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Succinate dehydrogenase, succinate, and superoxides: a genetic, epigenetic, metabolic, environmental explosive crossroad

Paule Bénit[1], Judith Goncalves[2], Riyad El Khoury[3], Malgorzata Rak[1], Judith Favier[2], Anne-Paule Gimenez-Roqueplo[4] and Pierre Rustin[1*]

[1] Université Paris Cité, Inserm, NeuroDiderot, F-75019 Paris, France; paule.benit@inserm.fr
[2] Université Paris Cité, Inserm, PARCC, F-75015 Paris, France
[3] Department of Pathology and Laboratory Medicine, Neuromuscular Diagnostic Laboratory, American University of Beirut Medical Center, Beirut 1107 2020, Lebanon; re70@aub.edu.lb
[4] Département de Médecine Génomique des Tumeurs et des Cancers, AP-HP, Hôpital Européen Georges Pompidou, F-75015 Paris, France. anne-paule.gimenez-roqueplo@aphp.fr
* Correspondence: pierre.rustin@inserm.fr

Abstract: Research focused on succinate dehydrogenase (SDH) and its substrate, succinate, culminated in the 50's accompanying the rapid development of research dedicated to bioenergetics and intermediary metabolism. This allowed to uncover the implication of the SDH in both the mitochondrial respiratory chain and the Krebs cycle. Nowadays this theme is experiencing a real revival following the discovery of the role of SDH and succinate in a subset of tumors and cancers in human. The aim of this review is to enlighten the many questions yet unanswered, ranging from fundamental to clinically oriented aspects, up to the danger of the current use of SDH as a target for a sub class of pesticides.

Keywords: Mitochondria; Respiratory chain; Krebs cycle; Succinate; Cancer; Encephalopathy; SDH; SDHI; pesticides

1. Introduction

Similarly to other Krebs cycle enzymopathies, succinate dehydrogenase (SDH) deficiency has long been held incompatible with human life [1]. Yet, thanks to genetic investigations grounded themselves on clinical features and biochemical evidences of enzyme deficiencies, one year after the fumarase deficiency in 1994 [2], SDH deficiency was recognized as a pathology, although considered to be extremely rare [3]. Nowadays in 2022, SDH deficiency has been finally identified in a whole spectrum of human diseases resulting in unexpected and distant phenotypes [4]. In turn, this shed light on unanticipated roles of SDH and its substrate, succinate, in human physiology and more generally in living organisms. Over the years, a number of reviews have covered most aspects of the physiological and pathological features associated with this enzyme and the related metabolic segments in a large collection of living organisms [5-36].

Prompted by recent developments in somewhat distant scientific fields, we thought useful to gather and analyze accessible data to highlight questions that remain open. Thus, we cover in this review from the biochemical, metabolic, genetic, epigenetic, clinical aspects related to SDH and succinate, up to the danger that represent the use of SDH-poisoning substances which, at variable concentration, impregnate nature and the organisms living therein.

2. The Succinate crossroad: Enzymes and metabolites stakeholders

Thanks to the work of several outstanding biochemists in the 50's, the main functions of the SDH (EC 1.3.5.1) in the mitochondria of aerobic organisms were unraveled. SDH was found to play a role in the Krebs cycle and in the respiratory chain (RC), [32, 37-39]. In animals, as in most living organisms, SDH has no cellular isoform unlike most enzymes of the Krebs cycle but citrate synthase and

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succinyl CoA ligase. In case of a SDH dysfunction or deficiency, this reduces the possibility for metabolic bypasses. In most microorganisms and animals, the enzyme is made up of four subunits (Figure 1A; SDHA to D or SDH1 to 4 according to organisms) all encoded by nuclear genes, typically conserved through evolution. These are organized as an operon in many prokaryotes [40]. Presumably a further illustration of the archaebacterial origin of mitochondria [41-43], SDH3 and SDH4, are however still encoded in mitochondrial DNA in many but not all land plants [44]. Noticeably, the composition and function of the plant enzyme has been elusive and differs from the well-characterized enzymes in mammals and bacteria [45]. It appears to include up to four additional proteins [44, 46], the function of which being yet unknown. Noticeably, the enzyme has also been reported to be associated with the thylakoid membranes of cyanobacteria [47], and with the chloroplasts of *Chlamydomonas* [48]. In higher plants, SDH identification in chloroplast preparations was attributed to the presence of contaminating mitochondria [49]. Noticeably, SDH is involved in several specific key roles in plants such as stomatal function and nitrogen assimilation [50].

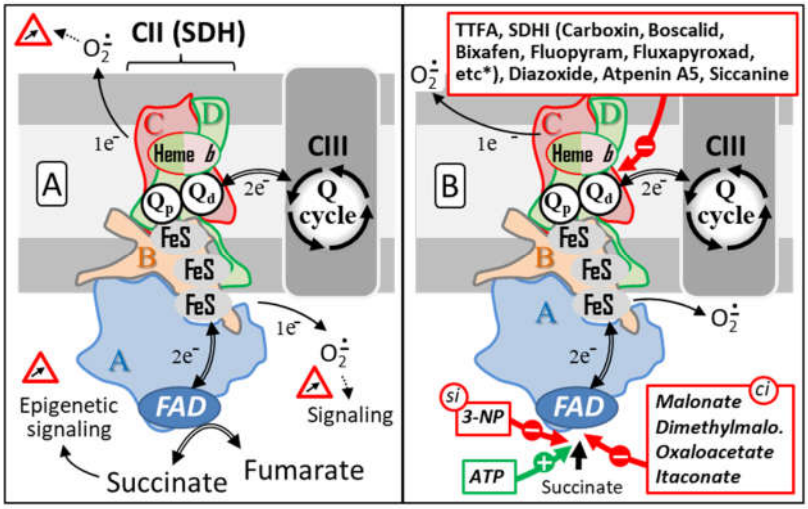

Figure 1. The succinate dehydrogenase (A) and some of its inhibitors (B).

A: The four subunits (A-D) SDH conserved during evolution from microorganism to human, the plant enzyme has four subunits (E-H) whose function remains to be defined [44]. The animal enzyme, as its plant counterpart, reduces a specific pool of ubiquinone which competes with other kinetically distinct quinone pools reduced by various mitochondrial dehydrogenases for the reduction of CIII-associated quinones. Depending on the state of reduction of the redox centers of the enzyme, it will generate variable amounts of superoxides used for cell signaling but which, in excess, can lead to multiple deleterious effects. B: This part of the diagram shows a series of the best-known SDH inhibitors (red arrows). A part of these (bottom) through their binding to the A subunit of the enzyme, acting either as competitive inhibitors (ci) or as suicide inhibitor (si). ATP also capable of binding to this same subunit exerts, on the contrary, an activating effect (green arrow). Another series of inhibitors act through their link with the endogenous quinone binding site (Top). This type of inhibitor includes a long series of molecules distributed as fungicides in agriculture (*a more complete list of SDHI inhibitors can be found at http://endsdhi.com/wp-content/uploads/2022/04/SDHI-structure-15-Avril-22.pdf). ATP, Adenosine triphosphate; CII, CIII, respiratory chain complex II and III; FeS, iron-sulfur cluster; FAD, SDH A-bound flavin adenine dinucleotide; 3-NP, 3 nitropropionic acid; Q, ubiquinone with variable length isoprenoid side chain according to species (CoQ10 in human).

SDHA subunit, also known as the flavoprotein (Fp) subunit, harbors the catalytic/regulatory site(s) binding organic substrates, succinate or fumarate, and various effectors, e.g., oxaloacetate, ATP, etc [51]. SDHA is localized to the inner mitochondrial membrane where it faces, and protrudes into, the mitochondrial matrix space. Together with SDHB, they catalyze the phenazine methosulfate - dichlorophenol indophenol (PMS-DCPIP) reductase activity without involving SDHC and D subunits. These latter subunits are embedded in the inner membrane and bind a b-type cytochrome whose function(s) is

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not yet clearly determined [52]. SDHC and D subunits are often referred as the anchoring subunits ensuring electron flow to ubiquinone. Noticeably, while other complexes of the respiratory chain may have gained additional subunits or contrariwise have lost some or all subunits (e.g., CI in *Saccharomyces cerevisiae*), SDH has a preserved composition across species with few exceptions. In addition to the four constitutive subunits, several supplemental assembly factors are required for the maturation of individual subunits and for the correct assembly and function of SDH. SDHAF1 (or LYRM8; SDH6 in yeast) is a chaperone that is involved in maturation of SDHB by recruiting and inserting the iron-sulfur cluster [53, 54] while simultaneously affording protection from oxidants. It was suggested that SDHAF1 acts together with SDHAF3 (SDH7 in yeast) to perform its function [54]. SDHAF2 (SDH5 in yeast) appears required for the flavination of the SDHA subunit [55, 56]. SDHAF4 (SDH8 in yeast) is known to bind to the SDHA subunit after flavination of this latter, affording protection from oxidants, while catalyzing the proper assembly of the catalytic dimer with the SDHB [57]. The activity of a set of additional proteins ensures the proper functioning of SDH. These proteins encompass those involved in the assembly of iron-sulfur clusters, such as Frataxin [58]. The deficiency of Frataxin is known to cause Friedreich's ataxia. This ataxia is hallmarked by a generalized deficiency of mitochondrial iron-sulfur cluster containing proteins, including SDH [59, 60].

In mitochondria, under aerobic conditions, SDH catalyzes the oxidation of succinate to fumarate in the context of the metabolons that are known to orchestrate the protein-rich mitochondrial matrix (up to 500 mg/ml, *i.e.*, close of that found in a protein crystal [61]). In this dense medium, enzymes and cofactors are functionally clustered, but not necessarily constantly and uniformly throughout the matrix space [62-65]. Electrons derived from succinate oxidation to fumarate are passed through a series of redox centers to a closely located quinone pool. Contrarily to other RC complexes, electron transfer through SDH occurs without proton pumping in the intermembrane space (Figure 1).

SDH appears to play largely similar essential roles in all living organisms, which echoes its evolutionary conserved composition across species. This remarkable conservation undoubtedly accounts for the shared sensitivity of the enzyme to a wide variety of inhibitors regardless of the organisms from which it originates [66]. Markedly, SDH is among the respiratory chain enzymes whose activities have been shown to be surprisingly heat resistant [67]. Accordingly, cellular meltome studies identified SDH subunits among the cell heat resistant proteins [68]. Clustering of heat-releasing mitochondria in cells allows for heat-requiring processes to take place [69]. With a melting point close to 60°C, SDH subunits have therefore the intrinsic capacity to work stably in a hot mitochondrial background reaching 50°C; a temperature experimentally verified in a growing series of cell lines [70]. Interestingly, the gap between the high temperature values locally measured in mitochondria and the theoretical predictions is gradually fulfilled by growing evidence of unexpected intracellular thermal conductivity variations [71]. This might fit with the idea that transient temperature spikes occur in order to allow for significant heat differential between mitochondria, or part of it, and the surrounding cytosol under specific conditions [72].

SDH, also known as RC complex II, is not part of identified RC supercomplexes that involve varying combinations and ratios of complexes I, III and IV [73]. Nevertheless, along with mitochondrial ATP-binding cassette protein 1 (mABC1), phosphate carrier, adenine nucleotide translocator, and ATP synthase, complex II or a portion of it, has been reported to be part of a multiprotein complex (mitoK$_{ATP}$) conferring ATP-sensitive K+ channel activity to mitochondria [74]. It should be noted that the composition of RC supercomplexes may vary with physiological conditions and can be even heterogeneous within one mitochondrion [75, 76]. Identification of supercomplexes kinetically insulating ubiquinone pools supposes redox exchanges through spatially distant pools of ubiquinone [77]. The level of reduction of a given quinone pool is furthermore dependent on the kinetic properties of the dehydrogenase connected to the pool. The occurrence of kinetically distinct quinone pools does not oppose their diffusion/exchange especially in the presence of inhibitors. Such kinetic compartmentation presumably holds true for all

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dehydrogenases, *e.g.*, SDH (CII), glycerol 3 phosphate dehydrogenase, dihydroorotate dehydrogenase, NADH dehydrogenase (CI), dehydrogenases implicated in fatty acid oxidation, etc. (Figure 2). When active, all these dehydrogenases are susceptible to feed electrons to proton pumping RC supercomplexes for an ultimate reduction of oxygen to water by complex IV. Compartmentation also implies the coexistence of variably reduced ubiquinone sub pools within one mitochondrion. A locally highly reduced pool of ubiquinone may favor direct interaction with oxygen and the subsequent production of superoxides by auto oxidation reaction. High reduction of quinone may also render thermodynamically possible the reverse electron-flow through redox centers as long as an electron acceptor is quantitatively available. Typically, this type of reaction is typically favored by hypoxic conditions favoring quinone over reduction. Thus, driven by succinate, the reduction of ubiquinone by the SDH can reverse electron flow through CI resulting in the reduction of NAD^+ [78]. On the other hand, in the presence of fumarate and reduced quinones, redox equilibria can convert the reaction of the SDH ($\Delta G0$ = 0 kJ/mol) into a fumarase reductase leading to the production of succinate [79].

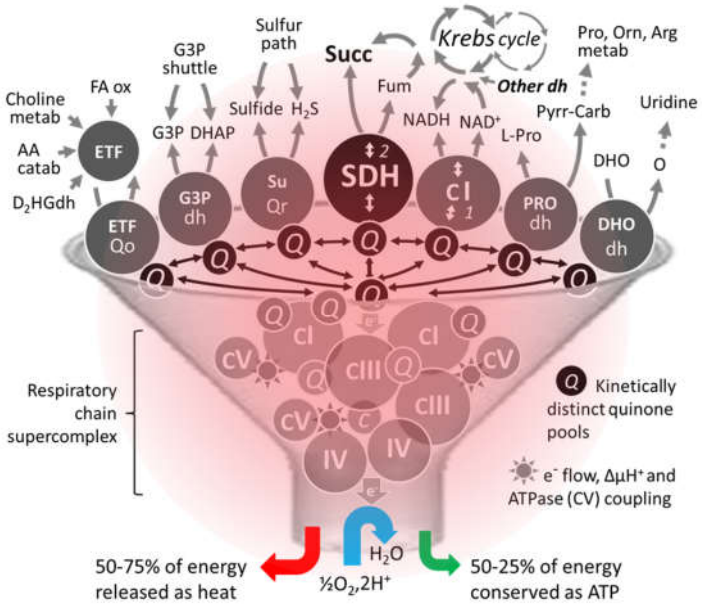

Figure 2. An unconventional schematic diagram featuring the succinate dehydrogenase among some of its competitors/coworkers for access to supercomplexes existing in the heating respiratory chain.

The number of dehydrogenases capable of reducing part of mitochondrial ubiquinone varies between organisms, while, in particular in humans, their activity and proportion can differ markedly depending on the organ. Due to this competition, small variation (<) can result in significant pathological consequences in humans. Abbreviations/symbols: AA catab, amino acid catabolism; Arg, arginine; dh, dehydrogenase; DHO, dihydroorotate; DHAP, dihydroxyacetone phosphate; D2HGdh, D-2-hydroxyglutarate dehydrogenase; ETF, electron transfer flavoprotein; FA ox, β fatty acid oxidation; Fum, fumarase; G3P, glycerol-3-phosphate; H2S, hydrogen sulfide; metab, metabolism; Orn, ornithine; O, orotate; Pro, proline; Pyrr-Carb, pyrroline 5-carboxylate; Q, (ubi)quinone pool; Qo, quinone oxidase; Qr, quinone reductase; Succ, succinate; SuQr, sulfide quinone reductase; I-V, respiratory chain complexes I-V.

As depicted in Figure 2, this also implies that, simultaneously to metabolic cooperation between substrates, dehydrogenases somehow compete for transferring electrons to the terminal part of the RC [80]. This also impacts the variable distribution of electrons from dehydrogenases to various terminal oxidases (obviously when these are present) as early reported in plant mitochondria [81]. Such kinetic compartmentation would be readily lost

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in the presence of an overpowered electron sink (*e.g.*, high alternative oxidase, AOX, expression).

Unlike the other RC complexes, SDH is activated under conditions where ubiquinone pools of the RC are rather reduced, such as under anaerobiosis. In addition, the activity of SDH is regulated by various co-factors. For instance, ATP binding to the SDHA is known to upregulate SDH activity when mitochondria are under what is referred as mitochondrial state 4 (high ATP/ADP ratio) that is marked by an increased proton motive force [82]. Conversely, SDH activity is downregulated or even fully inhibited by the oxaloacetate (OAA) that is produced by the malate dehydrogenase under state 3 (high ADP/ATP), OAA binding competitively to the succinate binding site.

SDH thus has the particularity of working just as much in the reduced state (state 4) as in the oxidized state (state 3). This will impact the status of the redox centers (1 Flavin, 3 FeS, 1 haem) that might well be partially reduced under both state 3 and 4. The reduction level of such redox centers is known to control the production of superoxides by the enzyme. Thus, rather than the rate of electron flux through the enzyme, it is the extent of the enzyme reduction level that determines the magnitude of superoxide production [83]. Accordingly, a similar inhibition of electron flux brought about by malonate (binding on the substrate site on Fp subunit) and thenoyltrifluoroacetone (TTFA) (binding on the quinone-binding site of the enzyme) (Figure 1B) results in opposite effect in term of superoxide production due to opposite effect on the status of the redox centers. This potential uncoupling between rates of electron flow and of superoxide production makes it hazardous to predict and quantify the actual consequence of genetic mutations or chemical inhibitors.

An abundant literature is devoted to the role of superoxide in cell biology, ranging from physiology to pathology [84]. Thus, through the production of superoxides, SDH may intervene in numerous key cellular processes. Moreover, in contrast to many RC-linked mitochondrial processes that tend to slow down under reduced conditions, which promote superoxides production, succinate dehydrogenase maintains a sustained activity and represents a perennial source of superoxides. However, this involvement in superoxide production must be closely regulated since superoxides are involved in many vital cellular processes including cell differentiation, cell proliferation, and cell death. Dealing with the peculiarities of superoxide production by SDH, different studies have been focused on the existence of micro domains involved in the production of superoxides at an enzymatic level [83]. On this occasion, it was shown that reverse electron flow through CI is not at the origin of superoxide production linked to succinate metabolism [86].

Like many enzymes of the intermediary metabolism, succinate dehydrogenase activity varies according to organisms and organs, and to their variable and fluctuating tissue- and cell-type specific metabolic demands [87]. Indeed, compared to the activities of the other complexes of the respiratory chain which appear to be mostly conserved between tissues, complex II activity varies in significant proportions among tissues [88]. IFor instance, SDH activity in the liver or in the kidney is greater than that of complex III which allows the SDH to remain in the reduced state, even when complex III is activated (phosphorylation state 3). Activities of other components of the RC may also varies among tissues (e.g. the glycerol 3-phosphate dehydrogenase which presence is even not generalized [87]), but their activities do not exceed the capacity of the RC to cope with their reducing activity. These enzymes presumably remain oxidized when the RC is freely working. The rate of quinone diffusion in the mitochondrial membrane between various dehydrogenases and RC complexes should also be taken into account. This rate may differ between tissues according to mitochondrial membrane composition and organization, being moreover affected by the long-ignored existence of super complexes in the respiratory chain. This discussion takes place because the reduction state of the redox centers of all these enzymes, up to eleven sites listed in mitochondria including the SDH and the G3Pdh, determines their propensity to generate superoxides so important in cellular physiology [89].

3. Mitochondrial and cellular entangling

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SDH links the activity of a segment of the Krebs cycle to that of the respiratory chain (Fig. 2). However, since, on one hand, electron flow through the SDH is not associated with a proton motive activity, the SDH does not participate in the supercomplexes of the respiratory chain, can initiate various reverse transfers of electrons that can escape the chain in the form of superoxides, the link with Krebs cycle appears to be particularly flexible. In contrast to most RC-linked enzymes, SDH activity is enhanced by a reduced environment favoring reverse electron transfer through complex I and the conversion of OAA, a major physiological inhibitor of the SDH, to malate. SDH activity is also enhanced by the binding of ATP to the enzyme, while in most segments of the respiratory chain ATP promotes the reduction the electron transfer activity by preventing the dissipation of the proton gradient across the ATP synthase.

The segment of the Krebs cycle in which SDH is engaged (Figure 3; [90]) represents a path through which glutamate (possibly arising from glutamine) can be substituted for acetyl-CoA (possibly arising from pyruvate) as a carbon source for the Krebs cycle. The multiplicity of metabolic pathways and the spatial organization of matrix proteins into metabolons have been studied and described in mitochondria from only some tissues of few species. Indeed, this intra-mitochondrial complexity further foreshadows the multiple possibilities of interaction within the changing cellular metabolic context in which mitochondria work. Hence reducing the functioning of the mitochondria to a simple scheme that can be generalized to all situations, all organisms and their organs is unreasonable. Some functional elements will however be found in all mitochondria but their layouts and activity levels vary in very significant proportions.

A rapid survey of the scientific literature of the last twenty years shows that succinate level and the activity of the enzymes and proteins producing it, using or being targeted by this metabolite, as well as the succinate-dependent redox signals, are all very tightly controlled in most living organisms. This shows the peculiarity and strategic role both the organic acid itself and associated enzymes play in cellular life.

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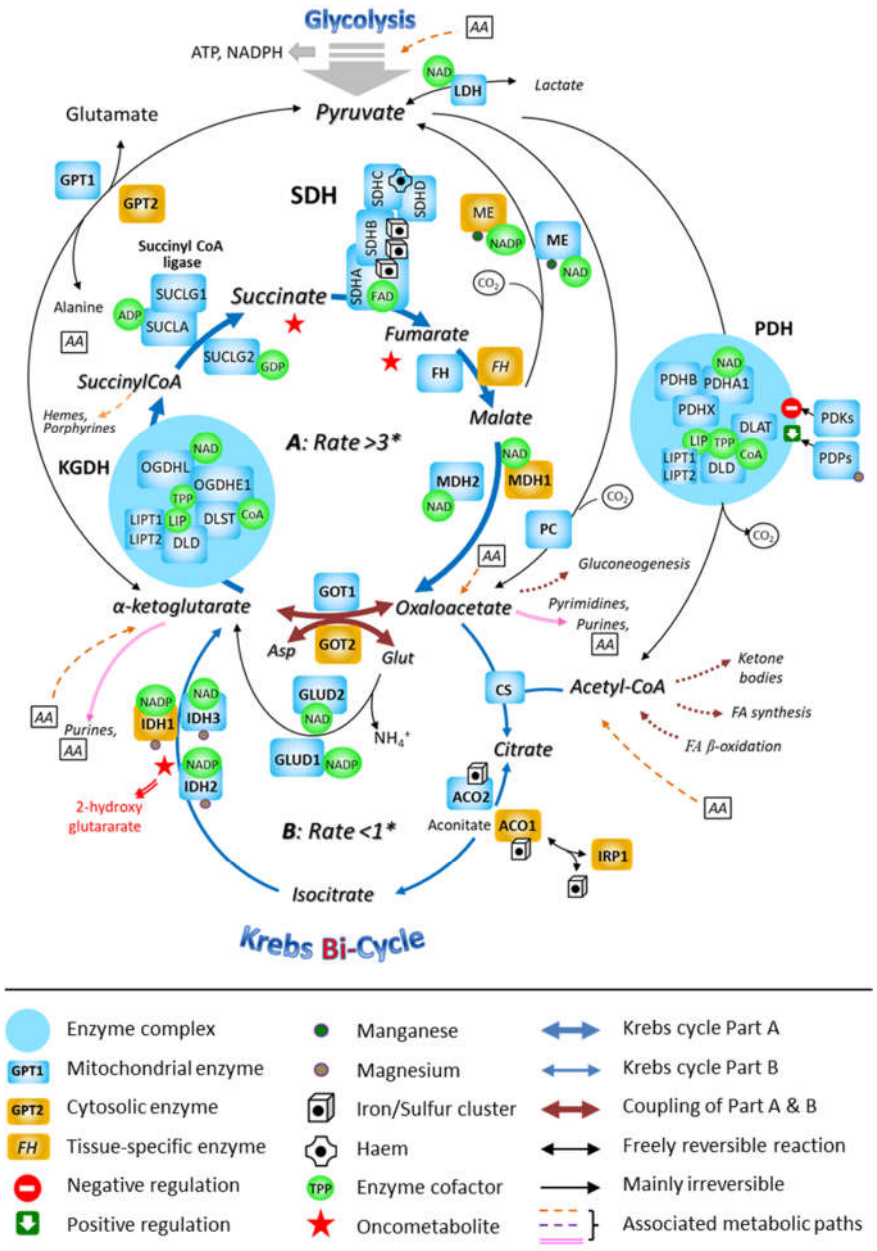

Figure 3. The double involvement of the mitochondrial SDH in the respiratory chain (respiratory chain complex II) and in the fast segments of the Krebs bi-cycle.

The Krebs cycle is represented as consisting of two parts due to different flow velocities that have been measured between each part A and B. * Respective fluxes through the segments A and B of the bi-cycle in nmol/min/mg prot [90]. Abbreviations: ACO, aconitase; ADP, adenosine-diphosphate; FA, fatty acid; CoA, coenzyme A; CS, citrate-synthase; DLAT, dihydrolipoamide-S-acetyl-transferase (E2); DLD, dihydrolipoamide-dehydrogenase (E3); DLST, dihydrolipoamide-S-succinyl-transferase (E2); FH, fumarate-hydratase; GDP, guanosine-diphosphate; GPT, glutamate-pyruvate-transaminase; GOT, glutamate-oxaloacetate-transaminase; GLUD, glutamate-dehydrogenase; IDH, isocitrate-dehydrogenase; IRP, iron-responsive protein; LDH, lactate-dehydrogenase; LIP, lipoic acid; LIPT1, 2: lipoyltransferase; MDH, malate-dehydrogenase; ME, malic enzyme; NAD, nicotinamide-adenine-dinucleotide; NADP, nicotinamide-adenine-dinucleotide-phosphate ; OGDH, oxoglutarate-dehydrogenase (α-ketoglutarate-dehydrogenase; OGDHE1: E1; OGDHL, E1-like); PC, pyruvate-carboxylase; PDH, pyruvate-dehydrogenase (PDHA1 and PDHB, α and β subunits of E1); PDHX, pyruvate-dehydrogenase compound X ; PDKs, pyruvate-dehydrogenase-kinases; PDP, pyruvate-dehydrogenase-phosphatase; TPP, thiamine-pyrophosphate; SDH, succinate-

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dehydrogenase; SUCL, succinyl Coa-ligase.The number of dehydrogenases capable of reducing part of mitochondrial ubiquinone varies between organisms, while, in particular in humans, their activity and proportion can differ markedly depending on the organ. Due to this competition, small variation can result in significant pathological consequences in humans. Abbreviations/symbols: AA catab, amino acid catabolism; Arg, arginine; dh, dehydrogenase; DHO, dihydroorotate; DHAP, dihydroxyacetone phosphate; D2HGdh, D-2-hydroxyglutarate dehydrogenase; ETF, electron transfer flavoprotein; FA ox, β fatty acid oxidation; Fum, fumarase; G3P, glycerol-3-phosphate; H2S, hydrogen sulfide; metab, metabolism; Orn, ornithine; O, orotate; Pro, proline; Pyrr-Carb, pyrroline 5-carboxylate; Q, (ubi)quinone pool; Qo, quinone oxidase; Qr, quinone reductase; Succ, succinate; SuQr, sulfide quinone reductase; I-V, respiratory chain complexes I-V.

4. Genetics and epigenetics: SDH and succinate Discussion

The genes encoding SDH subunits have now been sequenced in many species, ranging from microorganisms to higher mammals. Comparative sequence analysis indicated a spectacular conservation of the genes encoding the four core subunits of the enzyme with slight noted variations [66]. Paralogs of *SDH* genes (*Sdh3* and *Sdh4*) have been reported in *Saccharomyces cerevisiae* [91], while expression of a dispensable *Sdh*3 paralog has been shown to account for resistance to SDH inhibitor (SDHI) in another fungus, *Zymoseptoria tritici* [92]. Various mechanisms, *i.e.,* alternative splicing [93], promoter usage [94], or post-transcriptional modifications [95], contribute significantly to the large variation in expression patterns of SDH limited number of subunits, which confers a huge flexibility to SDH to adapt to the various physiological conditions. Alternative splicing of *SDHC* has for instance been described in human and reported to play a role in rare human pathologies [93].

Metabolism sustains cellular life. In turn, cell must sense the nutritional status, making use of multiple signaling networks. Accordingly, numerous chromatin modifying enzymes respond to metabolites and metabolic enzyme cofactors. For instance, it has been shown that Histone Acetyltransferase uses Acetyl-CoA as cofactor, while PARPs and Sirtuins activity are dependent on Nicotinamide Adenine Dinucleotide (NAD), Lysine demethylase 1 on Flavin Adenine Dinucleotide (FAD), DNA and Histone methyltransferases on S-adenosyl-methionine (SAM) [96]. Finally, the activities of several dioxygenases catalyzing α-ketoglutarate-dependent hydroxylation of proteins (*e.g.*, HIF 1 α), as well as the activities of the TET-Family DNA demethylases and of Jumonji C-family Histone demethylases are sensitive to α-ketoglutarate/succinate balance. In humans, the specific role of succinate in these crucial reactions was brought to light when mutations in genes encoding various SDH subunits were uncovered. Mitochondrial metabolites and cofactors have until now been seen primarily as sources and regulators of cellular energy through ATP synthesis. We now know that they play infinitely more complex roles that are still largely to be dissected [5]. These long-ignored mechanisms control through epigenome remodeling, and in a very coordinated manner, cell proliferation and death as well as cell differentiation in all living organisms.

Most chromatin-modifying enzymes use as cofactors important intermediates of cellular metabolism. Depending on dietary intake, the level and distribution of metabolites will vary and affect the expression of a large number of genes by modulating the activity of epigenetic pathways and associated chromatin modifying enzymes. Considering that the methylation of DNA and each post-translational modification (PTM) of proteins can be affected by many metabolic pathways, the epigenome might act as a sensor of the whole metabolic network.

As a result of a decreased activity of any of the Krebs cycle enzymes, the overall activity of the cycle may decrease, as long as the affected enzyme controls the rate of the segment where it acts. Simultaneously, a subset of the cycle intermediates may escape the mitochondrial matrix and accumulate in the cytosol resulting in an imbalance of organic acids within mitochondria and more generally in the cell. In case of SDH blockade, it was

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established in as early as 2005 that succinate accumulated in cells to an extent that disrupted the equilibrium with α-ketoglutarate, which altered the activity of the prolyl-hydroxylase that controls the hypoxia-sensitive pathway. This disruption of the transcriptomic program is now recognized as triggering tumorigenesis and cancer.

5. An unexpected spectrum of human diseases

Heterozygous germline mutations in the SDH complex (*SDHx*) genes (*SDHA, SDHB, SDHC, SDHD* and *SDHAF2*), which act as tumor suppressor genes, predispose to pheochromocytomas and paragangliomas (PPGLs) and rarely to gastrointestinal stromal tumors (GIST), renal cell carcinoma and pituitary adenomas. *SDHx* genes associated germline mutations (substitution or deletion, large copy number variation, promoter hypermethylation or epimutation) have an autosomal-dominant inheritance, which implies the occurrence of a secondary somatic genetic event at specific gene locus (loss of the wild type allele or loss of heterozygosity, another genetic variant in trans allele, promoter hypermethylation or epimutation) to trigger tumorigenesis. The first mutations in a *SDHx* gene (*SDHD*) have been reported in patients with PPGL in 2000 [97]. Today, it is well known that around half of patients with PPGL carry a germline mutation in genes encoding tricarboxylic acid enzymes such as succinate dehydrogenase (*SDHA, SDHB, SDHC, SDHD, SDHAF2*), fumarate hydratase (*FH*), malate dehydrogenase (*MDH2*) and dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex (*DLST*), as well as components of the malate-aspartate shuttle such as the oxoglutarate/malate carrier (*SLC25A11*) or the mitochondrial aspartate aminotrans-ferase (*GOT2*), which all lead to cancer via different mechanisms including epigenetic modifications [98].

Paragangliomas are rare neuroendocrine tumors that can develop in parasympathetic and sympathetic paraganglia, from the skull base to the pelvic region, while pheochromocytomas arise in adrenal medulla. Catecholamine-producing tumors (pheochromocytoma and functional paraganglioma) are severe disorders, sometimes revealed by life-threatening emergencies or by hypertension and/or cardiovascular morbidities. Non secreting paragangliomas are most frequently located in the head and neck and revealed by a cervical mass or a hearing loss. International recommendations stated that affected patients should be referred to multidisciplinary expert centers for imaging, treatment, and management. Surgical resection or therapeutic radiations are usually discussed as first options [99]. PPGL become metastatic in around 15% of the cases [100]. [100].

Large international cohorts of patients with *SDHx*-related PPGL have revealed interesting phenotype-genotype correlations. Patients with *SDHD* pathogenic variants develop predominantly multiple head and neck tumors whereas patients carrying *SDHB* pathogenic variants develop usually extra-adrenal, retroperitoneal, or pelvic paragangliomas. The identification of a germline mutation in *SDHB* gene is a risk factor for malignancy. Nevertheless, whatever the mutated *SDHx* gene, mutation-carriers can present all the disease spectrum. Since large and metastatic tumors at diagnosis are difficult to treat, it is recommended to propose genetic counselling in first relatives to detect small asymptomatic tumors in mutation carriers [99]. An international consensus on initial screening and follow-up of asymptomatic *SDHx* mutation carriers was published in 2021. A first tumor screening including clinical evaluation and total body magnetic resonance imaging and assessment of metanephrines (catecholamine metabolites) in plasma or urine is recommended between 6 and 10 years of age for asymptomatic *SDHB* mutation carriers and between 10 and 15 years of age in asymptomatic *SDHA, SDHC* and *SDHD*-paternal inherited mutation carriers that should be repeated every two or three years [101].

Another *SDHx*-related neoplasia is the gastrointestinal stromal tumors commonly referred to as succinate dehydrogenase (SDH)-deficient GISTs. Around 50% of SDH-deficient GISTs are caused by hypermethylation of the *SDHC* promoter (epimutation), 30% by a germline mutation in *SDHA* and 20% to 30% in *SDHB, SDHC*, or *SDHD*. SDH-deficient GISTs are usually diagnosed in children and young adults. They appear to be

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specifically located in the stomach. Frequently indolent, they can be multifocal and metastatic. SDH-deficient renal cell carcinoma is rare, accounting for less than 1% of renal carcinomas. In most of cases, they are caused by a *SDHB* gene mutation and diagnosed in a young adult [102]. SDH-deficient pituitary adenomas are benign tumors, treated by surgery and/or dopamine agonists, which can produce prolactin and/or growth hormone or to be non-functional. Only in very few cases, the causality between SDHx variants and pituitary adenoma has been clearly established [103]. SDHB immunohistochemistry is a well-validated tool for detecting SDHs-related neoplasia after surgery. Whatever the mutated SDH subunit, a loss of protein B expression is observed in the tumor tissue. Furthermore, SDHA immunohistochemistry detect SDHA-related tumors specifically [104]. Recently, an *in vivo* approach has been developed to confirm the *SDHx*-mutated status of a tumor by proton magnetic resonance spectroscopy, which is able to detect a succinate peak testifying to the succinate accumulation in the tumor resulting from succinate dehydrogenase inactivation [105].

The last forms of SDH-deficient diseases are mitochondrial complex II deficiencies causing early-onset progressive neurodegenerative autosomal recessive disorders due to homozygous or heterozygous composite *SDHx* genetic variants. Most clinically affected individuals reported in the literature harbor genetic variants within the *SDHA* gene and present with a Leigh syndrome, epileptic encephalopathy, and cardiomyopathy [106]. Less common, pathogenic variants involving the *SDHB, SDHD* and *SDHAF1* genes have also been reported [25, 107]. The first recessive *SDHA* gene mutation was reported in two siblings diagnosed in the early childhood for a typical Leigh syndrome, hallmarked by the hypo density of the white matter revealed by a computer tomography scan (CT scan). They both presented rather similar disease course, developed normally up to 10 months, when they rapidly presented marked rigidity, bilateral pyramidal tract signs, cortical blindness, and difficulties in swallowing fluids. They died in their second year of life. The mutation caused a severe, yet partial deficiency of the SDH activity, resulting in a tissue-specific involvement [3]. Bi-allelic genetic variants were reported in *SDHB* in a dozen patients developing infantile leukoencephalopathy [108]. Compound heterozygous mutations in the *SDHD* gene were first reported in a girl with encephalomyopathy and biochemical evidence of isolated mitochondrial complex II deficiency who died at 10 years and homozygous *SDHD* mutation was found in an infant with fatal cardiomyopathy and mitochondrial complex II deficiency [109-110].

6. A worrisome environmental context

Succinic acid is a natural constituent of basically all organic tissues, varying according to time and conditions. Its distribution is not only limited to organisms but also extends to their biological products, *e.g.,* honey [111]. Succinate plays fundamental roles in many key molecular, cellular and physiological processes. Modulating the supply of succinate represent therefore a mean by which the physiology of organisms may be affected. Thus, succinate made available to gut microorganisms can also affect the physiology of their host [112, 113]. Succinate is quite stable. Baltic amber, resulting mainly from the fossilization of the conifer resin, contains up to 8% of succinic acid in its surface, hence the name amber acid sometimes given to succinate [111]. It is also worth mentioning that a significant number of virtues especially in term of human health have been attributed to succinate, which enabled a sustained trade for many years. Currently succinate is commercially produced and approved by the US Food & Drugs Administration.

6.1. Natural SDH inhibitors

Considering the crucial role of succinate metabolism, it is not surprising that many organisms, including humans, attempt to get rid of predators and pests by targeting their ability to metabolize succinate. Many plants produce, among various mitotoxic

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compounds targeting diverse mitochondrial functions, a subgroup of compounds susceptible to act on the SDH enzyme. Thus, malonate accumulates in several plant families, frequently in leguminous plants. In chickpea, malonate is the predominant acid in roots and nodules, but not in leaves. This has been taken as an indication of a defensive role is these plant parts [114]. On the other hand, several bacteria, fungi, mammals, and plants possess the ability to metabolize malonate [115, 116]. Malonate might also have beneficial effect. Intracoronary-injected malonate, as well as dimethyl malonate which can be found in several plants (*e.g.*, *Ananas comosis*, *Myrtus communis*, *Astragalus sp.*), affords cardio protection against ischemia-reperfusion injury. However, malonate having simultaneously toxic effects its systemic administration in animals is precluded [117] [118]. Another substance, Atpenin A5 is known as an antifungal antibiotic acting through SDH inhibition, being quite active as well again nematode and human SDH (respective IC_{50} = 12 and 3.7 nM). It has been initially extracted from *Penicillium sp*. As malonate or dimethyl malonate, Atpenin A5 is cardio protective. The effect of these SDH inhibitors has been ascribed to an activation effect on mitochondrial K+ channels which relationship with SDH remains obscure [119, 120]. Moreover, the modulation of SDH activity by these effectors has an unpredictable cardioprotective effect [121]. Noticeably, these molecules do not similarly bind to the SDH. While malonate is well known as a competitive inhibitor of the enzyme at the substrate site, Atpenin A5 is a highly specific ubiquinone-binding site inhibitor of the SDH enzyme (IC_{50} 12 and 3.7 nM in nematode and mammalian mitochondria, respectively). Binding to these two opposite sites on SDH has been reported to have opposite effects in terms of superoxide production. As malonate and Atpenin act on the two opposite inhibitor sites of the SDH (Figure 1B), it appears difficult to envision modulation of superoxide production (by SDH) as instrumental in cardio protection, since these two inhibitors have a similar effect.

Several other SDH inhibitors have been identified, mostly in plants or microorganisms. 3-nitropropionate, also known as Bovinocidin, or Hiptagenic acid, a glycoside found in numerous fungi and upper part of plants, is readily liberated in the gut of herbivores, allowing it to exert its toxic effect [122] [123]. Nitro propionate is noticeably present in the poisonous plant *Indigofera tinctoria* used to prepare indigo dyeing [124]. It is a suicide inhibitor of the SDH which displays antimycobacterial properties and is used for skincare. This toxin can also originate from food contaminated by *Aspergillus sp*. It has been used to model Huntington's disease. This neurodegenerative disease often presents subtle problems with mood or mental abilities followed by a general lack of coordination and an unsteady gait [125]. Used to mimic Huntington's disease in rat [126], it causes behavioral anomalies, with somnolence and general depressed activity, ultimately resulting in coma. It has also been used in mouse, causing ataxia [127], *drosophila* [128] and baboons [129]. Other intended use of another SDH inhibitor, Siccanin, initially isolated from a pathogenic fungus has been recently proposed as a new chemotherapeutic [130]. Yet another use, that of Promysalin a secondary metabolite targeting SDH produced by the bacteria *Pseudomonas putida* has been proposed to inhibit the growth of other *Pseudomonas* as the Gram-negative pathogen *Pseudomonas aeruginosa* [131, 132]. SDH inhibitors are also found as secondary metabolites in various strains of *Penicilium roqueforti* used in the famous French Roquefort cheese.

6.2. Chemical inhibitors: Poisons and medicines

Beside these naturally occurring SDH-inhibitors, all with quite limited spreading, agrochemical industry produces and recommends the widespread use of SDH inhibitors (SDHI) to counteract fungi proliferation on seeds, plants and their products, fruits, and vegetables. SDHI just in a few tens of years have been massively disseminated in nature, from now on impregnating living organisms and the entire biosphere. The dissemination of unspecific pesticides such as SDHI has already devastating consequences, illustrated by the major collapse of insect, bird populations to only mention the most visible concerned organisms. It is important to note that it is not really feasible to quantify the impact on the soil microorganisms in terms of their populations and respective equilibria.

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Concerning human health, it has become a nightmare to estimate the impact of any substance, including SDHI molecule, taking into account the number of molecules utilized, their frequent changes, and the variable exposition of the population.

Resulting from their generalized use, SDH inhibitors are now omnipresent in the various compartments of the biosphere (earth, water, air) including protected areas [133]. Their generally low concentration may be functionally counterbalanced by the simultaneous presence of several tens, or more, of toxic molecules [134], in particular compounds affecting the use of oxygen or the detoxification of reactive oxygen derivatives. The role of this pesticide mixture is regularly pointed out in the biodiversity loss. It may also have a role in the evolution of various human diseases where mitochondrial, especially SDH, activity is impaired [135]. Indeed, while human diseases originating from SDH gene mutations/losses are now fully recognized, the mechanism of disease progression is far to be understood. As in most mitochondrial diseases, variable expression, or variable times of onset, even within a given family, has been observed. Thus, beside individual genetic factors, this leaves room for disease modulation by additional factors.

Thus, to only speak of the diseases where the SDH is affected, altitude has been identified through population studies, as a phenotypic modifier in hereditary paraganglioma type 1, resulting from SDH gene mutations, evidence pointing to an oxygen-sensing defect [136]. Dysregulation of HIF1α has been further recognized as instrumental for this oxygen-sensitive process. Oxygen has also been recognized as a phenotype-modifier in a mitochondrial disease such as Friedreich ataxia where SDH is involved and signaling of cell antioxidant defenses is being weakened. More generally, environmental-induced oxidative stress is known as key actor in the course of mitochondria-associated neurodegenerative disorders and aging [137].

The widespread use of SDHI is known to result in the rapid apparition, in few years, of numerous fungi mutants. This makes the long usage of each SDHI problematic. This has implied a consistently faster turnover of SDHI molecules of the last generation, additionally interfering with other ubiquinone-binding proteins such as those involved in complex III. On the other hand, the widespread use of SDHI increases the risk of appearance of dangerous drug-resistant microorganisms, including fungi [138-140]. These, with potential growth advantages, could in the future become multi-drug resistant and have uncontrollable broadening targets, from plant to other living organisms.

7. Concluding remarks

The conclusion that emerges from this review covering a wide field of scientific knowledge is that we face more questions than answers despite the colossal work conducted by so many scientists on SDH and its substrate succinate for nearly a century. This stands true whatever the angle from which the subject is approached, whether starting from the enzyme or its substrate.

To start from the most fundamental aspect: the transfer of electrons through the enzyme still retains a part of the mystery. For example, the function of the haem com-ponent of SDH is hypothetical. Does it act as a source or a trap vis-à-vis superoxides, or both? Moreover, several redox centers have the capacity to generate superoxides but which one of them is involved in this production is not known in many conditions. Furthermore, the production of superoxides depends on the redox state of these centers, but what is the extent of the relationship between the speed of the flow of electrons through the enzyme and the reduction state, in other words the relationship between the activity level of the complex and the production of superoxides remains to be established.

Another unsettled question concerns the interactions of SDH with other complexes and supercomplexes of RC, or other constituents of the inner mitochondrial membrane and the matrix. It should be however emphasized that such interactions may vary over time depending on the conditions, on the organisms, the organs or even depending on the location of the enzyme in the heterogeneous mitochondrial space. The oversimplification of these aspects often implemented for honorable pedagogical reasons, ends in unrealistic

structural and functional models that do not correspond in any way to the numerous physiological realities.

Concerning the dichotomy of clinical presentations associated with mutations in the various SDH encoding genes, primarily discussed on the basis of the SDH subunit genes found mutated, [141], it should be noted that additional clinical presentations have also been reported, e.g., isolated cardiomyopathy [110, 142]. It is worth Then, it is fair to note that mentioning that similar contrasting clinical presentations have been reported associated with mutations in most Krebs cycle of the enzymes of the Krebs cycle, including fumarase, a tetrameric enzyme encoded by… a single gene [2, 143].

Regarding the substrate of SDH, succinate, it has been known for long as a key intermediate of mitochondrial metabolism and consequently of the whole intermediary metabolism. Succinate has also a well-established role in the mitochondrial oxygen-dependent energy production in the form of heat or ATP. Once again, this role is variable according to the organisms, organs, and situations, until constituting a tangle that is difficult to reduce to oversimplified metabolic diagrams. The question is far from academic at a time when computer-assisted in silico representation of metabolism based on such diagrams are proposed to shed light on the effect of inhibitors on metabolism [144]. Thus, it has been "demonstrated" that a blockade of SDH (in this instance by SDHI) in human should not result in succinate accumulation, ignoring the large number of studies that have measured this accumulation resulting from inhibitors or genetic anomalies [96, 107, 145-151]. Such an accumulation is now used routinely to detect tumors linked to SDH defects [105].

We now also know that succinate is a key oncometabolite in both cell differentiation and proliferation, due particularly to its effect on several methylases involved in controlling the expression of the genome. In this way the metabolic balances involving succinate play a crucial role during the development of living organisms. Through succinate-dependent prolyl hydroxylases, it also controls the response to factors important for aerobic organisms and their various responses to hypoxia. Again, the entanglement of functions involving succinate, even considering only a single organism, appears such that it cannot be summarized in over simplified diagrams.

In such a context, one should understand, as Singer and colleagues did 40 years ago [152, 153], the major risk for the entire biosphere, including man [154], of using this metabolic crossroad as a target for any large scale or long duration treatment.

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