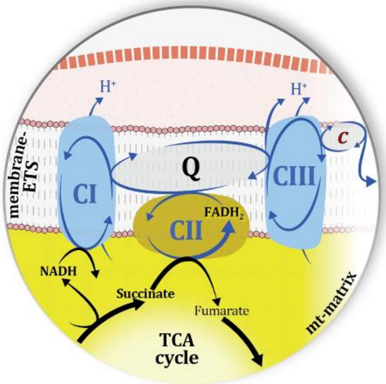
Theoretical Communication

Cite
Gnaiger E (2023) Complex II ambiguities ─ FADH2 in the electron transfer system. MitoFit Preprints 2023.3. https://doi.org/10.26124/mitofit:2023-0003

Conflicts of interest
The author declares no conflict of interest.



Received 2023-03-24
Accepted 2023-03-24
Online 2023-03-24

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

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

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Summary

The current narrative that the reduced coenzymes NADH and $FADH_2$ feed electrons from the tricarboxylic acid (TCA) cycle into the mitochondrial electron transfer system can create ambiguities around respiratory Complex CII. Succinate dehydrogenase or CII reduces FAD to $FADH_2$ in the canonical forward TCA cycle. However, some graphical representations of the membrane-bound electron transfer system (ETS) depict CII as the site of oxidation of $FADH_2$. This leads to the false believe that $FADH_2$ generated by electron transferring flavoprotein (CETF) in fatty acid oxidation and mitochondrial glycerophosphate dehydrogenase (CGpDH) feeds electrons into the ETS through CII. In reality, NADH and succinate produced in the TCA cycle are the *substrates* of Complexes CI and CII, respectively, and the reduced flavin groups $FMNH_2$ and $FADH_2$ are downstream *products* of CI and CII, respectively, carrying electrons from CI and CII into the Q-junction. Similarly, CETF and CGpDH feed electrons into the Q-junction but not through CII. The ambiguities surrounding Complex II in the literature call for quality control, to secure scientific standards in current communications on bioenergetics and support adequate clinical applications.

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; Arnold, Finley 2023). TCA cycle metabolites are (*1*) oxidized while reducing NAD^+ to NADH in the forward cycle, or (*2*) transported into the cytosol (Murphy MP, O'Neill LAJ 2018). Succinate dehydrogenase (succinate:quinone oxidoreductase, Complex CII) plays a key role in metabolic remodeling in cancer tissue (DeBerardinis, Chandel 2016; Schöpf et al 2020). The reversed TCA cycle has gained interest in studies ranging from anaerobic metabolism (Hochachka, Somero 2002), thermodynamic efficiency of anoxic and aerobic ATP production (Gnaiger 1993), reversed electron transfer and ROS production (Tretter et al 2016; Robb et al 2018), hypoxia and ischemia-reperfusion injury (Couchani et al 2014), to pathway evolution (Lane 2022).

Complex CII participates both in the membrane-bound electron transfer system (membrane-ETS) and TCA cycle (matrix-ETS plus CII; Gnaiger et al 2020). Branches of electron transfer from the reduced coenzyme NADH of nicotinamide adenine dinucleotide N and succinate S converge at coenzyme Q (Q-junction; 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 to Q from CII and CI, respectively, just as succinate and NADH are the comparable reduced substrates of CII and CI, respectively (Hatefi 1962; Tzagoloff 1982; Gnaiger 2020). In CII the oxidized form FAD is reduced by succinate to the product $FADH_2$ while fumarate is formed as the oxidized product in the TCA cycle. In CI FMN is reduced by NADH forming (red) $FMNH_2$ and (ox) NAD^+. $FADH_2$ and $FMNH_2$ are reoxidized downstream in CII and CI by electron transfer to Q in the membrane-bound ETS (Figure 1b).

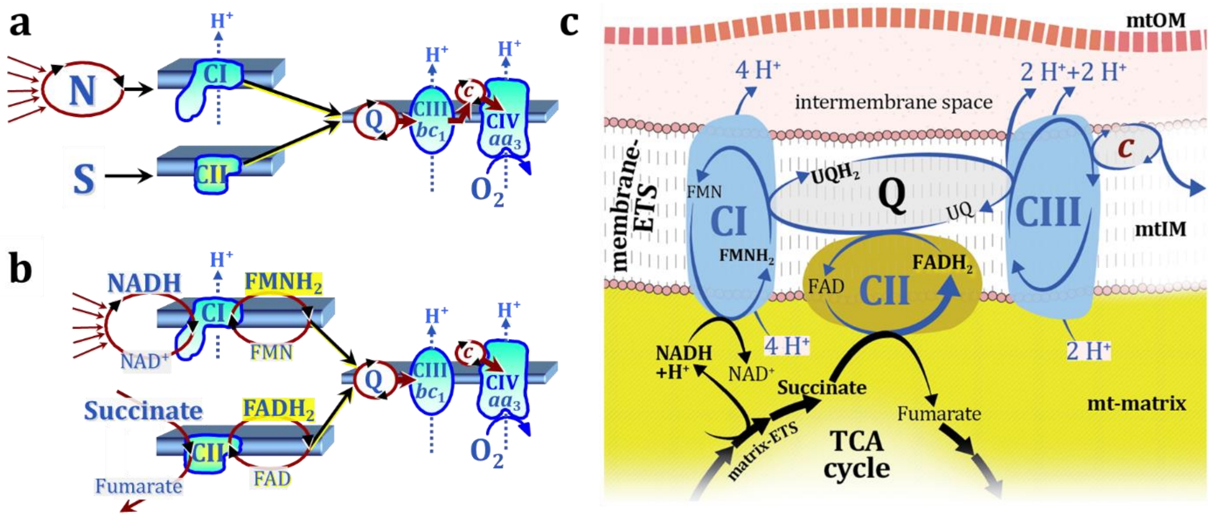

Figure 1. Convergent electron transfer from NADH and succinate to the Q-junction. **(a)** Electron flow catalyzed by dehydrognases localized in the mitochondrial (mt) matrix reduces nicotinamide adenine dinucleotide N and converges at the N-junction. Electron flow through Complexes CI and CII converges at the Q-junction. Modified from Gnaiger (2020). **(b)** NADH and succinate are substrates of CI- and CII-catalyzed redox reactions, respectively. $FMNH_2$ and $FADH_2$ are products in CI and CII, respectively. **(c)** Complex CII is integrated in the membrane-bound electron transfer system (membrane-ETS in the mt-inner membrane mtIM) and the TCA cycle (matrix-ETS). Electron flow [2 H] +0.5 $O_2 \dashrightarrow H_2O$ from succinate reduces FAD to $FADH_2$. [2 H] from $FADH_2$ reduces ubiquinone UQ to ubiquinol UQH_2. Complex CIII passes electrons to cytochrome *c*.

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 1c) may be communicated as a divergence from an established truth. The following quotes from Cooper (2000) provide an example (Figure 2).

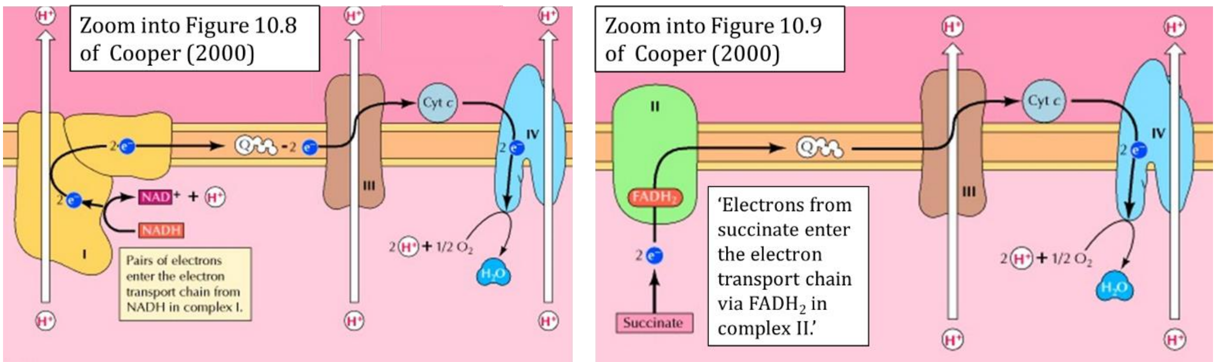

Figure 2. Electron flow into Complexes CI (left) and CII (right). Zoom into figures of Cooper (2000).

(*1*) The standard comparison is made between NADH (linked to CI) and $FADH_2$ (linked to CII): '*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 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 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 1c). (*b*) If electrons enter the '*electron transport chain* via *$FADH_2$ in complex II*', then CII 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).

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 error.

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

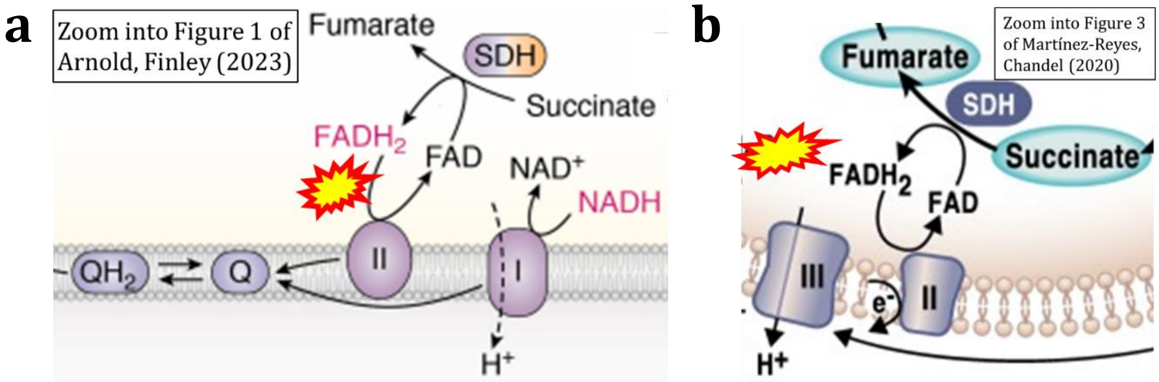

Figure 3. FADH2 depicted as product and substrate of Complex II. Zoom into figures by **(a)** Arnold, Finley (2023) and **(b)** Martínez-Reyes, Chandel (2020).

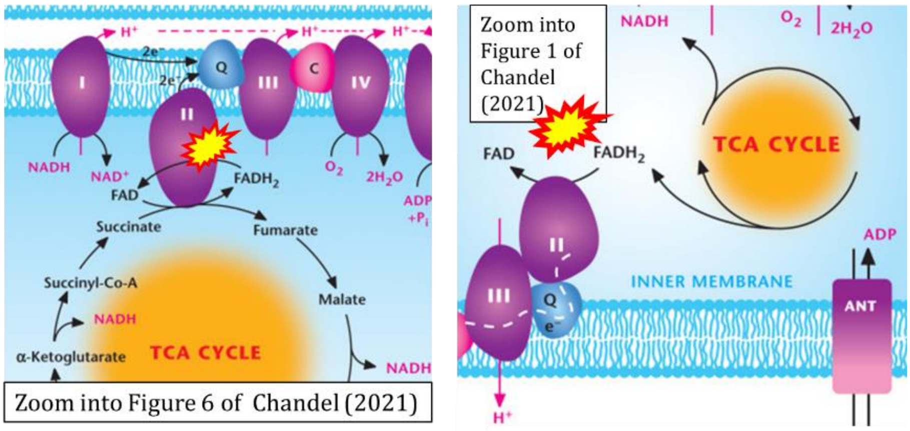

Figure 4. Evolving disarrangement in graphical representations of FADH2 as a product or substrate of Complex II. From ambiguity to error in figures 6 and 1 of Chandel (2021).

(2) Evolution from ambiguity to error in graphical representations (Figure 4).

(3) Discrepancy between erroneous graphical representation (Figure 5) and correct text. '*Reducing equivalents (NADH, FADH2) provide electrons that flow through complex I, the ubiquinone cycle (Q/QH2), complex III, cytochrome c, complex IV, and to the final acceptor O2 to form water*' (Fisher-Wellman, Neufer 2012).

(*4*) Simple graphical errors (Figure 6).

(*5*) Error propagation from graphical representation (Figure 3a) to text: '*SDH reduces FAD to FADH2, which donates its electrons to complex II*'; '*each complete turn of the TCA cycle generates three NADH and one FADH2 molecules, which donate their electrons to complex I and complex II, respectively*'; '*complex I and complex II oxidize NADH and FADH2, respectively*' (Arnold, Finley 2023).

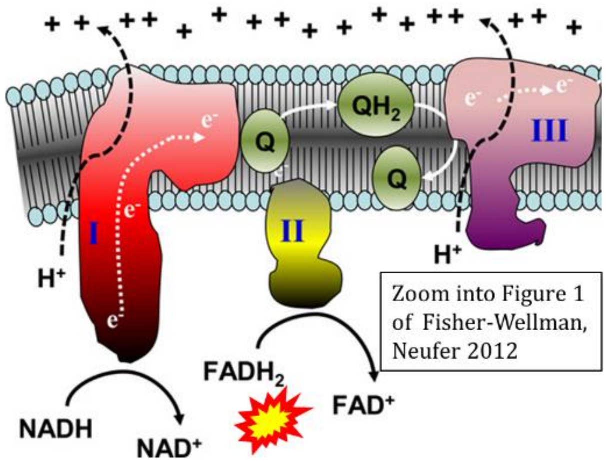

Figure 5. FADH2 is shown as the substrate of Complex II. This graphical representation contradicts the text that clarifies that FADH2 provides electron flow through the Q-cycle (Fisher-Wellman and Neufer 2012).

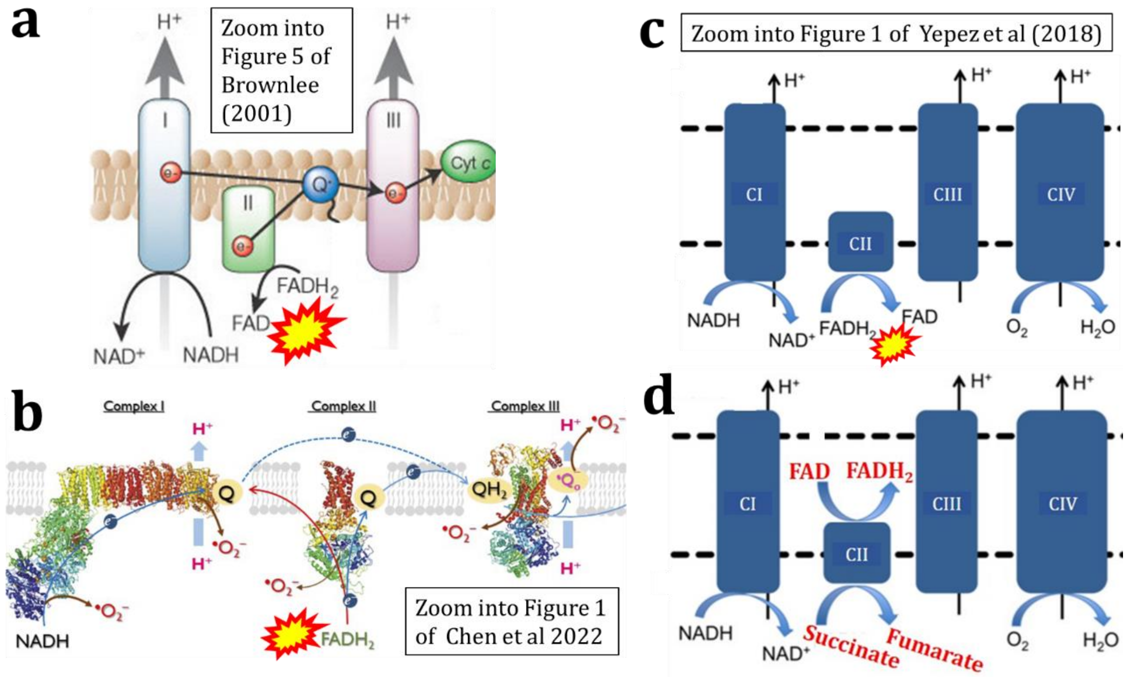

Figure 6. FADH2 is shown as the substrate of Complex II. Zoom into figures from **(a)** Brownlee (2001), **(b)** Chen et al (2022), **(c)** Yépez et al (2018). **(d)** Correction showing succinate and FAD as substrates of Complex II.

The 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. 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).

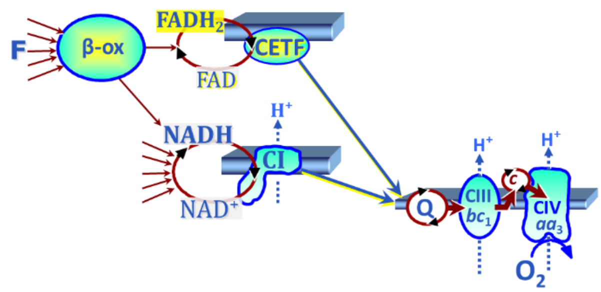

Figure 7. Fatty acid oxidation through the β-oxidation cycle (β-ox), electron transferring flavoprotein CETF, and Complex CI with convergent electron transfer into the Q-junction. Modified after Gnaiger (2020).

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 #36). 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).

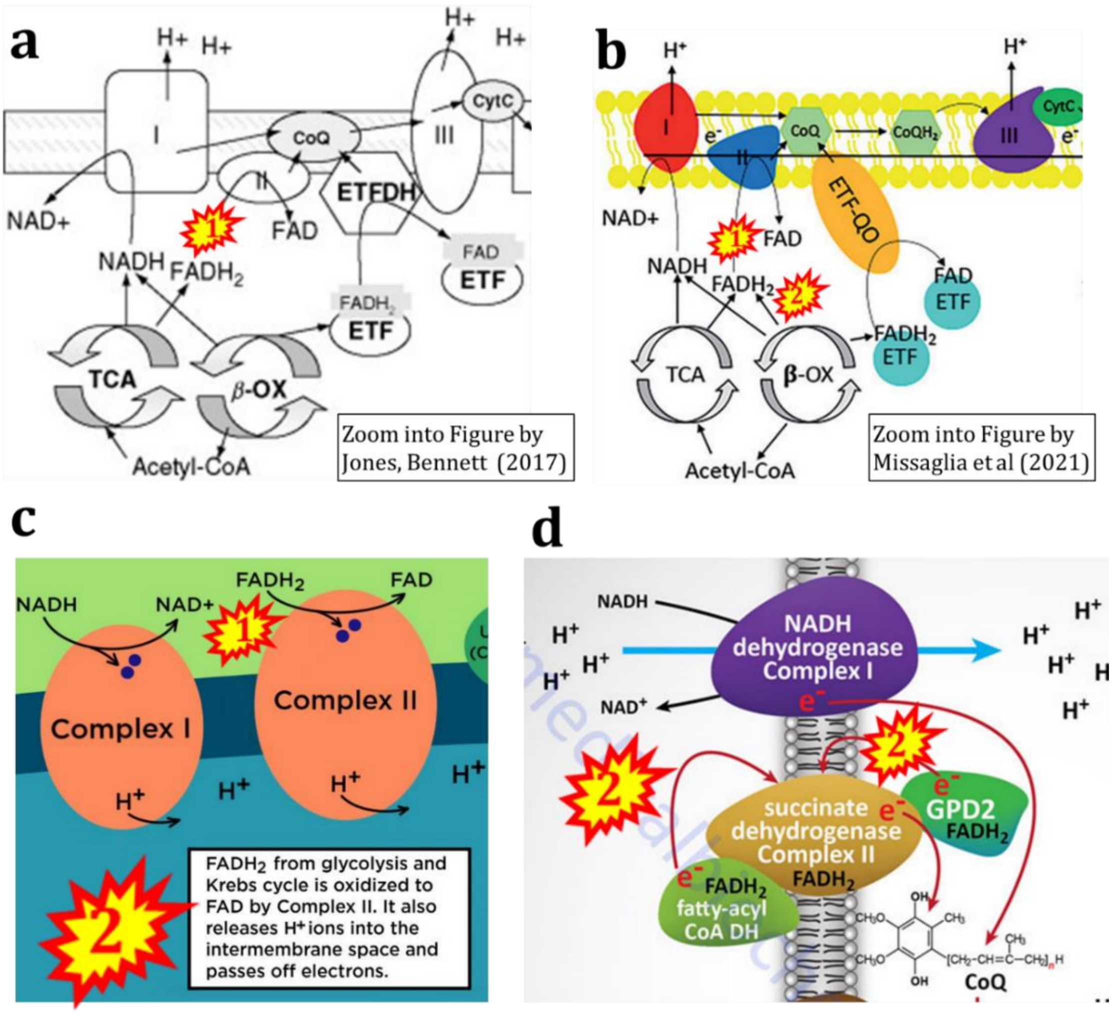

Figure 8. When $FADH_2$ is erroneously shown as a substrate of CII (1), a role of CII in oxidation of $FADH_2$ from glycolysis and fatty acid oxidation is suggested as a consequence (2). Zoom into figures by **(a)** Jones, Bennett (2017); **(b)** Missaglia et al (2021); **(c)** https://www.expii.com/t/electron-transport-chain-summary-diagrams-10139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

4. Conclusions

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. 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

C1	Complex I	FAO	fatty acid oxidation
CII	Complex II	$FMNH_2$	reduced flavin mononucleotide
CETF	electron transferring flavoprotein	$NADH_2$	reduced nicotinamide adenine dinucleotide
$FADH_2$	reduced flavin adenoside dinucleotide	TCA cycle	tricarboxylic acid cycle

Acknowledgements

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

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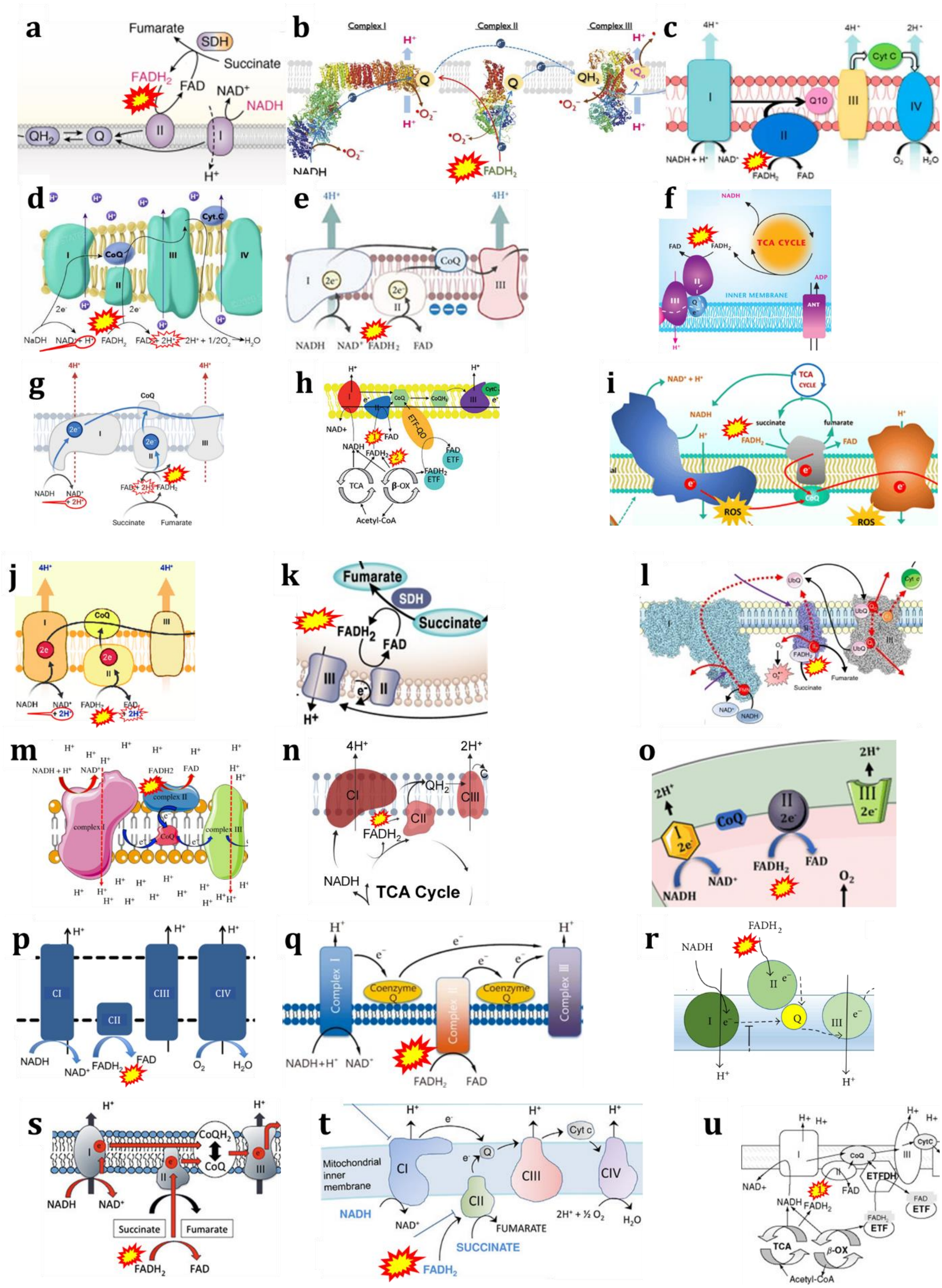
Supplement Figure S1



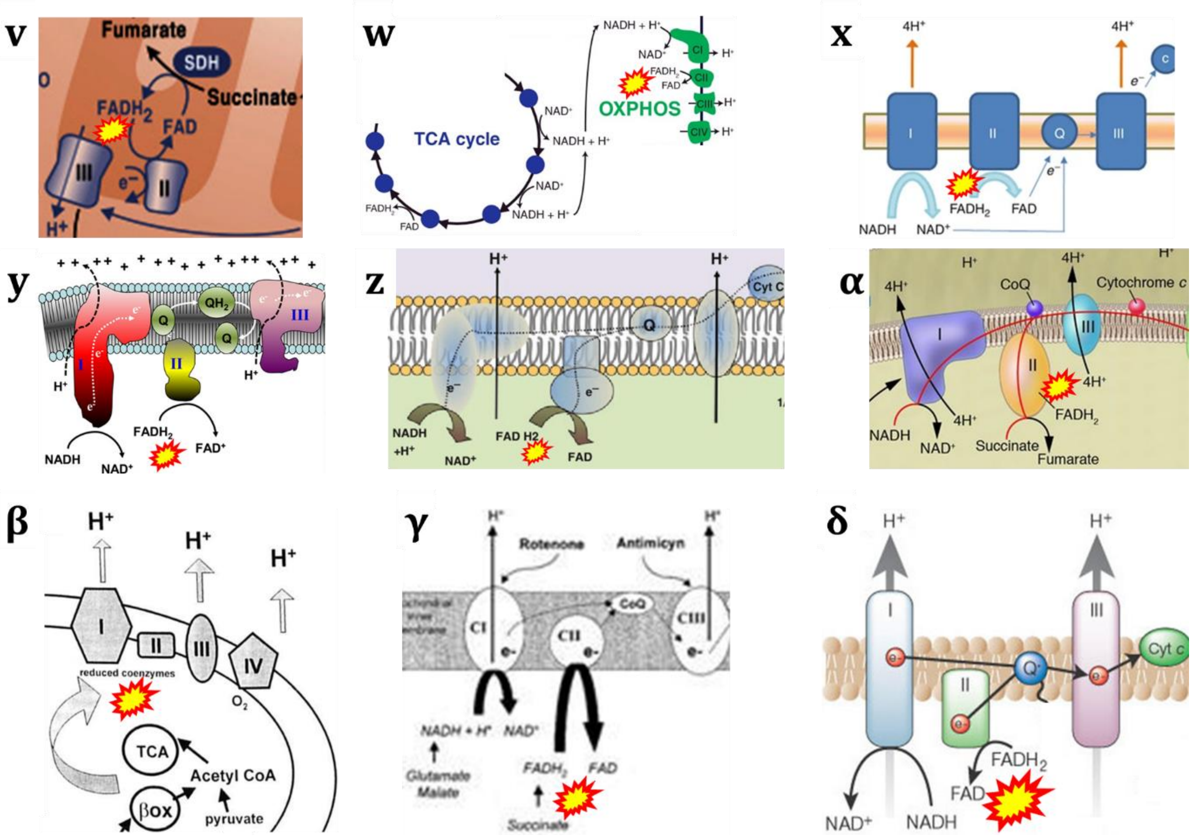


Figure S1. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. Chronological sequence of publications from 2001 to 2023. See References for Figure S1.

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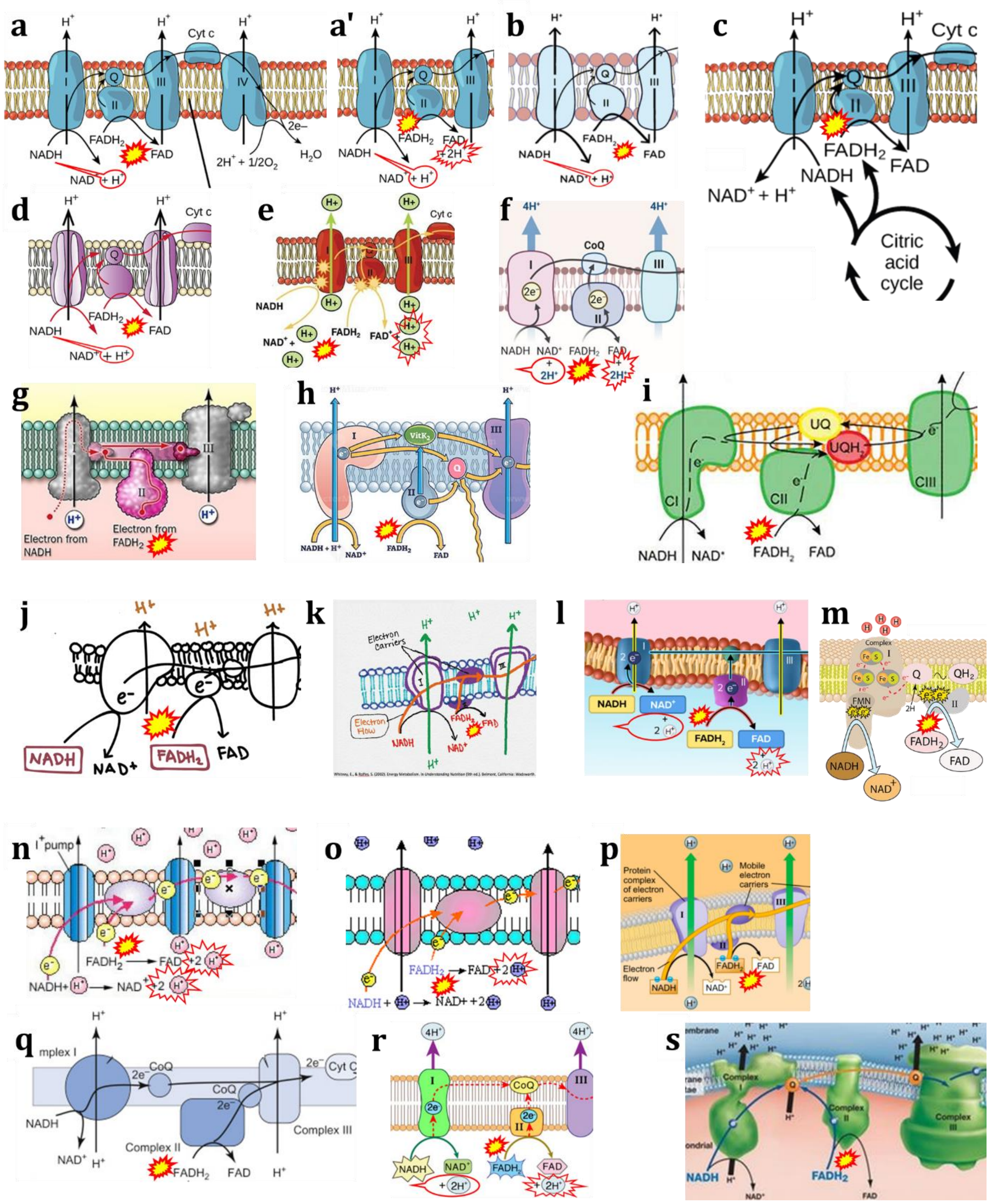
Supplement Figure S2



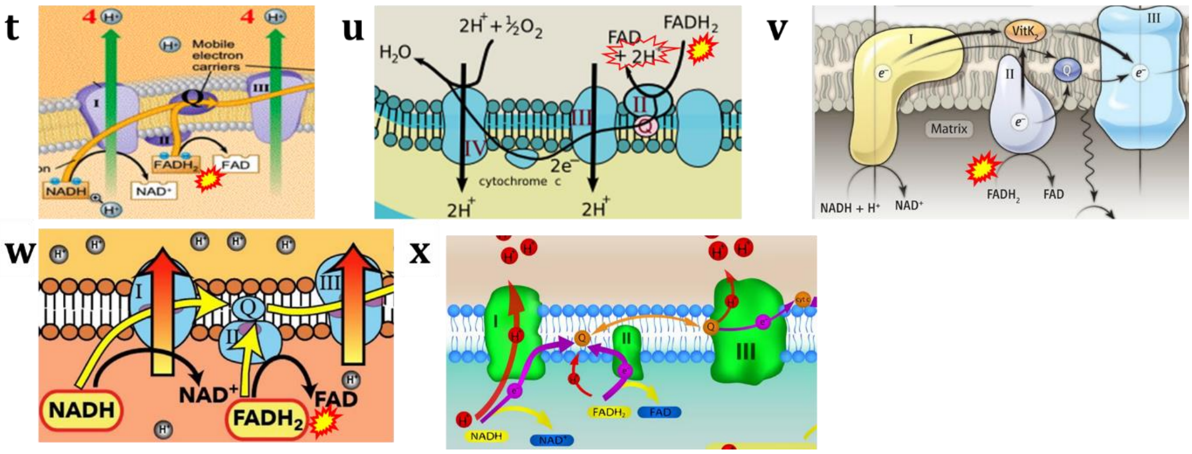


Figure S2. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. Weblinks (#): **a** 1-5; **a'** 6-7; **b** 8; **c** 1, 6, 7, 9; **d** 10; **e** 4, 9, 11-16; **f** 17-18; **g** 19; **h** 20-21; **i** 22; **j** 6-7; **k** 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** 15, 17.

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

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

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3 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 - Figure 7.11.1

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

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7 https://learn.saylor.org/mod/page/view.php?id=32815 -Saylor Academy

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9 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

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Supplement S3

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

35 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 $FADH_2$.*

36 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).

37 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'.