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FORUM EDITORIAL

Oxidation and Reduction of Cysteines in the Intermembrane Space of Mitochondria: Multiple Facets of Redox Control

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Abstract

Eukaryotic cells employ a large variety of protein modifications to integrate individual protein activities into regulatory or signaling networks. Thereby, different compartments prefer specific types of protein modifications. For example, protein phosphorylation is a highly frequent modification in the cytosol and the nucleus, whereas in the lumen of the endoplasmic reticulum, protein functions may be predominantly regulated by protein oxidation or glycosylation. On the hundreds of mitochondrial proteins, only very few modifications were reported until very recently. This is particularly true for proteins of the intermembrane space, the compartment between the outer and inner membrane. However, studies over the last 5 years suggest that the introduction of disulfide bonds might regulate a variety of processes in this compartment. The different processes for which such redox regulations were shown or proposed include the import and folding of proteins and the assembly of cofactors of respiratory chain complexes. Although the understanding of the molecular functions underlying these processes is rapidly increasing, we still do hardly understand how these redox activities are used to coordinate mitochondrial activities with cellular functions such as apoptosis, reactive oxygen species homeostasis, or aging. *Antioxid. Redox Signal.* 13, 1323–1326.

The Intermembrane Space

During a conference on mitochondria a couple of years ago, it was vividly discussed why mitochondria still contain an outer and an inner membrane. The argument was that because of the porins in the outer membrane, which allow the diffusion of components between the cytosol and the intermembrane space, this compartment was anyway not tightly sealed off by an impermeable membrane. Thus, its physicochemical properties should be comparable to those of the cytosol and the outer membrane therefore should be dispensable. There was a long controversial debate about the relevance of the outer membrane and the cellular role of the intermembrane space.

At this time, the intermembrane space was regarded to be a small lumen between two membranes that is almost devoid of proteins and functions. This view has clearly changed considerably over the last years, and about 50 different proteins that are involved in various functions are now known in this compartment (21) (Fig. 1, point 1). These components comprise factors that trigger apoptosis as soon as they become released into the cytosol, transport factors for metabolites, metals, lipids, or ions, proteases and other enzymes, and a large number of assembly factors of respiratory chain complexes. This plethora of functions suggests that the intermembrane space is far from being dispensable. Moreover, despite the presence of porins, the redox milieu of the intermembrane space clearly differs from that of the cytosol and the matrix and is considerably more oxidizing (23). Recent evidence suggests that the oxidation and reduction of cysteines is controlled by a complex system of factors, which will be introduced in this issue of *Antioxidants & Redox Signaling*.

Protein Oxidation in the Intermembrane Space

Their outstanding chemical reactivity sets cysteine residues apart from the other 19 amino acid residues (8, 12, 17, 22). To prevent the unwanted oxidation of cysteine residues to disulfide bonds, the cytosol of eukaryotic cells and bacteria contains a high concentration of reduced glutathione (about 10 mM) and a variety of reducing enzymes (32). As a consequence, under physiological conditions most cytosolic cysteines are reduced (20). In contrast, protein thiols are efficiently oxidized in the endoplasmic reticulum, presumably to increase the stability of secretory proteins and protein complexes (35, 40). Thiol oxidation is mediated by two classes of proteins: sulfhydryl oxidases, which generate disulfide bonds *de novo* (15, 18, 39), and protein disulfide isomerases, which

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shuffle disulfide bonds from sulfhydryl oxidases to substrates (13). In the intermembrane space of mitochondria, a comparable oxidation system was identified recently. This system consists likewise of two players: the sulfhydryl oxidase Erv1 (10, 28) and Mia40, which transfers disulfide bonds to its substrates (9, 31, 37). Both components constitute the mitochondrial disulfide relay system, which introduces disulfide bonds in a number of newly synthesized proteins during or subsequent to their passage from the cytosol into the intermembrane space. The structure of Mia40 was recently solved in two pioneering studies (1, 2), allowing interesting insights into its substrate binding and electron transfer mechanism. The relevance of these findings is discussed in detail in the review article by Endo and coworkers (14) (Fig. 1, point 2).

The sulfhydryl oxidase Erv1 is an FAD-binding protein. It consists of two domains: an N-terminal shuttle domain that accepts electrons from Mia40 and transfers these electrons to the second domain that contains the FAD cofactor. The FAD domain functions as the "disulfide bond generator", which uses the cofactor to transfer the electrons onto cytochrome *c* and the respiratory chain (6, 11). The interplay between both domains was analyzed by detailed *in vitro* and *in vivo* studies (1, 4, 29, 38) (Fig. 1, point 3). However, it is not fully understood why the FAD domain does not directly interact with Mia40 or substrates of the disulfide relay system. Most likely, the N-terminal transfer domain contributes to the substrate specificity of Erv1 and ensures that it prefers to oxidize Mia40 rather than other proteins of the intermembrane space (4, 19).

The mitochondrial disulfide relay not only oxidizes *bona fide* mitochondrial proteins but also some factors that distribute between the intermembrane space and mitochondria. One example for such a dual distribution are copper-zinc superoxide dismutase and its assembly factor Ccs1 (24, 33, 36) (Fig. 1, point 4). The role of Mia40 and Erv1 in the import reaction of both proteins is not well understood and is discussed in detail in a review article by Kawamata and Manfredi in this issue (25).

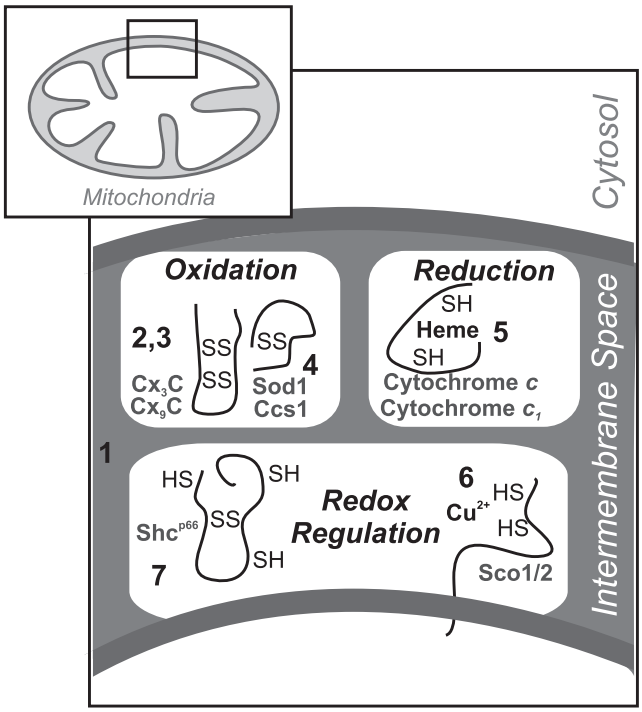

FIG. 1. In the intermembrane space of mitochondria, cysteine-based redox processes play critical roles in protein biogenesis. In the case of twin Cx_3C and twin Cx_9C proteins as well as for Sod1 and Ccs1, the formation of specific disulfide bonds (SS) is coupled to their import into mitochondria and their subsequent folding. On the other hand, the thiol groups of cysteine residues need to be reduced (SH) in proteins such as *c*-type cytochromes, which employ cysteine residues for heme binding. Some proteins might even cycle through reduction and oxidation reactions, presumably to control their binding to metal ions (Sco1/Sco2) or to regulate their activity (Shc^{p66}). The examples depicted here are described in detail in the articles in this issue of *Antioxidants & Redox Signaling*.

Protein Reduction in the Intermembrane Space

To which extent proteins are oxidized in the intermembrane space is unknown. But clearly, there are a number of proteins in this compartment that contain reduced cysteines (34). Examples are *c*-type cytochromes, which use reduced cysteine residues to bind heme cofactors. Mitochondria contain complex machineries that mediate the biogenesis of *c*-type cytochromes (7) (Fig. 1, point 5). In the bacterial periplasm, the reduction of apocytochromes is mediated by the reductase CcmG (30), which counteracts apocytochrome oxidation by the bacterial oxidation machinery. For mitochondrial apocytochrome proteins, it is unknown whether and how they escape oxidation by the disulfide relay or whether reductases convert them into the reduced state to allow heme insertion. The flavoprotein Cyc2 was recently identified as a critical heme insertion factor (3), and it was suggested that it might reduce the cysteine thiols of *c*-type cytochromes in mitochondria.

Thiol-Disulfide Redox Regulation of Mitochondrial Activities

In the cytosol of bacteria and eukaryotes, the controlled formation or reduction of disulfide bonds can be used to adapt protein activities to specific redox conditions (8, 17, 26). It is not known whether similar redox-based regulation mechanisms are employed by mitochondria, but it was proposed that mitochondrial activities might be adapted to the specific levels of molecular oxygen or of oxygen radicals by the use of disulfide bond formation (5, 34). Two examples are the copper transfer to cytochrome oxidase (27) (Fig. 1, point 6) and the biogenesis of Shcp66, a component influencing the lifespan in mammals (16) (Fig. 1, point 7). Both examples are discussed in detail in this issue.

With these seven articles, we tried to cover many of the exciting aspects of thiol oxidation in the intermembrane space of mitochondria. This is a recently emerging field, and therefore, the complexity and relevance of redox processes in this compartment are difficult to estimate. However, the number of different aspects of redox processes increased substantially over the last years, and therefore, it is likely that what was unraveled so far only represents the tip of an iceberg

that awaits further exploration. It is our pleasure to thank all authors, the referees, and the staff of *Antioxidants & Redox Signaling* for their invaluable work that made this issue possible.

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Abbreviations Used

FAD = flavin adenine dinucleotide
Sod1 = superoxide dismutase

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