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FOCUSED REVIEW

Mitochondrial complex II of plants: subunit composition, assembly, and function in respiration and signaling

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SUMMARY

Complex II [succinate dehydrogenase (succinate-ubiquinone oxidoreductase); EC 1.3.5.1; SDH] is the only enzyme shared by both the electron transport chain and the tricarboxylic acid (TCA) cycle in mitochondria. Complex II in plants is considered unusual because of its accessory subunits (SDH5–SDH8), in addition to the catalytic subunits of SDH found in all eukaryotes (SDH1–SDH4). Here, we review compositional and phylogenetic analysis and biochemical dissection studies to both clarify the presence and propose a role for these subunits. We also consider the wider functional and phylogenetic evidence for SDH assembly factors and the reports from plants on the control of SDH1 flavination and SDH1–SDH2 interaction. Plant complex II has been shown to influence stomatal opening, the plant defense response and reactive oxygen species-dependent stress responses. Signaling molecules such as salicyclic acid (SA) and nitric oxide (NO) are also reported to interact with the ubiquinone (UQ) binding site of SDH, influencing signaling transduction in plants. Future directions for SDH research in plants and the specific roles of its different subunits and assembly factors are suggested, including the potential for reverse electron transport to explain the succinate-dependent production of reactive oxygen species in plants and new avenues to explore the evolution of plant mitochondrial complex II and its utility.

Keywords: plant mitochondria, succinate dehydrogenase, complex II, assembly factors, reactive oxygen species, stress signaling.

INTRODUCTION

Plant mitochondria are involved in numerous cellular processes, most notably oxidative phosphorylation leading to ATP synthesis, respiratory carbon metabolism and the production of reactive oxygen species (ROS) (Millar *et al.*, 2011; Huang *et al.*, 2016). The electron transport chain (ETC) and tricarboxylic acid (TCA) cycle are the key metabolic pathways responsible for these functions. The mitochondrial ETC is composed of four respiratory complexes (complexes I–IV). Complex II (succinate dehydrogenase) oxidizes succinate to fumarate and is a component of both the ETC and the TCA cycle. Understanding this protein complex in plants has been achieved by two distinct but important areas of research. In the first area, an integrated understanding of the composition of complex II in plants is being aided by recent advances in our understanding of the evolution of its subunits and its assembly machinery across kingdoms (Box 1). In the second area, function studies are linking the operation of complex II with diverse aspects of plant function and responses to the environment (Box 1).

COMPLEX II SUBUNITS SDH1–SDH4

SDH consists of four classical subunits that contribute to its catalytic function, and these are named differently across species (Bullis and Lemire, 1994; Daignan-Fornier *et al.*, 1994; Iverson *et al.*, 2012; Huang and Millar, 2013b) (Table 1). In Arabidopsis they are referred to as SDH1, SDH2, SDH3 and SDH4. The structure of SDH was first resolved from *Escherichia coli* using X-ray crystallography (Yankovskaya *et al.*, 2003). A similar structure was

Box 1 Main findings on complex II of plants.

- Complex II (succinate dehydrogenase) is a central component of both the electron transport chain and the tricarboxylic acid cycle.
- Dysfunction of succinate dehydrogenase causes disorder in the mitochondrial metabolism via the accumulation of succinate and decreased energy production.
- Plant complex II has four additional SDH subunits and the biogenesis of plant complex II requires assembly factors.
- Complex II is a key player in the production of mitochondrial reactive oxygen species (mtROS), and contributes to signaling in downstream stress and pathogen responses.

confirmed for the animal mitochondrial SDH isolated from pig and chicken (Sun *et al.*, 2005; Huang *et al.*, 2006). SDH1 contains a flavin adenine dinucleotide (FAD) cofactor that is located at its N-terminus. SDH2 harbors three Fe–S clusters – [2Fe–2S], [3Fe–4S] and [4Fe–4S] – with [2Fe–2S] ligated to its N-terminus, whereas the others are attached to the C-terminus. SDH1 and SDH2 protrude into the mitochondrial matrix and together form the succinate dehydrogenase domain of complex II (Figure 1a). SDH1/2 are bound to the inner mitochondrial membrane by a small membrane domain formed by SDH3 and SDH4. These membrane proteins are structurally and evolutionarily related, and include three membrane-spanning helices each, forming a conserved six-helix bundle that binds a heme group (Sun *et al.*, 2005).

During succinate oxidation to fumarate by SDH1, the FAD cofactor accepts two electrons. These electrons are transferred from FAD via the [2Fe–2S], [3Fe–4S] and [4Fe–4S] clusters in SDH2 to finally reduce ubiquinone (UQ) to UQH_2 at the Qp site in SDH3/4. Based on SDH crystal structure and biochemical analysis, there is no direct role of the heme group in electron transfer to UQ (Sun *et al.*, 2005; Oyedotun *et al.*, 2007; Tran *et al.*, 2007). There are two UQ binding sites in SDH, one (Qp) on the matrix side face of the IMM and a second (Qd) on the opposite side of the membrane from the Qp site, distal from the matrix (Yankovskaya *et al.*, 1996; Oyedotun and Lemire, 1999, 2001; Sun *et al.*, 2005). A structure of SDH bound to the SDH inhibitor 2-thenoyltrifluoroacetone (TTFA) showed the inhibitor tightly bound to the Qp pocket, suggesting a high binding affinity for this site (Sun *et al.*, 2005). A second molecule of TTFA was also found to bind to the Qd site (Hagerhall, 1997; Sun *et al.*, 2005). The higher binding affinity of TTFA at the Qp site than at the Qd site indicated the existence of one strong and one weaker inhibitor binding site for UQ reduction (Yankovskaya *et al.*, 1996; Sun *et al.*, 2005).

Genes encoding mitochondrial complex II subunits SDH1–SDH4 in eukaryotes were inherited from the Alphaproteobacterial progenitor of mitochondria. Each of SDH1–SDH4 is conserved across eukaryotic diversity (Figure 2), although the amino acid sequences of SDH3 and SDH4 are considerably more divergent than those of SDH1 and SDH2. This reflects the strong structure-dependent functional basis of succinate oxidation that constrains the evolution of SDH1 and SDH2, in contrast to SDH3 and SDH4, which are primarily membrane-embedded anchors, albeit with UQ binding sites.

Over the course of eukaryotic evolution, most mitochondrial genes were transferred to the nucleus (Gray

Table 1 List of SDH subunits and assembly factors in Arabidopsis and their orthologs in humans and yeast

SDH subunits and AFs	Arabidopsis (Arabidopsis *thaliana*)	Human (*Homo sapiens*)	Yeast (*Saccharomyces cerevisiae*)
SDH1	At5g66760; At2g18450	SDHA (P31040)	SDH1 (Q00711)
SDH2	At3g27380; At5g40650; At5g65165	SDHB (P21912)	SDH2 (P21801)
SDH3	At5g09600; At4g32210	SDHC (Q61Q2)	SDH3 (P33421)
SDH4	At2g46505	SDHD (O14521)	SDH4 (P37298)
SDH5	At1g47420		
SDH6	At1g08480		
SDH7	At3g47833; At5g62575		
SDH8	At2g46390		
SDHAF1	At2g39725*	SDHAF1 (A6NFY7)	SDH6 (Q3E785)
SDHAF2	At5g51040	SDHAF2 (Q9NX18)	SDH5 (Q08230)
SDHAF3	n.d.	SDHAF3 (Q9NRP4)	SDH7 (Q04401)
SDHAF4	At5g67490	SDHAF4 (Q5VUM1)	SDH8 (P38345)

The designations SDH5, SDH6, SDH7 and SDH8 were introduced for the four extra subunits present in complex II of flowering plants (Eubel *et al.*, 2003; Millar *et al.*, 2004). At a later stage, the same designations were introduced for complex II assembly factors in fungi and mammals (Hao *et al.*, 2009; Van Vranken *et al.*, 2015). The two sets of SDH5–SDH8 proteins are not related.
n.d., not detected.
*Experimental evidence is required to prove its function.

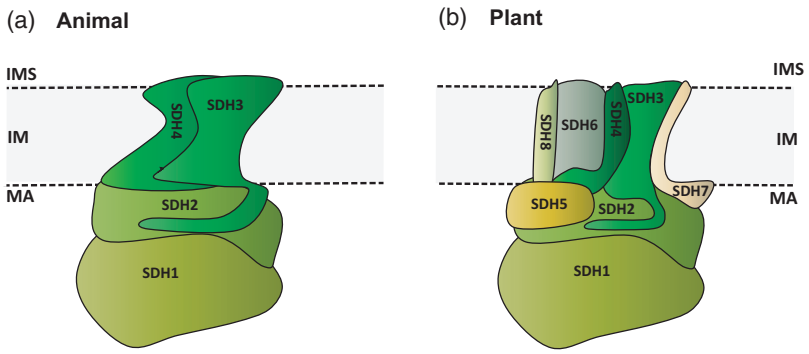

Figure 1. Topological model of mitochondrial complex II of animal (*Gallus gallus*) (a) and plant (*Arabidopsis thaliana*) (b) (from Schikowsky *et al.*, 2017; modified). The subunit arrangement for *G. gallus* is taken from the crystal structure (Huang *et al.*, 2006); the arrangement for *A. thaliana* is based on the biochemical results presented by Schikowsky *et al.* (2017). The lower part of the complex, which protrudes into the mitochondrial matrix, includes SDH1 and SDH2. The upper part of the complex anchors SDH1 and SDH2 to the inner mitochondrial membrane. Besides SDH3 and SDH4 it includes the plant-specific subunits SDH6 and SDH7. SDH7 is suggested to interact with SDH3 and SDH6 is suggested to interact with SDH4 based on biochemical and evolutionary considerations (Schikowsky *et al.*, 2017). The localization of SDH5 is probably at the interface between the succinate dehydrogenase and the membrane domain, but could also be found at another position. The localization of SDH8, which is only 4.9 kDa in size and includes one membrane-spanning helix, is not known. Abbreviations: IM, inner membrane; IMS, intermembrane space; MA, matrix.

et al., 1999), and this has happened to a different extent for different complex II subunits. *SDH1* is not found in the mitochondrial DNA (mtDNA) of any eukaryotic lineage described thus far, indicating an ancient transfer event (Lang *et al.*, 1999). *SDH2* is rarely mtDNA encoded, but is found in the exceptionally gene-rich mtDNAs of jakobids (Burger *et al.*, 2013), along with red algae (Gray *et al.*, 1998). In contrast, *SDH3* and *SDH4* are found in either mitochondrial or nuclear genomes (Gray *et al.*, 1998); in animals and fungi, all four *SDH*s are nucleus encoded. In the plant lineage, gene transfer to the nucleus is a continuing process (Palmer *et al.*, 2000), and this is reflected by *SDH3* and *SDH4*. SDH3 is mtDNA encoded in charophytes (*Chara* and *Klebsormidium*), basal plants such as *Marchantia*, *Physcomitrella* and *Selaginella*, and some dicots, but is nucleus encoded in monocots, and in dicots such as Arabidopsis and *Medicago* (Figure 2). *SDH4* follows a very similar pattern to *SDH3* (Figure 2), and is also present as a pseudogene in some angiosperm mitochondrial genomes (Giege *et al.*, 1998). In fact, studies of angiosperms suggested that *SDH3* and *SDH4* were transferred to the nucleus four and three times, respectively (Adams *et al.*, 2001). Phylogenetic analysis of subunits SDH1 and SDH2 recovers members of Streptophyta (Embryophyta + Charophyta) as a monophyletic grouping, although typically not with strong statistical support (Figure S1). For SDH3 and SDH4, streoptophyte monophyly is not recovered perfectly (Figure S1), likely reflecting mutational saturation and the small size of these proteins. In parallel with the transfer of *SDH3* and *SDH4* from the mitochondrial genome to the nuclear genome in some plants, SDH3 and SDH4 became truncated and more hydrophilic. Using sequence comparisons of SDH3 and SDH4 proteins from various plants it was shown that the truncation of SDH4 preceded the truncation of SDH3 during the evolution of Streptophyta (Embryophyta and Charophyta) (Schikowsky *et al.*, 2017).

SDH1–SDH4 thus represent two subcomplexes that come together to form a holo-complex and that have co-evolved differently. SDH1 and SDH2 are nuclear encoded in almost all eukaryotes and their sequences are highly conserved. This portion of the complex is responsible for succinate oxidation and the transfer of electrons to cofactor groups within the enzyme. In contrast, SDH3 and SDH4 have been moved from mitochondrial to nuclear genomes repeatedly, and have diverged in sequence. Although it could be argued that this reflects a more structural membrane-anchoring role, it also indicates greater diversity in the UQ binding site of the enzyme. The presence of the heme group bound by SDH3 and SDH4 subunits but with no clear role in the catalytic path may indicate a loss of function associated with this complex history or an unknown function associated with SDH3 and SDH4 in complex II.

COMPLEX II SUBUNITS SDH5–SDH8

Whereas the SDH complex of bacteria and animals consists of these four classical subunits alone, and has a mass of ~110 kDa, in various plants four additional subunits have been identified, and a native mass of ~160 kDa has been reported (Eubel *et al.*, 2003; Millar *et al.*, 2004; Huang *et al.*, 2010). The designations SDH5, SDH6, SDH7 and SDH8 were introduced for the four extra subunits present in complex II of flowering plants (Eubel *et al.*, 2003; Millar *et al.*, 2004) (Table 1). At a later stage, the same designations were also introduced for complex-II assembly factors in fungi and mammals (Hao *et al.*, 2009; Van Vranken *et al.*, 2015); however, these two sets of SDH5–SDH8 proteins are not related, and later SDH assembly factors were renamed with SDHAF designations. The four additional

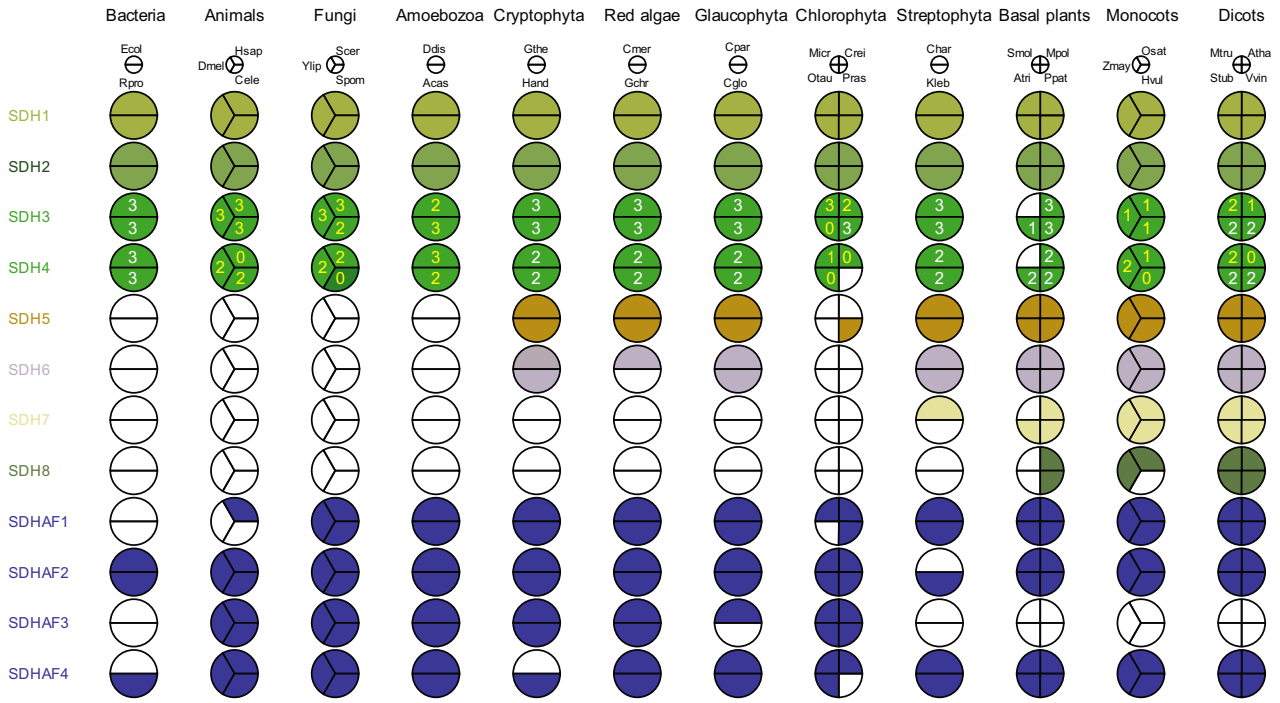

Figure 2. Evolutionary conservation of complex II subunits and assembly factors. Numbers on the SDH3 and SDH4 pie wedges indicate the number of predicted transmembrane helices; numbers in yellow depict nucleus-encoded proteins, whereas numbers in white refer to proteins encoded in mitochondrial or bacterial genomes. Complex II in animals, fungi and bacteria is composed of four conserved subunits (SDH1–SDH4), in contrast to plant complex II, which includes four additional subunits (SDH5–SDH8). SDH5–SDH8 are less conserved through evolution, and some subunits are plant specific. SDH5 and SDH6 were first identified in land-plant complex II, and have only been biochemically characterized in plants; however, homologs are also encoded in the genomes of related lineages, such as cryptophytes, red algae, glaucophytes and streptophyte (green) algae. SDH7 is found in plants and some streptophytes (*e.g. Chara*), whereas SDH8 is only found in plants and is challenging to identify bioinformatically because of its small size (46 amino acids in Arabidopsis). Complex II assembly factors 2 and 4 (SDHAF2 and SDHAF4) are present in bacteria and are well conserved across eukaryotes; SDHAF1 is similarly well conserved but is not present in bacteria. SDHAF3 is present in most eukaryotes, but homologs cannot be identified in plants. Whether plant SDHAF3 has been functionally replaced by another protein, lost altogether, or has diverged beyond recognition is unknown. Interestingly, the loss of plant SDHAF3 appears to coincide with the appearance of SDH7. Abbreviations: Acas, *Acanthamoeba castellanii*; Atha, *Arabidopsis thaliana*; Atri, *Amborella trichopoda*; Cele, *Caenorhabditis elegans*; Cglo, *Cyanoptyche gloeocystis*; Char, *Chara braunii*; Cmer, *Cyanidioschyzon merolae*; Cpar, *Cyanophora paradoxa*; Crei, *Chlamydomonas reinhardtii*; Ddis, *Dictyostelium discoideum*; Dmel, *Drosophila melanogaster*; Ecol, *Escherichia coli*; Gchr, *Graciliariopsis chorda*; Gthe, *Guillardia theta*; Hand, *Hemiselmis andersenii*; Hsap, *Homo sapiens*; Hvul, *Hordeum vulgare*; Kleb, *Klebsormidium nitens*; Micr, *Micromonas pusilla*; Mpol, *Marchantia polymorpha*; Mtru, *Medicago trunculata*; Osat, *Oryza sativa*; Otau; *Ostreococcus tauri*; Ppat, *Physcomitrella patens*; Pras, *Prasinoderma* (including *P. singularis* and *P. coloniale*); Rpro, *Rickettsia prowazekii*; Scer, *Saccharomyces cerevisiae*; Smol, *Selaginella moellendorffii*; Spom, *Schizosaccharomyces pombe*; Stub, *Solanum tuberosum*; Vvin, *Vitis vinifera*; Ylip, *Yarrowia lipolytica*; Zmay, *Zea mays*. The maximum-likelihood phylogenetic analyses of proteins were given in Figure S1.

subunits in flowering plants, SDH5–SDH8 (Table 1) do not show clear functional domains in their sequence and are subunits of SDH rather than assembly factors.

The subunit topology of the 160-kDa complex II from *Arabidopsis thaliana* (Figure 1b) was recently investigated biochemically using controlled destabilization of the holocomplex by treatment with detergent (Schikowsky *et al.*, 2017). Complex II from plants only remains intact upon membrane solubilization with very mild detergents, e.g. digitonin (Eubel *et al.*, 2003). In the presence of the detergent dodecylmaltoside, which allows the isolation of intact complex II from other groups of organisms (Schägger and von Jagow, 1991; Schägger and Pfeiffer, 2000), complex II from Arabidopsis is dissected into two subcomplexes (Schikowsky *et al.*, 2017). One subcomplex consists of the soluble SDH1 and SDH2 proteins; the other complex includes the membrane anchor subunits SDH3 and SDH4 together with SDH6 and SDH7 (Figure 1b). The SDH5 subunit is hydrophilic and was not bound to either of the subcomplexes under the conditions applied. The SDH5 subunit is most probably located at the interface of the succinate dehydrogenase domain (SDH1 and SDH2) and the membrane anchor domain (SDH3, SDH4, SDH6 and SDH7) (Figure 1b), and is liberated from complex II by destabilization using dodecylmaltoside (Schikowsky *et al.*, 2017). It should be noted that complex II of plants is clearly larger than complex II of other groups of organisms. This may indicate that the plant-specific subunits are involved in additional so-far unknown functions implemented into plant complex II. Similar discoveries of additional functions have been reported for other protein complexes of the OXPHOS system of plant mitochondria (Braun *et al.*, 1992; Sunderhaus *et al.*, 2006).

Plant SDH3 and SDH4 sequences lack stretches that in other organisms contribute to the binding of SDH1, which

might explain the reduced stability of plant complex II upon detergent solubilization (Schikowsky *et al.*, 2017). In attempts to explain these losses, it was noted that SDH6 and SDH7 subunits are predicted to include one and two membrane-spanning helices that might substitute the missing helices in SDH3 and SDH4 (Schikowsky *et al.*, 2017). Similar cases of mitochondrial protein truncation have been reported previously (Morales *et al.*, 2009; Gawryluk and Gray, 2010). SDH6 and SDH7 exhibit some low but significant sequence similarity to the sequence stretches of SDH3 and SDH4 in *Escherichia coli*, which are absent in SDH3 and SDH4 from plants (Schikowsky *et al.*, 2017). It was suggested from biochemical studies that a solution to the puzzle could be that Arabidopsis SDH7 corresponds to the C-terminus of SDH3 from *E. coli*, and that Arabidopsis SDH6 corresponds to the N-terminus of SDH4 from *E. coli* (Schikowsky *et al.*, 2017) (Figure 1b).

A deeper analysis of the eclectic pattern of presence and absence of these additional subunits across eukaryotes show that SDH5 and SDH6 are indeed not plant-specific subunits of complex II, as originally thought (Eubel *et al.*, 2003; Millar *et al.*, 2004), but are also present in distantly related lineages, such as red algae, glaucophytes and cryptophytes (Schikowsky *et al.*, 2017) (Figure 2), indicating that they may be ancient components of complex II or another enzyme. In contrast, SDH7 is found in charophytes, basal plants, monocots and dicots (Figure 2). The occurrence of SDH6 (suggested to represent the N-terminal part of *E. coli* SDH4 absent in plant SDH4; see above) preceded the occurrence of SDH7 (suggested to resemble the C-terminus of *E. coli* SDH3 absent in some plants).

Interestingly, genes encoding SDH5, SDH6 and SDH7 are absent in Chlorophyta (Schikowsky *et al.*, 2017), although a SDH5 homolog was found in the prasinophyte *Prasinoderma* (Figure 2), from the MMETSP transcriptome project (Keeling *et al.*, 2014). To exclude the possibility that *SDH5*–*SDH7* have been overlooked as a result of low sequence similarity in chlorophytes, complex II from *Polytomella* spp. (close relatives of *Chlamydomonas*) has been biochemically dissected by Blue Native (BN)/sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), confirming a classical four-subunit composition (Schikowsky *et al.*, 2017). Genes encoding SDH6 have been found in the genomes of some cryptophytes, glaucophytes and rhodophytes, although SDH6 homologs have not been validated as components of complex II outside of plants. Unlike in plants, however, SDH3 in these lineages is not truncated. This implies that SDH6 and full-length SDH3 can co-exist for over a billion years, suggesting that SDH6 may do more than substitute for lost helical regions of SDH3. Current models of plant complex II may have to wait for extended genome sequence information on the various clades of Plantae.

The SDH8 subunit (4.9 kDa) could not be assigned to a complex II subcomplex by Schikowsky *et al.* (2017). It is the smallest known subunit of any of the complexes in the ETC and has been biochemically described in Arabidopsis only (Millar *et al.*, 2004). Homologs of SDH8 have been identified in the genomes of Brassicaceae and monocotyledoneous plants (Schikowsky *et al.*, 2017); putative homologs can also be found in *Marchantia* and *Physcomitrella* via profile-based queries (Figure 2). No putative role for SDH8 is currently proposed and its short sequence will hinder identification in incomplete genome sequencing efforts across Plantae.

COMPLEX II ASSEMBLY MACHINERY SDHAF1–SDHAF4

Considering the central role of SDH in both the mitochondrial TCA cycle and electron transport chain, understanding the biogenesis of SDH is of great interest. Based on studies undertaken in yeast, *Drosophila* and mammalian cells, the pathway of SDH biogenesis was recently reviewed and an assembly model was presented (Bezawork-Geleta *et al.*, 2017). To date, four assembly factors have been reported to play a role in assembling the mature SDH holo-complex. Here, these are named SDHAF1, SDHAF2, SDHAF3 and SDHAF4 to indicate their designation as assembly factors and to differentiate them from the varying number of SDH subunits in different organisms (Table 1).

The FAD cofactor plays a vital role in SDH catalysis and the insertion of FAD into SDH1 is an essential step in the maturation of this subunit. This process depends on the maintenance of FAD levels in the mitochondrial matrix, and therefore synthesis and stabilization of free FAD plays an important role in SDH biogenesis (Kim and Winge, 2013). In yeast, riboflavin kinase (Fmn1), FAD synthetase (Fad1) and a putative mitochondrial transporter protein (Flx1) are involved in FAD biosynthesis and delivery to mitochondria (Santos *et al.*, 2000). So far it is not clear how FAD levels in plant mitochondria are regulated. Unlike some bacterial SDH1 orthologs, eukaryotic SDH1 does not self-flavinate, indicating that an assembly factor is required to insert FAD into SDH1 (Robinson and Lemire, 1996; Kounosu, 2014). This hypothesis was validated by the discovery of yeast Sdh5, later renamed SDHAF2 (Hao *et al.*, 2009). SDHAF2 directly interacts with SDH1 and is required for FAD insertion while at the same time SDH1 protein is needed for SDHAF2 stabilization (Hao *et al.*, 2009). The knock-out of *SDH2* in yeast led to an increase in SDHAF2 levels, probably as a result of the accumulation of free SDH1, showing that SDHAF2 acts at an early stage of SDH assembly (Hao *et al.*, 2009; Kim *et al.*, 2012). Homologs of SDHAF2 are also found in diverse proteobacteria (named SDHE), and are required for the flavinylation of bacterial SDH1 (McNeil *et al.*, 2012). Recent analysis of the structure of SdhE in *E. coli* (equivalent to SDHAF2) in complex with

SdhA (equivalent to SDH1) showed that SdhE makes a direct interaction with the FAD-linked residue His45 in SdhA, and maintains the capping domain of SdhA in an 'open' conformation (Maher *et al.*, 2018). The FAD-linked His residue in SDH1/SDHA is conserved across species (Maher *et al.*, 2018), indicating similar mechanisms of interaction of SDHAF2 with SDH1. An *SDHAF2* ortholog gene in Arabidopsis was identified and characterized, showing it to be an SDH assembly factor in plants (Huang *et al.*, 2013) (Figure 3). The knock-out of *SDHAF2* in Arabidopsis was lethal, indicating that it has an essential role for plant development (Huang *et al.*, 2013). *SDHAF2* knockdown lines (*sdhaf2*) showed a short root phenotype as well as lower SDH activity (Huang *et al.*, 2013). The level of FAD-bound SDH1 protein in *sdhaf2* was reduced, indicating its function in promoting the incorporation of FAD into this subunit (Huang *et al.*, 2013) (Figure 3). Arabidopsis SDHAF2 has only 30% and 32% identity with human and yeast SDHAF2, respectively, over a region corresponding to positions 69–165 in Arabidopsis (Huang *et al.*, 2013). Phylogenetic analysis of SDHAF2-like proteins separates plant-lineage proteins from those of animals and fungi (Figure S1), although known species relationships within plants are not recovered precisely. In human SDHAF2, the glycine at position 78 (G78R) is essential for the SDHAF2–SDH1 interaction and the accumulation of flavinylated SDH1 (Hao *et al.*, 2009), and this glycine residue is conserved across all species (Huang and Millar, 2013a). Mutation of G78R in *Serratia* SdhE also inhibited the accumulation of flavinylated SDH1 (McNeil *et al.*, 2012).

Following FAD insertion into SDH1, assembly with SDH2 is needed as it is SDH2 that then binds to the membrane subunits of SDH. The assembly factor SDHAF4 was first identified in yeast and was reported to interact specifically with flavinated SDH1 to promote its assembly with SDH2 (Van Vranken *et al.*, 2014). In yeast, the deletion of *SDHAF4* decreased SDH2 abundance but did not affect SDH1 abundance. Loss of *SDHAF4* in *Drosophila* showed destabilization of SDH1 as well as SDH2, indicating that SDHAF4 is required for SDH1 stabilization, at least in *Drosophila* (Van Vranken *et al.*, 2014). The SDHAF4 ortholog in Arabidopsis (Table 1) was recently characterized and shown to be required for SDH1 assembly to SDH2 (Belt *et al.*, 2018) (Figure 3). Free SDH1 accumulated as soluble protein in the

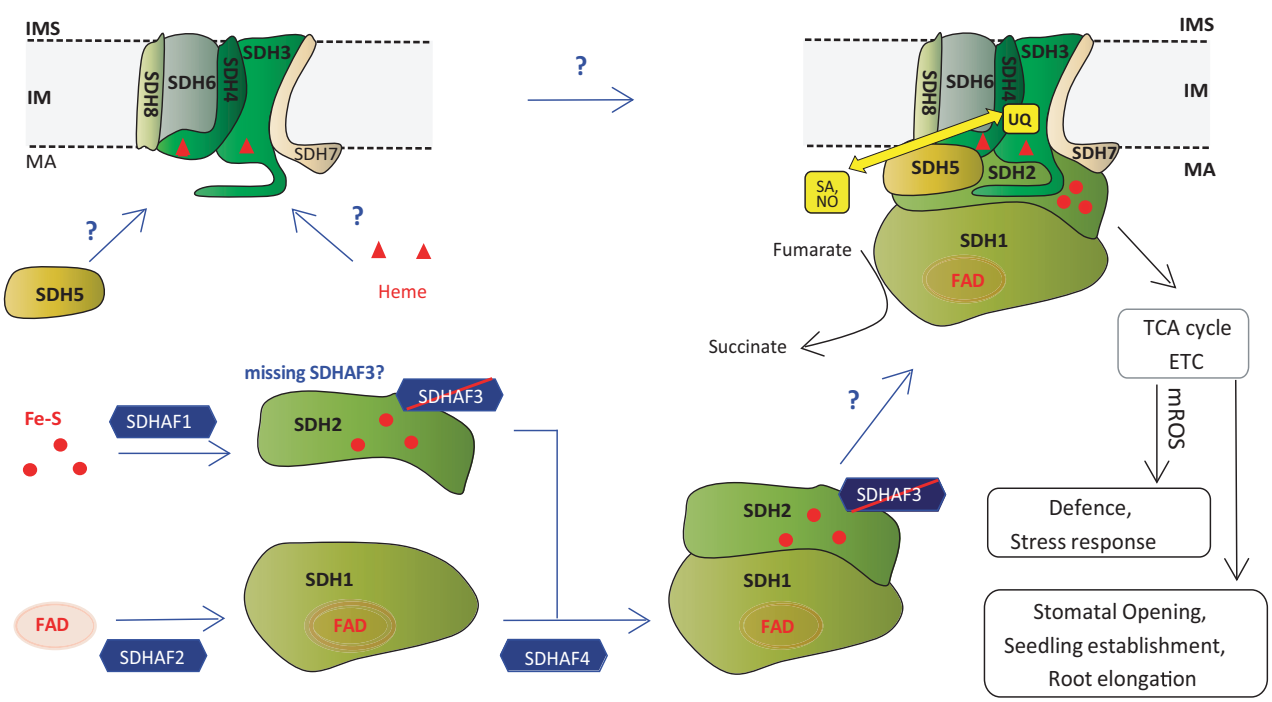

Figure 3. Schematic diagram for step-wise biogenesis of plant complex II assisted by assembly factors and its physiological roles. Flavination of SDH1 requires the assembly factor SDHAF2. After flavination, SDHAF4 binds to SDH1-FAD and assists further insertion of SDH1 into SDH2. Plants have an SDHAF1-like protein that was first discovered in mammals and yeast, but its proposed function in the insertion of Fe–S clusters into SDH2 requires experimental evidence to validate this hypothesis. In mammals and yeast, SDHAF3 facilitates the formation of the SDH1–SDH2 subcomplex to allow the full assembly of complex II. Higher plants do not have an SDHAF3-like protein based on analysis of plant genomes, however. The process of the assembly of hemes into the membrane-anchored SDH3 and SDH4 subcomplex is unknown in any organism to date. The process of assembly of transmembrane SDH6, 7 and 8 subunits and SDH5 subunit to form a holoenzyme with the classical four subunits of SDH is unknown. Signaling molecules such as salicyclic acid (SA) and nitric oxide (NO) interact with the UQ binding sites of SDH for downstream signaling transduction in plants, linking SDH function with plant responses to the environment. Mutants studies show plant mitochondrial complex II plays roles in the regulation of defense, stress responses, early seedling establishment and root elongation by modifications of its subunits or assembly factors. Abbreviations: IM, inner membrane; IMS, intermembrane space; MA, matrix.

isolated mitochondria from *sdhaf4*, indicating that SDH1 is a stable protein without further assembly assisted by SDHAF4 in Arabidopsis (Belt *et al.*, 2018). SDHAF2 levels were increased in *sdhaf4* whereas SDH1 levels remained the same (Belt *et al.*, 2018). This observation was similar to the results obtained from *SDH2*-deficient mutants in yeast (Hao *et al.*, 2009; Kim *et al.*, 2012), demonstrating that SDHAF2 is likely to promote the stabilization of SDH1 in Arabidopsis. Arabidopsis SDHAF4 has only 28% identity with human and yeast SDHAF4 in a conserved region of approximately 25 amino acids located near the C-terminus (Belt *et al.*, 2018); this region corresponds to DUF1674, which is conserved in bacteria. Phylogenetic analysis of SDHAF4-like proteins fails to recover a monophyletic plant group, probably because of low sequence conservation (Figure S1). Potential homologs of SDHAF4, containing a DUF1674 domain, are also present in some proteobacteria, but this has not been previously reported (Figure 2).

SDH2 contains the Fe–S clusters of SDH and insertion of these Fe–S clusters also requires assistance from assembly factors. Studies in yeast and genetic mutations occurring in families suffering from infantile leukoencephalopathy led to the identification of SDHAF1 and SDHAF3 (Ghezzi *et al.*, 2009; Na *et al.*, 2014). SDHAF1 contains an LYR protein domain that is a signature for Fe–S interacting proteins (Ghezzi *et al.*, 2009). The eukaryote-specific LYR-domain protein superfamily are typically mitochondrial (Na *et al.*, 2014; Angerer, 2015). Mutation of the yeast homolog SDHAF1 as well as the expression of variants corresponding to human mutants result in a reduction in SDH activity and the failure of OXPHOS-dependent growth (Ghezzi *et al.*, 2009). Mutations in *SDHAF1* affect its interaction with SDH2, resulting in an altered biogenesis of the SDH holo-enzyme (Bezawork-Geleta *et al.*, 2014; Na *et al.*, 2014; Maio *et al.*, 2016) (Figure 3). Detailed analysis revealed that SDHAF1 transiently binds to aromatic peptides of SDH2 through an arginine-rich region in its C-terminus and specifically engages an Fe–S donor complex, consisting of the scaffold, holo-ISCU and the co-chaperone–chaperone pair, HSC20–HSPA9, through an LYR motif near its N-terminal domain (Maio *et al.*, 2016). An Arabidopsis *SDHAF1* ortholog has been identified (Table 1) and is predicted to be located in mitochondria (http://suba.live). It is unclear, however, whether AtSDHAF1 is part of the SDH assembly pathway in plants as no data are yet available about the function of this gene (Figure 3).

SDHAF1 and SDHAF3 have been proposed to act together to promote SDH2 maturation by binding to an SDH1/SDH2 intermediate in order to protect it from oxidants (Ghezzi *et al.*, 2009; Na *et al.*, 2014; Maio *et al.*, 2016). SDHAF3 assists the binding of SDHAF1 to SDH2, which promotes the transfer and incorporation of Fe–S clusters into SDH2 (Na *et al.*, 2014). Once the SDH1/SDH2 intermediate is formed, the membrane-bound subunits SDH3 and SDH4 anchor these two subunits to the inner membrane. However, the number of assembly factors that might be involved in SDH3/SDH4 membrane anchoring remains unknown. Chaperones or assembly factors for the maturation of SDH3 and SDH4 as well as the incorporation and function of the heme group has not yet been identified in any organism (Figure 3). The deletion of either *SDH1* or *SDH2* causes almost a complete loss of SDH3 and SDH4 in yeast (Kim *et al.*, 2012; Na *et al.*, 2014). Therefore, the assembly or stability of the SDH3/SDH4 membrane anchor domain is likely to be somehow connected to the assembly of SDH1/SDH2.

SDHAF1 was identified in human and yeast (Ghezzi *et al.*, 2009), and Arabidopsis At2g39725 is likely to be an SDHAF1 ortholog, with 39% sequence identity near the N-terminus. SDHAF1-like proteins are found across eukaryotes, and phylogenetic analysis separated plant homologs from other eukaryotes (Figure S1). SDHAF3 assists the binding of SDHAF1 to SDH2, which promotes the transfer and incorporation of Fe–S clusters into SDH2 (Na *et al.*, 2014). Yeast SDHAF3 (Q04401) contains an LYR motif, but human SDHAF3 (Q9NRP4) contains an LYK motif instead; LYR motifs aren't strictly conserved for SDHAF1 either, with variants including FHR in the fission yeast *Schizosaccharomyces*, and LHR in *Marchantia*. Using diverse SDHAF3 sequences as BLASTP queries, we were unable to identify putative charophyte or plant homologs in the National Center for Biotechnology Information (NCBI) sequence database, although homologs from chlorophyte algae were readily found (Figure 2). Similarly, profile-based queries with HMMER3 (Mistry *et al.*, 2013) did not reveal candidate SDHAF3 homologs in charophytes or plants (Figure 2).

Although no SDHAF3 ortholog gene is readily identifiable in plants (Figure 2; Table 1), it is difficult to conclude definitively that SDHAF3 has been lost in the plant lineage. It may be that plant SDHAF3 has, for unknown reasons, undergone extensive sequence divergence since charophytes and plants shared a common ancestor with chlorophytes. If this is the case, it will be important to characterize LYR motif proteins of unknown function that are localized to mitochondria and phylogenetically restricted to plants. Alternatively, if SDHAF3 has indeed been lost, it will be important to understand how plant complex II copes without an otherwise conserved assembly factor. Intriguingly, the presence of SDH7 correlates well with the absence of SDHAF3 (Figure 2). Is it possible that the acquisition of this novel plant-specific subunit has somehow made SDHAF3 surplus to requirements? Obviously, further experiments are required to distinguish between these scenarios. Alternatively, maturation of SDH2 in plants might be regulated differently than in yeast or mammalian systems and this possibility needs to be investigated.

The assembly process of subunits SDH5, SDH6, SDH7 and SDH8 into plant complex II is currently unknown. It has been speculated that SDH5 is located at the interface of the succinate dehydrogenase domain (SDH1 and SDH2) and the membrane domain (composed of SDH3, SDH4, SDH6, and SDH7 in plants) (Figures 1 and 3). SDH5 might be necessary for the assembly of the two domains but is not strictly an assembly factor because it is present in the holo-complex II. The only clue we currently have to this process is in data from complexome profiling (Senkler *et al.*, 2017), which show an association of SDH1 and SDH5 (Figure S2).

COMPLEX II PRODUCTION OF, AND RESPONSE TO, SIGNALING MOLECULES IN PLANTS

The production of mitochondrial ROS (mtROS) in plants can influence redox signaling, retrograde signaling, plant hormone action, programmed cell death and defense against pathogens (Huang *et al.*, 2016). SDH is a significant source for mtROS production in both mammalian and plant systems (Gleason *et al.*, 2011; Quinlan *et al.*, 2012; Jardim-Messeder *et al.*, 2015). In mammals, SDH can generate superoxide (O_2^-) or hydrogen peroxide (H_2O_2) at high rates, exceeding the maximum rates of complexes I and III, when complexes I and III are inhibited and the succinate concentration is low (Quinlan *et al.*, 2012). The highest rates of ROS production have been observed at low succinate concentration and a so called 'ping-pong' mechanism has been suggested in which ROS is only generated where dicarboxylate-free reduced enzyme interacts with oxygen (Grivennikova *et al.*, 2017). ROS generated by SDH has been shown to originate from both the forward reaction, when electrons are provided by succinate oxidation, as well as the reverse reaction, when electrons are supplied from UQH2 (Quinlan *et al.*, 2012). A highly reduced UQH2 pool and a large proton-motive force together drive electrons backwards through complex I (reverse electron transport) and lead to a dramatic increase in ROS production (Murphy, 2009). SDH influences reperfusion injury in mammals through mtROS production during reverse electron transport via complex I after succinate accumulation under anoxia (Chouchani *et al.*, 2014). It is not clear whether plants can produce mtROS via reverse electron transport or not, but the high accumulation and then oxidation of succinate during transient periods of waterlogging, flooding or anoxia in plants (Shingaki-Wells *et al.*, 2011; Huang *et al.*, 2018) is similar to the scenario of reperfusion in mammals. Very recently, succinate-dependent SDH initiation of mtROS has been shown to selectively drive thermogenic respiration in brown adipose tissue, independently of cyclic AMP–protein kinase A signaling (Mills *et al.*, 2018).

Studies in *A. thaliana* and *Oryza sativa* have demonstrated that SDH is a direct source of mtROS and that the induction of ROS production by specific SDH inhibitors can impair plant growth (Jardim-Messeder *et al.*, 2015). This effect was accompanied by the downregulation of cell cycle genes and the upregulation of stress-related genes, indicating a possible role of SDH in both plant development and stress responses (Jardim-Messeder *et al.*, 2015). Mitochondrial complex II derived superoxide has also been shown to be the primary source of mercury toxicity in the root tip of *Hordeum vulgare* (barley). Mercury inhibited SDH activity in barley root tips, in a similar manner to non-competitive SDH inhibitors, and caused a rapid increase in superoxide generation *in vivo* and *in vitro* (Tamás and Zelinová, 2017). *In vitro*, the mercury-induced superoxide production was inhibited by competitive inhibitors of SDH (Tamás and Zelinová, 2017).

Analysis of the Arabidopsis SDH1 mutants *dsr1* and *sdhaf2* showed that SDH is involved in a ROS-induced stress signaling pathway that is likely to be triggered by salicylic acid (SA) (Gleason *et al.*, 2011; Belt *et al.*, 2017). This stress signaling pathway could be partially rescued in *sdhaf2*, but not in *dsr1*, by supplementing plant growth media with succinate (Belt *et al.*, 2017). Kinetic characterization showed that low concentrations (10–50 μM) of either SA or UQ binding site inhibitors (TTFA or carboxin) increased SDH activity and induced mitochondrial H_2O_2 production (Belt *et al.*, 2017). The effect of stimulation of SDH activity by low SA concentrations acting at the UQ binding site (potentially at Qp site with high affinity to TTFA) could lead to reactions between electron and oxygen to form ROS. Both *dsr1* and *sdhaf2* showed lower rates of SA-dependent H_2O_2 production *in vitro*, in line with their low SA-dependent stress signaling responses *in vivo* (Belt *et al.*, 2017). These data provided quantitative and kinetic evidence that SA acts at or near the ubiquinone binding site of SDH (Figure 3) to stimulate activity and contribute to plant stress signaling by increased rates of mitochondrial H_2O_2 production, leading to part of the SA-dependent transcriptional response in plant cells. The SA concentration-dependent transcriptional response was also observed in suspension cells of *Nicotiana tabacum* (tobacco). SA at high concentration (3 mM) failed to induce the expression of *Aox1a* and led to cell death, but SA at low concentration (100 μM) induced *Aox1a* and reduced cell death (Cvetkovska and Vanlerberghe, 2012). In tobacco leaf, an incompatible plant–bacteria interaction that produced high SA levels and the hypersensitive response was associated with low levels of AOX, whereas an incompatible interaction that produced only low SA levels with defense induction, but no hypersensitive response, was associated with high levels of AOX (Cvetkovska and Vanlerberghe, 2012). The coordination of the expression of AOX and MonSOD to avoid and scavenge ROS production was proposed to determine cell fate, but it is also possible that complex II plays a role in ROS production in an SA-concentration-dependent manner.

There are contradictions in reports of the sites of autoxidation responsible for ROS production by SDH. Some studies point to the FAD site within SDH (Imlay, 1995; Messner and Imlay, 1999, 2002), whereas others indicate ubisemiquinone and Fe–S centers to be the responsible sites (Guo and Lemire, 2003; Liang and Patel, 2004; Huang and Lemire, 2009). ROS can theoretically be generated by solvent-accessible FAD, which may oxidize substrates and be auto-oxidized by O_2, forming superoxide (Messner and Imlay, 2002; Guzy *et al.*, 2008). How ROS production from SDH might interact with the assembly process is an interesting line of enquiry. Studies in mammalian cells showed that the knock-down of *SDH2* but not *SDH1* caused an increase in ROS production (Ishii *et al.*, 1998; Guzy *et al.*, 2008). Subsequently, SDHAF4 has been reported to prevent the production of ROS during assembly of SDH1 to SDH2 in *Drosophila* and the high abundance of free flavinated SDH1 in *sdhaf4* might therefore be expected to have toxic effects (Van Vranken *et al.*, 2014). In Arabidopsis, however, the measurement of SDH activity as well as ROS production in soluble fractions from *sdhaf4* mitochondria showed no increase, despite the accumulation of soluble flavinated SDH1 (Belt *et al.*, 2018). Assembly of SDH1 and its unassembled catalytic capability thus appear to be an unlikely source of mtROS in plants. In Arabidopsis, it is probable that Fe–S clusters or the UQ site form the reactive site or a combination of FAD and Fe–S centers (Belt *et al.*, 2018). Aligned with this is evidence that complex II in plants is not only a site for mtROS production but also a target site for reactive nitrogen species. Nitric oxide (NO) inhibits succinate-dependent O_2 consumption in the presence of ADP but not in the absence of ADP in *Solanum tuberosum* (potato) tuber mitochondria (Simonin and Galina, 2013). In an FAD reduction assay, NO blocks electron flow through SDH in much the same way as the SDH non-competitive inhibitor TTFA (Simonin and Galina, 2013). It is suggested that NO inhibits SDH at its UQ site or at its Fe–S centers (Simonin and Galina, 2013).

PHYSIOLOGICAL ROLES OF SDH IN PLANTS

Mutations of SDH subunits and assembly factors in plants or the use of SDH inhibitors have been reported to affect plant development, to change rates of respiration or photosynthesis and the accumulation of succinate, and to altere mitochondrial ROS production rates and stress responses (Leon *et al.*, 2007; Fuentes *et al.*, 2011; Gleason *et al.*, 2011; Huang *et al.*, 2013). By combining these observations we can build a more detailed understanding of the impact of SDH on plant function (Figure 3).

Mutants affecting SDH1 or its assembly show the widest range of effects on plant function. SDH1 is encoded by two genes in Arabidopsis (Table 1). *SDH1-2* is only expressed at very low levels and the knock-out of this gene does not show any effect on growth or development of Arabidopsis (Leon *et al.*, 2007). The knock-out of *SDH1-1*, on the other hand, is embryo lethal (Leon *et al.*, 2007), as is the knock-out of *SDHAF2* (Huang *et al.*, 2013); however, studies on knock-down lines for both SDH1-1 and SDHAF2 have provided an array of phenotypes. The knock-down of *SDH1-1* showed pollen abortion and reduced seed set (Leon *et al.*, 2007). Heterozygous *SDH1-1/sdh1-1* plants showed low SDH activity but increased photosynthesis and improved growth in nitrogen-limiting conditions through alterations in their stomata conductance (Fuentes *et al.*, 2011). It is speculated that the lower SDH1 levels in these mutants cause the changes in stomatal function and photosynthesis performance. An *SDH1-1* point mutation line (*dsr1*) reduced SDH activity, interrupted the SA-dependent stress signal response and demonstrated a higher susceptibility to specific bacterial (*Pseudomonas syringae* Pst DC3000) and fungal (*Alternaria brassicicola* and *Rhizoctonia solani*) pathogens (Gleason *et al.*, 2011). Studies on the SDHAF2 knock-down line *sdhaf2* showed a specific decrease of root elongation but normal leaf development and no effect on photosynthetic rate or stomatal conductance (Huang *et al.*, 2013). Therefore, different strategies targeted with SDH1-1 caused phenotypic variations, even though there are common observations with the accumulation of succinate and the decrease of SDH enzymatic activity. One possible explanation for phenotypic variations is that disruptions of SDH1 at the different sites cause the change of SDH enzymatic kinetics. For example, *dsr1* (point mutation of SDH1-1) had a higher K_m for succinate but a lower SDH catalytic efficiency when compared with the *sdhaf2* line (i.e. the knock-down of SDHAF2 with reduced SDH1-1 level) (Belt *et al.*, 2017). Another possible explanation is that disruptions of SDH1 at different sites may induce differential downstream signaling patterns, such as ROS production intensity, related to the growth/development or stress response of the different tissues.

SDH2, the Fe–S subunit, is encoded by three genes in Arabidopsis named *SDH2-1* (At3g27380), *SDH2-2* (At5g40650) and *SDH2-3* (At5g65165) (Table 1). In Arabidopsis, *SDH2-1* and *SDH2-2* show distinct cell-specific expression patterns; in fact, only *SDH2-2* is expressed in root tips at high levels (Elorza *et al.*, 2004). Knock-out of *SDH2-1* does not result in any phenotype. *SDH2-3* is specifically expressed in the embryo during seed development (Elorza *et al.*, 2006), and its disruption alone was shown to cause delayed seed germination (Roschzttardtz *et al.*, 2009). Recent analysis indicated that *SDH2-3* expression in seed is regulated by ABA, with the promoter region containing three ABA-responsive elements (Restovic *et al.*, 2017). In *Solanum lycopersicum* (tomato), RNA interference lines of *SDH2-2* showed increased rates of photosynthesis and growth caused by their higher stomatal aperture (Araujo *et al.*, 2011), similar to the reported *SDH1-1*/*sdh1-1* plants mentioned above (Fuentes *et al.*, 2011). To

date it is not clear how and if SDH is directly involved in stomata regulation; nevertheless, a model exists suggesting that SDH may be involved by altering malate and fumarate levels (Araujo *et al.*, 2011).

The SDH inhibitors are also important sources of information on the role of SDH in plants. Partial inhibition of SDH by the competitive inhibitor malonate or non-competitive inhibitor TTFA decrease shoot and root growth rate in Arabidopsis (Jardim-Messeder *et al.*, 2015). SDH inhibitors completely block hypocotyl elongation in the dark and seedling establishment in the light, indicating that complex II plays a role in the acquisition of photosynthetic competence and the transition from heterotrophy to autotrophy (Restovic *et al.*, 2017). SDH inhibitors are also widely used worldwide as fungicides to prevent the proliferation of molds in cereal crops. Commercial SDHIs target the UQ binding sites of fungal SDH. As a result of the sequence divergence of SDH3 and SDH4 (Figure 2) at the UQ site, this has been a valuable source of inhibitors that differentially target fungal metabolism, but it is expected that SDHIs also interact with plant SDH, albeit with lower affinity. The application of the SDHI bixafen has great efficacy against many cereal pathogens, but also improved yield formation by delaying senescence with increased transpiration rate in *Triticum aestivum* (wheat; Berdugo *et al.*, 2012, 2013). By contrast, foliar application of another SDHI, fluxapyroxad, was found to reduce the leaf transpiration rate, resulting in a higher water use efficiency in both glasshouse- and field-grown wheat (Smith *et al.*, 2013). Under dry field conditions, the application of benzovindiflupyr during pre-anthesis in wheat improved water-use efficiency and resulted in better seed setting and filling (Kuznetsov *et al.*, 2018). Although further research is required on the impact of SDHI fungicides on crop physiology and the kinetics of plant SDH, there are intriguing similarities between SDH knock-down phenotypes and the effect of some SDHI fungicides on plants.

FUTURE DIRECTIONS

Recent studies show that SDH is being revealed as an important regulator in mitochondrial and cellular metabolism as well as in the mitochondrial stress response and cellular signaling. But some key questions on complex II's assembly and functions remain unanswered (Box 2). In humans, a variety of SDH mutations could be linked to tumors and neurodegeneration diseases, and in plants SDH mutations have been linked to pathogen sensitivity. Further studies will be necessary in order to elucidate the following questions.

(i) What are the *in vivo* functions of SDH5 and SDH8 in plants? Analysis of knock-out/knock-down lines for *SDH5* and *SDH8* as well as tandem affinity purification could be useful to investigate functions and protein interactions with other enigmatic subunits of SDH.

Box 2 Key questions of plant complex II research.

- Does reverse electron transport in complex I for succinate-dependent reactive oxygen species (ROS) production occur in plants?
- How has the plant mitochondrial complex II evolved?
- What are the functions of the extra SDH subunits in plants?
- Do SDHAF1 and SDHAF3 assembly factors exist in plants?

(ii) Are SDH5- and SDH6-like proteins part of complex II in distantly related red algae, glaucophyte and cryptophtes? Experimental analysis using Blue Native gel separation of complexes in isolated mitochondria from those species will provide the evidence needed for the ancient components of complex II.

(iii) Is SDH3 and SDH4 assembled prior to or after the formation of the SDH1/SDH2 intermediate in plants? And which assembly factors are involved? In humans, SDH assembly factors are often identified by mutations occurring in patients suffering from a specific disease, but in plants it will be challenging to screen for a specific phenotype as SDH mutations affect a variety of plant functions.

(iv) Do functional orthologs of SDHAF1 and SDHAF3 exist in plants, if not, how is Fe–S insertion into SDH2 regulated in plants? A broad assessment of LYR domain proteins in plants may be needed to find functional equivalents of SDHAF1 and SDHAF3, as sequence orthologs for SDHAF3 have not been found in Streptophyta, basal plants or higher plants (Figure 2), and it is unclear whether the orthologs of SDHAF1 identified in higher plants are indeed SDH assembly factors. An *SDHAF1* mutant line in Arabidopsis would be the best starting place for further analysis.

(v) How does SA interact with SDH? Although evidence to date suggests that it is likely to bind at the UQ site, physical evidence of binding or protein interaction of SA with either SDH3 or SDH4 is needed to confirm this hypothesis. To identify SA binding proteins, SA antibodies together with mass spectrometry could be used on mitochondria extracts to identify those proteins. Alternatively, mitochondrial proteins could be separated by SA affinity chromatography (Chen and Klessig, 1991; Manohar *et al.*, 2014).

(vi) Why does SA increase SDH activity at the UQ site at low concentrations, but act as an inhibitor at higher concentrations? Assuming that SA is able to bind to a UQ site, it is unclear how this interaction would increase enzyme activity at low concentrations but inhibit enzyme activity at higher concentrations. Given the existence of two UQ sites and previous studies that showed different affinities for UQ binding (Sun *et al.*, 2005), it seems plausible that SA

might bind with a different affinity to these sites. This possible explanation for the increase in activity at low concentrations and the inhibitory effect at higher levels of SA could be tested experimentally.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Maximum-likelihood (ML) phylogenetic analyses of proteins from Figure 2. Proteins were aligned with MAFFT-LINSI (Katoh and Standley, 2013), and RAXML 8.2.11 (Stamatakis, 2014) was used to generate phylogenies using the PROTGAMMALGF model of evolution. Bootstrap support values derived from 100 replicates were mapped onto the highest likelihood ML tree generated from 100 independent heuristic searches: A, SDH1; B, SDH2; C, SDH3; D, SDH4; E, SDH5; F, SDH6; G, SDH7; H, SDHAF1; I, SDHAF2; J, SDHAF3; K, SDHAF4. Sequences from members of Streptophyta (Embryophyta and Charophyta) are highlighted, and only bootstrap values of ≥50 are shown.

Figure S2. Abundance profiles of complex II subunits of *Arabidopsis thaliana* along a one-dimensional Blue Native gel stripe.

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