



# Respiratory electron transfer pathways in plant mitochondria

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The respiratory electron transport chain (ETC) couples electron transfer from organic substrates onto molecular oxygen with proton translocation across the inner mitochondrial membrane. The resulting proton gradient is used by the ATP synthase complex for ATP formation. In plants, the ETC is especially intricate. Besides the “classical” oxidoreductase complexes (complex I–IV) and the mobile electron transporters cytochrome c and ubiquinone, it comprises numerous “alternative oxidoreductases.” Furthermore, several dehydrogenases localized in the mitochondrial matrix and the mitochondrial intermembrane space directly or indirectly provide electrons for the ETC. Entry of electrons into the system occurs via numerous pathways which are dynamically regulated in response to the metabolic state of a plant cell as well as environmental factors. This mini review aims to summarize recent findings on respiratory electron transfer pathways in plants and on the involved components and supramolecular assemblies.

**Keywords:** plant mitochondria, electron transport chain, dehydrogenase, alternative oxidase, respiratory supercomplex

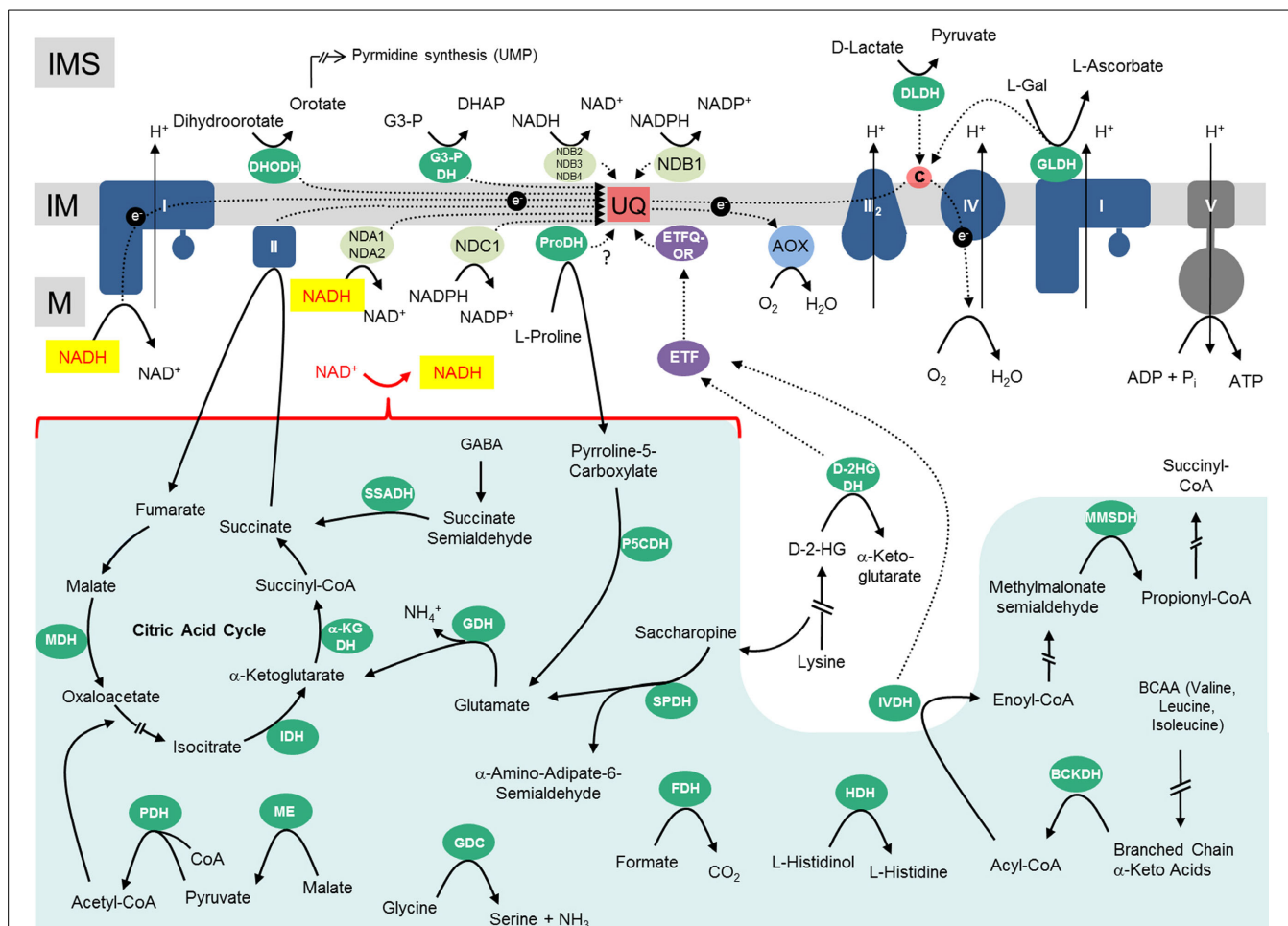
## INTRODUCTION

During cellular respiration, organic compounds are oxidized to generate usable chemical energy in the form of ATP. The respiratory electron transport chain (ETC) of mitochondria is at the center of this process. Its core consists of four oxidoreductase complexes, the NADH dehydrogenase (complex I), the succinate dehydrogenase (complex II), the cytochrome c reductase (complex III) and the cytochrome c oxidase (complex IV), as well as of two mobile electron transporters, cytochrome c, and the lipid ubiquinone. Overall, electrons are transferred from the coenzymes NADH or FADH<sub>2</sub> onto molecular oxygen which is reduced to water. Three of the four oxidoreductase complexes (complexes I, III and IV) couple their electron transfer reactions with proton translocation across the inner mitochondrial membrane. As a result, a proton gradient is formed which can be used by the ATP synthase complex (complex V) for the phosphorylation of ADP. In its classically described form, cellular respiration is based on a linear ETC (from NADH via complexes I, III, and IV to molecular oxygen). However, electrons can enter and leave the ETC at several alternative points. This is especially true for the plant ETC system, which is highly branched. In this review we aim to integrate current knowledge on the ETC system in plants with respect to its components, electron transport pathways and supramolecular structure.

## COMPONENTS OF THE PLANT ETC SYSTEM

The “classical” oxidoreductase complexes of the respiratory chain (given in dark blue in **Figure 1**) resemble their homologues in animal mitochondria but at the same time have some clear distinctive features (reviewed in Millar et al., 2008, 2011; Rasmusson and Moller, 2011; van Dongen et al., 2011; Jacoby et al., 2012). *Complex I* is especially large in plant mitochondria and includes

nearly 50 different subunits (Braun et al., 2014). Compared to its homologs from bacteria and other eukaryotic lineages it has an extra domain which includes carbonic anhydrase-like proteins. The function of this additional domain is currently unclear but it has been suggested to be important in the context of an intercellular CO<sub>2</sub> transfer mechanism to provide mitochondrial CO<sub>2</sub> for carbon fixation in chloroplasts (Braun and Zabaleta, 2007; Zabaleta et al., 2012). *Complex II* is composed of four subunits in bacteria and mitochondria of animals and fungi. In plants complex II includes homologs of these subunits but additionally four extra proteins of unknown function (Millar et al., 2004; Huang and Millar, 2013). In contrast, the subunit composition of *complex III* from plants is highly similar to the ones in yeast and bovine mitochondria (Braun and Schmitz, 1995a). The two largest subunits of this protein complex, termed “core proteins” in animals and fungi, are homologous to the two subunits of the mitochondrial processing peptidase (MPP) which removes pre-sequences of nuclear-encoded mitochondrial proteins after their import into mitochondria. In animal mitochondria, the core proteins are proteolytically inactive. Instead, an active MPP is present within the mitochondrial matrix. In contrast, the core subunits of complex III from plants have intact active sites (Braun et al., 1992; Glaser et al., 1994). Indeed, complex III isolated from plant mitochondria efficiently removes pre-sequences of mitochondrial pre-proteins. The differing functional states of complex III in diverse eukaryotic lineages might reflect different evolutionary stages of this protein complex (Braun and Schmitz, 1995b). Also *complex IV* has some extra subunits in mitochondria of plants (Millar et al., 2004). Eight subunits are homologous to complex IV subunits from other groups of eukaryotes and another six putative subunits represent proteins of unknown functions.



**FIGURE 1 | Mitochondrial dehydrogenases and the respiratory chain.**

Within the mitochondrial matrix (M) numerous dehydrogenases generate NADH by oxidizing various carbon compounds. NADH subsequently is re-oxidized at the inner mitochondrial membrane (IM) by the respiratory electron transfer chain (ETC). The electrons of NADH can enter the ETC through complex I or at the ubiquinone level via alternative NAD(P)H-dehydrogenases. Besides, some dehydrogenases of the mitochondrial matrix transfer electrons to ubiquinone via the ETF/ETFQOR system. Proline dehydrogenase possibly directly transfers electrons onto ubiquinone. In the intermembrane space (IMS), electrons from NAD(P)H generated in the cytoplasm can be inserted into the ETC via alternative NAD(P)H dehydrogenases. Furthermore, some dehydrogenases of the IMS can directly transfer electrons onto ubiquinone or cytochrome c. Color code—dark blue, protein complexes of the ETC; blue, AOX; purple, ETF/ETFQOR system; light green, alternative NAD(P)H dehydrogenases of the ETC; green, dehydrogenases; red, ubiquinone and cytochrome c; yellow, NADH produced by dehydrogenases of the mitochondrial matrix/NADH re-oxidized by complex I or internal alternative NADH dehydrogenases; dark gray, ATP synthase complex; light green background, NADH producing

dehydrogenases of the mitochondrial matrix. Abbreviations—*alphabetically ordered*. I, complex I; II, complex II; III, complex III; IV, complex IV; V, complex V;  $\alpha$ -KGDH,  $\alpha$ -ketoglutarate dehydrogenase; AOX, alternative oxidase; BCKDH, branched-chain  $\alpha$ -ketoacid dehydrogenase complex; c, cytochrome c; D-2HGDH, D-2-hydroxyglutarate dehydrogenase; DHODH, dihydroorotate dehydrogenase; DLDH, D-lactate dehydrogenase; ETF, electron transfer flavoprotein; ETFQOR, electron transfer flavoprotein ubiquinone oxidoreductase; FDH, formate dehydrogenase; GDC, glycine dehydrogenase; NDA1/2, NDB2/3/4, alternative NADH dehydrogenase; NDC1, NDB1, alternative NADPH dehydrogenase; P5CDH, pyrroline-5-carboxylate dehydrogenase; PDH, pyruvate dehydrogenase; ProDH, proline dehydrogenase; SPDH, saccharopine dehydrogenase; SSADH, succinic semialdehyde dehydrogenase; UQ, ubiquinone. For further information of the enzymes see **Table 1**.

The ETC of plant mitochondria additionally includes several so-called “alternative oxidoreductases”: the alternative oxidase (AOX; light blue in **Figure 1**) and several functionally distinguishable alternative NAD(P)H dehydrogenases (alternative NDs, light green in **Figure 1**). Findings on their functional roles have been reviewed recently (Rasmusson et al., 2008; Rasmusson and Moller, 2011; Moore et al., 2013). AOX directly transfers

electrons from ubiquinol to molecular oxygen and therefore constitutes an alternative electron exit point of the ETC. As a result, complexes III and IV are excluded from respiratory electron transport. The alternative NAD(P)H dehydrogenases serve as alternative electron entry points of the plant ETC and may substitute complex I. They differ with respect to co-factor requirement and localization at the outer or inner surface of

**Table 1 | Mitochondrial dehydrogenases in *Arabidopsis thaliana*<sup>a</sup>.**

| <b>Enzyme</b>                               | <b>Accession no.<sup>b</sup></b><br>subunits<br>isoforms etc.   | <b>Catalysed reaction</b>  | <b>Oligomeric state</b><br>Native mass/monomer mass according<br>to GelMap <sup>c</sup> (according to other data in<br>the literature)     | <b>Publication<sup>d</sup></b><br>for <i>Arabidopsis</i><br>(for other plants)   |
|---|---|--|--|--|
| Malate<br>dehydrogenase                     | At1g53240<br>At3g15020  | Malate + NAD <sup>+</sup><br>⇌ Oxaloacetate + NADH   | At1g53240: 89 kDa/42 kDa<br>At3g47520: 157 kDa/38 kDa  | Journet et al., 1981<br>Gietl, 1992<br>Krömer, 1995<br>Nunes-Nesi et al., 2005<br>Lee et al., 2008<br>Tomaz et al., 2010   |
| Isocitrate<br>dehydrogenase                 | At4g35260<br>At5g14590<br>At4g35650<br>At3g09810<br>At5g03290<br>At2g17130  | Isocitrate + NAD <sup>+</sup><br>⇌ α-Ketoglutarate + CO <sub>2</sub><br>+ NADH   | At4g35260: 89 kDa/42 kDa<br>At5g14590: 140 kDa/53 kDa<br>At3g09810: 138 kDa/40 kDa<br>At5g03290: 138 kDa/40 kDa                            | Behal and Oliver, 1998<br>Lancien et al., 1998<br>Lin et al., 2004<br>Lemaitre and Hodges, 2006<br>Lemaitre et al., 2007   |
| α-Ketoglutarate<br>dehydrogenase<br>complex | At3g55410 (E1)<br>At5g65750 (E1)<br>At4g26910 (E2)<br>At5g55070 (E2)<br>At3g17240 (E3)<br>At1g48030 (E3)<br>At3g13930 (E3)                            | α-Ketoglutarate +<br>coenzyme A + NAD <sup>+</sup><br>⇌ succinyl-CoA + CO <sub>2</sub> +<br>NADH   | At5g65750: 207 kDa/91 kDa<br>At3g55410: 207 kDa/91 kDa<br><br>(1.7 MDa complex)  | Poulsen and Wedding, 1970<br>Wedding and Black, 1971a,b<br>Dry and Wiskich, 1987<br>Millar et al., 1999<br>Araújo et al., 2008<br>Araújo et al., 2013                        |
| Glutamate<br>dehydrogenase                  | At5g18170<br>At5g07440<br>At3g03910   | Glutamate + H <sub>2</sub> O + NAD <sup>+</sup><br>⇌ α-Ketoglutarate + NH <sub>4</sub> <sup>+</sup><br>+ NADH                            | At5g18170: 209 kDa/48 kDa<br>At5g07440: 209 kDa/48 kDa<br>At3g03910: 209 kDa/48 kDa  | Yamaya et al., 1984<br>Turano et al., 1997<br>Aubert et al., 2001<br>Miyashita and Good, 2008a,b<br>Fontaine et al., 2012<br>Tarasenko et al., 2013<br>Fontaine et al., 2012 |
| Malic enzyme                                | At2g13560<br>At4g00570<br>At1g79750   | Malate + NAD <sup>+</sup> ⇌<br>Pyruvate + NADH + CO <sub>2</sub>   | At2g13560: 370 kDa/63 kDa<br>At4g00570: 370 kDa/63 kDa   | Jenner et al., 2001<br>Tronconi et al., 2008<br>Tronconi et al., 2010<br>Tronconi et al., 2012   |
| Pyruvate<br>dehydrogenase<br>complex        | At1g59900 (E1)<br>At1g24180 (E1)<br>At5g50850 (E1)<br>At3g52200 (E2)<br>At1g54220 (E2)<br>At3g13930 (E3)<br>At3g17240 (E3)<br>At1g48030 (E3)          | Pyruvate + coenzyme A +<br>NAD <sup>+</sup> ⇌ Acetyl-CoA +<br>CO <sub>2</sub> + NADH   | At3g13930: 1500 kDa/54 kDa<br>At1g24180: 470 kDa/41 kDa<br>At5g50850: 150 kDa/39 kDa<br>At1g59900: 138 kDa/44 kDa<br><br>(9.5 MDa complex) | Luethy et al., 1994<br>Grof et al., 1995<br>Zou et al., 1999<br>Tovar-Méndez et al., 2003<br>Szurmak et al., 2003<br>Yu et al., 2012   |
| Glycine<br>dehydrogenase<br>complex         | At4g33010 (P)<br>At2g26080 (P)<br>At1g32470 (H)<br>At2g35120 (H)<br>At2g35370 (H)<br>At1g11860 (T)<br>At4g12130 (T)<br>At3g17240 (L)<br>At1g48030 (L) | Glycine + H <sub>4</sub> folate +<br>NAD <sup>+</sup> ⇌ methylene-H <sub>4</sub><br>folate + CO <sub>2</sub> + NH <sub>3</sub> +<br>NADH | At4g33010: 144 kDa/91 kDa<br>At2g26080: 209 kDa/91 kDa<br>At1g11860: 148 kDa/46 kDa<br><br>(1.3 MDa complex)                               | Somerville and Ogren, 1982<br>Oliver et al., 1990<br>Oliver, 1994<br>Srinivasan and Oliver, 1995<br>Douce et al., 2001   |

(Continued)

Table 1 | Continued

| Enzyme  | Accession no. <sup>b</sup><br>subunits<br>isoforms etc.  | Catalysed reaction  | Oligomeric state<br>Native mass/monomer mass according<br>to GelMap <sup>c</sup> (according to other data in<br>the literature) | Publication <sup>d</sup><br>for Arabidopsis<br>(for other plants)   |
|---|--|---|---|---|
| Branched-chain alpha<br>keto acid<br>dehydrogenase<br>complex | At5g09300 (E1)<br>At1g21400 (E1)<br>At1g55510 (E1)<br>At3g13450 (E1)<br>At3g06850 (E2)<br>At3g13930 (E3)<br>At3g17240 (E3)<br>At1g48030 (E3) | Branched chain alpha<br>keto-acids + CoA + NAD <sup>+</sup><br>⇌ Acyl-CoA + NADH  | At1g55510: 150 kDa/39 kDa<br><br>(0.95 MDa complex)   | Fujiki et al., 2000<br>Mooney et al., 2000<br>Fujiki et al., 2001<br>Fujiki et al., 2002<br>Taylor et al., 2004<br>Binder, 2010   |
| Formate<br>dehydrogenase                                      | At5g14780  | Formate + NAD <sup>+</sup> ⇌ CO <sub>2</sub><br>+ NADH  | (200 kDa complex)   | Halliwell, 1974<br>Colas des Francs-Small et al.,<br>1993<br>Hourton-Cabassa et al., 1998<br>Jansch et al., 1996<br>Bykova et al., 2003<br>Baack et al., 2003<br>Olson et al., 2000<br>Alekseeva et al., 2011 |
| Methylmalonate<br>semialdehyde<br>dehydrogenase               | At2g14170  | (S)-methylmalonate-<br>semialdehyde + coenzyme<br>A + NAD <sup>+</sup> + H <sub>2</sub> O ⇌<br>propanoyl-CoA +<br>bicarbonate + NADH                | At2g14170: 200 kDa/59 kDa   | Oguchi et al., 2004<br>Tanaka et al., 2005<br>Kirch et al., 2004  |
| Isovaleryl-CoA<br>dehydrogenase                               | At3g45300  | Isovaleryl-CoA + acceptor<br>(ETF) ⇌<br>3-methylbut-2-enoyl-CoA +<br>reduced acceptor (ETF)<br>(also considerable activity<br>with other acyl-CoAs) | At3g45300: 132 kDa/46 kDa<br><br>(homodimeric complex)  | Däschner et al., 1999<br>Reinard et al., 2000<br>Favre-Nitschke et al., 2001<br>Däschner et al., 2001<br>Goetzman et al., 2005<br>Araújo et al., 2010   |
| D-2-Hydroxyglutarate<br>dehydrogenase                         | At4g36400  | D-2-hydroxyglutarate +<br>acceptor (ETF) ⇌<br>2-oxoglutarate + reduced<br>acceptor (ETF)  | (homodimeric complex)   | Engqvist et al., 2009<br>Araújo et al., 2010<br>Engqvist et al., 2011   |
| Saccharopine<br>dehydrogenase                                 | At5g39410  | Saccharopine + NAD <sup>+</sup> +<br>H <sub>2</sub> O ⇌ Glutamate +<br>-Amino adipate<br>semialdehyde + NADH  | not known   | Zhu et al., 2000<br>Heazlewood et al., 2003   |
| Pyrroline-5-<br>carboxylate<br>dehydrogenase                  | At5g62530  | Pyrroline-5-carboxylate +<br>NAD <sup>+</sup> ⇌ Glutamate<br>(Glutamate-5-<br>semialdehyde) +<br>NADH   | At5g62530: 158 kDa/59 kDa   | Forlani et al., 1997<br>Deuschle et al., 2001<br>Deuschle et al., 2004<br>Miller et al., 2009   |
| Proline<br>dehydrogenase                                      | At3g30775<br>At5g38710   | L-Proline ⇌<br>Pyrroline-5-Carboxylate  | not known   | Elthon and Stewart, 1981<br>Verbruggen et al., 1996<br>Kiyosue et al., 1996<br>Mani et al., 2002<br>Szabados and Savouré, 2010<br>Funck et al., 2010<br>Sharma and Verslues, 2010<br>Schertl et al., in press |

(Continued)

Table 1 | Continued

| Enzyme  | Accession no. <sup>b</sup><br>subunits<br>isoforms etc.                                 | Catalysed reaction  | Oligomeric state<br>Native mass/monomer mass according<br>to GelMap <sup>c</sup> (according to other data in<br>the literature) | Publication <sup>d</sup><br>for Arabidopsis<br>(for other plants)   |
|---|---|---|---|---|
| L-Galactono-1,4-lactone dehydrogenase   | At3g47930   | L-Galactono-1,4-Lactone $\leftrightarrow$ L-Ascorbate                 | (420 kDa, 470 kDa, 850 kDa complexes)   | Mapson and Breslow, 1958<br>Siendones et al., 1999<br>Leferink et al., 2008<br>Pineau et al., 2008<br>Leferink et al., 2009<br>Schertl et al., 2012 |
| D-Lactate dehydrogenase   | At5g06580   | D-Lactate $\leftrightarrow$ Pyruvate                                  | (homodimeric complex)   | Bari et al., 2004<br>Atlante et al., 2005<br>Engqvist et al., 2009<br>Wienstroer et al., 2012   |
| Glycerol-3-phosphate dehydrogenase  | At3g10370   | Glycerol 3-phosphate $\leftrightarrow$ Dihydroxyacetonephosphate      | At3g10370: 160 kDa/65 kDa   | Shen et al., 2003<br>Shen et al., 2006  |
| Dihydroorotate dehydrogenase  | At5g23300   | Dihydroorotate $\leftrightarrow$ Orotate                              | At5g23300: 156 kDa/49 kDa   | Ullrich et al., 2002<br>Doremus and Jagendorf, 1985<br>Miersch et al., 1987   |
| Succinic semialdehyde dehydrogenase   | At1g79440   | Succinic semialdehyde $\leftrightarrow$ Succinate                     | At1g79440: 163 kDa/55 kDa   | Busch and Fromm, 1999<br>Bouché et al., 2003<br>Kirch et al., 2004<br>Toyokura et al., 2011   |
| Histidinol dehydrogenase  | At5g63890   | L-histidinol + NAD <sup>+</sup> $\leftrightarrow$ L-histidine + NADH  | At5g63890: 115 kDa/51 kDa   | Nagai and Scheidegger, 1991<br>Ingle, 2011  |
| Alternative NAD(P)H dehydrogenases (NDA1, NDB4, NDA2, NDB2, NDB3, NDB1, NDC1) | At1g07180<br>At2g20800<br>At2g29990<br>At4g05020<br>At4g21490<br>At4g28220<br>At5g08740 | NAD(P)H + UQ $\leftrightarrow$ NAD(P) <sup>+</sup> + UQH <sub>2</sub> | At2g20800: 160 kDa/65 kDa<br>At2g29990: 163 kDa/55 kDa<br>At4g05020: 160 kDa/65 kDa   | Escobar et al., 2004<br>Rasmusson et al., 2004<br>Rasmusson et al., 2008<br>Wulff et al., 2009<br>Wallström et al., 2014a,b                         |

<sup>a</sup>Mitochondrial dehydrogenases without complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) of the respiratory chain. This list corresponds to the dehydrogenases shown in **Figure 1**.

<sup>b</sup>Accession numbers in accordance with *The Arabidopsis Information Resource (TAIR)*.

<sup>c</sup>Oligomeric state: native mass and monomer mass according to GelMap (<https://gelmap.de/231>).

<sup>d</sup>Key publications for Arabidopsis (other plants).

the inner mitochondrial membrane (external alternative NDs, internal alternative NDs). Some of the genes encoding alternative NDs are activated by light (Rasmusson et al., 2008; Rasmusson and Moller, 2011). The latter enzymes are considered to be important during photorespiration and all alternative enzymes during various stress conditions. Since none of the alternative oxidoreductases couple electron transfer with proton translocation across the inner mitochondrial membrane, their enzymatic function is believed to be important in the context of an overflow protection mechanism for the ETC which is especially relevant during high-light conditions.

Finally, dehydrogenases (dark green in **Figure 1**; **Table 1**) can directly or indirectly insert electrons into the respiratory chain (Rasmusson et al., 2008; Rasmusson and Moller, 2011). Numerous dehydrogenases of the mitochondrial matrix

generate NADH which is re-oxidized by complex I and the internal alternative NDs. However, some dehydrogenases directly transfer electrons onto ubiquinone [dihydroorotate dehydrogenase (DHODH), glyceraldehyde 3-phosphate dehydrogenase (G3-PDH) and possibly proline dehydrogenase (ProDH)] or onto cytochrome c [L-galactone-1,4-lactone dehydrogenase (GLDH) and D-lactate dehydrogenase (DLDH)]. Furthermore, at least two dehydrogenases [isovaleryl-coenzyme A dehydrogenase (IVDH) and D-2-hydroxyglutarate dehydrogenase (D-2HGDH)] transfer electrons onto ubiquinone via a short electron transfer chain composed of the “electron transfer flavoprotein” and the “electron transfer flavoprotein-ubiquinone oxidoreductase” (ETF and ETFQ-OR, purple in **Figure 1**) (Ishizaki et al., 2005, 2006; Araújo et al., 2010). IVDH is involved in the branched chain amino acid catabolism and D-2HGDH in the catabolism of lysine. In

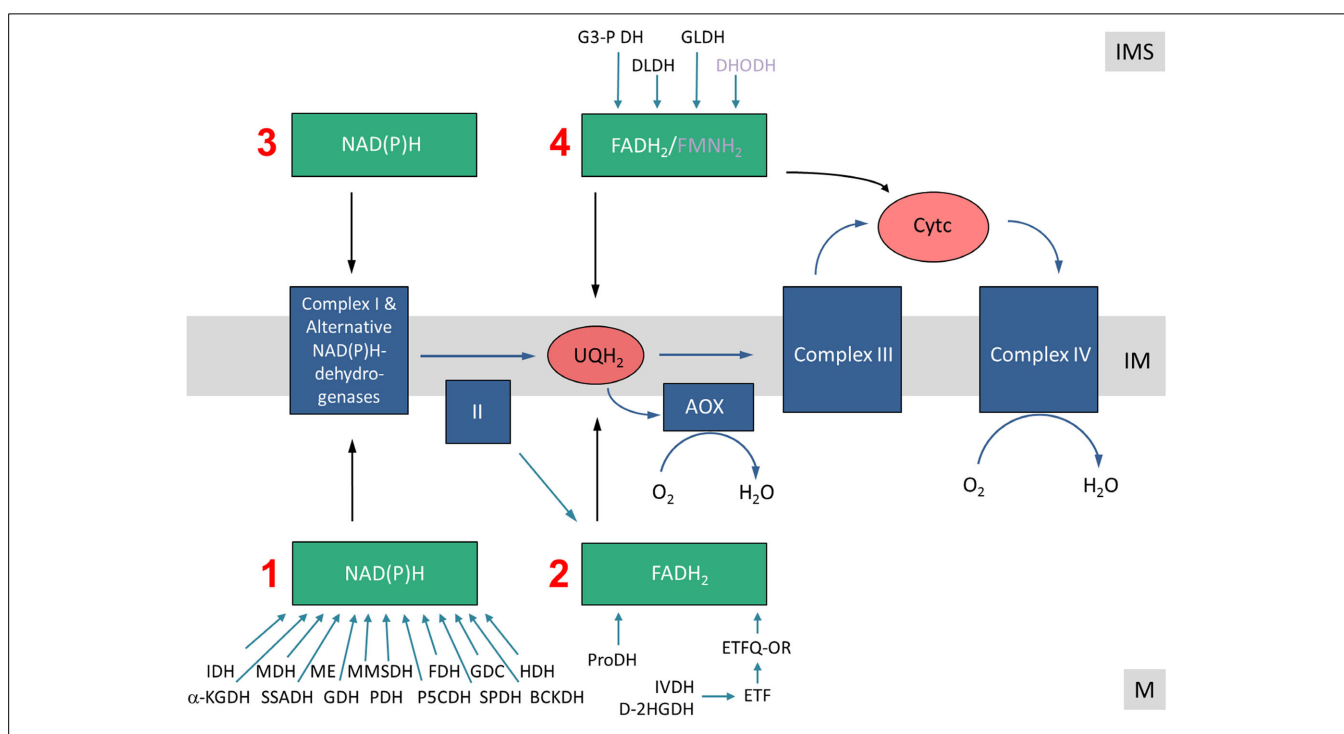
plants, degradation of amino acids for respiration was shown to be especially important during carbon starvation conditions, e.g., extended darkness (Araújo et al., 2011). In contrast to animal mitochondria, fatty acid oxidation does not take place in plant mitochondria and the involved dehydrogenases consequently are absent. Instead, additional metabolic pathways occur in plants, e.g., the final step of an ascorbic acid biosynthesis pathway, which is catalyzed by GLDH. Electrons of L-galactono-1,4-lactone (GL) oxidation are inserted into the ETC via cytochrome c (Bartoli et al., 2000). Proline, besides being a building block for protein biosynthesis, is used as an osmolyte in plant cells. Proline is catabolized in mitochondria by a two-step process involving pyrroline-5-carboxylate dehydrogenase (P5CDH) and ProDH (Szabados and Savouré, 2010). P5CDH produces NADH, whereas ProDH represents a flavoenzyme which is assumed to transfer electrons directly or indirectly onto ubiquinone. Some additional dehydrogenases occur in plant mitochondria in the mitochondrial matrix and the intermembrane space

which also contribute electrons to the ETC (Figure 1, Table 1). However, in some cases their mitochondrial localization is not entirely certain and should be further investigated by future research.

### ELECTRON ENTRY PATHWAYS INTO THE ETC

All electrons enter the ETC via NAD(P)H (generated by a variety of dehydrogenases in the mitochondrial matrix or the intermembrane space/the cytoplasm) or via flavine nucleotides (FADH<sub>2</sub>, FMNH<sub>2</sub>), which generally are bound to proteins termed flavoproteins. Consequently, the following electron entry pathways into the ETC can be defined: (i) the Matrix NAD(P)H pathway, (ii) the Matrix-FADH<sub>2</sub> pathway, (iii) the Intermembrane-space-NAD(P)H pathway, and (iv) the Intermembrane-space-FADH<sub>2</sub>/FMDH<sub>2</sub> pathway (Figure 2).

Different metabolic processes, which vary depending on the physiological state of the plant cell, contribute to the four electron entry pathways. During stable carbohydrate conditions, electrons



**FIGURE 2 | Electron entry pathways into the mitochondrial electron transport chain in plants.** Electrons enter the respiratory chain via four different pathways. (1) The Matrix-NAD(P)H pathway. Various dehydrogenases oxidize carbon compounds in the mitochondrial matrix. Electrons are transferred in the form of NADH to the ETC. NADH is re-oxidized by complex I or the internal alternative NAD(P)H dehydrogenases. (2) The Matrix-FADH<sub>2</sub> pathway. FAD-containing enzymes oxidize carbon compounds in the mitochondrial matrix and directly (ProDH?) or indirectly (via the ETF/ETFQO system) transfer electrons to the ubiquinone pool. (3) The IMS-NAD(P)H pathway. Cytoplasmically formed NAD(P)H is re-oxidized via external alternative dehydrogenases. (4) The IMS-FADH<sub>2</sub> pathway. FAD/FMN-containing enzymes oxidize carbon compounds in the mitochondrial intermembrane space. Electrons are transferred either to the ubiquinone or the cytochrome c. M, matrix; IM, inner membrane; IMS, intermembrane space. Abbreviations—*alphabetically ordered*. I,

complex I; II, complex II; III, complex III; IV, complex IV; α-KGDH, α-ketoglutarate dehydrogenase; AOX, alternative oxidase; BCKDH, branched-chain α-ketoacid dehydrogenase complex; Cyt c, cytochrome c; D-2HGDH, D-2-hydroxyglutarate dehydrogenase; DHODH, dihydroorotate dehydrogenase; DLDH, D-lactate dehydrogenase; ETF, electron transfer flavoprotein; ETFQOR, electron transfer flavoprotein ubiquinone oxidoreductase; FDH, formate dehydrogenase; GDC, glycine dehydrogenase; GDH, glutamate dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; G3-P DH, glyceraldehyde 3-phosphate dehydrogenase; HDH, histidinol dehydrogenase; IDH, isocitrate dehydrogenase; IVDH, isovaleryl-coenzyme A dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; MMSDH, methylmalonate-semialdehyde dehydrogenase; P5CDH, pyrroline-5-carboxylate dehydrogenase; PDH, pyruvate dehydrogenase; ProDH, proline dehydrogenase; SPDH, saccharopine dehydrogenase; SSADH, succinic semialdehyde dehydrogenase; UQH<sub>2</sub>, ubiquinol.

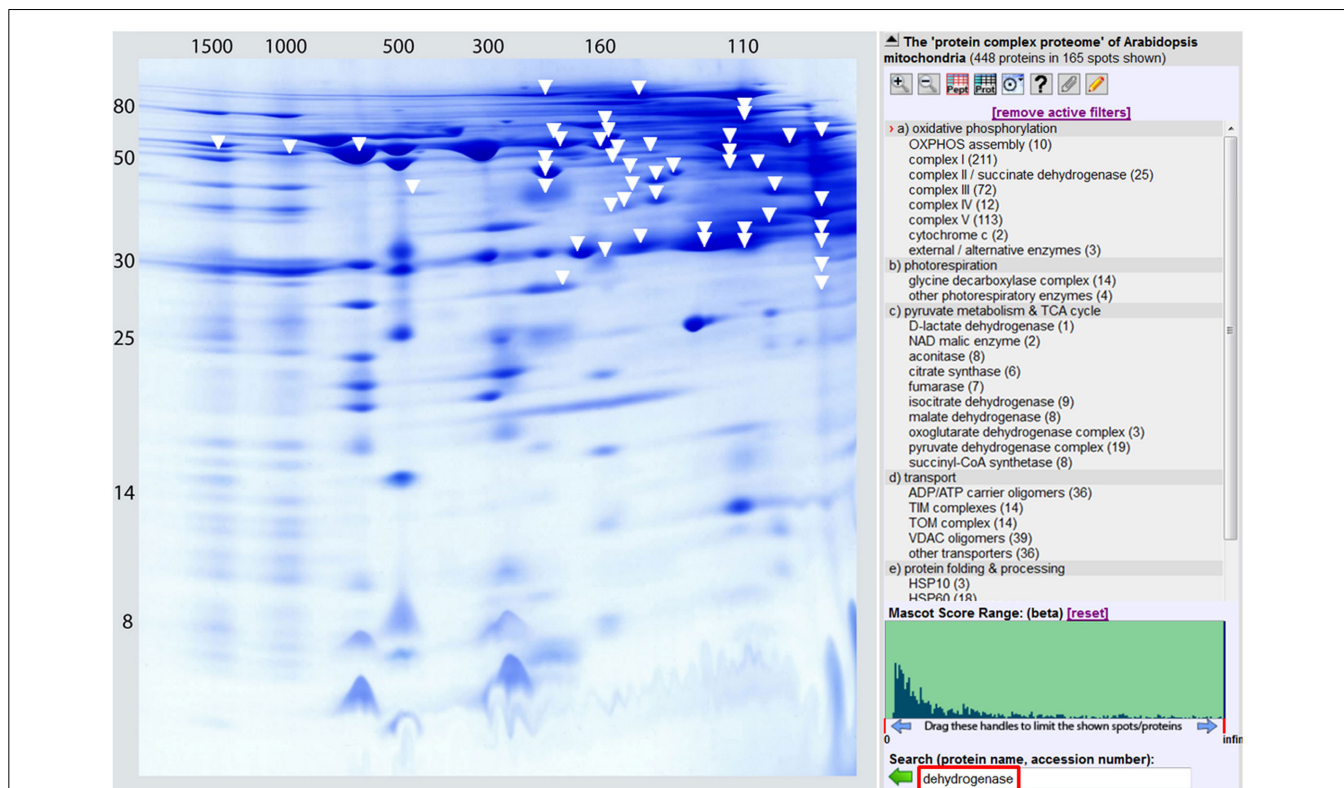
for the respiratory chain can be supplied by NADH and FADH<sub>2</sub> provided by the tricarboxylic acid (TCA) cycle. This is believed to be the standard mode of cellular respiration in non-green plant tissues or green tissues at night and resembles the basic situation in animal cells. However, during photosynthesis, NADH generation of the TCA cycle is reduced because some of its intermediates are used for anabolic reactions (reviewed in Sweetlove et al., 2010). Furthermore, the pyruvate dehydrogenase (PDH) complex is deactivated in plant mitochondria in the light by phosphorylation (Budde and Randall, 1990). At the same time photorespiration leads to an increase in NADH formation in the mitochondrial matrix by the activity of the glycine dehydrogenase complex (GDC). Indeed, at high-light conditions, NADH formed by GDC is believed to be the main substrate of the ETC, and not the NADH formed by the enzymes of the TCA cycle. At the same time, plant cells might become over-reduced in the presence of high-light. In this situation alternative oxidoreductases can insert excess electrons into the respiratory chain without contributing to the proton gradient. Upon carbon starvation conditions (e.g., extended darkness) electrons from the breakdown of amino acids are provided to the ETC (Araújo et al., 2011). Especially after release of salt stress the amino acid proline is used as an electron source (Szabados and Savouré, 2010). In summary, electron entry into the ETC is a highly flexible process in plants

which much depends on light, the metabolic state of the cell as well as environmental stress factors.

### SUPRAMOLECULAR STRUCTURE OF THE ETC SYSTEM

The ETC is based on defined protein-protein interactions. Most stable interactions occur within the four “classical” oxidoreductase complexes of the respiratory chain. Indeed, complexes I to IV can be isolated in intact form by various biochemical and electrophoretic procedures. Furthermore, several lines of evidence indicate that complexes I, III and IV interact within the inner mitochondrial membrane forming respiratory supercomplexes (reviewed in Dudkina et al., 2008). Complex I as well as complex IV associate with dimeric complex III (I + III<sub>2</sub> and IV<sub>2</sub> + III<sub>2</sub> supercomplexes). An even larger supercomplex includes complexes I, III<sub>2</sub>, and IV and was proposed to be called “respirasome” because it can autonomously catalyze the overall ETC reaction in the presence of ubiquinone and cytochrome c. The alternative oxidoreductases of the plant ETC seem not to be part of the respiratory supercomplexes. However, alternative NDs were found to be part of other protein complexes of about 160 kDa (Klodmann et al., 2011) or 150–700 kDa (Rasmusson and Agius, 2001).

Experimental data also indicate that several of the mitochondrial dehydrogenases form protein complexes. TCA cycle



**FIGURE 3 | The dehydrogenase subproteome of plant mitochondria.**

Mitochondrial proteins from *Arabidopsis thaliana* were separated by 2D Blue native/SDS PAGE and displayed via GelMap (<https://gelmapp.de/231#>). Protein separation under native condition was from left to right, protein separation in the presence of SDS from top

to bottom. Molecular masses of standard proteins are given to the left/above the 2D gel. All proteins annotated as “dehydrogenase” are indicated by white arrows. Exception: The subunits of complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) are not indicated on the figure.

enzymes can assemble forming multienzyme clusters (Barnes and Weitzman, 1986). In addition, some of the mitochondrial dehydrogenases interact with ETC complexes, e.g., malate dehydrogenase has been reported to interact with complex I in animal mitochondria (Fukushima et al., 1989; see Braun et al., 2014 for review). Information on the native state of mitochondrial dehydrogenases furthermore comes from the GelMap project (Klodmann et al., 2011). Using 2D Blue native/SDS PAGE and systematic protein identifications, various dehydrogenases were described (Figure 3, Table 1). Native molecular mass of the dehydrogenases in many cases much exceeds the molecular mass of the monomeric proteins (Table 1, column 3). This indicates that probably most mitochondrial dehydrogenases form part of defined higher order structures.

## CONCLUSION AND OUTLOOK

Cellular respiration in plants is an especially dynamic system. The classical protein complexes of the ETC have extra functions and several alternative oxidoreductases occur. A network of mitochondrial dehydrogenases directly or indirectly supplies electrons for the respiratory chain. Insertion of electrons via various pathways is highly dependent on the metabolic state of the plant cell. The regulation of electron entry pathways into the respiratory chain is only partially understood and might, besides others, depend on the formation of supramolecular structures. Non-invasive experimental procedures will be necessary to physiologically investigate the function of these structures by future research.

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