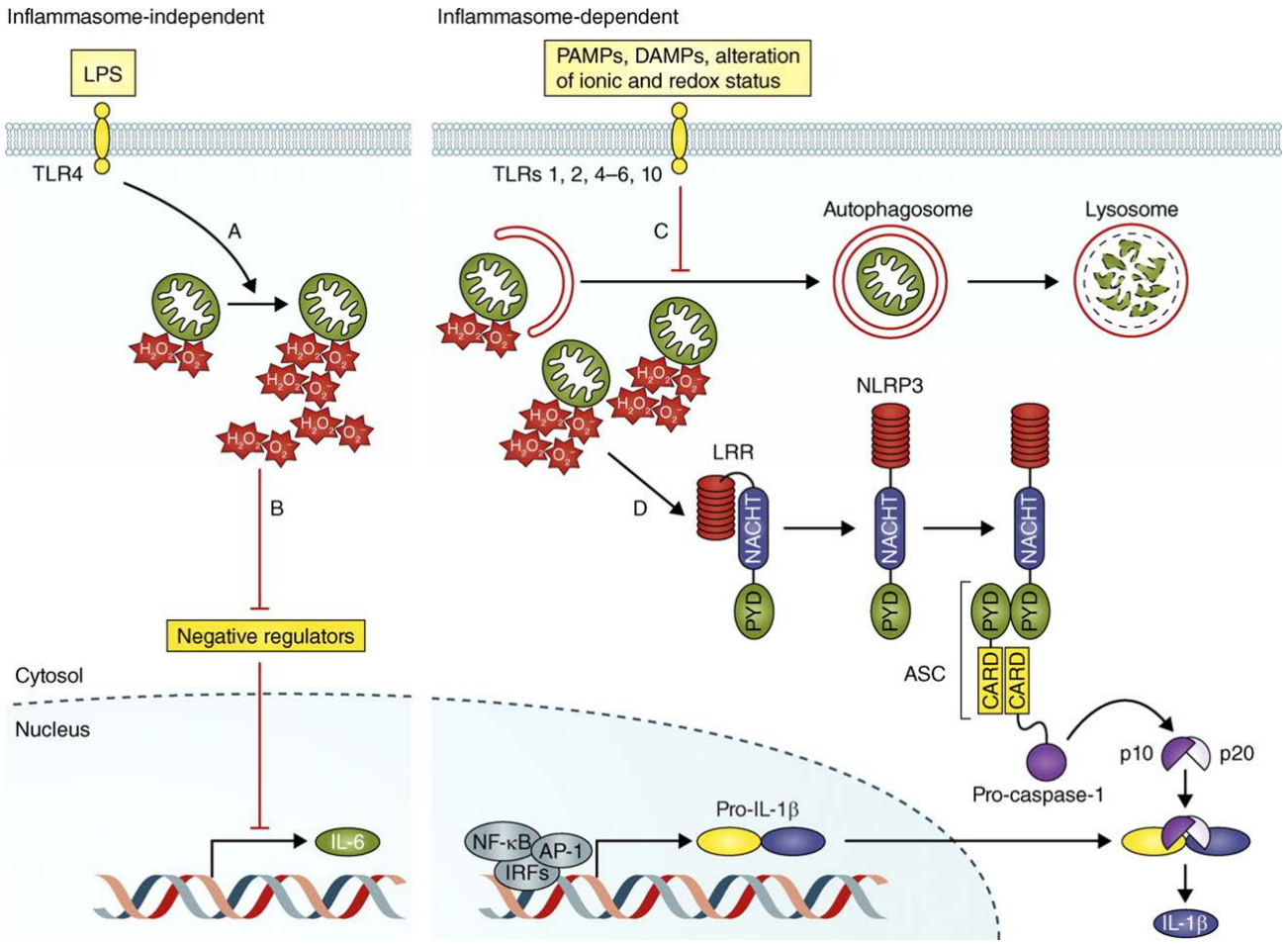
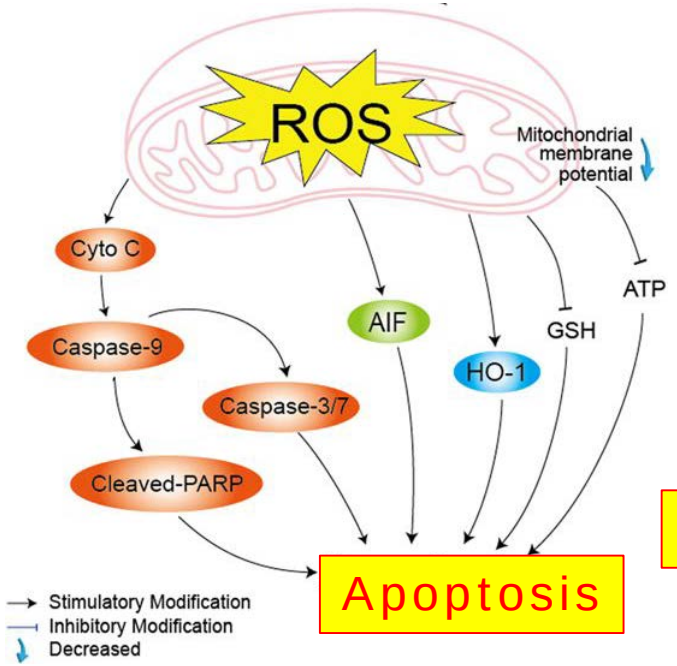


Inflammasome activation

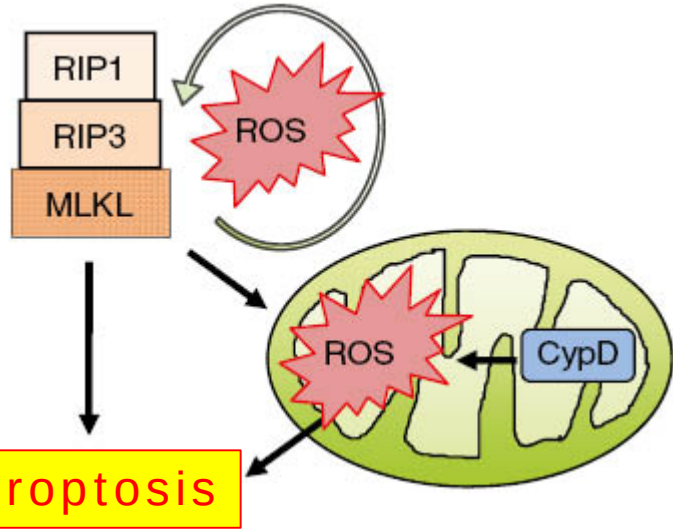
Nat Med. 2011 Nov 7;17(11):1391-401.

ROS: root of all evil



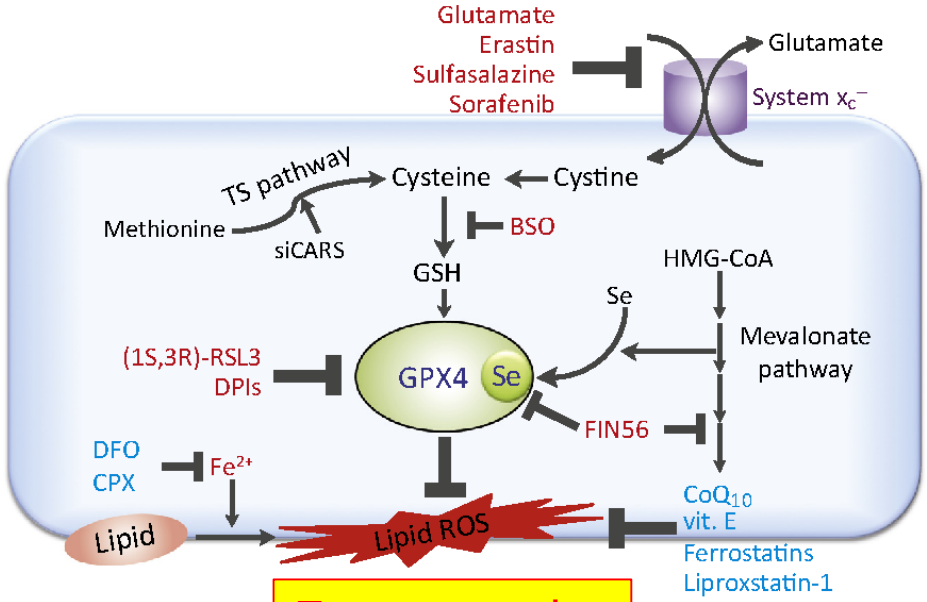
Apoptosis

Scientific Reports 5, 15104 (2015)



Necroptosis

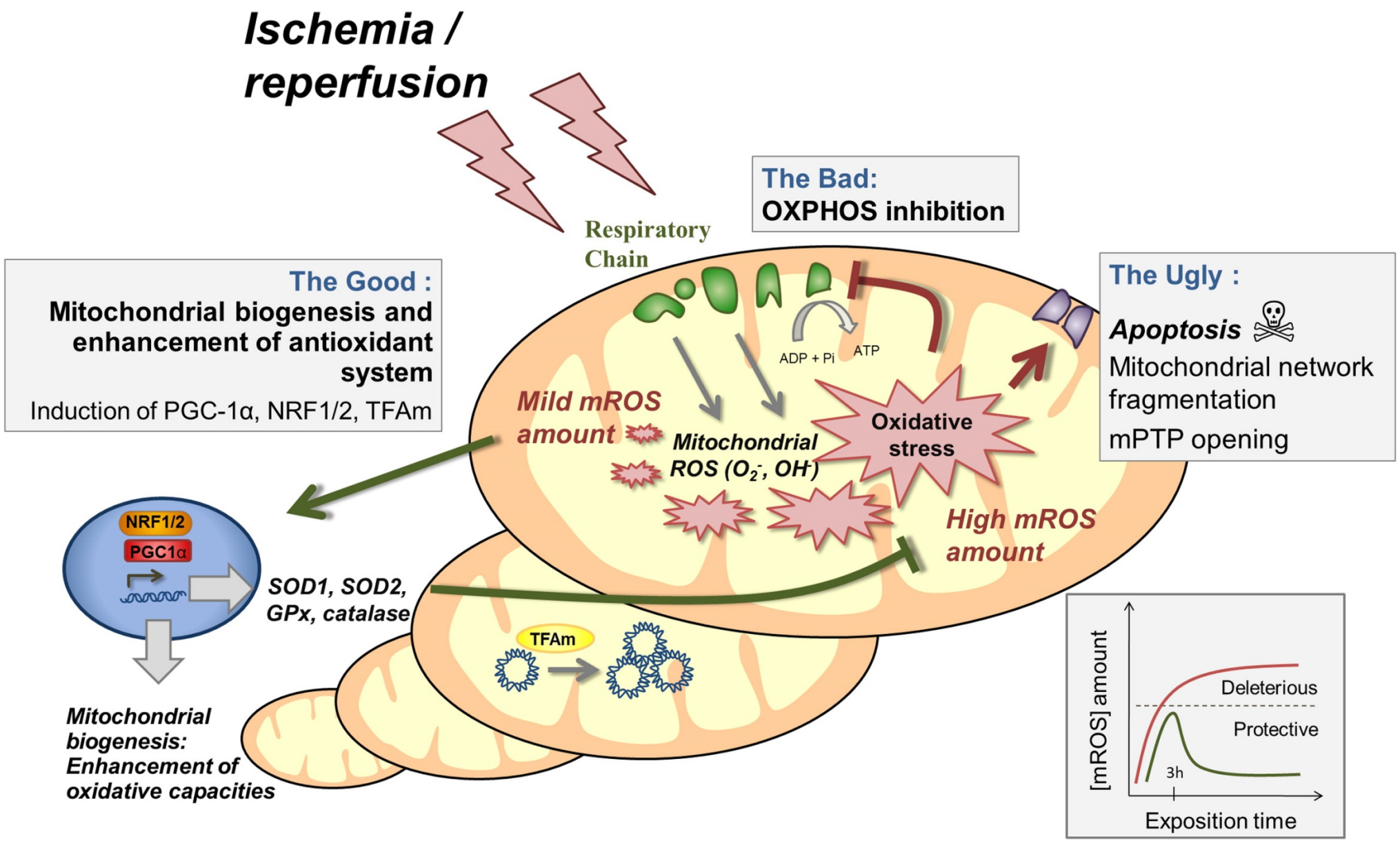
Biol. Chem. 2016; 397(7): 657–660



Ferroptosis

Cell. 2012 May 25;149(5):1060-72

Mitochondria and ROS



Ischemia/reperfusion injury (IRI)

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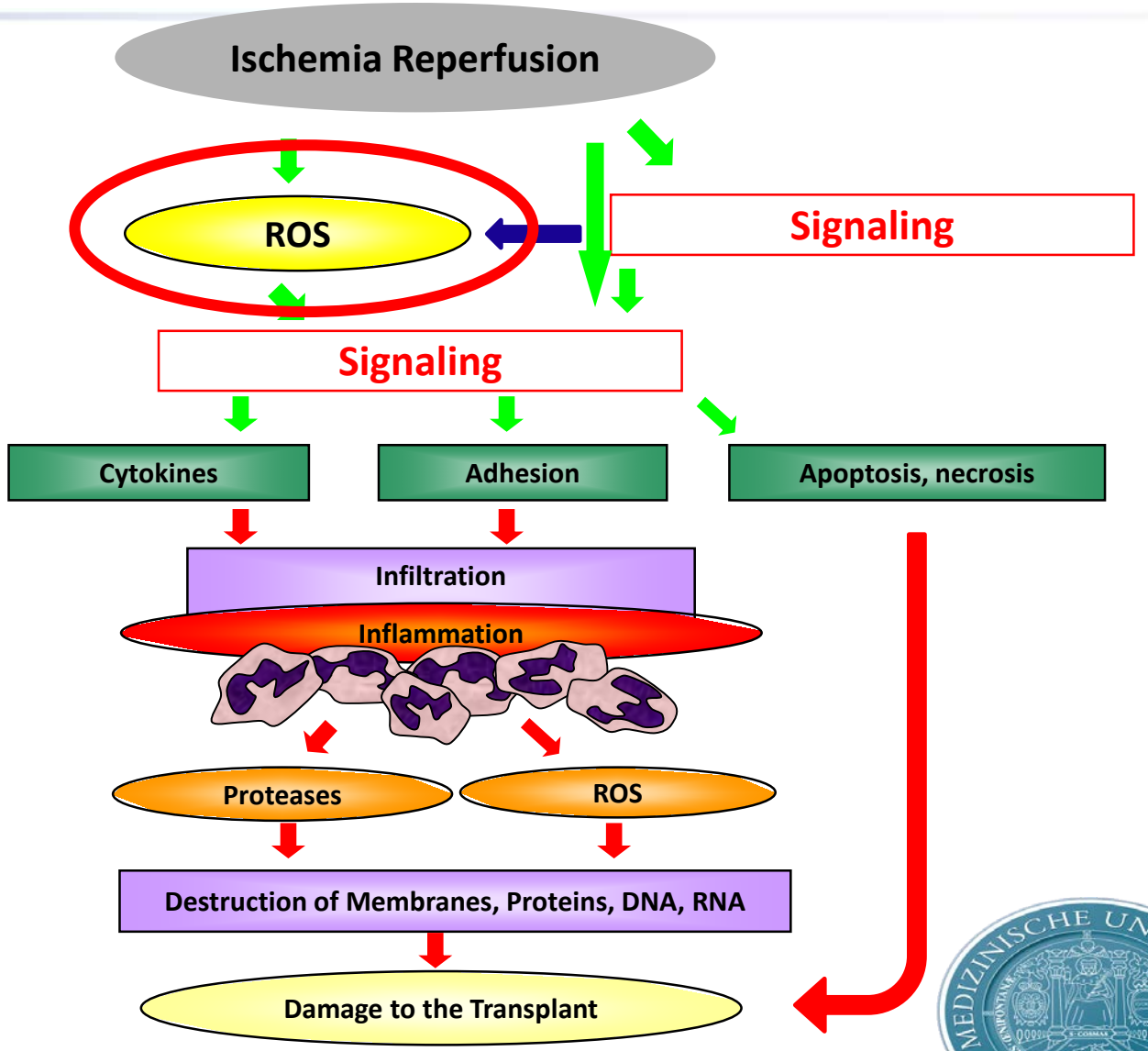
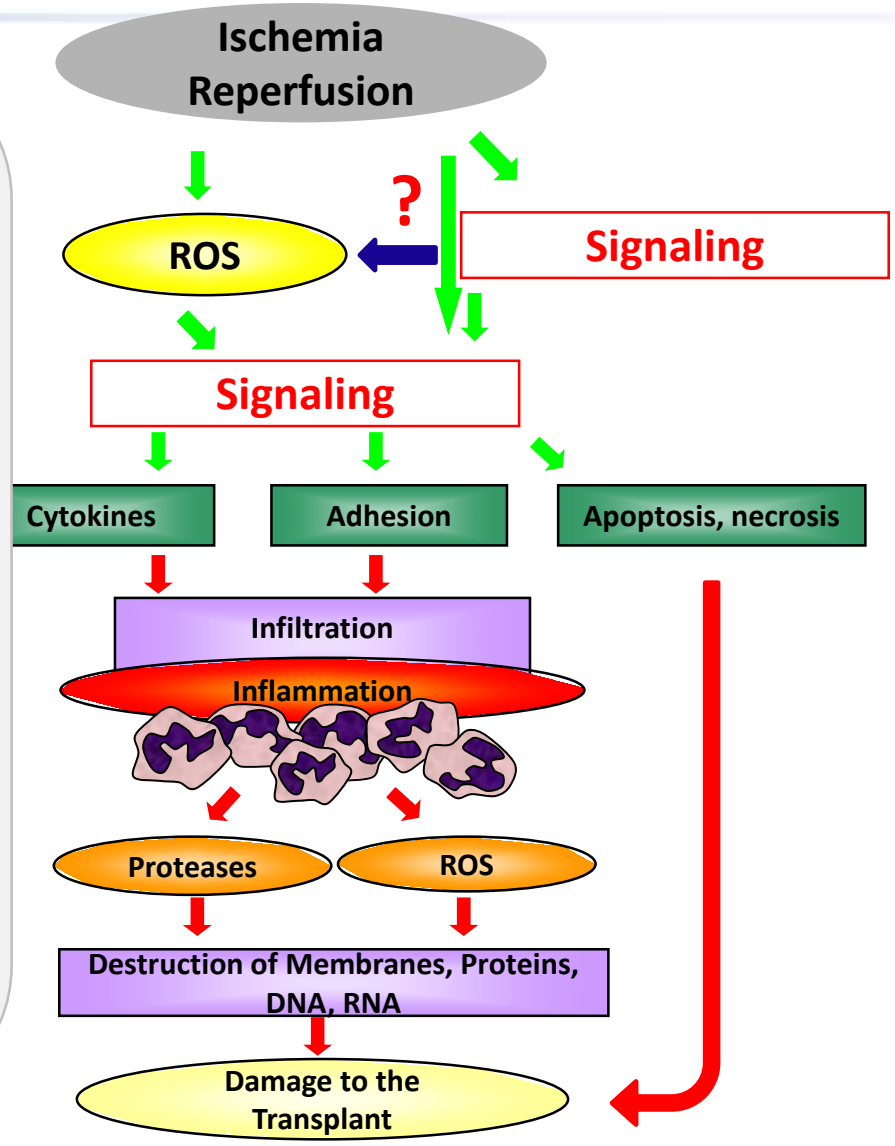


Ischemia/reperfusion injury (IRI)

Antioxidants fail in clinical routine.

The approach:

Limiting ROS levels by by defining its origin.



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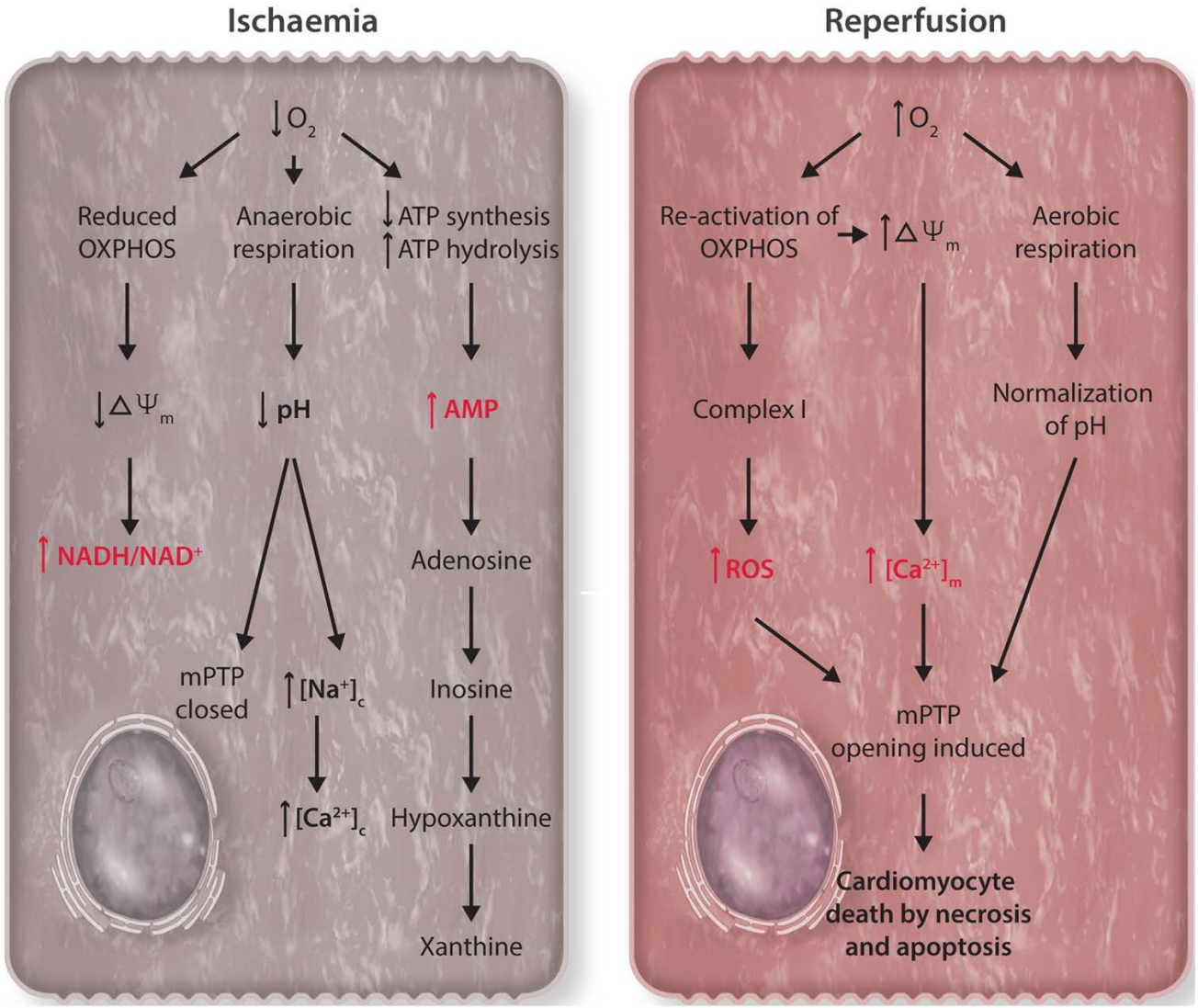
Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS

Edward T. Chouchani[1,2]*, Victoria R. Pell[2]*, Edoardo Gaude[3], Dunja Aksentijević[4], Stephanie Y. Sundier[5], Ellen L. Robb[1], Angela Logan[1], Sergiy M. Nadtochiy[6], Emily N. J. Ord[7], Anthony C. Smith[1], Filmon Eyassu[1], Rachel Shirley[7], Chou-Hui Hu[2], Anna J. Dare[1], Andrew M. James[1], Sebastian Rogatti[1], Richard C. Hartley[8], Simon Eaton[9], Ana S. H. Costa[3], Paul S. Brookes[6], Sean M. Davidson[10], Michael R. Duchen[5], Kourosh Saeb-Parsy[11], Michael J. Shattock[4], Alan J. Robinson[1], Lorraine M. Work[7], Christian Frezza[3], Thomas Krieg[2] & Michael P. Murphy[1]

nature

Which process leads to the generation of mito ROS?



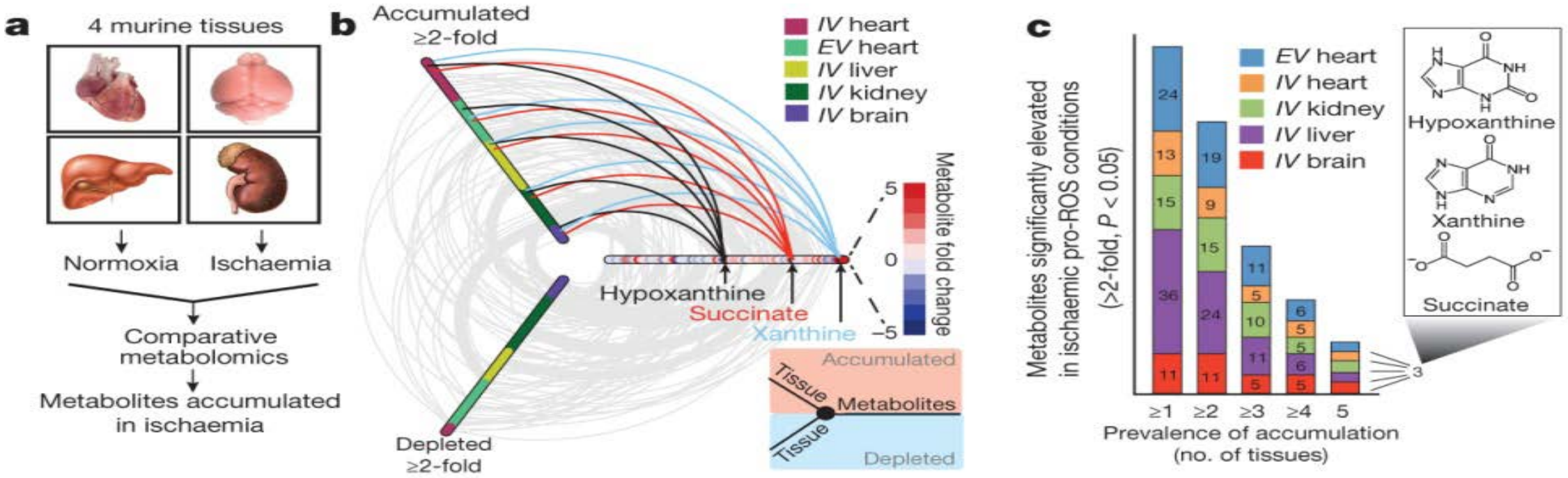
Which process leads to the generation of mito ROS?

Mitochondrial ROS production is a crucial early driver of ischaemia-reperfusion (IR) injury, but has been considered a nonspecific consequence of the interaction of a dysfunctional respiratory chain with oxygen during reperfusion.

Here we investigated an alternative **hypothesis**:
that mitochondrial ROS during IR are generated
by a specific metabolic process.

To do this, we developed a comparative metabolomics approach to identify **conserved metabolic signatures in tissues during IR that might indicate the source of mitochondrial ROS**.

Comparative metabolomics identifies **succinate** as a potential mitochondrial metabolite that drives reperfusion ROS production



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nature

Comparative metabolomics identifies **succinate** as a potential mitochondrial metabolite that drives reperfusion ROS production

Comparative analysis revealed that only three were increased across all tissues. Two metabolites were well-characterized by-products of ischaemic purine nucleotide breakdown, xanthine and hypoxanthine, corroborating the validity of our approach. Xanthine and hypoxanthine are metabolised by cytosolic xanthine oxidoreductase and do not contribute to mitochondrial metabolism. The third metabolite, the mitochondrial citric acid cycle (CAC) intermediate succinate, increased 3–19-fold to concentrations of 61–729 ng mg wet weight across the tested tissues, and was the sole mitochondrial feature of ischaemia that occurred universally in a range of metabolically diverse tissues. Therefore, we focused on the **potential role of succinate in mitochondrial ROS roduction during IR.**

Nature. 2014 Nov 20;515(7527):431-435.
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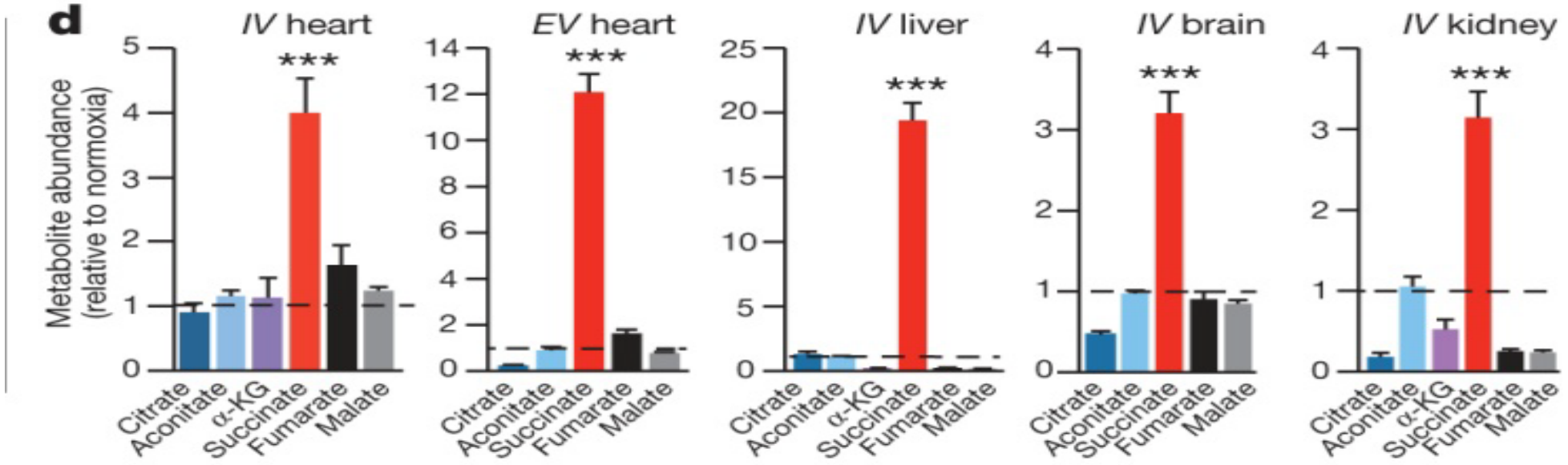

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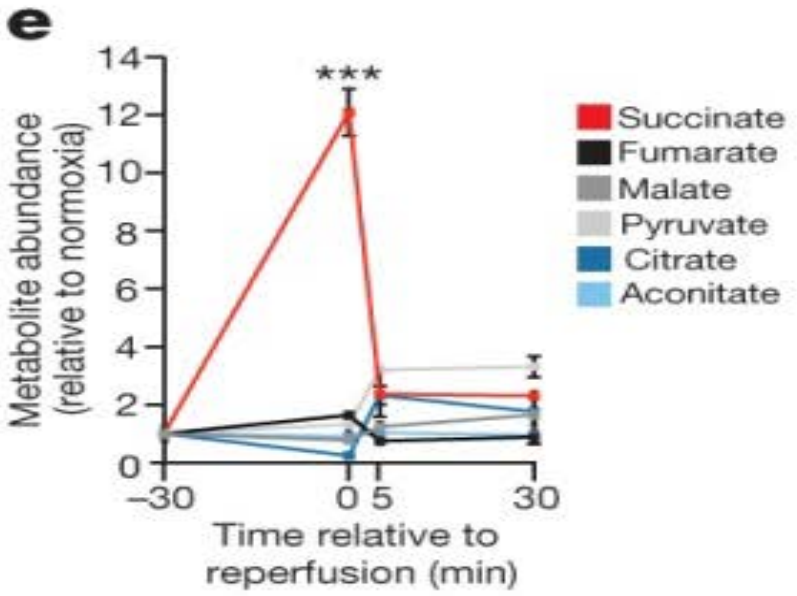
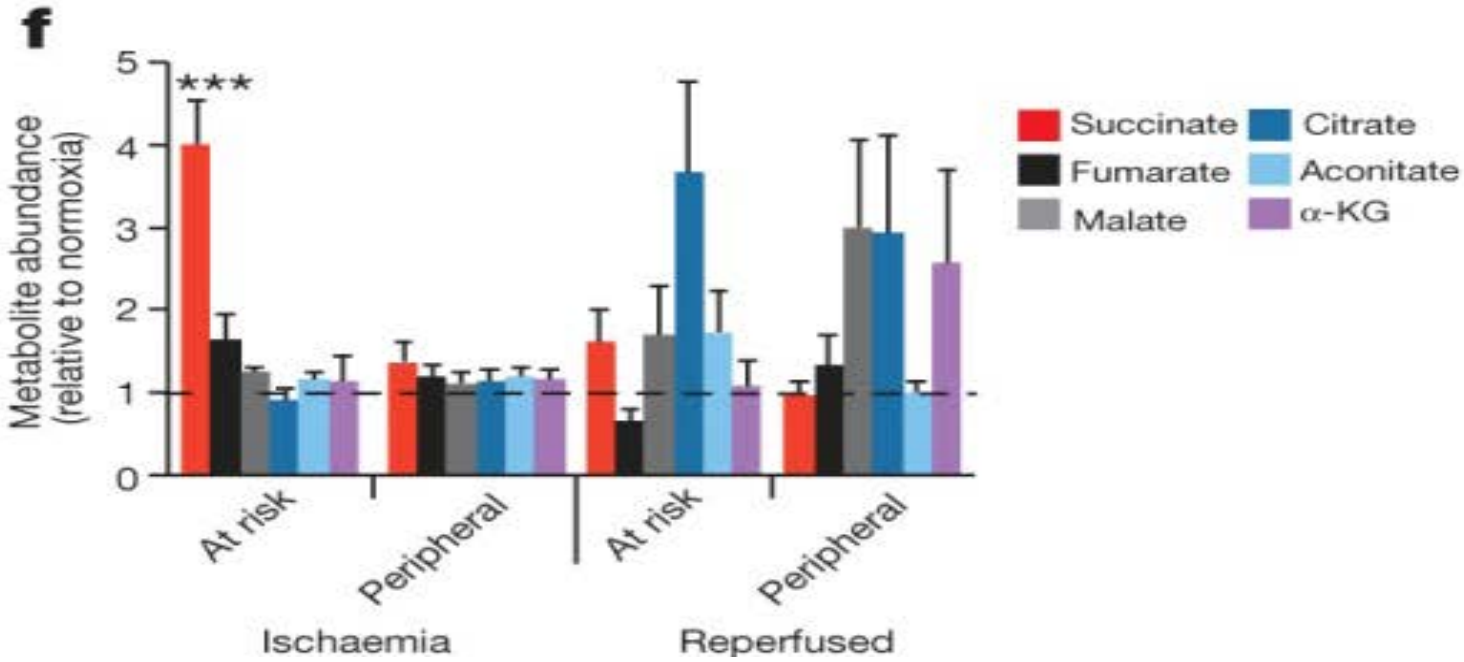
Comparative metabolomics identifies **succinate** as a potential mitochondrial metabolite that drives reperfusion ROS production



EV heart

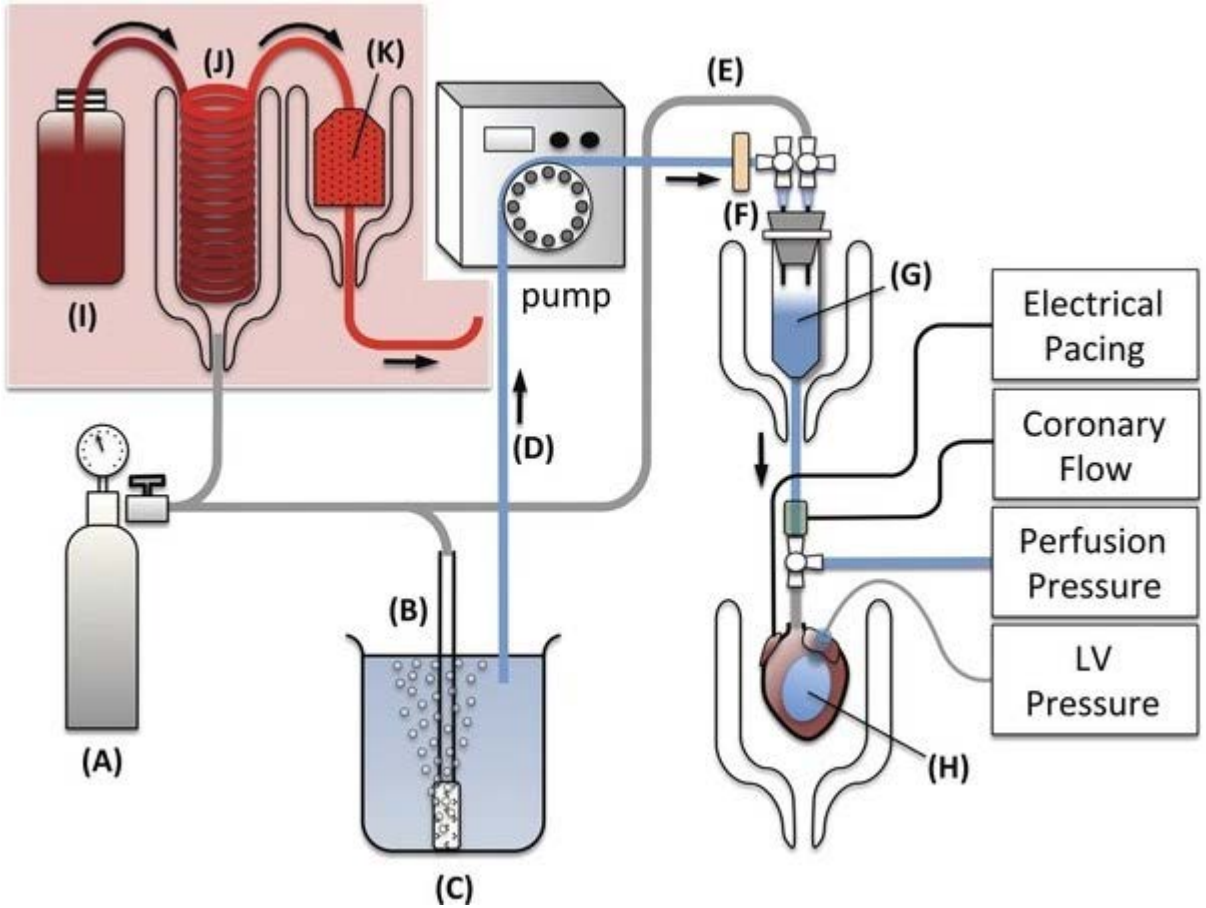


IV heart

ET Chouchani *et al. Nature* **000**, 1-5 (2014) doi:10.1038/nature13909

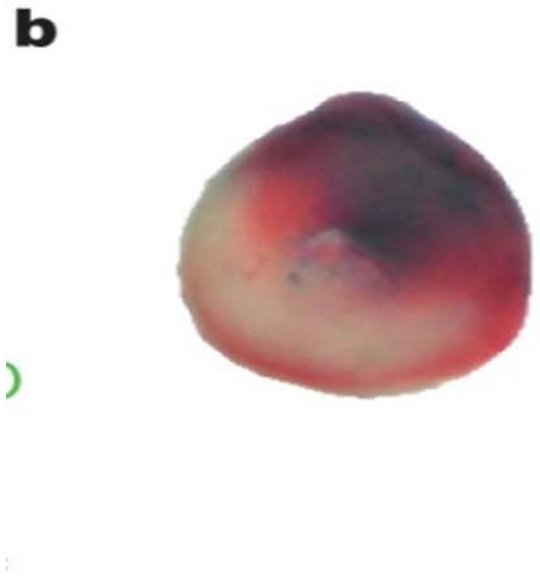
nature

Method



Langendorff apparatus

Method



Infarct size measurement by triphenyltetrazolium chloride staining: **infarcted areas appear white**

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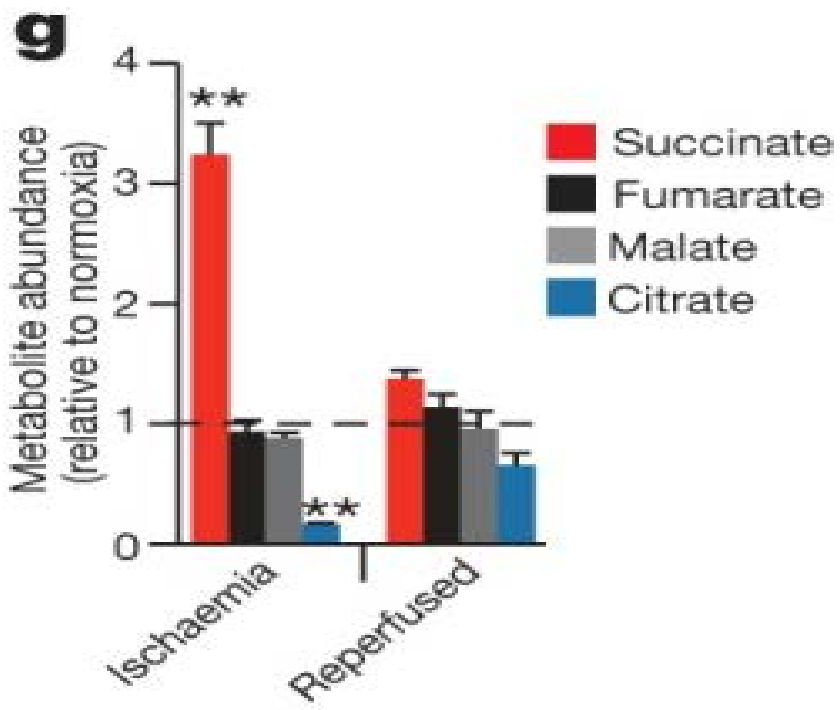
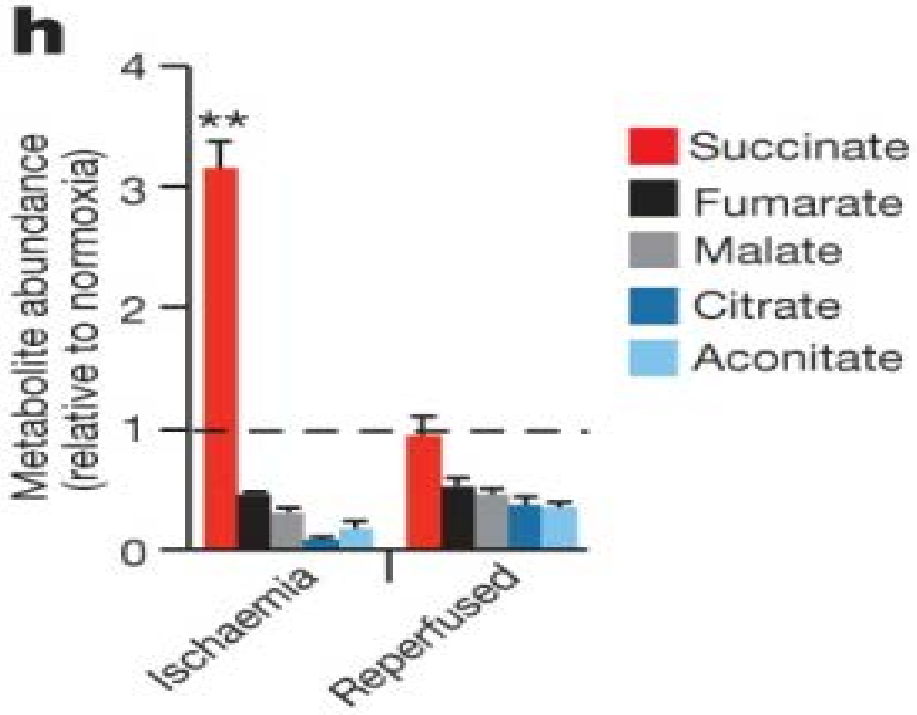


IV kidney



IV brain

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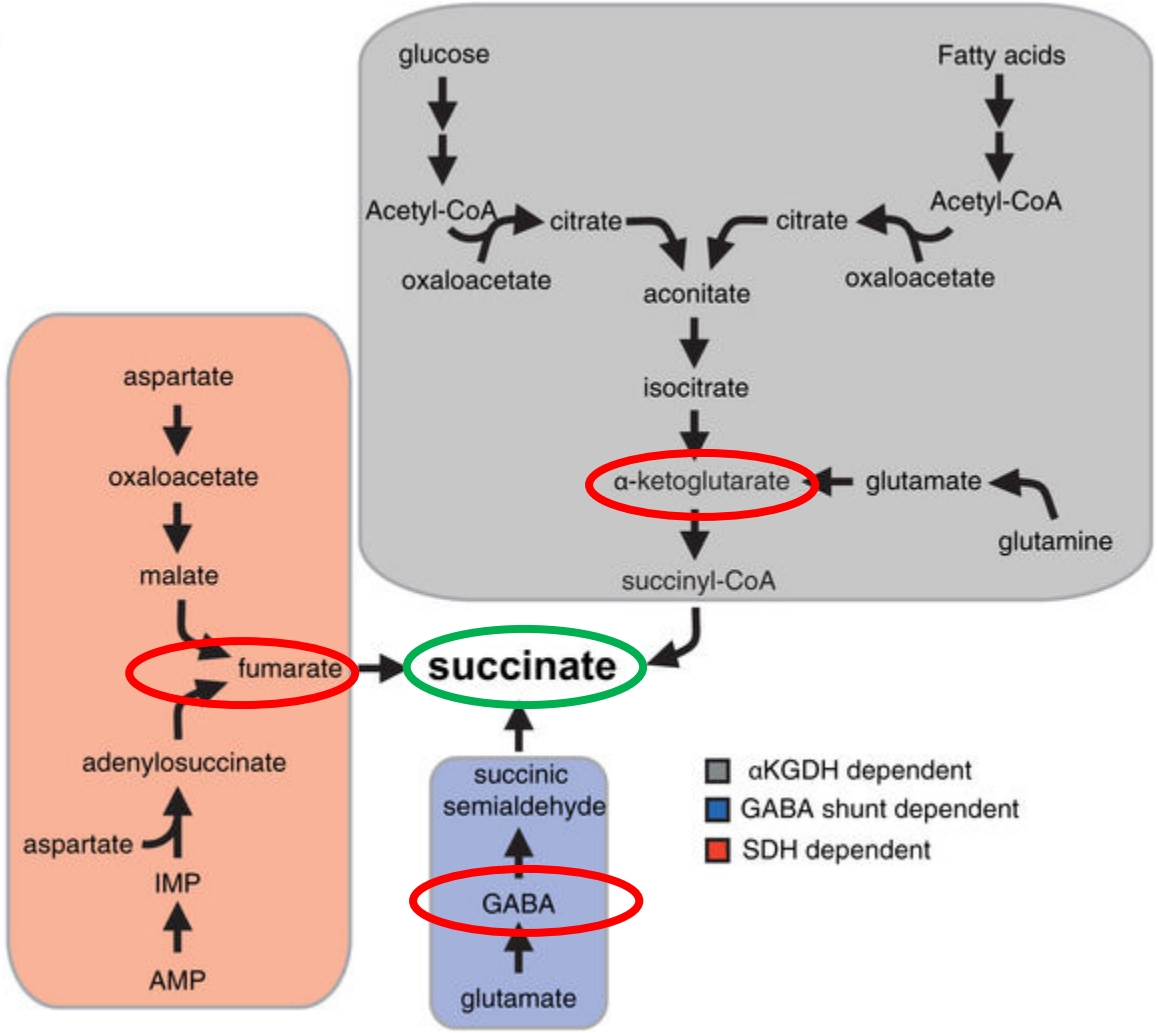
Summary of the three potential metabolic inputs for succinate-directed ischaemic flux:

Summary of the three potential metabolic inputs for succinate-directed ischaemic flux:

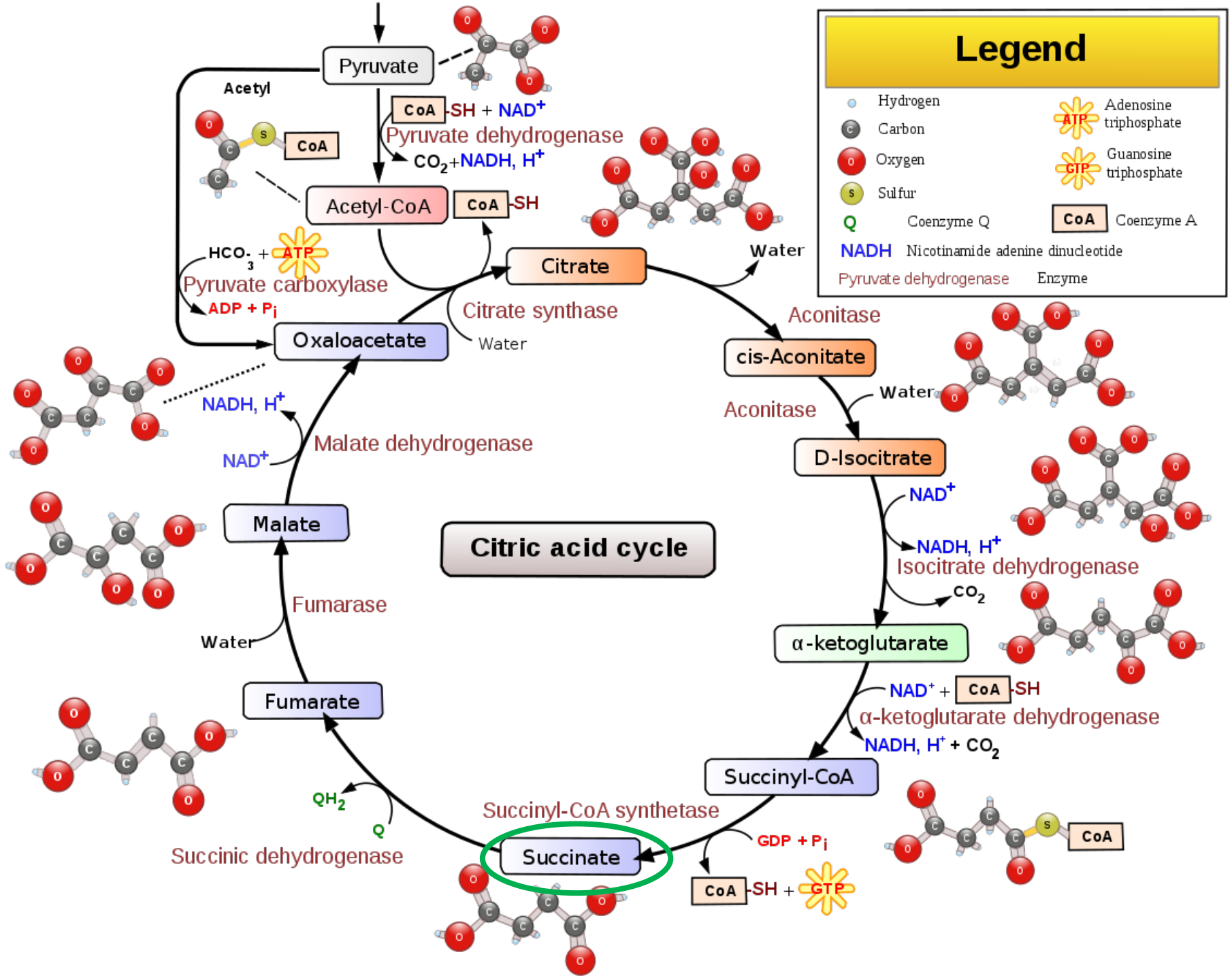
1. from a-ketoglutarate produced by the CAC, derived from glycolysis, fatty acid oxidation, and glutaminolysis (grey box),

2. from succinic semialdehyde produced from the **GABA shunt** (blue box),

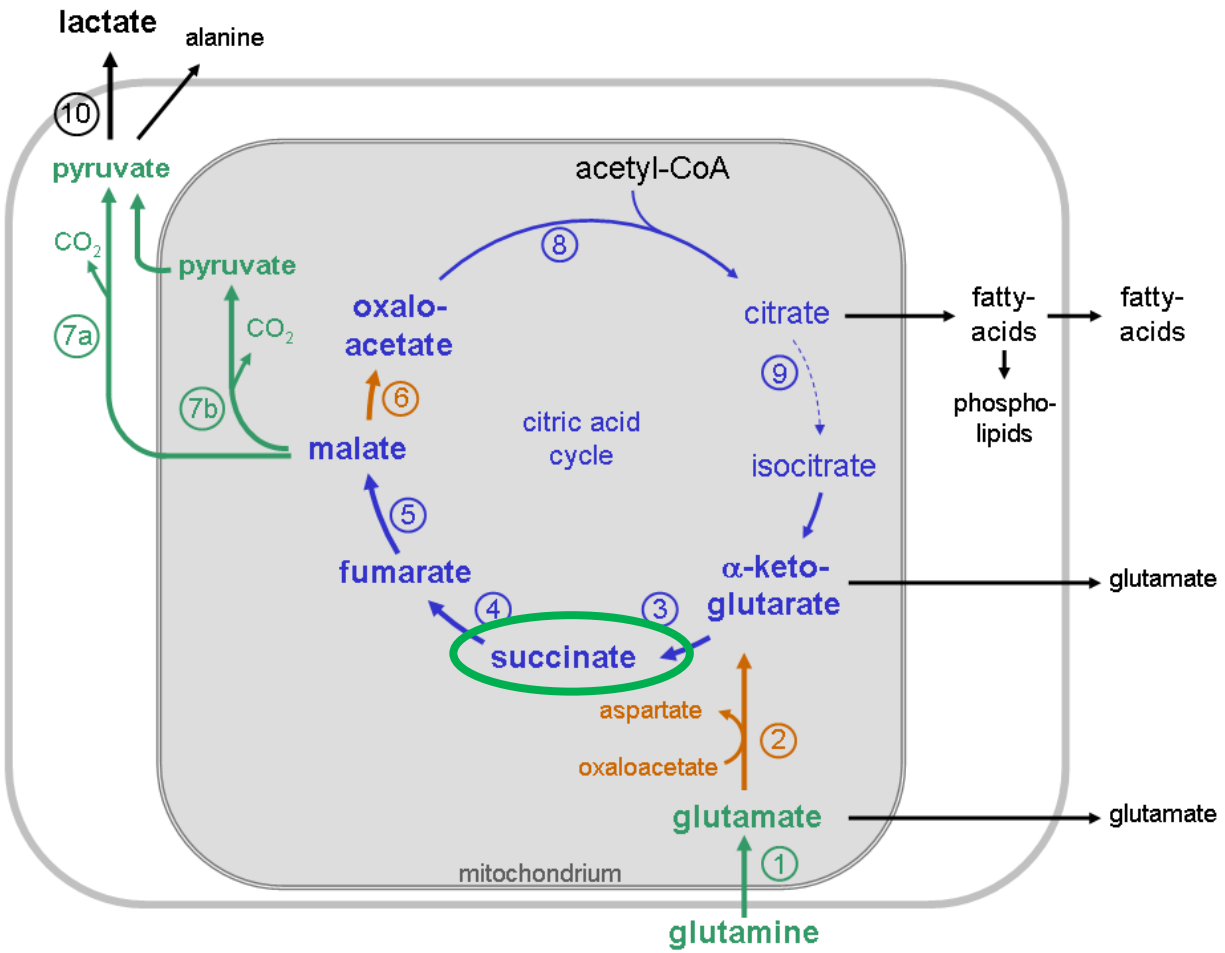
3. and from fumarate produced from the **malate-aspartate shuttle** and **purine nucleotide cycle - PNC** (red box) via the reversal of SDH.



citric acid cycle (CAC), tricarboxylic acid (TCA) cycle, Krebs cycle



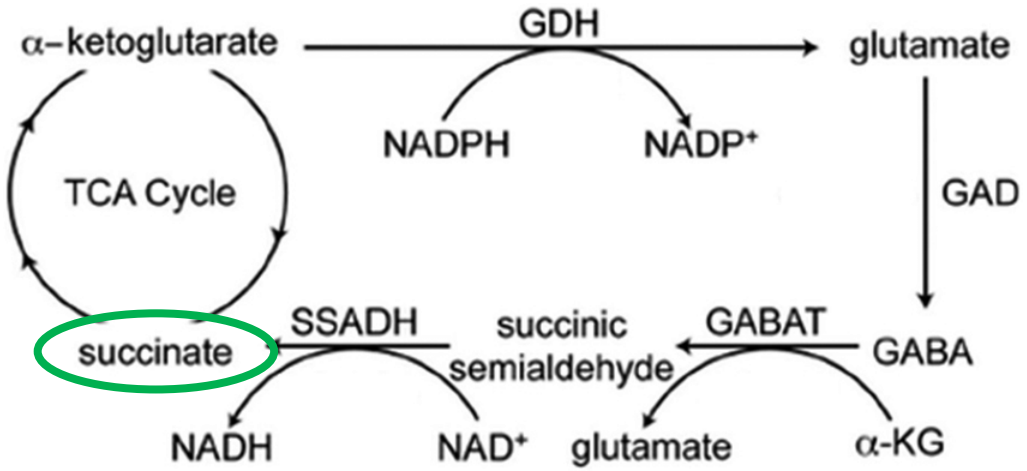
Glutaminolysis



In proliferating and tumor cells the lactate produced does not only derive from the conversion of glucose. Part of the lactate is also produced by the degradation of the amino acid glutamine. The conversion of the amino acid glutamine to lactate has been termed – in anology to glycolysis – as glutaminolysis.

GABA shunt

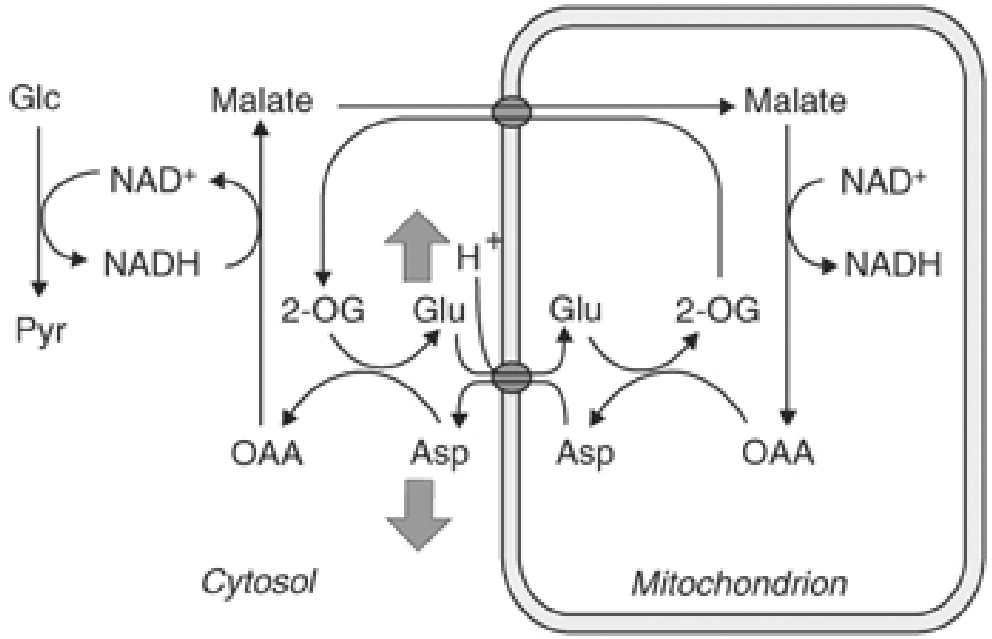
GABA: gamma-aminobutyric acid



L-glutamate is formed by transamination of 2-oxoglutarate (KG) from the tricarboxylic acid (TCA) cycle. The reaction is catalyzed by the mitochondrial glutamate dehydrogenase. L-glutamate is decarboxylated by cytosolic GAD (glutamate decarboxylase) to GABA which is transported into mitochondria where is deaminated by GABAT (GABA transaminase) to succinate semialdehyde. The last enzyme of GABA shunt is succinate semialdehyde dehydrogenase (SSADH) which catalyzes succinate semialdehyde oxidation to succinate. The latter compound enters the TCA cycle and GABA can be resynthesized from 2-oxoglutarate.

The GABA shunt serves two functions, it can mediate both the catabolism of GABA and channels L-glutamate to the TCA cycle, bypassing two steps of that cycle.

Malate-aspartate shuttle (MAS)



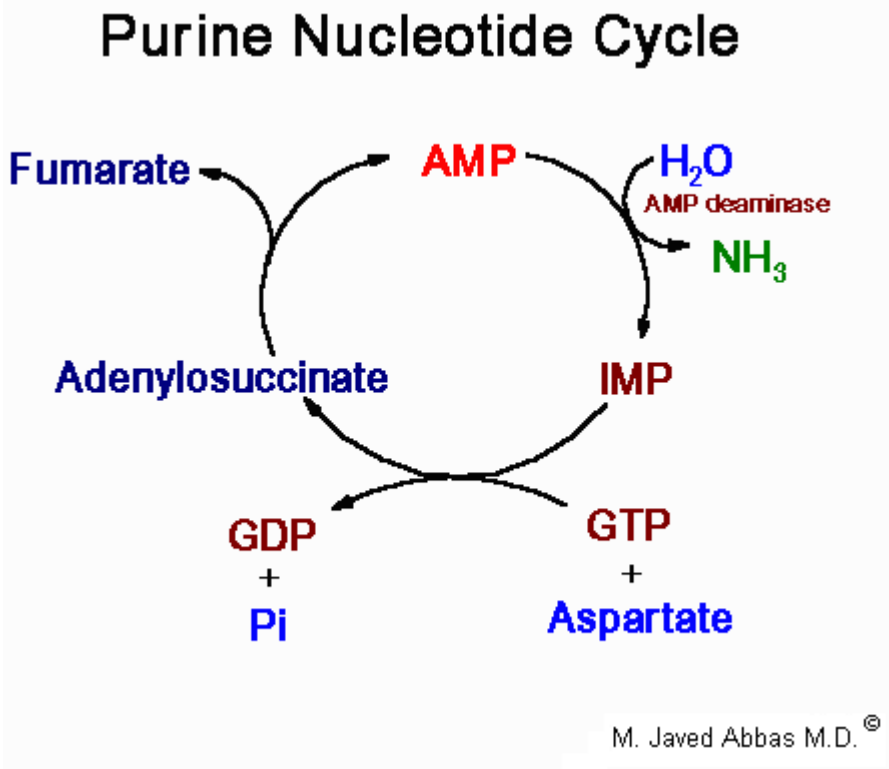
Our heart and liver cells use a process called the malate-aspartate shuttle to transport NADH molecules produced in glycolysis into the matrix of the mitochondria. We can break down this shuttle into seven steps:

(1) The NADH produced in glycolysis is used to reduce oxaloacetate into malate
(2) The malate then moves into the intermembrane space and then enters the matrix via an antiporter transport system in exchange for an alpha-ketoglutarate
(3) In the matrix, the malate is then oxidized back into oxaloacetate and the pair of electrons are collected by NAD+ to form an NADH molecule. This NADH can now be used by complex I of the electron transport chain
(4) The oxaloacetate cannot move across the inner mitochondrial membrane and so a transamination reaction is needed to convert it into aspartate
(5) The aspartate can now flow out of the inner membrane via an antiporter system in exchange for glutamate
(6) The glutamate that moves into the matrix transfers an amino group onto oxaloacetate to form aspartate and alpha-ketoglutarate
(7) The aspartate transported into the cytoplasm is deaminated to form oxaloacetate. The amino group is used to form glutamate from alpha-ketoglutarate.

Purine-nucleotide cycle (PNC)

The Purine Nucleotide Cycle is a metabolic pathway in which fumarate is generated from aspartate in order to increase the concentration of Krebs cycle intermediates. The pathway was first described by John Lowenstein, who demonstrated its role in increasing the rate of oxidative phosphorylation in skeletal muscle.

The purine nucleotide cycle serves an important function within exercising muscle. The generation of fumarate provides skeletal muscle with its' only source of anaplerotic substrate for the TCA cycle. In order for continued operation of the cycle during exercise, muscle protein must be utilized to supply the amino nitrogen for the generation of aspartate. The generation of asparate occurs by the standard transamination reactions that interconvert amino acids with a-ketoglutarate to form glutamate and glutamate with oxaloacetate to form aspartate.

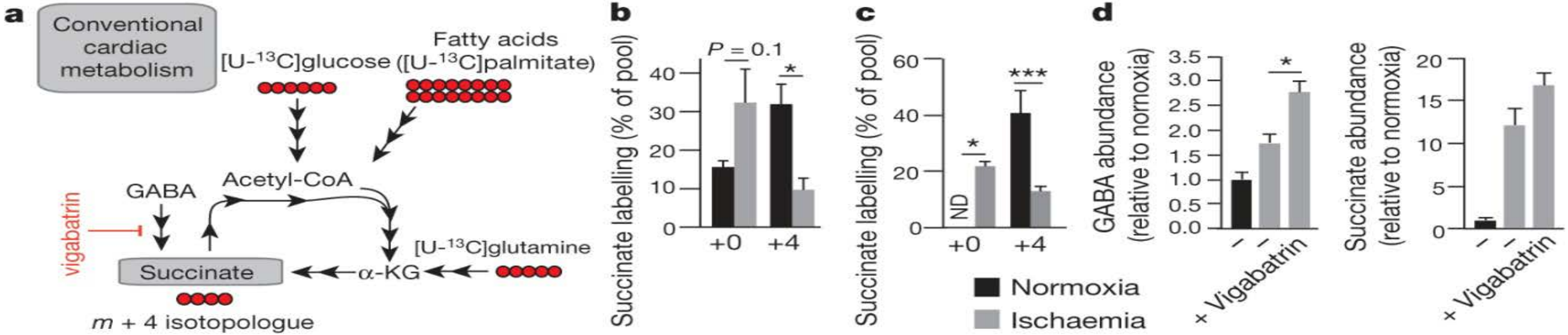


Reverse SDH activity drives ischaemic succinate accumulation by the reduction of fumarate.

13C-glucose

13C-palmitate

GABA shunt inhibited



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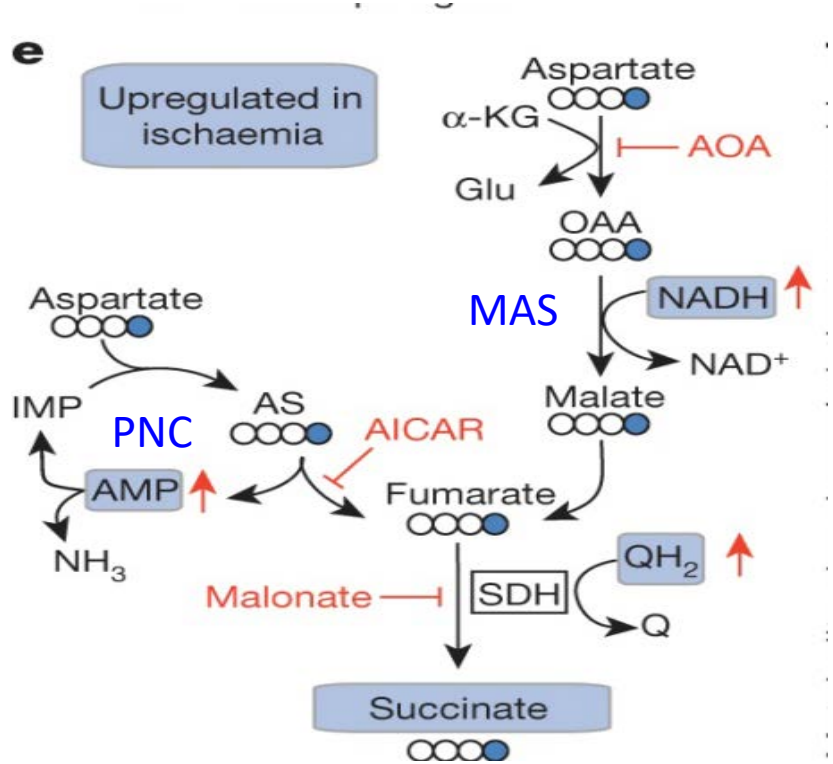
nature

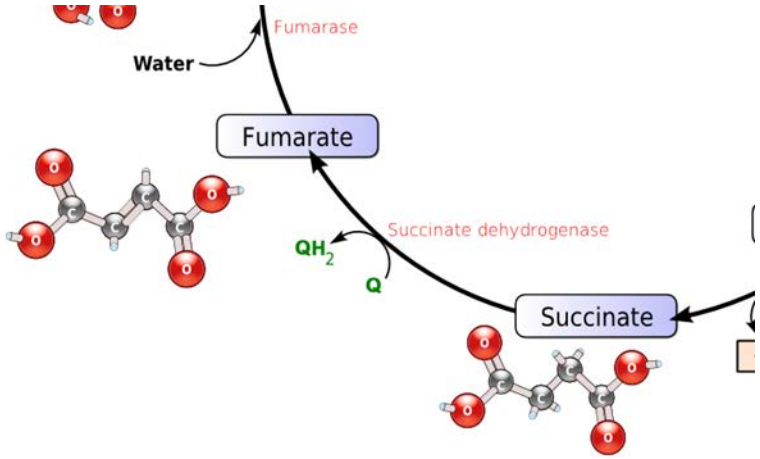
Also: Glutamine was not a major carbon source for CAC metabolites in normoxia or ischaemia and the minimal 13C-glutamine incorporation to α-ketoglutarate was decreased in ischaemia.

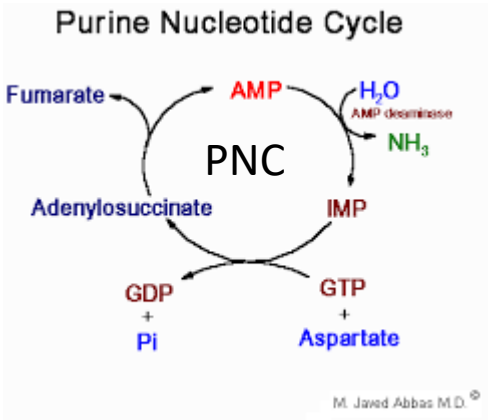
Together, these data demonstrate that the major carbon sources for the CAC under normoxia do not significantly contribute to the build-up of succinate during ischaemia, indicating that succinate accumulation is not caused by conventional operation of cardiac metabolism.

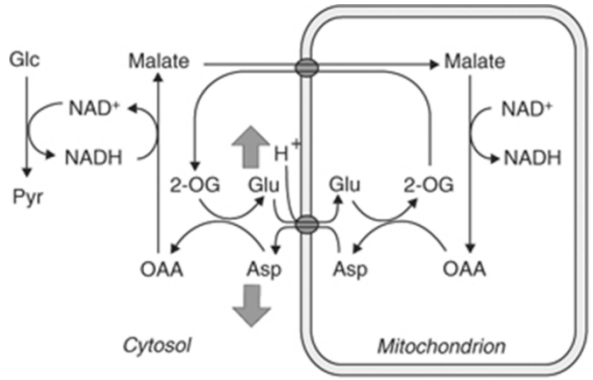
Nature. 2014 Nov 20;515(7527):431-435.
Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS.

To explore other mechanisms that could lead to succinate accumulation during ischaemia, we considered earlier speculations that **during anaerobic metabolism succinate dehydrogenase (SDH) might act in reverse to reduce fumarate to succinate**. Although SDH reversal has not been demonstrated in ischaemic tissues, *in silico* flux analysis determined succinate production by SDH reversal during ischaemia as the best solution to sustain proton pumping and ATP production when metabolites including fumarate, aspartate and malate were available The model predicted that **fumarate supply to SDH came from two converging pathways**: the malate/aspartate shuttle (**MAS**), in which the high NADH/NAD ratio during ischaemia drives malate formation that is converted to fumarate; andAMP-dependent activation of the purine nucleotide cycle (**PNC**) that drives fumarate production.









MAS

Nature. 2014 Nov 20;515(7527):431-435.
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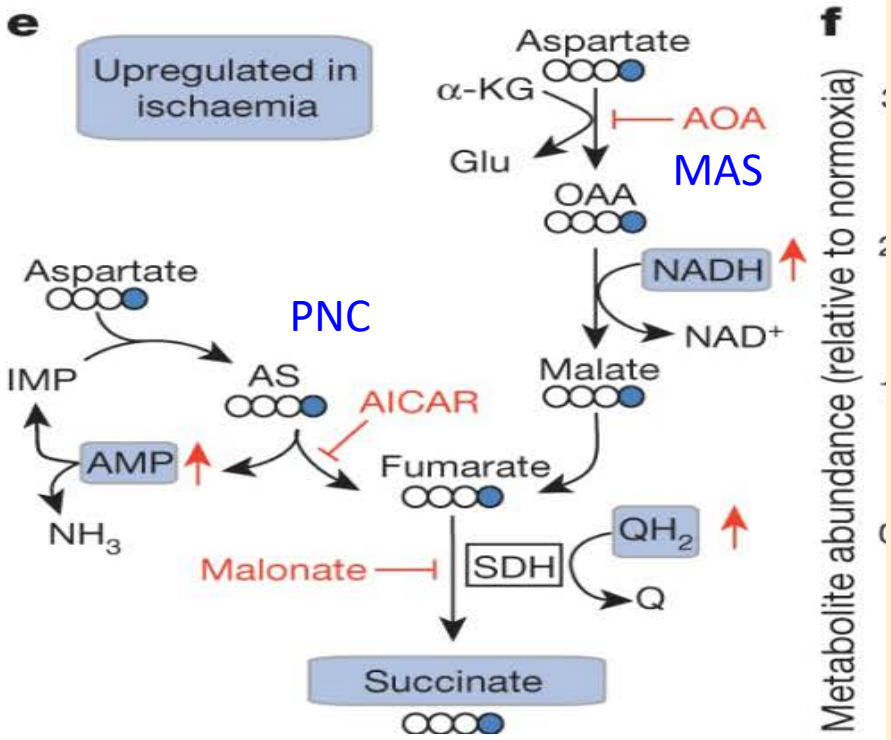
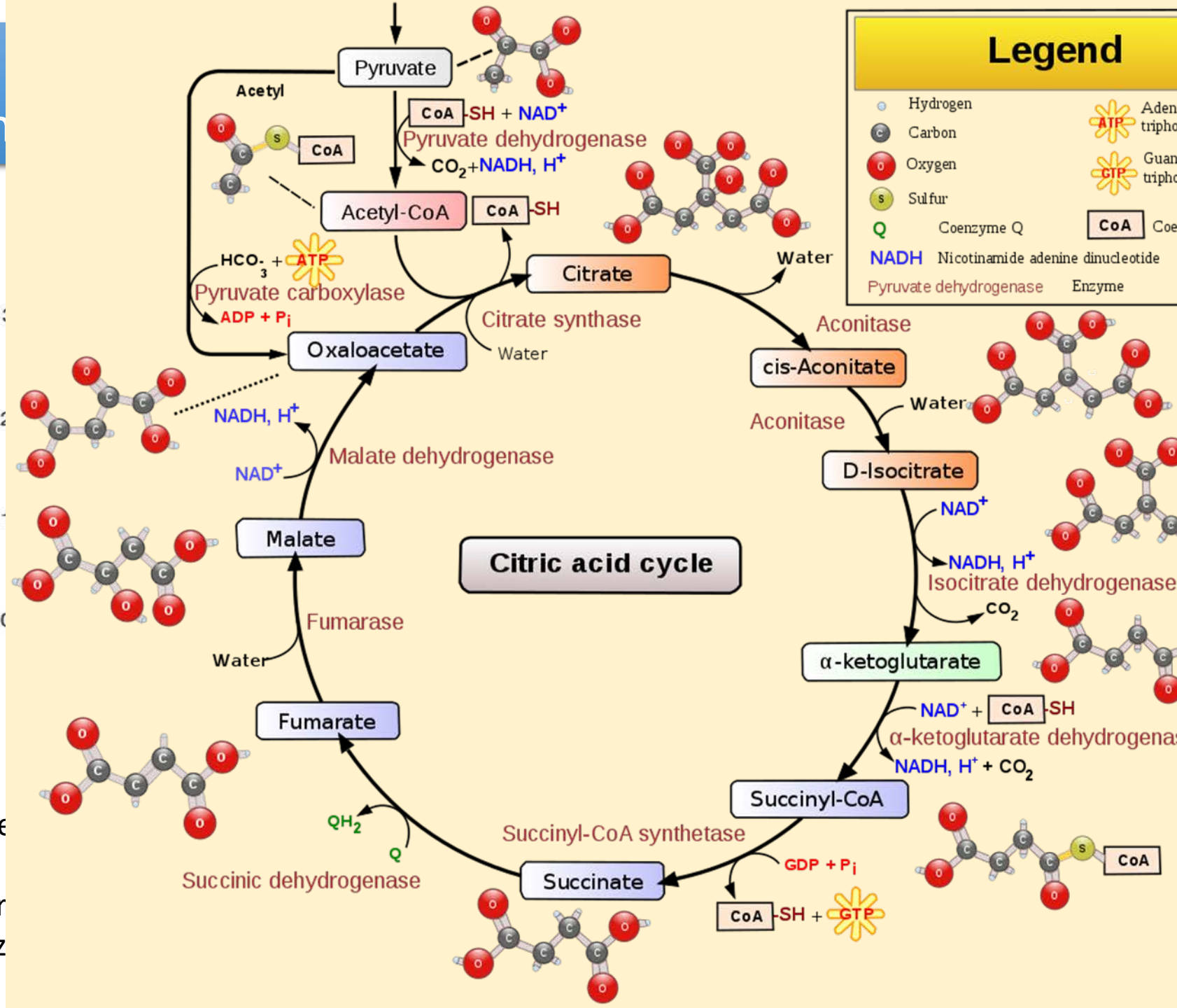
Reverse SDH activity
th



Dimethylmalonate: a membrane-permeable
inhibitor malonate.

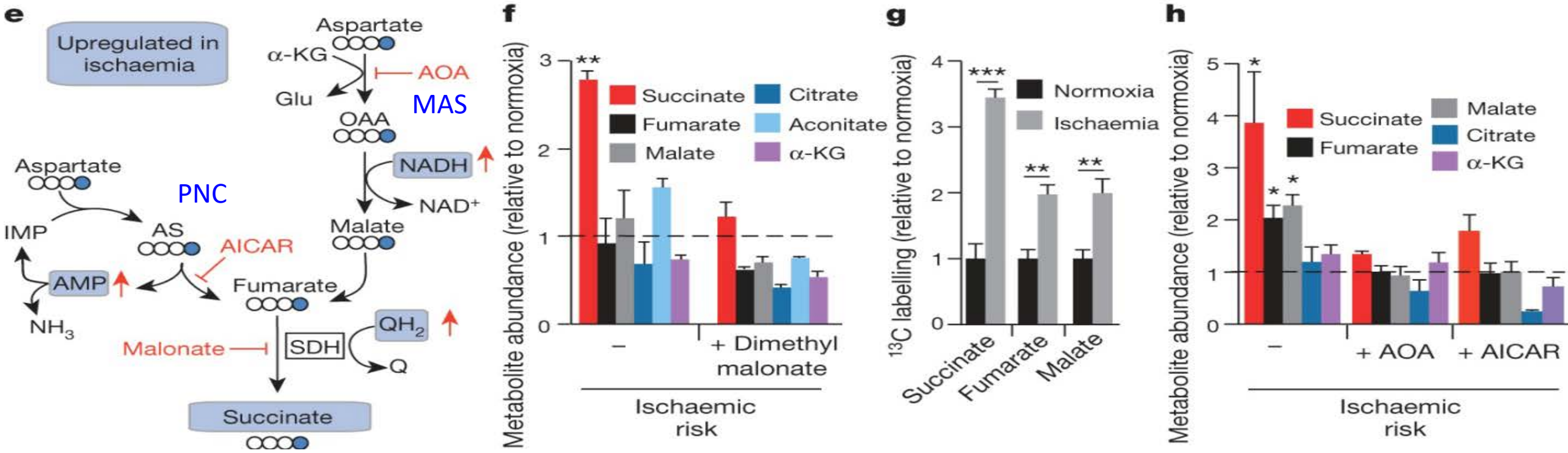
AOA: aminooxyacetate, which inhibits aspar

AICAR: 5-amino-1-b-D-ribofuranosyl-imidaz
adenylosuccinate lyase in the PNC.



Reverse SDH activity drives ischaemic succinate accumulation by the reduction of fumarate.

13C-aspartate



Dimethylmalonate: a membrane-permeable precursor of the SDH competitive inhibitor malonate.

AOA: aminooxyacetate, which inhibits aspartate aminotransferase in the MAS.

AICAR: 5-amino-1-b-D-ribofuranosyl-imidazole-4-carboxamide , which inhibits adenylosuccinate lyase in the PNC.

nature

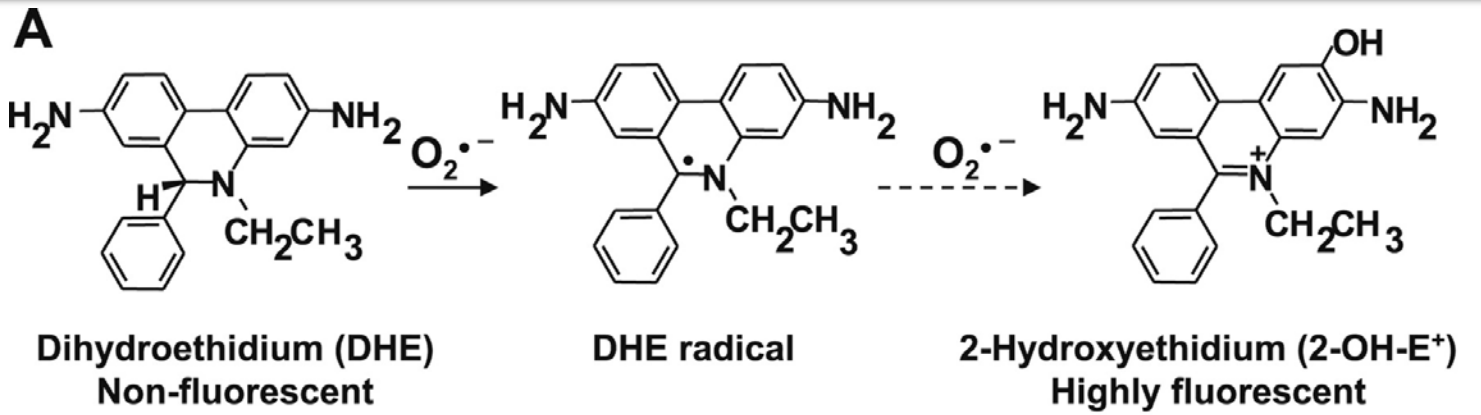
Method

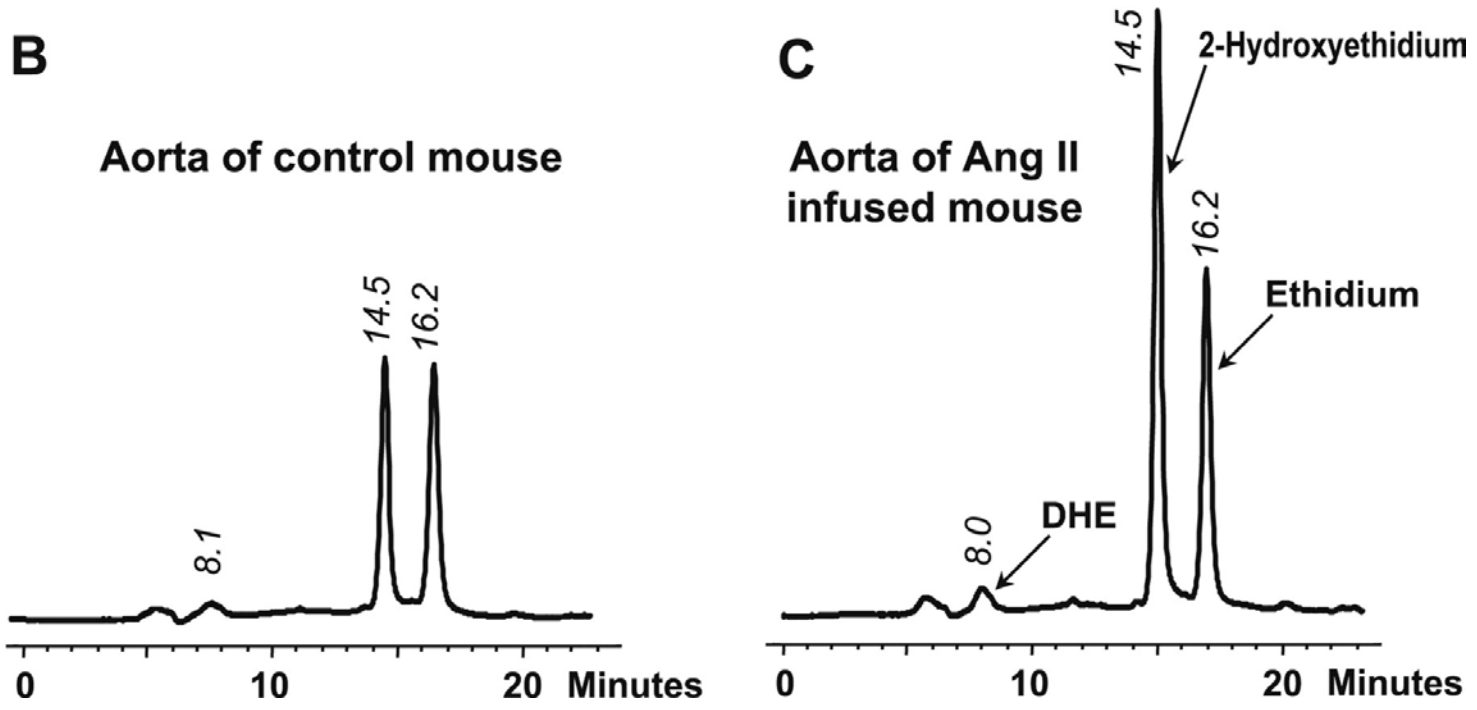
Effects on mitochondrial function:

To test whether the succinate accumulated during ischaemia could drive complex I RET on reperfusion, we tracked **mitochondrial ROS** with the fluorescent probe dihydroethidium (DHE), and **mitochondrial membrane potential** from the potential-sensitive fluorescence of tetramethylrhodamine methyl ester (TMRM), in a primary cardiomyocyte model of IR injury.

Mitochondrial membrane potential (ΔΨm). Mitochondria produce energy by establishing an electrochemical proton motive force (Δp) across their inner cell membrane, which in turn fuels the synthesis of ATP by driving the proton turbine F0F1 ATPase

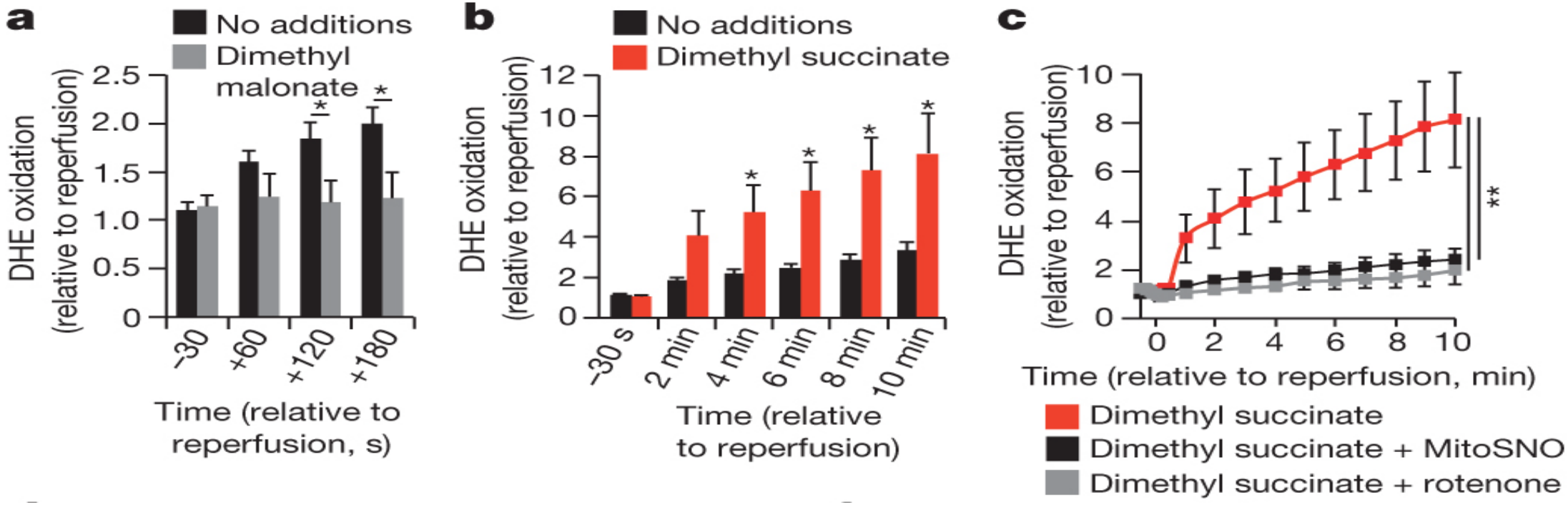
Method





Hypertension. 2007;49:717-727

Ischaemic succinate levels control ROS production in adult primary cardiomyocytes and in the heart *in vivo*.



primary cardiomyocytes

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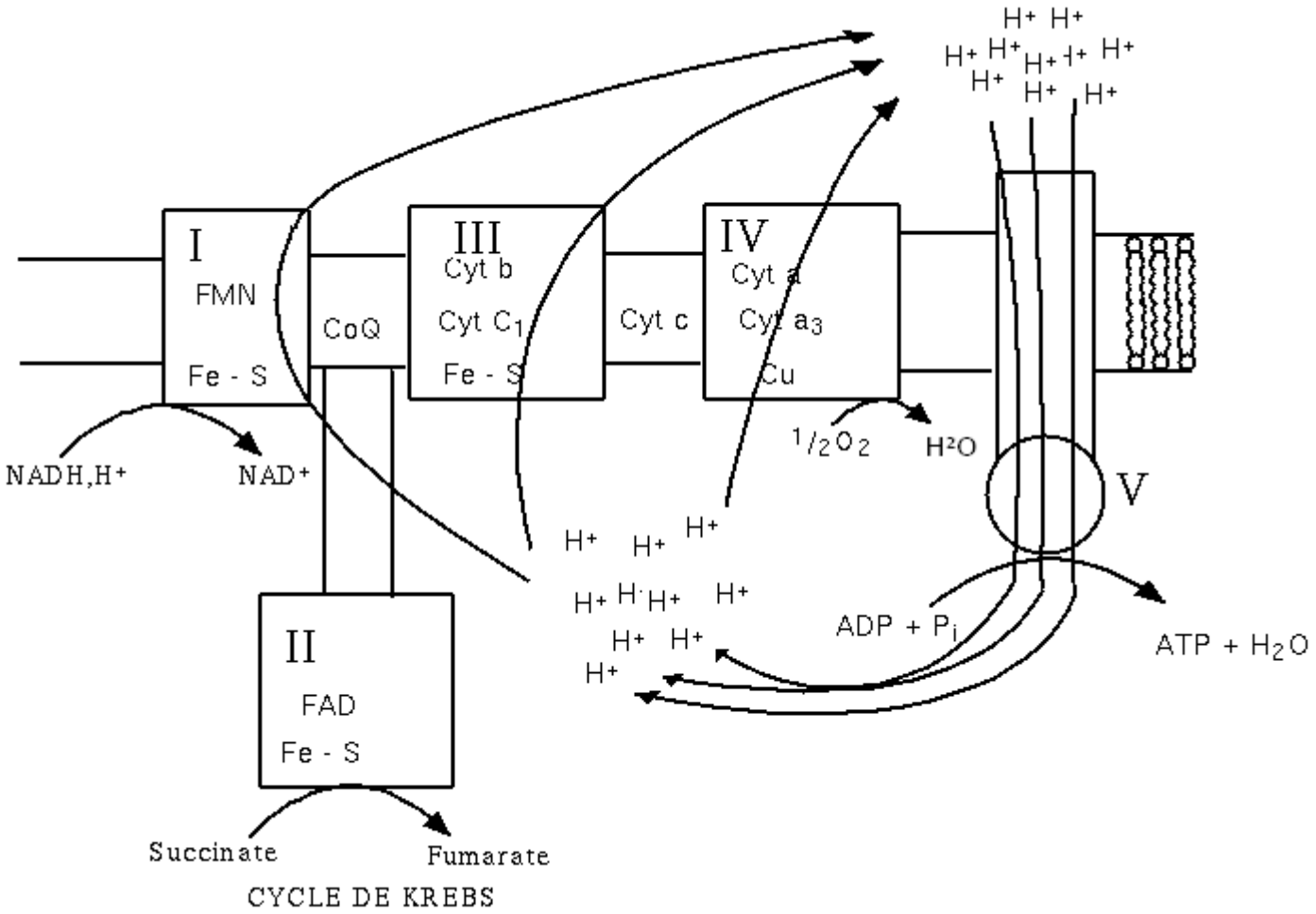
Dimethyl succinate, a cell-permeable derivative of succinate,, which is readily taken up by cells, where it is then hydrolysed thereby increasing succinate levels

nature

Method



Rotenone, a plant product used as insecticide and pesticide, block the ETC between NADH dehydrogenase (Complex I) and CoQ.

Method

MitoSNO works by briefly 'switching off' the mitochondria in the first few minutes after blood flow is returned to prevent a build-up of free radicals that can kill heart cells. To achieve this, MitoSNO is designed to accumulate inside heart mitochondria rapidly after its injection into the blood.

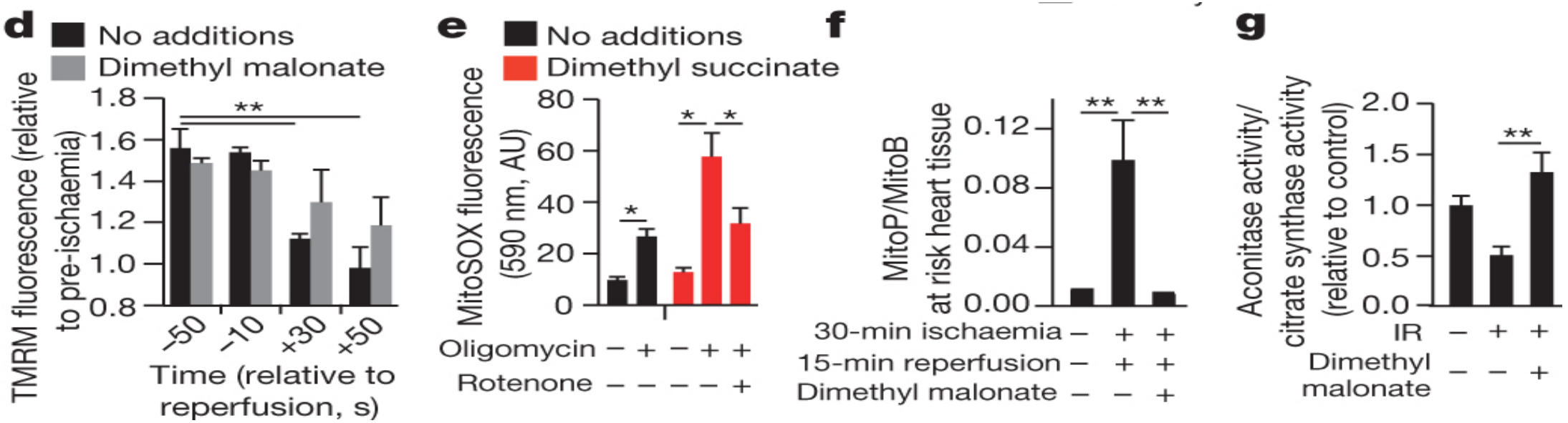
Dr Mike Murphy from the MRC Mitochondrial Biology Unit, who led the study, said: "When cells are starved of oxygen for any length of time, they begin to shut down. When blood rushes back the mitochondria go into over-drive, churning out free radicals that cause the cells to die. MitoSNO effectively flicks a switch in the mitochondria, slowing down reactivation during those critical first minutes when blood flow returns and protecting the heart tissue from further damage.

http://www.cam.ac.uk/research/news/new-drug-could-protect-from-tissue-damage-following-heart-attack

Nat Med. 2013 Jun;19(6):753-9.

Ischaemic succinate levels control ROS production in adult primary cardiomyocytes and in the heart *in vivo*.

C2C12myoblasts *heart*



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TMRM: mitochondrial membrane potential from the potential-sensitive fluorescence of tetramethylrhodamine methyl ester

nature

Method

Nat Protoc. 2012 Apr 19;7(5):946-58. doi: 10.1038/nprot.2012.035.

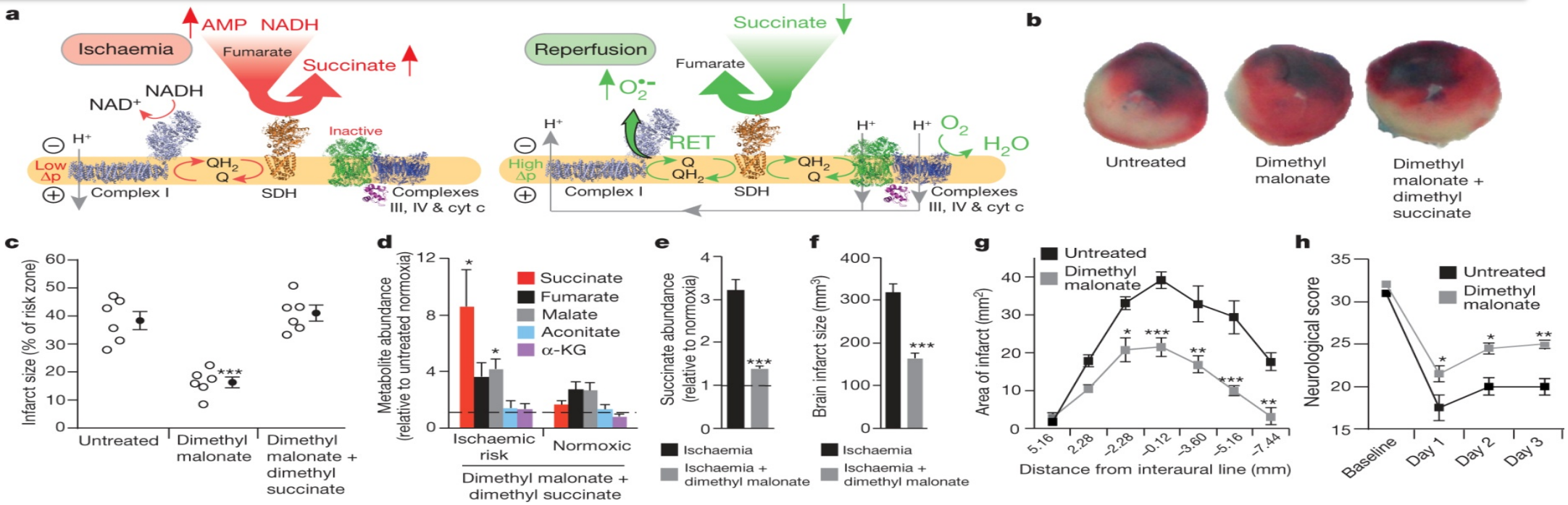
nature protocols

Using the mitochondria-targeted ratiometric mass spectrometry probe MitoB to measure H2O2 in living Drosophila.

Cochemé HM[1], Logan A, Prime TA, Abakumova I, Quin C, McQuaker SJ, Patel JV, Fearnley IM, James AM, Porteous CM, Smith RA, Hartley RC, Partridge L, **Murphy** MP.

The role of hydrogen peroxide (H(2)O(2)) in mitochondrial oxidative damage and redox signaling is poorly understood, because it is difficult to measure H(2)O(2) in vivo. Here we describe a method for assessing changes in H(2)O(2) within the mitochondrial matrix of living Drosophila. We use a ratiometric mass spectrometry probe, MitoB ((3-hydroxybenzyl)triphenylphosphonium bromide), which contains a triphenylphosphonium cation component that drives its accumulation within mitochondria. The arylboronic moiety of MitoB reacts with H(2)O(2) to form a phenol product, MitoP. On injection into the fly, MitoB is rapidly taken up by mitochondria and the extent of its conversion to MitoP enables the quantification of H(2)O(2). To assess MitoB conversion to MitoP, the compounds are extracted and the MitoP/MitoB ratio is quantified by liquid chromatography-tandem mass spectrometry relative to deuterated internal standards. This method facilitates the investigation of mitochondrial H(2)O(2) in fly models of pathology and metabolic alteration, and it can also be extended to assess mitochondrial H(2)O(2) production in mouse and cell culture studies.

NADH and AMP sensing pathways drive ischaemic succinate accumulation to control reperfusion pathologies *in vivo* through mitochondrial ROS production.



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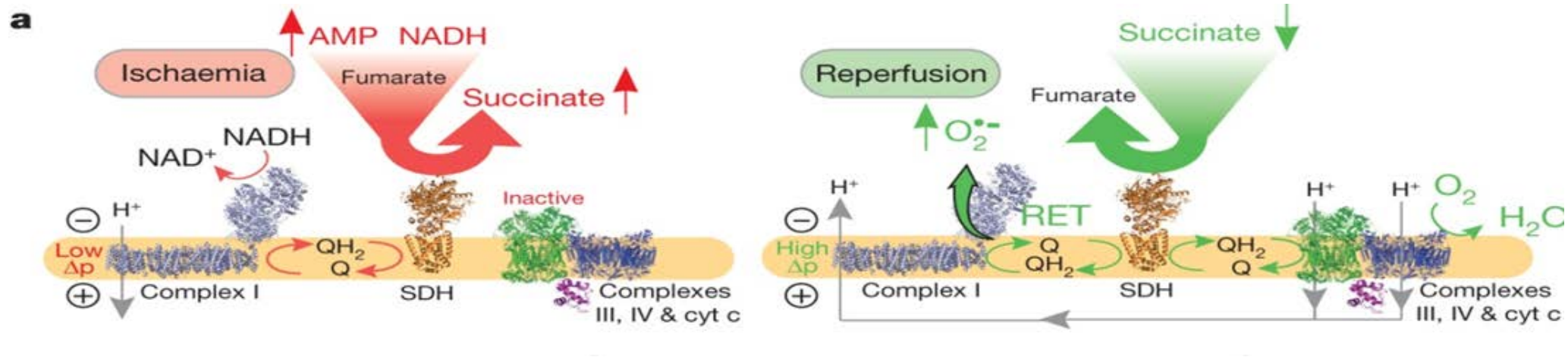
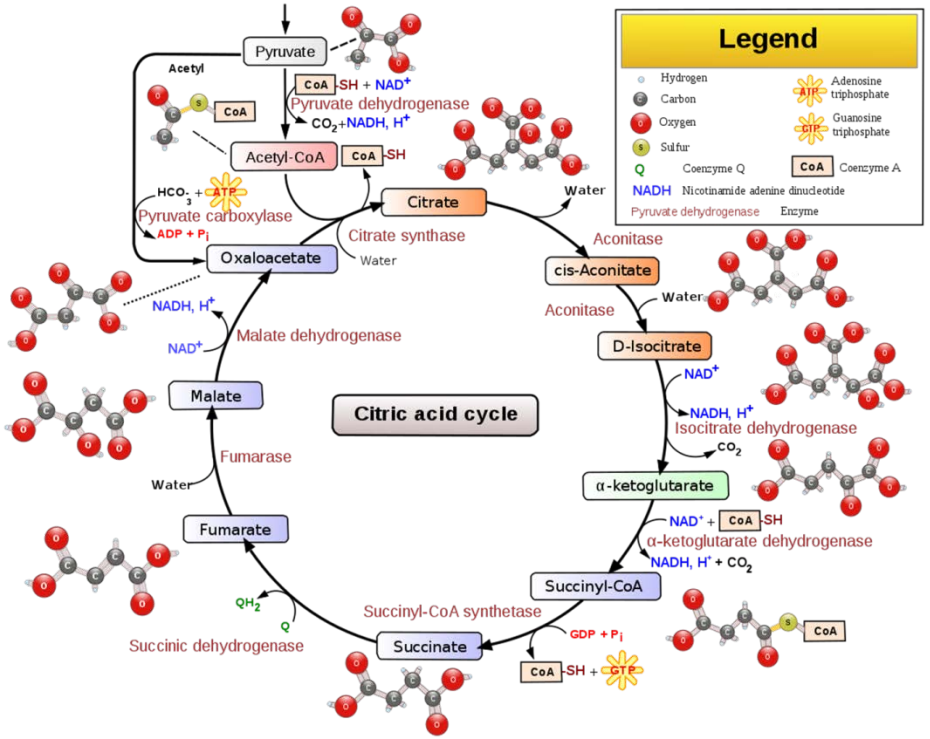
nature



Our findings suggest the following **model** (Fig. 4a): during ischaemia, fumarate production increases, through activation of the MAS and PNC, and is then reduced to succinate by SDH reversal. After reperfusion, the accumulated succinate is rapidly oxidized,
thereby sustaining a large proton-motive force by conventional electron transport through complexes III and IV to oxygen, while also driving RET at complex I to produce the mitochondrial ROS that initiate IR injury .
This model provides a unifying framework for many hitherto unconnected aspects of IR injury, such as the requirement for time-dependent priming during ischaemia to induce ROS upon reperfusion, protection against IR injury by the inhibition of complexes I and II,
and by mild uncoupling. RET is produced when electrons from ubiquinol are transferred back to respiratory complex I, reducing NAD+ to NADH. This process generates a significant amount of ROS.



Ischaemic succinate accumulation to control reperfusion pathologies *in vivo* through mitochondrial ROS production.

Notably, our model also generates an unexpected, but testable, **prediction**.

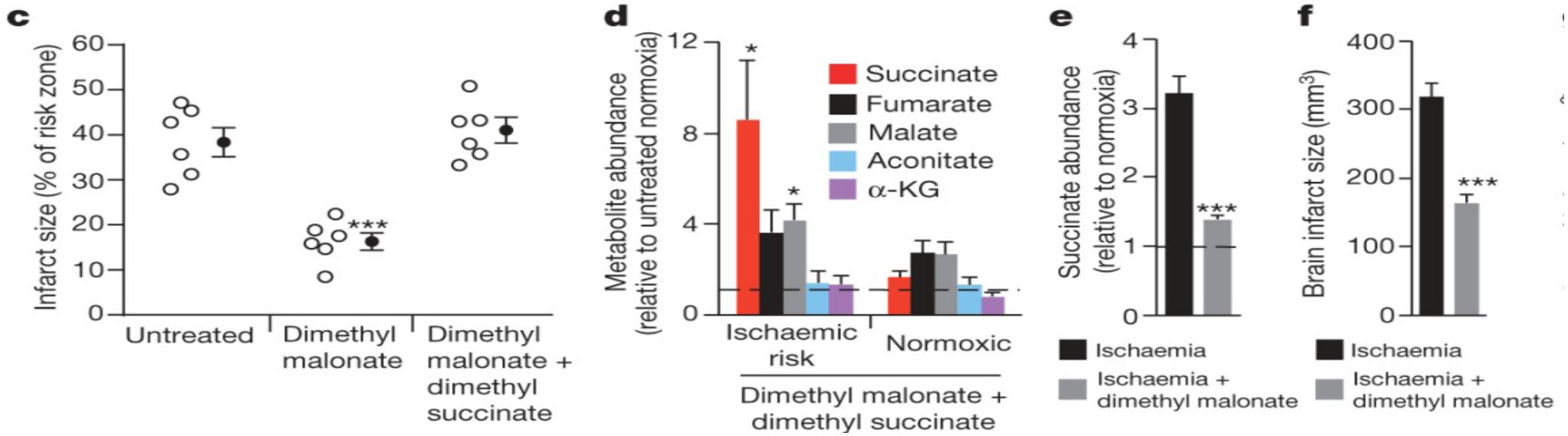
Manipulation of the pathways that increase succinate during ischaemia and oxidize it on reperfusion should determine the extent of IR injury.

Because the reversible inhibition of SDH blocks both succinate accumulation during ischaemia (see above) and its oxidation upon reperfusion, it should protect against IR injury in vivo.

Ischaemic succinate accumulation to control reperfusion pathologies *in vivo* through mitochondrial ROS production.

in vivo model of cardiac IR injury

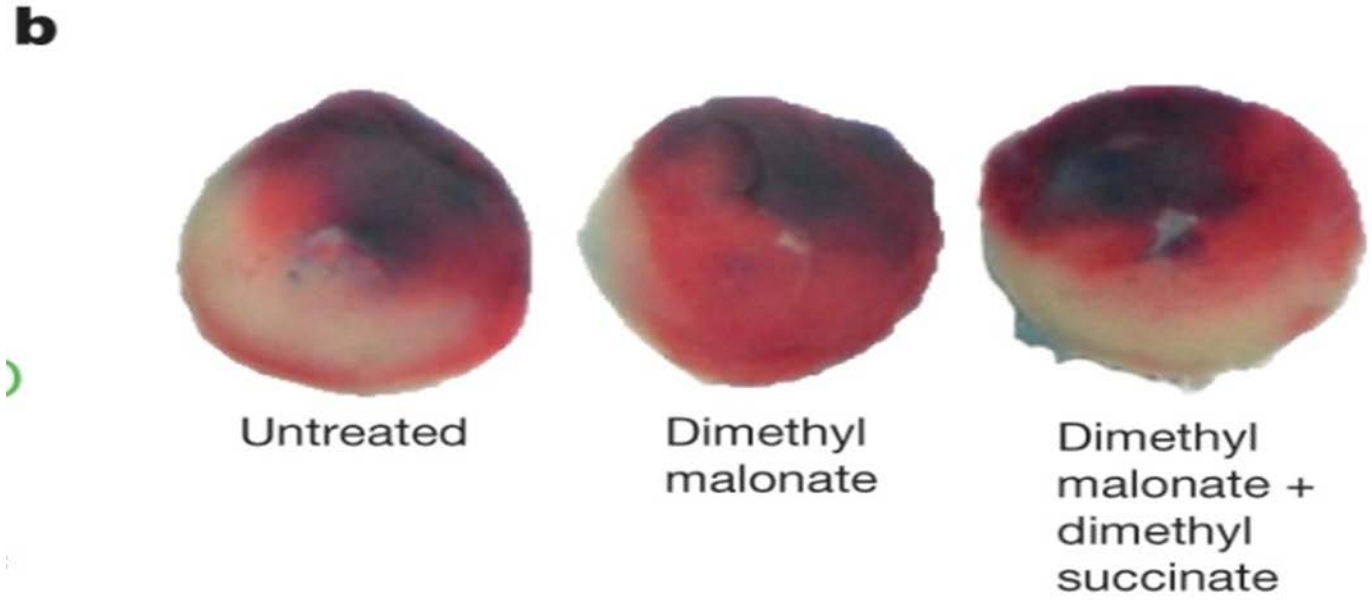
Ischaemic brain



ET Chouchani *et al. Nature* **000**, 1-5 (2014) doi:10.1038/nature13909

nature

Method

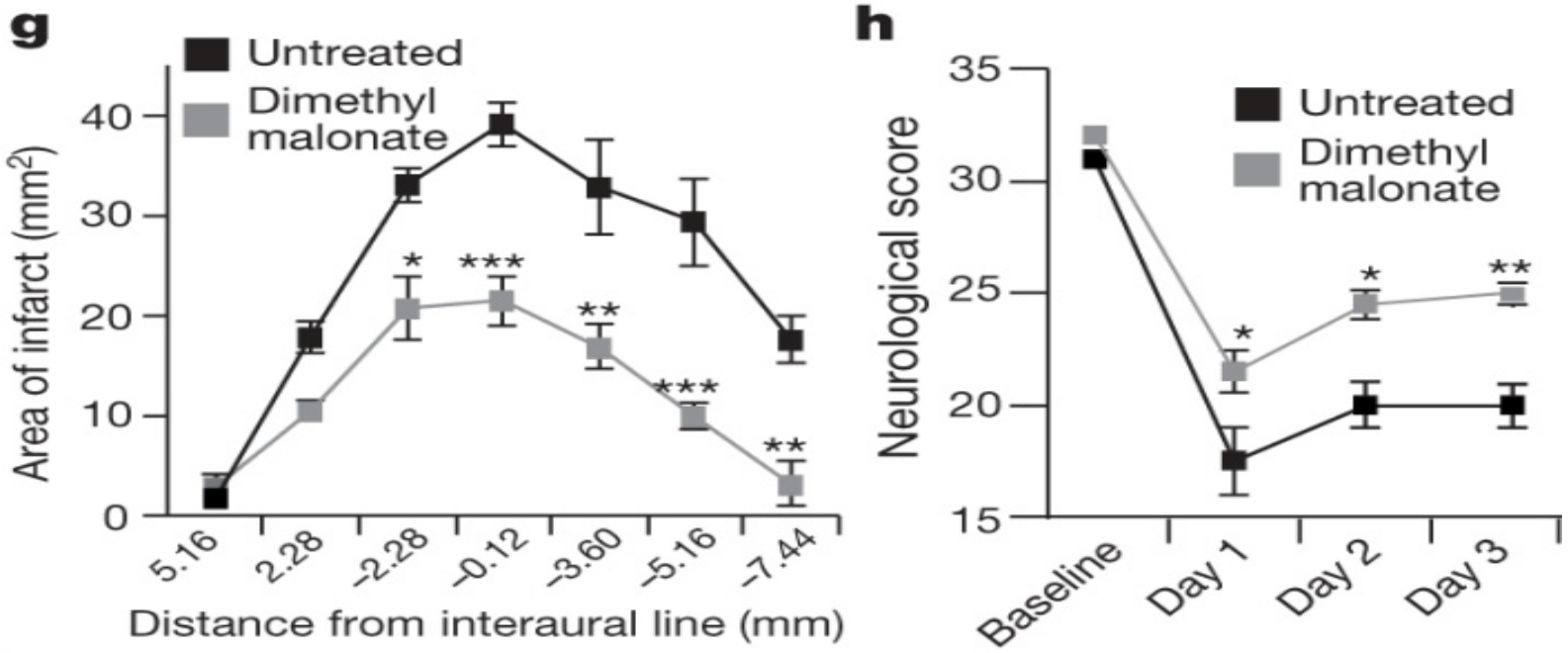


Infarct size measurement by triphenyltetrazolium chloride staining: **infarcted areas appear white**

Nature. 2014 Nov 20;515(7527):431-435.
Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS.

Ischaemic succinate accumulation to control reperfusion pathologies *in vivo* through mitochondrial ROS production.

Ischaemic brain



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Summary

Accumulation of succinate, via fumarate production and reversal of SDH, is a universal metabolic signature of ischaemia in vivo.

Succinate is a primary driver of the mitochondrial ROS production on reperfusion that underlies IR injury in a range of tissues.

Ischaemic accumulation of succinate may be of further relevance via its role in inflammatory and hypoxic signalling. Thus succinate could contribute to both the acute pathogenesis of IR injury by mitochondrial ROS, and then upon secretion also trigger inflammation and neovascularisation.

Mitochondrial ROS produced by RET at complex I may normally act as a redox signal from mitochondria that responds to changes in electron supply to the Q pool and ATP demand, but is grossly over-activated in IR injury.

Preventing succinate accumulation during ischaemia is protective against IR injury in vivo, suggesting novel therapeutic targets for IR injury in pathologies such as heart attack and stroke.

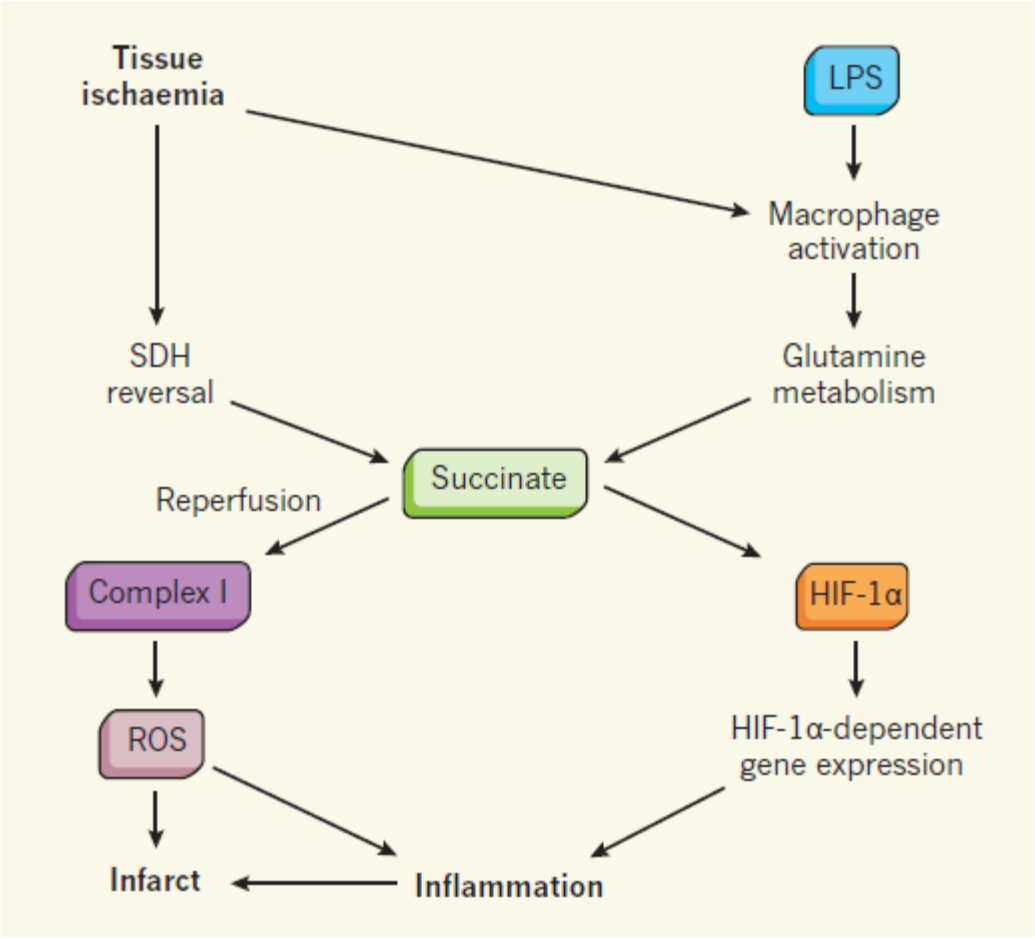

Figure 1 | Succinate in inflammation and infarct. Chouchani *et al.*[1] show that the metabolic intermediate succinate is markedly elevated during ischaemia — oxygen deprivation to a tissue as a result of blocked blood supply. This accumulation occurs through the reverse activity of the enzyme succinate dehydrogenase (SDH). On blood reperfusion, the succinate is oxidized, leading to reverse electron transport through complex 1 (a multiprotein enzyme complex), which generates reactive oxygen species (ROS) — molecules that mediate the infarct (damaged tissue) seen in strokes and heart attacks and that promote inflammation. Succinate has also been implicated in inflammation driven by macrophage cells that are activated when the receptor TLR4 is bound by the bacterial component lipopolysaccharide (LPS)[2] or, perhaps, by products of ischaemic tissue[10]. In this case, the succinate is generated from the metabolism of glutamine, and leads to activation of the transcription factor HIF-1α and expression of genes encoding pro-inflammatory proteins.

Nature. 2013 Apr 11;496(7444):238-42. doi: 10.1038/nature11986. Epub 2013 Mar 24.

Succinate is an inflammatory signal that induces IL-1β through HIF-1α.

Tannahill GM1, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA.