ASCORBATE SUBCELLULAR COMPARTMENTATION AND ITS DISEASES

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Abbreviations: AA, ascorbic acid; ATS, arterial tortuosity syndrome; DHA, dehydroascorbic acid; ER, endoplasmic reticulum; GLO, gulonolactone oxidase; HIF, hypoxia inducible factor; NE, nuclear envelope; 2OG, 2-oxoglutarate; PDI, protein disulfide isomerase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SVCT, sodium-dependent vitamin C transporter; TET, ten-eleven translocation.

Abstract

Beyond its general role as antioxidant, specific functions of ascorbate are compartmentalized within the eukaryotic cell. The list of organelle-specific functions of ascorbate has been recently expanded with the epigenetic role exerted as a cofactor for DNA and histone demethylases in the nucleus. Compartmentation necessitates the transport through intracellular membranes; members of the GLUT family and sodium-vitamin C cotransporters mediate the permeation of dehydroascorbic acid and ascorbate, respectively. Recent observations show that increased consumption and/or hindered entrance of ascorbate in/to a compartment results in pathological alterations partially resembling to scurvy, thus diseases of ascorbate compartmentation can exist. The review focuses on the reactions and transporters that can modulate ascorbate concentration and redox state in three compartments: endoplasmic reticulum, mitochondria and nucleus. By introducing the relevant experimental and clinical findings we make an attempt to coin the term of ascorbate compartmentation disease.

Keywords: ascorbate; dehydroascorbic acid; compartmentation; endoplasmic reticulum; mitochondria; nucleus; scurvy; arterial tortuosity syndrome; GLUT; SCVT.

Introduction

During the elapsed 80 years since the discovery of ascorbic acid (AA) as the antiscorbutic vitamin [1], the knowledge on its biological functions, absorption, distribution, catabolism and excretion has been notably expanded. Ascorbate functions started to be defined not only in the whole body, but also on cellular or even subcellular levels. Ascorbate deficiency results in the development of scurvy in species unable to synthesize this compound (*e.g.* in humans). It has been hypothesized that decreased transport of vitamin C into the cells may cause latent or intracellular scurvy. This theory already assumed the role of transporters in the pathogenesis. Recent observations suggest that subcellular scurvy may also exist; decreased ascorbate transport into or increased consumption within a compartment results in scurvy-like phenotype even in an ascorbate synthesizing species.

Almost all known functions of AA are based on its easy oxidability: ascorbate can undergo two consecutive, one-electron oxidations resulting in the formation of ascorbyl radical and dehydroascorbic acid (DHA). Being an excellent electron donor, AA distinguished itself as a major water-soluble antioxidant [2]. AA efficiently detoxifies reactive oxygen (ROS) and nitrogen (RNS) species, which are commonly formed during the metabolism and in stress situations, and could damage cellular components (Fig. 1). In addition, AA serves as a reducing cofactor for many enzymes, including copper-containing monooxygenases [3] and Fe(II)/2-oxoglutarate (2OG)-dependent dioxygenases [4] (Fig. 1). Through these enzymatic reactions AA is required for the synthesis of carnitine [5] and catecholamines [6], as well as the posttranslational modification of extracellular matrix proteins, including collagen [7,8]. Moreover, AA-dependent regulatory processes have been also emerged, such as normoxia-hypoxia response by hypoxia inducible factor (HIF) hydroxylation [9] and epigenetically relevant oxidation reactions affecting methylated histones and nucleic acids [10].

AA utilizing reactions can be found in all subcellular compartments of mammalian cells. The antioxidant function is presumably present everywhere with an emphasis on the oxidative organelles such as mitochondria, peroxisomes and endoplasmic reticulum (ER). The occurrence of AA-dependent enzymes has been reported in different compartments, *e.g.* HIF prolyl hydroxylases are present in the cytosol, collagen prolyl/lysil hydroxylases in the ER lumen [7,8], dopamine β-monooxygenase and peptidylglycine α-hydroxylating monooxygenase in the chromaffin granules, synaptic and secretory vesicles [11], histone and DNA demethylases in the nucleoplasm. Since AA and its oxidized products are charged and

water-soluble molecules, transporters are crucial to keep vitamin C concentrations optimal in the organelles. AA/DHA transporters have been essentially characterized in the plasma membrane (Fig. 2). AA can be taken up by a secondary active transport mediated by sodium-dependent vitamin C transporters SVCT1 (*SLC23A1*) and SVCT2 (*SLC23A2*). SVCT1 is expressed in epithelial tissues, contributing to the maintenance of whole-body vitamin C levels, whereas the expression of SVCT2 is relatively widespread (for a recent review see [12]). DHA, the oxidized form of AA is taken up by facilitated diffusion mediated by the members of the GLUT family [13]. For example, in SVCT1 knockout mice the absorption of vitamin C can be normalized by adding DHA to the diet, which crosses the intestinal epithelium *via* GLUTs of the plasma membrane [14]. Nonetheless, under normal circumstances DHA transport is thought to be less significant since DHA is unstable and therefore present at low concentrations in extra/intracellular compartments. However, GLUT-dependent uptake becomes more intense in oxidative stress and can be stimulated by extracellular oxidants or by a local oxidase activity [15]. Although the presence and functioning of these transporters in the plasma membrane is well documented, little is known about their distribution in the endomembranes. Table I show the most important characteristics of GLUTs and SVCTs possibly involved in AA/DHA transport in the subcellular compartments.

Endoplasmic reticulum: ascorbate in dual role

In vertebrates AA is synthesized by the ER-resident integral membrane protein L-gulonolactone oxidase (GLO with the production of equimolar hydrogen peroxide as byproduct [32], or taken up with the diet. Indeed, in certain species including humans, GLO is mutated and fully inactive [33] and the presence of vitamin C in the diet is essential. Hepatic AA synthesis is accompanied with intraluminal oxidation of glutathione [34-36] and protein thiols [37,38] likely due to hydrogen peroxide formation within the ER. These findings, together with the predicted membrane topology of GLO suggest the intraluminal orientation of its active site. Thus, the ER in all cells of vitamin C-dependent species needs transport mechanisms to ensure the substrate supply of intraluminal vitamin C utilizing enzymes. Moreover, being GLO prevalently expressed in liver [39,40], transport mechanisms for vitamin C should be operative in the ER of all extrahepatic cells. In spite of these considerations, AA/DHA transporters have not been characterized at molecular level in the

ER. A functional study found a preferential DHA uptake in mammalian ER-derived vesicles and the characteristics of the transport suggested the involvement of GLUT-type transporter(s) [41]. In line with this assumption, it was observed that AA hardly crosses the ER membrane [41] and its oxidation (*i.e.* DHA formation) is a prerequisite of the uptake [42]. An AA oxidase activity associated with the ER fraction has been also reported [43], as a possible promoter of the uptake. More recently, GLUT10 has been proposed as an ER DHA transporter [26], but the fact that its inherited deficiency is restricted to certain cell types only (see below), suggests that other ER DHA transporters likely exist (Fig. 3). To give a comprehensive view of ascorbate transport in the ER it should be mentioned that partial colocalization of SVCT2 and protein disulfide isomerase (PDI) as a protein marker for ER has been shown recently. The colocalization results were supported by the copurification of SVCT2 with calnexin [29]. Further functional characterization of ascorbic acid transport in the ER is required to estimate its contribution to vitamin C uptake by the organelle.

Luminal AA is required for local antioxidant defense in the ER. During the electron transfer in the ER-resident cytochrome P450 system, ROS can be formed as a result of chemical accidents [44]. The terminal oxidase of the oxidative protein folding (Ero1) also generates hydrogen peroxide in the lumen [45,46]. High local concentration of AA is probably important to balance these prooxidant events. Moreover, at least in cells involved in collagen/elastin production, ER luminal Fe(II)/2OG-dependent dioxygenases (*e.g.*, prolyl hydroxylases and lysyl hydroxylases) also require AA for maintaining the redox state of ferrous iron at the active site of the enzymes (Fig. 3, reaction as in Fig.1c) (see [8,47] for recent reviews).

In the reactions above AA behaves as an electron donor (Fig. 1). However, a pro-oxidant role of AA might also be present in the ER. DHA, either formed in local reactions or transported into the lumen of the ER can be reduced by PDI, oxidizing the active central dithiols of the enzyme; oxidized PDI reacts with reduced substrate proteins yielding protein disulfides and catalytically regenerating PDI (Fig. 3) [48]. Alternatively, DHA can rapidly react with dithiols in unfolded or partially folded proteins in a PDI-independent manner [49].

In vivo findings also prove that luminal electron donor and electron acceptor functions of ascorbate mutually supports each other. Results gained in scorbutic guinea pigs showed that not only prolyl- and lysyl-hydroxylation was disturbed, but the missing prooxidant effect led to ER stress, presumably due to an impairment of oxidative protein folding [50]. Moreover, compromising the oxidative protein folding in mice with combined loss-of-function mutations in genes of the key enzymes (ERO1α, ERO1β, and peroxiredoxin 4) resulted also

in decreased hydroxyproline formation and defective intracellular maturation of procollagen. A noncanonical form of scurvy, *i.e.* ascorbate depletion was also observed, due to the consumption of ascorbate as a reductant for sulfenylated protein intermediates of oxidative folding aberrantly formed in the absence of ERO1α, ERO1β, and peroxiredoxin 4 [51,52].

Vitamin C and mitochondria

Although the first report on mitochondrial AA/DHA transport was published more than 30 years ago [53], many details of the transport became evident in the last few years (see Fig. 4 for a schematic representation of AA/DHA transporters in mitochondria). The previous findings that vitamin C can enter plant mitochondria as DHA [54] was confirmed by Golde and co-workers [17] in animal cells. A stereo-selective mitochondrial D-glucose uptake mechanism, which competes with the transport of DHA, was found in mitochondria from mammalian cells. In accordance with the computational analysis of the N-terminal sequences of human GLUT isoforms, the presence of GLUT1 in mitochondrial membrane was verified by mitochondrial expression of GLUT1-EGFP, immunoblot analysis and by cellular immunolocalization [17].

More recently, GLUT10 was also reported to be localized to the mitochondria of mouse aortic smooth muscle cells and insulin-stimulated adipocytes [24]. GLUT10 enhanced the entry of radiolabeled DHA into mitochondria, which was accompanied with the reduction of ROS levels in H_2O_2-treated smooth muscle cells. This protecting effect could be abolished by glucose pretreatment or by RNA interference of GLUT10 mRNA expression in smooth muscle cells [24].

Up to now DHA was considered to be the transported form of vitamin C through the mitochondrial inner membrane. Mitochondrial uptake and accumulation of the reduced form, AA could not be observed in mitochondria from human kidney cells or from rat liver tissue [17,55]. A recent report on the mitochondrial expression of the sodium dependent ascorbic acid transporter, SVCT2 in U937 cells raised the possibility of the existence of ascorbic acid transport through the inner mitochondrial membrane [28,56]. Very recently the relevance of this transport mechanism has been proven by further experimental results. Confocal colocalization experiments and immunoblotting of proteins extracted from highly purified mitochondrial fractions of HEK-293 cells confirmed that SVCT2 protein was associated mainly with mitochondria. A substantial level of SVCT2 colocalization could be also

observed with the endoplasmic reticulum marker PDI and a very low level of colocalization with the plasma membrane protein marker GLUT1 [29]. This localization pattern was observed in 16 different human cell lines including normal, neoplastic and primary cell cultures. The silencing of SVCT2 expression by SVCT2-siRNA caused the decrease of mitochondrial protein abundance of SVCT2 by at least 75%. Consistent with the decrease of protein abundance the capacity of ascorbate transport of mitochondria from siRNA-treated cells was only the one fourth of control cells [29]. The mitochondrial localization of GLUT1 could not be reinforced in the case of HEK-293 cells, thus the general role of GLUT1 in mitochondrial vitamin C transport can be queried. The same research group did not find GLUT10 expression in HEK-293 cells [29]. Hence it is hard to say anything about the contribution of this and another GLUT isoforms to the transport of vitamin C through the mitochondrial membrane.

Irrespectively of the way of vitamin C uptake, mitochondria must have an efficient mechanism for ascorbate recycling, otherwise DHA taken up or generated in the matrix would be lost within minutes under physiological circumstances [57]. Addition of DHA to mitochondria resulted in a reduction of DHA and mitochondrial AA concentration reached the millimolar range [55]. Various mechanisms have been suggested for intramitochondrial AA recycling. Mitochondria are capable of reducing DHA to AA in an α-lipoic acid dependent manner [58]. Thioredoxin reductase in mitochondria could also reduce DHA. However, the inability to detect a significant decrease in DHA reduction by mitochondria isolated from selenium deficient animals suggested that it is a small component in the mitochondrial DHA reduction machinery [55]. Since DHA loading caused a significant decrease in mitochondrial GSH content and the depletion of mitochondrial GSH content caused significant impairment in DHA reduction, it is likely that GSH dependent reduction of DHA is one of the major AA recycling reactions in mammalian mitochondria [55]. Using specific substrates and inhibitors, the role of mitochondrial electron transport chain in DHA reduction was also described and the site of AA sparing was localized to complex III [59].

The elevated level of ROS can induce the collapse of the mitochondrial membrane potential, leading to cell death. The mitochondrial membrane potential could be partially conserved and the denaturation and mitochondrial release of cytochrome c upon H_2O_2 treatment could also be avoided by DHA pretreatment in HL-60 cells [60], after FAS-induced apoptosis in monocytes [61] or in cells undergoing hypoxia-reperfusion [62]. These findings suggest that mitochondrial AA is an important component in the maintenance of the mitochondrial membrane potential and that AA exerts its anti-apoptotic effect through ROS scavenging

[55,60-64]. Furthermore, it was clearly demonstrated that AA protected mtDNA against the ROS-induced elevation of 8-oxo-dGuanidine and apurinic/apyrimidinic sites. AA preload could also significantly attenuate the hydrogen peroxide induced shearing of mtDNA [55]. These findings were confirmed by the significant reduction of hydrogen peroxide induced lesions in mtDNA by vitamin C in retinal pigment epithelium cells [65]. Thus, experimental data suggest that AA is predominantly utilized as an antioxidant in the organelle.

Ascorbate in the nucleus: regulation of gene expression

It was clearly evident from the previous ample literature that AA from one hand is able to affect proliferation and differentiation, and from the other hand to modulate gene expression in a variety of cell types. AA induces *in vitro* cell proliferation/differentiation of fibroblasts, osteoblasts, chondrocytes, myoblasts, bone-marrow mesenchymal stem cells (see [66] and refs therein) and, generally speaking, promotes the cell reprogramming process [67]. AA not only permits collagen hydroxylation, but also upregulates procollagen mRNA transcription [68,69], which has been ascribed to an additional effect of AA on mRNA stabilization [70,71]. Moreover, AA treatment upregulates genes mainly involved in neurogenesis in embryonic stem cells [72,73], and affects an array of genes in cultured fibroblast [74] as well as in the liver of wild-type [75] and gulonolactone oxidase-deficient [76] mice.

Recent findings may provide an explanation of effects exerted by AA on gene expression. Importantly, AA present in the nuclear compartment can well regulate the activity of a variety of AA-dependent enzymes catalyzing epigenetically relevant reactions in the nucleus. These enzymes are Fe(II)/2OG-dependent dioxygenases, which catalyze the demethylation of histones and nucleic acids as well as the hydroxylation of certain histones [77]. Moreover, several Fe(II)/2OG-dependent dioxygenases can also participate in DNA repairing processes.

The nuclear Fe(II)/2OG-dependent dioxygenases that demethylate histones mainly belong to the Jumonji protein family conserved from yeast to humans with a common jmjC functional domain [78]. These enzymes appear to be involved in a wide variety of biopathological processes (reviewed in [10]), including cell proliferation and senescence [79], carcinogenesis [78,80-82], hepatic gluconeogenesis [83], and somatic cell reprogramming. It has been recently proposed that Jumonji proteins can also produce stable hydroxylated histones [84]. Actually, the Jumonji domain-6 protein appears also to hydroxylate the splicing factor U2

small nuclear ribonucleoprotein auxiliary factor 65-kilodalton subunit (U2AF65) [85], as well as lysine residues of histones H2A/H2B and H3/H4 [86], in a Fe(II)/2OG-dependent fashion.

Active DNA demethylation refers to an enzymatic process that removes or modifies the methyl group from 5-methylcytosine as illustrated in [87]. The TET (ten-eleven translocation) family comprises at least three Fe(II)/2OG-dependent dioxygenases, Tet1, Tet2 and Tet3 (see [10] for references), which hydroxylate DNA 5-methylcytosine yielding the 'sixth base' 5-hydroxymethylcytosine. Moreover, TET can iteratively oxidize 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine, although the levels of the latter two products are at least an order of magnitude less than those of 5-methylcytosine [88,89]. A wide literature [10,87,90-93] indicates that DNA demethylation is a crucial tool to modulate gene expression during embryogenesis, cell reprogramming and stem cell differentiation, as well as in cancer cells. TET may also participate in DNA repairing processes by acting in concert with thymine DNA glycosylase (reviewed in [87]). Indeed, this latter enzyme can directly excise the Tet products 5-formylcytosine and 5-carboxylcytosine [89,94], finally leading to the substitution of the original 5-methylcytosine with the non-methylated base.

AA appears to enhance 5-hydroxymethylcytosine formation by acting as a cofactor for TET Fe(II)/2OG-dependent dioxygenases to hydroxylate 5-methylcytosine (as in Fig. 1c). It can be assumed that the role of AA in the TET-mediated oxidations is to supply the reducing potential necessary to keep Fe (II) reduced, as is the case with prolyl-4-hydroxylase-mediated hydroxylation. In cultured mouse embryonic fibroblasts, it has been recently observed that the effect of AA on 5-methylcytosine hydroxylation cannot be vicariated by other electron donors such a GSH, is fully independent of the cellular uptake of iron, enhanced expression of TET, or changes in the production of 2OG [95,96]. Contrarily, the size of the intracellular (nuclear?) pool of AA appears to be critical for inducing the generation of 5-hydroxymethylcytosine [97].

Several nuclear enzymes of the AlkB family proteins are involved in repairing damage caused by DNA/RNA alkylation. Originally discovered in *E. coli,* alkylation protein B (AlkB) belongs to the superfamily of Fe(II)/2OG-dependent dioxygenases; in mammals, there are at least nine AlkB homologs: ABH1–ABH8 and FTO (fat mass and obesity associated) (see [9] and [98] for references). These enzymes repair the damages caused by DNA/RNA alkylation, through a direct oxidative dealkylation mechanism that is dependent on 2OG, Fe^{2+} and ascorbate (see Fig. 1d). AlkB and ABH3 preferentially repair single-stranded DNA lesions and can also repair methylated bases in RNA, while ABH2 has a

primary role in repairing lesions in double-stranded DNA. FTO has been shown to catalyze the dealkylation of 3-methylthymine in single-stranded DNA [99].

Against this background, some questions logically arise. Since vitamin C is imported from extracellular fluids or synthesized in the ER compartment, how AA/DHA can reach the nuclear compartment? Which is the AA/DHA concentration and redox status and how are they maintained/regulated in the nuclear environment? Little experimental evidence exists to answer these questions.

As to the first question, although we cannot presently exclude a direct entry of cytosolic AA (and DHA) into the nucleus across the nuclear pore, one can speculate that DHA or AA present in nuclear envelope (NE) - that is a subdomain of ER - can enter into the nucleus across specific transporters located at the inner membrane of the NE itself. ER GLUT transporters (GLUT10?) may mediate DHA entry (Fig. 5); AA transporters might also be selectively expressed at the NE inner membrane, since its protein composition is known to be different from that of the NE outer membranes [100]. This phenomenon is not unprecedented for charged compounds (such as DHA and AA) since it is known that both cations and anions can permeate the inner membrane of the NE *via* several transporters [101,102]. It should be noted that even assuming a passage of DHA through the nuclear pore, the larger size of ER/NE DHA pool – as compared to the cytosolic one – and the large surface of the NE would favor DHA transport from the ER/NE lumen to replenish a nuclear pool.

As to the nuclear AA/DHA concentrations and redox status, the absolute concentrations are unknown but several compounds capable to ultimately affect the redox couple – GSH [103], NADH [104] or FAD [105] – are present in the nucleoplasm. Nonetheless, data on enzymatic connections among them and the AA/DHA redox couple are presently missing. Irrespectively of the way of getting into, ascorbate concentration in the nucleoplasm can be an important regulator of epigenetic events [92,97].

Ascorbate compartmentation diseases?

Scurvy, the disease of ascorbate deficiency, has been known long before the discovery of AA (see *e.g.* [106]). Although scurvy is thought to be primarily due to impaired collagen hydroxylation and production, the patients present generalized symptoms.

Scurvy at cellular level can be manifested even at normal intake of vitamin C. The competition between glucose and DHA for GLUT transporters can reduce tissue AA levels in

hyperglycemia [107,108]. This "latent“ or “tissue“ scurvy thought to be present in insulin dependent diabetes mellitus and can be resulted in endothelial dysfunction and the development of atherosclerosis [109].

Recent studies revealed that subcellular scurvy could also exist, due to either increased consumption of AA in a cellular compartment or defective transport into an organelle. Thus, disturbances of AA compartmentation can be manifested in compartmentation diseases. Zito and coworkers studied the effect of the combined loss-of-function mutations in genes encoding the ER thiol oxidases ERO1α, ERO1β, and peroxiredoxin 4, participating in the electron transfer connected to oxidative protein folding [51]. Surprisingly, minor alterations only were found in disulfide bond formation, suggesting the presence of alternative electron transfer routes. Contrarily, low tissue AA concentrations and decreased 4-hydroxyproline content of procollagen were observed in the mice. It was found that in the absence of ER thiol oxidases, cysteinyl thiol groups were oxidized to sulfenic acid through an alternative hydrogen peroxide dependent way. Sulfenylated proteins consumed AA as their reductant [52]. The competition between sulfenic groups and prolyl hydroxylases for AA in the ER lumen ultimately resulted in impaired procollagen hydroxylation and in the alterations of extracellular matrix, *i.e.* in an unorthodox form of scurvy. Although AA concentration was not measured in the ER lumen, it is obvious that the primary cause of AA shortage is the enhanced local utilization. Thus, neither AA synthesis (mice are able to produce AA) nor AA transport could keep pace with the increased consumption.

Another possible example for the importance of AA compartmentation comes from the human disease arterial tortuosity syndrome (ATS). This rare congenital connective tissue disorder is characterized by elongation and tortuosity of the major arteries [110]. ATS is due to the mutation of a transporter from the GLUT family, GLUT10 [25]. The transporter is present in the endomembranes; its existence has been reported in the mitochondria, the ER and the Golgi [24-26]. GLUT10 can transport DHA, as it was shown in mouse mitochondria [24]. In the cells presenting pathological signs in ATS (*i.e.*, arterial fibroblasts and smooth muscle cells) impaired mitochondrial uptake of DHA should cause AA shortage in the matrix, and a consequent defective elimination of ROS, leading to cell injury. It should be noted, however, that GLUT10 knockout mice do not present any ATS-like pathology [111], which raises doubts on the aforementioned mitochondrial hypothesis. It has been also suggested that the absence of GLUT10 in the ER may result in defective collagen/elastine hydroxylation, which may be responsible for the defects of extracellular matrix [26]. Thus,

shortage of AA in a subcellular compartment, let it be either the mitochondrial matrix or/and the ER lumen, may be in the background of the pathomechanism of ATS.

Although the combined loss-of-function mutations of ER thiol oxidases has not been observed in patients and the etiology of ATS has not been fully elucidated, the above findings clearly show that disturbances in AA compartmentation can be an important factor in the pathomechanism of different human diseases. Compartment-level detection of ascorbate concentrations by biochemical approaches has been an unresolved problem. However, a successful organelle-specific detection of ascorbate has been recently reported, by using immunocytohistochemistry combined with computer supported transmission electron microscopy in plant cells [112,113]. The technique allows the simultaneous investigation of changes in the subcellular distribution of ascorbate in all compartments of the cell in one experiment. Subcellular mapping of AA concentrations, determination of the redox state of the AA/DHA redox couple and the exploration of AA/DHA transport mechanisms in the organelles, both under physiological and pathological situations, are future challenges, which will likely lead to a more detailed understanding of AA compartmentation in health and disease.

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Legend to Figures

Figure 1. Ascorbate as electron donor

Ascorbate (AA) can react with free radicals and other reactive species in spontaneous non-enzymatic reactions (a). AA is an electron donor for copper-containing monooxygenases, such as dopamine β-hydroxylase and peptidylglycine α-hydroxylating monooxygenase. Ascorbate reduces the copper sites to the catalytically active Cu^{+} form (b). AA is required for the regeneration of Fe(II) in catalysis by Fe(II)/2OG-dependent dioxygenases of hydroxylation (c) or demethylation (d) of different substrates.

Figure 2. Possible mechanisms of ascorbate transport through membranes

Ascorbate anion (AA^{-}) can be transported by secondary active transport by using the concentration gradient formed by a cation pump (a). Facilitated diffusion of dehydroascorbic acid (DHA) can be mediated by different GLUT transporters (b). Since cellular DHA concentrations are extremely low, DHA transport might be facilitated by an ascorbate oxidase on the *cis* and a DHA reductase on the *trans* side of the membrane (c). Note that only mechanisms (a) and (c) can generate an AA gradient.

Figure 3. Ascorbate in the ER

Dehydroascorbic acid (DHA), presumably taken up by a GLUT-type transporter, can efficiently oxidize protein- and non-protein thiols in the ER lumen. Ascorbate (AA) formed in these enzymatic or non-enzymatic reactions supports the catalytic function of luminal Fe(II)/2OG dependent dioxygenases: prolyl- and lysyl-hydroxylases.

Figure 4. Ascorbate in the mitochondria

Both SVTC and GLUT dependent uptake processes have been described in mitochondria. Dehydroascorbic acid (DHA) is reduced back to ascorbic acid (AA) by various electron donors (glutathione, lipoic acid, pyridine nucleotides) in the matrix. AA is predominantly utilized as an antioxidant in the organelle.

Figure 5. Ascorbate in the nucleus

Hypothetical route of entry of dehydroascorbic acid (DHA) from a pool present in the endoplasmic reticulum (ER) subdomain nuclear envelope (NE) through GLUT type transporters (including GLUT10) into the nucleus. In the nucleus, DHA is supposed to be enzymatically (?) reduced back to ascorbic acid (AA) by undetermined electron donors (R-H). AA, in turn, supports the catalytic function of Fe(II)/2OG-dependent dioxygenases involved in oxidative modifications of histones and nucleic acids.

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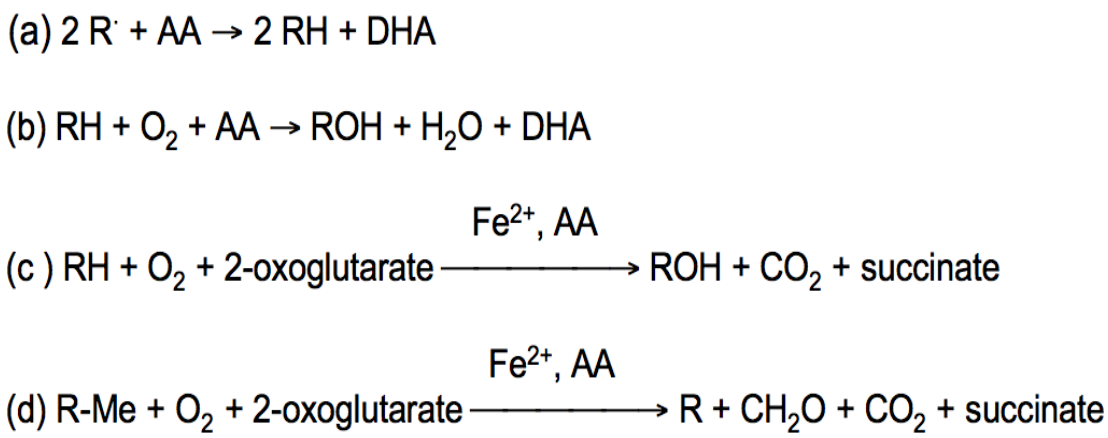
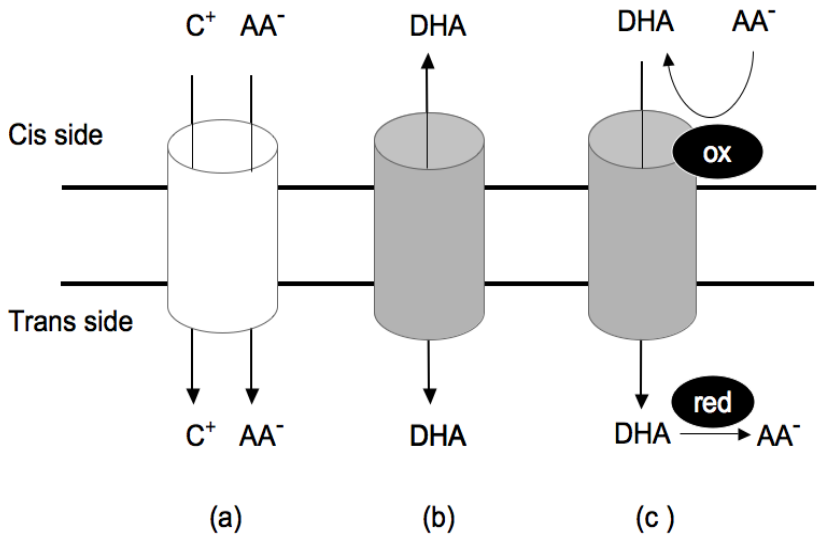

(a) 2 R· + AA → 2 RH + DHA
(b) RH + O2 + AA → ROH + H2O + DHA
(c) RH + O2 + 2-oxoglutarate Fe2+, AA ROH + CO2 + succinate
(d) R-Me + O2 + 2-oxoglutarate Fe2+, AA R + CH2O + CO2 + succinate

Fig. 1.



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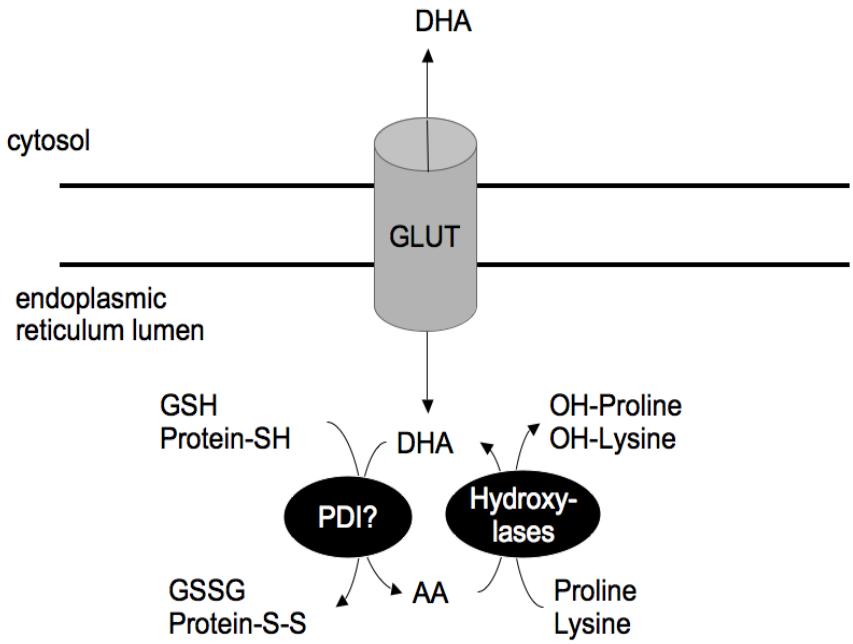
DHA
cytosol
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Fig. 3.

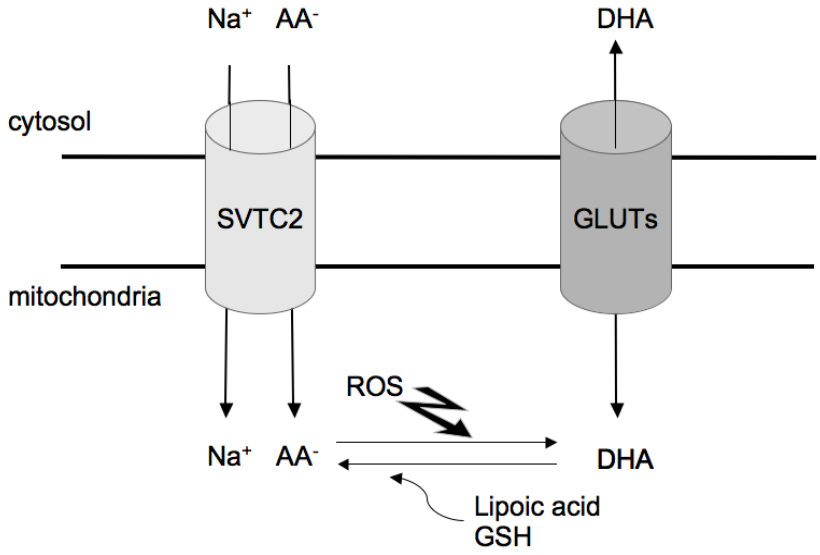
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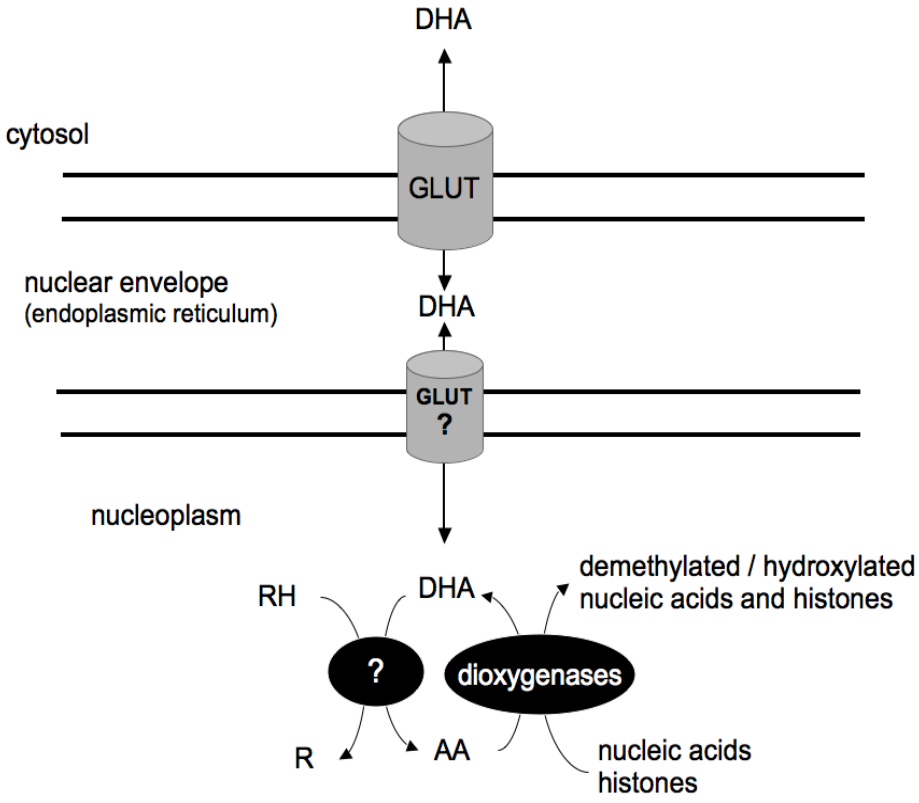
Fig. 4.


DHA
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demethylated / hydroxylated
nucleic acids and histones
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dioxygenases
R
AA
nucleic acids
histones

Fig. 5.