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Succinate dehydrogenase: the complex roles of a simple enzyme

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Succinate dehydrogenase (SDH) oxidises succinate to fumarate as a component of the tricarboxylic acid cycle and ubiquinone to ubiquinol in the mitochondrial electron transport chain. Studies of SDH mutants have revealed far-reaching effects of altering succinate oxidation in plant cells. The plant SDH complex composition, structure and assembly are all beginning to be understood but the implications of the divergence across eukaryotes is still unclear. We propose an integration of the reported physiological roles of SDH in plants which influence photosynthesis, the function of stomata, root elongation and fungal defence. Future SDH research needed in plants should involve tissue-specific studies of mutants, analysis of the pathways induced by succinate-dependent reactive oxygen species generation and assessment of the impact of succinate accumulation on metabolism.

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Introduction

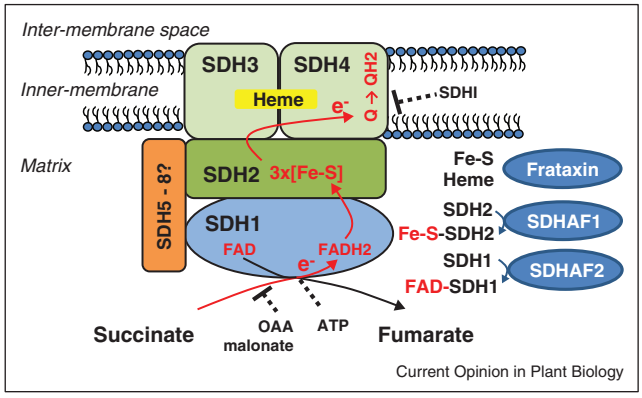
Succinate dehydrogenase (SDH EC 1.3.99.1 and complex II, EC 1.3.5.1) has a central role in mitochondrial metabolism as the only enzyme that is a component of both the TCA cycle and the electron transport chain. SDH catalyzes the oxidation of succinate to fumarate in the mitochondria matrix and transfers electrons to ubiquinone without pumping protons across the mitochondrial inner membrane. SDH is allosterically regulated by the binding of oxaloacetate and is activated by ATP in the process that does not involve phosphorylation ([1] and refs therein). Distinct diseases have been shown to be associated with mutations in different subunits of SDH in humans [2]. Recently, the reasons for these phenotypic differences in human SDH mutations are starting to be revealed by in-depth studies of the consequences of interrupting succinate oxidation at different points in the catalytic operation of SDH [3]. In plants, mutations effecting different SDH subunits differentially affect roots, leaves and plant responses to the environment [4••,5•,6••,7•]. In this review we attempt to integrate recent advances into the composition, assembly and physiological roles played by SDH in plants into a broader framework of the role of SDH in eukaryotes, and discuss key directions for exploring the roles of SDH in plants.

Composition and assembly of SDH in plants

The apparent diversity of SDH complex size and assembly among different organisms may underlie differences in the physiological function of SDH in these species. SDH in eukaryotes is classically comprised of four subunits (Figure 1), a flavoprotein (SDH1) that contains a bound FAD cofactor, an iron–sulfur (Fe–S) protein (SDH2) that contains three Fe–S clusters, and two small integral membrane proteins (SDH3 and SDH4) that bind heme to form the *b*-type cytochrome [8,9]. The amino acid sequences of SDH1 and SDH2 share 80% identity across eukaryotes in key regions needed for succinate, FAD and Fe–S binding, showing the tight structure/function relationship in succinate oxidation. However, somewhat surprisingly, the sequences of SDH3 and SDH4 subunits and the ubiquinone binding regions have greatly diverged in plants, fungi and mammals (Figure 2), perhaps in part due to a complex genetic history of their switching from being encoded in nuclear and mitochondrial genes [10,11].

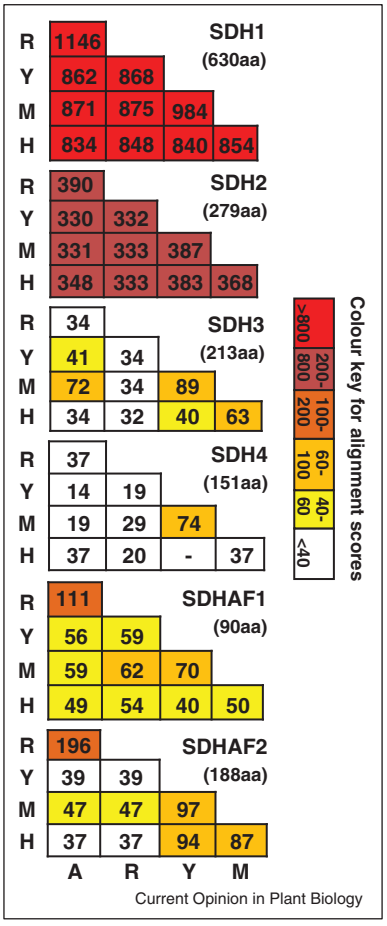
Despite much research on assembly of other complexes of the mitochondrial respiratory chain, very little was known until recently about the process of assembly of SDH. Two assembly factors for SDH, SDHAF1 and SDHAF2 (Figure 1), were recently reported in mammals as the cause of different human diseases [12,13]. SDHAF1 and SDHAF2 are proposed to function to insert Fe–S and FAD in SDH2 and SDH1, respectively [12,13]. An ortholog of human SDHAF2 was recently identified as an unknown function mitochondrial protein in the model plant Arabidopsis [5,14]. This plant SDHAF2 has low amino acid sequence similarity to human and yeast SDHAF2, except in a small conserved domain present in all SDHAF2 sequences (Figure 2). Knockdown plants for this Arabidopsis SDHAF2 had decreased SDH enzymatic activity and a lowered amount of flavinated SDH1 in isolated mitochondria [5•]. A putative Arabidopsis ortholog for human SDHAF1 exists, but also has low amino acid sequence similarity outside one key region of the protein (45% identity in a 40 AA window, Figure 2). This protein is predicted to be targeted to mitochondria but has not been experimentally identified in plant mitochondria to date [5•]. Other plant genomes have orthologs of the

Figure 1



SDH composition, assembly factors and inhibitors. The plant mitochondrial succinate dehydrogenase (SDH, complex II), like its mammalian counterpart, is composed of four subunits: two hydrophilic matrix facing proteins (SDH1 and SDH2) and two largely hydrophobic proteins (SDH3 and SDH4) anchoring the complex in the membrane. SDH1 is a flavoprotein containing a covalently bound FAD, SDH2 is an iron–sulfur (Fe–S) protein which contains three Fe–S clusters. SDH3 and 4 are two integral membrane proteins that binding heme to form the b-type cytochrome. In addition, the plant mitochondrial SDH complex contains four plant-specific hydrophilic subunits (SDH5–8) with unknown functions. Two SDH assembly factors, SDHAF1 and SDHAF2, are required for insertion of Fe–S and FAD into SDH2 and SDH1, respectively. Frataxin has a role in Fe–S and/or heme synthesis for SDH. During the oxidation of succinate to form fumarate, electrons (e^-) are passed through FAD, Fe–S centres and then reduce ubiquinone (Q) to ubiquinol (QH_2). The succinate pocket is acted upon by substrate-level inhibitors (such as the succinate-analogues: malonate, malate, oxaloacetate (OAA) and also by ATP that activates the enzyme by inhibitor removal. A different class of inhibitors (SDHI) bind in the ubiquinone pocket (*e.g.*, carboxin, which is used as a fungicide on crop plants).

Figure 2



Similarity of SDH subunits and assembly factors in selected eukaryotes. Protein sequences of SDH subunits and its assembly factors from the model plant *Arabidopsis thaliana* (A), the model crop plant *Oryza sativa*, rice (R), the model fungi *Saccharomyces cerevisiae*, yeast (Y), and human (H) were compared with BLASTP. The recently sequenced fungus *Mycosphaerella graminicola* (M) was included because it is a major disease of crop plants and succinate dehydrogenase inhibitors (SDHI) are widely used to control its spread. The numbers in boxes represent alignment bit scores between paired protein sequences from BLAST analysis. Amino acid length of *Arabidopsis* proteins is given in the upper right side of individual panel. Rice Os08g17650 and Os11g32480 are potential candidates for SDHAF1, SDHAF2 in this species, and *Arabidopsis* At2g39725 is a potential candidate for SDHAF1 based on sequence similarity but all three require further experimental evidence to definitively prove their function. The SDH1–4, SDHAF1 and SDHAF2 accession numbers used for sequence alignment are: for Arabidopsis, At5g66760, At3g27380, At5g09600, At2g46505, At2g39725, At5g51040; for Rice, Os07g04240, Os08g02640, Os02g02940, Os01g70980, Os08g17650, Os11g32480; for Yeast, Q00711, P21801, P33421, P37298, Q3E785, Q08230; for *Mycosphaerella graminicola*, XP_003857174.1, XP_003850753.1, XP_003850451.1, XP_003853609.1, XP_003857429.1, XP_003857639.1; and for human, P31040, P21912, Q6IAQ2, O14521, A6NFY7, NP_060311, respectively.

SDHAF2 and the putative SDHAF1 of Arabidopsis. Another Arabidopsis protein, frataxin, has been experimentally implicated in assembly of Fe–S in SDH in plants [15,16•]. However, there are three different Fe–S clusters in SDH2, and it is still not clear whether frataxin and SDHAF1 are responsible for independent insertion of different Fe–S clusters, or whether they work together in Fe–S insertion. To complicate matters further, frataxin's role is not specific to SDH, but has a broad role in assembly of Fe–S clusters for proteins in plant mitochondria [15,16•].

The four classical SDH1–4 subunits found in all eukaryotes form ~110 kDa native complexes. The plant SDH native complex appears to have a series of unknown function accessory subunits and a native mass of ~160 kDa (Figure 1). These subunits have been resolved by blue native (BN)-SDS-PAGE in a number of dicotyledonous plants and identified in Arabidopsis by peptide mass spectrometry [17,18]. Termed SDH5–8, these subunits have no known function and their sequences do not contain clear functional motifs, but together they may represent a secondary peripheral activity of the SDH complex in plants [18]. This could be analogous to the secondary functions already found for accessory and integral subunits of plant respiratory complexes I and III [19,20]. The genome of rice contains clear orthologs of SDH5, SDH6 and SDH7, but to date no SDH8 ortholog