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Huang, S., & Millar, H. (2013). Succinate dehydrogenase: the complex roles of a simple enzyme. Current Opinion in Plant Biology, 16(3), 344-349. DOI: 10.1016/j.pbi.2013.02.007

Published in:
Current Opinion in Plant Biology

DOI:
10.1016/j.pbi.2013.02.007

Document Version
Peer reviewed version

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Highlights

- The composition of plant SDH complex is different to those in other eukaryotes
- SDH mutation in plants changes organic acids, respiration and ROS generation
- SDH mutation alters photosynthesis, stomata, root elongation and fungal defence
- Tissue-specific phenotypes, ROS and succinate increases are key research topics

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Abstract

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.

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 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, 6, 7 and 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 complex I and III [19,20]. The genome of rice contains

clear orthologs of SDH5, SDH6 and SDH7, but to date no SDH8 ortholog has been defined in this any other plant species [1]. Interestingly, BN/SDS-PAGE separations of SDH from monocotyledonous plants, including rice and barley, typically observe a smaller SDH native complex than in Arabidopsis, potato and bean [1,17,21]. This may indicate a difference in the stability or composition of the larger plant SDH complex between plants. Recently, a native SDH complex in the monocot *Symplocarpus renifolius* was reported to be nearly 340 kDa in size. It contained a range of smaller molecular mass subunits [22] and is the largest native SDH complex reported to our knowledge .

The effect of SDH loss on plant phenotypes

A series of mutants in different subunits have been recently reported that show the impact of lowering or removing SDH function from plant cells. These reports have shown that differential changes in organic acid levels, altered respiration rates in specific tissues and changes in mitochondrial ROS generation characteristics are likely to be the key reasons for this diversity of SDH dysfunction phenotypes.

The flavoprotein SDH1 is the site of succinate binding, is the basis for the allosteric activation of SDH by ATP and houses the first cofactor of SDH - a flavin covalently attached to a histidine residue. There are two genes for SDH1 in Arabidopsis, *SDH1-1* (At5g66760) and *SDH1-2* (At2g18450). Knockout of *SDH1-2* did not affect the growth or development of Arabidopsis [23], *SDH1-2* was expressed at a very low transcript level and has not been definitively identified at the protein level. In contrast, knockout of S*DH1-1* led to failure of gameophyte development. Decrease of *SDH1-1* by RNA interference resulted in pollen abortion and a reduced seed set [23]. Heterozygous *SDH1-1/sdh1-1* plants were recently reported to have low SDH activity but improved photosynthesis as well as improved growth in nitrogen-limiting conditions due to altered stomatal conductance and nitrogen assimilation, respectively (**Figure 3, green boxes**) [7•]. The stomatal aperture and density in leaves were higher in both *SDH1-1/sdh1-1* plants and *sdh1-1* RNAi plants [7•]. Metabolite analysis revealed succinate was increased by 2-fold in these mutants, while some amino acids such as proline and glutamine were significantly lower in abundance [7•]. It has been speculated that metabolic alterations caused by less SDH1 could be the key basis for the significant changes in stomatal function,

photosynthesis and nitrogen assimilation in these mutants [7•] (**Figure 3**). In contrast, knockdown of Arabidopsis SDHAF2 (At5g51040) which is the assembly factor for SDH1, showed a specific inhibition of root growth, but normal leaf growth (**Figure 3, blue boxes**) [5•]. The low SDH activity in *sdhaf2*, did not affect photosynthetic rate or stomatal conductance [5•], which is very different from SDH1 deficient lines in Arabidopsis (**Figure 3**). The decrease of primary root elongation rate may be explained by the decreased rate of root tip respiration in these plants [5•]. Knockout of SDHAF2 in Arabidopsis lead to seed abortion, indicating its essential role early in seed development [5•] perhaps akin to the strong phenotype of SDH1-1 knockouts in Arabidopsis [23].

The iron-sulfur subunit (SDH2) of SDH in Arabidopsis is encoded by three genes, *SDH2-1* (At3g27380), *SDH2-2* (At5g40650) and *SDH2-3* (At5g65165). *SDH2-1* and *SDH2-2* in Arabidopsis have distinct cell specific expression patterns and promoter activities, in fact only the *SDH2-2* isoform is expressed in root tips at a high level [24]. The knockout of *SDH2-1* did not have any phenotype, indicating functional redundancy between *SDH2-1* and *SDH2-2* [24]. *SDH2-3* is specifically expressed in the embryo during seed development [25] and the disruption of *SDH2-3* alone delayed seed germination [26]. In tomato plants, RNA interference of *SDH2* increased the rate of net photosynthesis and growth (**Figure 3, green boxes**), mainly due to greater stomatal aperture in these lines [4••]. This observation is similar to that reported for the Arabidopsis heterozygous *SDH1-1/sdh1-1* plants mentioned above [7•]. The inhibition of the *SDH2-2* expression using a guard cell–specific MYB60 promoter had no effect on changes in stomatal aperture or rates of photosynthesis in the mutant, suggesting an unexpected role for SDH in mesophyll cells that controlled stomatal function [4••]. A model has been proposed of SDH regulation of stomatal aperture by alteration of malate and fumarate levels [4••].

The direction and extent of changes in organic acid abundances in SDH1 and SDH2 plant mutants vary widely between reports and the tissues studied (**Figure 3, white boxes**). Also, SDH is more abundant in non-green tissues, such as roots, while other respiratory complexes such as complex I are more abundant in green tissues, such as leaves [27]. This could indicate a potential difference in the metabolic requirements in leaves and roots for these two major protein complexes of

mitochondrial respiration. Complex I mutants are often small plants with equally reduced growth of both root and shoot [28-30], which clearly contrasts with the plant SDH mutants that have been reported with normal or enhanced leaf growth. In humans, the impact of organic acid changes following SDH inhibition has not been linked to the consequences of fumarate depletion, but to rather succinate accumulation and its impact as a competitive inhibitor of α-ketoglutarate-dependent enzymes [31]. These succinate-dependent inhibitions of other enzymes spread the impact of SDH inhibition from a respiration limitation to a wider impact on pseudohypoxia induction [32,33], sulphur metabolism and histone demethylation [31]. Details of how elevated succinate levels might inhibit plant cell functions are still unknown and require investigation.

Complexes I and III and the UQ cycle are generally considered as the main sites of reactive oxygen species (ROS) production in the eukaryotic respiratory chain [34], however, evidence is emerging that succinate-dependent H_2O_2 production by mitochondria is also very significant [35] and can be modulated independently of H_2O_2 production from other sites in the electron transport chain [36]. Structural analysis of SDH has proposed that SDH can produce ROS at the FAD- or UQ-binding sites though single electron reductions of O_2 (**Figure 3**) [37]. Recent *in vitro* experiments have proved this is true in both the forward and reverse reactions of the enzyme, i.e. as a succinate oxidase and as a fumarate reductase [38]. The mutation of SDH subunits that raise ROS formation from the complex have caused oxidative stress and ageing in nematodes [39] and caused cancers in humans [3]. In plants, a point mutation in *SDH1-1* (*dsr1*), at a conserved region in the substrate binding pocket, led to the significant reduction of SDH activity and paradoxically a significant lowering of mitochondrial ROS production (**Figure 3, yellow boxes**) [6••]. This mutation interrupted salicylic acid-mediated expression of specific stress responsive transcripts and defence genes in Arabidopsis (**Figure 3**), indicating the role of SDH-derived ROS in plant defense gene expression. These mitochondrial ROS-deficient plants exhibited increased susceptibility to specific bacterial pathogen (*P. syringae Pst DC3000*) and fungal (*A. brassicicola*, *R. solani*) (**Figure 3**) as a result [6••]. Coupling these discoveries with the recent deployment of SDH inhibitors as pesticides against major fungal pathogens of crops [40, 41•, 42] raised questions

about whether inhibitor actions across plant and fungi SDH may contribute to the success of these pesticides.

Collectively these observations are allowing us to build an integrated working model of how SDH dysfunction impacts plant function, and ipso facto, the wider roles of SDH function in plant growth and development (**Figure 3**). Some aspects of the four main effects observed in plants (**blue, yellow, green and white boxes**) have clear mammalian counterparts (**grey boxes**), while others still remain as plant-specific consequences of altered succinate oxidation.

Conclusions

Loss or mutation of SDH subunits in plants can result in the decrease of SDH activity and respiration rate, accumulation of succinate, changes to the abundance of other organic acids, and variations in the production of mitochondrial ROS. As a research community, we still don't understand how tissue specific effects in SDH mutants may be linked to the clear tissue specific expression patterns of SDH isoforms in plants or how these change SDH kinetics or function. Furthermore, investigations on how different plant SDH mutants influence ROS production and downstream signals may provide new clues to explain the observed differences that mitochondrial ROS play in cellular REDOX signal transduction and also in plant mutant phenotypes. Future analysis of the functional importance of the variation of SDH3 and SDH4 in plants, combined with SDH UQ binding inhibitor studies, will enhance our understanding of how these subunits diverge and also define any side-effects on plants that may be caused by the use of SDH inhibitors as crop fungicides (**Figure 1**). Clearly it seems that SDH, once considered a simple and well known enzyme, plays a complicated role in plants that is only now beginning to be understood.

Figure captions:

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, 6, 7 and 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 are 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, 2, 3, 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.

Figure 3. Mutations, effects and phenotypes of SDH loss in plants and animals. Dysfunction of SDH in plants, such as tomato *sdh2* antisense lines [4••], Arabidopsis *SDH1-1*/*sdh1-1* heterozygous lines [7•], *sdhaf2* [5•], and *dsr1* [6••], result in the reduction of SDH activities and the accumulation of succinate. However, these lines do not show the same phenotypes. Tomato *sdh2* antisense lines and Arabidopsis *sdh1-1* heterozygotes have higher organic acid contents, increased stomatal conductance and higher rates of photosynthesis (**Green box**). A knockdown line of *sdhaf2* had low root tip respiration and inhibited primary root elongation, without any impact on stomata or photosynthesis (**Blue box**). The *dsr1* mutant (carrying a point mutation of SDH1-1) had reduced mitochondrial reactive oxygen species (ROS) production and affected expression of nuclear genes (such as GSTs and HSPs), making plants susceptible to bacterial and fungi diseases (**Yellow box**). In humans, dysfunction of SDH either causes neurological and blood disorders linked to lowered respiration rate or tumorigenesis linked to elevated mitochondrial reactive oxygen species (ROS) formation [2,3] (**Grey box**). The accumulation of succinate rather than changes in the abundance of fumarate and malate have been linked to organic acid based impact of SDH inhibition in humans. Elevated succinate inhibits α-ketoglutarate-dependent enzymes and propyl hydroxylases then cause downstream cellular toxicity [31,32] (**Grey box**).

Acknowledgements:

The work of SH and AHM is funded by the Australian Research Council through the ARC Centre of Excellence in Plant Energy Biology (CE0561495). AHM was funded as an ARC Australian Future Fellow (FT110100242).

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• of special interest

•• of outstanding interest

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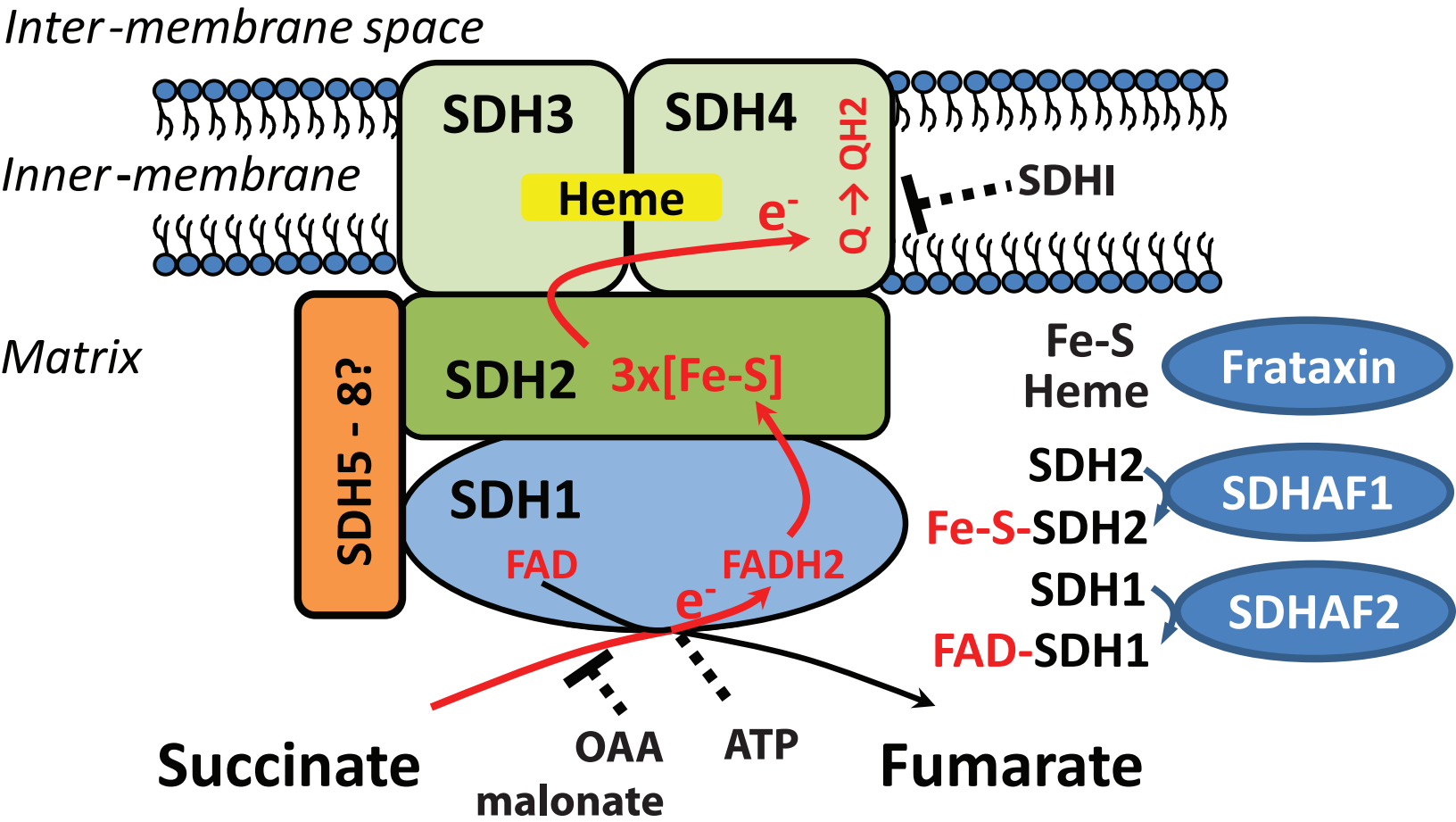

Figure 1

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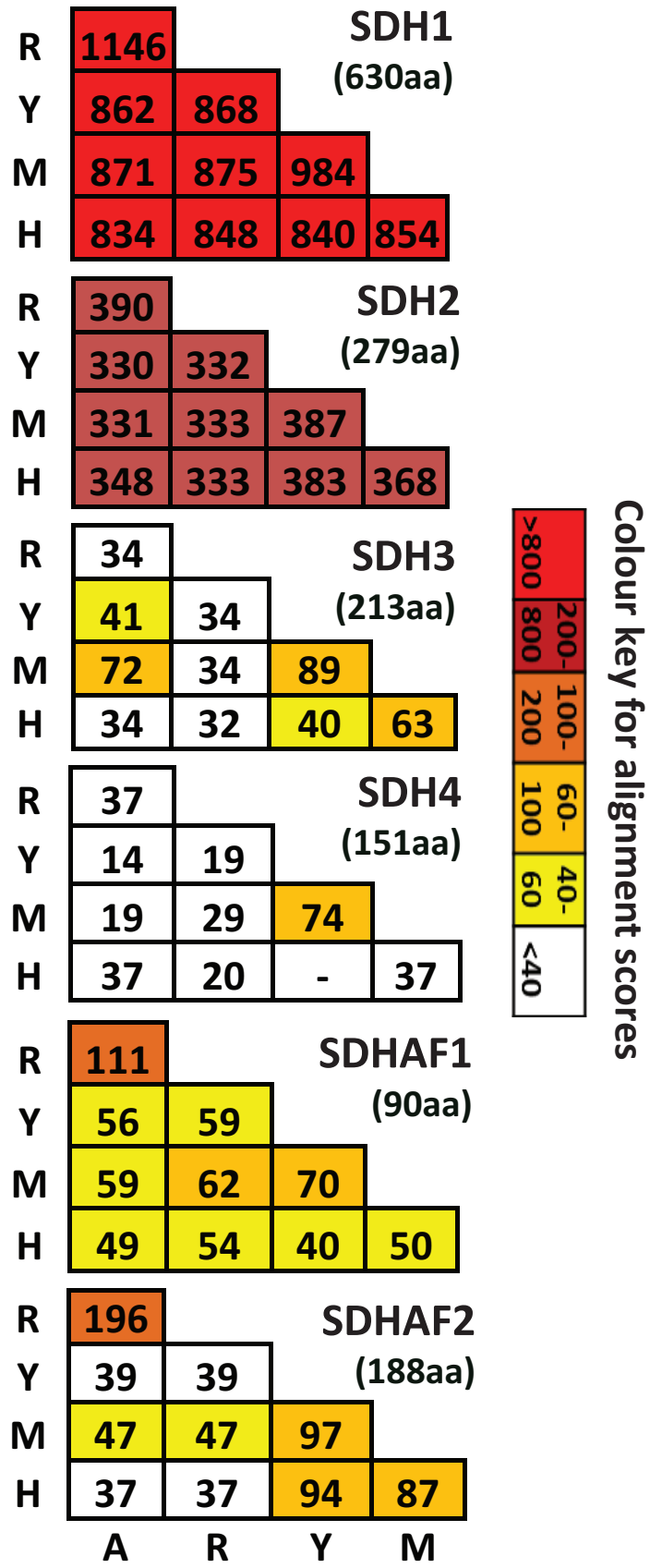

Figure 2

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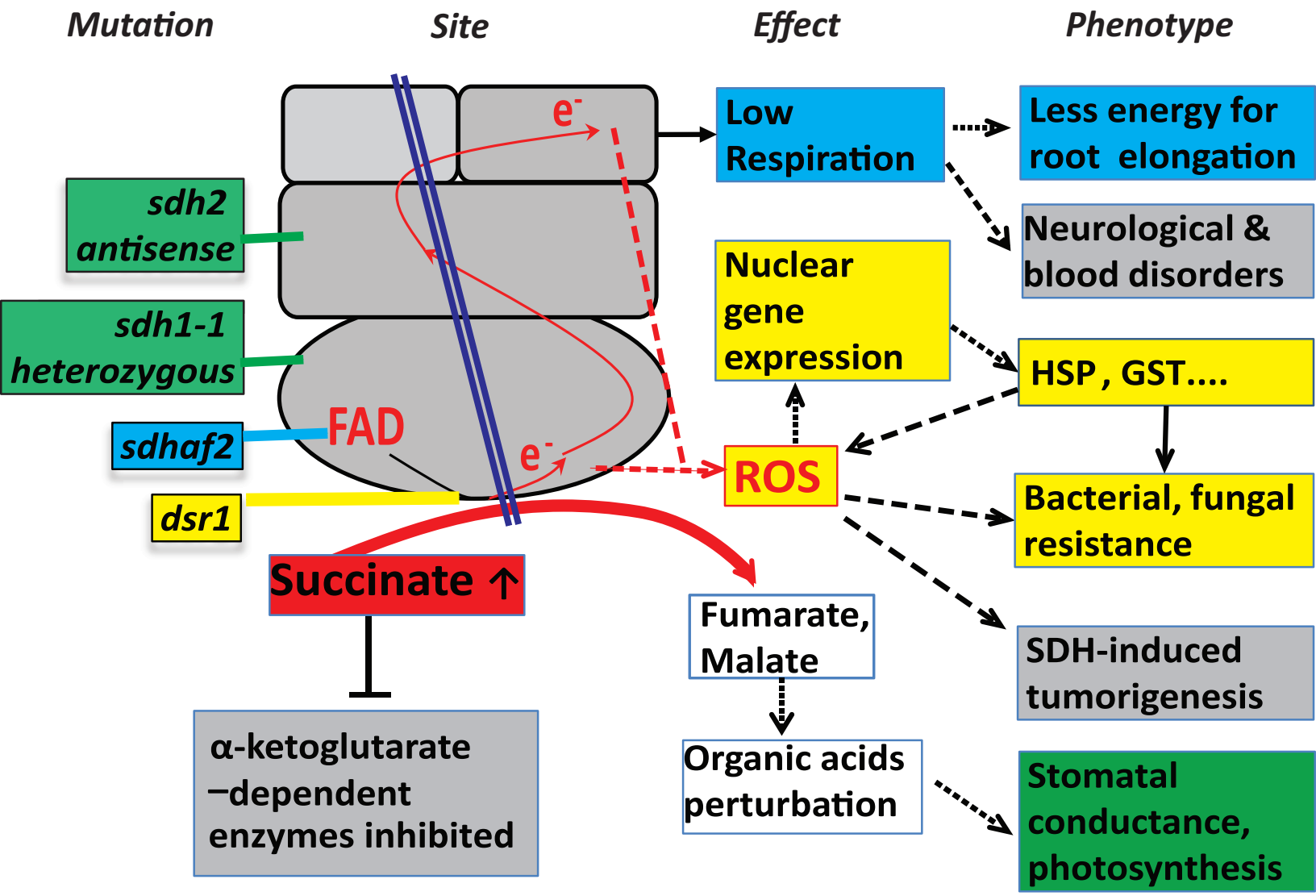

Figure 3