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 FADH2 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; Rasmussen and Moller, 2011; van Dongen et al., 2011; Jacoby et al., 2012). Each of these complexes has more than 30 subunits. In animal mitochondria, the core complexes contain 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 inner-mitochondrial CO2 transfer mechanism to provide mitochondrial CO2 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.
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; α-KGDH, α-ketoglutarate dehydrogenase; AOX, alternative oxidase; BCKDH, branched-chain α-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 oxireductase; FDH, formate dehydrogenase; GDC, glycine dehydrogenase; GDH, glutamate dehydrogenase; GLDH, L-galactono-1,4-lactone dehydrogenase; G3-PDH, 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; 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 Møller, 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.

| Enzyme                        | Accession no. | Catalysed reaction | Oligomeric state | Publication for Arabidopsis | Publication for other plants |
|-------------------------------|---------------|---------------------|------------------|----------------------------|------------------------------|
| Malate dehydrogenase         | At1g53240     | Malate + NAD⁺ ⇔ Oxaloacetate + NADH | At1g53240: 89 kDa/42 kDa | Journet et al., 1981        | Gietl, 1992                  |
|                               | At3g15020     |                     | At3g47520: 157 kDa/38 kDa | Krömer, 1995                | Nunes-Nesi et al., 2005      |
|                               |               |                     |                  | Lee et al., 2008             | Tomaz et al., 2010           |
| Isocitrate dehydrogenase      | At4g35260     | Isocitrate + NAD⁺ ⇔ α-Ketoglutarate + CO₂ + NADH | At4g35260: 89 kDa/42 kDa | Behal and Oliver, 1998      | Lancien et al., 1998         |
|                               | At5g14590     |                     | At5g14590: 140 kDa/53 kDa | Lin et al., 2004             | Lemaitre and Hodges, 2006    |
|                               | At4g35650     |                     | At3g09810: 138 kDa/40 kDa | Lemaitre et al., 2007        | Lemaitre et al., 2007        |
|                               | At3g09810     |                     | At5g03290: 138 kDa/40 kDa |                         |                              |
|                               | At2g317130    |                     |                  |                         |                              |
| α-Ketoglutarate dehydrogenase complex | At3g55410 (E1) | α-Ketoglutarate + coenzyme A + NAD⁺ ⇔ succinyl-CoA + CO₂ + NADH | At3g55410: 207 kDa/91 kDa | Poulsen and Wedding, 1970    |                              |
|                               | At5g05750 (E1) |                     |                  | Wedding and Black, 1971a,b  |                              |
|                               | At4g26910 (E2) |                     |                  | Dry and Wiskich, 1987       |                              |
|                               | At3g55070 (E2) |                     |                  | Millar et al., 1999         |                              |
|                               | At3g17240 (E3) |                     |                  | Araújo et al., 2008         |                              |
|                               | At1g48030 (E3) |                     |                  | Araújo et al., 2013         |                              |
|                               | At3g13930 (E3) |                     |                  |                              |                              |
| Glutamate dehydrogenase      | At5g18170     | Glutamate + H₂O + NAD⁺ ⇔ α-Ketoglutarate + NH₄⁺ + NADH | At5g18170: 209 kDa/48 kDa | Yamaya et al., 1984         | Turano et al., 1997          |
|                               | At5g07440     |                     | At5g07440: 209 kDa/48 kDa | Aubert et al., 2001         | Fontaine et al., 2012        |
|                               | At3g03910     |                     | At3g03910: 209 kDa/48 kDa | Miyashita and Good, 2008a,b | Fontaine et al., 2012        |
|                               |               |                     |                  |                              |                              |
| Malic enzyme                 | At2g13560     | Malate + NAD⁺ ⇔ Pyruvate + NADH + CO₂ | At2g13560: 370 kDa/63 kDa | Jenner et al., 2001         | Tronconi et al., 2008        |
|                               | At4g00570     |                     | At4g00570: 370 kDa/63 kDa | Tronconi et al., 2010       | Tronconi et al., 2012        |
|                               | At1g79750     |                     |                  |                              |                              |
| Pyruvate dehydrogenase       | At1g59900 (E1) | Pyruvate + coenzyme A + NAD⁺ ⇔ Acetyl-CoA + CO₂ + NADH | At1g59900: 1500 kDa/54 kDa | Luethy et al., 1994         | Grof et al., 1995            |
|                               | At1g24180 (E1) |                     | At1g24180: 470 kDa/41 kDa | Zou et al., 1999            | Tovar-Méndez et al., 2003    |
|                               | At3g50850 (E1) |                     | At1g50850: 150 kDa/39 kDa | Srinivasan and Oliver, 1995 | Szurmack et al., 2003        |
|                               | At3g52200 (E2) |                     | At1g59900: 138 kDa/44 kDa |                              | Yu et al., 2012              |
|                               | At1g54220 (E2) |                     |                  |                              |                              |
|                               | At3g13930 (E3) |                     |                  |                              |                              |
|                               | At3g17240 (E3) |                     |                  |                              |                              |
|                               | At1g48030 (E3) |                     |                  |                              |                              |
| Glycine dehydrogenase        | At4g33010 (P) | Glycine + H₂ folate + NAD⁺ ⇔ methylene-H₂ folate + CO₂ + NH₄⁺ + NADH | At4g33010: 144 kDa/91 kDa | Somerville and Ogren, 1982  | Oliver et al., 1990          |
|                               | At2g26080 (P) |                     | At2g26080: 209 kDa/41 kDa | Oliver, 1994                | Oliver, 1994                 |
|                               | At1g32470 (H) |                     | At1g11860: 148 kDa/46 kDa | Srinivasan and Oliver, 1995 |                              |
|                               | At2g35120 (H) |                     |                  | Doucet et al., 2001         |                              |
|                               | At2g35370 (H) |                     |                  |                              |                              |
|                               | At1g11860 (T) |                     |                  |                              |                              |
|                               | At4g12130 (T) |                     |                  |                              |                              |
|                               | At3g17240 (L) |                     |                  |                              |                              |
|                               | At1g48030 (L) |                     |                  |                              |                              |

(Continued)
| Enzyme | Accession no. | Catalysed reaction | Oligomeric state | Publication |
|--------|---------------|-------------------|------------------|-------------|
| Branched-chain alpha keto acid dehydrogenase complex | At5g09300 (E1) At1g21400 (E1) At1g55510 (E1) At3g13450 (E1) At3g06850 (E2) At3g13930 (E3) At3g17240 (E3) At1g48030 (E3) | Branched chain alpha keto-acids + CoA + NAD⁺ ⇔ Acyl-CoA + NADH | At1g55510: 150 kDa/39 kDa (0.95 MDa complex) | Fujiki et al., 2000 Mooney et al., 2000 Fujiki et al., 2001 Fujiki et al., 2002 Taylor et al., 2004 Binder, 2010 |
| Formate dehydrogenase | At5g14780 | Formate + NAD⁺ ⇔ CO₂ + NADH | (200 kDa complex) | Halliwell, 1974 Colas des Francs-Small et al., 1993 Hourton-Cabassa et al., 1998 Jänsch et al., 1996 Bykova et al., 2003 Baack et al., 2003 Olson et al., 2000 Alekseeva et al., 2011 |
| Methylmalonate semialdehyde dehydrogenase | At2g14170 | (S)-methylmalonate-semialdehyde + coenzyme A + NAD⁺ + H₂O ⇔ propanoyl-CoA + bicarbonate + NADH | At2g14170: 200 kDa/59 kDa | Oguchi et al., 2004 Tanaka et al., 2005 Kirch et al., 2004 |
| Isovaleryl-CoA dehydrogenase | At3g45300 | Isovaleryl-CoA + acceptor (ETF) ⇔ 3-methylbut-2-enoyl-CoA + reduced acceptor (ETF) (also considerable activity with other acyl-CoAs) | At3g45300: 132 kDa/46 kDa (homodimeric complex) | Däschner et al., 1999 Reinard et al., 2000 Faivre-Nitschke et al., 2001 Däschner et al., 2001 Goetzman et al., 2005 Araújo et al., 2010 |
| D-2-Hydroxyglutarate dehydrogenase | At4g36400 | D-2-hydroxyglutarate + acceptor (ETF) ⇔ 2-oxoglutarate + reduced acceptor (ETF) (homodimeric complex) | | Engqvist et al., 2009 Araújo et al., 2010 Engqvist et al., 2011 |
| Saccharopine dehydrogenase | At5g39410 | Saccharopine + NAD⁺ + H₂O ⇔ Glutamate + -Amino adipate semialdehyde + NADH | not known | Zhu et al., 2000 Headlewood et al., 2003 |
| Pyrroline-5-carboxylate dehydrogenase | At5g62530 | Pyrroline-5-carboxylate + NAD⁺ ⇔ Glutamate (Glutamate-5-semialdehyde) + NADH | At5g62530: 158 kDa/59 kDa | Forlani et al., 1997 Deuschle et al., 2001 Deuschle et al., 2004 Miller et al., 2009 |
| Proline dehydrogenase | At3g30775 At5g38710 | L-Proline ⇔ Pyrroline-5-Carboxylate | not known | Elthon and Stewart, 1981 Verbruggen et al., 1996 KiyoSue et al., 1996 Mani et al., 2002 Szabados and Savouré, 2010 Funck et al., 2010 Sharma and Verslues, 2010 Schertl et al., in press |
Table 1 | Continued

| Enzyme                                | Accession no. | Catalysed reaction | Oligomeric state | Publication |
|---------------------------------------|---------------|--------------------|------------------|-------------|
| L-Galactono-1,4-lactone dehydrogenase | At3g47930     | L-Galactono-1,4-Lactone ⇔ L-Ascorbate | (420 kDa, 470 kDa, 850 kDa complexes) | Mapson and Breslow, 1958 |
|                                       |               |                    |                  | Siendones et al., 1999 |
|                                       |               |                    |                  | Leferink et al., 2008 |
|                                       |               |                    |                  | Pineau et al., 2008 |
|                                       |               |                    |                  | Leferink et al., 2009 |
|                                       |               |                    |                  | Schertl et al., 2012 |
| D-Lactate dehydrogenase               | At5g06580     | D-Lactate ⇔ Pyruvate | Homodimeric complex | Bari et al., 2004 |
|                                       |               |                    |                  | Atlante et al., 2005 |
|                                       |               |                    |                  | Engqvist et al., 2009 |
|                                       |               |                    |                  | Wienstroer et al., 2012 |
| Glycerol-3-phosphate dehydrogenase    | At3g10370     | Glycerol 3-phosphate ⇔ Dihydroxyacetonephosphate | At3g10370: 160 kDa/65 kDa | Shen et al., 2003 |
|                                       |               |                    |                  | Shen et al., 2006 |
| Dihydroorotate dehydrogenase          | At5g23300     | Dihydroorotate ⇔ Orotate | At5g23300: 156 kDa/49 kDa | Ullrich et al., 2002 |
|                                       |               |                    |                  | Doremus and Jagendorf, 1985 |
|                                       |               |                    |                  | Miersch et al., 1987 |
| Succinic semialdehyde dehydrogenase   | At1g79440     | Succinic semialdehyde ⇔ Succinate | At1g79440: 163 kDa/55 kDa | Busch and Fromm, 1999 |
|                                       |               |                    |                  | Bouché et al., 2003 |
|                                       |               |                    |                  | Kirch et al., 2004 |
|                                       |               |                    |                  | Toyokura et al., 2011 |
| Histidinol dehydrogenase              | At5g63890     | L-histidinol + NAD⁺ ⇔ L-histidine + NADH | At5g63890: 115 kDa/51 kDa | Nagai and Scheidegger, 1991 |
|                                       |               |                    |                  | Ingle, 2011 |
| Alternative NAD(P)H dehydrogenases    | At1g07180     | NAD(P)H + UQ ⇔ NAD(P)⁺ + UQH₂ | At1g07180: 160 kDa/65 kDa | Escobar et al., 2004 |
| (NDA1, NDB4, NDA2, NDB2, NDB3, NDB1, NDC1) | At2g20800     |                    | At2g20800: 163 kDa/55 kDa | Rasmusson et al., 2004 |
|                                       | At2g29990     |                    | At2g29990: 163 kDa/55 kDa | Rasmusson et al., 2008 |
|                                       | At4g06020     |                    | At4g06020: 160 kDa/65 kDa | Wulff et al., 2009 |
|                                       | At4g21490     |                    | At4g21490: 160 kDa/65 kDa | Wallström et al., 2014a,b |
|                                       | At4g28220     |                    | At4g28220: 160 kDa/65 kDa | |
|                                       | At5g08740     |                    | At5g08740: 160 kDa/65 kDa | |

*a 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.*

*b Accession numbers in accordance with The Arabidopsis Information Resource (TAIR).*

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

*d 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-galactono-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 ETF-Q-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$_2$, FMNH$_2$), 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$_2$ pathway, (iii) the Intermembrane-space-NAD(P)H pathway, and (iv) the Intermembrane-space-FADH$_2$/FMNH$_2$ 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...
for the respiratory chain can be supplied by NADH and FADH$_2$ 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$_2$ and IV$_2$ + III$_2$ supercomplexes). An even larger supercomplex includes complexes I, III$_2$, and IV and was proposed to be called “respirasome” because it can autonomously catalyzes 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...
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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