Vaishali Manikrao Patil · Dileep Kumar · Satya P. Gupta *Editors*

Deciphering The Role of Succinate Dehydrogenase in Drug Discovery

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Preface

In the evolving landscape of biochemistry and medical research, succinate dehydrogenase (SDH) has moved from a relatively niche area to become a focal point of inquiry. Once primarily recognized as a key enzyme within the citric acid cycle and the electron transport chain, SDH is now understood to play critical roles across a range of biological processes, with implications for human health, agriculture, and for the development of therapeutics. This book explores expanding role of SDH across various scientific domains, offering readers a comprehensive view of its functions, pathological relevance, and applications.

The journey begins in Chapter 1, which provides a structural and functional overview of SDH, highlighting its role as Complex II in the electron transport chain and its involvement in the citric acid cycle. Through this, SDH could facilitate crucial electron transfer steps via its subunits, namely, SDHA, SDHB, SDHC, and SDHD. These processes are essential for cellular energy production, and the chapter details how disruptions in SDH activity can lead to metabolic imbalances and disease. Recent research has shed light on SDH's assembly and regulation, showing it as an enzyme whose mutations can lead to various disorders.

In Chapter 2, SDH's involvement in cellular respiration is examined in greater depth, with a focus on how it influences hypoxic response and gene expression. Dysfunction in SDH, caused by either genetic mutations or environmental stressors, disrupts cellular energy balance, leading to conditions that range from benign metabolic disorders to severe pathologies.

Chapter 3 explores SDH's role in maintaining cellular homeostasis, emphasizing how it prevents oxidative stress and supports normal physiological function. Importance of proper SDH activity is essential for energy production. Any type of disruptions in this enzyme can lead to cellular damage and further impacts overall health.

The significance of SDH as a therapeutic target in neglected diseases is examined in Chapter 4. Enzymes that participate in energy metabolism, like SDH, are pivotal for parasite survival, making them attractive drug targets. This chapter explores the potential of SDH inhibitors for treating diseases such as trypanosomiasis, schistosomiasis, and leishmaniasis, which disproportionately affect low-income populations. Current research on SDH-targeted therapies highlights its potential as a treatment target in diseases often overlooked in mainstream drug development.

In Chapter 5, the focus shifts to mitochondrial disorders linked to SDH dysfunction. The chapter provides an analysis of how disruptions in SDH activity contribute to mitochondrial pathologies, which in turn affect cellular respiration and energy production. As mitochondrial diseases are often complex, this chapter delves into how SDH dysfunction interacts with other mitochondrial components, leading to multifactorial pathophysiological effects.

Chapter 6 examines SDH's influence on cardiovascular health, detailing how defects in SDH contribute to conditions like ischemia and Barth syndrome. Using animal models, the chapter investigates how inhibiting SDH during reperfusion can have protective effects on the heart by preventing production of reactive oxygen species and mitochondrial permeability transitions. SDH inhibitors like diazoxide and 3-nitropropionic acid are explored for their cardioprotective potential, alongside discussion on how SDH dysfunction can exacerbate ischemic damage, suggesting new therapeutic strategies for cardiovascular conditions.

In Chapter 7, SDH's connection to cancer is explored, where it functions as a metabolic checkpoint with implications for tumorigenesis. Beyond its enzymatic activity, SDH acts as a tumor suppressor, with its substrate succinate classified as an oncometabolite. SDH mutations lead to succinate accumulation, affecting processes like epigenetic regulation, angiogenesis, and cancer progression. SDH's role in cancer diagnosis is also discussed, presenting it as a biomarker for various cancers.

Chapter 8 focuses on neurodegenerative diseases, explaining how SDH dysfunction can impair mitochondrial function and contribute to conditions like Alzheimer's, Parkinson's, and Huntington's diseases. The chapter explores the enzyme's critical role in the brain's high-energy demand and its potential in neuroprotection, with SDH emerging as a regulator in managing neurodegenerative disease progression.

Chapter 9 further explores SDH's potential in treating neurodegenerative disorders. Given SDH's role in energy homeostasis, the chapter investigates how SDH dysfunction contributes to lipid synthesis abnormalities and excitotoxicity, worsening neurodegenerative conditions. The chapter underscores SDH's significance in developing neuroprotective therapies.

Chapter 10 examines the use of SDH inhibitors in agriculture, particularly as fungicides for crop protection. The chapter provides an overview of SDH inhibitors, their classification, and mechanism of action. These fungicides play a critical role in combating fungal diseases in crops. However, the chapter also addresses resistance in fungal pathogens, environmental concerns, and health risks from long-term SDH inhibitor use. Sustainable approaches are discussed to ensure the efficacy and safety of these fungicides in agriculture.

Chapter 11 highlights importance of SDH in oxidative phosphorylation and the tricarboxylic acid cycle, emphasizing its relevance in metabolic diseases. Known also as electron transport chain complex II, SDH has garnered attention due to its role in regulating mitochondrial metabolism. This chapter explores SDH's involvement in metabolic reprogramming and energy balance, as well as its effects on glucose-induced oxidative phosphorylation. The role of SDH as a potential generator of reactive oxygen species, especially in obesity and inflammatory bowel diseases, is also discussed, alongside its impact on immune system inflammation. By

examining SDH's biological and pathological functions, this chapter emphasizes the enzyme's importance in understanding metabolic disorders.

Throughout these chapters, this book offers a deep understanding of SDH's complex biology and its broad impact on human and environmental health. By bridging research across cellular biology, medicine, and agriculture, it highlights SDH's centrality in essential processes and its therapeutic potential. This volume will serve as a resource for scientists, clinicians, and researchers interested in unraveling the role of SDH across disease treatment, drug discovery, and agriculture. Through this exploration, we hope to inspire continued research into SDH and its roles in maintaining cellular health and addressing pressing health and environmental challenges.

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Satya P. Gupta is presently a Professor Emeritus at Meerut Institute of Engineering and Technology (MIET), Meerut, India, after retiring as Professor in the Department of Applied Sciences at National Institute of Technical Teachers' Training and Research (NITTTR), Bhopal, India. Earlier he served at Tata Institute of Fundamental Research (TIFR), Mumbai, Birla Institute of Technology and Science (BITS), Pilani, and then Meerut Institute of Engieering and Technology as its Director-cum Professor of Eminence in the Department of Pharmacy. Professor Gupta has a very long standing of teaching quantum chemistry, pharmaceutical chemistry, biophysics, and drug design. He had obtained his M.Sc. and D.Phil degrees from University of Allahabad, Allahabad, India, in 1967 and 1971, respectively. For his work in drug design, he has bagged several honors and awards to his credit, Professor Gupta has more than 200 research publications in highly reputed national and international journals and several reviews in prestigious periodicals as *Chemical Reviews* (American Chemical Society), *Progress in Drug Research* (Birkhäuser Verlag Basel, Switzerland), and *Current Medicinal Chemistry* (Bentham Science, Netherlands), and has been on editorial board of several international journals.

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Succinate Dehydrogenase: Structure, Function and Significance

1

Dileep Kumar, Aditya Ganeshpurkar, and Ankit Ganeshpurkar

Abstract

The chapter underscores succinate dehydrogenase (SDH) as a pivotal enzyme fundamental to cellular life, tracing its historical evolution and structural intricacies. Its central role in the Krebs cycle, efficiently converting succinate to fumarate, is pivotal for electron transport chain function and ATP production. Regulation of SDH activity is crucial for cellular health maintenance. Beyond its metabolic role, SDH dysfunction is linked to various diseases like hereditary paraganglioma–pheochromocytoma syndrome, leiomyomatosis and neurodegeneration, opening avenues for further investigation. Understanding how mutations or altered SDH activity contribute to these conditions can inform novel therapeutic approaches. In summary, SDH transcends its metabolic function, offering rich potential for future research and therapeutic development. Further exploration promises insights into healthcare pathways and a deeper comprehension of cellular dynamics. SDH's complexity and significance ensure ongoing intrigue and inspiration in scientific pursuits.

Keywords

Succinate dehydrogenase · Electron transport chain · ATP · Succinate · Ubiquonoe

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Abbreviations

AMS	Acute mountain sickness
ATP	Adenosine triphosphate
CoQ	Coenzyme Q
Cyt c	Cytochrome c
DCPIP	2,6-Ddichlorophenolindophenol
ETC	Electron transport chain
FAD	Flavin adenine dinucleotide
FADH2	Flavin adenine dinucleotide
GIST	Gastrointestinal stromal tumour
HLRCC	Hereditary leiomyomatosis and renal cell cancer
IMS	Intermembrane space
NADH	Nicotinamide adenine dinucleotide
NPA	3-Nitro propionic acid
SDH	Succinate dehydrogenase
TCA	Tricarboxylic acid

1.1 Introduction

Mitochondria, the powerhouse of cells, plays a crucial role in cellular metabolism, particularly in the 'tricarboxylic acid' (TCA) cycle, also known as the 'citric acid cycle or Krebs cycle'. The TCA cycle takes place within the mitochondrial matrix. The cycle serves as a primary metabolic pathway for the oxidation of acetyl-CoA obtained from metabolic processes, including fatty acid oxidation, glycolysis and amino acid catabolism (Martínez-Reyes and Chandel 2020).

In the TCA cycle, acetyl-CoA is chemically added to oxaloacetate forming citric acid, which further undergoes a series of enzymatic reactions, ultimately regenerating oxaloacetate. This process generates reducing equivalents viz. reduced form of 'nicotinamide adenine dinucleotide' (NADH) and 'flavin adenine dinucleotide' ($FADH_2$) (Fernie et al. 2004). These reducing equivalents are then utilized by the 'electron transport chain' (ETC) to generate 'adenosine triphosphate' (ATP) through 'oxidative phosphorylation'. Additionally, the intermediates formed in the TCA cycle are utilized for various biosynthetic anabolic pathways, including the synthesis of nucleotides, amino acids and the production of heme rings (Chandel 2021). Furthermore, the TCA cycle is tightly regulated by allosteric regulators, substrate availability and post-translational modifications to ensure proper metabolic flux and energy production in response to cellular demands (Mayes and Bender 2003).

Succinate dehydrogenase (SDH), a pivotal enzyme in cellular respiration, plays a crucial role in energy metabolism and redox balance within biological systems. It is a key component of both the TCA cycle and ETC and is anchored in the inner mitochondrial membrane (Moosavi et al. 2019). The unique structural composition of SDH allows its biochemical catalytic function and also facilitates the electron transfer from 'succinate to ubiquinone', contributing to ATP generation. It is of

paramount importance in various biological processes, serving as a critical enzyme with diverse functions. It has significance spanning across cellular respiration, energy production, redox balance and the regulation of key metabolic pathways (Kregiel 2012).

The primary function of SDH lies in its involvement in the TCA cycle, where it catalyses the oxidation of 'succinate to fumarate'. Simultaneously, SDH transfers electrons to ubiquinone facilitating the flow of electrons in the ETC. The enzyme's catalytic mechanism involves succinate oxidation and the subsequent reduction of flavin adenine dinucleotide (FAD) to $FADH_2$. The electrons are then transferred to ubiquinone, contributing to the proton motive force and ATP synthesis (Marín-García and Marín-García 2013).

Aberrations in SDH have clinical implications, particularly in hereditary cancers and neurodegenerative diseases. Mutations in subunits of the SDH enzyme are linked to certain cancers, such as paragangliomas and pheochromocytomas, emphasizing its role as a tumour suppressor. Understanding the clinical relevance of SDH provides insights into potential therapeutic targets for diseases associated with mitochondrial dysfunction (Rasheed and Tarjan 2018). SDH stands at the junctions of vital biological processes, influencing energy metabolism, redox balance and overall cellular health. Its complex involvement in both the TCA cycle and the ETC emphasizes its indispensability for sustaining life and maintaining cellular functionality. The multifaceted importance of SDH extends from fundamental bioenergetics to clinical implications, making it a crucial player in the intricate web of cellular processes.

1.2 Historical Perspectives

1.2.1 Early Discoveries

The origins of SDH's discovery date back to the late nineteenth century when researchers such as Eduard Buchner were pioneering the study of cellular respiration. Buchner's experiments with cell-free extracts revealed fermentation, showcasing the role of enzymes in sugar breakdown (Buchner 1897). This breakthrough encouraged deeper investigations into the specific enzymes involved in cellular metabolic processes. During a similar period, researchers such as Otto Warburg were focusing on isolating and studying individual enzymes implicated in respiration. Warburg's investigations with minced muscle tissue were pivotal in setting the stage for identifying SDH (Otto 2016). However, the formal discovery and characterization of SDH are commonly credited to scientists like Hans Thunberg in the early twentieth century. Thunberg's studies on tissue dehydrogenase activity offered compelling evidence for the presence of an enzyme targeting succinate, a critical component in cellular respiration (Holmes 1991).

In the early 1900s, Krebs and his contemporaries identified the series of chemical reactions involved in the breakdown of nutrients to produce energy, providing a conceptual framework for subsequent discoveries (Gasmi et al. 2021). This

provided the groundwork for understanding cellular respiration and the TCA cycle. In the 1930s and 1940s, researchers focused on isolating and characterizing the enzymes associated with the TCA cycle. The initial investigations identified enzymes like citrate synthase and aconitase, shedding light on the sequential steps of the cycle. The observation of SDH, the conversion of 'succinate to fumarate', became a crucial clue in understanding the TCA cycle. Scientists noticed this enzymatic activity and recognized the significance of a specific enzyme in facilitating this process. Subsequent experiments and assays specifically aimed at SDH identified the presence of an enzyme responsible for catalysing this reaction. The isolation and characterization of this enzyme marked a pivotal point in the discovery of SDH. This discovery cemented SDH's place as a core component of cellular energy production.

As research progressed in the 1950s and 1960s, the integration of TCA cycle components with the ETC became apparent. SDH was identified not only as a participant in the TCA cycle but also as a crucial component linking it to the ETC. Advances in biochemical techniques allowed researchers to purify and characterize SDH. Biochemical assays, chromatography and biophysical methods provided insights into the enzyme's structure, function and cofactor involvement.

1.3 Structural Insights

1.3.1 Molecular Components of SDH

The structural insights and molecular composition of SDH have been extensively studied. It is a 'heterotetrameric protein complex' composed of four nuclear-encoded subunits that link the TCA with the ETC. In humans, SDH is a multi-subunit enzyme present in the inner mitochondrial membrane. It consists of four subunits: 'SDHA, SDHB, SDHC and SDHD'. SDHA and SDHB form the catalytic domain, while SDHC and SDHD constitute the membrane-anchor domain.

1.3.2 SDHA (Flavoprotein Subunit)

SdhA is the largest subunit and contains a FAD cofactor covalently linked to the protein subunit. It hosts a catalytic site that binds to organic molecules such as succinate or fumarate. Numerous effector molecules also bind to these subunits *viz*. ATP or oxaloacetate. FAD is essential for the initial dehydrogenation reaction in which succinate is oxidized to fumarate (Gutman and Silman 1975). The electrons derived from succinate are transferred to FAD during the catalytic process. SDHA is situated on the inner membrane, positioning itself to interface and extend into the space of the mitochondrial matrix (Van Vranken et al. 2015). SDHA adopts a 'Rossmann-type fold' comprising four distinct domains: an 'FAD-binding domain (residues 52-267 and 355-439)', a 'capping domain (residues 268-354)', a 'helical domain (residues 440-537)' and a 'C-terminal domain'. A covalent bond is present

between FAD and 'His99' with additional coordination of FAD by several residues. These residues establish a structured hydrogen bonding network (Sun et al. 2005). 3-Nitro propionic acid (NPA) was used as a probe to identify the putative succinate binding site. The study indicated that NPA binds with the FMN portion of FAD, along with Glu261, Glu246, Thr260, His248, Arg403 and His359. FAD serves as a vital cofactor for SDH1. It originates from dietary riboflavin, also known as vitamin B_2, and its conversion involves 'two ATP-dependent enzymes', a 'riboflavin kinase' (Fmn1) and 'FAD synthetase' (Fad1). Fmn1 phosphorylates tricyclic isoalloxazine ring to produce FMN.

Putative mitochondrial FAD transporter (Flx1) plays a vital role in the insertion of FAS in enzymes. Flx1-deficient cells display decreased levels of FAD in the matrix, resulting in diminished activity of 'two matrix flavoproteins': 'SDH' and 'lipoamide dehydrogenase'. This impairment stems from a defect in flavinylation, the process of attaching FAD to these enzymes (Kim et al. 2012; Tzagoloff et al. 1996). SDH1 migrates into the mitochondrial matrix as an apoprotein and this process is allosterically affected by succinate along with several other intermediates of the TCA cycle (Brandsch and Bichler 1989; Robinson et al. 1994). SDH5 is another crucial protein that is responsible for the flavinylation of SDH1. The deletion of SDH5 gene in yeast inhibited growth dependent on respiration and led to a significant decrease in oxygen consumption (Hao et al. 2009; Kim et al. 2012). The binding of FAD is encouraged by, although not dependent upon, the presence of the 'iron–sulphur subunit' and intermediates of the TCA cycle, such as malate, succinate or fumarate (Robinson and Lemire 1996).

1.4 SDHB (Iron–Sulphur Subunit)

The 'N-terminal domain (residues 37-142)' comprises a small 'α-helix' along with a five-strand 'β-sheet'. This domain contains the FAD-proximal 2Fe-2S centre, coordinated by 'Cys93, Cys98, Cys101 and Cys113'. On the other hand, the 'C-terminal domain' (residues 142–280) consists of six 'α-helices', 4Fe-4S and 3Fe-4S centres. These centres are coordinated by 'Cys186, Cys189 and Cys192', 'Cys253 and Cys196' and 'Cys243 and Cys249', respectively. SDHB is a critical subunit for the enzyme activity that contains 'iron–sulphur clusters', including a [2Fe-2S] and a [4Fe-4S] cluster. Adjacent to the FAD site is a 2Fe-2S centre, followed by a neighbouring 4Fe-4S centre, and subsequently, a 3Fe-4S centre (Read et al. 2021). Consequently, the terminal 3Fe-4S centre is strategically positioned in proximity to ubiquinone, facilitating the transfer of ultimate electrons (Sun et al. 2005). It also abridges the membrane anchoring and catalytic domains of the enzyme (Hägerhäll 1997). These clusters act as electron carriers, aiding in the electron transfer to ubiquinone from succinate. The Fe-S clusters also play a role in the electron transport chain, assisting in the creation of a proton gradient across the inner mitochondrial membrane (Rouault and Maio 2017). These clusters are synthesized within the matrix of mitochondria utilizing a complex scaffold (ISU), composed of four proteins: Isd11, Yfh1, Nfs1 and Isu1 (Lill et al. 2012; Schmucker et al.

2011; Tsai and Barondeau 2010). Sulphide ions, essential for cluster formation, are supplied by the 'Nfs1 cysteine desulfurase', in conjunction with its associated proteins 'Isd11 and Yfh1' (Pandey et al. 2012; Pandey et al. 2013). Additionally, the Yah1 ferredoxin reductant also supports this process (Sheftel et al. 2010). Before being transferred to client proteins, Fe(II) and sulphide ions combine to form '2Fe-2S clusters' on the scaffold proteins *viz*. Isu1 (or Isu2). The preformed cluster then binds to the monothiol Grx5, a Fe-S shuttle protein. This binding is facilitated by the DnaJ protein Jac1, which interacts with Isu1, leading to the dissociation of the ISU complex (Majewska et al. 2013). Additionally, the Hsp70 enzyme Ssq1 is recruited during this process. Mutations in mitochondrial protein BolA3 have been found to cause malfunctions in respiratory complexes as well as 2-oxoacid dehydrogenases, akin to Nfu1 mutations (Baker et al. 2014; Cameron et al. 2011). This suggests that BolA3 might be involved in the later phases of mitochondrial Fe-S biogenesis or transfer (Li and Outten 2012).

1.5 SDHC (Membrane Anchor Subunit)

SDHC is a transmembrane protein composed of four helices, serving to anchor the SDH complex firmly to the inner mitochondrial membrane. It contains two 'heme b' groups, aiding in the coordination of electrons during the electron transfer. The 'heme b' groups in SDHC also contribute to the overall function of SDH in the mitochondrial ETC (Rutter et al. 2010; Van Vranken et al. 2015).

1.6 SDHD (Membrane Anchor Subunit)

SDHD is another transmembrane subunit containing five helices that assist in the attachment of the complex to the inner mitochondrial membrane. It interacts with SDHC to ensure proper localization and is crucial for the integrity and stability of the SDH complex within the membrane of the mitochondria. 'N-terminal helix' of SDHD resides within the matrix of mitochondria and facilitates the hydrophilic dimer localization through interaction with SDHB.

SDHC and SDHD interact with the porphyrin ring of the 'heme B cofactor' situated at the core four-helix bundle interface. Within this arrangement, conserved histidine residues of both subunits coordinate with the iron of the heme group. Additionally, two arginine residues, one from each subunit, along with SDHC another histidine residue, interact with the propionate groups of heme moiety (Sun et al. 2005).

1.7 Active Sites and Catalytic Mechanisms

The hydrophilic head comprises two subunits, SDHA and SDHB, which together form the catalytic core in mammals. The catalytic core hosts the redox cofactors essential for the transfer of electrons to ubiquinone. SDHA houses the FAD cofactor

and hosts the succinate binding site. SDHB encompasses the three Fe-S centres responsible for facilitating electron transfer to ubiquinone (Hägerhäll 1997; Sun et al. 2005). SDHB connects the catalytic and membrane anchor domains within the complex. The interaction between SDHB and both SDHA and SDHC involves a similar surface area, indicating that the catalytic core does not function as an independent dimer without a membrane anchor. SDHC and SDHD contribute two transmembrane helices each to form a 'four-helix bundle', constituting the membrane anchor core (Hao et al. 2009). Each subunit contributes a transmembrane helix that encircles the core (Van Vranken et al. 2015). Within the membrane domain, a heme b moiety is bound at the interface of the subunits SDHC and SDHD, with each subunit providing one of the two axial His ligands. Additionally, two separate ubiquinone binding sites have been detected in mammalian SDH complexes (Sun et al. 2005; Yankovskaya et al. 2003). The residues Thr254, His354 and Arg399 within subunit A provide stabilization to the molecule. Meanwhile, FAD undergoes oxidation and transports the electrons to the initial iron–sulphur cluster, [2Fe-2S] (Kenney 1975).

The 'succinate-binding site' and 'ubiquinone-binding site' are connected through a sequence of redox centres, including FAD and iron–sulphur clusters. This chain extends over 40 Å across the enzyme monomer (Yankovskaya et al. 2003). The high-affinity ubiquinone site (QP-proximal) is located on the matrix side of the inner mitochondrial membrane (IM) and involves residues from SDHB, SDHC and SDHD. Within proximity of 7 Å to the '3Fe–4S redox centre', the QP site serves as the primary ubiquinone binding site (Oyedotun and Lemire 2001; Silkin et al. 2007). In contrast, the second, less tightly bound ubiquinone site (QD-distal) is located closer to the intermembrane space (IMS) side of the inner membrane. Ubiquinone reduction occurs through two individual electron reactions, differing from the two-electron reduction of FAD. The QP site notably stabilizes the partially reduced semiquinone, aiding in its full reduction to ubiquinol. The QP site notably enhances the stability of the partially reduced semiquinone, enabling its complete reduction to ubiquinol (Yankovskaya et al. 2003). The role of the conserved heme group in eukaryotic SDHs and the distant QD site remains unclear. While the heme is not essential for ubiquinone reduction at the QP site, it may aid in electron transfer to the distant QD site. SDH complexes capable of succinate reduction at heme might also produce ubiquinol at the QD site, but definitive proof is lacking. Unlike the bc1 Complex III, SDH lacks a Q cycle and does not actively transport protons, despite having two Q sites.

1.8 Functional Significance

1.8.1 Role in Cellular Respiration

SDH is an important membrane dehydrogenase allied with the respiratory chain and is part of the TCA cycle. Its primary role is to catalyse the oxidation of succinate to fumarate. This reaction involves the removal of two hydrogen atoms from succinate,

which are transferred to FAD, a prosthetic group, which is tightly attached to the SDH enzyme. Various structural analyses displayed that arginine residue, for instance, Arg 286 of *E. coli*, serves as a proton shuttle for the reaction. It is followed by two possible eliminations, *i.e.* E_2 or E_{1cb}. In the E_2 reaction, a basic amino acid or cofactor removes a proton from the α-carbon, while FAD accepts a hydride from the β-carbon, leading to the oxidation of succinate. On the other hand, an enolate is formed before accepting the proton by FAD in the E_{1cb} reaction (Fig. 1.1). The product formed loses affinity for the active site and the depart forms the enzyme cavity (Dorota 2012).

1.8.2 Contribution to the Electron Transport Chain

Electron transport through SDH involves a series of redox reactions within the enzyme complex. Initially, electrons are extracted from succinate during its oxidation to fumarate. These electrons are transferred to the FAD cofactor, leading to its reduction to $FADH_2$. Subsequently, electrons are shuttled through a series of 'iron–sulphur clusters', i.e. traversing the [Fe-S] relay until reaching the [3Fe-4S] cluster present within SDH. The clusters serve as electron carriers, facilitating the transfer of electrons along the enzyme complex. Ultimately, the electrons are transferred to a mobile electron carrier named ubiquinone present in the mitochondrial membrane. It is postulated that electrons received by ubiquinone through the 3Fe-4S cluster oscillate between the 'heme moiety' and 'ubiquinone' intermediate resulting in the development of an electron sink. The orientation and reduction of ubiquinone within SDH involve specific interactions with amino acid residues within the enzyme complex. Initially, hydrogen bonds between ubiquinone and specific residues guide its positioning at the active site. Upon the arrival of electrons from the 'iron–sulphur cluster', ubiquinone undergoes a 'conformational change', leading to further interactions with the enzyme. This enables stepwise reduction of ubiquinone to ubiquinol, involving the formation of a semiquinone radical species followed by the addition of a 'second electron' to complete the reduction process. The electron transfer process within SDH links succinate oxidation in the TCA cycle to the ETC, aiding in the formation of a proton gradient across the inner mitochondrial membrane (Fig. 1.2) (Moser et al. 2006).

1.8.3 Impact on ATP Production

In contrast to other respiratory complexes, SDH does not actively pump protons across the inner mitochondrial membrane. Instead, it indirectly contributes to proton gradient generation by reducing ubiquinone to ubiquinol. Ubiquinol then transfers electrons to complex III consisting of 'cytochrome c reductase' and complex IV made up of 'cytochrome c oxidase', which directly transfers protons across the inner membrane (Tretter et al. 2016). This results in the build-up of the necessary

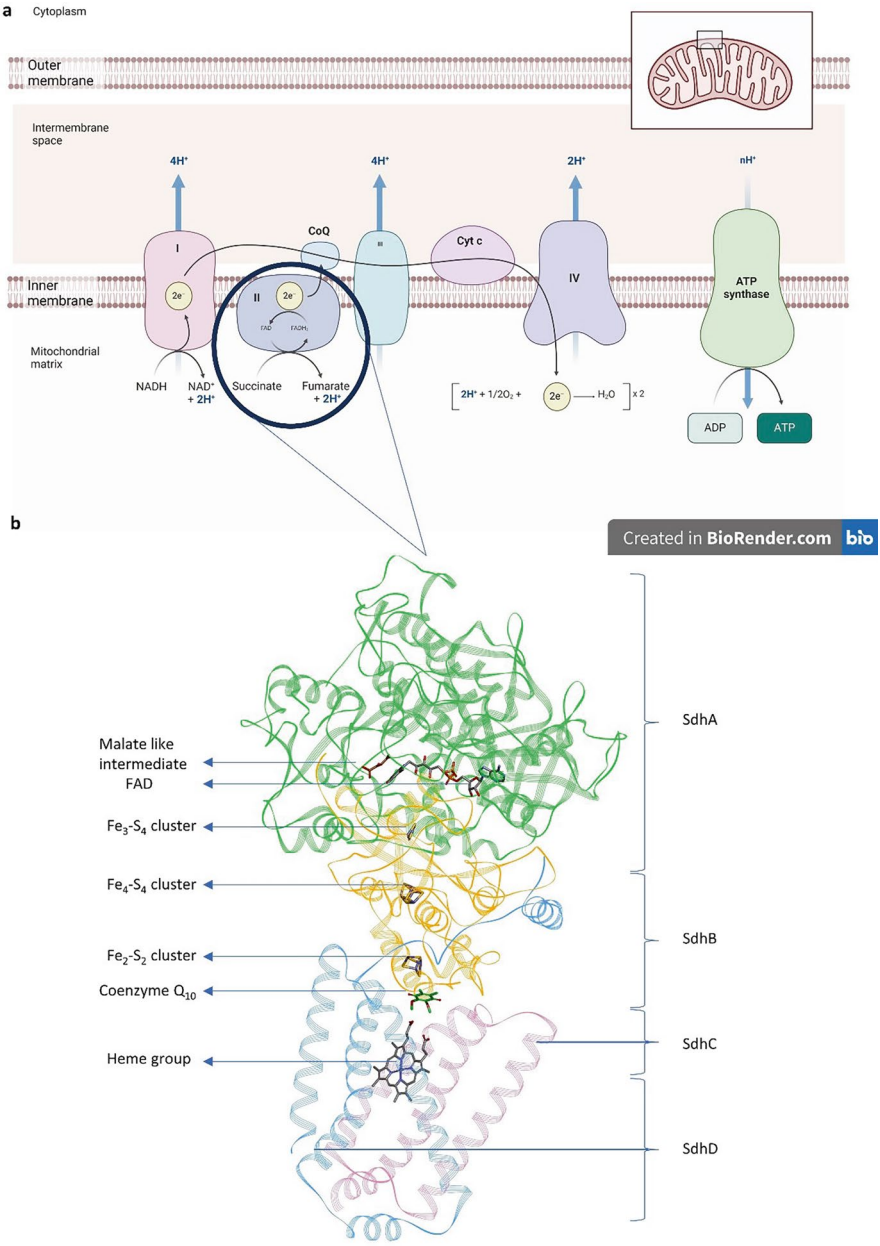

Fig. 1.1 (**a**) Electron transport chain, within the mitochondria, showcasing the sequential flow of electrons through respiratory complexes, leading to the generation of ATP and the establishment of a proton gradient across the inner mitochondrial membrane. (**b**) Representation of the subunit arrangement within the SDH enzyme complex, highlighting the interaction between various subunits and cofactors essential for its catalytic activity. (Created from BioRender.com)

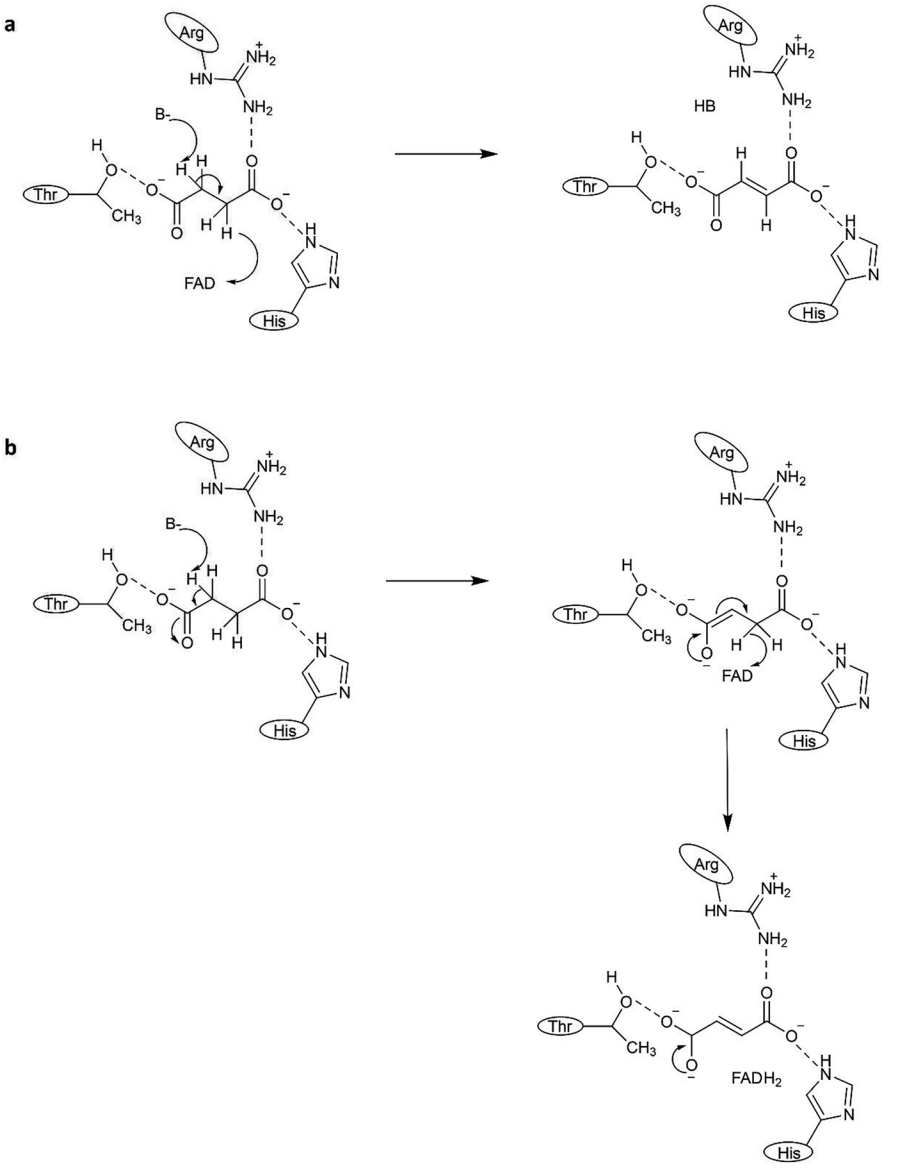

Fig. 1.2 (**a**) E_2 and (**b**) E_{1cb} mechanisms involved in the conversion of fumarate to malate, depicting the stepwise enzymatic reactions and the intermediates formed during the process

proton gradient for ATP synthesis. The gradient hence produced powers the F_1F_0 ATP synthase, enabling the phosphorylation of ADP for the synthesis of ATP. This enzyme is driven by protons, harnessing the energy stored within the proton gradient, to facilitate the phosphorylation of ADP to ATP (Rustin et al. 2002).

1.9 Regulation of Enzyme

Post-translational modifications play a crucial role in regulating SDH activity. The acetylation and phosphorylation of the active site cause inhibition of activity. Studies demonstrated that reversible acetylation at numerous lysine residues within mouse SDH1 leads to the reduction of its catalytic activity (Cimen et al. 2010). On the other hand, sirtuins, notably SIRT3, facilitates the deacetylation of SDH1's active site, thereby regulating the acetylation level and activity of SDH. Further, the phosphorylation of the SDH1 subunit also deactivates the enzyme similar to acetylation (Tomitsuka et al. 2009). In vitro experiments have demonstrated that Fgr tyrosine kinase can phosphorylate tyrosine residues of SDH1 in two instances. However, the biological relevance of this modification by Fgr remains unexplored (Salvi et al. 2007). The enzymatic function of SDH is affected by Krebs cycle intermediates, with oxaloacetate serving as a strong inhibitor. Conversely, succinate triggers the release of oxaloacetate from SDH, activating the enzyme. This oxaloacetate-mediated inhibition may contribute to the modulation of SDH activity depending on the mitochondrial metabolic state (Gutman and Silman 1975).

'Necrosis Factor Receptor-Associated Protein 1' (TRAP1) is also an inhibitor of SDH activity of the complex II through 'activity down-modulation'. It triggers pseudohypoxia by stabilizing HIF1α and also inhibits the 'ROS-dependent' opening of the 'mitochondrial permeability transition pores', reducing oxidative damage in tumour cells (Guzzo et al. 2014). Malonate, a substrate analogue of succinate, competitively inhibits the SDH complex. It interacts with positively charged amino acid residues within the active site, akin to succinate. However, the absence of ethylenic linkage of malonate, necessary for dehydration, prevents it from oxidation (Hajjawi 2010; Hajjawi and Hider 2009).

1.10 Determination of SDH Activity

Succinate is an efficient energy substrate making the SDH activity assay a vital method for assessing yeast and other cells' vitality, particularly monitoring various fermentation processes. SDH activity can be assessed both in vitro, using cell lysates or mitochondrial fractions, and in situ, within individual cells (Kregiel et al. 2008). Its localization on the inner mitochondrial membrane makes it readily isolable alongside mitochondria using various techniques such as 'sucrose density gradient ultracentrifugation', 'free-flow electrophoresis' or commercially available 'kit-based methods'. The mitochondrial fraction obtained by the above methods represents the primary source of this enzyme (Hartwig et al. 2009).

The feasibility of using fumarate or succinate for the estimation of the enzyme activity is not very favourable; hence, synthetic electron donors are employed. Additionally, the normal flow of electrons should be blocked in ETC for such estimation. Poisons such as sodium azide and potassium cyanide block electron transfer from cytochrome a_3 to oxygen (electron acceptor). Consequently, the preceding cytochromes and 'coenzyme Q' are unable to facilitate the transfer of electrons. 2,6-Dichlorophenolindophenol (DCPIP), an artificial electron acceptor, captures

electrons instead of $FADH_2$. The reduced product of DCPIP can be measured spectrometrically to colourless from the blue product.

$$SDH\text{-}FADH_2 + DCPIP_{oxid} \rightarrow SDH\text{-}FAD + DCPIP_{red} + 2H^+$$

The alteration in absorbance, quantified at '600 nm', is employed to track the progression of the reaction throughout its duration. The rate at which the blue colour diminishes correlates directly with the enzyme concentration. By monitoring the change in absorbance of the mixture over time, the enzyme concentration can be accurately determined from the resulting data (Mahesha 2014).

Various tetrazolium salts also act as substrates for SDH. The metabolically active cells reduce different tetrazolium salts by dehydrogenases, resulting in the formation of intensely coloured end products known as formazans, thus making in situ estimation of SDH feasible. In the case of in situ estimation of SDH enzymatic activity, the plasma membrane acts as a barrier and reduces the cellular penetration of these tetrazolium salts (Kregiel et al. 2008). Hence, cell permeabilization, such as with digitonin, is suggested as an alternative approach to investigate intracellular enzyme activities. Berlowska et al. demonstrated the efficacy of digitonin in 'membrane permeabilization' without adverse effects on cell morphology. After treatment with digitonin, formazan crystals were visible inside the yeast cells but not outside (Berlowska et al. 2006). The formazan products, while insoluble in water, readily diffuse out of cells once solubilized in dimethyl sulfoxide.

1.11 Role of SDH in Diseases

SDH plays a significant role in mitochondrial diseases by contributing to mitochondrial dysfunction. Dysregulation of SDH activity can lead to disruptions in energy production, increased oxidative stress and impaired cellular function, contributing to the pathogenesis of various 'mitochondrial disorders'. These disorders encompass a wide range of conditions, including Leigh syndrome, mitochondrial encephalomyopathy and mitochondrial myopathy, among others. SDH dysfunction may result from mutations in genes encoding its subunits or regulatory proteins, leading to a cascade of biochemical abnormalities that compromise mitochondrial function and cellular health (Table 1.1).

1.12 Hereditary Paraganglioma–Pheochromocytoma Syndrome

'Hereditary paraganglioma–pheochromocytoma syndrome' is an inherited disorder marked by tumour growth in paraganglia, clusters of cells near nerve ganglia. These tumours, known as paragangliomas, may include pheochromocytomas, which arise in the adrenal glands responsible for stress hormone production (Else et al. 1993). Paragangliomas primarily occur in the 'head and neck', particularly at the 'carotid

Table 1.1 Diseases associated with succinate dehydrogenase deficiency

Disease	Associated genes	Signs and symptoms	Effects	References
Leigh syndrome	SDHA, SDHB, SDHC, SDHD	Early-onset encephalopathy, developmental delay, exercise intolerance, muscle weakness, seizures, vision problems, lactic acidosis.	Severe brain damage, intellectual disability, movement problems, heart failure.	Karimzadeh et al. (2020)
Hereditary paraglioma/ pheochromocytoma syndrome (HAPP)	SDHB, SDHC, SDHD	Increased risk of paragangliomas (tumours of the nervous system), pheochromocytomas (tumours of the adrenal gland), headaches, sweating, palpitations, high blood pressure.	Cancers, hormonal imbalances, severe hypertension.	Nazar et al. (2019)
Gastrointestinal stromal tumours (GISTs)	SDHB	Abdominal pain, bleeding, nausea, early satiety.	Cancers of the digestive tract.	Nazar et al. (2019)
Renal cell carcinoma	SDHB (less common)	Blood in urine, flank pain, weight loss, fatigue.	Kidney cancer.	Nazar et al. (2019)
Leukodystrophy	SDHB (limited studies)	Progressive loss of coordination, muscle weakness, vision problems, seizures, dementia.	Degeneration of white matter in the brain, leading to severe neurological disability.	Moreno et al. (2020)
Ataxia	SDHB (limited studies)	Difficulty walking, speaking and coordinating movements, tremors.	Loss of balance and coordination.	Moreno et al. (2020)
Optic atrophy	SDHB (limited studies)	Vision loss, decreased colour vision, night blindness.	Degeneration of the optic nerve, leading to permanent vision impairment.	Moreno et al. (2020)
Acute mountain sickness (AMS)	Likely epigenetic modifications of SDHA/SDHB	Headache, nausea, vomiting, dizziness, fatigue, shortness of breath at high altitudes.	Difficulty acclimatizing to high altitude, potentially life-threatening.	Moreno et al. (2020)
Carney-Stratakis syndrome	SDHB, SDHC	Gastrointestinal stromal tumours (GISTs), paragangliomas, pheochromocytomas, skin pigmentation changes, myxomas (benign tumours).	Increased risk of multiple tumours in various organs.	Negro et al. (2017)
Neuroblastoma	SDHB (rare)	Abdominal mass, pain, fatigue, weight loss, bone pain.	Aggressive childhood cancer.	Rapizzi et al. (2014)

bifurcation (80%)', 'jugular bulb or tympanic nerve (17.5%)' and 'vagal nerve (4.5%)' (Lack 1997). In recent times, genetic mutations linked to the mitochondrial SDH complex have been demonstrated to lead 'head and neck paragangliomas', 'extra-adrenal paragangliomas' and 'pheochromocytomas'. Gene SDHD, situated at 11q23, encodes the transmembrane subunit of Complex II in the ETC and displays 110 distinct mutations related to the disease. These tumours predominantly manifest in the 'head and neck' region, specifically within the parasympathetic trunk (Baysal et al. 2000).

SDHB, situated at 1p36.1-p35, is responsible for encoding the catalytic subunit of iron–sulphur protein within Complex II. With 175 distinct mutations identified, SDHB mutations are linked to tumours found in abdominal/thoracic paraganglia, adrenal glands and the sympathetic trunk (Astuti et al. 2001). SDHC, located at 1q23.3, is responsible for encoding another transmembrane subunit of 'Complex II'. It has been associated with 34 distinct mutations. These tumours primarily emerge in the 'head and neck' region, particularly in the parasympathetic trunk (Mannelli et al. 2007). SDHAF2, at 11q12.2, is linked to hereditary paraganglioma/pheochromocytoma syndrome, with one unique mutation reported. Tumours associated with SDHAF2 mutations are located in the 'head and neck', particularly in the parasympathetic trunk (Bayley et al. 2010). SDHA, at 5p15, contributes to hereditary paraganglioma/pheochromocytoma syndrome, with one unique mutation identified. Tumours associated with SDHA mutations predominantly occur in the abdominal paraganglia and the sympathetic trunk (Burnichon et al. 2010).

1.13 Hereditary Leiomyomatosis and Renal Cell Cancer

'Hereditary leiomyomatosis and renal cell cancer' (HLRCC) is an 'autosomal dominant' tumour susceptibility syndrome. It is characterized by a predisposition to benign leiomyomas of the skin and uterus (Kiuru and Launonen 2004). Genetic mutations associated with SDHB and SDHD genes, which encode subunits of SDH, are linked to HLRCC. These mutations interfere with normal cellular functions, promoting tumourigenesis through mechanisms such as pseudohypoxia (Kiuru and Launonen 2004; Nazar et al. 2019). Individuals with HLRCC who harbour mutations in SDHB and SDHD genes are at an increased risk of developing aggressive types of kidney cancer. Regular screening is crucial for early detection and effective management of renal cell carcinoma in these individuals.

1.14 Gastrointestinal Stromal Tumours

SDH-deficient gastrointestinal stromal tumours (GISTs) exhibit a scarcity of SDH, resulting in a distinct multinodular/plexiform architecture and epithelioid cell composition. Typically found in the stomach, these tumours display unique morphological traits like multinodular growth patterns and lymphovascular involvement

(Ibrahim and Chopra 2019; Lv et al. 2021). Patients with SDH-deficient GISTs often present at a young age, with symptoms like gastrointestinal bleeding, abdominal pain and fatigue.

1.15 Renal Oncocytomas

Renal oncocytomas present as well-defined tumours characterized by typical gross features, including a 'tan or mahogany coloured mass' with a 'central stellate scar'. Microscopically, they demonstrate a nested architecture with 'bland cytology', regular nuclei featuring 'prominent central nucleoli' and 'eosinophilic cytoplasm'. These tumours primarily consist of oncocytes, characterized by an abnormal abundance of mitochondria (Lieber et al. 1981). Studies have identified cases where renal oncocytomas initially diagnosed based on histological features showed characteristic histologic features of 'SDH-deficient renal cell carcinomas'. Loss of SDHB gene expression by immunohistochemistry was observed in these cases, indicating a potential link between SDH alterations and the development of oncocytomas (Gupta et al. 2019).

1.16 Cardiomyopathy

Cardiomyopathy encompasses a group of heart muscle diseases that impair the heart's ability to pump blood effectively. 'Dilated cardiomyopathy' involves the enlargement of one of the ventricles and is more prevalent in males. 'Hypertrophic cardiomyopathy' is characterized by thickening of the heart muscle posing a risk of sudden death, particularly in young athletes. 'Arrhythmogenic cardiomyopathy' causes irregular heartbeats or rhythms and can lead to sudden cardiac death, especially in children and young adults (Wexler et al. 2009). SDH plays a vital role in myocardial homeostasis by overseeing fatty acid oxidation and glycolysis. Mice deficient in SDH show disturbances in critical metabolic pathways for cardiac function, underscoring SDH's involvement in epigenetic and metabolic regulation in the heart (Li et al. 2023). Research has revealed a specific shortage of SDH in the heart, notably in conditions such as Barth syndrome, a genetic ailment linked to cardiomyopathy. This insufficiency prompts structural alterations in respiratory chain complexes, diminishing enzymatic capabilities and elevating reactive oxygen species production, which exacerbates cardiac issues (Dudek et al. 2016).

1.17 Neurodegeneration

Research highlights that oxidative stress and inflammation are major contributors to neurodegeneration. SDH deficiency can exacerbate oxidative stress within neurons, leading to cellular damage and dysfunction. Disruption in SDH function may result in mitochondrial dysfunction, reduced energy generation and heightened oxidative

stress, thereby playing a role in neuronal injury and neurodegeneration seen in conditions such as 'Alzheimer's, Parkinson's and Huntington's disease' (Farshbaf and Kiani-Esfahani 2018). Neurodegenerative disorders are marked by aberrant protein aggregation and subsequent cell demise. Changes in SDH function might impact protein misfolding occurrences, thus playing a role in the neuronal decline seen in diseases such as 'amyotrophic lateral sclerosis', multiple system atrophy and tauopathies.

1.18 Conclusion

SDH, a multi-subunit enzyme nestled within the inner mitochondrial membrane, stands as a pivotal player in cellular respiration. It bridges the gap between the TCA cycle and the ETC, performing the seemingly simple yet vital task of oxidizing succinate to fumarate. However, this seemingly straightforward reaction lies at the core of a complex task of influencing energy metabolism, redox balance and overall cellular health. SDH's intricate structure, with its unique blend of subunits and cofactors, facilitates its diverse functions. The FAD-bound SDHA subunit catalyses the initial dehydrogenation reaction, while the iron–sulphur clusters housed within SDHB act as electron carriers, shuttling electrons to ubiquinone. SDHC and SDHD, the membrane anchor subunits, ensure proper localization within the mitochondrial membrane.

Beyond its role in the TCA cycle, SDH contributes significantly to the ETC. Electrons extracted from succinate are transferred through a series of redox centres, ultimately reaching ubiquinone, a mobile electron carrier. This electron transfer helps establish the proton gradient across the inner mitochondrial membrane, a crucial step in ATP synthesis. The activity of SDH is tightly regulated by various mechanisms, including post-translational modifications and metabolite availability. Understanding these regulatory mechanisms sheds light on how cellular demands are met and on potential therapeutic targets for diseases associated with mitochondrial dysfunction. The importance of SDH extends far beyond basic cellular processes. Mutations in genes encoding SDH subunits have been linked to various cancers, including paragangliomas, pheochromocytomas and certain kidney cancers. These findings highlight the role of SDH as a tumour suppressor and underscore the potential for SDH-based therapeutic strategies.

In conclusion, SDH stands as a remarkable example of how a seemingly simple enzyme can orchestrate a multitude of complex cellular functions. Its role in energy production, redox balance and disease development makes it a captivating target for further research and a potential key to unlocking new avenues in medicine.

Conflict of Interest The authors declare that they have no conflict of interest.

References

Astuti D, Latif F, Dallol A, Dahia PL, Douglas F, George E, Sköldberg F, Husebye ES, Eng C, Maher ER (2001) Gene mutations in the succinate dehydrogenase subunit SDHB cause susceptibility to familial pheochromocytoma and to familial paraganglioma. Am J Hum Genet 69(1):49–54. https://doi.org/10.1086/321282

Baker PR, Friederich MW, Swanson MA, Shaikh T, Bhattacharya K, Scharer GH, Aicher J, Creadon-Swindell G, Geiger E, MacLean KN (2014) Variant non ketotic hyperglycinemia is caused by mutations in LIAS, BOLA3 and the novel gene GLRX5. Brain 137(2):366–379. https://doi.org/10.1093/brain/awt328

Bayley J-P, Kunst HP, Cascon A, Sampietro ML, Gaal J, Korpershoek E, Hinojar-Gutierrez A, Timmers HJ, Hoefsloot LH, Hermsen MA (2010) SDHAF2 mutations in familial and sporadic paraganglioma and phaeochromocytoma. Lancet Oncol 11(4):366–372. https://doi.org/10.1016/S1470-2045(10)70007-3

Baysal BE, Ferrell RE, Willett-Brozick JE, Lawrence EC, Myssiorek D, Bosch A, Mey AVD, Taschner PE, Rubinstein WS, Myers EN (2000) Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. Science 287(5454):848–851. https://doi.org/10.1126/science.287.5454.848

Berlowska J, Kregiel D, Klimek L, Orzeszyna B, Ambroziak W (2006) Novel yeast cell dehydrogenase activity assay in situ. Pol J Microbiol 55(2):127–131. https://www.ncbi.nlm.nih.gov/pubmed/17419290

Brandsch R, Bichler V (1989) Covalent cofactor binding to flavoenzymes requires specific effectors. Eur J Biochem 182(1):125–128. https://doi.org/10.1111/j.1432-1033.1989.tb14808.x

Buchner E (1897) Alkoholische gärung ohne hefezellen. Ber Dtsch Chem Ges 30(1):1110–1113

Burnichon N, Brière J-J, Libé R, Vescovo L, Riviere J, Tissier F, Jouanno E, Jeunemaitre X, Bénit P, Tzagoloff A (2010) SDHA is a tumor suppressor gene causing paraganglioma. Hum Mol Genet 19(15):3011–3020. https://doi.org/10.1093/hmg/ddq206

Cameron JM, Janer A, Levandovskiy V, MacKay N, Rouault TA, Tong W-H, Ogilvie I, Shoubridge EA, Robinson BH (2011) Mutations in iron-sulfur cluster scaffold genes NFU1 and BOLA3 cause a fatal deficiency of multiple respiratory chain and 2-oxoacid dehydrogenase enzymes. Am J Hum Genet 89(4):486–495. https://doi.org/10.1016/j.ajhg.2011.08.011

Chandel NS (2021) Amino Acid Metabolism. Cold Spring Harb Perspect Biol 13(4). https://doi.org/10.1101/cshperspect.a040584

Cimen H, Han M-J, Yang Y, Tong Q, Koc H, Koc EC (2010) Regulation of succinate dehydrogenase activity by SIRT3 in mammalian mitochondria. Biochemistry 49(2):304–311. https://doi.org/10.1021/bi901627u

Dorota K (2012) Succinate dehydrogenase of saccharomyces cerevisiae – the unique enzyme of TCA cycle – current knowledge and new perspectives. In: Rosa Angela C (ed) Dehydrogenases. IntechOpen, p 9. https://doi.org/10.5772/48413

Dudek J, Cheng IF, Chowdhury A, Wozny K, Balleininger M, Reinhold R, Grunau S, Callegari S, Toischer K, Wanders RJ, Hasenfuß G, Brügger B, Guan K, Rehling P (2016) Cardiac-specific succinate dehydrogenase deficiency in Barth syndrome. EMBO Mol Med 8(2):139–154. https://doi.org/10.15252/emmm.201505644

Else T, Greenberg S, Fishbein L (1993) Hereditary paraganglioma-pheochromocytoma syndromes. University of Washington, Seattle. http://europepmc.org/abstract/MED/20301715

Farshbaf MJ, Kiani-Esfahani A (2018) Succinate dehydrogenase: prospect for neurodegenerative diseases. Mitochondrion 42:77–83. https://doi.org/10.1016/j.mito.2017.12.002

Fernie AR, Carrari F, Sweetlove LJ (2004) Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. Curr Opin Plant Biol 7(3):254–261. https://doi.org/10.1016/j.pbi.2004.03.007

Gasmi A, Peana M, Arshad M, Butnariu M, Menzel A, Bjørklund G (2021) Krebs cycle: activators, inhibitors and their roles in the modulation of carcinogenesis. Arch Toxicol 95(4):1161–1178. https://doi.org/10.1007/s00204-021-02974-9