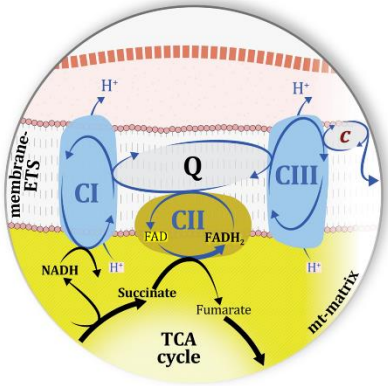
MitoFit Preprints 2023.3.v2
https://doi.org/10.26124/mitofit:2023-0003.v2
Open Access

Theoretical Communication

Cite
Gnaiger E (2023) Complex II ambiguities — $FADH_2$ in the electron transfer system. MitoFit Preprints 2023.3.v2. https://doi.org/10.26124/mitofit:2023-0003.v2

Conflicts of interest
The author declares no conflict of interest.



Online (v1) 2023-03-24
Online (v2) 2023-04-04

Keywords
coenzyme Q junction, Q-junction
Complex II, CII
electron transfer system, ETS
fatty acid oxidation, FAO
flavin adenine dinucleotide, $FADH_2$/FAD
nicotinamide adenine dinucleotide, NADH/NAD^+
succinate dehydrogenase, SDH
tricarboxylic acid cycle, TCA

Complex II ambiguities — $FADH_2$ in the electron transfer system

Erich Gnaiger

Oroboros Instruments, Innsbruck, Austria.

Correspondence: erich.gnaiger@oroboros.at

Summary

The current narrative that the reduced coenzymes NADH and $FADH_2$ feed electrons from the tricarboxylic acid cycle into the mitochondrial electron transfer system creates ambiguities around respiratory Complex II (CII). The succinate dehydrogenase subunit SDHA of CII oxidizes succinate and reduces covalently bound FAD to $FADH_2$ in the canonical forward tricarboxylic acid cycle. However, several graphical representations of the membrane-bound electron transfer system (ETS) depict $FADH_2$ in the mitochondrial matrix to be oxidized by CII. This leads to the false conclusion that $FADH_2$ feeds electrons into the ETS through CII, including $FADH_2$ from the tricarboxylic acid cycle, the β-oxidation cycle in fatty acid oxidation, and the glycerophosphate shuttle. In reality, FAD and succinate are the *substrates* of SDHA at the ETS-entry into CII. The reduced flavin groups $FADH_2$ and $FMNH_2$ are *products* downstream within CII and CI, respectively. Further electron transfer converges at the coenzyme Q-junction. Similarly, electron transferring flavoprotein and mitochondrial glycerophosphate dehydrogenase feed electrons into the Q-junction but not through CII. The ambiguities surrounding Complex II in the literature and educational tools call for quality control, to secure scientific standards in current communications on bioenergetics and ultimately support adequate clinical applications.

1. Introduction

The tricarboxylic acid (TCA) cycle — the citric acid cycle or Krebs cycle — sparked a renaissance of interest in cellular and mitochondrial bioenergetics (Gnaiger et al 2020; Bénit et al 2022; Arnold, Finley 2023). TCA cycle metabolites are oxidized while reducing NAD^+ to NADH in the forward cycle, or transported into the cytosol (Murphy, O'Neill 2018). Respiratory Complex II (CII, succinate dehydrogenase SDH; succinate-ubiquinone oxidoreductase; EC 1.3.5.1) has a unique position in both the TCA cycle and the

mitochondrial inner membrane-bound electron transfer system (membrane-ETS). CII is not a proton pump in contrast to respiratory Complexes CI, CIII and CIV. All genes for CII are nuclear encoded, with exceptions in red algae and land plants (Huang et al 2019; Moosavi et al 2019). Succinate:quinone oxidoreductases (SQRs, succinate dehydrogenases SDH) favour oxidation of succinate and reduction of quinone in the canonical forward direction of the TCA cycle and electron transfer into the Q-junction. Operating in the reverse direction, quinol:fumarate reductases (QFRs, fumarate reductases, FRD) reduce fumarate and oxidize quinol (Iverson 2013; Maklashina et al 2022). The reversed TCA cycle has gained interest in studies ranging from metabolism in anaerobic animals (Hochachka, Somero 2002), thermodynamic efficiency of anaerobic and aerobic ATP production (Gnaiger 1993), reversed electron transfer and ROS production (Tretter et al 2016; Robb et al 2018; Spinelli et al 2021), hypoxia and ischemia-reperfusion injury (Couchani et al 2014), to evolution of metabolic pathways (Lane 2022). In cancer tissue CII plays a key role in metabolic remodeling (DeBerardinis, Chandel 2016; Schöpf et al 2020).

The coenzyme NAD^+ is reduced to $NADH+H^+$ during the oxidation of pyruvate and through redox reactions catalyzed by the TCA cycle enzymes, including isocitrate dehydrogenase, oxoglutarate (α-ketoglutarate) dehydrogenase, and malate dehydrogenase. In turn, coenzyme FAD is reduced to $FADH_2$ during oxidation of succinate by succinate dehydrogenase (CII). This has lead to confusion, when NADH and $FADH_2$ are considered the reduced compounds feeding electrons from the TCA cycle into the 'respiratory chain' — rather than NADH and succinate (Gnaiger 2020). This 'Complex II ambiguitiy' has penetrated publications on bioenergetics without sufficient quality control. Therefore, a critical literature survey is needed to draw attention to widespread ambiguities, particularly in graphical representations of the mitochondrial electron transfer system, to ensure scientific standards in communications on bioenergetics.

2. Experimental evidence

Complex II is a flavoprotein with a covalently bound flavin adenine dinucleotide as documented in early reports (Kearney 1960) and summarized in classical textbooks (Lehninger 1970; Tzagoloff 1982). Microscopic detail on the structure and function of CII has expanded our knowledge on the mechanism of enzyme assembly (Maklashina et al 2022), enzyme structure (Vercellino, Sazanov 2022), kinetic regulation of CII activity (Mills et al 2018; Fink et al 2022), and associated pathologies (Bénit et al 2022).

The reversible oxidoreduction of succinate and fumarate is catalyzed in the soluble domain of CII extending from the mitochondrial inner membrane (mtIM) into the mt-matrix. Succinate donates electrons — i.e. two hydrogen ions and two electrons ($2\{H^+ + e^-\}$) — to the cofactor FAD which is tightly bound to the subunit SDHA. In SDHA the oxidized yellow (450 nm) form FAD functions as hydrogen acceptor from succinate to the reduced product $FADH_2$ while fumarate is formed as the oxidized product in the TCA cycle. Like in most flavin-linked dehydrogenases, the flavin nucleotide remains tightly bound to the enzyme during the catalytic cycle. $FADH_2$ relays electrons further through a series of iron-sulfur redox centers in SDHB to ubiquinone in the membrane domain harboring SDHC and SDHD (Moosavi et al 2019) (Figure 1a).

The reduced flavin groups $FADH_2$ of flavin adenine dinucleotide and $FMNH_2$ of flavin mononucleotide are at functionally comparable levels in the electron transfer in CII and

CI, respectively, to the Q-junction (Figure 1b). FMN in CI is reduced by NADH forming (reduced) $FMNH_2$ and (oxidized) NAD^+. $FADH_2$ and $FMNH_2$ are reoxidized downstream in CII and CI, respectively, by electron transfer to coenzyme Q (Figure 1b).

The branches of electron transfer from NADH and succinate converge through CI and CII at the Q-junction. The convergent architecture of the electron transfer system (ETS; in contrast to a linear electron transfer chain) is emphasized in Figure 1c (Hatefi 1962; Gnaiger 2020). Comparable to CII, several additional respiratory Complexes are localized in the mtIM which catalyze electron transfer converging at the Q-junction, including electron transferring flavoprotein (CETF) in fatty acid oxidation, glycerophosphate dehydrogenase (CGpDH), sulfide-ubiquinone oxidoreductase, choline dehydrogenase, dihydro-orotate dehydrogenase, and proline dehydrogenase (Gnaiger 2020; Bénit et al 2022; Pallag et al 2022).

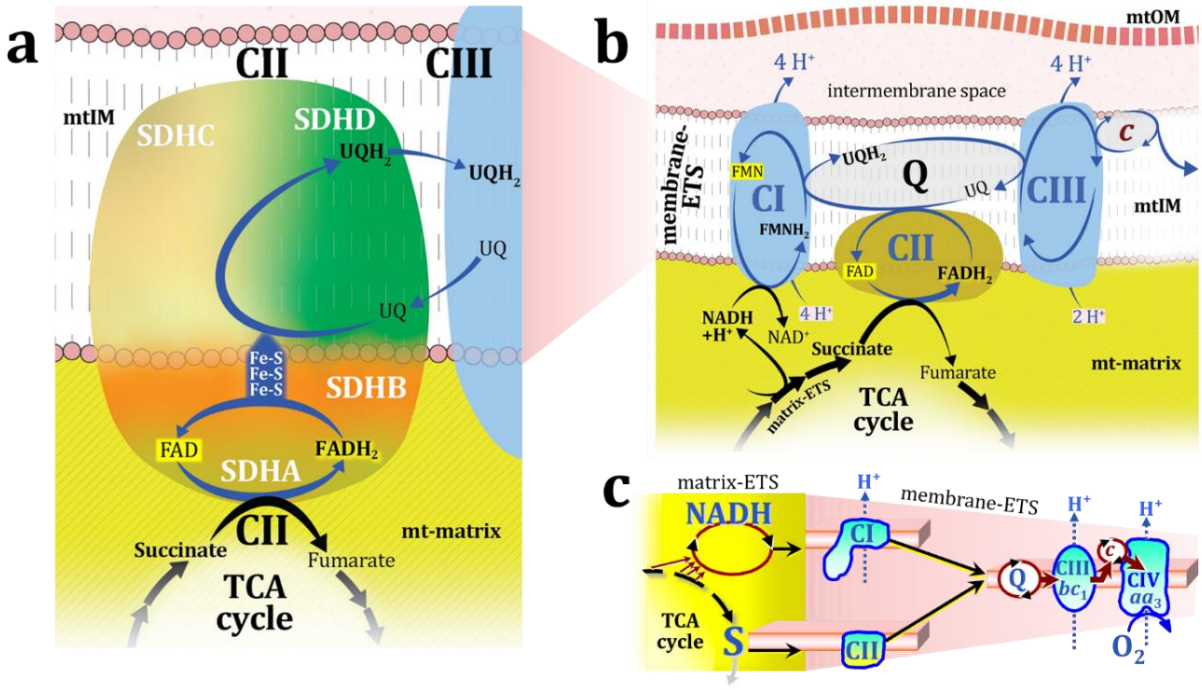


Figure 1. Complex II bridges electron transfer from the TCA cycle to the mitochondrial inner membrane. Graphical representations of the electron transfer system ETS with successive emphasis on pathway architecture and concomitant loss of detail. CII is integrated in the TCA cycle (matrix-ETS) and the membrane-bound electron transfer system (membrane-ETS in the mt-inner membrane mtIM). Joint half-circular arrows indicate electron transfer $2\{H^+ + e^-\}$, distinguished from hydrogen ion H^+ transport across the mtIM. **(a)** In the soluble domain of CII, the flavoprotein SDHA catalyzes the oxidation succinate → fumarate+$2\{H^+ + e^-\}$ and reduction FAD+$2\{H^+ + e^-\}$ → $FADH_2$. The iron–sulfur protein SDHB transfers electrons through Fe-S clusters to the mtIM domain where ubiquinone UQ is reduced with $2\{H^+ + e^-\}$ to ubiquinol UQH_2 in SDHC and SDHD. **(b)** NADH and succinate are substrates of redox reactions in CI and CII, respectively, with $FMNH_2$ and $FADH_2$ as the corresponding products. Succinate and fumarate indicate the chemical entities irrespective of ionization, whereas the charges are shown in NADH, NAD^+, and H^+. **(c)** Electron flow catalyzed by dehydrognases localized in the mitochondrial (mt) matrix converges at the N-junction, reducing NAD^+ to NADH. Electron flow from NADH and succinate S to molecular oxygen, $2\{H^+ + e^-\}+0.5\ O_2 \dashrightarrow H_2O$, converges through CI and CII at the Q-junction. CIII passes electrons to cytochrome *c* and in CIV to O_2.

3. Results and discussion

3.1. The source and consequence of Complex II ambiguities

> '*No representation is ever perfectly expressive, for if it were it would not be a representation but the thing itself*' (Grosholz 2007).

Ambiguities emerge if the representation of a concept is vague to an extent that allows for equivocal interpretations. As a consequence, even a basically clear concept (Figure 1) may be communicated as a divergence from an established truth. The following quotes from Cooper (2000) provide an example (Figure 2).

(*1*) The standard comparison is made between NADH (linked to CI) and $FADH_2$ (linked to CII; Figure 2): '*Electrons from NADH enter the electron transport chain in complex I, .. A distinct protein complex (complex II), which consists of four polypeptides, receives electrons from the citric acid cycle intermediate, succinate (Figure 10.9). These electrons are transferred to $FADH_2$, rather than to NADH, and then to coenzyme Q.*'

(*2*) '*In contrast to the transfer of electrons from NADH to coenzyme Q at complex I, the transfer of electrons from $FADH_2$ to coenzyme Q is not associated with a significant decrease in free energy and, therefore, is not coupled to ATP synthesis.*' Note that CI is in the path of electron transfer from NADH to coenzyme Q. In contrast, electron transfer from $FADH_2$ to coenzyme Q is downstream of succinate oxidation by CII. Thus even a large Gibbs force ('*decrease in free energy*') in $FADH_2 \rightarrow Q$ would fail to drive the coupled process of proton translocation through CII. The Gibbs force (Gnaiger 2020) in $S \rightarrow FADH_2$ must be accounted for. (In parentheses: None of these steps are directly coupled to ATP synthesis. Redox-driven proton translocation must be distinguished from phosphorylation of ADP driven by the protonmotive force).

(*3*) CII receives electrons from succinate, yet it is suggested that '*electrons from succinate enter the electron transport chain via $FADH_2$ in complex II.*' The ambiguity is caused by a lack of unequivocal definition of the electron transfer system (*electron transport chain*). Two contrasting definitions are implied of the '*electron transport chain*' or ETS. (*a*) CII is part of the ETS. Hence electrons enter the ETS from succinate but not from $FADH_2$ — from the matrix-ETS to the membrane-ETS (Figure 1b,c). (*b*) If electrons enter the '*electron transport chain* via *$FADH_2$ in complex II*', then subunit SDHA would be upstream and hence not part of the ETS (to which conclusion obviously nobody would agree). There remains the ambiguity of electron entry into CII from succinate (Figure 1) or from $FADH_2$ as the product of succinate dehydrogenase in the TCA cycle (Figure 3).

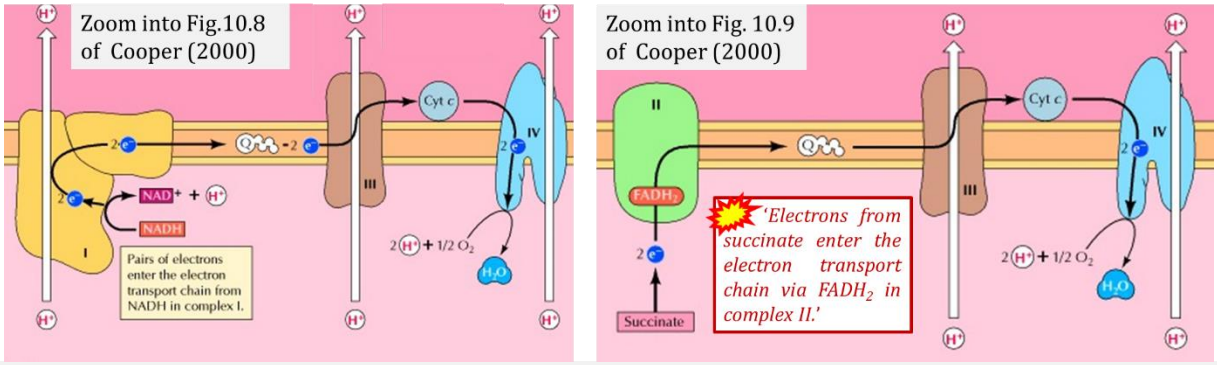


Figure 2. Electron flow into Complexes CI (left) and CII (right). Zoom into figures of Cooper (2000), with marked quote inserted from the legend to Figure 10.9.

3.2. The $FADH_2$ - FAD confusion in the succinate-pathway

The narrative that the reduced coenzymes NADH and $FADH_2$ feed electrons from the TCA cycle into the mitochondrial electron transfer system causes confusion. As a consequence, $FADH_2$ appears in several publications erroneously as the substrate of CII in the ETS linked to succinate oxidation. This error is widely propagated (Supplement S1 and S2) and requires clarification (Gnaiger 2020; page 48). The following examples illustrate the transition from ambiguity to misunderstanding.

(1) Ambiguities appear in graphical representations, where $FADH_2$ is the product and substrate of CII in the same figure (Figure 3).

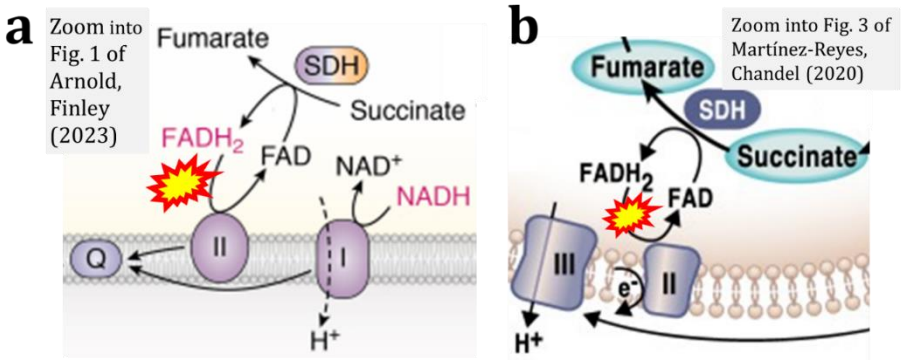


Figure 3. $FADH_2$ depicted as product and substrate of Complex II. Zoom into figures by **(a)** Arnold, Finley (2023) and **(b)** Martínez-Reyes, Chandel (2020).

(2) Ambiguity evolved to misconception in graphical representations (Figure 4).

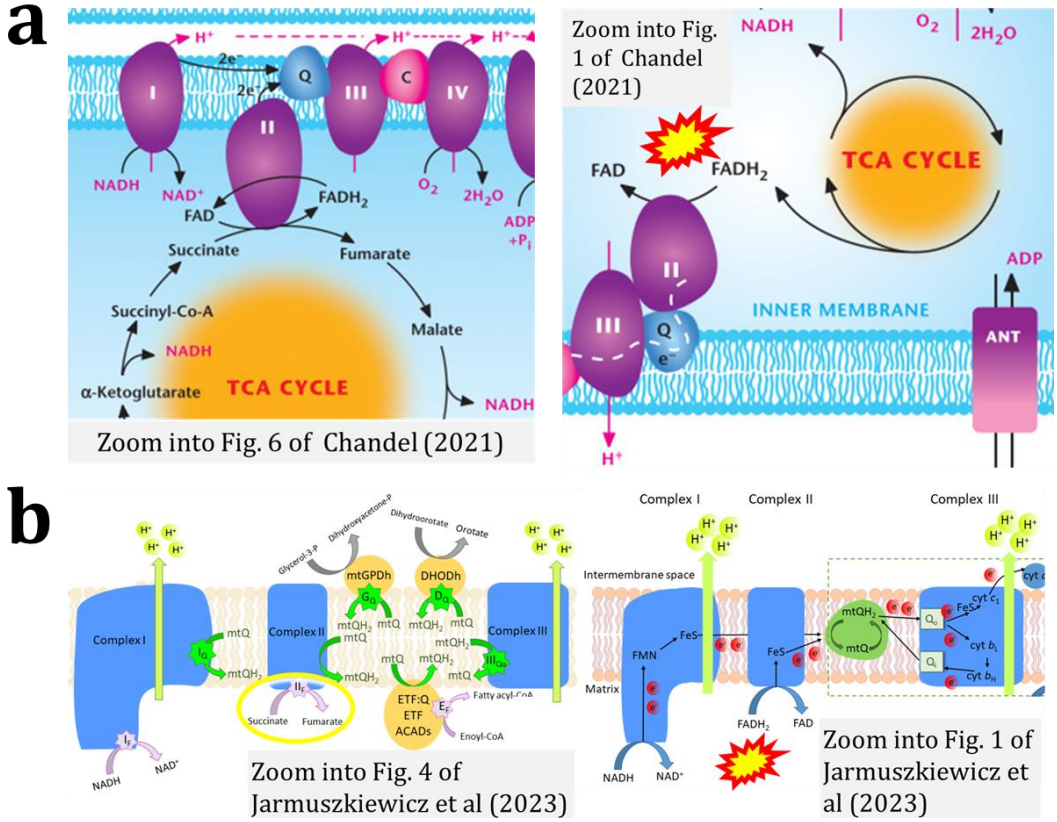


Figure 4. Evolving disarrangement in graphical representations of $FADH_2$ as a substrate of CII. (a) From ambiguity to misconception in Fig. 6 and 1 of Chandel (2021). **(b)** Succinate or $FADH_2$ as substrates of CII in Fig. 4 and 1 of Jarmuszkiewicz et al (2023).

(3) Discrepancies are apparent between erroneous graphical representation (Figure 5) and correct text. '*Reducing equivalents (NADH, $FADH_2$) provide electrons that flow through complex I, the ubiquinone cycle (Q/QH_2), complex III, cytochrome c, complex IV, and to the final acceptor O_2 to form water*' (Fisher-Wellman, Neufer 2012).

(*4*) Graphical errors remain without comment in the text (Figure 6).

(*5*) Error propagation from graphical representation (Figure 3a) to misunderstanding in the text: '*SDH reduces FAD to $FADH_2$, which donates its electrons to complex II*'; '*each complete turn of the TCA cycle generates three NADH and one $FADH_2$ molecules, which donate their electrons to complex I and complex II, respectively*'; '*complex I and complex II oxidize NADH and $FADH_2$, respectively*' (Arnold, Finley 2023).

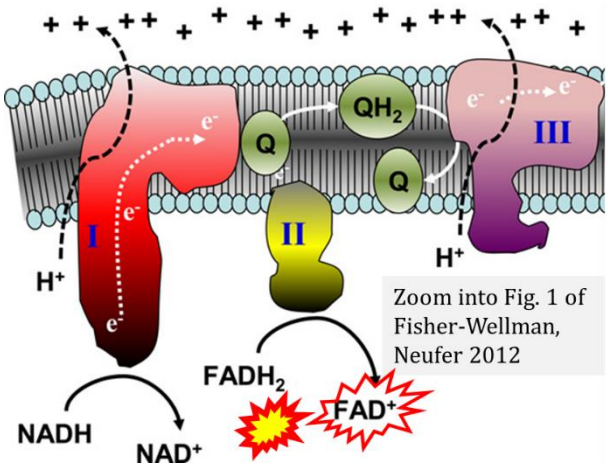


Figure 5. $FADH_2$ is shown as the substrate of CII. This graphical representation takes the NADH→NAD^+ analogy to the erroneous charge of FAD, but contradicts the text that clarifies that $FADH_2$ provides electron flow through the Q-cycle (Fisher-Wellman, Neufer 2012).

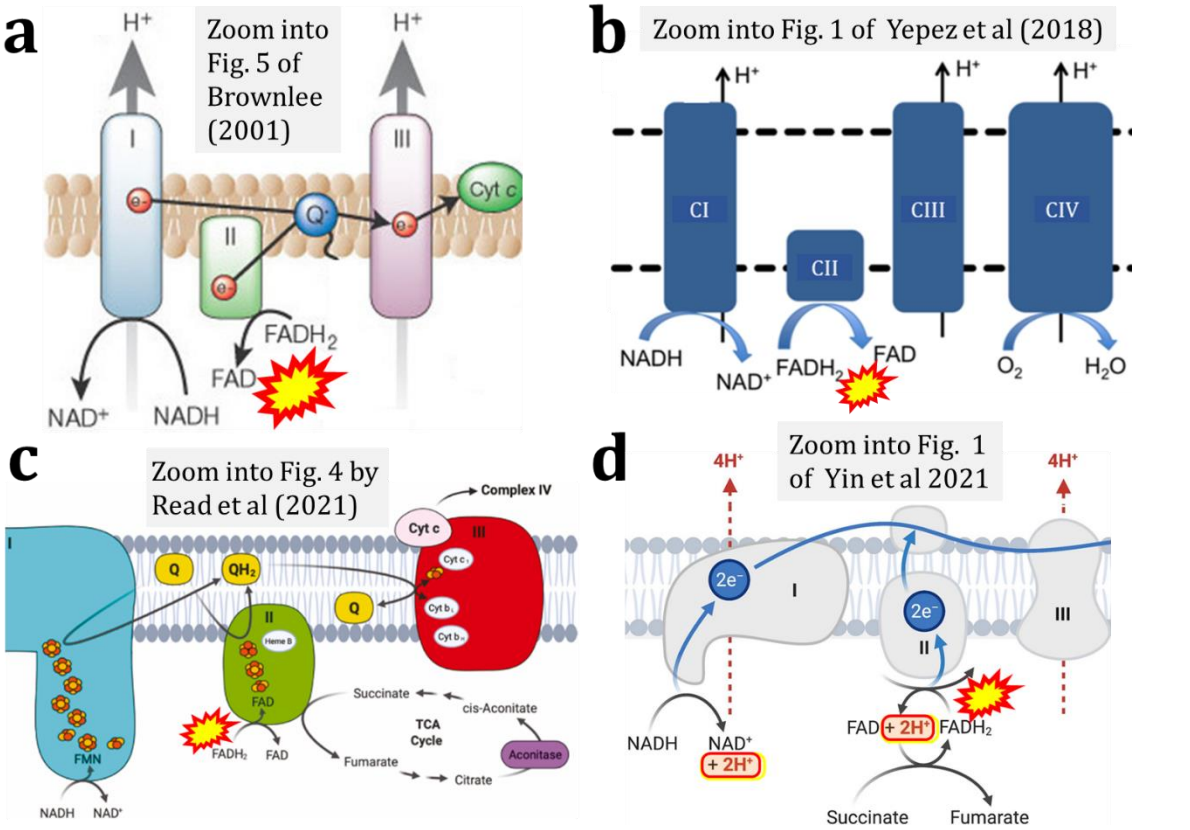


Figure 6. $FADH_2$ shown as substrate of CII. Zoom into figures from **(a)** Brownlee (2001); **(b)** Yépez et al (2018); **(c)** Read et al (2021) showing FAD as product in CII and the mt-matrix; **(d)** Yin et al (2021) with unjustified indication of $2H^+$ formation in the mt-matrix.

3.3. Oxidation of $FADH_2$ to FAD and $2\{H^+ + e^-\}$ transfer

Electron transfer from succinate in the TCA cycle to coenzyme FAD can be written as redox reactions, where oxidation (ox) of succinate yields two hydrogen ions and two electrons $2\{H^+ + e^-\}$ which are donated in the reduction (red) of FAD to $FADH_2$,

ox: $Succinate^{2-} \longrightarrow Fumarate^{2-} + 2\{H^+ + e^-\}$ (Eq. 1a)
red: $2\{H^+ + e^-\} + SDHA\text{-}FAD \longrightarrow SDHA\text{-}FADH_2$ (Eq. 1b)

which yields the net equation

redox: $Succinate^{2-} + SDHA\text{-}FAD \longrightarrow SDHA\text{-}FADH_2 + Fumarate^{2-}$ (Eq. 1)

Commonly the charges of succinate, fumarate, and other metabolites are not shown explicitly in graphical representations of metabolic pathways, but NAD^+ is clearly distinguished from FAD (Figure 1b). Taking oxidation of malate by malate dehydrogenase for comparison,

ox: $Malate^{2-} \longrightarrow Oxaloacetate^{2-} + 2\{H^+ + e^-\}$ (Eq. 2a)
red $2\{H^+ + e^-\} + NAD^+ \longrightarrow NADH + H^+$ (Eq. 2b)
redox: $Malate^{2-} + NAD^+ \longrightarrow NADH + H^+ + Oxaloacetate^{2-}$ (Eq. 2)

H^+ from Eq. 2 is frequently omitted to simplify graphical representations (Figures 3 and 4). However, the rationale for $NADH \longrightarrow NAD^+ + 2H^+$ and $FAD \longrightarrow FAD + 2H^+$ is unclear (Figure 6d; Supplement 1 and 2). Caution is warranted to distinguish in figures electron or $2\{H^+ + e^-\}$ *transfer* from coupled H^+ *transport* across the mtIM.

The frequent presentation of electron transfer from $FADH_2$ to CII (Figure 6; Supplement Figures S1 and S2) has a logical consequence. Electron transferring flavoprotein in β-oxidation and mitochondrial glycerophosphate dehydrogenase generate $FADH_2$. If $FADH_2$ would donate electrons to CII, then CII can be seen as an enzyme involved downstream of $FADH_2$ in FAO and the glycerophosphate shuttle. This topic requires clarification.

3.4. Complex II and fatty acid oxidation

Electron transferring flavoprotein CETF and CI are the respiratory Complexes involved in convergent electron entry into the Q-junction during FAO (Figure 7).

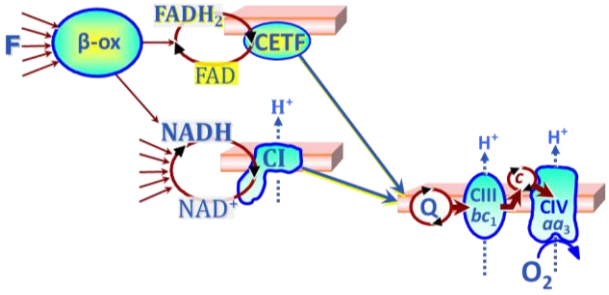


Figure 7. Fatty acid oxidation through the β-oxidation cycle (β-ox), electron transferring flavoprotein (CETF), and Complex I (CI) with convergent electron transfer into the Q-junction. Modified after Gnaiger (2020).

In the β-oxidation cycle of FAO, acetyl-CoA and the reducing equivalents $FADH_2$ and NADH are formed in reactions catalyzed by acyl-CoA dehydrogenases and hydroxyacyl-CoA dehydrogenases, respectively, in the mitochondrial matrix (Houten et al 2016). When $FADH_2$ is erroneously shown as a substrate of CII, a dubious role of CII in FAO is suggested as a consequence (Figure 8a,b). Confused electron transfer pathways are described in Figure 8c (Supplement 2, Weblink #9) and Figure 8d (Supplement 3, Weblink #44). Lemmi et al (1990) noted: '*mitochondrial Complex II also participates in the oxidation of*

fatty acids'. This holds for the oxidation of acetyl-Co in the TCA cycle, forming NADH and succinate with downstream electron flow through CI and CII, respectively, into the Q-junction (Figure 1). In contrast, electron transfer from $FADH_2$ formed during β-oxidation proceeds through electron transferring flavoprotein CETF and entry into the Q-junction independent of CII (Figure 7).

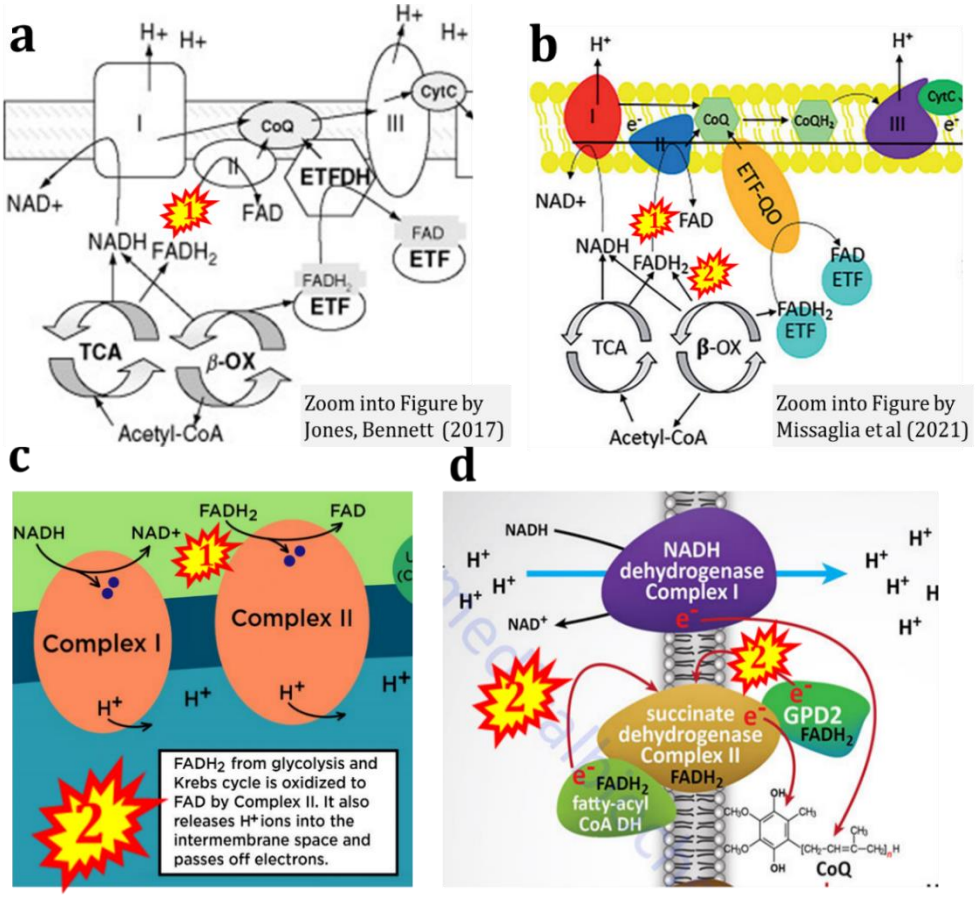


Figure 8. When $FADH_2$ is erroneously shown as a substrate of CII (1), a role of CII in oxidation of $FADH_2$ from glycolysis and fatty acid oxidation is suggested as a consequence (2). Zoom into figures by **(a)** Jones, Bennett (2017); **(b)** Missaglia et al (2021); **(c)** https://www.expii.com/t/electron-transport-chain-summary-diagrams-10139 (accessed 2023-03-21); **(d)** https://themedicalbiochemistrypage.org/oxidative-phosphorylation-related-mitochondrial-functions/ (accessed 2023-03-21).

4. Conclusions

There is currently ambiguity surrounding the precise role of Complex II in fatty acid oxidation. While Complex II is not essential for fatty acid oxidation, it plays a regulatory role by sensing changes in metabolic demand and activating the TCA cycle for oxidation of acetyl-Co depending on the metabolic conditions. This regulatory function may be particularly important during periods of low oxygen availability or high energy demand. The integration of FAO with the membrane-bound ETS (Wang et al 2019) has significant implications for understanding and treating disorders related to β-oxidation and oxidative phosphorylation. Using precisely defined terminology can prevent misunderstandings (Gnaiger et al 2020; footnotes in Supplement 4). Clarification instead

of perpetuation of Complex II ambiguities helps to maintain the high scientific standards required for translating knowledge on metabolism into clinical solutions for mitochondrial diseases.

Abbreviations

$2\{H^+ + e^-\}$	redox equivalents in electron transfer	$NADH_2$	reduced nicotinamide adenine dinucleotide
CI	Complex I	NAD^+	oxidized nicotinamide adenine dinucleotide
CII	Complex II	Q	ETS-reactive coenzyme Q, oxidation state is not implied
CETF	electron transferring flavoprotein	QFR	mena-quinol-fumarate oxidoreductase
$FADH_2$	reduced flavin adenoside dinucleotide	SQR	succinate-ubiquinone oxidoreductase
FAD^+	oxidized flavin adenoside dinucleotide	SDH, SDHABCD	succinate dehydrogenase, CII
FAO	fatty acid oxidation	TCA cycle	tricarboxylic acid cycle
$FMNH_2$	reduced flavin mononucleotide		
mt-matrix	mitochondrial matrix		
mtIM	mitochondrial inner membrane		

Acknowledgements

I thank Luiza H Cardoso and Sabine Schmitt for stimulating discussions, and Paolo Cocco for expert help on the graphical abstract and Figures 1a and b. Contribution to the European Union's Horizon 2020 research and innovation program Grant 857394 (FAT4BRAIN).

References

Arnold PK, Finley LWS (2023) Regulation and function of the mammalian tricarboxylic acid cycle. https://doi.org/10.1016/j.jbc.2022.102838

Bénit P, Goncalves J, El Khoury R, Rak M, Favier J, Gimenez-Roqueplo AP, Rustin P (2022) Succinate dehydrogenase, succinate, and superoxides: a genetic, epigenetic, metabolic, environmental explosive crossroad. https://doi.org/10.3390/biomedicines10081788

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. https://doi.org/10.1038/414813a

Chandel NS (2021) Mitochondria. https://doi.org/10.1101/cshperspect.a040543

Cooper GM (2000) The cell: a molecular approach. 2nd edition. Sunderland (MA): Sinauer Associates Available from: https://www.ncbi.nlm.nih.gov/books/NBK9885/

Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord ENJ, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa ASH, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP (2014) Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. https://doi.org/10.1038/nature13909

DeBerardinis RJ, Chandel NS (2016) Fundamentals of cancer metabolism. https://doi.org/10.1126/sciadv.1600200

Fink BD, Rauckhorst AJ, Taylor EB, Yu L, Sivitz WI (2022) Membrane potential-dependent regulation of mitochondrial complex II by oxaloacetate in interscapular brown adipose tissue. https://doi.org/10.1096/fba.2021-00137

Fisher-Wellman KH, Neufer PD (2012) Linking mitochondrial bioenergetics to insulin resistance via redox biology. https://doi.org/10.1016/j.tem.2011.12.008

Gnaiger E (1993) Efficiency and power strategies under hypoxia. Is low efficiency at high

glycolytic ATP production a paradox? In: Surviving hypoxia: Mechanisms of control and adaptation. Hochachka PW, Lutz PL, Sick T, Rosenthal M, Van den Thillart G (eds) CRC Press, Boca Raton, Ann Arbor, London, Tokyo:77-109.

Gnaiger E (2020) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. https://doi.org/10.26124/bec:2020-0002

Gnaiger E et al - MitoEAGLE Task Group (2020) Mitochondrial physiology. https://doi.org/10.26124/bec:2020-0001.v1

Grosholz ER (2007) Representation and productive ambiguity in mathematics and the sciences. Oxford Univ Press:312 pp.

Hatefi Y, Haavik AG, Fowler LR, Griffiths DE (1962) Studies on the electron transfer system XLII. Reconstitution of the electron transfer system. https://doi.org/10.1016/S0021-9258(19)73804-6

Huang S, Braun HP, Gawryluk RMR, Millar AH (2019) Mitochondrial complex II of plants: subunit composition, assembly, and function in respiration and signaling. https://doi.org/10.1111/tpj.14227

Hochachka PW, Somero GN (2002) Biochemical adaptation: mechanism and process in physiological evolution. Oxford Univ Press, New York:466 pp.

Houten SM, Violante S, Ventura FV, Wanders RJ (2016) The biochemistry and physiology of mitochondrial fatty acid β-oxidation and its genetic disorders. https://doi.org/10.1146/annurev-physiol-021115-105045

Iverson TM (2013) Catalytic mechanisms of complex II enzymes: a structural perspective. https://doi.org/10.1016/j.bbabio.2012.09.008

Jarmuszkiewicz W, Dominiak K, Budzinska A, Wojcicki K, Galganski L (2023) Mitochondrial coenzyme Q redox homeostasis and reactive oxygen species production. https://doi.org/10.31083/j.fbl2803061

Jones PM, Bennett MJ (2017) Chapter 4 - Disorders of mitochondrial fatty acid β-oxidation. Elsevier In: Garg U, Smith LD , eds. Biomarkers in inborn errors of metabolism. Clinical aspects and laboratory determination:87-101. https://doi.org/10.1016/C2014-0-03841-5

Kearney EB (1960) Studies on succinic dehydrogenase. XII. Flavin component of the mammalian enzyme. J Biol Chem 235:865-77.

Lane N (2022) Transformer: the deep chemistry of life and death. Profile Books:400 pp. ISBN-10: 0393651487

Lehninger AL (1970) Biochemistry. The molecular basis of cell structure and function. Worth Publishers, New York:833 pp.

Lemmi CA, Pelikan PC, Geesaman B, Seamon E, Koyle M, Rajfer J (1990) Kinetics of cyclosporine A-induced inhibition of succinate-coenzyme Q dehydrogenase in rat renal cortical mitochondria. https://doi.org/10.1016/0885-4505(90)90027-x

Maklashina E, Iverson TM, Cecchini G (2022) How an assembly factor enhances covalent FAD attachment to the flavoprotein subunit of complex II. https://doi.org/10.1016/j.jbc.2022.102472

Martínez-Reyes I, Chandel NS (2020) Mitochondrial TCA cycle metabolites control physiology and disease. https://doi.org/10.1038/s41467-019-13668-3

Mills EL, Pierce KA, Jedrychowski MP, Garrity R, Winther S, Vidoni S, Yoneshiro T, Spinelli JB, Lu GZ, Kazak L, Banks AS, Haigis MC, Kajimura S, Murphy MP, Gygi SP, Clish CB, Chouchani ET (2018) Accumulation of succinate controls activation of adipose tissue thermogenesis. https://doi.org/10.1038/s41586-018-0353-2

Missaglia S, Tavian D, Angelini C (2021) ETF dehydrogenase advances in molecular genetics and impact on treatment. https://doi.org/10.1080/10409238.2021.1908952

Moosavi B, Berry EA, Zhu XL, Yang WC, Yang GF (2019) The assembly of succinate dehydrogenase: a key enzyme in bioenergetics. https://doi.org/10.1007/s00018-019-03200-7

Murphy MP, O'Neill LAJ (2018) Krebs cycle reimagined: the emerging roles of succinate and itaconate as signal transducers. https://doi.org/10.1016/j.cell.2018.07.030

Pallag G, Nazarian S, Ravasz D, Bui D, Komlódi T, Doerrier C, Gnaiger E, Seyfried TN, Chinopoulos

C (2022) Proline oxidation supports mitochondrial ATP production when Complex I is inhibited. https://doi.org/10.3390/ijms23095111

Read AD, Bentley RE, Archer SL, Dunham-Snary KJ (2021) Mitochondrial iron-sulfur clusters: Structure, function, and an emerging role in vascular biology. https://doi.org/10.1016/j.redox.2021.102164

Robb EL, Hall AR, Prime TA, Eaton S, Szibor M, Viscomi C, James AM, Murphy MP (2018) Control of mitochondrial superoxide production by reverse electron transport at complex I. https://doi.org/10.1074/jbc.RA118.003647

Schöpf B, Weissensteiner H, Schäfer G, Fazzini F, Charoentong P, Naschberger A, Rupp B, Fendt L, Bukur V, Giese I, Sorn P, Sant'Anna-Silva AC, Iglesias-Gonzalez J, Sahin U, Kronenberg F, Gnaiger E, Klocker H (2020) OXPHOS remodeling in high-grade prostate cancer involves mtDNA mutations and increased succinate oxidation. https://doi.org/10.1038/s41467-020-15237-5

Spinelli JB, Rosen PC, Sprenger HG, Puszynska AM, Mann JL, Roessler JM, Cangelosi AL, Henne A, Condon KJ, Zhang T, Kunchok T, Lewis CA, Chandel NS, Sabatini DM (2021) Fumarate is a terminal electron acceptor in the mammalian electron transport chain. https://doi.org/10.1126/science.abi7495

Tretter L, Patocs A, Chinopoulos C (2016) Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. https://doi.org/10.1016/j.bbabio.2016.03.012

Tzagoloff A (1982) Mitochondria. Plenum Press, New York 342 pp.

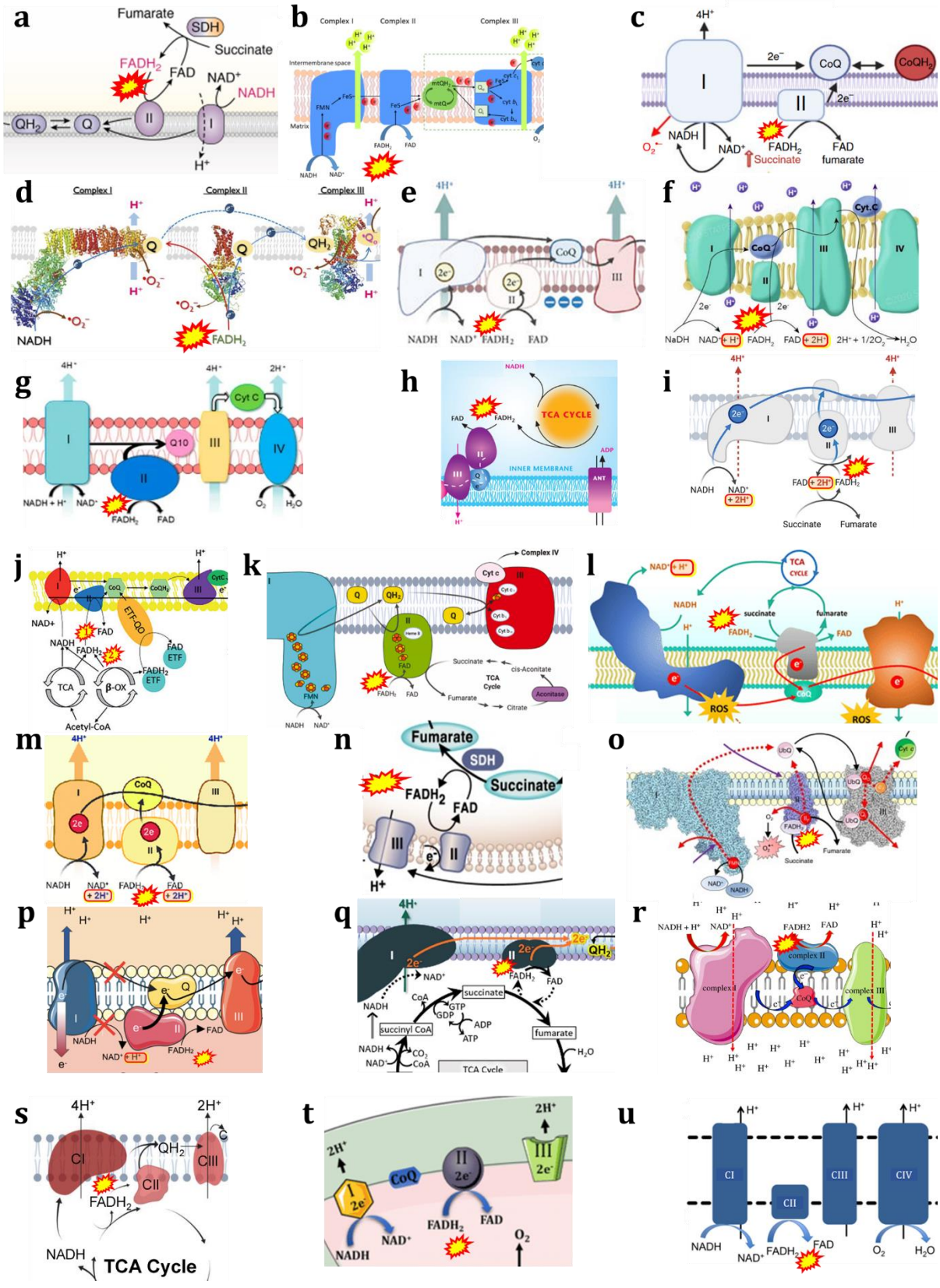
Vercellino I, Sazanov LA (2022) The assembly, regulation and function of the mitochondrial respiratory chain. https://doi.org/10.1038/s41580-021-00415-0

Wang Y, Palmfeldt J, Gregersen N, Makhov AM, Conway JF, Wang M, McCalley SP, Basu S, Alharbi H, St Croix C, Calderon MJ, Watkins S, Vockley J (2019) Mitochondrial fatty acid oxidation and the electron transport chain comprise a multifunctional mitochondrial protein complex. https://doi.org/10.1074/jbc.RA119.008680

Yépez VA, Kremer LS, Iuso A, Gusic M, Kopajtich R, Koňaříková E, Nadel A, Wachutka L, Prokisch H, Gagneur J (2018) OCR-Stats: Robust estimation and statistical testing of mitochondrial respiration activities using Seahorse XF Analyzer. https://doi.org/10.1371/journal.pone.0199938

Yin M, O'Neill LAJ (2021) The role of the electron transport chain in immunity. https://doi.org/10.1096/fj.202101161R

Supplement Figure S1



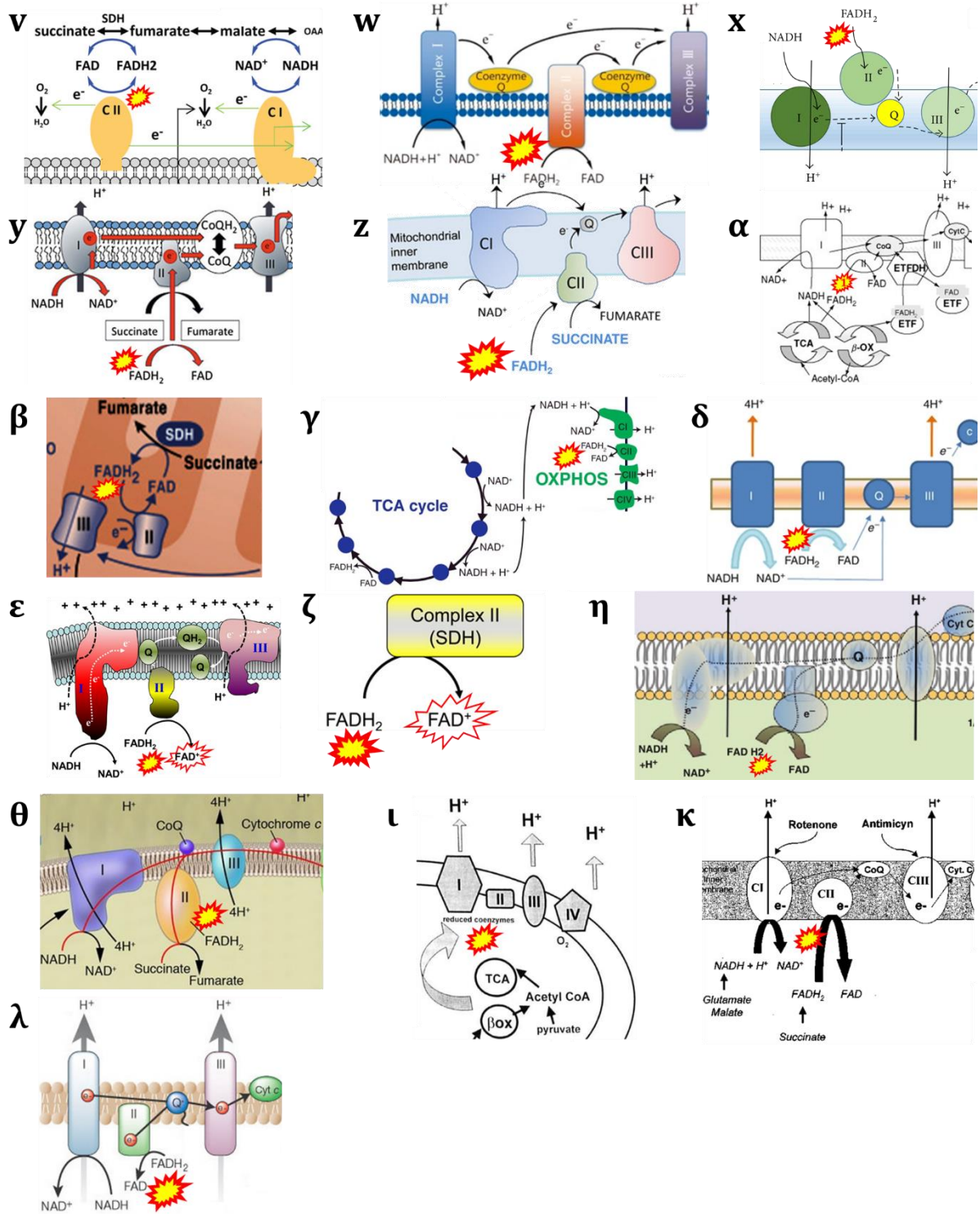


Figure S1. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. Chronological sequence of publications from 2001 to 2023. NADH → NAD^+ is frequently written in graphs without showing the H^+ on the left side of the arrow, except for (**g**, **w**, **γ**, **η**, **κ**). However, NADH → NAD^++H^+ (**f**, **l**, **p**) and NADH → NAD^++$2H^+$ (**i**, **m**) should be corrected to NADH+H^+ → NAD^+ (Eq. 2). $FADH_2$ → FAD+$2H^+$ (**f**, **i**, **m**) should be corrected to $FADH_2$ → FAD (Eq. 1). Perhaps FAD received a false positive charge (**ε**, **ζ**) from comparing Eq. 1 with Eq. 2. See References for Figure S1.

References for Figure S1

a Arnold PK, Finley LWS (2023) Regulation and function of the mammalian tricarboxylic acid cycle. **J Biol Chem** 299:102838. https://doi.org/10.1016/j.jbc.2022.102838

b Jarmuszkiewicz W, Dominiak K, Budzinska A, Wojcicki K, Galganski L (2023) Mitochondrial coenzyme Q redox homeostasis and reactive oxygen species production. **Front Biosci (Landmark Ed)** 28:61. https://doi.org/10.31083/j.fbl2803061

c Billingham LK, Stoolman JS, Vasan K, Rodriguez AE, Poor TA, Szibor M, Jacobs HT, Reczek CR, Rashidi A, Zhang P, Miska J, Chandel NS (2022) Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation. **Nat Immunol** 23:692-704. https://doi.org/10.1038/s41590-022-01185-3

d Chen CL, Zhang L, Jin Z, Kasumov T, Chen YR (2022) Mitochondrial redox regulation and myocardial ischemia-reperfusion injury. **Am J Physiol Cell Physiol** 322:C12-23. https://doi.org/10.1152/ajpcell.00131.2021

e Yuan Q, Zeng ZL, Yang S, Li A, Zu X, Liu J (2022) Mitochondrial stress in metabolic inflammation: modest benefits and full losses. **Oxid Med Cell Longev** 2022:8803404. https://doi.org/10.1155/2022/8803404

f Ahmad M, Wolberg A, Kahwaji CI (2022) Biochemistry, electron transport chain. **StatPearls Publishing** StatPearls [Internet]. Treasure Island (FL). https://www.ncbi.nlm.nih.gov/books/NBK526105/

g Turton N, Cufflin N, Dewsbury M, Fitzpatrick O, Islam R, Watler LL, McPartland C, Whitelaw S, Connor C, Morris C, Fang J, Gartland O, Holt L, Hargreaves IP (2022) The biochemical assessment of mitochondrial respiratory chain disorders. **Int J Mol Sci** 23:7487. https://doi.org/10.3390/ijms23137487

h Chandel NS (2021) Mitochondria. **Cold Spring Harb Perspect Biol** 13:a040543. https://doi.org/10.1101/cshperspect.a040543

i Yin M, O'Neill LAJ (2021) The role of the electron transport chain in immunity. **FASEB J** 35:e21974. https://doi.org/10.1096/fj.202101161R

j Missaglia S, Tavian D, Angelini C (2021) ETF dehydrogenase advances in molecular genetics and impact on treatment. **Crit Rev Biochem Mol Biol** 56:360-72. https://doi.org/10.1080/10409238.2021.1908952

k Read AD, Bentley RE, Archer SL, Dunham-Snary KJ (2021) Mitochondrial iron-sulfur clusters: Structure, function, and an emerging role in vascular biology. **Redox Biol** 47:102164. https://doi.org/10.1016/j.redox.2021.102164

l Gasmi A, Peana M, Arshad M, Butnariu M, Menzel A, Bjørklund G (2021) Krebs cycle: activators, inhibitors and their roles in the modulation of carcinogenesis. **Arch Toxicol** 95:1161-78. https://doi.org/10.1007/s00204-021-02974-9

m Turton N, Bowers N, Khajeh S, Hargreaves IP, Heaton RA (2021) Coenzyme Q10 and the exclusive club of diseases that show a limited response to treatment. **Expert Opinion Orphan Drugs** 9:151-60. https://doi.org/10.1080/21678707.2021.1932459.

n Martínez-Reyes I, Chandel NS (2020) Mitochondrial TCA cycle metabolites control physiology and disease. **Nat Commun** 11:102. https://doi.org/10.1038/s41467-019-13668-3

o Raimondi V, Ciccarese F, Ciminale V (2020) Oncogenic pathways and the electron transport chain: a dangeROS liaison. **Br J Cancer** 122:168-81. https://doi.org/10.1038/s41416-019-0651-y

p Risiglione P, Leggio L, Cubisino SAM, Reina S, Paternò G, Marchetti B, Magrì A, Iraci N, Messina A (2020) High-resolution respirometry reveals MPP+ mitochondrial toxicity mechanism in a cellular model of parkinson's disease. **Int J Mol Sci** 21:E7809. https://doi.org/10.3390/ijms21217809

q Nolfi-Donegan D, Braganza A, Shiva S (2020) Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. **Redox Biol** 37:101674. https://doi.org/10.1016/j.redox.2020.101674

r Morelli AM, Ravera S, Calzia D, Panfoli I (2019) An update of the chemiosmotic theory as suggested by possible proton currents inside the coupling membrane. **Open Biol** 9:180221. https://doi.org/10.1098/rsob.180221

s Lewis MT, Kasper JD, Bazil JN, Frisbee JC, Wiseman RW (2019) Quantification of mitochondrial oxidative phosphorylation in metabolic disease: application to Type 2 diabetes. **Int J Mol Sci** 20:5271. https://doi.org/10.3390/ijms20215271

t Sarmah D, Kaur H, Saraf J, Vats K, Pravalika K, Wanve M, Kalia K, Borah A, Kumar A, Wang X, Yavagal DR, Dave KR, Bhattacharya P (2019) Mitochondrial dysfunction in stroke: implications of stem cell

therapy. **Transl Stroke Res**. https://doi.org/10.1007/s12975-018-0642-y

u Yépez VA, Kremer LS, Iuso A, Gusic M, Kopajtich R, Koňaříková E, Nadel A, Wachutka L, Prokisch H, Gagneur J (2018) OCR-Stats: Robust estimation and statistical testing of mitochondrial respiration activities using Seahorse XF Analyzer. **PLOS ONE** 13:e0199938. https://doi.org/10.1371/journal.pone.0199938

v Fink BD, Bai F, Yu L, Sheldon RD, Sharma A, Taylor EB, Sivitz WI (2018) Oxaloacetic acid mediates ADP-dependent inhibition of mitochondrial complex II-driven respiration. **J Biol Chem** 293:19932-41. https://doi.org/10.1074/jbc.RA118.005144

w Zhang H, Feng YW, Yao YM (2018) Potential therapy strategy: targeting mitochondrial dysfunction in sepsis. **Mil Med Res** 5:41. https://doi.org/10.1186/s40779-018-0187-0

x Roy Chowdhury S, Banerji V (2018) Targeting mitochondrial bioenergetics as a therapeutic strategy for chronic lymphocytic leukemia. **Oxid Med Cell Longev** 2018:2426712. https://doi.org/10.1155/2018/2426712

y de Villiers D, Potgieter M, Ambele MA, Adam L, Durandt C, Pepper MS (2018) The role of reactive oxygen species in adipogenic differentiation. **Adv Exp Med Biol** 1083:125-144. https://doi.org/10.1007/5584_2017_119

z Polyzos AA, McMurray CT (2017) The chicken or the egg: mitochondrial dysfunction as a cause or consequence of toxicity in Huntington's disease. **Mech Ageing Dev** 161:181-97. https://doi.org/10.1016/j.mad.2016.09.003

α Jones PM, Bennett MJ (2017) Chapter 4 - Disorders of mitochondrial fatty acid β-oxidation. **Elsevier** In: Garg U, Smith LD , eds. Biomarkers in inborn errors of metabolism. Clinical aspects and laboratory determination:87-101. https://doi.org/10.1016/C2014-0-03841-5

β DeBerardinis RJ, Chandel NS (2016) Fundamentals of cancer metabolism. **Sci Adv** 2:e1600200. https://doi.org/10.1126/sciadv.1600200

γ Nsiah-Sefaa A, McKenzie M (2016) Combined defects in oxidative phosphorylation and fatty acid β-oxidation in mitochondrial disease. **Biosci Rep** 36:e00313. https://doi.org/10.1042/BSR20150295

δ Prochaska LJ, Cvetkov TL (2013) Mitochondrial electron transport. In: Roberts, G.C.K. (eds) Encyclopedia of biophysics. **Springer**, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-16712-6_25

ε, ζ Fisher-Wellman KH, Neufer PD (2012) Linking mitochondrial bioenergetics to insulin resistance via redox biology. **Trends Endocrinol Metab** 23:142-53. https://doi.org/10.1016/j.tem.2011.12.008

η Benard G, Bellance N, Jose C, Rossignol R (2011) Relationships between mitochondrial dynamics and bioenergetics. In: Lu Bingwei (ed) Mitochondrial dynamics and neurodegeneration. **Springer** ISBN 978-94-007-1290-4:47-68.

θ Nussbaum RL (2005) Mining yeast in silico unearths a golden nugget for mitochondrial biology. **J Clin Invest** 115:2689-91. https://doi.org/10.1172/JCI26625

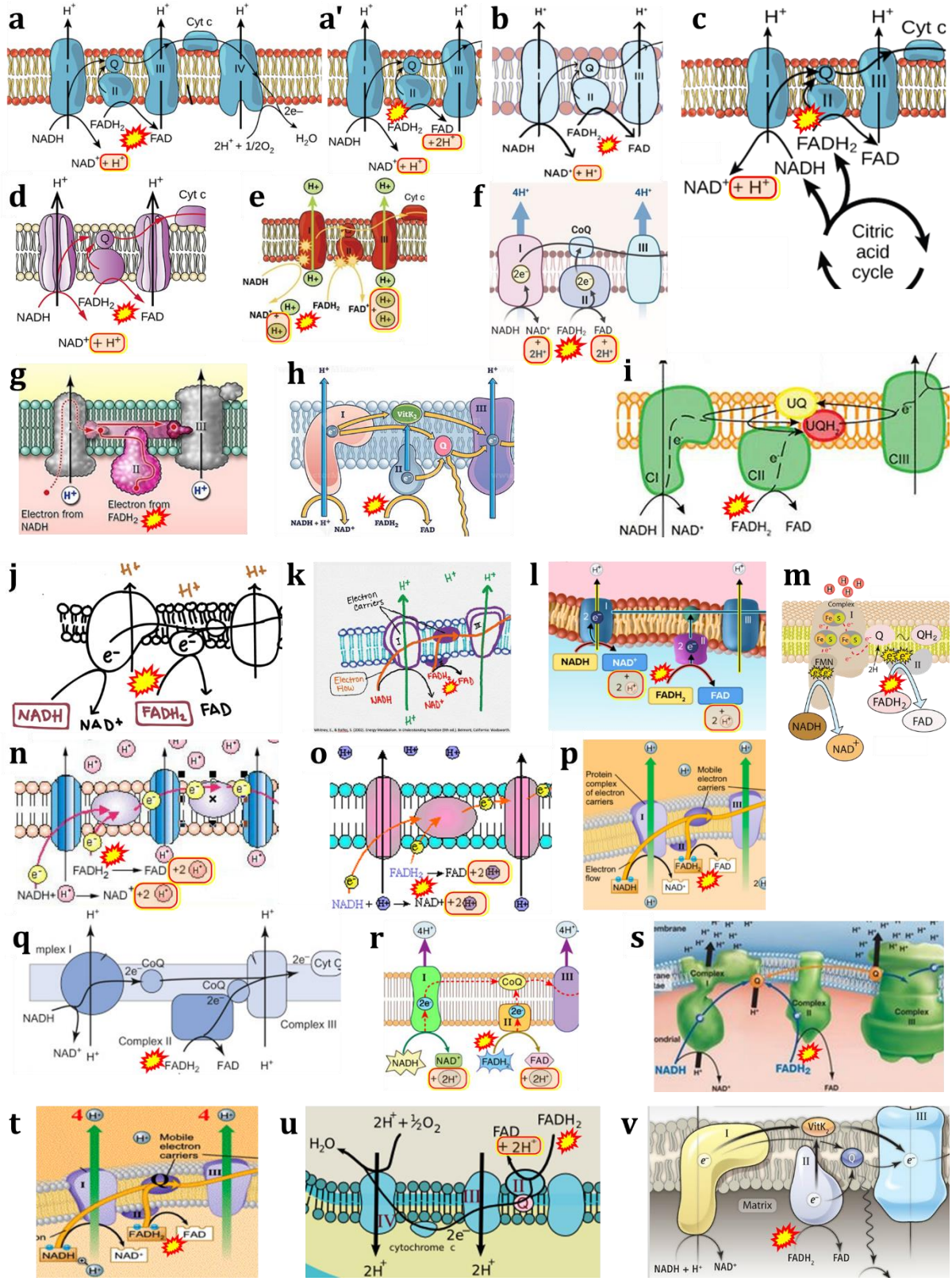
ι Himms-Hagen J, Harper ME (2001) Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis. **Exp Biol Med (Maywood)** 226:78-84. https://doi.org/10.1177/153537020122600204

κ Sanchez H, Zoll J, Bigard X, Veksler V, Mettauer B, Lampert E, Lonsdorfer J, Ventura-Clapier R (2001) Effect of cyclosporin A and its vehicle on cardiac and skeletal muscle mitochondria: relationship to efficacy of the respiratory chain. **Br J Pharmacol** 133:781-8. https://doi.org/10.1038/sj.bjp.0704129

λ Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. **Nature** 14:813-20. https://doi.org/10.1038/414813a

- Copied by: Arden GB, Ramsey DJ (2015) Diabetic retinopathy and a novel treatment based on the biophysics of rod photoreceptors and dark adaptation. In: Kolb H, Fernandez E, Nelson R, eds. **Webvision**: The organization of the retina and visual system [Internet]. Salt Lake City (UT): University of Utah Health Sciences Center; 1995-. https://www.ncbi.nlm.nih.gov/books/NBK310272/

Supplement Figure S2



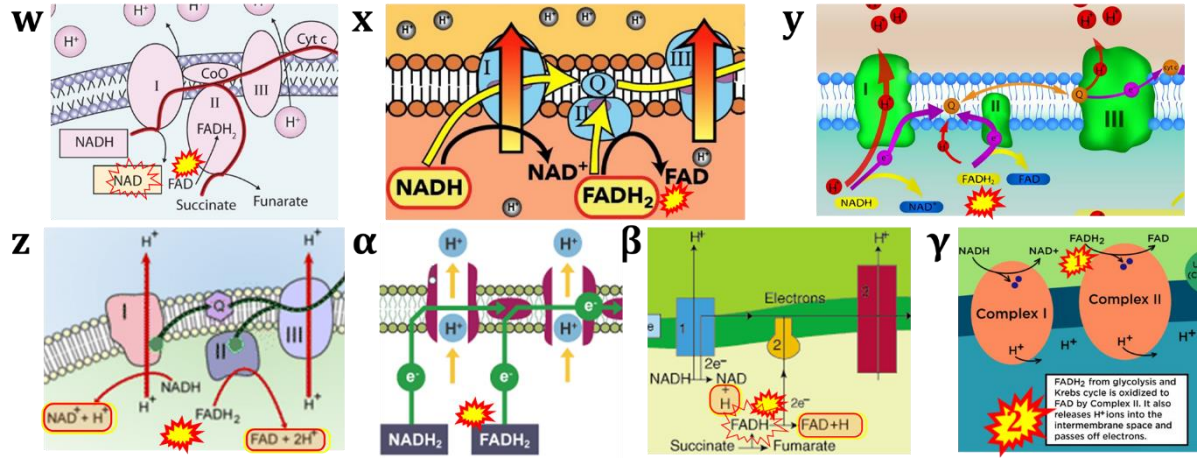


Figure S2. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. NADH → NAD^+ is frequently written in graphs without showing the H^+ on the left side of the arrow, except for (**h**, **v**). However, NADH → NAD^++H^+ (**a-e**, **z**, **β**), NADH → NAD^++$2H^+$ (**f**, **l**, **r**), NADH+H^+ → NAD^++$2H^+$ (**n**, **o**), and NADH → NAD (**w**) should be corrected to NADH+H^+ → NAD^+ (Eq. 2). $FADH_2$ → FAD+$2H^+$ (**a'**, **e**, **f**, **l**, **n**, **o**, **r**, **u**, **z**) and FADH → FAD+H (**β**) should be corrected to $FADH_2$ → FAD (Eq. 1). Weblinks (#): (**a**) 1-5, 37-40; (**a'**) 6-7; (**b**) 8; (**c**) 1, 6, 7, 9, 37, 39; (**d**) 10; (**e**) 4, 9, 11-16; (**f**) 17-18; (**g**) 19; (**h**) 20-21; (**i**) 22; (**j**) 6-7; (**k**) 9; (**l**) 23; (**m**) 24; (**n**) 25; (**o**) 26; (**p**) 27; (**q**) 28; (**r**) 29; (**s**) 30; (**t**) 31; (**u**) 9, 32; (**v**) 33; (**w**) 34; (**x**) 35; (**y**) 15, 17; (**z**) 36; (**α**) 41; (**β**) 42; (**γ**) 9.

Weblinks for Figure S2 (retrieved 2023-03-21 to 2023-04-04)

1 (**a,c**) https://openstax.org/books/biology/pages/7-4-oxidative-phosphorylation - OpenStax Biology (CC BY 3.0) - Fig. 7.10 / Fig. 7.12

2 (**a**) https://opentextbc.ca/biology/chapter/4-3-citric-acid-cycle-and-oxidative-phosphorylation/ - Concepts of Biology - 1st Canadian Edition by Charles Molnar and Jane Gair - Fig. 4.19a

3 (**a**) https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/07%3A_Cellular_Respiration/7.11%3A_Oxidative_Phosphorylation_-_Electron_Transport_Chain - LibreTexts Biology – Fig. 7.11.1

4 (**a,e**) https://courses.lumenlearning.com/wm-biology1/chapter/reading-electron-transport-chain/ - lumen Biology for Majors I - Fig. 1 / Fig. 3

5 (**a**) https://www.pharmaguideline.com/2022/01/electron-transport-chain.html - Pharmaguideline

6 (**a',c,j**) https://www.khanacademy.org/science/ap-biology/cellular-energetics/cellular-respiration-ap/a/oxidative-phosphorylation-etc - Khan Academy - Image modified from "Oxidative phosphorylation: Fig. 1", by OpenStax College, Biology (CC BY 3.0) / Image modified from "Oxidative phosphorylation: Fig. 3," by Openstax College, Biology (CC BY 3.0)

7 (**a',c,j**) https://learn.saylor.org/mod/page/view.php?id=32815 -Saylor Academy

8 (**b**) https://jackwestin.com/resources/mcat-content/oxidative-phosphorylation/electron-transfer-in-mitochondria - Jack Westin MCAT Courses

9 (**c,e,k,u,γ**) https://www.expii.com/t/electron-transport-chain-summary-diagrams-10139 - expii - Image source: By CNX OpenStax / By OpenStax College CC BY 3.0, via Wikimedia Commons / Whitney, Rolfes 2002 / By User:Rozzychan CC BY-SA 2.5, via Wikimedia Commons

10 (**d**) https://www.labxchange.org/library/items/lb:LabXchange:005ad47f-7556-3887-b4a6-66e74198fbcf:html:1 - Labxchange - Figure 8.15 credit: modification of work by Klaus Hoffmeier
11 (**e**) https://commons.wikimedia.org/w/index.php?curid=30148497 - wikimedia 30148497 - Anatomy & Physiology, Connexions Web site. http://cnx.org/content/col11496/1.6/, 2013-06-19
12 (**e**) https://biologydictionary.net/electron-transport-chain-and-oxidative-phosphorylation/ - biologydictionary.net 2018-08-21
13 (**e**) https://www.quora.com/Why-does-FADH2-form-2-ATP - Quora
14 (**e**) https://teachmephysiology.com/biochemistry/atp-production/electron-transport-chain/ - TeachMePhysiology - Fig. 1. 2023-03-13
15 (**e,y**) https://www.thoughtco.com/electron-transport-chain-and-energy-production-4136143 - ThoughtCo / extender01 / iStock / Getty Images Plus
16 (**e**) https://www.toppr.com/ask/question/short-long-answer-types-whatis-the-electron-transport-system-and-what-are-its-functions/ - toppr
17 (**f,y**) https://researchtweet.com/mitochondrial-electron-transport-chain-2/ - researchtweet
18 (**f**) https://microbenotes.com/electron-transport-chain/ - Microbe Notes
19 (**g**) https://biochemden.com/electron-transport-chain-mechanism/ - BiochemDen.com
20 (**h**) https://www.dreamstime.com/electron-transport-chain-as-respiratory-embedded-transporters-outline-diagram-electron-transport-chain-as-respiratory-embedded-image235345232 - dreamstime
21 (**h**) https://vectormine.com/item/electron-transport-chain-as-respiratory-embedded-transporters-outline-diagram/ - VectorMine
22 (**i**) https://www.creative-biolabs.com/drug-discovery/therapeutics/electron-transport-chain.htm - creative-biolabs
23 (**l**) https://flexbooks.ck12.org/cbook/ck-12-biology-flexbook-2.0/section/2.28/primary/lesson/electron-transport-bio/ - FlexBooks - CK-12 Biology for High School- 2.28 Electron Transport, Fig. 2
24 (**m**) http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/Complex1.html - hyperphysics
25 (**n**) https://theory.labster.com/Electron_Transport_Chain/ - Labster Theory
26 (**o**) https://www2.nau.edu/~fpm/bio205/u4fg36.html - nau.edu
27 (**p**) https://quizlet.com/245664214/electron-transport-chain-facts-of-cell-respiration-diagram/ - Quizlet
28 (**q**) https://www.google.com/imgres?imgurl=https%3A%2F%2Fars.els-cdn.com%2Fcontent%2Fimage%2F3-s2.0-B9780128008836000215-f21-07-9780128008836.jpg&imgrefurl=https%3A%2F%2Fwww.sciencedirect.com%2Ftopics%2Fengineering%2Felectron-transport-chain&tbnid=g3dD4u8Tvd6TWM&vet=12ahUKEwjc9deUprT9AhVxhv0HHXZbAd0QMygCegUIARDBAQ..i&docid=Moj_2_W0OpUDcM&w=632&h=439&q=FADH2%20is%20the%20substrates%20of%20Complex%20II&client=firefox-b-d&ved=2ahUKEwjc9deUprT9AhVxhv0HHXZbAd0QMygCegUIARDBAQ - ScienceDirect
29 (**r**) https://www.sciencefacts.net/electron-transport-chain.html - ScienceFacts
30 (**s**) https://sbi4uraft2014.weebly.com/electron-transport-chain.html - SNC1D - BIOLOGY LESSON PLAN BLOG
31 (**t**) https://www.unm.edu/~lkravitz/Exercise%20Phys/ETCstory.html - unm.edu
32 (**u**) https://commons.wikimedia.org/wiki/File:Mitochondrial_electron_transport_chain.png - Wikimedia
33 (**v**) https://www.google.com/imgres?imgurl=https%3A%2F%2Fi.ytimg.com%2Fvi%2FLsRQ5_EmxJA%2Fmaxresdefault.jpg&tbnid=6w-0DVPMw7vOdM&vet=12ahUKEwjw2YO5--T9AhUwpCcCHduuDVgQMygDegUIARDzAQ..i&imgrefurl=https%3A%2F%2Fwww.youtub

e.com%2Fwatch%3Fv%3DLsRQ5_EmxJA&docid=bZxQYNch1Ys-VM&w=1280&h=720&q=electron%20transport%20chain&hl=en-US&client=firefox-b-d&ved=2ahUKEwjw2YO5--T9AhUwpCcCHduuDVgQMygDegUIARDzAQ - YouTube Dirty Medicine Biochemistry - Uploaded 2019-07-18

34 (**w**) https://chem.libretexts.org/Courses/Saint_Marys_College_Notre_Dame_IN/CHEM_118_(Under_Construction)/CHEM_118_Textbook/12%3A_Metabolism_(Biological_Energy)/12.4%3A_The_Citric_Acid_Cycle_and_Electron_Transport - LibreTexts Chemistry - The Citric Acid Cycle and Electron Transport – Fig. 12.4.3

35 (**x**) https://www.google.com/imgres?imgurl=https%3A%2F%2Fi.ytimg.com%2Fvi%2FVER6xW_r1vc%2Fmaxresdefault.jpg&tbnid=Brshl0oN9LyYnM&vet=12ahUKEwjjlKSKpOX9AhWjmycCHbvGC34QMygWegUIARDWAQ..i&imgrefurl=https%3A%2F%2Fwww.youtube.com%2Fwatch%3Fv%3DVER6xW_r1vc&docid=VgTgrLf24Lzg4M&w=1280&h=720&itg=1&q=FADH2%20is%20the%20substrates%20of%20Complex%20II&hl=en&client=firefox-b-d&ved=2ahUKEwjjlKSKpOX9AhWjmycCHbvGC34QMygWegUIARDWAQ - YouTube sciencemusicvideos - Uploaded 2014-08-19

36 (**z**) https://www.ck12.org/biology/electron-transport/lesson/The-Electron-Transport-Chain-Advanced-BIO-ADV/ - cK-12

37 (**a,c**) https://www.texasgateway.org/resource/74-oxidative-phosphorylation - Texas Gateway - Figure 7.11

38 (**a**) https://opentextbc.ca/biology/chapter/4-3-citric-acid-cycle-and-oxidative-phosphorylation/ - Charles Molnar and Jane Gair. 4.3 Citric Acid Cycle and Oxidative Phosphorylation. Concepts of Biology - 1st Canadian Edition, BCcampus

39 (**a,c**) https://opened.cuny.edu/courseware/lesson/639/overview -CUNY

40 (**a**) https://brainbrooder.com/lesson/254/7-4-1-electron-transport-chain - Brain Brooder

41 (**α**) https://www.bbc.co.uk/bitesize/guides/zdq9382/revision/5 - BBC BITESIZE

42 (**β**) https://www.sparknotes.com/biology/cellrespiration/oxidativephosphorylation/section2/ - SparkNotes

Supplement S3

Weblinks on FAO and CII (retrieved 2023-03-21)

43 https://conductscience.com/electron-transport-chain/ - Conduct Science: "*In Complex II, the enzyme succinate dehydrogenase in the inner mitochondrial membrane reduce $FADH_2$ to FAD^+. Simultaneously, succinate, an intermediate in the Krebs cycle, is oxidized to fumarate.*" - Comments: FAD does not have a postive charge. $FADH_2$ is the reduced form, it is not reduced. And again: *In CII, FAD is reduced to $FADH_2$.*

44 https://themedicalbiochemistrypage.org/oxidative-phosphorylation-related-mitochondrial-functions/ - The Medical Biochemistry Page: '*In addition to transferring electrons from the $FADH_2$ generated by SDH, complex II also accepts electrons from the $FADH_2$ generated during fatty acid oxidation via the fatty acyl-CoA dehydrogenases and from mitochondrial glycerol-3-phosphate dehydrogenase (GPD2) of the glycerol phosphate shuttle*' (Figure 8d).

45 https://www.chem.purdue.edu/courses/chm333/Spring%202013/Lectures/Spring%202013%20Lecture%2037%20-%2038.pdf - CHM333 LECTURES 37 & 38: 4/27 – 29/13 SPRING 2013 Professor Christine Hrycyna - Acyl-CoA dehydrogenase is listed under 'Electron transfer in Complex II'.

Supplement S4: Footnotes on terminology

Electron transfer: A distinction is necessary between electron *transfer* in redox reactions and electron *transport* in the diffusion of charged ionic species within or between cellular compartments.

Electron transfer system ETS: The *convergent* architecture of the electron transfer *system* is emphasized in contrast to *linear* electron transfer *chains* ETCs within segments of the ETS.

Matrix-ETS: Electron transfer and corresponding OXPHOS capacities are classically studied in mitochondrial preparations as oxygen consumption supported by various fuel substrates undergoing partial oxidation in the mt-matrix, such as pyruvate, malate, succinate, and others. Therefore, the *matrix* component of ETS (matrix-ETS) is distinguished from the ETS *bound to the mt-inner membrane* (membrane-ETS; Gnaiger et al 2020).

Membrane-ETS: Electron transfer is frequently considered as the segment of redox reactions linked to the mtIM. However, the *membrane*-ETS is only part of the total ETS, which includes the upstream *matrix*-ETS.

2{H^++e-}: The symbol [2 H] is frequently used to indicate redox equivalents in the transfer from hydrogen donors to hydrogen acceptors, which does not explicitly express that it applies equally to *electron* and *hydrogen* ion transfer. Brackets are avoided to exclude the confusion with their frequent application to indicate amount-of-substance concentrations.