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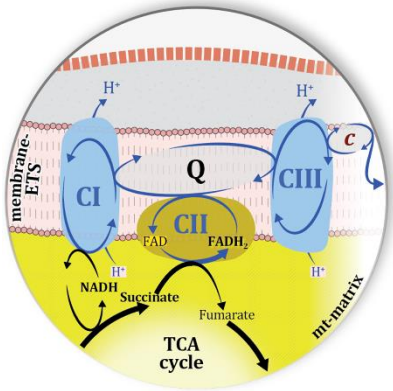

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tricarboxylic acid cycle, TCA

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 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 and the β-oxidation cycle in fatty acid oxidation. 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 are 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 membrane-bound electron transfer system (membrane-ETS). 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 (Cecchini 2003). 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 production of reactive oxygen species (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).

Two-electron transfer $2\{e^-\}$ from succinate to the oxidized flavin adenoside dinucleotide FAD is redox-coupled to the transfer of two hydrogen ions $2\{H^+\}$ with formation of $FADH_2$. This H^+-linked electron transfer (Hsu et al 2022) through CII is not coupled to H^+ translocation across the mitochondrial inner membrane (mtIM). Hence, CII is not a H^+ pump in contrast to the respiratory Complexes CI, CIII and CIV through which electron transfer drives and maintains the protonmotive force. The coenzyme NAD^+ is reduced to $NADH+H^+$ during the oxidation of pyruvate and through redox reactions catalyzed by 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). Confusion emerges, however, when NADH and $FADH_2$ are considered as 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 deeply penetrated the scientific literature 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 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. SDHA contains the catalytically active dicarboxylate binding site where succinate is oxidized to fumarate. 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 final electron transfer to coenzyme Q (Figure 1b).

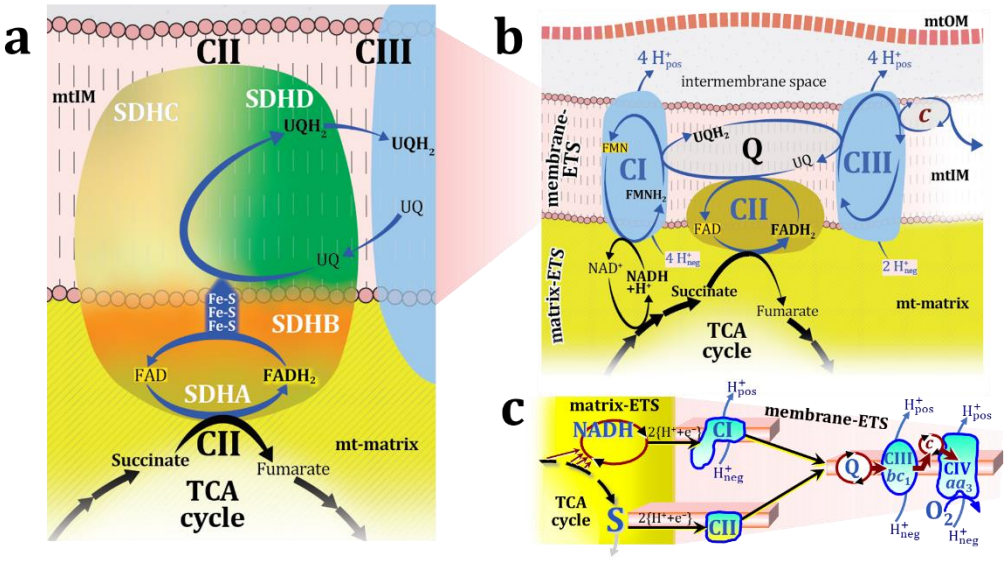


Figure 1. Complex II bridges electron transfer from the TCA cycle to the mitochondrial inner membrane. Graphical representations of the electron transfer system ETS with successive emphasis on pathway architecture and concomitant loss of detail. CII is integrated in the TCA cycle (matrix-ETS) and the membrane-bound electron transfer system (membrane-ETS of the mt-inner membrane mtIM). Joint pairs of half-circular arrows indicate electron transfer $2\{H^+ + e^-\}$, distinguished from vectorial hydrogen ion transport across the mtIM ($H^+_{neg} \rightarrow H^+_{pos}$) from the negatively to the positively charged compartment. **(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 $2\{H^+ + e^-\}$ transfer to CI and CII, respectively, with $FMNH_2$ and $FADH_2$ as the corresponding products. NADH+H^+ and NAD^+ cycle between matrix-dehydrogenases and CI, whereas FAD and $FADH_2$ cycle within the enzyme CII. 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 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 ⇢ H_2O, converges through CI and CII at the Q-junction. CIII passes electrons to cytochrome *c* and in CIV to O_2.

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).

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 comparison between NADH linked to CI and $FADH_2$ (instead of succinate) linked to CII leads us astray, as illustrated by the following quotes from Cooper (2000) (Figure 2).

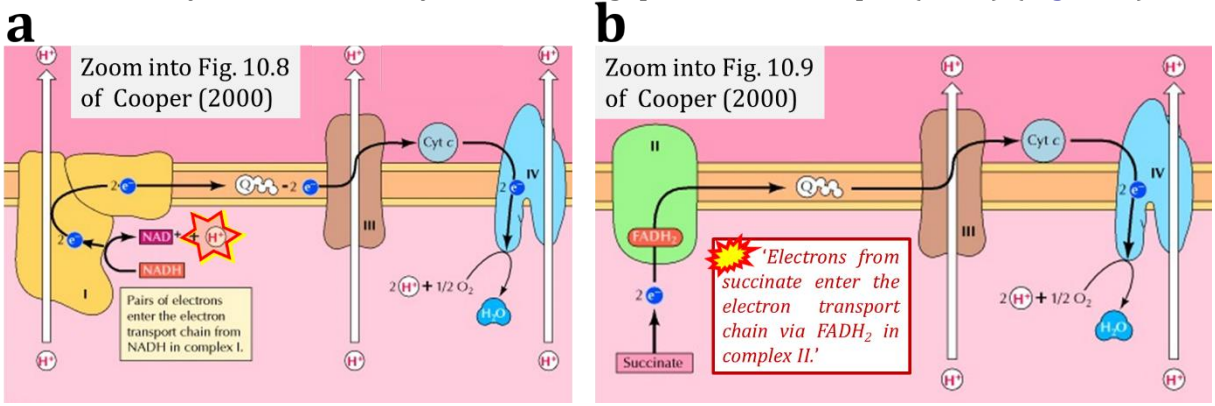


Figure 2. Electron flow into Complexes (a) CI and (b) CII. Zoom into figures of Cooper (2000). **(a)** The marked H^+ is consumed in H^+-linked electron transfer instead of being produced. **(b)** Marked quote inserted from the legend to Figure 10.9.

(*1*) '*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 total Gibbs force (Gnaiger 2020) in $S \rightarrow FADH_2 \rightarrow Q$ 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*'; Supplement 1). 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).

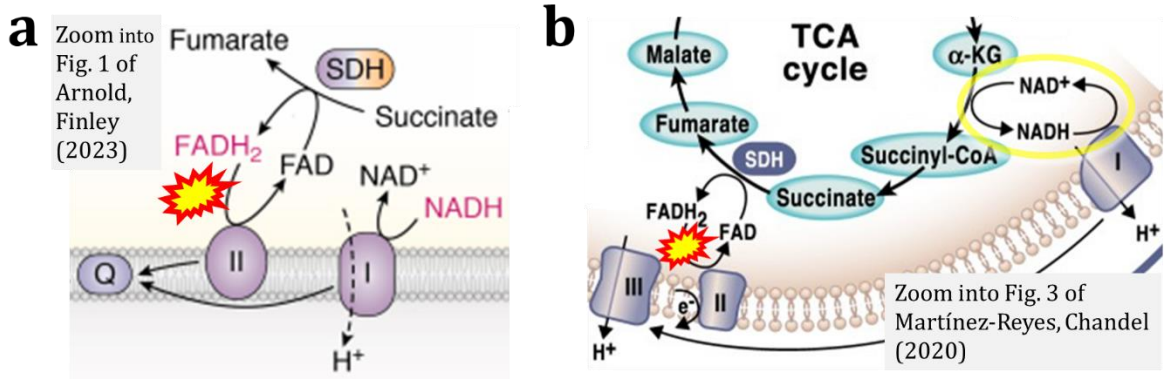


Figure 3. $FADH_2$ depicted as product and substrate of Complex II. Zoom into figures by **(a)** Arnold, Finley (2023) and **(b)** Martínez-Reyes, Chandel (2020). NADH and NAD^+ cycle between different types of enzymes (yellow circle), in contrast to the $FADH_2$-FAD cycle located within the same enzyme Complex (SDH and CII are synonyms).

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 in 98 publications found from 2001 to 2023 (Supplements 2 to 6) and educational websites (Supplement 7). Clarification is required (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 (synonymous with SDH) in the same figure (Figure 3; Suppl Figure S2).

(2) Correct representation or ambiguity evolved to misconception in graphical representations (Figure 4).

(*3*) Graphical errors on electron entry from $FADH_2$ into CII show up without comment in or context to the text (Figure 5; Suppl Figures S3). Instead of $NADH+H^+ \rightarrow NAD^+$ there appears $NADH \rightarrow NAD^+ + H^+$ (or $+2H^+$) and by analogy $FADH \rightarrow FAD + 2H^+$ (Figure 5d; Suppl Figure S4). The analogy $NADH \rightarrow NAD^+$ is taken further to include a charge for FAD or even writing $FADH^+$ as the product (Figure 6; Suppl Figure S5).

(*4*) 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).

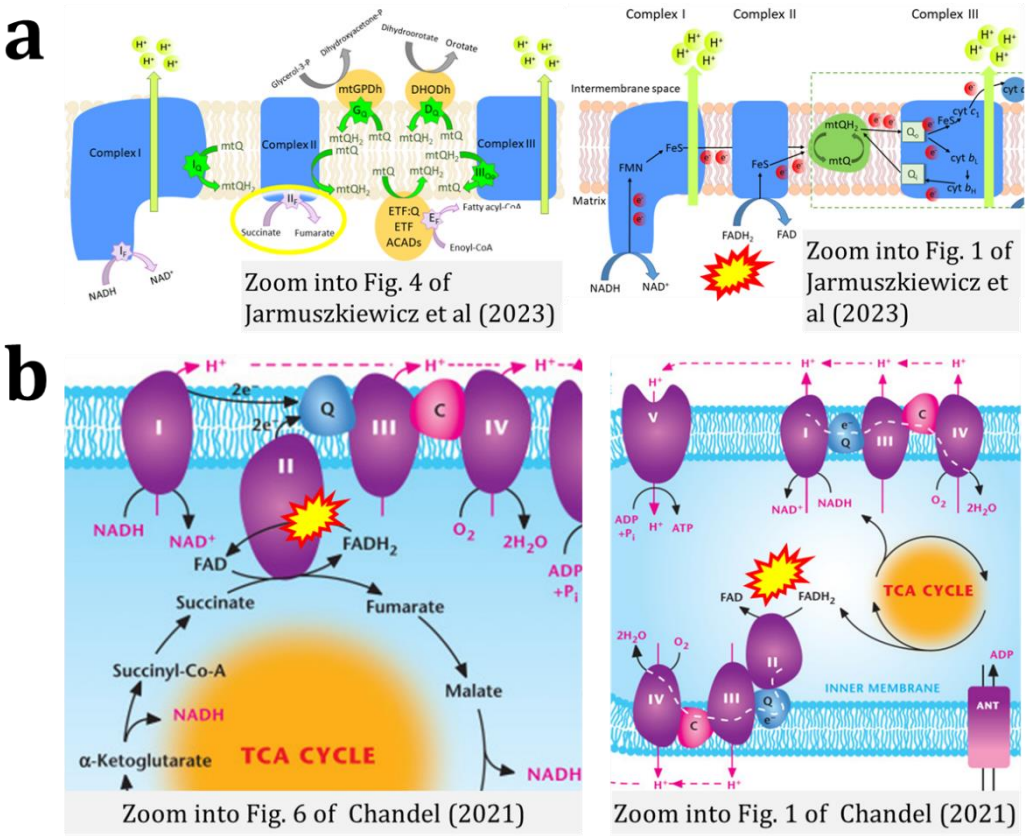


Figure 4. Evolving disarrangement in graphical representations of $FADH_2$ as a substrate of CII. (a) Succinate or $FADH_2$ as substrates of CII (Jarmuszkiewicz et al 2023). **(b)** From ambiguity to misconception (Chandel 2021).

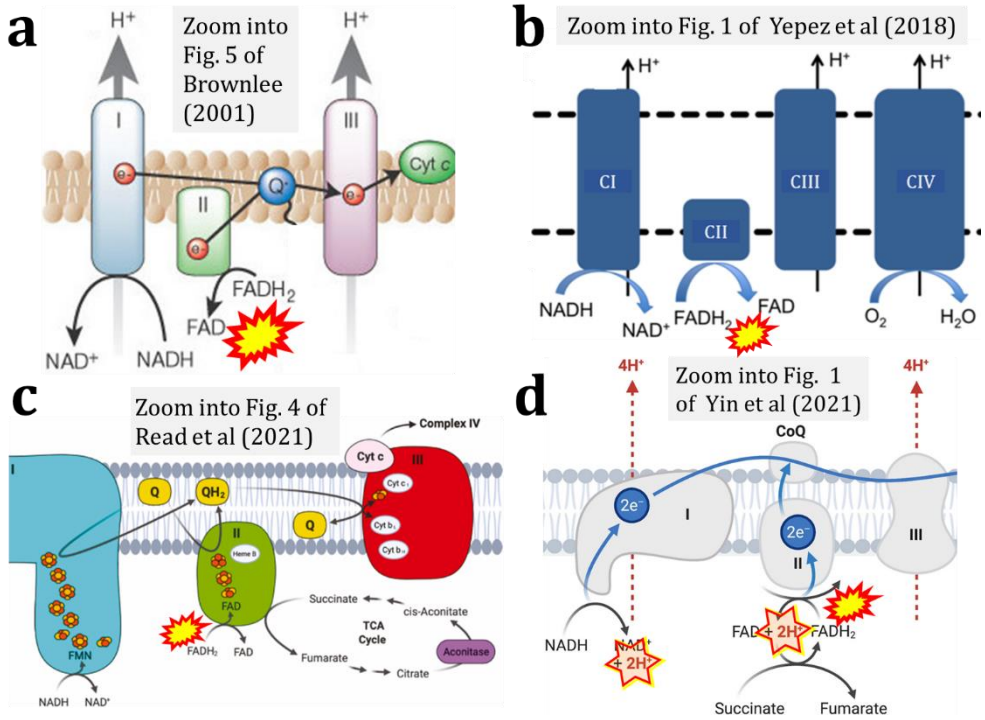
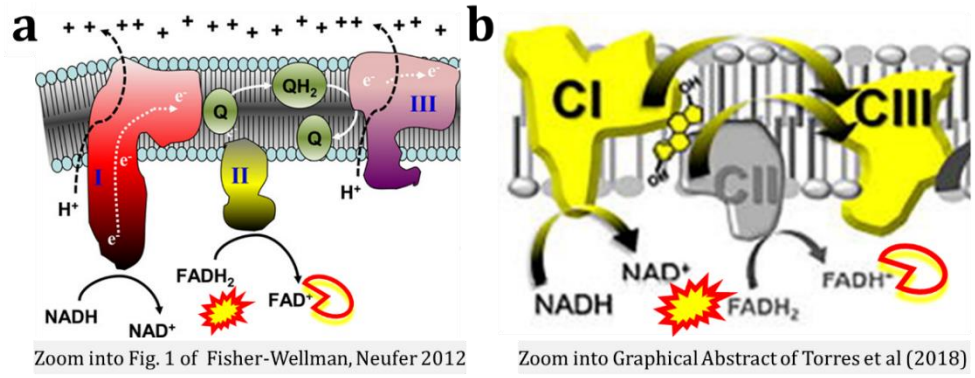


Figure 5. $FADH_2$ shown as substrate of CII. Zoom into figures from **(a)** Brownlee (2001); **(b)** Yépez et al (2018); **(c)** Read et al (2021) showing FAD as product in CII and the mt-matrix; **(d)** Yin et al (2021) with unjustified indication of $2H^+$ formation in the mt-matrix.



Zoom into Fig. 1 of Fisher-Wellman, Neufer 2012

Zoom into Graphical Abstract of Torres et al (2018)

Figure 6. $FADH_2$ is shown as the substrate of CII. These graphical representations take the NADH→NAD^+ analogy to the level of depicting FAD as **(a)** FAD^+ (Fisher-Wellman, Neufer 2012) or **(b)** $FADH^+$ (Torres et al 2018).

In summary, downstream of the dehydrogenases of the TCA cycle, NADH is oxidized by CI. Two-electron oxidation of succinate is redox-linked to reduction of FAD to $FADH_2$. In terms of electron entry into CII many publications show it in the wrong direction, i.e. $FADH_2$ as electron donor from the TCA cycle to CII (Figures 3 to 6; Suppl Figures S2 to S6).

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 a redox reaction, 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 redox reaction equation

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

Commonly the charges of succinate, fumarate (Eq. 1), 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)

In brief, oxidation of NADH and $FADH_2$ may be represented as

ox: $NADH + H^+ \longrightarrow NAD^+ + 2\{H^+ + e^-\}$ (Eq. 3a)
ox: $FADH_2 \longrightarrow FAD + 2\{H^+ + e^-\}$ (Eq. 3b)

H^+ in Eq. 3a is frequently omitted to simplify graphical representations, and a pair of rounded arrows – one external touching the enzyme and one internal within the enzyme – indicates H^+-linked electron transfer in terms of $2\{H^+ + e^-\}$ (Figures 1a, 1b, 3a, and 4). However, caution is warranted to distinguish (*1*) H^+ in chemical acid/base reactions, such as the hydrogencarbonate equilibrium $H_2CO_3 \leftrightarrow HCO_3^- + H^+$, (*2*) chemical H^+-linked electron *transfer* (Hsu et al 2022) indicated as $2\{H^+ + e^-\}$ in redox reactions (Eq. 1 and 2), and (*3*) coupled vectorial *transport* or translocation of H^+ across the mtIM ($H^+_{neg} \longrightarrow H^+_{pos}$; Figures 1b and c; Supplement 1). The equilibrium in Eq. 3a depends on pH, whereas Eq.

3b is independent of pH. The fundamental differece between H^+ and $2\{H^+ + e^-\}$ in Eq. 3a is lost in representations such as Figure 5d.

Disturbingly, oxidation of $FADH_2$ is shown in meaningless patterns in various figures, occasionally corresponding with analogous representations of oxidation of NADH (Figure 6; Table 1).

Table 1. Misconceptions in graphical representations of electron entry into CII.

Analogy with NADH		Suppl Figure	$FADH_2$		Suppl Figure
$NADH + H^+$	$\longrightarrow NAD^+$				
NADH	$\longrightarrow NAD^+ + H^+$	S3d,r,λ,π,φ	$FADH_2$	$\longrightarrow$ FAD	S2, S3
NADH	$\longrightarrow NAD^+ + H^+$	S4a,e,g	$FADH_2$	$\longrightarrow FAD + 2H^+$	S4a-i
NADH	$\longrightarrow NAD^+ + 2H^+$	S4c,f,h,i			
			$FADH_2$	$\longrightarrow FAD^+$	S5a-g
$NADH + H^+$	$\longrightarrow$ NADH	S6a	$FADH_2$	$\longrightarrow$ FADH	S6a-d
			$FADH_2$	$\longrightarrow FADH^+$	S6e
			FADH	$\longrightarrow$	S6f
NADH	$\longrightarrow NAD + H^+$	S4b	FADH	$\longrightarrow FAD^+$	S6g
NADH	$\longrightarrow NAD^+ + H^+$	S6h	FADH	$\longrightarrow FAD^+ + H^+$	S6h
NADH	$\longrightarrow NAD^+ + H^+$	S6i	FADH	$\longrightarrow FAD^+ + 2H^+$	S6i

The erroneous presentation of electron transfer from $FADH_2$ to CII has a logical consequence. β-oxidation generates $FADH_2$ (Figure 7). If $FADH_2$ would donate electrons to CII, then CII can be seen as an enzyme involved downstream of $FADH_2$ in FAO. 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).

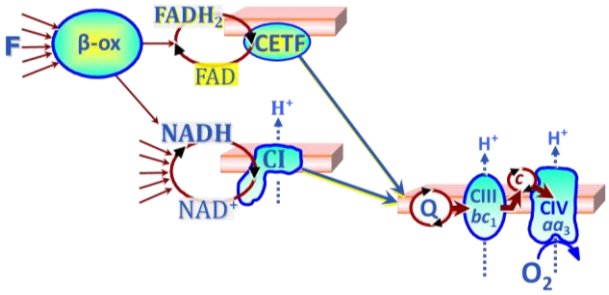


Figure 7. Fatty acid oxidation through the β-oxidation cycle (β-ox), electron transferring flavoprotein (CETF), and Complex I (CI) with convergent electron transfer into the Q-junction. Modified after Gnaiger (2020).

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 8; Supplement 8). 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). Fatty acylCoA dehydrogenase reduces FAD to $FADH_2$ in the mitochondrial matrix. The

$FADH_2$ of the of the fatty acyl-CoA dehydrogenase is reoxidized by the FAD-containing electron transfer flavoprotein Complex CETF. Thus $FADH_2$ can be seen as an internal substrate of CETF, comparable to NADH as a substrate of CI, succinate as a substrate of CII and glycerophosphate as a substrate of CGpDH.

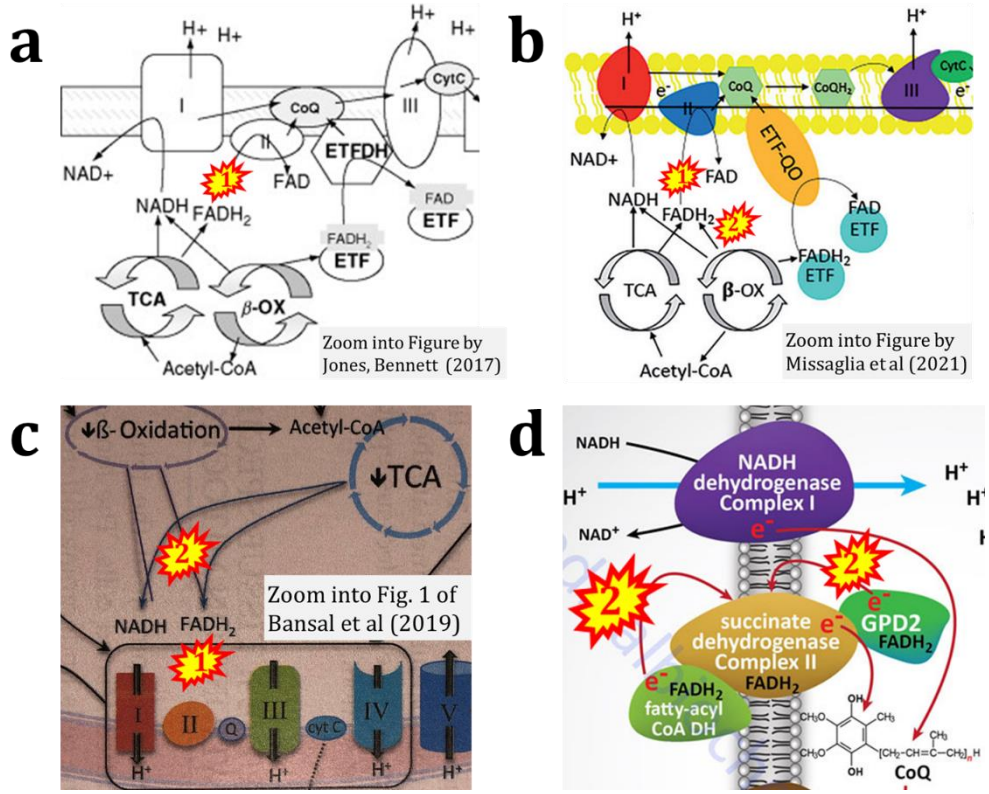


Figure 8. When $FADH_2$ is erroneously shown as a substrate of CII (1), a role of CII in oxidation of $FADH_2$ from fatty acid oxidation is suggested as a consequence (2). Zoom into figures from **(a)** Jones, Bennett (2017); **(b)** Missaglia et al (2021); **(c)** Bansal et al (2019); **(d)** https://themedicalbiochemistrypage.org/oxidative-phosphorylation-related-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 1). Do erroneous diagrams – from ambiguous electron transfer (Suppl Figures S2 to S8) to presentation of CII as a H^+ pump (Suppl Figure S9) – cast some doubts on the quality of the publication? Whether using iconic or symbolic elements in graphical representations, incorporating complementary text not only enhances the communication of intended meaning but diagrams will be improved in the process. When peer review provides insufficient help for corrections, post-peer review by editors and critical readers is required for revisions

of articles which may be re-published as living communications (Gnaiger 2021). Clarification instead of perpetuation of Complex II ambiguities leads to a better representation of fundamental concepts of bioenergetics and 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		

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References

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

Bansal A, Rashid C, Simmons RA (2019) Impact of fetal programming on mitochondrial function and susceptibility to obesity and type 2 diabetes. https://doi.org/10.1016/B978-0-12-811752-1.1.00014-6

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

Cecchini G (2003) Function and structure of Complex II of the respiratory chain. https://doi.org/10.1007/10.1146/annurev.biochem.72.121801.161700

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

Cooper GM (2000) The cell: a molecular approach. 2nd ed. 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 (2021) Beyond counting papers – a mission and vision for scientific publication. https://doi.org/10.26124/bec:2021-0005

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

Hsu CP, Hammarström L, Newton MD (2022) 65 years of electron transfer. https://doi.org/10.1063/5.0102889

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

Torres MJ, Kew KA, Ryan TE, Pennington ER, Lin CT, Buddo KA, Fix AM, Smith CA, Gilliam LA, Karvinen S, Lowe DA, Spangenburg EE, Zeczycki TN, Shaikh SR, Neufer PD (2017) 17β-estradiol directly lowers mitochondrial membrane microviscosity and improves bioenergetic function in skeletal muscle. https://doi.org/10.1016/j.cmet.2017.10.003

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 1

Footnotes on terminology

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.

Electron transfer: A distinction is necessary between electron *transfer* in redox reactions and electron *transport* (translocation) in the diffusion of charged ionic species within or between cellular compartments. The symbol $2\{H^+ + e^-\}$ is introduced to indicate H^+-linked electron transfer of two hydrogen ions and two electrons in a redox reaction.

H^+-linked electron transfer: The term H^+-coupled electron transfer (Hsu et al 2022) is replaced by H^+-*linked* electron transfer, to avoid confusion with *coupled* H^+ translocation.

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).

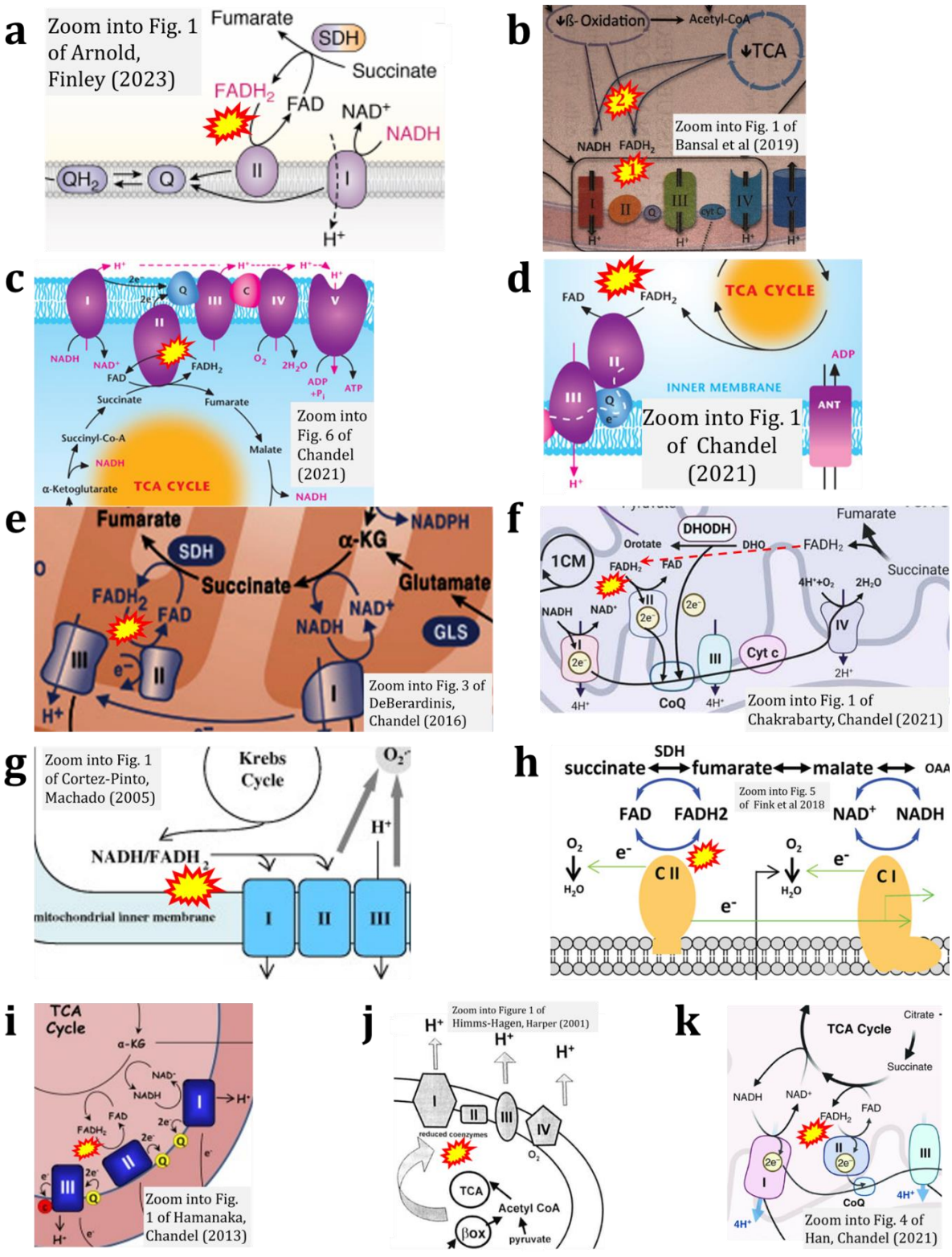
Membrane-ETS: Electron transfer is frequently considered as the segment of redox reactions linked to the mtIM. However, the *membrane*-ETS is only part of the total ETS, which includes the upstream *matrix*-ETS.

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

Supplement 2

FAD a substrate of SDH and $FADH_2$ a substrate of CII (Figure S2)

SDH: FAD ⟶ $FADH_2$
CII: $FADH_2$ ⟶ FAD (*1*)



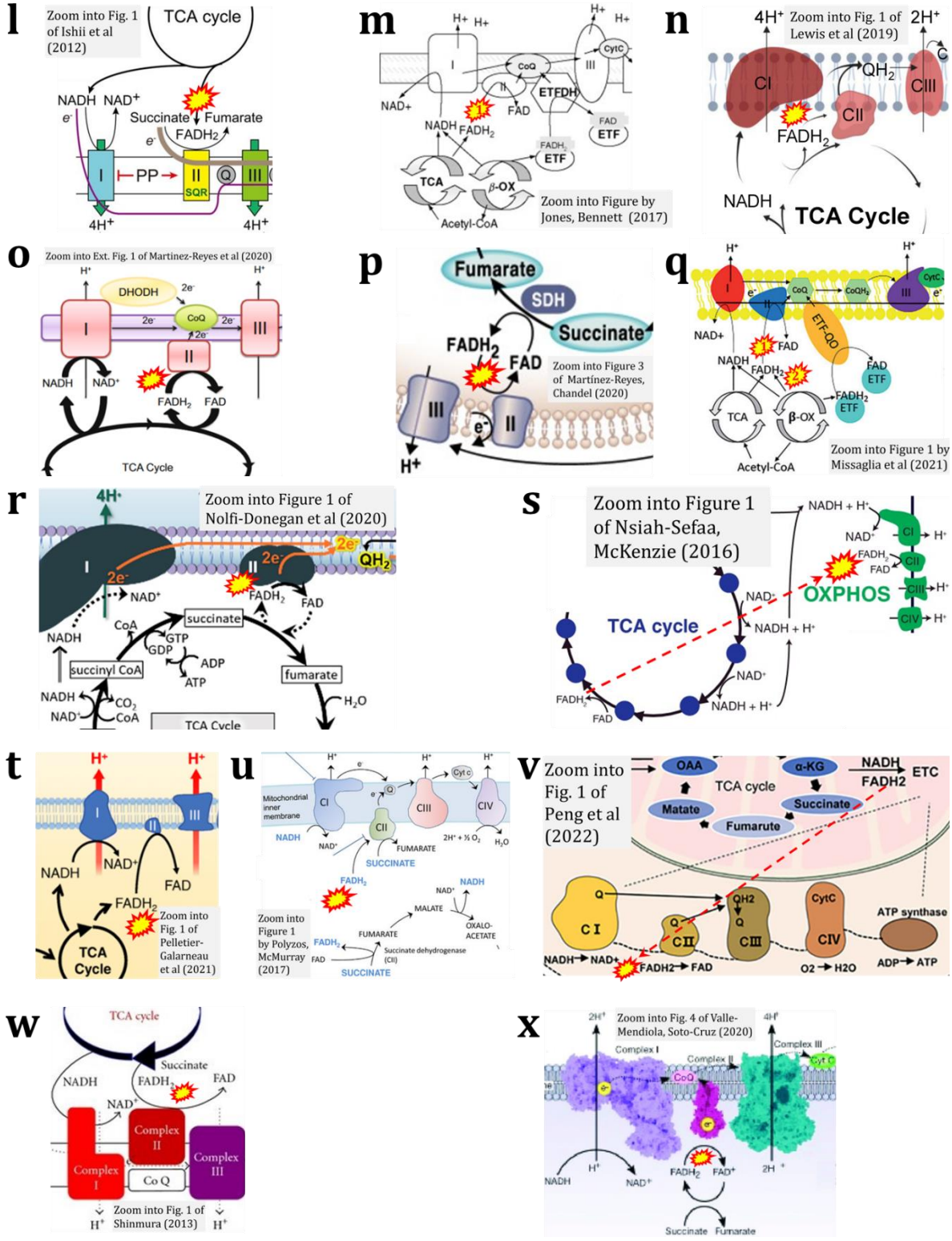


Figure S2. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. The TCA cycle reduces FAD to $FADH_2$ - in several cases shown to be catalyzed by SDH. Then $FADH_2$ is erroneously shown to feed electrons into CII. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S2.

References for Figure S2

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 Bansal A, Rashid C, Simmons RA (2019) Impact of fetal programming on mitochondrial function and susceptibility to obesity and type 2 diabetes. Academic Press In: Mitochondria in obesity and type 2 diabetes. Morio B, Pénicaud L, Rigoulet M (eds) **Academic Press.** https://doi.org/10.1016/B978-0-12-811752-1.1.00014-6

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

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

f Chakrabarty RP, Chandel NS (2021) Mitochondria as signaling organelles control mammalian stem cell fate. **Cell Stem Cell** 28:394-408. https://doi.org/10.1016/j.stem.2021.02.011

g Cortez-Pinto H, Machado MV (2009) Uncoupling proteins and non-alcoholic fatty liver disease. **J Hepatol** 50:857-60. https://doi.org/10.1016/j.jhep.2009.02.019

h 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

i Hamanaka RB, Chandel NS (2013) Mitochondrial metabolism as a regulator of keratinocyte differentiation. **Cell Logist** 3:e25456. https://doi.org/10.4161/cl.25456

j 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

k Han S, Chandel NS (2021) Lessons from cancer metabolism for pulmonary arterial hypertension and fibrosis. **Am J Respir Cell Mol Biol** 65:134-45. https://doi.org/10.1165/rcmb.2020-0550TR

l Ishii I, Harada Y, Kasahara T (2012) Reprofiling a classical anthelmintic, pyrvinium pamoate, as an anti-cancer drug targeting mitochondrial respiration. **Front Oncol** 2:137. https://doi.org/10.3389/fonc.2012.00137

m 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

n 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

o Martínez-Reyes I, Cardona LR, Kong H, Vasan K, McElroy GS, Werner M, Kihshen H, Reczek CR, Weinberg SE, Gao P, Steinert EM, Piseaux R, Budinger GRS, Chandel NS (2020) Mitochondrial ubiquinol oxidation is necessary for tumour growth. **Nature** 585:288-92. https://doi.org/10.1038/s41586-020-2475-6

p 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

q 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

r 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

s 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

t Pelletier-Galarneau M, Detmer FJ, Petibon Y, Normandin M, Ma C, Alpert NM, El Fakhri G (2021) Quantification of myocardial mitochondrial membrane potential using PET. **Curr Cardiol Rep** 23:70. https://doi.org/10.1007/s11886-021-01500-8

u 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

v Peng M, Huang Y, Zhang L, Zhao X, Hou Y (2022) Targeting mitochondrial oxidative phosphorylation eradicates acute myeloid leukemic stem cells. **Front Oncol** 12:899502. https://doi.org/10.3389/fonc.2022.899502

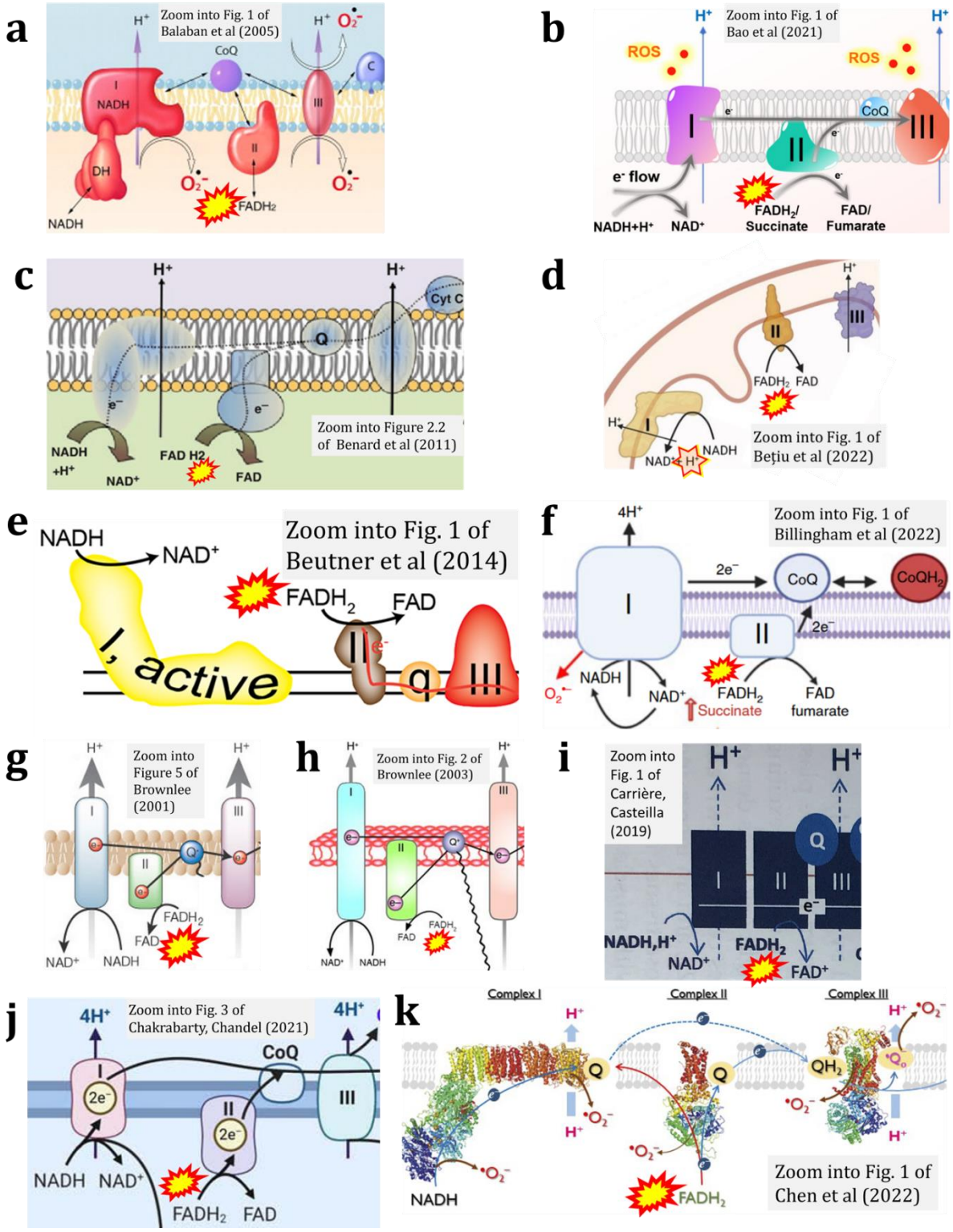
w Shinmura K (2013) Effects of caloric restriction on cardiac oxidative stress and mitochondrial bioenergetics: potential role of cardiac sirtuins. **Oxid Med Cell Longev** 2013:528935. https://doi.org/10.1155/2013/528935

x Valle-Mendiola A, Soto-Cruz I (2020) Energy metabolism in cancer: The roles of STAT3 and STAT5 in the regulation of metabolism-related genes. **Cancers (Basel)** 12:124. https://doi.org/10.3390/cancers12010124

Supplement 3

$FADH_2$ as substrate of CII (Figure S3)

$FADH_2 \longrightarrow FAD$ (*1*)



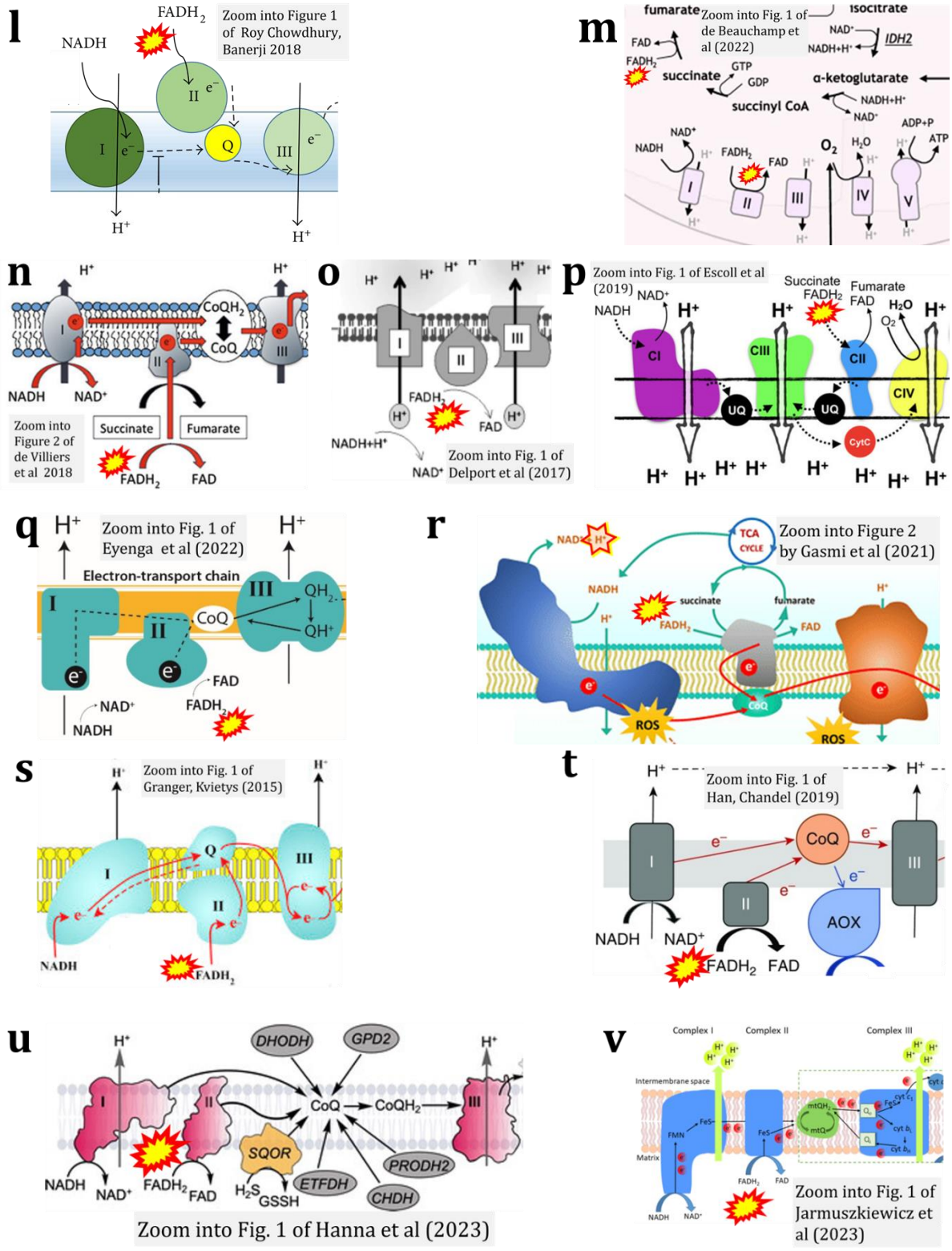


Figure S3. *Continued*

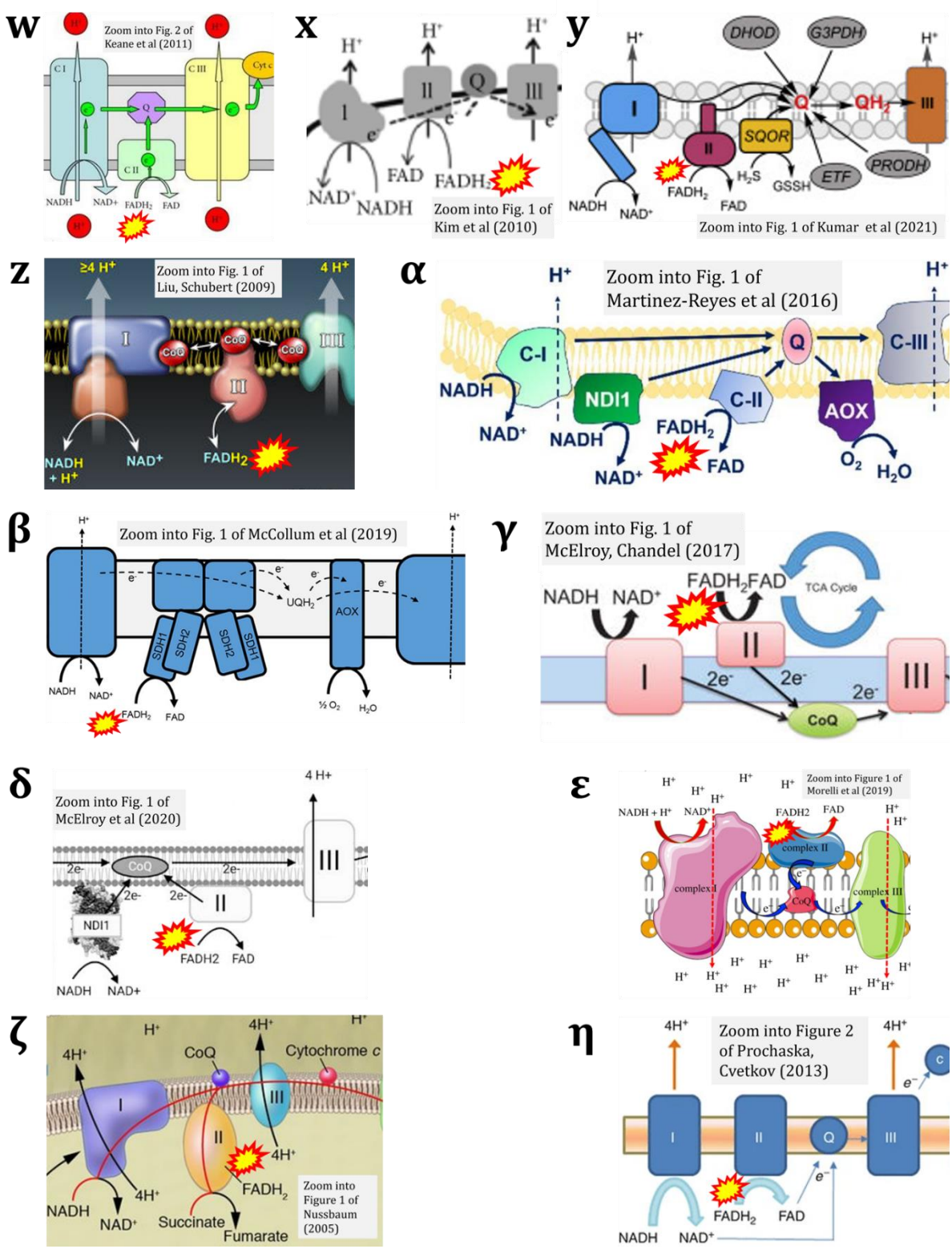


Figure S3. *Continued*

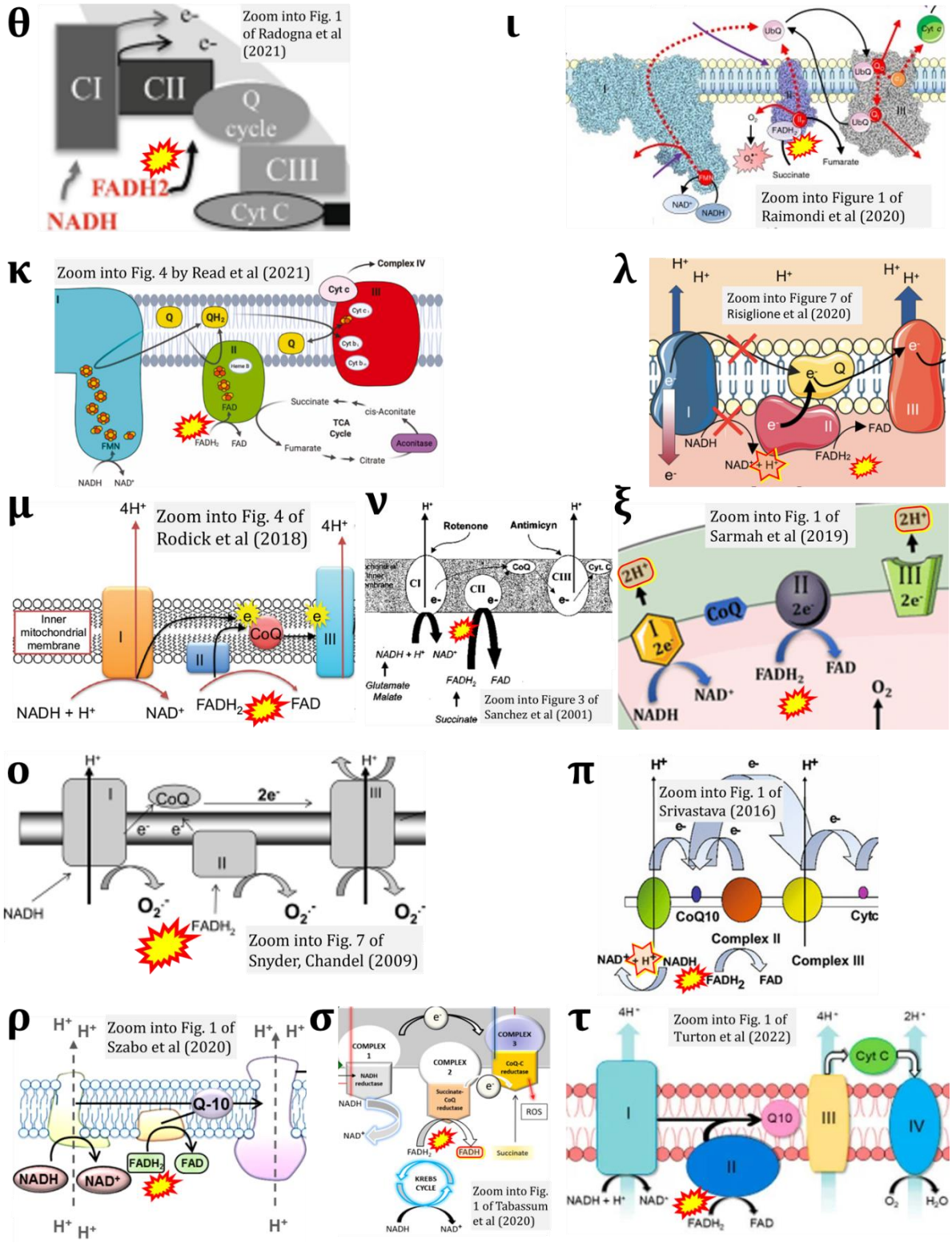


Figure S3. *Continued*

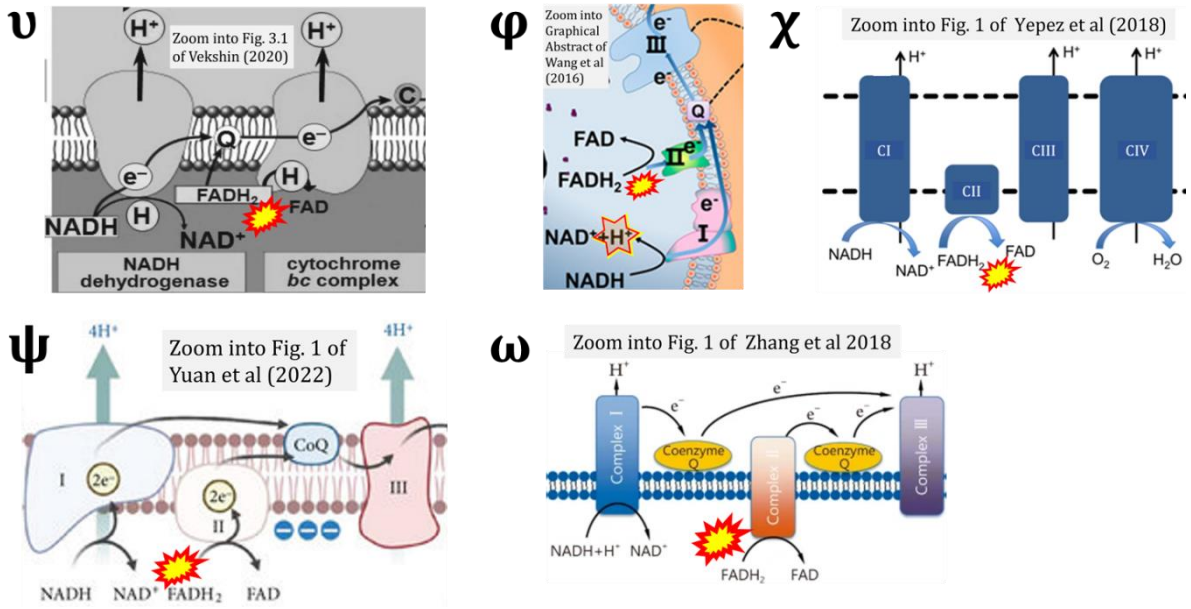


Figure S3. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S3.

References for Figure S3

a Balaban RS, Nemoto S, Finkel T (2005) Mitochondria, oxidants, and aging. **Cell** 120:483-95. https://doi.org/10.1016/j.cell.2005.02.001

b Bao MH, Wong CC (2021) Hypoxia, metabolic reprogramming, and drug resistance in liver cancer. **Cells** 10:1715. https://doi.org/10.3390/cells10071715

c 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.

d Beţiu AM, Noveanu L, Hâncu IM, Lascu A, Petrescu L, Maack C, Elmér E, Muntean DM (2022) Mitochondrial effects of common cardiovascular medications: the good, the bad and the mixed. **Int J Mol Sci** 23:13653. https://doi.org/10.3390/ijms232113653

e Beutner G, Eliseev RA, Porter GA Jr (2014) Initiation of electron transport chain activity in the embryonic heart coincides with the activation of mitochondrial complex 1 and the formation of supercomplexes. **PLoS One** 9:e113330. https://doi.org/10.1371/journal.pone.0113330

f 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

g 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/

h Brownlee M (2003) A radical explanation for glucose-induced beta cell dysfunction. **J Clin Invest** 112:1788-90. https://doi.org/10.1172/JCI20501

i Carriere A, Casteilla L (2019) Role of mitochondria in adipose tissues metabolism and

plasticity. Academic Press In: Mitochondria in obesity and type 2 diabetes. Morio B, Pénicaud L, Rigoulet M (eds) **Academic Press**. https://doi.org/10.1016/B978-0-12-811752-1.1.00007-9

j Chakrabarty RP, Chandel NS (2021) Mitochondria as signaling organelles control mammalian stem cell fate. **Cell Stem Cell** 28:394-408. https://doi.org/10.1016/j.stem.2021.02.011

k 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

l 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

m de Beauchamp L, Himonas E, Helgason GV (2022) Mitochondrial metabolism as a potential therapeutic target in myeloid leukaemia. **Leukemia** 36:1-12. https://doi.org/10.1038/s41375-021-01416-w

n 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

o Delport A, Harvey BH, Petzer A, Petzer JP (2017) Methylene blue and its analogues as antidepressant compounds. **Metab Brain Dis** 32:1357-82. https://doi.org/10.1007/s11011-017-0081-6

p Escoll P, Platon L, Buchrieser C (2019) Roles of mitochondrial respiratory Complexes during infection. **Immunometabolism** 1:e190011. https://doi.org/10.20900/immunometab20190011

q Eyenga P, Rey B, Eyenga L, Sheu SS (2022) Regulation of oxidative phosphorylation of liver mitochondria in sepsis. **Cells** 11:1598. https://doi.org/10.3390/cells11101598

r 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

s Granger DN, Kvietys PR (2015) Reperfusion injury and reactive oxygen species: The evolution of a concept. **Redox Biol** 6:524-551. https://doi.org/10.1016/j.redox.2015.08.020

t Han S, Chandel NS (2019) There is no smoke without mitochondria. **Am J Respir Cell Mol Biol** 60:489-91. https://doi.org/10.1165/rcmb.2018-0348ED

u Hanna D, Kumar R, Banerjee R (2023) A metabolic paradigm for hydrogen sulfide signaling via electron transport chain plasticity. **Antioxid Redox Signal** 38:57-67. https://doi.org/10.1089/ars.2022.0067

v 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

w Keane PC, Kurzawa M, Blain PG, Morris CM (2011) Mitochondrial dysfunction in Parkinson's disease. **Parkinsons Dis** 2011:716871. https://doi.org/10.4061/2011/716871

x Kim EH, Koh EH, Park JY, Lee KU (2010) Adenine nucleotide translocator as a regulator of mitochondrial function: implication in the pathogenesis of metabolic syndrome. **Korean Diabetes J** 34:146-53. https://doi.org/10.4093/kdj.2010.34.3.146

y Kumar R, Landry AP, Guha A, Vitvitsky V, Lee HJ, Seike K, Reddy P, Lyssiotis CA, Banerjee R (2021) A redox cycle with complex II prioritizes sulfide quinone oxidoreductase dependent H_2S oxidation. **J Biol Chem** 298:101435. https://doi.org/10.1016/j.jbc.2021.101435

z Liu Y, Schubert DR (2009) The specificity of neuroprotection by antioxidants. **J Biomed Sci** 16:98. https://doi.org/10.1186/1423-0127-16-98

α Martínez-Reyes I, Diebold LP, Kong H, Schieber M, Huang H, Hensley CT, Mehta MM, Wang

T, Santos JH, Woychik R, Dufour E, Spelbrink JN, Weinberg SE, Zhao Y, DeBerardinis RJ, Chandel NS (2016) TCA cycle and mitochondrial membrane potential are necessary for diverse biological functions. **Mol Cell** 61:199-209. https://doi.org/10.1016/j.molcel.2015.12.002

β McCollum C, Geißelsöder S, Engelsdorf T, Voitsik AM, Voll LM (2019) Deficiencies in the mitochondrial electron transport chain affect redox poise and resistance toward Colletotrichum higginsianum. **Front Plant Sci** 10:1262. https://doi.org/10.3389/fpls.2019.01262

γ McElroy GS, Chandel NS (2017) Mitochondria control acute and chronic responses to hypoxia. **Exp Cell Res** 356:217-22. https://doi.org/10.1016/j.yexcr.2017.03.034

δ McElroy GS, Reczek CR, Reyfman PA, Mithal DS, Horbinski CM, Chandel NS (2020) NAD+ regeneration rescues lifespan, but not ataxia, in a mouse model of brain mitochondrial Complex I dysfunction. **Cell Metab** 32:301-8.e6. https://doi.org/10.1016/j.cmet.2020.06.003

ε 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

ζ 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

η 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

θ Radogna F, Gerard D, Dicato M, Diederich M (2021) Assessment of mitochondrial cell metabolism by respiratory chain electron flow assays. **Methods Mol Biol** 2276:129-41. https://doi.org/10.1007/978-1-0716-1266-8_9

ι 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

κ 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

λ 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

μ Rodick TC, Seibels DR, Babu JR, Huggins KW, Ren G, Mathews ST (2018) Potential role of coenzyme Q10 in health and disease conditions. **Nutrition and Dietary Supplements** 10:1-11. https://doi.org/10.2147/NDS.S112119.

ν 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

ξ 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

ο Snyder CM, Chandel NS (2009) Mitochondrial regulation of cell survival and death during low-oxygen conditions. **Antioxid Redox Signal** 11:2673-83. https://doi.org/10.1089/ars.2009.2730

π Srivastava S (2016) Emerging therapeutic roles for NAD(+) metabolism in mitochondrial and age-related disorders. **Clin Transl Med** 5:25. https://doi.org/10.1186/s40169-016-0104-7

ρ Szabo L, Eckert A, Grimm A (2020) Insights into disease-associated tau impact on

mitochondria. **Int J Mol Sci** 21:6344. https://doi.org/10.3390/ijms21176344

σ Tabassum N, Kheya IS, Ibn Asaduzzaman SA, Maniha SM, Fayz AH, Zakaria A, Fayz AH, Zakaria A, Noor R (2020) A review on the possible leakage of electrons through the electron transport chain within mitochondria. **J Biomed Res Environ Sci** 1:105-13. https://doi.org/10.37871/jels1127

τ 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

υ Vekshin N (2020) Biophysics of mitochondria. Springer Cham:197 pp. https://doi.org/10.1007/978-3-030-33853-4

φ Wang G, Feng H, Gao A, Hao Q, Jin W, Peng X, Li W, Wu G, Chu PK (2016) Extracellular electron transfer from aerobic bacteria to Au-loaded TiO2 semiconductor without light: a new bacteria-killing mechanism other than localized surface plasmon resonance or microbial fuel cells. **ACS Appl Mater Interfaces** 8:24509-16. https://doi.org/10.1021/acsami.6b10052

χ 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

ψ 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

ω 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

Supplement 4

$FADH_2$ as substrate of CII and FAD + $2H^+$ as products (Figure S4)

$$FADH_2 \longrightarrow FAD + 2H^+ \quad (2)$$

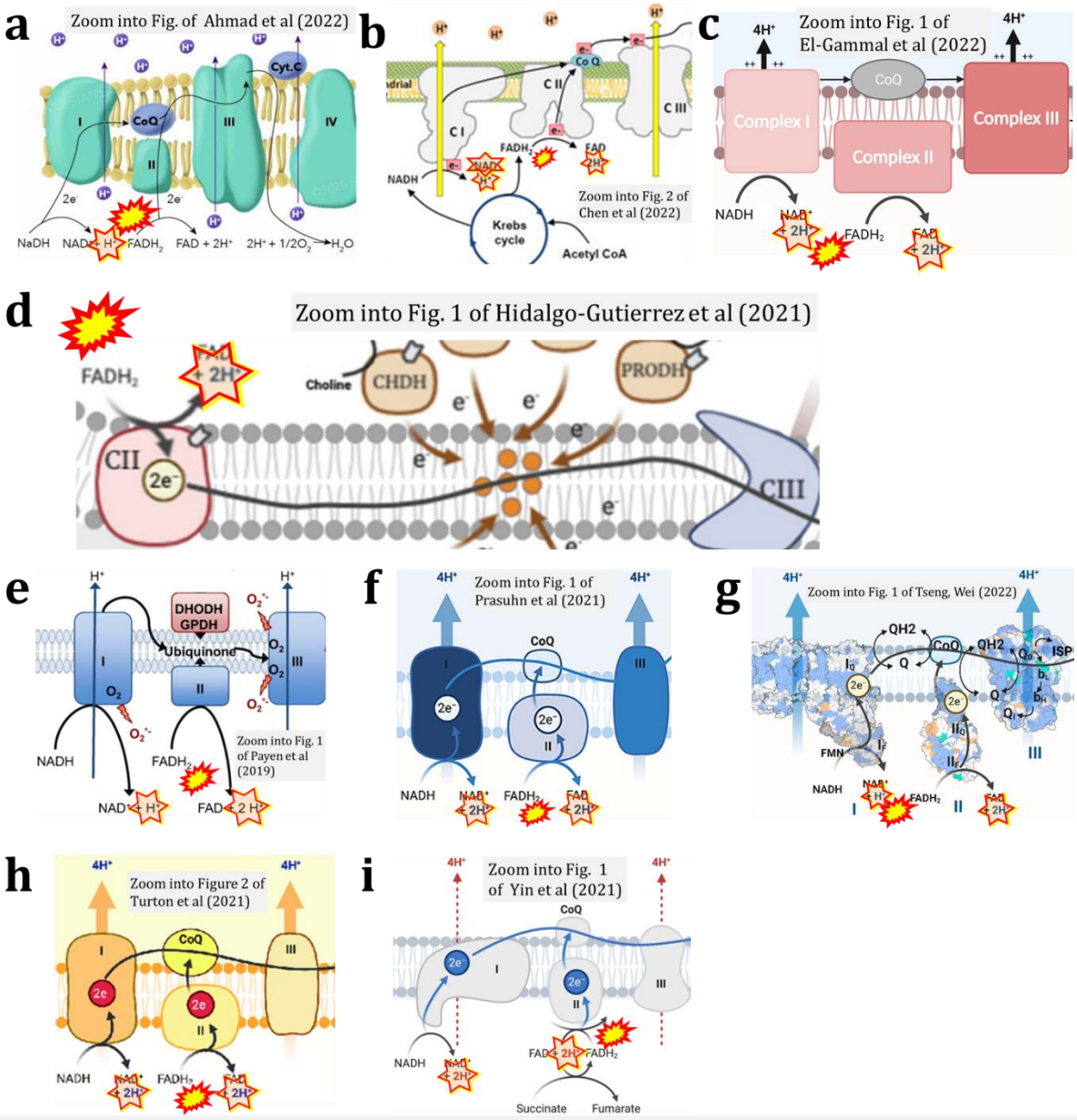


Figure S4. Complex II ambiguities: $FADH_2$ as substrate of CII and FAD + $2H^+$ as products. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S4.

References for Figure S4

a 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/

b Chen TH, Koh KY, Lin KM, Chou CK (2022) Mitochondrial dysfunction as an underlying cause of skeletal muscle disorders. **Int J Mol Sci** 23:12926.

https://doi.org/10.3390/ijms232112926

c El-Gammal Z, Nasr MA, Elmehrath AO, Salah RA, Saad SM, El-Badri N (2022) Regulation of mitochondrial temperature in health and disease. **Pflugers Arch** 474:1043-51. https://doi.org/10.1007/s00424-022-02719-2

d Hidalgo-Gutiérrez A, González-García P, Díaz-Casado ME, Barriocanal-Casado E, López-Herrador S, Quinzii CM, López LC (2021) Metabolic targets of coenzyme Q_{10} in mitochondria. **Antioxidants (Basel)** 10:520. https://doi.org/10.3390/antiox10040520

e Payen VL, Zampieri LX, Porporato PE, Sonveaux P (2019) Pro- and antitumor effects of mitochondrial reactive oxygen species. **Cancer Metastasis Rev** 38:189-203. https://doi.org/10.1007/s10555-019-09789-2

f Prasuhn J, Davis RL, Kumar KR (2021) Targeting mitochondrial impairment in Parkinson's disease: challenges and opportunities. **Front Cell Dev Biol** 8:615461. https://doi.org/10.3389/fcell.2020.615461

g Tseng W-W, Wei A-C (2022) Kinetic mathematical modeling of oxidative phosphorylation in cardiomyocyte mitochondria. **Cells** 11:4020. https://doi.org/10.3390/cells11244020

h 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.

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

Supplement 5

$FADH_2$ as substrate of CII and FAD^+ as product (Figure S5)

$FADH_2 \longrightarrow FAD^+$ (*3*)

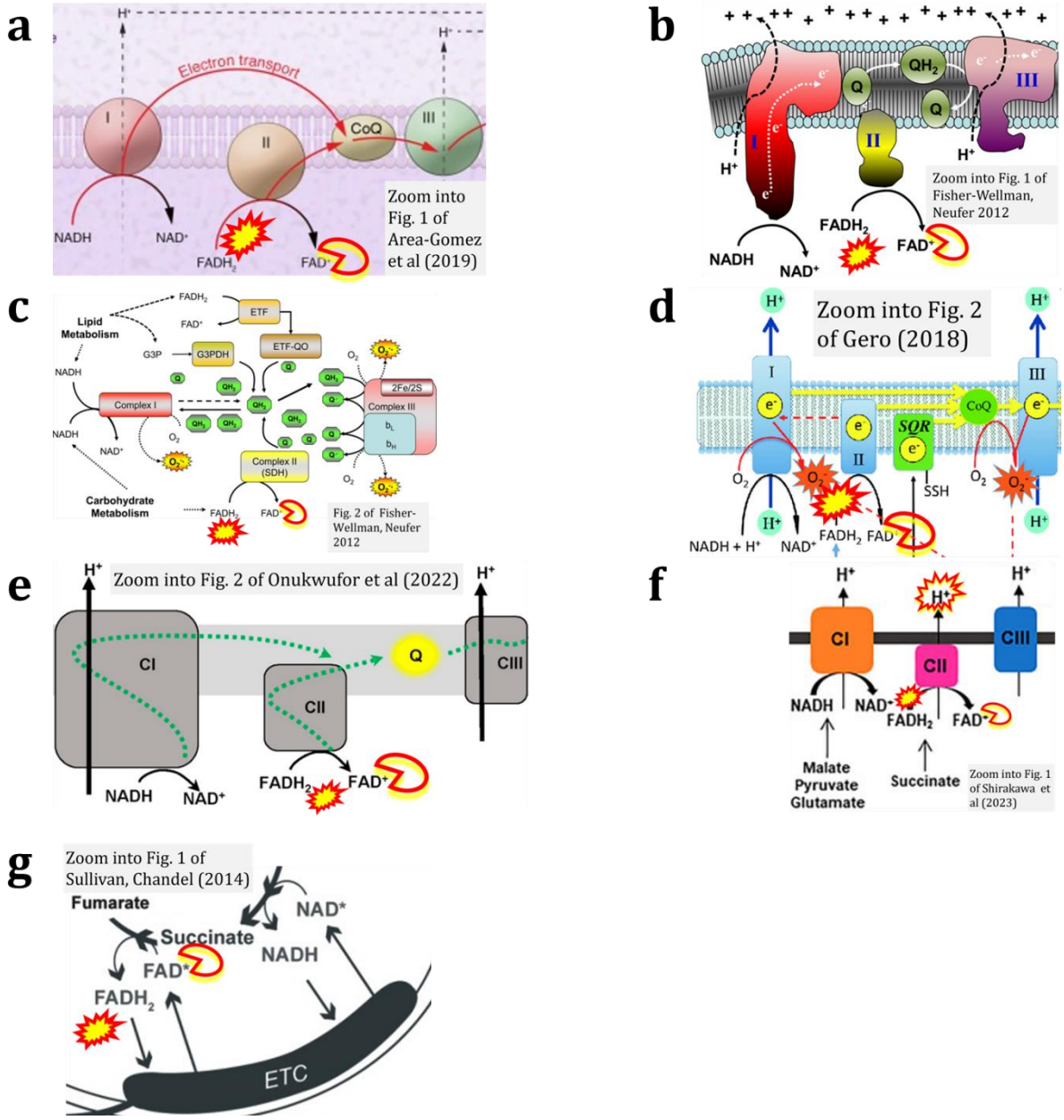


Figure S5. Complex II ambiguities: $FADH_2$ or FADH as substrate of CII and FAD^+ or $FAD^+ + H^+$ as products. Alphabetical sequence of publications from 2001 to 2023. See References for Figure S5.

References for Figure S5

a Area-Gomez E, Guardia-Laguarta C, Schon EA, Przedborski S (2019) Mitochondria, OxPhos, and neurodegeneration: cells are not just running out of gas. **J Clin Invest** 129:34-45. https://doi.org/10.1172/JCI120848

b,c 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

d Gero D (2023) Hyperglycemia-induced endothelial dysfunction. **IntechOpen** Chapter 8. http://dx.doi.org/10.5772/intechopen.71433

e Onukwufor JO, Dirksen RT, Wojtovich AP (2022) Iron dysregulation in mitochondrial dysfunction and Alzheimer's disease. **Antioxidants (Basel)** 11:692. https://doi.org/10.3390/antiox11040692

f Shirakawa R, Nakajima T, Yoshimura A, Kawahara Y, Orito C, Yamane M, Handa H, Takada S, Furihata T, Fukushima A, Ishimori N, Nakagawa M, Yokota I, Sabe H, Hashino S, Kinugawa S, Yokota T (2023) Enhanced mitochondrial oxidative metabolism in peripheral blood mononuclear cells is associated with fatty liver in obese young adults. **Sci Rep** 13:5203. https://doi.org/10.1038/s41598-023-32549-w

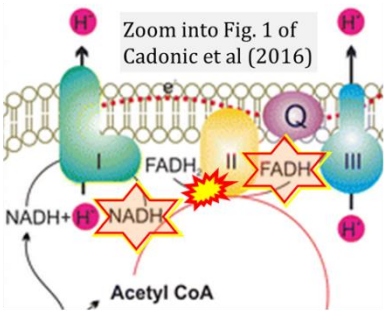
g Sullivan LB, Chandel NS. (2014) Mitochondrial metabolism in TCA cycle mutant cancer cells. **Cell Cycle** 13:347-8. https://doi.org/10.4161/cc.27513

Supplement 6

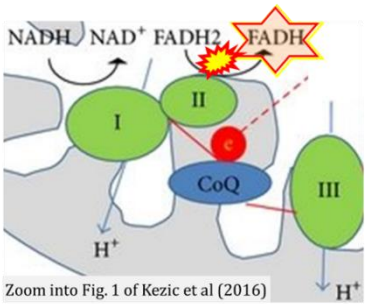
$FADH_2$ or FADH as substrate of CII and FADH, $FADH^+$, or FAD^+ as product (Figure S6)

a-d	$FADH_2$	⟶ FADH	(*4*)
e	$FADH_2$	⟶ $FADH^+$	(*5*)
f	FADH	⟶	(*6*)
g	FADH	⟶ FAD^+	(*7*)
h	FADH	⟶ FAD^+ + H^+	(*8*)
i	FADH	⟶ FAD^+ + $2H^+$	(*9*)

a



b



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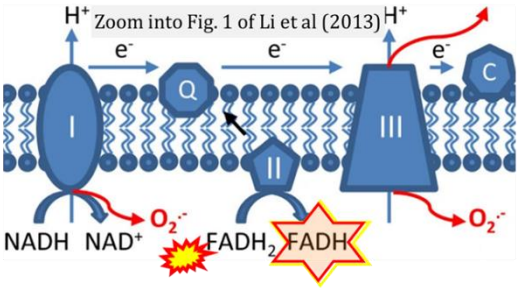

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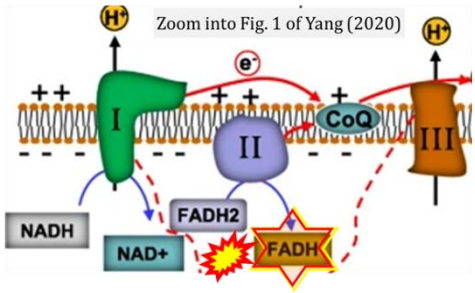
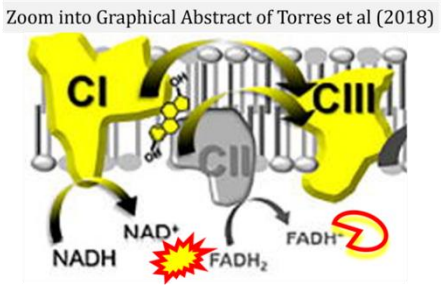


e



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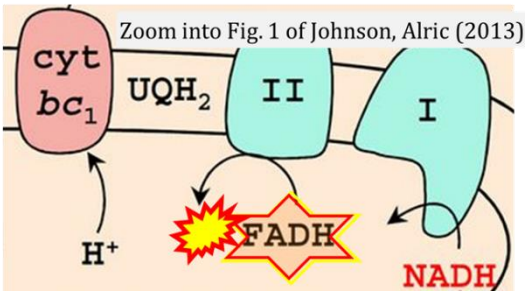

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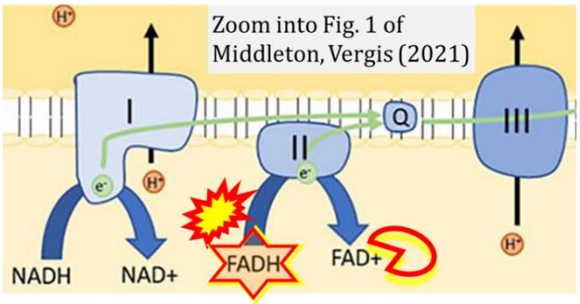
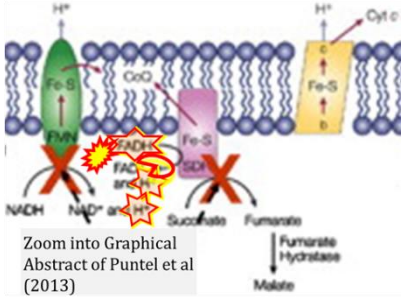


h



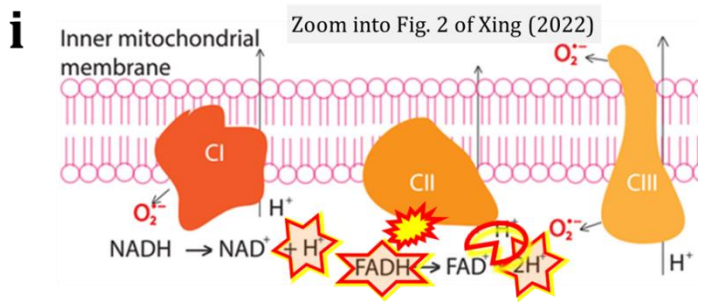


Figure S6. Complex II ambiguities: $FADH_2$ as substrate of CII and FADH or $FADH^+$ as product. Sequence of publications from 2001 to 2023 according to FAD-*a* to FAD-*h*. See References for Figure S6.

References for Figure S6

a Cadonic C, Sabbir MG, Albensi BC (2016) Mechanisms of mitochondrial dysfunction in Alzheimer's disease. **Mol Neurobiol** 53:6078-90. https://doi.org/10.1007/s12035-015-9515-5

b Kezic A, Spasojevic I, Lezaic V, Bajcetic M (2016) Mitochondria-targeted antioxidants: future perspectives in kidney ischemia reperfusion injury. **Oxid Med Cell Longev** 2016:2950503. https://doi.org/10.1155/2016/2950503

c Li X, Fang P, Mai J, Choi ET, Wang H, Yang XF (2013) Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. **J Hematol Oncol** 6:19. https://doi.org/10.1186/1756-8722-6-19

d Yang L, Youngblood H, Wu C, Zhang Q (2020) Mitochondria as a target for neuroprotection: role of methylene blue and photobiomodulation. **Transl Neurodegener** 9:19. https://doi.org/10.1186/s40035-020-00197-z

e Torres MJ, Kew KA, Ryan TE, Pennington ER, Lin CT, Buddo KA, Fix AM, Smith CA, Gilliam LA, Karvinen S, Lowe DA, Spangenburg EE, Zeczycki TN, Shaikh SR, Neufer PD (2017) 17β-estradiol directly lowers mitochondrial membrane microviscosity and improves bioenergetic function in skeletal muscle. **Cell Metab** 27:167-79. https://doi.org/10.1016/j.cmet.2017.10.003

f Johnson X, Alric J (2013) Central carbon metabolism and electron transport in Chlamydomonas reinhardtii: metabolic constraints for carbon partitioning between oil and starch. **Eukaryot Cell** 12:776-93. https://doi.org/10.1128/EC.00318-12

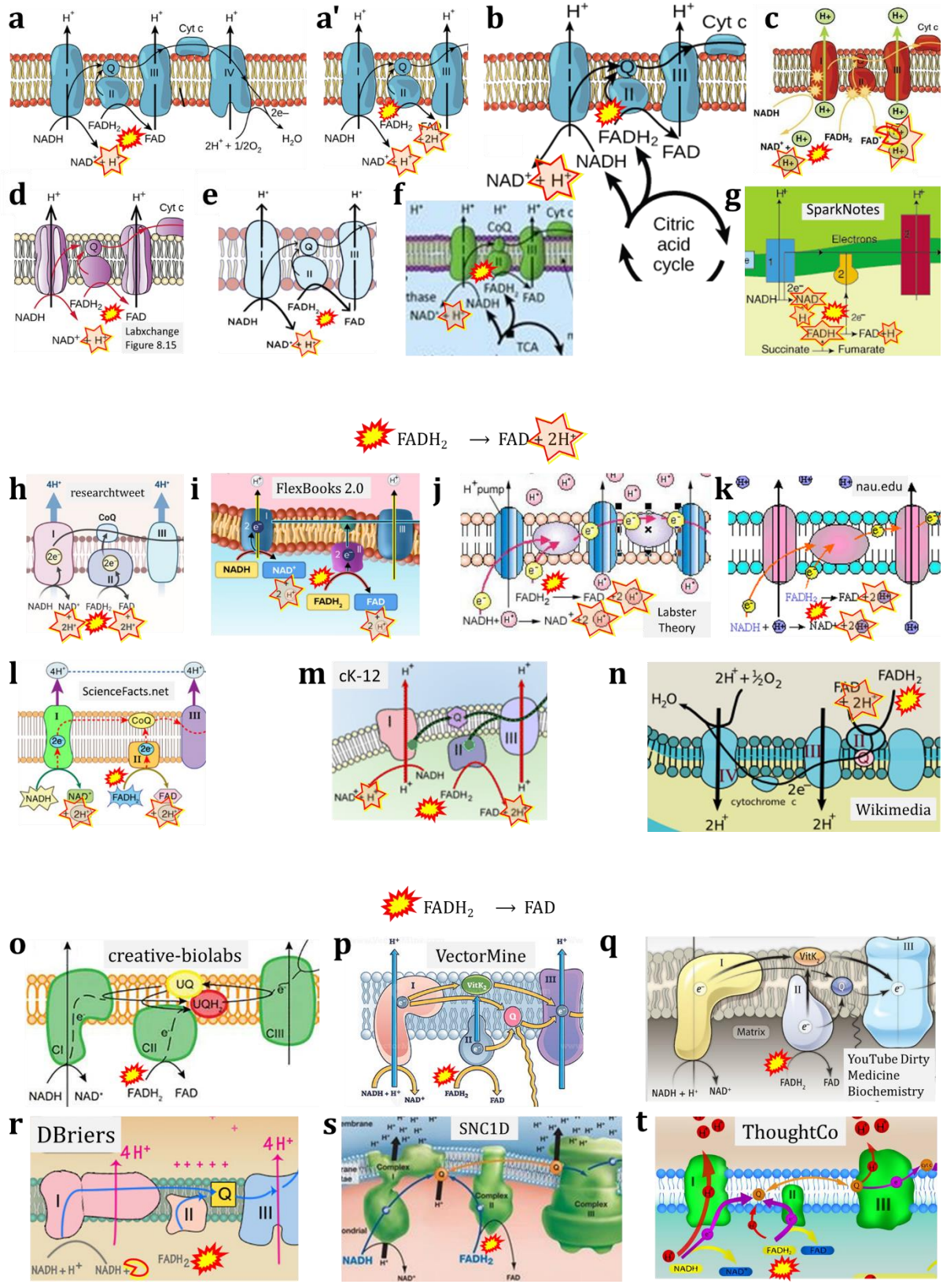
g Middleton P, Vergis N (2021) Mitochondrial dysfunction and liver disease: role, relevance, and potential for therapeutic modulation. **Therap Adv Gastroenterol** 14:17562848211031394. https://doi.org/10.1177/17562848211031394

h Puntel RL, Roos DH, Seeger RL, Rocha JB (2013) Mitochondrial electron transfer chain complexes inhibition by different organochalcogens. **Toxicol In Vitro** 27:59-70. https://doi.org/10.1016/j.tiv.2012.10.011

i Xing Yunxie (2022) Is genome instability a significant cause of aging? A review. **Atlantis Press**. https://doi.org/10.2991/assehr.k.220504.260

Supplement 7

$FADH_2$ or FADH as substrate of CII in websites (Figure S7)



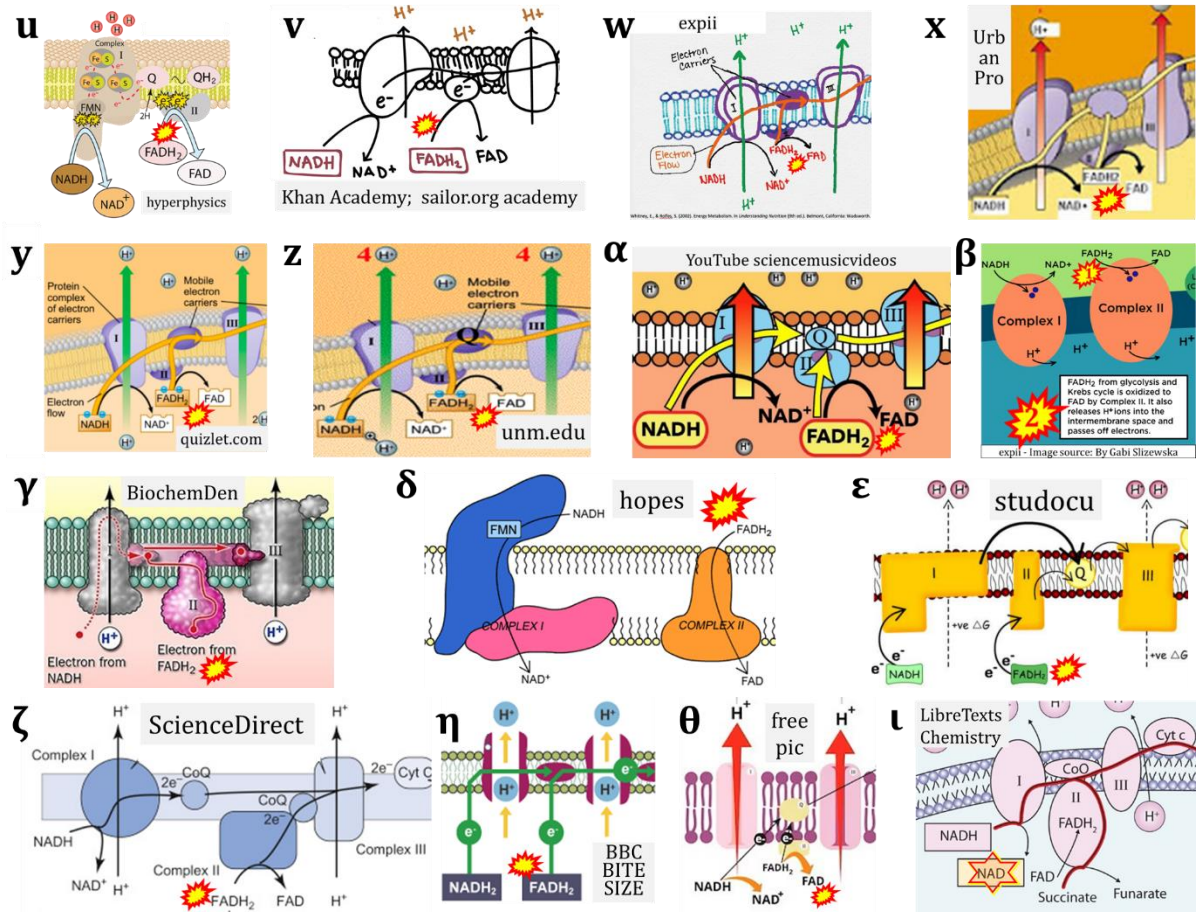


Figure S7. Complex II ambiguities in graphical representations on $FADH_2$ as a substrate of Complex II in the canonical forward electron transfer. FADH → FAD+H (**g**), $FADH_2$ → FAD+$2H^+$ (**a'**, **c**, **h-n**), and $FADH_2$ → FAD (**a**, **b**, **d-f**, **o-θ**) should be corrected to $FADH_2$ → FAD (Eq. 3b). NADH → NAD^+ is frequently written in graphs without showing the H^+ on the left side of the arrow, except for (**p-r**). NADH → NAD^++H^+ (**a-g**, **m**), NADH → NAD^++$2H^+$ (**h-l**), NADH+H^+ → NAD^++$2H^+$ (**j**, **k**), and NADH → NAD (**ι**) should be corrected to NADH+H^+ → NAD^+ (Eq. 3a). Weblinks #: (**a**) 1-5, 8-10; (**a'**) 6-7; (**b**) 6-9,11; (**c**) 10-16,35; (**d**) 17; (**e**) 18; (**f**) 19; (**g**) 20; (**h**) 21-22; (**i**) 23; (**j**) 24; (**k**) 25; (**l**) 26; (**m**) 27; (**n**) 11,28; (**o**) 29; (**p**) 30-31; (**q**) 32; (**r**) 33; (**s**) 34; (**t**) 22,35; (**u**) 36; (**v**) 6-7; (**w**) 11; (**x**) 37; (**y**) 38; (**z**) 39; (**α**) 40; (**β**) 11; (**γ**) 41; (**δ**) 42; (**ε**) 43; (**ζ**) 44; (**η**) 45; (**θ**) 46; (**ι**) 47.

Weblinks for Figure S7 (retrieved 2023-03-21 to 2023-05-04)

1 (**a**,**b**) 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/ - Charles Molnar, Jane Gair, Concepts of Biology - 1st Canadian Edition, BCcampus - 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**) https://www.pharmaguideline.com/2022/01/electron-transport-chain.html - Pharmaguideline

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

6 (**a',b,v**) 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',b,v**) https://learn.saylor.org/mod/page/view.php?id=32815 -Saylor Academy
8 (**a,b**) https://www.texasgateway.org/resource/74-oxidative-phosphorylation - Texas Gateway - Figure 7.11
9 (**a,b**) https://opened.cuny.edu/courseware/lesson/639/overview -CUNY
10 (**a,c**) https://courses.lumenlearning.com/wm-biology1/chapter/reading-electron-transport-chain/ - lumen Biology for Majors I - Fig. 1 / Fig. 3
11 (**b,c,w,n,β**) 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
12 (**c**) 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
13 (**c**) https://biologydictionary.net/electron-transport-chain-and-oxidative-phosphorylation/ - biologydictionary.net 2018-08-21
14 (**c**) https://www.quora.com/Why-does-FADH2-form-2-ATP - Quora
15 (**c**) https://teachmephysiology.com/biochemistry/atp-production/electron-transport-chain/ - TeachMePhysiology - Fig. 1. 2023-03-13
16 (**c**) https://www.toppr.com/ask/question/short-long-answer-types-whatis-the-electron-transport-system-and-what-are-its-functions/ - toppr
17 (**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
18 (**e**) https://jackwestin.com/resources/mcat-content/oxidative-phosphorylation/electron-transfer-in-mitochondria - Jack Westin MCAT Courses
19 (**f**) https://videodelivery.net/79e91c40bf96f9692560fa378c5086b6/thumbnails/thumbnail.jpg - videodelivery
20 (**g**) https://www.sparknotes.com/biology/cellrespiration/oxidativephosphorylation/section2/ - SparkNotes
21 (**h**) https://microbenotes.com/electron-transport-chain/ - Microbe Notes
22 (**h,t**) https://researchtweet.com/mitochondrial-electron-transport-chain-2/ - researchtweet
23 (**i**) 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 (**j**) https://theory.labster.com/Electron_Transport_Chain/ - Labster Theory
25 (**k**) https://www2.nau.edu/~fpm/bio205/u4fg36.html - nau.edu
26 (**l**) https://www.sciencefacts.net/electron-transport-chain.html - ScienceFacts
27 (**m**) https://www.ck12.org/biology/electron-transport/lesson/The-Electron-Transport-Chain-Advanced-BIO-ADV/ - cK-12
28 (**n**) https://commons.wikimedia.org/wiki/File:Mitochondrial_electron_transport_chain.png - Wikimedia
29 (**o**) https://www.creative-biolabs.com/drug-discovery/therapeutics/electron-transport-chain.htm - creative-biolabs

30 (**p**) https://www.dreamstime.com/electron-transport-chain-as-respiratory-embedded-transporters-outline-diagram-electron-transport-chain-as-respiratory-embedded-image235345232 - dreamstime
31 (**p**) https://vectormine.com/item/electron-transport-chain-as-respiratory-embedded-transporters-outline-diagram/ - VectorMine
32 (**q**) 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.youtube.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
33 (**r**) http://www.dbriers.com/tutorials/2012/04/the-electron-transport-chain-simplified/ - DBriers
34 (**s**) https://sbi4uraft2014.weebly.com/electron-transport-chain.html - SNC1D - BIOLOGY LESSON PLAN BLOG
35 (**c,t**) https://www.thoughtco.com/electron-transport-chain-and-energy-production-4136143 - ThoughtCo / extender01 / iStock / Getty Images Plus
36 (**u**) http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/Complex1.html - hyperphysics
37 (**x**) https://www.urbanpro.com/ba-tuition/oxidative-phosphorylation - UrbanPro
38 (**y**) https://quizlet.com/245664214/electron-transport-chain-facts-of-cell-respiration-diagram/ - Quizlet
39 (**z**) https://www.unm.edu/~lkravitz/Exercise%20Phys/ETCstory.html - unm.edu
40 (**α**) 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
41 (**γ**) https://biochemden.com/electron-transport-chain-mechanism/ - BiochemDen.com
42 (**δ**) https://hopes.stanford.edu/riboflavin/ - hopes, Huntington's outreach project for education, at Stanford
43 (**ε**) https://www.studocu.com/en-gb/document/university-college-london/mammalian-physiology/electron-transport-chain/38063777 - studocu, University College London
44 (**ζ**) 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
45 (**η**) https://www.bbc.co.uk/bitesize/guides/zdq9382/revision/5 - BBC BITESIZE
46 (**θ**) https://www.freepik.com/premium-vector/oxidative-phosphorylation-process-electron-transport-chain-final-step-cellular-respiration_29211885.htm - freepik
47 (**ι**) 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

Supplement 8

Weblinks on FAO and CII (retrieved 2023-03-21 to 2023-05-02)

48 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 $FADH_2$.*

49 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).

50 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 9

CII as a proton pump (Figure S9)

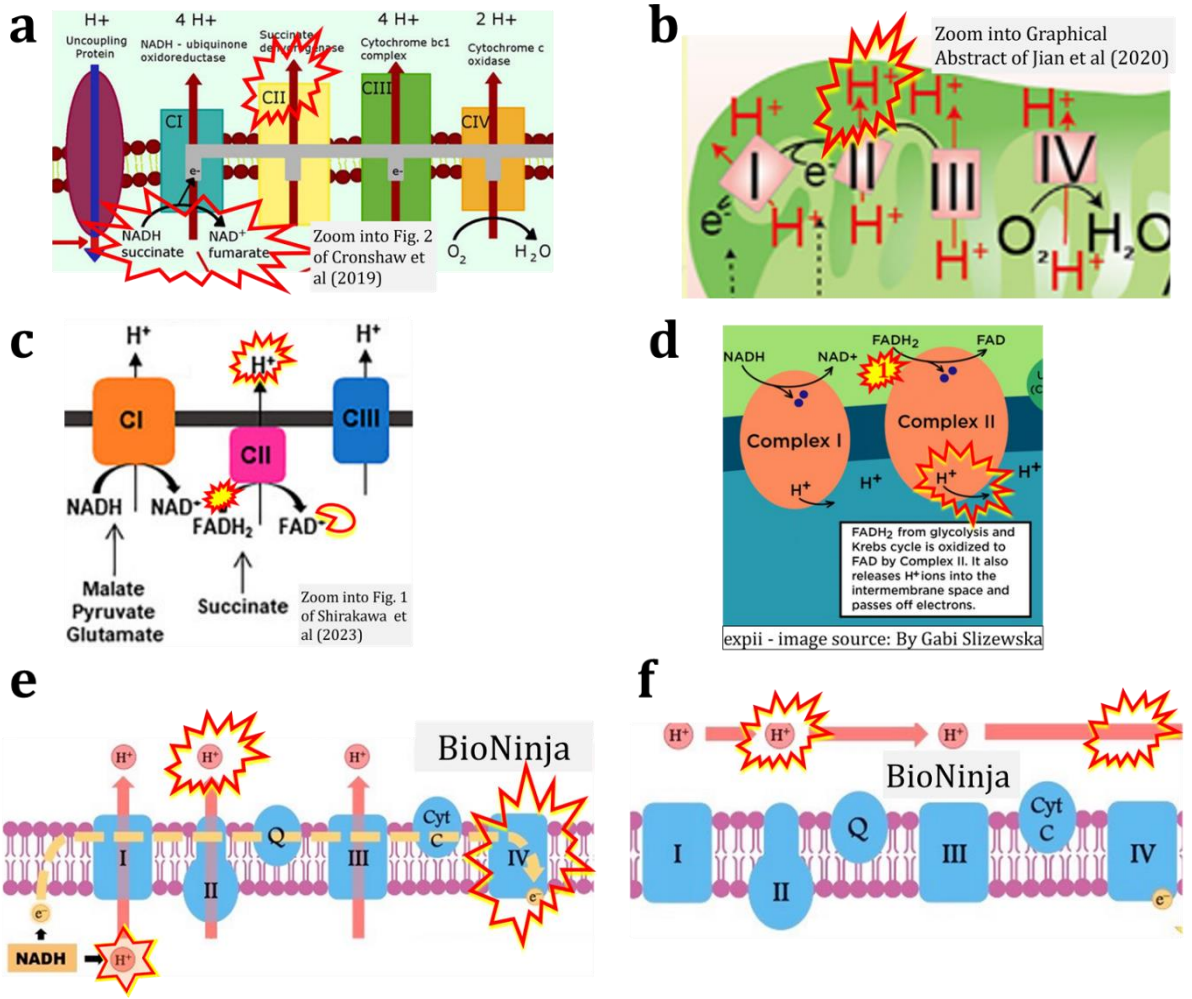


Figure S9. Complex II as a proton pump.

a Cronshaw M, Parker S, Arany P (2019) Feeling the heat: evolutionary and microbial basis for the analgesic mechanisms of photobiomodulation therapy. **Photobiomodul Photomed Laser Surg** 37:517-26. https://doi.org/10.1089/photob.2019.4684

b Jian C, Fu J, Cheng X, Shen LJ, Ji YX, Wang X, Pan S, Tian H, Tian S, Liao R, Song K, Wang HP, Zhang X, Wang Y, Huang Z, She ZG, Zhang XJ, Zhu L, Li H (2020) Low-dose sorafenib acts as a mitochondrial uncoupler and ameliorates nonalcoholic steatohepatitis. **Cell Metab** 31:892-908. https://doi.org/10.1016/j.cmet.2020.04.011

c Shirakawa R, Nakajima T, Yoshimura A, Kawahara Y, Orito C, Yamane M, Handa H, Takada S, Furihata T, Fukushima A, Ishimori N, Nakagawa M, Yokota I, Sabe H, Hashino S, Kinugawa S, Yokota T (2023) Enhanced mitochondrial oxidative metabolism in peripheral blood mononuclear cells is associated with fatty liver in obese young adults. **Sci Rep** 13:5203. https://doi.org/10.1038/s41598-023-32549-w

d https://www.expii.com/t/electron-transport-chain-summary-diagrams-10139 - expii - Image source: By Gabi Slizewska: '*$FADH_2$ from glycolysis and Krebs cycle is oxidized to FAD by Complex II. It also releases H+ ions into the intermembrane space and passes off electrons*' (retrieved 2023-05-04).

e,f https://ib.bioninja.com.au/higher-level/topic-8-metabolism-cell/untitled/electron-transport-chain.html - BioNinja (retrieved 2023-05-04).