Review article

Coenzyme Q-dependent functions of plasma membrane in the aging process

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Abstract

Coenzyme Q (Q) is reduced in plasma membrane and mitochondria by NAD(P)H-dependent reductases providing reducing equivalents to maintain both respiratory chain and antioxidant protection. Reactive oxygen species (ROS) are accumulated in the aging process originating mainly in mitochondria but also in other membranes, such as plasma membrane partially by the loss of electrons from the semiquinone. The reduction of Q by NAD(P)H-dependent reductases in plasma membrane is responsible for providing its antioxidant capacity, preventing both the lipid peroxidation chain and the activation of the ceramide-dependent apoptosis pathway. Both Q content and its reductases are decreased in plasma membrane of aging mammals. Calorie restriction, which extends mammal life span, increases the content of Q in the plasma membrane and also activates Q reductases in this membrane. Both lipid peroxidation and ceramide production are decreased in the plasma membrane in calorie-restricted animals. Plasma membrane is, then, an important cellular component to control the aging process through its concentration and redox state of Q.

Coenzyme Q (ubiquinone, CoQ) is an essential electron carrier in the mitochondrial respiratory chain (Crane et al. 1957). Whereas it is mainly located in the inner mitochondrial membrane, CoQ is also present in serum lipoproteins, endomembranes and the plasma membrane of eukaryotic cells (Turunen et al. 2004). CoQ is also a redox component of electron transport chains in extramitochondrial membranes, where it acts as a powerful antioxidant and a membrane stabilizer (Rauchova et al. 1995; Villalba et al. 1995, 1998). In addition, CoQ is an obligatory factor for the activation of uncoupling proteins (Echtay et al. 2001), and the regulation of the permeability transition pore opening (Fontaine et al. 1998).

Chemically, CoQ is constituted by a benzoquinone ring and a lipid side chain constructed with several isoprenoid units, its number being species specific. Different sizes of the isoprenoid side chain can be

found in model organisms used for aging research. *Saccharomyces cerevisiae* has six isoprene units (CoQ₆), *Caenorhabditis elegans* CoQ isoform contains nine isoprenoid units (CoQ₉), and mammalian species have different proportions of CoQ₉ and CoQ₁₀. A higher proportion of CoQ₁₀ correlates with increased longevity in different mammalian species (Lass et al. 1997).

Redox functions of CoQ are due to its ability to exchange two electrons in a redox cycle between the oxidized (ubiquinone, CoQ) and the reduced form (ubiquinol, CoQH₂). This redox reaction can be driven either by the simultaneous transfer of two electrons in a single step, or by two sequential steps of one electron transfer through a partially reduced semiquinone intermediate. CoQ is the only lipidic antioxidant that is synthesized in mammals by all cells, and its biosynthesis is a very complex process

which involves the participation of at least eight gene products in all species studied (Kawamukai 1998). Most of the proteins encoded by these genes have not yet been purified and the regulation of this biosynthesis pathway is still largely unknown (Turunen et al. 2004).

Cellular sources of ROS

Within a cell, mitochondria largely contribute to the production of ROS via the respiratory chain (Lenaz 1998, 2001). The relevance of mitochondrial production of ROS within a cell is indirectly revealed by the effects of a deficiency in mitochondrial antioxidant enzymes. Mitochondria contain an isozyme of superoxide dismutase (SOD-2) and glutathione peroxidase (GPx). The lack of SOD-2 (Melov et al. 1999) and of mitochondrial GPx (Esposito et al. 2000) is deleterious to cells.

It is now recognized that the major sites of ROS production are within Complexes I and III (Lenaz 2001), but most authors consider Complex I rather than Complex III as the main source of ROS (Kushnareva et al. 2002; Kudin et al. 2004). In addition, also glycerol-3-phosphate dehydrogenase has been shown to be a major source of ROS (Drahota et al. 2002).

Calorie restriction is the only non-genetic intervention that increases life span in mammals (Masoro 2001). It induces a decrease of both proton- and electron-leak in mitochondria preventing oxidative damage (Barja 2004).

In addition to mitochondria, however, other sources of ROS are present in the cell. In the endoplasmic reticulum, the NADPH cytochrome P_{450} reductase is involved in ROS generation during oxidation of unsaturated fatty acids and bioactivation of xenobiotics (Sauer et al. 2000). Additional intracellular sources of ROS are xanthine oxidase, lipoxygenase, cyclooxygenase and γ -glutamyl-transpeptidase (Sauer et al. 2001).

There is, however, increasing evidence that a NAD(P)H oxidase, similar but not identical to that present in phagocytic cells and responsible for the oxidative burst during phagocytosis, is present in the plasma membrane of a variety of cells. This NAD(P)H oxidase is regulated by ligand activation, substantially through the activation of PI3-kinase by growth factors and cytokines and subsequent mediation by Rac1 (Sauer et al. 2001). Similar to the

phagocytic enzyme, this NAD(P)H oxidase is a strong producer of superoxide radicals and a source of oxidative stress consequent to activation of signal transduction pathways (Thannickal et al. 2000; Arnold et al. 2001; Yang et al. 2001; Shiose et al. 2001; Droge 2002, 2003; Mahadev et al. 2004; Prata et al. 2004). Indeed, ROS cytotoxicity may be alleviated by the addition of redox reactants, such as pyruvate (Bassenge et al. 2000), indicating that the release of reducing power at the plasma membrane significantly inhibits ROS production.

Plasma membranes can also produce ROS by the trans-plasma membrane redox system that drives electrons from intracellular NAD(P)H to external antioxidants (del Castillo-Olivares et al. 2000). CoQ is the redox carrier in this system (Villalba et al. 1995; Sun et al. 1992; Kishi et al. 1999; Morré et al. 1999). The transport of electrons through this system involves ROS production (Kagan et al. 1998), which can be increased by its inhibition with CoQ antagonists (Wolvetang et al. 1996; Macho et al. 1999).

CoQ functions at the plasma membrane

The activity of the transplasma membrane redox system has been related to growth control and development (Crane et al. 1985). Different components and activities have been associated with this electron transport system, including NADH oxidase (Morré and Morré 2004), an isoform of VDAC that does not reduce DCIP (Baker et al. 2004; Malik et al. 2004), and a DCIP-reducing activity that is not yet structurally identified (Lenaz et al. 2002; Deleonardi et al. 2004).

NADH-driven trans-membrane reduction of electron acceptors in plasma membrane vesicles is mediated by endogenous reduced CoQ (Villalba et al. 1995; Sun et al. 1992; Kishi et al. 1999). The use of CoQ-deficient yeast strains has provided strong genetic evidence for the requirement of CoQ in transplasma membrane electron transport (Santos-Ocaña et al. 1998). Several enzymes have been reported to catalyze CoQ reduction in the plasma membrane of eukaryotic cells. NADH-cytochrome b_5 reductase can reduce CoQ through a one-electron reaction mechanism, and this enzyme could be responsible for the maintenance of basal levels of CoQH₂ in the plasma membrane (Navarro et al. 1995; Arroyo et al. 1998). The soluble enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-

diaphorase) can reduce quinones through a two-electron reaction mechanism (Lind et al. 1990) and it has also been reported to maintain the reduced state of hydrophobic CoQ homologues *in vitro* (Beyer et al. 1996). NQO1 is an inducible enzyme whose expression is increased by oxidative challenge (Rushmore et al. 1991). A distinct cytosolic NADPH-CoQ reductase which is separate from NQO1 has also been described (Takahashi et al. 1992, 1995). CoQH₂ maintained in plasma membrane by these enzymes would exert its antioxidant function against the progression of peroxidation reactions (Kagan and Tyurina 1998). In accordance, an inverse correlation between plasma membrane CoQ concentration and lipid peroxidation rates has been demonstrated (Lopez-Lluch et al. 1999).

CoQH₂ produced by plasma membrane oxidoreductases may also be important for regeneration of reduced forms of other antioxidants, such as α -tocopherol and ascorbate, thus contributing to enhance the general protection against oxidative stress. The regeneration of α -tocopherol from the reduction of its α -tocopheroxyl radical by CoQH₂ or ubisemiquinone is clearly demonstrated (Kagan et al. 1990, 1998). This mechanism may also be operative in the plasma membrane because both antioxidants, and also the CoQ reductases, are present in this membrane (Constantinescu et al. 1994). NADH-cytochrome *b*₅ reductase can regenerate Trolox, a soluble analogue of α -tocopherol, by reducing its phenoxyl radical in a process that requires NADH and CoQ₀, and partially involves superoxide anions (Kagan et al. 1998). Semiquinone radicals generated by the reaction of superoxide with CoQ might be partly responsible for the α -tocopherol regeneration observed (Stoyanovsky et al. 1995). Transplasma membrane NADH-AFR reductase may also have an antioxidant role through the regeneration of extracellular ascorbate. A role for CoQH₂ in the regeneration of extracellular ascorbate by extracellular AFR has been deduced from experimental evidence showing a requirement for CoQ in transmembrane NADH-AFR reductase activity, and from the ability of cells to decrease ascorbate oxidation rates and to scavenge AFR (Villalba et al. 1998; Santos-Ocaña et al. 1998; Gómez-Díaz et al. 1997). In addition, ascorbate can also regenerate α -tocopherol in membranes, liposomes and serum lipoproteins (Kagan et al. 1990; Sharma and Buettner 1993), although this relationship has not been confirmed *in vivo* (Liebler 1993).

The action of these enzymes would be important for the aging process if their expression and/or activities are modulated by changes in oxidative stress levels or other types of stress that occur during aging. There are different conditions that could cause oxidative stress in eukaryotic organisms and the *de novo* biosynthesis of CoQ would also be important to lead its protection. Thus, it is possible that breakdown products of CoQ produced by its catabolism, induced by the peroxidation of lipids, could play an important role in the enhancement of its biosynthesis (Turunen et al. 2004). Also, it has been observed that the removal of α -tocopherol and selenium from the rat diet induces a significant increase of both CoQ and CoQ-dependent reductases, such as cytochrome *b*₅ reductase and NQO1 in liver plasma membrane. NQO1 translocates from a soluble form to a plasma membrane-bound form under these stress conditions (Navarro et al. 1998). The CoQH₂/CoQ ratio, which is increased in these conditions, will transiently support the antioxidant protection of the plasma membrane instead of the antioxidant couple α -tocopherol/CoQ.

Regulation of ceramide signaling by plasma membrane CoQ

Ceramide is able to induce cell death after its intracellular accumulation (Obeid et al. 1993; Jayadev et al. 1995) by activating proteases of the caspase family (Mizushima et al. 1996). Thus, the increase of ceramide levels appears as an essential component in the stress response pathway (Hannun 1996). Ceramide accumulation in the cell can occur from *de novo* synthesis with the participation of ceramide synthase, or from the hydrolysis of sphingomyelin by a number of sphingomyelinases (SMase, sphingomyelin phosphodiesterase; E.C. 3.1.4.12). The complexity of ceramide generation upon cell stimulation can be illustrated by the fact that both pathways are apparently involved in apoptosis induced by the cytokine tumor necrosis factor α (TNF α) (Dbaiibo et al. 2001).

The plasma membrane of eukaryotic cells contains a magnesium-dependent neutral sphingomyelinase (nSMase), which is an integral plasma membrane protein (Chatterjee et al. 1999). The levels of nSMase activity in rat brain and liver plasma membrane increase with neuronal maturation and aging, suggesting a positive correlation between the expression

of the nSMase with the aging process (Spence and Burgess 1978; Petkova et al. 1988; Liu et al. 1997; Lightle et al. 2000). This enzyme has been purified from pig liver plasma membrane and shows a non-competitive inhibition by CoQ (Martin et al. 2001). This inhibition is more efficiently carried out by CoQH₂, which specifically inhibits the Mg²⁺-dependent enzyme in isolated liver plasma membranes, but no inhibition of the Mg²⁺-independent neutral sphingomyelinase was observed (Martin et al. 2002).

Interestingly, different experimental studies have indicated that the exogenous treatment with CoQ stimulates the immune response (Bentinger et al. 2003). This latter effect and the inhibition of nSMase by CoQ are factors to be considered that could be related to its beneficial effects on cells and organisms, beyond its participation in mitochondrial energy production or as an antioxidant.

Plasma membrane oxidoreductase in aging

According to the mitochondrial theory of aging (Lenaz 1998; Miquel et al. 1980; Linnane et al. 1989), somatic mutations of mitochondrial DNA (mtDNA) caused by ROS would induce damage to the polypeptides encoded by mtDNA in the respiratory complexes, with a consequent decrease in electron transfer, causing a progressive energy decline leading to cellular senescence and death. Another model developed to study the antioxidant functions of CoQ in the plasma membrane during the aging process is the development of ethidium bromide-induced mitochondria-defective ρ^0 cells that are depleted of a functional mitochondrial respiratory chain (King and Attardi 1989). Some of these cells, such as the fibroblast-derived ρ^0 701.2a cell line, are much more sensitive to serum withdrawal than parental cells and can be protected from apoptosis by the overexpression of Bcl-2 (Raff et al. 1993). However, ρ^0 cells derived from both Namalwa and HL-60 cell lines are more resistant to serum removal than parental cells (Larm et al. 1994; Barroso et al. 1997). These cells up-regulate the plasma membrane-associated redox activity during depletion of mtDNA, and the cells can maintain growth with the supplementation of growth media with oxidants. It has also been shown that ρ^0 HL-60 cells up-regulate plasma membrane redox activity as a consequence of a significant increase in CoQ₁₀ levels in the plasma membrane (Gómez-

Díaz et al. 1997). In agreement with these observations, the inhibition of CoQ-dependent activities in the plasma membrane by compounds such as vanilloids triggers reactive oxygen species (ROS)-dependent apoptosis, which is partially prevented by CoQ (Wolvetang et al. 1996). Interestingly, the increase of ROS occurs before the dissipation of the mitochondrial membrane potential, supporting then the participation of the plasma membrane in the induction of apoptosis (Macho et al. 1998, 1999). Similar results were observed with the anticancer drug adriamycin, which inhibits the trans-plasma membrane redox system, causing the accumulation of ROS and triggering the apoptotic process (Lenaz et al. 1998).

It may be possible that aging cells over-express plasma membrane oxidoreductase (PMOR) when mitochondrial functions are depressed (Morré et al. 2000). Also in pathological conditions such as insulin-dependent diabetes, the PMOR system is over-expressed in circulating lymphocytes (Lenaz et al. 2002). More recently Malik et al. (2004) have shown that up-regulation of PMOR is a property only of cells having strong ATP requirement for growth.

Also oxidative stress was found to stimulate PMOR (Deleonardi et al. 2004; Lopez-Lluch et al. 1999; Navarro et al. 1998) suggesting an antioxidant function for this enzyme obtained through the increased reduction of membrane-bound Coenzyme Q and ascorbate; moreover a protective effect of reduced CoQ appears to be exerted through the direct inhibition of plasma membrane sphingomyelinase (Martin et al. 2002). Although ROS production at the plasma membrane inhibits apoptosis and stimulates cancer cell growth in some systems (Brar et al. 2003; Vaquero et al. 2004), it may stimulate apoptosis in others (Macho et al. 1998; Martin-Romero et al. 2002; Saamhan-Arias et al. 2004).

The role of PMOR in aging can therefore be accounted for by the following mechanism. In aging, mitochondrial function is progressively impaired and extra-mitochondrial NADH accumulates either as a consequence of the compensatory stimulation of glycolysis by the Pasteur effect (D'Aurelio et al. 2001) or by way of reversal of the malate-aspartate shuttle (Bremer and Davis 1975) to export the excess of mitochondrial reducing power not used by respiration (De Grey 2002). Accumulation of extra-mitochondrial NADH or a concomitant consequence of mitochondrial dysfunction may be the stimulus for

the overexpression of PMOR at the plasma membrane. Studies on insulin-dependent diabetes mellitus (Lenaz et al. 2002) suggest that the increased activity of PMOR in diabetic patients is likely to be a real up-regulation and not increased activity due to saturation of substrate NADH; although there is no direct evidence, this may also be the case in aging.

There is some evidence that the direct cause of PMOR overexpression may be oxidative stress accompanying mitochondrial damage (Deleonardi et al. 2004). In fact, in two different kinds of cultured cells, the overexpression of PMOR (as DCIP reductase) was more directly linked to ROS production than to respiratory dysfunction *per se*. This hypothesis is likely true, since ROS are known to stimulate transcription of several genes through activation of specific regulatory elements for compensation purposes (Calabrese et al. 2004).

Thus, the PMOR activation is a compensation for disposal of excess reducing power, but has the drawback that it may be the cause of further production of superoxide at the plasma membrane itself. This has been pointed out by De Grey (1998, 2000, 2002), by Morré et al. (2000), and by Gómez-Díaz et al. (1997). The reason for this is not clear, since De Grey (2002) pointed out that caloric restriction also stimulates PMOR through mitochondrial NADH accumulation, reversal of the malate–aspartate shuttle and rise of extramitochondrial NADH, however it inhibits ROS production. Although de Grey attributes both the effect of a mitochondrial lesion and that of caloric restriction to different stressful conditions over the same PMOR enzyme, with production of superoxide only in the former case, it seems more reasonable to attribute the difference to the activation of two different enzyme systems.

Activation of plasma membrane bound NQO1 (DT-diaphorase) under conditions of oxidative stress have been also demonstrated (Navarro et al. 1998; Olsson et al. 1993). NQO1 is a NAD(P)H quinone oxidoreductase sensitive to the inhibitor dicoumarol and normally located mainly in the cytoplasm (Lind et al. 1990), where it may be involved in the reduction of CoQ to its antioxidant fully reduced form (Beyer et al. 1996). NQO1 is a two-electron quinone reductase that does not produce semiquinone radicals but fully reduced quinones, that exert the antioxidant effect of CoQ in the plasma membrane (Villalba et al. 1998). Thus, activation of DT-diaphorase is a bona fide antioxidant protectant for

the cell. In fact, it has recently been shown that caloric restriction induces the translocation of NQO1 to the plasma membrane in aging rats. CR increases the ratio Q10/Q9 and redox activities leading to CoQ reduction, which are higher than in non-restricted membranes, thereby showing a potentiation of antioxidant properties (de Cabo et al. 2004).

What has been described for aging may be true for other conditions when mitochondrial function is impaired, such as diseases with ischemia reperfusion damage, diabetes mellitus, mitochondrial cytopathies, neurodegenerative diseases, and cancer. Nevertheless this area of research is still in its infancy.

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