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Metabolism and function of coenzyme Q

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Abstract

Coenzyme Q (CoQ) is present in all cells and membranes and in addition to be a member of the mitochondrial respiratory chain it has also several other functions of great importance for the cellular metabolism. This review summarizes the findings available to day concerning CoQ distribution, biosynthesis, regulatory modifications and its participation in cellular metabolism. There are a number of indications that this lipid is not always functioning by its direct presence at the site of action but also using e.g. receptor expression modifications, signal transduction mechanisms and action through its metabolites. The biosynthesis of CoQ is studied in great detail in bacteria and yeast but only to a limited extent in animal tissues and therefore the informations available is restricted. However, it is known that the CoQ is compartmentalized in the cell with multiple sites of biosynthesis, breakdown and regulation which is the basis of functional specialization. Some regulatory mechanisms concerning amount and biosynthesis are established and nuclear transcription factors are partly identified in this process. Using appropriate ligands of nuclear receptors the biosynthetic rate can be increased in experimental system which raises the possibility of drug-induced upregulation of the lipid in deficiency. During aging and pathophysiological conditions the tissue concentration of CoQ is modified which influences cellular functions. In this case the extent of disturbances is dependent on the localizaion and the modified distribution of the lipid at cellular and membrane levels.

Keywords: Coenzyme Q; Antioxidant; Mevalonate pathway; Aging; Cardiovascular disease

Coenzyme Q (CoQ) was isolated and characterized by Festenstein et al. in 1955 [1] and it was established in 1957 by Crane et al. [2] that this compound functions as a member of the mitochondrial respiratory chain. Wolf et al. [3] determined its complex structure in 1958. CoQ was found to be an unusual lipid since the redox active benzoquinone ring is connected to a long isoprenoid side chain, requiring specific placements in a biological membrane. At this time only the redox function of this lipid was studied while its function as an antioxidant was investigated mainly during the past 15 years. In the initial period, the concept of CoQ distribution and synthesis was attributed exclusively to the inner mitochondrial membrane. This appeared to be reasonable since the only known function at this time was shuttling electrons from complexes I and II to complex III in the mitochondrial electron transfer system.

During the 1960s the obligatory role of CoQ in the respiratory chain was proven by several facts, such as depletion and reincorporation of the lipid into submitochondrial particles causing inactivation and reactivation of NADH and succinate dehydrogenas activities [4]. In 1975 Mitchell [5] proposed the protonmotive Q cycle, the cyclic electron transfer pathway through complex III involving ubisemiquinone, a proposal generally accepted. The role of CoQ in the mitochondrial respiratory chain and associated oxidative phosphorylation is studied in great details by many investigators and is not discussed here. There are recent and excellent reviews about this subject which are recommended to consult [6–9].

1. Functions of CoQ

In addition to its central role in the mitochondrial respiratory chain, CoQ is now involved in a number of aspects of cellular metabolism and we can expect that

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Table 1
Functions of coenzyme Q

- Participation as electron carrier in the mitochondrial respiratory chain
- Participation in extra-mitochondrial electron transport (PLM[a], lysosomes)
- Endogenously synthesized, lipid-soluble antioxidant
- Regulation of mitochondrial permeability transition pores
- Required for activation of mitochondrial uncoupling proteins
- Regulation of the physicochemical properties of membranes
- Modulation of the amount of β2-integrins on the surface of blood monocytes
- Improvement of endothelial dysfunction (probably by increasing nitrogen oxide)
- Oxidation of sulfide (in yeast)
- Introduction of disulfide bonds (in bacteria)

[a] Plasma membranes.

several new functions will also appear in the near future (Table 1).

1.1. The plasma membrane redox system

The plasma membrane of eukaryotic cells contains an NADH oxidase (NOX) that is involved in the transfer of electrons across the membrane (Fig. 1). The name of this enzyme was given in the initial period of studies, when it was believed that the function of the enzyme is the oxidation of the externally added NADH. The NOX protein is not a transmembranous protein but is located at the external surface of the plasma membrane [10]. It has both hydroquinone (or NADH) oxidase and protein-disulfide-thiol interchange activities that have been shown to respond to hormones and growth factors [11,12]. The NOX protein is not related to the NADPH oxidase found in neutrophils, which is not dependent of CoQ [13]. At the cytosolic surface, a quinone reductase is present that catalyzes the reduction of CoQ in the presence of NADH [14]. This system together with the enzymatic mechanisms discussed in Section 7 are responsible for regeneration of cellular reduced CoQ. The participation of CoQ in the plasma membrane electron transport was shown by the fact that the NOX activity was inhibited by removal of CoQ with heptane and reconstitution of the activity by CoQ addition [15]. Several terminal electron acceptors have been suggested, such as molecular oxygen, protein disulfides or ascorbyl radicals [15–17].

The function of NOX is proposed to be related to the control of cell growth and differentiation as well as maintaining extracellular ascorbate in reduced form [18,19]. Another possibility is that the CoQ-dependent NOX is involved in the regulation of the cytosolic NAD^+/NADH ratio. In fact, Larm et al. [20] and Lawen et al. [21] showed that ρ^0 cells, lacking mitochondrial DNA, accumulate NADH as a result of glycolytic production of ATP and that the plasma membrane oxidoreductase system is able to reoxidize the NADH in these cells. Finally, Crane et al. [22] proposed yet another two functions for CoQ in the plasma membrane electron transfer system. First, the redox state of CoQ may act to control a tyrosine kinase in a manner analogous to that found in *Escherichia coli* [23]. Second, oxidation of the semiquinone in the plasma membrane could generate H_2O_2 and it has been shown that activation of peroxide generation, by for instance xanthine oxidase, induces tyrosine kinase and early gene expression [24,25].

Recently it was also suggested that lysosomes contain an NADH-dependent CoQ reductase involved in translocation of protons into the lysosomal lumen [26]. Apparently, at this location the reduction of CoQ occurs in two subsequent one-electron transfer steps involving FAD and cytochrome b_5 with molecular oxygen as the terminal electron acceptor. Though intriguing, further research will be necessary to define the function of this CoQ-dependent lysosomal reductase.

1.2. Antioxidants and generation of reactive oxygen species (ROS)

Eighty percent of the normal mammalian oxygen consumption occurs in mitochondria, of which 80% is coupled to ATP production [27,28]. ROS account for 1–2% of the

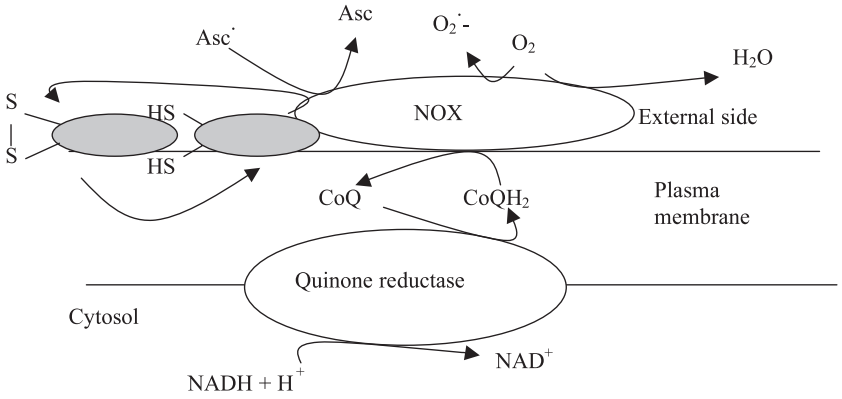

Fig. 1. Schematic representation of the CoQ-dependent trans-plasma membrane electron transport. At the cytosolic side of the plasma membrane, a quinone reductase is present that reduces CoQ to ubiquinol ($CoQH_2$). $CoQH_2$ shuttles the electrons to the NOX that is able to reduce extracellular ascorbyl radicals (Asc), produce superoxide (O_2^-), reduce O_2 to water and reduce protein disulfides. The figure is adopted from Morré et al. [317].

oxygen consumed by mitochondria and this compartment is considered to be the major source of $O_2^{\cdot-}$ and H_2O_2 production in the cell [29,30]. The leakage of electrons from the respiratory chain result in a 5–10-fold higher steady-state concentration of $O_2^{\cdot-}$ in the mitochondrial matrix than in the cytosolic and nuclear spaces [31]. The formation of mitochondrial ROS is dependent on the state of respiration, i.e. in state 4, when the mitochondrial membrane potential is high and the ADP levels are low, the production of ROS is high. In state 3, when the membrane potential is low and the ADP concentration is high, the production of ROS is low. Another source of ROS is the production of H_2O_2 by monoamine oxidase in mitochondria, the various flavin oxidases in peroxisomes and the leakage of electrons from cytochrome P-450 in the endoplasmic reticulum (ER) [32–34].

The free radicals formed in the cell are able to damage lipids, proteins and DNA, a concept known as oxidative stress. Lipid peroxidation is by far the most studied process and the initiation occurs by abstraction of a hydrogen atom from a methylene group of a polyunsaturated fatty acid (PUFA). The results of lipid peroxidation are carbon-centered free radical, peroxyl radical, lipid hydroperoxide, alkoxyl radical and degradation to hydrocarbons, alcohols, ethers, epoxides and aldehydes. The aldehydes formed are relatively long-lived and can therefore diffuse a longer distance from their site of origin than free radicals. Since they can also react with macromolecules, they further contribute to the damage caused by lipid peroxidation. A secondary defense mechanism involving phospholipase A_2 removes the peroxidized fatty acid that can be replaced by new fatty acids [35]. If the removed fatty acid is not replaced completely, the lysophospholipid formed acts as a detergent and damages the membrane. Other defense systems are the glutathione peroxidases, especially the phosphohydrolipid glutathione peroxidase, that scavenge the lipid hydroperoxides formed during lipid peroxidation [36].

Protein oxidation usually occurs at certain amino acid residues of a particular protein. Although some amino acids are more prone to oxidation than others, the selectivity of the initiating attack is attributed to protein-bound transition metals that react with "weak ROS" and form hydroxyl radicals at the metal-binding site [37,38]. The subsequent abstraction of a hydrogen atom and formation of peroxyl radicals and hydroperoxides are similar to lipid peroxidation. Propagation can occur within the protein, to another protein or to lipids. Oxidation of membrane proteins can also take place by lipid-derived free radicals [39]. Oxidized proteins are recognized by specific proteases and completely degraded to amino acids. Thus, replacement by de novo synthesis of proteins is the only repair mechanism [40,41].

DNA oxidation by ROS is believed to be dependent on transition metals in a similar manner to protein oxidation [42]. Due to the relatively high production of ROS in mitochondria, lack of protective histones and limited ability of repair, mitochondrial DNA has about 10-fold higher oxidative damage than nuclear DNA [43]. Oxidized DNA is repaired by two enzymatic mechanisms, removal and subsequent replacement of single oxidized deoxyribonucleotides and/or excision of 25–32 nucleotides long oligomers of oxidized bases that are replaced by DNA polymerase and subsequently sealed by DNA ligase [44].

Antioxidants are enzymes and nonenzymatic agents that can prevent the formation of, or remove ROS. Antioxidant enzymes include superoxide dismutase and various peroxidases such as glutathione peroxidase, catalase, thioredoxin reductase and peroxiredoxin [45,46]. Nonenzymatic agents include vitamins C and E, carotenoids, glutathione, α-lipoic acid, flavinoids and the reduced form of CoQ ($CoQH_2$) that all rely on a mechanism of regeneration in the cell.

$CoQH_2$ was originally described to inhibit lipid peroxidation in vitro using submitochondrial particles [47,48]. It is now well established that $CoQH_2$ is able to prevent lipid peroxidation in most subcellular membranes [49]. The lipid plays an important role as antioxidant also in the circulation [50,51]. Since $CoQH_2$ is oxidized during lipid peroxidation, reductive regeneration is a requirement for participation in antioxidative reactions. Mitochondrial $CoQH_2$ is efficiently regenerated by the respiratory chain and is normally kept in a highly reduced state [52]. As it will be discussed later (Section 7), the cell has effective systems to reduce CoQ at all intracellular locations. Studies performed on the mechanism of its action suggest that $CoQH_2$ acts by affecting the initiation process and preventing the formation of lipid peroxyl radicals (LOO) while vitamin E acts by quenching these radicals (Fig. 2) [53]. It appears that $CoQH_2$ reduces the initiating perferryl radical with the formation of ubisemiquinone and H_2O_2. It is also possible that it eliminates LOO directly; furthermore, it is established that $CoQH_2$ regenerates vitamin E from the α-tocopheroxyl radical. This appears to be favored in comparison with the alternative, which is regeneration by ascorbate. Lipid solubility, efficient continuous regeneration and involvement both in the initiation and propagation steps of lipid peroxidation explain why CoQ is considered as a highly efficient antioxidant against radicals produced in biological membranes. In fact, it appears that this antioxidant is considerably more efficient than vitamin E [54].

When phosphatidylcholine liposomes are oxidized with a water-soluble radical initiator in the presence of ascorbate, α-tocopherol (α-T) and CoQ the antioxidants are consumed in the order: ascorbate–CoQ–α-T [55]. When a lipid-soluble radical initiator is used, the antioxidants are consumed in the order: CoQ–ascorbate–α-T. Thus, α-T is efficiently spared in both cases and this kinetic data showed that the α-tocopheroxyl radical formed is reduced by $CoQH_2$. This sparing effect of α-T by $CoQH_2$ has also been observed in low density lipoprotein (LDL) [56,57]. The fact that the antioxidant function of $CoQH_2$ is not dependent on the presence of α-T, is evident from the fact that submitochondrial particles containing CoQ are protected from lipid peroxidation also in the absence of α-T [58].

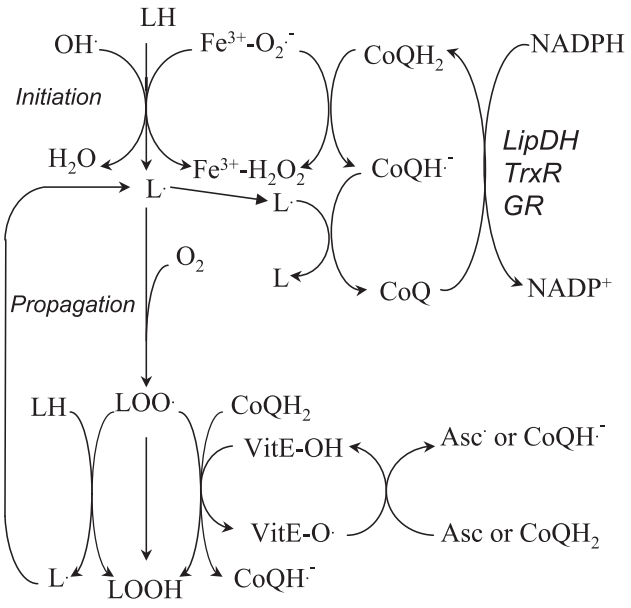

Fig. 2. Sites of action of CoQ, vitamin E and ascorbate on lipid peroxidation. Abbreviations: LH, polyunsaturated fatty acid; OH, hydroxyl radical; $Fe^3+O_2^-$, perferryl radical; $CoQH_2$, reduced coenzyme Q; $CoQH^-$, semiubiquinone; L, carbon-centered fatty acid radical; LOO, lipid peroxyl radical; VitE-OH, vitamin E (α-tocopherol); VitE-O, α-tocopheroxyl radical; Asc, ascorbate; Asc, ascorbyl radical; LOOH, lipid hydroperoxide; LipDH, lipoamide dehydrogenase; TrxR, thioredoxin reductase; GR, glutathione reductase.

Oxidation of membrane proteins can also be prevented by $CoQH_2$ [39,59,60]. DNA oxidation measured by 8-hydroxydeoxyguanosine formation in rat liver mitochondria and DNA strand breaks in human lymphocytes is also prevented by $CoQH_2$ administration both in vitro and in vivo [49,61,62]. Finally, reduced plastoquinone found in plant chloroplast membranes exerts an antioxidant activity similar to that shown for $CoQH_2$ [63].

1.3. Uncoupling proteins (UCPs)

UCPs are situated in the inner mitochondrial membrane and can translocate H^+ from the outside to the inside of the mitochondria. Thus, the proton gradient built by the respiratory chain is uncoupled from oxidative phosphorylation and heat rather than energy is produced [64–66]. UCPs occur in many animal and plant cells and form a subfamily of the mitochondrial carrier family. Five UCPs (1–5 in humans) are known and UCP1 is the most well characterized, present in brown adipose tissue where it participates in thermogenesis [67,68]. In other tissues where uncoupling is not a dominant feature, UCPs occur only in small amounts. UCP2 is expressed in most animal tissues and UCP3 is expressed most abundantly in human skeletal muscle [69]. In addition to thermogenesis, UCPs could be involved in suppression of oxygen radicals. There are also suggestions that altered UCP expression could be related to human diseases, such as obesity and diabetes.

Recently Echtay et al. [70,71] showed that CoQ is an obligatory cofactor for UCP function using bacterial over-expressed UCP1, -2 and -3 in liposomes. These liposomes were unable to transport H^+ in the absence of CoQ and H^+ transport was activated when CoQ was added to the membranes in the presence of fatty acids. The CoQ-stimulated activity could be inhibited by addition of ATP. Full activation of UCPs in reconstituted vesicles was achieved with CoQ/phospholipid ratio of 1:300 and CoQ/UCP ratio of 80:1. It was proposed that CoQ interact with UCP in the hydrophobic bilayer since maximal activity was achieved with CoQ10 and hydrophilic CoQs with none or a short isoprenoid side chain (0–2 isoprenes) were ineffective to activate UCPs (Fig. 3) [71]. It was also suggested that the interaction between CoQ and UCP3 occurred closer to the membrane surface, since UCP3 retains some of its activity with hydrophilic CoQs. Thus, CoQ, but not ubiquinol, could in an active way subtract H^+ from fatty acids and deliver them to the H^+ acceptor group of the UCP. Consequently, the oxidized form of CoQ has to be present in mitochondria for this activation.

1.4. The mitochondrial permeability transition pore (PTP)

The inner membrane of the mitochondria has a low permeability to ions and solutes in order to permit energy conservation in the form of an electron and a pH gradient over the membrane. In order to facilitate trans-membraneous transport, the inner membrane contains a number of macromolecule transporters and ion channels. However, under in vitro accumulation of Ca^{2+}, the mitochondria can undergo a generalized increase of permeability of the inner membrane, known as permeability transition. This enable macromolecules with a size of approximately 1500 Da to cross the membrane causing collapse of the protonmotive force,

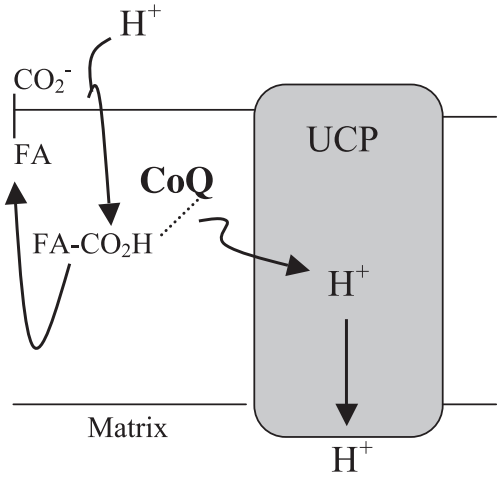

Fig. 3. Proposed role of coenzyme Q in UCP function. A proton (H^+) is delivered from a fatty acid (FA-CO_2H) to the UCP with the assistance of CoQ in the inner mitochondrial membrane. The UCP subsequently translocates the proton to the matrix. The figure is based on the proposals from Echtay et al. [71].

disruption of ionic status, loss of pyridine nucleotides, and hydrolysis of ATP [72]. The permeabilization of the membrane is due to the opening of an inner mitochondrial complex, the PTP. The PTP behaves like a voltage-dependent channel, favoring a closed conformation at high membrane potentials and an open conformation at low membrane potentials. Upon Ca^{2+} accumulation, more than 40 classes of unrelated factors can modulate the PTP to open or close, including the mitochondrial membrane potential, the matrix pH, cyclosporin A, phosphate, oxidized glutathione and pyridine nucleotides derived from oxidative stress. The permeability transition has been proposed to be an early key event in several models of apoptosis causing activation of the caspase cascade through release of cytochrome *c* [73]. Under physiological conditions, it is thought that the PTP serves as a fast Ca^{2+} release channel in the mitochondria [74,75].

Several analogues of CoQ have been shown to affect the PTP [76–78]. They all contain a benzoquinone ring with either none or a short (0–2 isoprenes) saturated or unsaturated side chain and can be divided into three functional classes; inhibitors, inducers and inactive quinones that counteract the effects of both the inhibitors and the inducers. The structure–function correlation of these analogues has suggested that minor changes in the isoprenoid side chain can turn an inhibitor into an inducer and that the methoxy groups are not essential for activity [77]. It has therefore been suggested that quinones modulate the PTP through a common binding site rather than through redox reactions. CoQ10 seems also to prevent PTP opening, since it was shown in a recent study that CoQ10 is able to counteract mitochondrial membrane potential depolarization, ATP depletion, cytochrome *c* release, caspase-9 activation and DNA fragmentation in keratinocytes upon apoptotic stimuli [79]. However, the actual PTP opening and the mechanism of action by CoQ10 were not investigated in this study.

1.5. Lymphocytes and monocytes

Isolated human blood lymphocytes increase their CoQ10 content several folds upon incubation with liposomes containing CoQ10 [61]. Supplementation of human subjects with 300 mg/day CoQ10 for 1 week elevates the lipid content in lymphocytes from 14 to 32 pmol/10^6 cells [62]. In vitro supplementation was proved to enhance DNA resistance for hydrogen peroxide-induced oxidation but it did not inhibit DNA strand break formation [61]. The in vivo enrichment of CoQ10 in lymphocytes was shown to inhibit oxidative DNA damage and enhanced the DNA repair enzyme activity in these lymphocytes [62]. Two months of CoQ10 administration to humans appears to affect the qualitative distribution of cells by increasing the ratio of T4/T8 lymphocytes in a significant extent [80,81]. An increase in the number of lymphocytes was also observed in a study after 3 months of dietary CoQ10 supplementation in humans [82]. An investigation of a healthy population in ages 90–106 years indicated that in women NK cell cytotoxicity is positively associated with plasma CoQ10 concentration [83].

Blood components are considered to play an important role during development of atherosclerosis. Monocytes initially attach and accumulate on the endothelial cells which are covering the arterial wall and appear as macrophages after migration [84,85]. According to this concept, the accumulation of oxidized cholesterol in macrophages is the main reason for the development of the atherosclerotic plaque. The process of monocyte–endothelial interaction is mediated by several substances and cellular components. One of the main factors is the adhesion molecules, the integrins, which upon stimulation are recruited to the surface of the cell [86,87]. Administration of CoQ10 to humans results in an uptake into monocytes but not into granulocytes (Table 2) [88]. Interestingly, α-T content is elevated in both monocytes and granulocytes. The phospholipid fatty acid composition displays a very selective modification, appearing as an increase of the arachidonic acid content. When the basal and stimulated levels of β2-integrin CD11b and complement receptor CD35 were investigated, the expression of these receptors on the cell surface was found to be decreased considerably. Again, these changes were restricted only to monocytes. The antiatherogenic effect of CoQ10 was established earlier from studies on apolipoprotein E deficient mice fed a high fat diet [89,90]. It was shown in these investigations that upon CoQ administration, in addition to the increased aortic CoQ and α-T levels and the decreased concentration of hydroperoxides in the atherosclerotic lesions, there was a clear decrease of lesion size in the whole aorta. This could not be attributed solely to an antioxidative effect since several oxidative stress markers did not decrease in this study. The decrease of adhesion factors upon dietary CoQ administration appears to inhibit the recruitment of monocytes to the

Table 2
Effects on human monocytes after coenzyme Q supplementation

	10-week CoQ treatment (% of 0 week)
CoQ	
Plasma	218
Mononuclear cells	149
Polynuclear cells	101
α-Tocopherol	
Plasma	108
Mononuclear cells	201
Polynuclear cells	158
Arachidonic acid in phospholipids	
Mononuclear cells	134
CD11b, mononuclear cells	
Resting	27
Stimulated	58
CD35, mononuclear cells	
Resting	38
Stimulated	79

Data taken from Turunen et al. [88].

atherosclerotic lesions and thereby counteract the progression of the disease. Consequently, other mechanisms than antioxidant protection only are involved in the anti-atherogenic effect of CoQ.

1.6. Endothelial function

One of the characteristic events in many diseases, such as diabetes and cardiovascular diseases, is endothelial dysfunction of the arteries which has serious consequences [91]. When the function of the brachial artery was measured as flow-mediated dilatation and glyceryl-trinitrate-mediated dilatation following dietary supplementation with CoQ10, a sizeable improvement of endothelial function was observed [92]. CoQ10 administration to type 2 diabetic patients also lowers systolic blood pressure and HbA_{1c} but it does not decrease F2-isoprostanes [93]. Increased endothelial release of nitric oxide is the most probable explanation for the arterial effect causing dilatation.

2. Distribution

2.1. Tissues

CoQ is present in all tissues and cells but, on weight basis, in variable amounts (Table 3A). The lipid is also found in all isolated lipoprotein fractions of human blood [94]. The main species in rat is CoQ9 that varies from 17 μg/g tissue in lung to 202 μg/g in heart [52]. About 10–20% of the total CoQ in rat has 10 isoprenes, CoQ10, with the exception of brain, spleen and intestines where 30–40% of the total is CoQ10. In human the main species is CoQ10 which ranges from 8 μg/g in lung to 114 μg/g in heart. Small amounts, 2–7% of CoQ9, are also found in all human tissues. By employing rapid extraction, partition and direct injection of the extract into HPLC, it is possible to estimate the degree of reduction, most probably existing also in vivo [95,302]. This shows that the degree of the reduced state is very high, more in human than in rat, with the exception of lung and brain. The reducing systems in various organs, as discussed later on, are highly efficient.

Table 3
(A) Coenzyme Q amount, type and extent of reduction in human and rat tissues

	Rat			Human		
	CoQ9	CoQ10	% Reduced	CoQ9	CoQ10	% Reduced
Heart	202	17	22	3	114	47
Kidney	124	22	42	3	67	73
Liver	131	21	87	2	55	95
Muscle	43	3	40	1	40	60
Brain	37	19	27	1	13	23
Pancreas	37	3	62	2	33	100
Spleen	23	9	18	1	25	87
Lung	17	2	12	1	8	24
Thyroidea	44	7	45	1	25	68
Testis	32	5	49	1	11	78
Intestine	51	19	67	1	12	93
Colon	48	8	52	1	11	83
Ventricle	56	5	52		12	59

(B) Coenzyme Q amount in different part of bovine brain

	CoQ10
Temporal cortex	10
Parietal cortex	15
Hippocampus	6
Striatum	25
Medulla oblongata	5
White matter	3

The values are given in μg/g tissue. Data taken from Åberg et al. [52] and Runquist et al. [96].

Table 4
Coenzyme Q, dolichol, cholesterol and α-tocopherol in subcellular organelles of rat liver

Organelle	CoQ	Dol+Dol-P (μg/mg protein)	Cholesterol	α-Tocopherol
Nuclear envelope	0.2	0.2	37.5	0.01
Mitochondria	1.4	0.09	2.3	0.04
Outer membranes	2.2	0.25	30	0.06
Inner membranes	1.9	0.009	5.0	0.04
Microsomes	0.2	0.4	28	0.07
Rough microsomes	0.2	0.5	16	0.04
Smooth microsomes	0.3	0.3	31	0.09
Lysosomes	1.9	4.7	38	0.18
Lysosomal membranes	0.4	0.6	6.1	
Golgi vesicles	2.6	1.7	71	0.48
Peroxisomes	0.3	0.8	6.4	0.02
Plasma membranes	0.7	0.8	128	0.008

CoQ, coenzyme Q; Dol, dolichol; Dol-P, dolichyl phosphate. Data taken from Löw et al. [110], Zhang et al. [209] and Ericsson and Dallner [301].

Table 3B [96]. Manifold differences are apparent between the individual regions, indicating both structural and functional specializations, e.g. histological structure and number of mitochondria.

2.2. Intracellular distribution

All organelles isolated upon subfractionation of the liver contain the different types of mevalonate pathway lipids, although in most variable amounts (Table 4). The highest amounts of CoQ (on protein basis) are found in the outer and inner mitochondrial membranes, lysosomes and Golgi vesicles. Dolichol is enriched in lysosomes and Golgi vesicles while cholesterol shows the characteristic gradient: rough microsomes→smooth microsomes→lysosomes→Golgi vesicles→plasma membranes. Golgi vesicles are the far most dominant organelle concerning α-T enrichment.

In most species, one chain length of CoQ is dominating and in a minor extent a lipid with a shorter or a longer side chain is also found. The situation is, however, quite different

from that of dolichol which is distributed in tissues as a family of polyisoprenoids with six to seven members [97]. It was proposed that the heterogeneity of CoQ is required for functional specializations, such as for mitochondrial respiration and for antioxidative action [98]. The experiences are, however, not in favor of such specialization. In rat brain where one-third of the CoQ has 10 isoprene residues, the distribution pattern is identical in various regions and also in subcellular organelles [99]. The effectiveness to serve as respiratory chain redox carrier or as an antioxidant agent is not influenced by the length of the isoprenoid side chain [100–102]. Upon reconstitution of extracted beef heart submitochondrial particles, either with CoQ9 or CoQ10 within the physiological range of lipid concentrations, no differences could be observed in the rate of superoxide generation [103]. In spite of these facts, in some cases the type of CoQ species is of importance. Activation of UCPs and regulation of the mitochondrial PTP have selective requirements [71,78]. It was observed that mumps virus infection of dorsal ganglion cells, which contain both CoQ9 and CoQ10, led to a decrease of these lipids and resulted in neuronal degeneration [104]. The cells could be rescued by addition of CoQ10 to the culture but not by addition of CoQ9.

2.3. Intramembranous localization

Lipids of the mevalonate pathway have distinct intramembranous localizations that has considerable consequences on membrane properties. Polyisoprenoid chains of CoQ, dolichol and dolichyl-P are present in the central hydrophobic region, between the double layers of phospholipid fatty acids (Fig. 4) [105–108]. They probably have a coiled formation and the three lipids saturate the available space, since membrane thickness is considered to be a characteristic of the membrane type investigated. The functionally active groups, the benzoquinone ring of CoQ and the phosphorylated α-isoprene of dolichol-P, turn out to the outer or inner surface of the membrane depending on the functional requirement. This central localization is considered to destabilize membranes and results in an increased fluidity and permeability. On the contrary, the other obligatory membrane component, cholesterol, is distributed between fatty acids on one side of the lipid leaflet, thereby causing stabilization with decreased fluidity and permeability [109].

This arrangement of isoprenoid-derived lipids has two major consequences. First, all membranes have to be saturated with the appropriate lipid for optimal function and the level of saturation is dependent on the structural organization of the membrane type. Second, if a membrane is deficient in an isoprenoid, the consequences for membrane function, e.g. fluidity, will be deleterious. In this case, one can expect an uptake from exogenous sources up to the limit of saturation. If the uptake continues, the excess lipid has to have a non-membranous distribution. In fact, lysosomes have a CoQ and dolichol content that exceeds several-fold the content of other membranes. After sonication, however, the major part of the lipid content is solubilized, including 80% of CoQ, and the lysosomal membranes exhibit a similar concentration of polyisoprenoids as the other cellular membranes [110]. Also, exogenous CoQ, taken up by the liver and appearing in organelles, can to a large extent be removed by mechanical treatment [111].

2.4. Removal of CoQ from membranes

There are limited possibilities to obtain an in vitro system with different CoQ contents and amounts of other neutral lipids. Extraction with selected organic solvents, preferentially with *n*-pentane, is one of the few ways for such studies [4]. The principle of this procedure is that membranes prepared in sucrose-free medium are lyophilized and repeatedly extracted with *n*-pentane. Thereafter, the extracted samples are equilibrated with *n*-pentane containing various amounts of CoQ (and/or cholesterol, dolichol and α-T). After drying the resuspended fraction, the preparation can be used for various measurements. The great advantage for using this

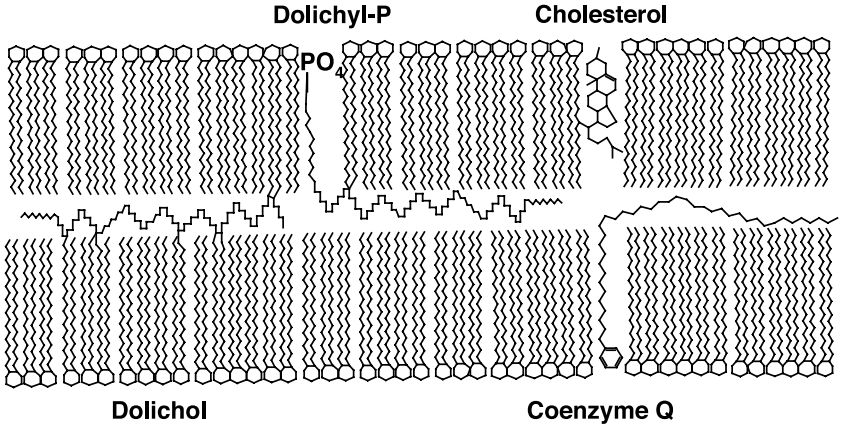

Fig. 4. Intramembraneous distribution of mevalonate pathway lipids.

procedure is that only neutral lipids but not phospholipids or proteins are extracted and most of the integral membrane enzymes are not denatured, and one can analyze enzyme and membrane properties in the presence of different CoQ content alone or together with other neutral lipids [112,113].

3. Biosynthesis

The biosynthesis of CoQ has been extensively investigated in bacteria and yeast but only studied to a limited extent in mammals. The terminal part of the bacterial CoQ biosynthesis differs from yeast in the order of synthetic events, i.e. in prokaryotes the modifications of the ring after attachment of the side chain proceeds via a decarboxylation prior to hydroxylation and methylation whereas in eukaryotes decarboxylation occurs after hydroxylation and methylation. All *COQ* genes isolated from yeast (*COQ1–8*) have a putative mitochondrial targeting signal and no isoenzymes have been identified [114]. However, CoQ is found in all subcellular compartments and, hence, a transport should exist from mitochondria to other membranes. In mammalian tissues, only two genes (*COQ3* and *COQ7*) have been isolated through complementary recognition with yeast [115,116].

3.1. The mevalonate pathway

The mevalonate pathway comprises the reactions starting from acetyl-coenzyme A (acetyl-CoA) and ending up with farnesyl pyrophosphate (FPP), the substrate for the biosynthesis of CoQ, cholesterol, dolichol and isoprenylated proteins (Fig. 5) [117]. Thus, the conversion of acetyl-CoA to FPP is common for all end-products. This organization is unusual since the biosynthetic sequence is identical for several lipids and one could expect that production of one lipid greatly influences the synthesis of the other lipids. However, the mevalonate pathway lipids are synthesized in highly different rates and amounts, which involves, in addition to the central regulation, a terminal regulation. The regulatory enzymes are probably the branch-point enzymes utilizing FPP. This fact makes the mevalonate pathway very complex in animal cells.

The initial part of the mevalonate pathway involves the condensation of three acetyl-CoA to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), which is mediated by two enzymes, acetoacetyl-CoA thiolase and HMG-CoA synthase. HMG-CoA reductase then converts HMG-CoA to mevalonate. This enzyme has been studied extensively and is considered to be the main regulatory enzyme in cholesterol biosynthesis [118]. Effective competitive inhibitors of this enzyme, compactin and mevinolin and their derivatives, are in the western world among the most commonly used drugs [119]. Interestingly, these inhibitors induce the synthesis of the reductase at the transcriptional level and, additionally, inhibit the degradation of the enzyme [120]. The action of inhibitors therefore leads to low enzyme activity but high amount of enzyme. Cholesterol mainly affects HMG-CoA reductase by feedback regulation but the enzyme is influenced by many other mechanisms,

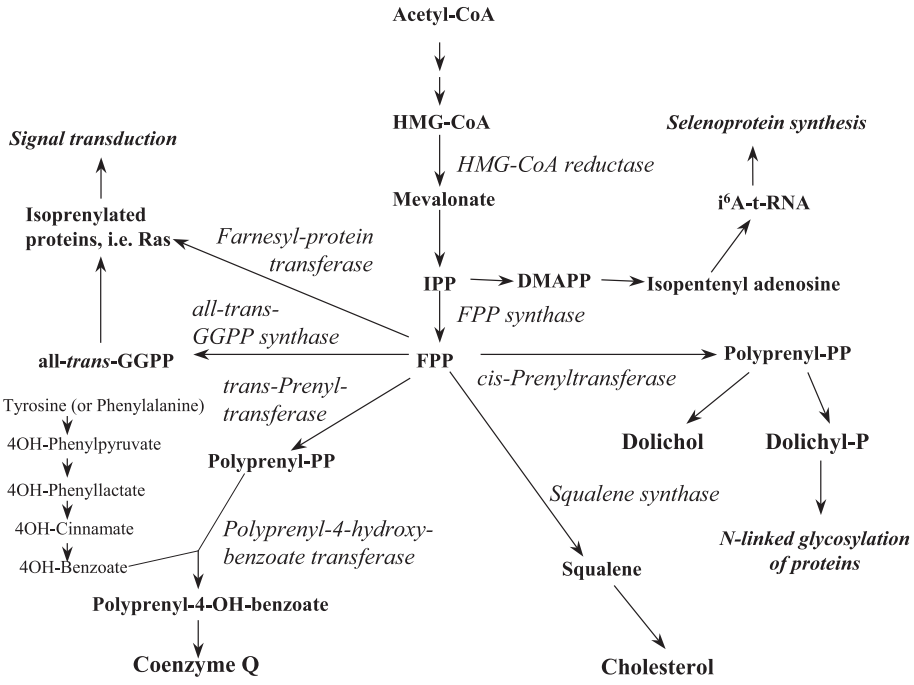

Fig. 5. The enzymatic conversion and condensation of acetate to farnesyl-PP and subsequent biosynthesis of CoQ, cholesterol and dolichol. Abbreviations: CoA, coenzyme A; DMAPP, dimethylallyl pyrophophate; FPP, farnesyl pyrophosphate; GGPP, geanylgeranyl pyrophosphate; HMG, 3-hydroxy-3-methylglutaryl; 4OH, 4-hydroxy; IPP, isopentenyl pyrophosphate. Key enzymes are indicated in italic.

e.g., diurnal rhythm, hormones and oxysterols [121,122]. One of the factors behind the rapid and reversible regulation of this enzyme can be inactivation–activation elicited by phosphorylation–dephosphorylation of the protein [123].

Mevalonate is subsequently phosphorylated in two steps by mevalonate kinase and phosphomevalonate kinase. Mevalonate phosphates appear, in addition to intermediates, to be regulators of cell proliferation and DNA synthesis [124]. Decarboxylation of mevalonate pyrophosphate yields isopentenyl pyrophosphate (IPP), which is not only a precursor of FPP but also the main building block in the polyisoprenoid biosynthesis of dolichol and the side chain of CoQ. Isomerization of IPP gives dimethylallyl pyrophosphate which is an intermediate in the biosynthetic process of isoprenoid lipids and, furthermore, also participates in the synthesis of isopentenyl $tRNA^{ser/sec}$ at the position six of an adenosine (i^6A) [125,126]. This isoprenylation of $tRNA^{ser/sec}$ is necessary for an effective selenoprotein synthesis.

Farnesyl pyrophosphate synthase utilizes IPP and its isomer dimethylallyl pyrophophate to make FPP with the formation of an intermediary product, geranyl pyrophosphate (GPP) [127]. This model involves GPP's presence only as an enzyme-bound intermediate. On the other hand, it was previously found that GPP—at least in in vitro conditions—is serving as the main precursor in solanesyl pyrophosphate and decaprenyl pyrophosphate synthesis both in rat liver and spinach homogenates [128,129]. If the major substrate for *trans*-prenyltransferase is GPP also in vivo, it should be a GPP pool for this purpose. Direct measurements of possible pools concerning mevalonate, FPP and GPP are, however, not yet made and therefore this question is unanswered. During various experimental conditions like fasting, cholesterol feeding, treatment with cholestyramine or mevinolin, FPP synthase is influenced in coordination with HMG-CoA synthase and HMG-CoA reductase activities [130]. The major part of hepatic FPP synthase is present in the cytoplasm but some activity is found in all organelles isolated [117]. The enzymatic activity at different locations exhibits different responses upon treatment of rats with inducers and drugs, which demonstrate that the enzyme is subjected to separate regulation depending on its location in cytosol, microsomes, mitochondria or peroxisomes [131].

The next enzymes in the biosynthesis of cholesterol, dolichol, CoQ and protein isoprenylation, i.e. squalene synthase, *cis*-prenyltransferase, *trans*-prenyltransferase and farnesyl-protein transferase, are considered to be the rate-limiting enzymes in the terminal part of their biosynthetic process. The isoprenoid biosynthetic enzymes in the mevalonate pathway are evolutionarily closely related. X-ray

Table 5
Isolated genes encoding the enzymes involved in the terminal biosynthesis of coenzyme Q

Enzyme	Gene	Isolated or cloned from	Reference
trans-Prenyltransferase	*COQ1*	*Saccharomyces cerevisiae*	[305]
	DSP	*Schizosaccharomyces pombe*	[306]
	ISPB	*Escherichia coli*	[307]
Polyprenyl-4-hydroxybenzoatetransferase	*COQ2*	*Saccharomyces cerevisiae*	[140]
	PPT1	*Schizosaccharomyces pombe*	[308]
	UBIA	*Escherichia coli*	[309]
Monooxygenase	*UBIB*	*Escherichia coli*	[167]
Unknown function	*COQ4*	*Saccharomyces cerevisiae*	[114]
2,3-Dihydroxy-5-polyprenylbenzoate-methyltransferase	*COQ3*	*Saccharomyces cerevisiae*	[149]
		Mouse	[154]
		Rat	[153]
		Human	[115]
	UBIG	*Escherichia coli*	[150]
Unknown function	*ABC1/COQ8*	*Saccharomyces cerevisiae*	[310]
			[166]
Decarboxylase	*UBID*	*Escherichia coli*	[311]
	UBIX	*Escherichia coli*	[312]
C-Hydroxylase	*COQ6*	*Saccharomyces cerevisiae*	[165]
	UBIH	*Escherichia coli*	[313]
C-Methyltransferase	*COQ5*	*Saccharomyces cerevisiae*	[314]
	UBIE	*Escherichia coli*	[315]
3-Methoxy-6-methyl-5-polyprenyl-benzoquinone-hydroxylase	*COQ7/CAT5*	*Saccharomyces cerevisiae*	[157]
			[158]
		Mouse and Rat	[160]
	UBIF	*Escherichia coli*	[316]
	CLK-1/COQ7	*Caenorhabditis elegans*	[161]
		Mouse	[159]
		Human	[163]
			[116]
Coq1-8p	*COQ1–8*	*Caenorhabditis elegans*	[184]

crystallization of several enzymes that use FPP or its derivatives as substrate have 3D structures similar to the avian FPP synthase [132].

3.2. The terminal part of CoQ biosynthesis

Although the genes in the terminal part of the CoQ biosynthesis have been cloned in bacteria and to some extent in yeast, there is still a limited amount of information about the biosynthetic enzymes in mammalian tissues. The genes so far identified as encoding the enzymes in CoQ synthesis are given in Table 5.

3.2.1. trans-Prenyltransferase, Coq1p

The *trans*-prenyltransferase catalyzes the condensation of FPP with several IPPs, all in *trans* configuration, to form a long isoprenoid chain. The enzyme belongs to class III enzymes in the trans-prenyltransferase family. While class I enzymes produce short isoprenoid chains and class II produces medium and long chains, class III only produces long isoprenoid chains. Although the short chain enzymes (e.g. FPP synthase) have been thoroughly characterized, the class III enzymes are still elusive. Only a few enzymes have been isolated, all in bacteria and yeast, and their characterization is based on complementary rescue of null mutants [133]. Most of them form homodimers and all require Mg^{2+} for activity. All classes share several conserved aspartic acid-rich sequence motifs and the length of the side-chain produced is species-specific. For instance, *Saccharomyces cerevisiae* make CoQ6, *E. coli* synthesizes CoQ8 and rodents and humans produce mainly CoQ9 and CoQ10, respectively. This specificity is retained within the gene sequence, since cross-expression of genes has shown that the length of the side chain is altered when a different *trans*-prenyltransferase encoding gene is expressed [134]. It is believed that Mg^{2+} serves to bind the substrate and that the region located around the first aspartic acid-rich motif regulates the length of the side chain produced. Especially the fifth amino acid, upstream the first aspartic acid-rich motif, seems to be important. If this amino acid is changed from alanine to an aromatic amino acid, the polyisoprenoid produced is shifted from a long chain to geranylgeranyl pyrophosphate [132]. The reverse has also been shown using FPP synthase and geranylgeranyl pyrophosphate synthase [135,136].

While the yeast *trans*-prenyltransferase is considered to be a mitochondrial protein, mammalian *trans*-prenyltransferase activity is mostly found in the ER, while lower activities are found in the mitochondria, Golgi vesicles and peroxisomes [128]. The rat *trans*-prenyltransferase also has a requirement for divalent cations but prefers *trans*-GPP and IPP as substrates. In vitro measurements of the rat and mouse *trans*-prenyltransferase have shown that the activity can be induced by administration of peroxisomal inducers [128,137].

3.2.2. Polyprenyl-4-hydroxybenzoate transferase, Coq2p

In the terminal part of CoQ synthesis two substrates are required, polyprenyl pyrophosphate and the ring structure, 4-hydroxybenzoate (Fig. 6). This latter substrate originates from tyrosine but theoretically it may also be deduced from phenylalanine either by hydroxylation to tyrosine or by enzymatic transformation similar to that of the tyrosine molecule. The intermediates given in Fig. 5 are produced by the action of transaminase, dehydrogenase, dehydratase and completed by a β-oxidation. It was found that this β-

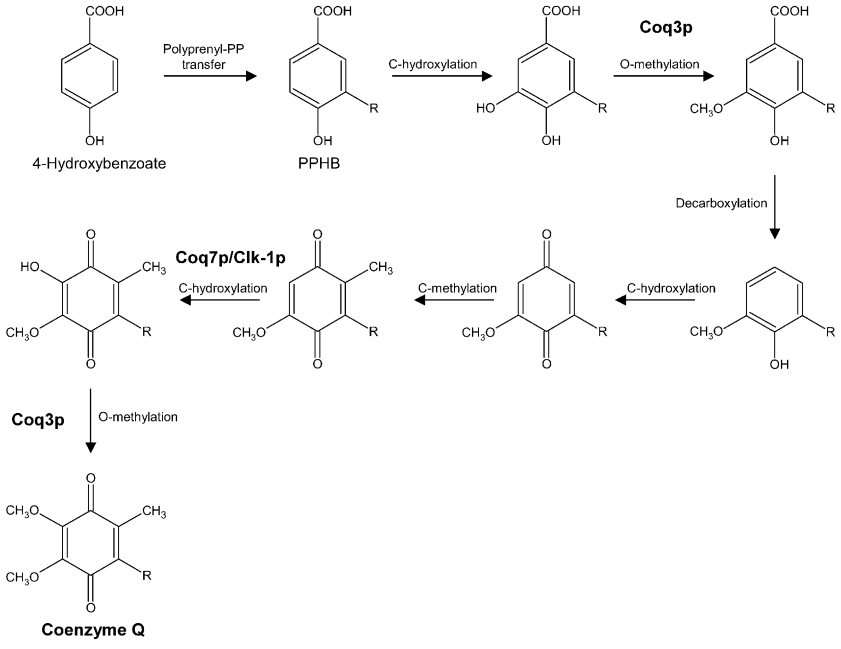

Fig. 6. Terminal reactions in the biosynthesis of CoQ. PPHB: polyprenyl-4-hydroxybenzoate.

oxidation is KCN-insensitive which is diagnostic for the peroxisomal oxidation, but the localization of biosynthesis is not analyzed in detail [138]. Since tyrosine is an essential amino acid in animal cells, contrary to bacteria and yeast, availability of the ring could be rate-limiting in an in vivo system. The experience is, however, that this substrate is in excess in tissues and the fact that 4-hydroxybenzoate is in 10-fold excess in the urine compared to the daily need for CoQ biosynthesis also supports this assumption [139]. Since 4-hydroxybenzoate is a common bacterial product in the intestinal system of animal organisms, it is possible that this supply is far exceeding that of endogenous production. The second step in the terminal part of CoQ biosynthesis is the condensation of the polyisoprenoid side-chain with 4-hydroxybenzoate (Figs. 5 and 6). The gene encoding polyprenyl-4-hydroxybenzoate transferase (*COQ2*) is cloned and characterized in yeast and *E. coli* (*UBIA*), but the mammalian *COQ2* homologue has not yet been identified. The predicted amino acid sequence of the yeast and *E. coli* gene exhibits two conserved aspartic rich motifs, which are believed to bind polyprenyl pyrophosphate [140,141]. The yeast *COQ2* contains a typical N-terminal mitochondrial leader sequence but the microsomal enzyme activity is nearly as high as the mitochondrial activity and both of these activities are absent in the *coq2* mutant [140]. The *UBIA* gene has been overexpressed and characterized in *E. coli*, giving a 3000-fold enrichment in activity [141]. The enzyme was not solubilized by hypertonic buffer or detergent and was shown to be Mg^{2+}-dependent. A broad spectrum of isoprenoids (GPP, FPP and solanesyl-PP) was accepted as substrates by this enzyme. The transferase can be inhibited in vitro by benzoate ring analogues such as 4-aminobenzoate and 4-chlorobenzoate, however, these inhibitors are not operating in vivo [142]. In cell culture, 4-aminobenzoate is competing for solanesyl-PP and is condensated with the polyprenol while no direct interaction is occurring with 4-chlorobenzoate. It appears that -SH groups are essential for the transferase activity since 4-hydroxymercuribenzoate is inhibitory. In vitro activity of the rat enzyme is found in the Golgi vesicles, but also in the ER, mitochondria and peroxisomes [112,137,143–145,177]. The enzymatic activity can be up-regulated by administration of the peroxisomal inducer clofibrate [137].

There are alternative possibilities to 4-hydroxybenzoate as acceptor of the polyisoprenoid side chain (Fig. 7). One of the proposals is that like fatty acids, the ring has to be activated with coenzyme A in order to interact with the side chain [146]. Modifications of the ring which are considered as subsequent reactions (hydroxylation and methylation) may precede the condensation reaction [138,144]. An interesting possibility is that products of the catecholamine metabolism, protocatechuic and vanillic acid, which are substituted derivatives of the 4-hydroxybenzoate, are qualified for completion to CoQ.

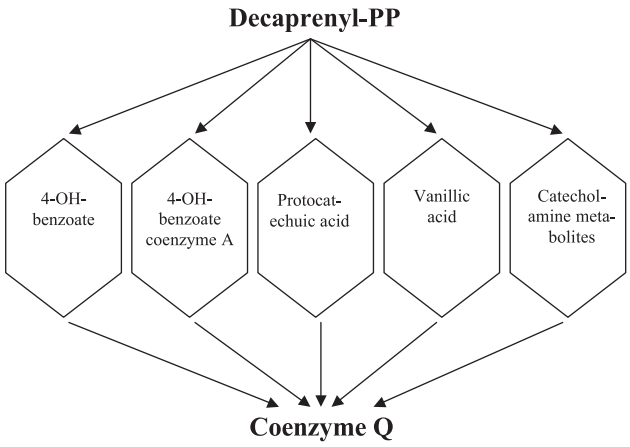

Fig. 7. Possible ring acceptors in CoQ biosynthesis.

3.2.3. 2,3-Dihydroxy-5-polyprenylbenzoate-methyltransferase, Coq3p

There are two *O*-methylations in the production of CoQ and the same enzyme presumably catalyzes both (Fig. 6). In eukaryotes, the first *O*-methylation occurs after polyprenyl-4-hydroxybenzoate has been hydroxylated. However, it has been suggested by Kang et al. [147] that the decarboxylation actually occurs before the first hydroxylation and methylation in rodents. The enzyme was first partially purified and characterized in yeast and it was concluded that *S*-adenosylmethionine (SAM) was the methyl donor [138]. Tzagoloff and Dieckmann isolated nine separate complementation groups in yeast containing reduced levels of CoQ, which led to the identification of *COQ3* in yeast and *UBIG* in *E. coli* [148–150]. The predicted amino acid sequence for the proteins of *COQ3* and *UBIG* contains four sequence motifs that are conserved in a large family of enzymes utilizing SAM as methyl donor [151,152]. The rat *COQ3* cDNA was recovered by its ability to rescue CoQ biosynthesis in yeast *coq3* mutants [153]. Its N-terminus shares the features common for mitochondrial import sequences. The rat cDNA has then been used to map the mouse *COQ3* to the proximal region of chromosome 4 and for the identification of the full-length human cDNA [115,154]. Using farnesylated analogues, 2,3-dihydroxy-5-polyprenyl-1,4-benzoic acid and demethylubiquinone, together with radiolabeled SAM, it was concluded that the same enzyme could possibly perform both *O*-methylations and that Coq3p or UbiGp is required for in vitro activity in yeast and *E. coli* [155,156]. The activity of Coq3p was found in yeast mitochondria and cell-free extracts of *E. coli*. Using [^{3}H]SAM, we have also found an in vitro methylation activity of the farnesylated analogues (supplied by C.F. Clarke, UCLA) in rat liver microsomes and mitochondria. However, the major part of the activity was recovered from the cytosol and therefore it is unclear to what extent the cytosolic catechol-*O*-methyltransferase contributes to these activities. Catechol-*O*-methyltransferase shares several features with the Coq3p, since they are both dependent on a divalent cation and utilize SAM. Their amino acid

sequences, however, fail to show any homology with yeast *COQ3* outside the sequence motifs for SAM binding that are conserved for all SAM-dependent enzymes.

3.2.4. 3-Methoxy-6-methyl-5-polyprenyl-benzoquinone-hydroxylase, Coq7p

The second out of two mammalian genes identified in the terminal part of the CoQ biosynthesis is *COQ7* [116]. The enzyme hydroxylates 3-methoxy-6-methyl-5-polyprenyl-benzoquinone (demethoxy-ubiquinone) yielding 2-hydroxyubiquinone [157]. The yeast gene was independently isolated as *CAT5*, and was thought to be encoding a protein required for release of gluconeogenic genes from glucose repression [158]. Later studies, however, showed that this gene is directly involved in CoQ biosynthesis and that the defect in gluconeogenetic gene activation could be restored by CoQ supplementation [159]. CoQ biosynthesis in *S. cerevisiae coq7* null mutants could be restored with the complementary *COQ7* genes from rat, humans or the *CLK-1* gene from *C. elegans* [116,160,161]. The *clk-1* mutants show early developmental arrest and sterility, and this phenotype can be rescued by a dietary source of CoQ [162]. The mouse *clk-1* mutant accumulates demethoxy-ubiquinone in heart and liver mitochondria. This product is redox active and can partially compensate for some of the defective respiratory functions seen in *C. elegans clk-1* mutants [163]. It appears, however, to lack capacity as antioxidant. The *CLK-1* is a gene determining the life span and was found to be conserved among eukaryotes, such as yeast, rodents and humans [161,164]. Consequently, the interest for this gene is accentuated and the fact that the *CLK-1* homologue *COQ7* is participating in CoQ biosynthesis gives great possibilities for hypotheses concerning connections between life span and CoQ metabolism.

In a recent study using RNA inteference, eight genes of CoQ synthesis (*COQ1–8*) were identified in *C. elegans* [184]. It was demonstrated that not only *CLK-1* but all the other genes are of importance for life span in *C. elegans*. In silencing experiments of the individual genes, there were unchanged mitochondrial respiratory activities but superoxide production was decreased and life span were extended.

3.2.5. Other CoQ biosynthetic enzymes

There are eight modifications of the benzoate ring and only two mammalian genes are isolated and their enzymes partly characterized [115,116]. Coq3p catalyzes two *O*-methylations and coq7p catalyzes one hydroxylation. Out of the remaining five reactions (C-methylation, decarboxylation and three hydroxylations) only some have been characterized in yeast and bacteria. The *COQ5* gene has been attributed to the C-methylation of the ring and the *COQ6* gene is suggested to be involved in one of the hydroxylations [165,311]. *COQ4* and *ABC1/COQ8* have been isolated although their function is unknown [114,166]. The *UBIB* gene of *E. coli* was recently identified and proposed to be involved in the first monooxygenase step [167]. Although the genes are now identified, much of the work on the biosynthesis of CoQ is still inconclusive and further work on a protein level is needed before the *COQ* genes can truly be attributed to the individual steps in CoQ biosynthesis. Identification and characterization of the individual steps, however, may turn out to be more difficult than it appears at present. The studies of Hsu et al. [168] have established that the major sequence of CoQ synthesizing enzymes, products of *COQ3–COQ8*, is organized in a multi-subunit complex in yeast. Consequently, the individual coq null mutants do not necessarily produce the appropriate intermediate which can be used both for identification and as substrates in enzymatic studies, since the presence of all polypeptides may be required for the proper function and stability of the complex. In fact, the different yeast coq mutants (*coq3–coq8*) fail to produce CoQ and all of them accumulate mainly or exclusively 3-hexaprenyl-4-hydroxybenzoic acid. This compound is the product of the protein controlled by the *COQ2* gene, which parallels the Coq1p by not participating in the complex. It appears probable that in animal tissues the organization of the biosynthetic system is similar, since both isolated perfused beating rat heart and mitochondria prepared from rat heart accumulate nonaprenyl- and decaprenyl-4-hydroxybenzoate [169,170].

4. Intracellular transport

Independently of the locations of CoQ synthesis, the lipid has to be transported within the cell since all cellular membranes contain CoQ. In animal cells the mitochondrial inner membrane, but not necessarily the outer membrane, probably receives all of its CoQ from the synthetic machinery located in the matrix–inner membrane space [138]. Liver perfusion studies established that VLDL assembled in the ER–Golgi system contain newly synthesized CoQ [171]. It is known that all components of the lipoprotein have to be synthesized at the location of the assembly. Newly synthesized CoQ is part of the lipoprotein, necessary for antioxidant protection. The way of transport of the lipid from the ER–Golgi system to other cellular compartments is not established. For lipid transport in general, three mechanisms are described: vesicular transport, carrier protein-bound transport and transport involving micellar formation, all of which are possible transport ways also for CoQ [172]. Using in vivo labeling and cell fractionation, it was found that in spinach leaves CoQ is transported from the ER to other compartments through a vesicle-mediated process, which involves the Golgi system [173]. Monensin and brefeldin are perturbing the Golgi system, resulting in an accumulation of the lipid in the ER. In this system the transport of CoQ is ATP-dependent but the cytoskeletal network is not involved. Similar conclusions were attained by applying a reconstituted cell-free system using mem-

brane fractions isolated from dark-grown spinach seedlings [174].

Transport also occurs in the opposite direction since exogenous CoQ is translocated through the plasma membrane and further intracellular transport again requires an appropriate mechanism. After administration of exogenous [^{3}H]CoQ to rats, followed by subcellular fractionation, the lipid was recovered from transport vesicles found in the fatty layer and from possible carrier proteins in the cytosol [111]. Recently, a new system was described which may be of great help in studying the transport mechanism. CoQ is absent in the electron transport chain of some yeast mutants which, however, are able to take up external CoQ into the plasma membrane and subsequently transport it into mitochondria [175]. In this way it is possible to reconstitute the deficient electron transport chain. In some other mutant strains the exogenous CoQ remains in the plasma membrane since there is a defect in the transport mechanism and the lipid is not delivered to mitochondria. Using this approach, yeast mutants may be useful for detection and characterization of specific transport vesicles in the cytoplasm as this mechanism probably is involved in the translocation process in other species.

5. Compartmentalization of the mevalonate pathway

It is common that a single biological process or enzymatic sequence has several locations in the animal cell contrary to one-cell organisms where functions are often concentrated to a single membrane system. The mevalonate pathway in rat liver is a good example for the complexity of cellular organization (Fig. 8). Acetyl-CoA is the initial substrate for the product which is FPP. The major part of enzymes mediating the reactions of the pathway is present in the cytoplasm with the exception of HMG-CoA reductase which is located in microsomes and peroxisomes. However, FPP synthase is present also at other compartments than in the cytosol. Both mitochondria and peroxisomes can utilize IPP for FPP synthesis, which means that both organelles contain the isomerase and FPP synthase. Since several enzymes of the mevalonate pathway are found in peroxisomes, it is also possible that peroxisomes contain the complete pathway in spite of the fact that earlier substrates than IPP cannot be used for FPP synthesis under in vitro conditions. Microsomal synthesis of the isoprenoid sidechain of CoQ requires GPP rather than FPP as substrate in both plants and rat liver, which is not explained so far, since FPP synthase mediates a two-step reaction from dimethylallyl pyrophophate and GPP is supposed to be present only as an enzyme-bound intermediate. It is possible that some of the terminal reactions distributed in various subcellular organelles of rat liver require FPP to be synthesized in the organelles themselves in order to be utilized by the branch-point enzymes of the same organelle. An alternative possibility is that a part of the synthase has structural association with the appropriate membrane by hydrophobic or charge forces and FPP is directly channeled into the membrane without releasing the product, to serve FPP requiring reactions.

CoQ biosynthesis is studied in mitochondria and in the ER–Golgi system of both liver and spinach leaf [129,138,145,176]. Recently, *trans*-prenyltransferase and nonaprenyl-4-hydroxybenzoate transferase were also found in peroxisomes [177] and continued studies are required to establish the presence of other CoQ biosynthetic enzymes in this organelle. The presence of CoQ synthesis at several locations in animal tissues is not surprising, since other mevalonate end products are also produced at several locations. Cholesterol and dolichol are synthesized in both

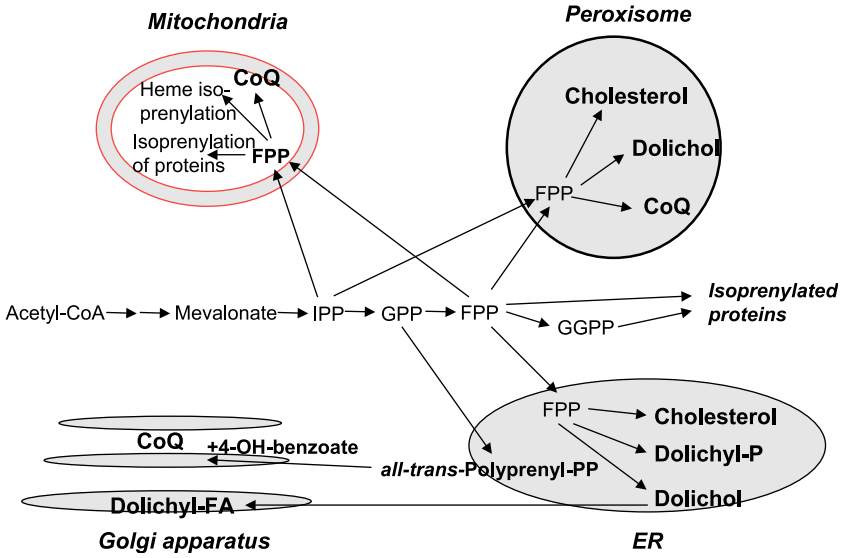

Fig. 8. Compartmentalization of the mevalonate pathway and associated reactions after the branch-point. Abbreviations: CoA, coenzyme A; CoQ, coenzyme Q; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; GPP, geranyl pyrophosphate; IPP, isopentenyl pyrophosphate.

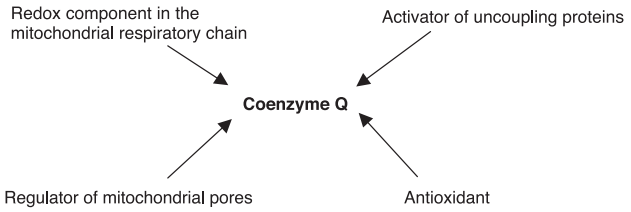

Fig. 9. Possible functional pools in mitochondria.

microsomes and peroxisomes, while protein isoprenylation takes place in mitochondria and microsomes, in addition to cytosol [178–180].

In general, the synthesis in two organelles does not mean a simple doubling of the reactions but is rather related to a specialization. β-Oxidation of fatty acids in mitochondria and peroxisomes are utilizing different substrates and the two organelles are cooperating in the total oxidation [181]. Mitochondria are probably producing CoQ for the respiratory chain and for other mitochondrial functions. On the other hand, mitochondria cannot deliver CoQ for blood lipoproteins and newly synthesized VLDL, during the transport from the ER–Golgi system to the blood, has to acquire this lipid from local synthesis. Also, ER–Golgi CoQ is the most reasonable source of this lipid in other cellular membranes, including plasma membranes, since transport mechanisms from this system to other membrane compartments are well established.

CoQ is present in various amounts in different cellular organelles, reflecting a compartmentalization. In fact, the function of this lipid in mitochondria, plasma membrane and in microsomes is certainly different. Another type of specific distribution is that of lysosomes, i.e. the presence of the lipid in both the membrane and the vesicle lumen [110]. This luminal CoQ is not necessarily a storage compartment, it may exert functional effects. The possibility that the lipid is compartmentalized also within the membrane is raised repeatedly [182,183]. In the mitochondrial inner membrane CoQ has at least four different functions, redox carrier, activating UCPs, PTP regulation and antioxidant, which makes it improbable that only one common pool is operating (Fig. 9). UCP requires an association with oxidized CoQ10 for activation which hardly allows this pool to cooperate with either those portions participating in the respiratory chain or with those acting as antioxidant [70]. The fact that the decrease of the CoQ content by 60–70% in *C. elegans* does not effect CoQ-dependent mitochondrial electron transport is also supporting the concept of compartmentalization [184]. It would be of great interest to study also other CoQ-dependent mitochondrial functions in this system.

6. Regulation of the mevalonate pathway

The main regulatory enzyme of the mevalonate pathway is HMG-CoA reductase, preferentially affecting cholesterol synthesis. This is according to the classical flow diversion hypothesis, in which the size of the FPP pool mainly influences cholesterol synthesis [185]. Squalene synthase has a high K_m for its substrate, FPP, and already a limited decrease in concentration leads to insufficient saturation of the enzyme and thereby reduction of cholesterol synthesis. Since the K_m for the other branch point enzymes, *cis*- and *trans*-prenyltransferases and farnesyl-protein transferase, are lower, these enzymes are saturated even at lower substrate concentrations. This kinetic arrangement is the basis for the use of our most common drug, statins, inhibitors of the HMG-CoA reductase. However, we do not know the actual K_m for the various enzymes, with the exception of farnesyl-protein transferase [186], since not all of them are isolated and no values are available for direct comparison. Most probably, the differences in K_m are not as great as it is assumed. Statin treatment, both in rats and humans, appears to affect the amount and synthesis of all mevalonate pathway lipids [128,187–191]. CoQ amounts are decreased in heart, muscle and liver after mevinolin treatment of rats, indicating the importance of a decreased FPP pool on the *trans*-prenyltransferase reaction [192]. Inhibition of squalene synthase by squalestatin-1, which increases the FPP pool, elevates CoQ synthesis both in tissue culture cells and in rats in vivo [193,194].

Metabolites and intermediates function commonly as regulatory substances for metabolic pathways, but none is described so far for CoQ biosynthesis. A good candidate for such a role is farnesol or one of its metabolites, e.g., farnesyl esters, hydroxyfarnesol, farnesoic acid or dicarboxylic acids. Farnesol is suggested to be involved in cell growth, phosphatidylcholine synthesis, ROS generation, apoptosis, HMG-CoA reductase degradation, regulation of protein kinase C and Ca^{2+} channels, and additional functions will most probably appear in the future [195–197]. There are also other candidates for a regulatory function in CoQ biosynthesis. These are the biosynthetic intermediates or their modified forms such as the partially substituted ring structure without side chain, like vanillic acid. Metabolites are also produced upon ultraviolet irradiation and after lipid peroxidation in membranes, if the CoQ is present in oxidized form [60,198]. Some of these products may be of considerable interest in future studies.

Hormones are the well-established regulators of a large number of tissue functions and CoQ is not an exception from this rule. Very few studies are made in this field but it is known that growth hormone, thyroxine, cortisone, adrenaline and dehydroepiandrosterone treatments increase the CoQ amount in rat liver [199]. Administration of thiouracil, inhibitor of thyroid hormone production, decreases the lipid concentration in liver [200]. Exposure of rodents to cold temperature at 4 °C increases liver CoQ [201]. It remains to be investigated whether this increase is under hormonal control.

Rats which receive protein-free diet exhibit a decreased CoQ content in liver and heart but not in kidney, spleen and

brain [202]. From the experimental point of view, the problem is naturally that these changes are not selective but affecting a number of other biosynthetic systems and, consequently, it may be difficult to relate functional modifications to CoQ deficiency.

Endurance exercise training increases CoQ concentration in rat muscle on weight basis [203,204]. It appears that this elevation is mainly explained by the increase of the mitochondrial mass. In human, 4 days of high intensity training does not elevate CoQ content in the exposed muscles [205]. Dietary CoQ10 appears in the plasma but not in the skeletal muscle or in mitochondria isolated from the muscle of the control or training group.

Contrary to the situation with cholesterol, dietary and diurnal variations do not appear to modify the tissue CoQ content. Dietary vitamin A deficiency increases while vitamin A administration decreases CoQ amount in liver [200,206]. Selenium deficiency in rat decreases CoQ content in the liver by 50%, and some decrease also occurs in heart and kidney but not in muscle [207]. Interestingly, the decrease in liver does not affect plasma membranes which exhibit an elevated CoQ content [208]. α-T and CoQ levels in blood and tissues appear to be modified in a parallel fashion which is probably expected because of the close functional relationships between these two lipids. Upon dietary administration of vitamin E, not only the vitamin itself but also CoQ is increased in blood and liver [209]. On the other hand, vitamin E-deficient diet decreases the concentration of both lipids. When these lipids are supplied together in the diet, the efficiency of the uptake is increased in liver and spleen but no uptake was observed in heart, kidney, muscle and brain [210]. On the other hand, higher amount of CoQ was found in mouse heart, skeletal muscle, kidney, brain and liver upon CoQ administration and the uptake of CoQ was lowered when not only this lipid but also α-T was included in the diet [211]. The differences seen in these studies could be due to the method of administration, composition of basal diet and the strain used. In hyperlipidemic patients not only blood triglycerides but also both CoQ and α-T are increased [212]. When these patients were treated with gemfibrozil, blood lipids were normalized and a parallel decrease of both CoQ and α-T was observed. CoQ diet in humans increases not only this administered lipid in monocytes but also that of α-T [88]. In prion diseases of mice both lipids are greatly increased in the brain [213]. The close relationship in the parallel modifications of the two antioxidant lipids is probably a biological necessity. CoQ can regenerate α-T in vitro and thereby protect it from being decomposed by a second radical attack, and it is therefore reasonable that these two antioxidants act in concert [214].

7. Enzymatic reduction of CoQ

For the antioxidant function of reduced CoQ, it is of great importance that the reduced form can be regenerated at all cellular locations [303,304]. Several investigators have so far studied the regeneration of CoQ and different CoQ reductases have been suggested as reduction enzymes. It was proposed that there is a cytosolic NADPH-dependent CoQ reductase which is different from the mitochondrial NADH-CoQ reductase and DT diaphorase as it is rotenone- and dicumarol-resistant [215,216]. The most studied enzyme so far is DT-diaphorase, a cytosolic homodimeric enzyme [217,218]. This enzyme has been shown to possess the capacity to reduce CoQ but it is less efficient for longer isoprenoid side chain length, i.e. those with 9 or 10 isoprene units [219].

Recently, it has been shown in vitro that the enzymes lipoamide dehydrogenase, thioredoxin reductase and glutathione reductase efficiently could reduce coenzyme Q10 [220–222] (Nordman, T. Björnstedt, M. Olsson, J.M., unpublished data). These FAD-containing enzymes are homodimeric with subunits that have a molecular weight of about 55 kDa and belong to a family of pyridine nucleotide disulfide oxidoreductases [223–225]. Lipoamide dehydrogenase is mainly known to participate in the three α-ketoacid complexes located in the inner mitochondrial membrane [226], however, this enzyme and the other two oxidoreductases are present also in the extra-mitochondrial space. All three enzymes can reduce lipoic acid to its antioxidant form dihydrolipoic acid. In turn, dihydrolipoic acid was demonstrated to be able to reduce CoQ [227]. Although thioredoxin reductase belongs to the same family as lipoamide dehydrogenase, there are crucial structural differences affecting the functions of this enzyme. The C-terminal part of thioredoxin reductase is in comparison with lipoamide dehydrogenase elongated with about 20 amino acids that contain a cysteine and a selenocysteine adjacent to each other [228]. The incorporation of selenium as selenocysteine has been suggested to be facilitated by isopentenylation of the $tRNA^{ser/sec}$ via the mevalonate pathway (see above) [125]. The exceptionally broad substrate specificity is probably connected with this selenenylsulfide as it is located close to the active disulfide site, consisting of the conserved sequence Cys-Val-Asn-Val-Gly-Cys, at the N-terminal of the other subunit [228]. It is obvious that these enzyme systems are connected and contribute to an elevated level of resistance against oxidative stress in the cell.

8. Dietary uptake

CoQ is taken up from the intestine into the circulation with a low rate, only about 2–4% can be recovered [229]. The mechanism of uptake is not studied in detail but since it is found in the various lipoproteins, redistribution in the circulation appears to occur. In the case of α-T, the liver possesses a specific binding protein which delivers it back to the circulation via VLDL for further transport to different organs [230]. The limited amount of information available indicates that VLDL synthesized in the ER–Golgi system

contains only de novo synthesized CoQ in an amount which is required for antioxidant protection but not for redistribution to tissues [171]. Tritium-labeled dietary CoQ in liver is either metabolized or excreted into the bile and thereafter removed from the body [111]. In liver, spleen, adrenals, ovaries, arterial endothelium, blood monocytes and lymphocytes from rat, CoQ is taken up from the circulation while the uptake by heart, pancreas, pituitary gland, testis and thymus is very limited. No uptake is visible in kidney, muscle, brain, thyroid gland and blood polynuclear cells. Uptake into the brain was observed in an investigation and it cannot be excluded that the particular conditions used in this study influence the results [232]. Species difference is probably a deciding factor as in mice dietary CoQ appear in various organs [231].

We have tried to improve the uptake of CoQ by administering two synthesized derivatives, disuccinylated (SQ) and diacetylated CoQ (AQ) [233]. This method of administration has been described for α-T earlier [234]. The objective was to maximize the plasma concentration of CoQ and hence induce CoQ uptake into tissues. The plasma levels of CoQ were indeed increased when SQ or AQ were administered in comparison with non-derivatized CoQ. However, none of the treatments showed any increase of the lipid uptake into various tissues. Furthermore, SQ and AQ were recovered in the plasma, liver and spleen as underivatized CoQ, indicating the presence of highly effective esterases in the circulation and the intestine.

The uptake mechanism of CoQ from the blood into tissues is not yet studied. Like cholesterol and α-T, LDL-receptor may be one of the mediators since organs such as adrenals and ovaries are rich in this receptor. This mechanism of uptake is probably not the only one since in some organs the receptor level and the degree of uptake are different. Another possibility is that a CoQ-specific receptor or binding protein is operating.

The limited uptake of external CoQ by animal tissues is explained by its distribution, cellular location and functional requirements. The localization of the lipid in the central hydrophobic portion make membranes saturated and there is no functional requirement or possibility to place more lipids into the limited space. Since all type of cells have the CoQ biosynthetic pathway, no external lipid is required under normal conditions [235]. In fact, labeled CoQ taken up by the liver does not appear in the mitochondria where more than 80% of the endogenous lipid is found [111]. It is distributed mainly in non-membranous luminal compartments, preferentially in lysosomes [110]. The situation is however different when the lipid is missing and the membrane has the capacity, at least theoretically, to accept the lipid. This situation is well documented in children with genetic deficiency of CoQ synthesis [236]. These cases, where there is almost complete lack of the lipid in fibroblasts and lymphoblasts, are associated with severe neuronal and muscular symptoms. Dietary CoQ improves dramatically the physical and intellectual capacities by restoring deficient mitochondrial respiration. Another case is patients with cardiomyopathy who exhibit low levels of CoQ in heart biopsy samples [237–239]. After CoQ supplementation, the uptake is higher in severe heart disease in comparison with the uptake in mild disease. Consequently, uptake and proper tissue and cellular distribution of external CoQ take place also in animal and humans, presupposing that there is a requirement. If this is not the case, CoQ is either not taken up, or it appears in cells mainly in the lumen of some organelles. It is, however, possible that this non-membranous lipid is functionally active. It is not clear how CoQ deficiency is defined and when a decrease in amount affects a function. In 1-year-old rats the amount of CoQ is decreased in most tissues but the uptake into organs upon dietary administration is not higher than in young rats [233].

Administration of an exogenous substance may have consequences for the production of the endogenous product. In vivo labeling of endogenous CoQ with ^{3}H-mevalonate demonstrated that there is no product inhibition upon dietary administration of the lipid [192]. In some studies, obviously depending on the experimental conditions, an increase in endogenous CoQ9 in rodents was described upon supplementation of CoQ10 [231,232,241]. A possible explanation is that the exogenous lipid is metabolized and some of the breakdown products act as stimulatory agents on the biosynthetic mechanism.

The above discussion dealt with the direct organ uptake of CoQ and its consequences. However, one has to keep in mind that direct cellular uptake is not the only possibility for action. The established way of action for many substances does not require entrance into the cell. Signal transduction, redox signaling, acting as primary ligand or secondary transducer are the possibilities for such action by CoQ. Influence on organ circulation and effect on the vascular system are other possibilities since this lipid is taken up to the blood. CoQ in the circulation may effect the production of cytokines and interleukines, change the levels of adhesion molecules and modify the production of prostaglandin and leukotriene metabolites. The presence of this antioxidant in the gastrointestinal system is an efficient way to prevent formation of interactive free radicals. Even small amounts of metabolites originating from CoQ, either from normal catabolism or by other ways (e.g. radiation or oxidation), may have considerable influence on many processes considering the effects of many drugs after metabolism. In this way, it may turn out that in spite of the limited organ uptake, dietary CoQ has an important influence on organ metabolism and function.

9. Catabolism

CoQ has a high catabolic rate which is indicated by the relatively short half-life, $T_{1/2}$ (Table 6). The half-life varies among tissues and it ranges between 49 and 125 h [242].

Table 6
Half-life of CoQ in rat tissues and spinach

	Hours
Liver	79
Heart	59
Kidney	125
Stomach	72
Thyroid	49
Colon	54
Muscle	50
Intestine	54
Pancreas	94
Testis	50
Thymus	104
Spleen	64
Brain	90
Spinach	30

Data taken from Thelin et al. [242] and Wanke et al. [244].

The $T_{1/2}$ of the mevalonate pathway lipids is similar in most tissues which is, however, not the case in brain [243]. In this organ both cholesterol and dolichol have a very high $T_{1/2}$, 4080 and 1010 h, respectively, while it is 90 h for CoQ. This finding demonstrates the importance of CoQ in the brain and also shows the operation of terminal regulation in the mevalonate pathway. Despite of the same initial reactions leading to FPP, the rates of biosynthesis for the three lipids are completely different. The relatively rapid breakdown of CoQ is not only an event in animal cells but also occurs in plants [244].

The catabolism of CoQ has been studied only to a limited extent. Breakdown products isolated from urine and feces of rat and guinea pig were characterized using mass spectrometry and it was found that the main products have an intact, fully substituted ring [245,246]. Using dietary CoQ7 in rat and guinea pig, it has been suggested that the liver degrades CoQ by initial ω-oxidation and subsequent β-oxidations of the side chain. The main product identified in the urine had an aromatic ring, the side chain was shortened to 5–7 carbon atoms and the ω-end was carboxylated. When [^{3}H]CoQ10 was injected to rats, radioactive water-soluble metabolites could be isolated from all those organs which take up the administered lipid [111]. The metabolites were found mainly in the urine, but also recovered in the feces (discharged through the bile), where even considerable amounts of non-metabolized [^{3}H]CoQ10 were found (Fig. 10).

At HPLC analysis of the urine extracts, two main metabolites were isolated and both of them gave a signal at *m*/*z* 389 in mass spectrometry. Upon fragmentation, two signals at *m*/*z* 79 and 80 appeared, corresponding to PO_3 (*m*/*z* 79) and HPO_3 (*m*/*z* 80). The main metabolite in the urine, isolated by Nakamura et al. [246], in the phosphorylated form has a calculated molecular weight of 389 which is probably the major breakdown product of CoQ (Fig. 10, insert). The two major products isolated on HPLC with identical molecular weight are most probably the same products but phosphorylated either at carbon 4 or alternatively at carbon 1.

By analysis of metabolites in the bile of guinea pig, it was found that conjugation occurred by glucuronidation which derivatives appeared in the feces [246]. In contrast to many other organs, the liver has a high glucuronidation capacity and this type of conjugation obviously takes place also in the liver for CoQ metabolites [247]. Conjugation of breakdown products by phosphorylation is not a common mechanism, but it is necessary in this case since this

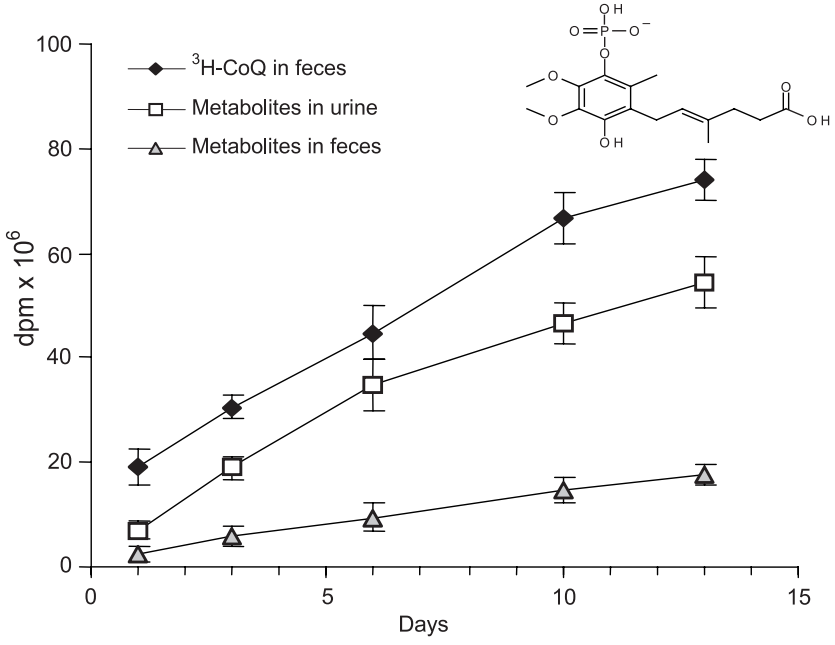

Fig. 10. Metabolites of [^{3}H]CoQ in urine and feces. The insert in upper right corner shows the main phosphorylated metabolite. Data taken from Bentinger et al. [111].

mechanism has a broad distribution and CoQ catabolism occurs in all tissues. It is also possible that minor products are conjugated by other mechanisms, such as sulfation or glutathione conjugation. Further studies will be necessary to establish the mechanism of the catabolism and identification of enzymes participating in this process.

Both gas chromatography-mass fragmentography and HPLC analyses have demonstrated that the metabolites isolated after injection of labeled CoQ are identical with the metabolites present in the urine produced after endogenous catabolism [111,246]. CoQ is synthesized in all cells of the animal organism and the lipid is also broken down in all cells. Before the products are discharged to the circulation, they are conjugated by phosphorylation to make them more hydrophilic. These metabolites are transported in the blood to the kidney where they are excreted by the glomerular–tubular system. In liver the main form of conjugation is glucuronidation and the discharge system is the bile–feces route.

10. Induction of CoQ biosynthesis

Lipid metabolism is regulated in a great extent by peroxisomes and this organelle has a high ability of proliferation. A large number of structurally different natural and synthetic compounds are known as peroxisomal inducers, such as hypolipidemic and anti-inflammatory drugs, some solvents, herbicides, plasticizers, surfactants and food flavors [248,249]. Increased numbers of peroxisomes are also observed at high-fat diet, vitamin E deficiency, cold adaptation, diabetes and at increased level of dehydroepiandrosterone. Fibrates are commonly used drugs for treatment of hyperlipidemia to decrease mainly blood triglycerides. In humans there is also an induction of some peroxisomal enzymes but no increase in the number of peroxisomes [250].

When rodents are treated with peroxisomal inducers, CoQ amount increases in various organs [251,252]. Among the most effective inducers in rat is the plasticizer di(ethylhexyl)phthalate (DEHP), which after 6 weeks of administration in the diet increases CoQ amount in the liver by five times (Fig. 11). There is also an increase in other organs, such as heart, muscle and blood but not in brain. Other inducers, clofibrate, salicylic acid and dehydroepiandrosterone, also give similar but less pronounced effects. The inducer is species-specific and in mouse the highest induction is obtained by administration of perfluorooctanoic acid [248]. The elevated levels of CoQ are the result of increased synthesis while the breakdown rate is unaffected [240]. In agreement with the enhanced biosynthesis is the finding that in vitro activities of *trans*-prenyltransferase and nonaprenyl-4-hydroxybenzoate transferase are up-regulated [253]. This is of importance since one of these enzymes is considered to be rate-limiting in the biosynthesis of CoQ. In young rats there is a manifold activation of fatty acid β-oxidation in the liver upon DEHP treatment which remains high also in old (17-month-old) animals [240]. Contrarily, the induction of CoQ amount of 42-day-old rats is as high as sevenfold, but in 17-month-old rats the induction of CoQ is completely absent. Consequently, other factors that are also altered during aging control the induction of CoQ biosynthesis by peroxisomal inducers.

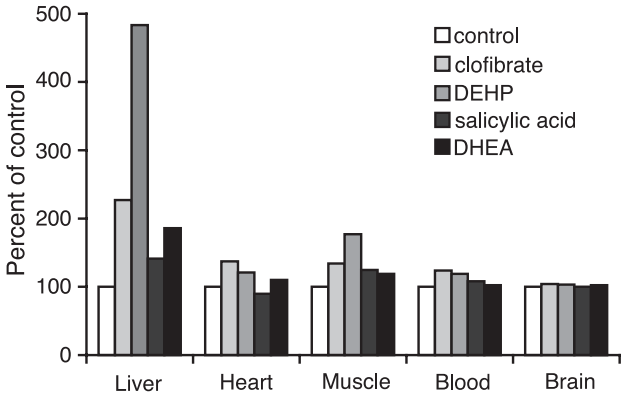

Fig. 11. Effect of a 6-week treatment with peroxisomal inducers on CoQ content in various organs. Data taken from Åberg et al. [251,252].

Cold exposure of rats elevates the amounts of CoQ in the liver threefold after 3 weeks [201]. In mice the induction is 60% and restricted to the liver [254]. In vivo incorporation of [^{3}H]mevalonate demonstrates that the changes are elicited by an increased biosynthesis of the lipid.

10.1. Transcriptional regulation of lipid biosynthesis

Nuclear hormone receptors are ligand-dependent DNA binding transcription factors that exert control on gene expression. They make up a superfamily of more than 150 members that are divided into two groups, the steroid receptor family and the non-steroid receptor family. Nuclear hormone receptors probably arose from preexisting protein modules during evolution and can be linked back structurally to Pex11p, a peroxisomal membrane protein in unicellular eukaryotes [255]. Nuclear receptors share common structure/function domains, i.e. a variable N-terminal region, a conserved DNA binding domain (DBD), a conserved ligand binding domain and a variable C-terminal region [256]. The DBD targets the receptor to specific DNA sequences or response elements and is composed of two highly conserved zinc fingers generated by two cysteine-rich motifs. Nuclear receptors form monomers, homodimers and heterodimers and regulate gene expression by altering chromatin structure, by interacting directly with the components of the pre-initiation complex or by recruiting cofactors. These cofactors lack DBD but are able to activate or repress transcription by enhancing or inhibiting interactions between the DNA binding transcription factors and the pre-initiation complex.

Peroxisomal inducers are a class of compounds that act as ligands to the steroid receptor family of peroxisomal

proliferator activated receptors (PPARα, β and γ) [257]. When ligands bind to PPAR, it dimerizes with another receptor, the retinoid X receptor (RXRα, β and γ) and the binding of the ligand–receptor complex to its cognate response element can activate or repress specific transcription [258]. PPARα affects genes including those encoding apolipoproteins, lipoprotein lipase, fatty acid transport protein, peroxisomal and mitochondrial fatty acid metabolizing enzymes and bile acid synthesizing enzymes [259–261]. Several chemicals such as hypolipidemic drugs, phthalates and fatty acids can act as ligands to PPARα while the ligand to RXR is 9-*cis* retinoic acid. RXR is able to form heterodimers with a variety of hormone and orphan receptors [262–265]. The dimerization partner includes RXR itself, the retinoic acid receptor, the vitamin D receptor, the thyroid hormone receptor, PPAR and orphan receptors. These heterodimers bind to direct repeat elements with spacings varying from one to five nucleotides [266]. RXR probably exists in cells as monomers in equilibrium with heterodimerized RXRs. Under in vivo conditions, the different nuclear hormone receptors compete for binding to RXR, bringing about a possible mechanism for crosstalk. Silencing of gene transcription can occur both in the absence of ligands and in the presence of additional co-repressors [267].

10.2. Influence of PPAR and RXR on CoQ biosynthesis

The experiments performed with peroxisomal inducers clearly indicate that nuclear receptors are involved in the regulation of CoQ metabolism. By utilizing PPARα-null mice, it was possible to investigate the involvement of PPARα in activation of CoQ biosynthesis [253]. Disruption of the PPARα gene results in mice that are refractory to the pleiotropic responses to inducers, but does not exhibit any gross phenotypic defects [268]. Both the PPARα-null and wild-type mice had similar amounts of tissue CoQ and also cholesterol and dolichol [253]. Upon administration of DEHP, there was no induction of CoQ in the null mice. As described before, the rate of biosynthesis rather than the rate of breakdown was altered by administration of inducers [240]. Also *trans*-prenyltransferase and nonaprenyl-4-hydroxybenzoate transferase activities were up-regulated after peroxisomal induction in the wild type, but not in the PPARα-null mice. The induction of CoQ in different tissues followed the expression of PPARα in these tissues [269]. Since neither of these two genes nor their promoters are identified in mammals, it was not possible to investigate whether PPARα-RXR acted directly on the CoQ genes or if the response to peroxisomal inducers was indirect.

Recently, a hepatocyte-specific RXRα-deficient mouse (RXRα-def) was produced [270]. These mice are different from the PPARα-null mice in several aspects. RXRα deficiency results in an induction of mRNA for several apolipoproteins, serum cholesterol and triglyceride levels, which is not seen in the PPARα-null mice [271]. Investigations of these mice revealed that RXRα-def mice have only half of the CoQ concentration in the liver in comparison with the controls while both cholesterol and dolichol levels were unchanged [254]. Also, the RXRα-def mice have a decreased in vivo incorporation of [^{3}H]mevalonate into CoQ when compared to wild-type mice. On the other hand, the induction of hepatic CoQ after exposure to DEHP is present in the RXRα-def mice. In the kidney, which does not have a dysfunctional RXR, CoQ concentration and biosynthesis is equal to the control in all aspects.

Table 7
RXRα and PPARα involvement in coenzyme Q metabolism

	Constitutive biosynthesis	Induction by the peroxisomal inducer DEHP	Induction by cold exposure
RXRα	required	not required	required
PPARα	not required	required	not required

Nuclear receptors are also involved in the CoQ induction upon cold exposure. PPARα-null mice had an increased amount of this lipid in the liver after cold treatment while the RXRα-def mice did not respond to the cold treatment [254]. These data further support the suggested presence of one or several response elements in the promoters of CoQ biosynthetic genes.

The role of nuclear receptors in CoQ metabolism is summarized in Table 7. RXRα regulates the amount of CoQ in liver and probably is required for the basal transcription of genes involved in CoQ biosynthesis and for its induction upon cold treatment, however, it is not involved in the elevated biosynthesis during treatment with peroxisomal inducer. PPARα is neither required for the basal biosynthesis of the lipid nor for its induction by cold exposure, but is necessary for the increase at treatment with peroxisomal inducer. RXR is operating as a heterodimer, but we have at present no information on the exact nature of the other receptor. Some of the candidates are the liver X and farnesol X receptors since both are related to lipid metabolism associated with the mevalonate pathway. Further studies have to answer on a number of questions concerning the involvement of transcription factors in CoQ biosynthesis. It appears also that neither PPARα nor RXRα is involved in the basic synthesis of cholesterol and dolichol.

11. From *E. coli* to human

Almost all knowledge in the field of CoQ distribution, metabolism, synthesis and function originate from studies performed on bacteria, yeast, *C. elegans* and, to some limited extent, on rats. A single cell or less complex organism is always advantageous to identify and characterize cellular processes and in most cases it is the only way to

approach a problem. One has to be, however, very cautious to extrapolate the findings to human for four reasons.

1. The metabolism of CoQ in higher organisms obviously differs in a great extent from that of single-cell organisms. Even if the synthesis and breakdown may follow a similar pattern, considerable differences are established concerning individual steps and, in particular, the regulation of these reactions.
2. In higher organisms the uptake of dietary CoQ is very limited under normal conditions and the uptake from the blood to the organs is also restricted, especially into mitochondria. In one-cell systems the uptake is not regulated by a complex mechanism but follows an uptake mechanism valid to most of lipids present in the environment.
3. Animal tissues have a number of efficient systems to reduce oxidized CoQ, which maintains a high antioxidant level. This is probably not the case in *C. elegans*, where dietary CoQ shortens life-span and, additionally, probably increases electron transport, resulting in release of ROS [272]. However, life-long administration of CoQ does not affect life-span of rats and mice [273,274].
4. Like other active cellular components, the action of CoQ is not restricted to only a few actions such as serving as redox mediator and antioxidant. CoQ interferes with β2-integrins and complement receptors of monocytes, changes hormone levels, affects uptake of α-T, may modify the production of leukotrienes and prostaglandins and regulates signaling systems by metabolites. In this way, contrary to single-cell organisms, a variety of metabolic interferences occur in mammals as a result of species-specific response.

12. Aging

Changes in lipid composition during aging are of great interest since these modifications have significant impact on the physico-chemical structure of membranes and function of the integral enzymes and their metabolic processes. The mevalonate pathway lipids display a characteristic behavior during aging [275]. In Table 8, human heart and pancreas from a 2-day old newborns are compared with persons of age 20 and 80 years. The CoQ content is greatly increased in the first 20 years of life, followed by a decrease in a variable extent and in some organs at 80 years of age may be lower than at birth. Both dolichol and dolichyl-P increase during the whole life at a great extent. An important question that is not yet addressed is, however, to which extent these modifications represent a real deficiency in the membrane structure or are attributed to a change in cellular organization. Only a limited extent of decrease of CoQ content was observed in synaptic and non-synaptic mitochondria from different brain regions, and in mitochondria in liver and heart of rats during aging [276,277]. Mitochondria prepared from tissues of aged mice do not exhibit changes in CoQ levels with the exception of skeletal muscle [278]. It would be of great importance to establish whether CoQ is decreased in individual cellular membranes or if the modification is the consequence of a decrease in number of mitochondria. CoQ in adult tissues is found mostly in reduced form but the extent of reduction in aged tissue is not yet determined. If the oxidized portion is increased, this may favor the catabolic activities leading to an accelerated breakdown and decrease in the amount of CoQ in the tissue.

13. Diseases

The CoQ content is altered in a number of diseases, of which the decrease in cardiomyopathies and degenerative muscle diseases are the most studied [279–282]. CoQ is commonly used for treatment of cardiomyopathy and there is substantial evidence that heart function is improved upon administration of the lipid [283]. CoQ reaches the heart via the blood but is taken up only to a limited extent, and the uptake is probably not sufficient to account for all of the observed improvements. Other mechanisms, either of signaling type or production of mediators, appear to exist which do not require the entrance of CoQ into the cell.

Hyperplastic noduli is the first stage during development of chemically induced hepatocellular cancer in rat and during this first stage the amount of CoQ increases (Table 9) [284,285]. Interestingly, the amount of CoQ in the mitochondria is stable, and the change in CoQ concentrations is attributed to the extra-mitochondrial compartments. During the first stage of the disease there is increased oxidative stress which has been suggested to induce an adaptive response, resulting in that the cell protects itself by raising the concentrations of antioxidants [286]. In the

Table 8
Mevalonate pathway lipids in human heart and pancreas during aging

Organ	Age (years)	Coenzyme Q	Dolichol (% of 2-day-old human)	Dolichyl-P	Cholesterol	Phospholipids
Heart	20	300	132	400	59	82
	80	129	605	2364	82	109
Pancreas	20	228	5450	176	50	140
	80	71	17,502	500	68	181

Data taken from Kalen et al. [275].

Table 9
Mevalonate pathway lipids in various pathological conditions

	CoQ	Dolichol	Dolichyl-P	Cholesterol	References
	(% of control)				
Noduli, rat liver	196	671	175	148	[284,285]
Noduli					[284]
Mitochondria	104	116		52	
Microsomes	693	474		83	
Lysosomes	261	84		106	
Cancer, rat liver	76	105	204	190	(Olsson, J.M., unpublished data)
Cancer, human liver	40	889	94	212	[287]
Cardiomyopathy, human heart	40-80				[237–239]
Alzheimer's disease, human brain	130	56	172	102	[293]
Prion disease, mice brain	250	67	158	103	[213]
Niemann–Pick type C disease, mouse liver	65	95	267	1100	[296]
Diabetes, type 2, rat liver	140				[298]
Diabetes, type 2, rat testis, mitochondria	125				[297]
Mevinolin treatment of rat					[192]
Heart	82	72	60	104	
Muscle	83	75	68	92	
Liver	89	134	91	85	

noduli also dolichol and dolichyl-P levels are elevated. Upon progression of the disease, manifest cancer develops and the amount of CoQ decreases to only 40% in human and 76% in rat [287] (Olsson, J.M., unpublished data). Cholesterol is doubled in fully developed cancerous liver.

In biopsy samples from human heart, a significant decrease of the CoQ content in cardiomyopathy was found and the decrease could be related to the severity of the disease [237–239]. In these biopsy samples, uptake of CoQ was found after dietary treatment with the lipid.

CoQ has been suggested to be involved in neurodegenerative diseases such as Huntington's disease, Parkinson's disease and amyotrophic lateral sclerosis [288–290]. In both experimental models and human patients, beneficial effects have been observed after supplementation with CoQ [291,292]. Changes in lipid concentrations are extensive in Alzheimer's disease in humans and in scrapie-caused prion disease in mice [213,293]. The amount of CoQ is greatly increased in both instances, especially in the prion disease, about 250%. α-T, the other main lipid-soluble antioxidant, is also increased under these conditions. It is now generally accepted that increased production of free radicals is an important feature during the development of these diseases, and it is not surprising that cellular protection is induced to counteract these radicals [294]. It has also been suggested that Alzheimer's and prion diseases—in spite of their different appearances—have much in common in etiology and development [295]. This possibility is further strengthened by the behavior of other mevalonate pathway lipids. In both diseases, dolichol decreases, dolichyl-P increases and cholesterol is unchanged throughout the process.

In a murine model of Niemann–Pick type C disease, there is a massive accumulation of cholesterol in the liver. In this disease dolichyl-P is greatly increased and CoQ is lowered in a significant extent [296]. In experimental diabetes, the concentration of CoQ in rat liver and in testis mitochondria is elevated [297,298]. Statins are of great interest as the most commonly used drugs for treatment of hypercholesterolemia and it is recently established that they are additionally decreasing the level of inflammatory cytokines [299]. These drugs, however, have unwanted effects by not only lowering CoQ levels in the blood but also by causing other symptoms such as muscle disturbances [300]. The explanation is that statins interfere with various enzymatic steps of the mevalonate pathway lipid biosynthesis since CoQ, dolichol and dolichyl-P in heart and muscle are decreased upon treatment [192].

The importance of CoQ in the life of the living organisms is illuminated most clearly by the number of reports describing the genetic disorders in which CoQ synthesis is impaired. Lowered lipid content in organs causes serious metabolic disturbances but CoQ supplementation reestablishes mitochondrial and other functions. Our primary goal in the near future should be to establish an animal model with deficiency of the biosynthetic system. Studies in such model could give valuable information on the physiological role of CoQ and answer questions concerning its complex involvement in various aspects of the living organism.

References

[1] G.N. Festenstein, F.W. Heaton, J.S. Lowe, R.A. Morton, A constituent of the unsaponifable portion of animal tissue lipids (λmax 272 mμ.), Biochem. J. 59 (1955) 558–566.
[2] F.L. Crane, Y. Hatefi, R.L. Lester, C. Widmer, Isolation of a quinone from beef heart mitochondria, Biochim. Biophys. Acta 25 (1957) 220–221.
[3] D.E. Wolf, C.H. Hoffman, N.R. Trenner, B.H. Arison, C.H. Shunk, B.O. Linn, J.F. McPherson, K. Folkers, Coenzyme Q. Structure studies on the coenzyme Q group, J. Am. Chem. Soc. 80 (1958) 4750–4752.
[4] L. Ernster, I.Y. Lee, B. Norling, B. Persson, Studies with ubiquinone-depleted submitochondrial particles. Essentiality of ubiquinone for the interaction of succinate dehydrogenase, NADH dehydrogenase, and cytochrome *b*, Eur. J. Biochem. 9 (1969) 299–310.
[5] P. Mitchell, Protonmotive redox mechanisms of cytochrome *b*-c_1 complex in the respiratory chain: protonmotive ubiquinone cycle, FEBS Lett. 56 (1975) 1–6.
[6] B.E. Schultz, S.I. Chan, Structures and proton-pumping strategies of mitochondrial respiratory enzymes, Annu. Rev. Biophys. Biomol. Struct. 30 (2001) 23–65.

[7] J. Smeitink, L. van den Heuvel, S. DiMauro, The genetics and pathology of oxidative phosphorylation, Nat. Rev., Genet. 2 (2001) 342–352.
[8] S.J.R. Heales, M.E. Gregg, J.B. Clarke, Oxidative phosphorylation: structure, function, and intermediary metabolism, Int. Rev. Neurobiol. 53 (2002) 25–56.
[9] G. Lenaz, C. Bovina, M.M. Pich, M. D'Aurelio, R. Fato, G. Formiggini, M.L. Genova, G. Guiliano, U. Paolucci, G.P. Castelli, B. Ventura, Role of mitochondria in oxidative stress and aging, Ann. N.Y. Acad. Sci. 959 (2002) 199–213.
[10] T. DeHahn, R. Barr, D.J. Morré, NADH oxidase activity present on both the external and internal surfaces of soybean plasma membranes, Biochim. Biophys. Acta 1328 (1997) 99–108.
[11] A.O. Brightman, J. Wang, R.K. Miu, I.L. Sun, R. Barr, F.L. Crane, D.J. Morré, A growth factor- and hormone-stimulated NADH oxidase from rat liver plasma membrane, Biochim. Biophys. Acta 1105 (1992) 109–117.
[12] D.J. Morré, The hormone- and growth factor-stimulated NADH oxidase, J. Bioenerg. Biomembranes 26 (1994) 421–433.
[13] T.G. Gabig, The NADPH-dependent O_2^- generating oxidase from human neutrophils. Identification of a flavoprotein component that is deficient in a patient with chronic granulomatous disease, J. Biol. Chem. 258 (1983) 6352–6356.
[14] T. Kishi, D.M. Morré, D.J. Morré, The plasma membrane NADH oxidase of HeLa cells has hydroquinone oxidase activity, Biochim. Biophys. Acta 1412 (1999) 66–77.
[15] I.L. Sun, E.E. Sun, F.L. Crane, D.J. Morré, A. Lindgren, H. Löw, Requirement for coenzyme Q in plasma membrane electron transport, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 11126–11130.
[16] P.J. Chueh, D.M. Morré, C. Penel, T. DeHahn, D.J. Morré, The hormone-responsive NADH oxidase of the plant plasma membrane has properties of a NADH: protein disulfide reductase, J. Biol. Chem. 272 (1997) 11221–11227.
[17] C. Gómez-Díaz, J.C. Rodríguez-Aguilera, M.P. Barroso, J.M. Villalba, F. Navaro, F.L. Crane, P. Navas, Antioxidant ascorbate is stabilized by NADH-coenzyme Q10 reductase in the plasma membrane, J. Bioenerg. Biomembranes 29 (1997) 251–257.
[18] F.L. Crane, I.L. Sun, M.G. Clark, C. Grebing, H. Löw, Transplasma membrane redox systems in growth and development, Biochim. Biophys. Acta 811 (1985) 233–264.
[19] M.I. Burón, J.C. Rodríguez-Agluilera, F.J. Alcaín, P. Navas, Transplasma membrane redox system in HL-60 cells is modulated during TPA-induced differentiation, Biochem. Biophys. Res. Commun. 192 (1993) 439–445.
[20] J.A. Larm, F. Vaillant, A.W. Linnane, A. Lawen, Up-regulation of the plasma membrane oxidoreductase as a prequisite for the viability of human Namalwa ρ0 cells, J. Biol. Chem. 269 (1994) 30097–30100.
[21] A. Lawen, R.D. Martinus, G.L. McMullen, P. Nagley, F. Vaillant, E.J. Wolvetang, A.W. Linnane, The universality of bioenergetic disease: the role of mitochondrial mutation and the putative inter-relationship between mitochondria and plasma membrane NADH oxidoreductase, Mol. Aspects Med. 15 (1994) 13–27.
[22] F.L. Crane, I.L. Sun, R.A. Crowe, F.J. Alcain, H. Löw, Coenzyme Q10, plasma membrane oxidase and growth control, Mol. Aspects Med. 15 (1994) 1–11.
[23] S. Iuchi, E.C. Lin, Adaptation of *Escherichia coli* to respiratory conditions: regulation of a gene expression, Cell 66 (1991) 5–7.
[24] M. Shibunama, T. Kuroki, K. Nose, Stimulation by hydrogen peroxide of DNA synthesis, competence family gene expression and phosphorylation of a specific protein in quiescent Balb/3T3 cells, Oncogene 5 (1990) 1025–1032.
[25] F. Stirpe, T. Higgins, P.L. Tazzari, E. Rozengurt, Stimulation by xanthine oxidase of 3T3 Swiss fibroblasts and human lymphocytes, Exp. Cell Res. 192 (1991) 635–638.
[26] L. Gille, H. Nohl, The existence of a lysosomal redox chain and the role of ubiquinone, Arch. Biochem. Biophys. 375 (2000) 347–354.
[27] S. Papa, Mitochondrial oxidative phosphorylation changes in the life span. Molecular aspects and physiopathological implications, Biochim. Biophys. Acta 1276 (1996) 87–105.
[28] D.F. Rolfe, G.C. Brown, Cellular energy utilization and molecular origin of standard metabolic rate in mammals, Physiol. Rev. 77 (1997) 731–758.
[29] S. Papa, V.P. Skulachev, Reactive oxygen species, mitochondria, apoptosis and aging, Mol. Cell. Biochem. 174 (1997) 305–319.
[30] V.P. Skulachev, Uncoupling: new approaches to an old problem of bioenergetics, Biochim. Biophys. Acta 1363 (1998) 100–124.
[31] E. Cadenas, K.J.A. Davis, Mitochondrial free radical generation, oxidative stress, and aging, Free Radic. Biol. Med. 29 (2000) 222–230.
[32] B. Chance, H. Sies, A. Boveris, Hydroperoxide metabolism in mammalian organs, Physiol. Rev. 59 (1979) 527–605.
[33] B. Halliwell, J.M.C. Cutteridge, Oxygen toxicity, oxygen radicals, transition metals and disease, Biochem. J. 219 (1984) 1–14.
[34] E. Cadenas, P. Hochstein, L. Ernster, Pro- and antioxidant functions of quinones and quinone reductases in mammalian cells, Adv. Enzymol. Retat. Areas Mol. Biol. 65 (1992) 97–146.
[35] F.J.M.G. van Kuijk, A. Sevanian, G.J. Handelman, E.A. Dratz, A new role for phosopholipase A2: protection of membranes from lipid peroxidation damage, Trends Biochem. Sci. 12 (1987) 31–33.
[36] R. Brigelius-Flohé, Tissue-specific functions of individual glutathione peroxidases, Free Radic. Biol. Med. 27 (1999) 951–965.
[37] E.R. Stadtman, Metal ion-catalyzed oxidation of proteins: biochemical mechanism and biological consequences, Free Radic. Biol. Med. 9 (1990) 315–325.
[38] E.R. Stadtman, B.S. Berlett, Fenton chemistry. Amino acid oxidation, J. Biol. Chem. 266 (1991) 17201–17211.
[39] P. Forsmark-Andrée, G. Dallner, L. Ernster, Endogenous ubiquinol prevents protein modification accompanying lipid peroxidation in beef heart submitochondrial particles, Free Radic. Biol. Med. 19 (1995) 749–757.
[40] T. Grune, T. Reinheckel, K.J.A. Davis, Degradation of oxidized proteins in mammalian cells, FASEB J. 11 (1997) 526–534.
[41] O. Ullrich, T. Reinheckel, N. Sitte, R. Hass, T. Grune, K.J.A. Davis, Poly-ADP ribose polymerase activates nuclear proteasome to degrade oxidatively damaged histones, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 6223–6228.
[42] B. Halliwell, O.I. Auroma, DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems, FEBS Lett. 281 (1991) 9–19.
[43] T. Ozawa, Genetic and functional changes in mitochondria associated with aging, Physiol. Rev. 77 (1997) 425–464.
[44] R.D. Wood, DNA repair in eukaryotes, Annu. Rev. Biochem. 65 (1996) 135–167.
[45] A. Holmgren, M. Björnstedt, Thioredoxin and thioredoxin reductase, Methods Enzymol. 252 (1995) 199–208.
[46] Z.A. Wood, L.B. Poole, P.A. Karplus, Peroxiredoxin evolution and the regulation of hydrogen peroxide signaling, Science 300 (2003) 650–653.
[47] A. Mellors, A.L. Tappel, The inhibition of mitochondrial peroxidation by ubiquinone and ubiquinol, J. Biol. Chem. 241 (1966) 4353–4356.
[48] R. Takayanagi, K. Takeshige, S. Minakami, NADH- and NADPH-dependent lipid peroxidation in bovine heart submitochondrial particles. Dependence on the rate of electron flow in the respiratory chain and an antioxidant role of ubiquinol, Biochem. J. 192 (1980) 853–860.
[49] L. Ernster, G. Dallner, Biochemical, physiological and medical aspects of ubiquinone function, Biochim. Biophys. Acta 1271 (1995) 195–204.
[50] A. Romagnoli, A. Oradei, C. Destito, A. Iacocagni, A.W. Marin, G.P. Littarru, Protective role in vivo of coenzyme Q10 during reperfusion of ischemic limbs, Mol. Aspects Med. 15 (1994) 177–185.
[51] R. Alleva, M. Tomasetti, M. Battino, G. Curatola, G.P. Littarru, K.

Folkers, The roles of coenzyme Q10 and vitamin E on the peroxidation of human low density lipoprotein subfractions, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 9388–9391.
[52] F. Åberg, E.L. Appelkvist, G. Dallner, L. Ernster, Distribution and redox state of ubiquinones in rat and human tissues, Arch. Biochem. Biophys. 295 (1992) 230–234.
[53] L. Ernster, P. Forsmark-Andree, Ubiquinol: an endogenous antioxidant in aerobic organisms, Clin. Investig. 71 (1993) 60–65.
[54] B. Frei, M.C. Kim, B.N. Ames, Ubiquinol-10 is an effective lipid-soluble antioxidant at physiological concentrations, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 4879–4883.
[55] H. Shi, N. Noguchi, E. Niki, Comparative study on dynamics of antioxidative action of alpha-tocopheryl hydroquinone, ubiquinol, and alpha-tocopherol against lipid peroxidation, Free Radic. Biol. Med. 27 (1999) 334–346.
[56] R. Stocker, V.W. Bowry, B. Frei, Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does alpha-tocopherol, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 1646–1650.
[57] S.R. Thomas, J. Neuzil, D. Mohr, R. Stocker, Co-antioxidants make α-tocopherol an efficient antioxidant for low-density lipoprotein, Am. J. Clin. Nutr. 62 (1995) 1357–1364.
[58] P. Forsmark, F. Åberg, B. Norling, K. Nordenbrand, G. Dallner, L. Ernster, Inhibition of lipid peroxidation by ubiquinol in submitochondrial particles in the absence of vitamin E, FEBS Lett. 285 (1991) 39–43.
[59] P. Forsmark-Andrée, B. Persson, R. Radi, G. Dallner, L. Ernster, Oxidative modification of nicotinamide nucleotide transhydrogenase in submitochondrial particles: effect of endogenous ubiquinol, Arch. Biochem. Biophys. 336 (1996) 113–120.
[60] P. Forsmark-Andrée, C.P. Lee, G. Dallner, L. Ernster, Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochondrial particles, Free Radic. Biol. Med. 22 (1997) 391–400.
[61] M. Tomasetti, G.P. Littarru, R. Stocker, R. Alleva, Coenzyme Q10 enrichment decreases oxidative DNA damage in human lymphocytes, Free Radic. Biol. Med. 27 (1999) 1027–1032.
[62] M. Tomasetti, R. Alleva, B. Borghi, A.R. Collins, In vivo supplementation with coenzyme Q10 enhances the recovery of human lymphocytes from oxidative DNA damage, FASEB J. 15 (2001) 1425–1427.
[63] T. Hundal, P. Forsmark-Andrée, L. Ernster, B. Andersson, Antioxidant activity of reduced plastoquinone in chloroplast thylakoid membranes, Arch. Biochem. Biophys. 324 (1995) 117–122.
[64] D.G. Nicholls, R.M. Locke, Thermogenic mechanisms in brown fat, Physiol. Rev. 64 (1984) 1–64.
[65] J. Nedergaard, B. Cannon, The uncoupling protein thermogenin and mitochondrial thermogenesis, New Compr. Biochem. 23 (1992) 385–420.
[66] M. Klingenberg, S.G. Huang, Structure and function of the uncoupling protein from brown adipose tissue, Biochim. Biophys. Acta 1415 (1999) 271–296.
[67] M. Klingenberg, K.S. Echtay, Uncoupling proteins: the issues from a biochemist point of view, Biochim. Biophys. Acta 1504 (2001) 128–143.
[68] J. Nedergaard, V. Golozoubova, A. Matthias, A. Asadi, A. Jacobsson, B. Cannon, UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency, Biochim. Biophys. Acta 1504 (2001) 82–106.
[69] C. Pecqueur, E. Couplan, F. Bouillaud, D. Ricquier, Genetic and physiological analysis of the role of uncoupling proteins in human energy homeostasis, J. Mol. Med. 79 (2001) 48–56.
[70] K.S. Echtay, E. Winkler, M. Klingenberg, Coenzyme Q is an obligatory cofactor for uncoupling protein function, Nature 408 (2000) 609–613.
[71] K.S. Echtay, E. Winkler, K. Frischmuth, M. Klingenberg, Uncoupling proteins 2 and 3 are highly active H+ transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone), Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 1416–1421.
[72] E. Fontaine, P. Bernardi, Progress on the mitochondrial permeability transition pore: Regulation by complex I and ubiquinone analogs, J. Bioenerg. Biomembranes 31 (1999) 335–345.
[73] D.R. Green, J.C. Reed, Mitochondria and apoptosis, Science 281 (1998) 1309–1312.
[74] F. Ichas, L.S. Jouaville, J.P. Mazat, Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals, Cell 89 (1997) 1145–1153.
[75] V. Petronilli, G. Miotto, M. Canton, M. Brini, R. Colonna, P. Bernardi, F. Di Lisa, Transient and long-lasting openings of the mitochondrial permeability transition pore can be monitored directly in intact cells by changes in mitochondrial calcein fluorescence, Biophys. J. 76 (1999) 725–734.
[76] E. Fontaine, F. Ichas, P. Bernardi, A ubiquinone-binding site regulates the mitochondrial permeability transition pore, J. Biol. Chem. 273 (1998) 25734–25740.
[77] L. Walter, V. Nogueira, X. Leverve, M.P. Heitz, P. Bernardi, E. Fontaine, Three classes of ubiquinone analogs regulate the mitochondrial permeability transition pore through a common site, J. Biol. Chem. 275 (2000) 29521–29527.
[78] L. Walter, H. Miyoshi, X. Leverve, P. Bernardi, E. Fontaine, Regulation of the mitochondrial permeability transition pore by ubiquinone analogs, A progress report, Free Radic. Res. 36 (2002) 405–412.
[79] L. Papucci, N. Schiavone, E. Witort, M. Donnini, A. Lapucci, A. Tempestini, L. Formigli, S.Z. Orlandini, G. Orlandini, G. Carella, R. Brancato, S. Capaccioli, Coenzyme Q10 prevents apoptosis by inhibiting mitochondrial depolarization independently of its free radical scavenging property, J. Biol. Chem. 278 (2003) 28220–28228.
[80] K. Folkers, T. Hanioka, L.J. Xia, J.T. McRee, P. Langsjoen, Coenzyme Q10 increases T4/T8 ratios of lymphocytes in ordinary subjects and relevance to patients having the AIDS related complex, Biochem. Biophys. Res. Commun. 176 (1991) 786–791.
[81] K. Folkers, M. Morita, J. McRee, The activities of coenzyme Q10 and vitamin B6 for immune responses, Biochem. Biophys. Res. Commun. 193 (1993) 88–92.
[82] B. Barbieri, B. Lund, B. Lundström, F. Scaglione, Coenzyme Q10 administration increases antibody titer in hepatitis B vaccinated volunteers—A single blind placebo-controlled and randomized clinical study, Biofactors 9 (1999) 351–357.
[83] G. Ravaglia, P. Forti, F. Maioli, L. Bastagli, A. Facchini, E. Mariani, L. Savarino, S. Sassi, D. Cucinotta, G. Lenaz, Effect of micronutrient status on natural killer cell immune function in healthy free-living subjects aged ≥90 y, Am. J. Clin. Nutr. 71 (2000) 590–598.
[84] J.D. Smith, E. Trogan, M. Ginsberg, C. Grigaux, J. Tian, M. Miyata, Decreased atherosclerosis in mice deficient in both macrophage colony stimulating factor (op) and apolipoprotein E, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 8264–8268.
[85] J.H. Qiao, J. Tripathi, N.K. Mishra, Y. Cai, S. Tripathi, X.P. Wang, S. Fishbein, M.C. Fishbein, S.K. Clinton, P. Libby, A.J. Lusis, T.B. Rajavashisth, Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteoporotic mice, Am. J. Pathol. 150 (1997) 1687–1699.
[86] D.H. Adams, S. Shaw, Leucocyte-endothelial interactions and regulation of leucocyte migration, Lancet 343 (1994) 831–836.
[87] A.K. Hubbard, R. Rothlein, Intracellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascades, Free Radic. Biol. Med. 28 (2000) 1379–1386.
[88] M. Turunen, L. Wehlin, M. Sjöberg, J. Lundahl, G. Dallner, K. Brismar, P.J. Sindelar, beta2-Integrin and lipid modifications indicate a non-antioxidant mechanism for the anti-atherogenic effect of dietary coenzyme Q10, Biochem. Biophys. Res. Commun. 296 (2002) 255–260.
[89] P.K. Witting, K. Pettersson, J. Letters, R. Stocker, Anti-atherogenic

effect of coenzyme Q10 in apolipoprotein E gene knockout mice, Free Radic. Biol. Med. 29 (2000) 295–305.
[90] S.R. Thomas, S.B. Leichtweis, K. Pettersson, K.D. Croft, T.A. Mori, A.J. Brown, R. Stocker, Dietary cosupplementation with vitamin E and coenzyme Q(10) inhibits atherosclerosis in apolipoprotein E gene knockout mice, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 585–593.
[91] J. Calles-Escandon, M. Cipolla, Diabetes and endothelial dysfunction: a clinical perspective, Endocrine Rev. 22 (2001) 36–52.
[92] G.F. Watts, D.A. Playford, K.D. Croft, N.C. Ward, T.A. Mori, V. Burke, Coenzyme Q(10) improves endothelial dysfunction of the brachial artery in type II diabetes mellitus, Diabetologia 45 (2002) 420–426.
[93] J.M. Hodgson, G.F. Watts, D.A. Playford, V. Burke, K.D. Croft, Coenzyme Q10 improves blood pressure and glycaemic control: a controlled clinical trial in subjects with type 2 diabetes, Eur. J. Clin. Nutr. 56 (2002) 1137–1142.
[94] M. Tomasetti, R. Alleva, M.D. Solenghi, G.P. Littarru, Distribution of antioxidants among blood components and lipoproteins: significance of lipids/CoQ10 ratio as a possible marker of increased risk for atherosclerosis, Biofactors 9 (1999) 231–240.
[95] Y. Yamamoto, S. Yamashita, Plasma ubiquinone ratio in patients with hepatitis, cirrhosis, and hepatoma, and in patients treated with percutaneous transluminal coronary reperfusion, Biofactors 9 (1999) 241–246.
[96] M. Runquist, I. Parmryd, A. Thelin, T. Chojnacki, G. Dallner, Distribution of branch point prenyltransferases in regions of bovine brain, J. Neurochem. 65 (1995) 2299–2306.
[97] T. Chojnacki, G. Dallner, The biological role of dolichol, Biochem. J. 251 (1988) 1–9.
[98] T. Matsura, K. Yamada, T. Kawasaki, Changes in the content and intracellular distribution of coenzyme Q homologs in rabbit liver during growth, Biochim. Biophys. Acta 1083 (1991) 277–282.
[99] Y. Zhang, E.L. Appelkvist, K. Kristensson, G. Dallner, The lipid compositions of different regions of rat brain during development and aging, Neurobiol. Aging 17 (1996) 869–875.
[100] G. Lenaz, D.G. Daves, K. Folkers, Organic structural specificity and sites of coenzyme Q in succinoxidase and DPNH-oxidase systems, Arch. Biochem. Biophys. 123 (1968) 539–550.
[101] V.E. Kagan, E.A. Serbinova, G.M. Koynova, S.A. Kitanova, V.A. Tyurin, T.S. Stoytchev, P.J. Quinn, L. Packer, Antioxidant action of ubiquinol homologues with different isoprenoid chain length in biomembranes, Free Radic. Biol. Med. 9 (1990) 117–126.
[102] G. Lenaz, Quinone specificity of complex I, Biochim. Biophys. Acta 1364 (1998) 207–221.
[103] A. Lass, S. Agarwal, R.S. Sohal, Mitochondrial ubiquinone homologues, superoxide radical generation, and longevity in different mammalian species, J. Biol. Chem. 272 (1997) 19199–19204.
[104] C. Edlund, K. Holmberg, G. Dallner, E. Norrby, K. Kristensson, Ubiquinone-10 protects neurons from virus-induced degeneration, J. Neurochem. 63 (1994) 634–639.
[105] C. Valtersson, G. van Duyn, A.J. Verkleij, T. Chojnacki, B. de Kruijff, G. Dallner, The influence of dolichol, dolichol esters and dolichyl phosphate on phospholipid polymorphism and fluidity in model membranes, J. Biol. Chem. 260 (1985) 2742–2751.
[106] B.A. Cornell, M.A. Keniry, A. Post, R.N. Robertson, L.E. Weir, P.W. Westerman, Location and activity of ubiquinone 10 and ubiquinone analogues in model and biological membranes, Biochemistry 26 (1987) 7702–7707.
[107] G. van Duijn, A.J. Verkleij, B. de Kruijff, C. Valtersson, G. Dallner, T. Chojnacki, Influence of dolichols on lipid polymorphism in model membranes and the consequences for phospholipid flip-flop and vesicle fusion, Chem. Scr. 27 (1987) 95–100.
[108] G. Lenaz, B. Samori, R. Fato, M. Battino, G. Parenti Castelli, I. Domini, Localization and preferred orientations of ubiquinone homologs in model bilayers, Biochem. Cell. Biol. 70 (1992) 504–514.
[109] P.W. van Dijck, B. de Kruijff, L.L. van Deenen, J. de Gier, R.A. Demel, The preference of cholesterol for phosphatidylcholine in mixed phosphatidylcholine-phosphatidylethanolamine bilayers, Biochim. Biophys. Acta 455 (1976) 576–587.
[110] P. Löw, E. Peterson, C. Edlund, U. Brunk, E.L. Appelkvist, Non-membrane associated dolichol in rat liver, Lipids 27 (1992) 1–9.
[111] M. Bentinger, G. Dallner, T. Chojnacki, E. Swiezewska, Distribution and breakdown of labeled coenzyme Q(10) in rat, Free Radic. Biol. Med. 34 (2003) 563–575.
[112] A. Kalen, B. Norling, E.L. Appelkvist, G. Dallner, Ubiquinone biosynthesis by the microsomal fraction from rat liver, Biochim. Biophys. Acta 926 (1987) 70–78.
[113] Å. Jakobsson-Borin, F. Åberg, G. Dallner, Lipid peroxidation of microsomal and mitochondrial membranes extracted with *n*-pentane and reconstituted with ubiquinol, dolichol and cholesterol, Biochim. Biophys. Acta 1213 (1994) 159–166.
[114] G.I. Belogrudov, P.T. Lee, T. Jonassen, A.Y. Hsu, P. Gin, C.F. Clarke, Yeast COQ4 endocodes a mitochondrial protein required for coenzyme Q synthesis, Arch. Biochem. Biophys. 392 (2001) 48–58.
[115] T. Jonassen, C.F. Clarke, Isolation and functional expression of human COQ3, a gene encoding a methyltransferase required for ubiquinone biosynthesis, J. Biol. Chem. 275 (2000) 12381–12387.
[116] Z. Vajo, L.M. King, T. Jonassen, D.J. Wilkin, N. Ho, A. Munnich, C.F. Clarke, C.A. Francomano, Conservation of the *Caenorhabditis elegans* timing gene clk-1 from yeast to human: a gene required for ubiquinone biosynthesis with potential implications for aging, Mamm. Genome 10 (1999) 1000–1004.
[117] J. Grünler, J. Ericsson, G. Dallner, Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins, Biochim. Biophys. Acta 1212 (1994) 259–277.
[118] M.S. Brown, J.L. Goldstein, A receptor-mediated pathway for cholesterol homeostasis, Science 232 (1986) 34–47.
[119] A.W. Alberts, Discovery, biochemistry and biology of lovastatin, Am. J. Cardiol. 62 (1988) 10–15.
[120] P.A. Edwards, S.F. Lan, A.M. Fogelman, Alterations in the rates of synthesis and degradation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase produced by cholestyramine and mevinolin, J. Biol. Chem. 258 (1983) 10219–10222.
[121] C.F. Clarke, A.M. Fogelman, P.A. Edwards, Diurnal rhythm of rat liver mRNAs encoding 3-hydroxy-3-methylglutaryl coenzyme A reductase. Correlation of functional and total mRNA levels with enzyme activity and protein, J. Biol. Chem. 259 (1984) 10439–10447.
[122] J.L. Goldstein, M.S. Brown, Regulation of the mevalonate pathway, Nature 343 (1990) 425–430.
[123] P.R. Clarke, D.G. Hardie, Regulation of HMG-CoA reductase: identification of the site phosphorylated by the AMP-activated protein kinase in vitro and in intact rat liver, EMBO J. 9 (1990) 2439–2446.
[124] J.A. Cuthbert, P.E. Lipsky, Negative regulation of cell proliferation by mevalonate or one of the mevalonate phosphates, J. Biol. Chem. 266 (1991) 17966–17971.
[125] G.J. Warner, M.J. Berry, M.E. Moustafa, B.A. Carlson, D.L. Hatfield, J.R. Faust, Inhibition of selenoprotein synthesis by selenocysteine tRNA[Ser]Sec lacking isopentenyladenosine, J. Biol. Chem. 275 (2000) 28110–28119.
[126] D.M. Driscoll, P.R. Copeland, Mechanism and regulation of selenoprotein synthesis, Annu. Rev. Nutr. 23 (2003) 17–40.
[127] H.C. Rilling, Eukaryotic prenyltransferases, Methods Enzymol. 110 (1985) 145–152.
[128] H. Teclebrhan, J. Olsson, E. Swiezewska, G. Dallner, Biosynthesis of the side chain of ubiquinone: *trans*-prenyltransferase in rat liver microsomes, J. Biol. Chem. 268 (1993) 23081–23086.
[129] E. Swiezewska, G. Dallner, B. Andersson, L. Ernster, Biosynthesis of ubiquinone and plastoquinone in the endoplasmic reticulum–

Golgi membranes of spinach leaves, J. Biol. Chem. 268 (1993) 1494–1499.
[130] D.S. Rosser, M.N. Ashby, J.L. Ellis, P.A. Edwards, Coordinate regulation of 3-hydroxy-3-methylglutaryl-coenzyme A synthase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and prenyltransferase synthesis but not degradation in HepG2 cells, J. Biol. Chem. 264 (1989) 12653–12656.
[131] M. Andersson, J. Ericsson, E.L. Appelkvist, S. Schedin, T. Chojnacki, G. Dallner, Modulations in hepatic branch-point enzymes involved in isoprenoid biosynthesis upon dietary and drug treatments of rats, Biochim. Biophys. Acta 1214 (1994) 79–87.
[132] K. Wang, S. Ohnuma, Chain-length determination mechanism of isoprenyl diphosphate synthases and implications for molecular evolution, Trends Biochem. Sci. 24 (1999) 445–451.
[133] K. Ogura, T. Koyama, Enzymatic aspects of isoprenoid chain elongation, Chem. Rev. 98 (1998) 1263–1276.
[134] K. Okada, K. Suzuki, Y. Kamiya, X. Zhu, S. Fujisaki, Y. Nishimura, T. Nishino, T. Nakagawa, M. Kawamukai, H. Matsuda, Polyprenyl diphosphate synthase essentially defines the length of the side chain of ubiquinone, Biochim. Biophys. Acta 1302 (1996) 217–223.
[135] S. Ohnuma, T. Nakazawa, H. Hemmi, A.M. Hallberg, T. Koyama, K. Ogura, T. Nishino, Conversion from farnesyl diphosphate synthase to geranylgeranyl diphosphate synthase by random chemical mutagenesis, J. Biol. Chem. 271 (1996) 10087–10095.
[136] S. Ohnuma, K. Hirooka, H. Hemmi, C. Ishida, C. Ohto, T. Nishino, Conversion of product specificity of archaebacterial geranylgeranyldiphosphate synthase. Identification of essential amino acid residues for chain length determination of prenyltransferase reaction, J. Biol. Chem. 271 (1996) 18831–18837.
[137] H. Teclebrhan, A. Jakobsson-Borin, U. Brunk, G. Dallner, Relationship between the endoplasmic reticulum–Golgi membrane system and ubiquinone biosynthesis, Biochim. Biophys. Acta 1256 (1995) 157–165.
[138] R.E. Olson, H. Rudney, Biosynthesis of ubiquinone, Vitam. Horm. 40 (1983) 1–43.
[139] S. Ranganathan, T. Ramasarma, The regulation of the biosynthesis of ubiquinone in the rat, Biochem. J. 148 (1975) 35–39.
[140] M.N. Ashby, S.Y. Kutsunai, S. Ackerman, A. Tzagoloff, P.A. Edwards, *COQ2* is a candidate for the structural gene encoding *para*-hydroxybenzoate: polyprenyltransferase, J. Biol. Chem. 267 (1992) 4128–4136.
[141] M. Melzer, L. Heide, Characterization of polyprenyldiphosphate:4-hydroxybenzoate polyprenyltransferase from *Escherichia coli*, Biochim. Biophys. Acta 1212 (1994) 93–102.
[142] S.S. Alam, A.M. Nambudiri, H. Rudney, 4-Hydroxybenzoate:polyprenyl transferase and the prenylation of 4-aminobenzoate in mammalian tissues, Arch. Biochem. Biophys. 171 (1975) 183–190.
[143] K. Momose, H. Rudney, 3-Polyprenyl-4-hydroxybenzoate synthesis in the inner membrane of mitochondria from *p*-hydroxybenzoate and isopentenylpyrophosphate. A demonstration of isoprenoid synthesis in rat liver mitochondria, J. Biol. Chem. 247 (1972) 3930–3940.
[144] A.M. Nambudiri, D. Brockman, S.S. Alam, H. Rudney, Alternate routes for ubiquinone biosynthesis in rats, Biochem. Biophys. Res. Commun. 76 (1976) 282–287.
[145] A. Kalen, E.L. Appelkvist, T. Chojnacki, G. Dallner, Nonaprenyl-4-hydroxybenzoate transferase, an enzyme involved in ubiquinone biosynthesis, in the endoplasmic reticulum–Golgi system of rat liver, J. Biol. Chem. 265 (1990) 1158–1164.
[146] G.H. Dialameh, H.G. Nowicki, K.G. Yekundi, R.E. Olson, Involvement of *p*-OH-benzoyl-coenzyme A in the biosynthesis of ubiquinone-9 in the rat, Biochem. Biophys. Res. Commun. 40 (1970) 1063–1069.
[147] D. Kang, K. Takeshige, R. Isobe, S. Minakami, Evidence that the decarboxylation reaction occurs before the first methylation in ubiquinone biosynthesis in rat liver mitochondria, Eur. J. Biochem. 198 (1991) 599–605.
[148] A. Tzagoloff, C.L. Dieckmann, PET genes of *Saccharomyces cerevisiae*, Microbiol. Rev. 54 (1990) 211–225.
[149] C.F. Clarke, W. Williams, J.H. Teruya, Ubiquinone biosynthesis in *Saccharomyces cerevisiae*. Isolation and sequence of COQ3, the 3,4-dihydroxy-5-hexaprenylbenzoate methyltransferase gene, J. Biol. Chem. 266 (1991) 16636–16644.
[150] G. Wu, H.D. Williams, M. Zamanian, F. Gibson, R.K. Poole, Isolation and characterization of *Escherichia coli* mutants affected in aerobic respiration: The cloning and nucleotide sequence of ubiG. Identification of an S-adenosylmethionine-binding motif in protein, RNA, and small molecule methyltransferases, J. Gen. Microbiol. 138 (1992) 2101–2112.
[151] R.M. Kagan, S. Clarke, Widespread occurrence of three sequence motifs in diverse *S*-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes, Arch. Biochem. Biophys. 310 (1994) 417–427.
[152] A. Niewmierzycka, S. Clarke, *S*-Adenosylmethionine-dependent methylation in *Saccharomyces cerevisiae*. Identification of a novel protein originine methyltransferase, J. Biol. Chem. 274 (1999) 814–824.
[153] B.N. Marbois, A. Hsu, R. Pillai, J. Colicelli, C.F. Clarke, Cloning of a rat cDNA encoding dihydroxypolyprenylbenzoate methyltransferase by functional complementation of a *Saccharomyces cerevisiae* mutant deficient in ubiquinone biosynthesis, Gene 138 (1994) 213–217.
[154] B.N. Marbois, Y.R. Xia, A.J. Lusis, C.F. Clarke, Ubiquinone biosynthesis in eukaryotic cells: tissue distribution of mRNA encoding 3,4-dihydroxy-5-polyprenylbenzoate methyltransferase in the rat and mapping of the COQ3 gene to mouse chromosome 4, Arch. Biochem. Biophys. 313 (1994) 83–88.
[155] A.Y. Hsu, W.W. Poon, J.A. Shepherd, D.C. Myles, C.F. Clarke, Complementation of coq3 mutant yeast by mitochondrial targeting of the *Escherichia coli* UbiG polypeptide: evidence that UbiG catalyzes both *O*-methylation steps in ubiquinone biosynthesis, Biochemistry 35 (1996) 9797–9806.
[156] W.W. Poon, R.J. Barkovich, A.Y. Hsu, A. Frankel, P.T. Lee, J.N. Shepherd, D.C. Myles, C.F. Clarke, Yeast and rat Coq3 and *Escherichia coli* UbiG polypeptides catalyze both *O*-methyltransferase steps in coenzyme Q biosynthesis, J. Biol. Chem. 274 (1999) 21665–21672.
[157] B.N. Marbois, C.F. Clarke, The COQ7 gene encodes a protein in *Saccharomyces cerevisiae* necessary for ubiquinone biosynthesis, J. Biol. Chem. 271 (1996) 2995–3004.
[158] M. Proft, P. Kotter, D. Hedges, N. Bojunga, K.D. Entian, CAT5, a new gene necessary for derepression of gluconeogenic enzymes in *Saccharomyces cerevisiae*, EMBO J. 14 (1995) 6116–6126.
[159] T. Jonassen, M. Proft, F. Randez-Gil, J.R. Schultz, B.N. Marbois, K.D. Entian, C.F. Clarke, Yeast Clk-1 homologue (Coq7/Cat5) is a mitochondrial protein in coenzyme Q synthesis, J. Biol. Chem. 273 (1998) 3351–3357.
[160] T. Jonassen, B.N. Marbois, L. Kim, A. Chin, Y.-R. Xia, A.J. Lusis, C.F. Clarke, Isolation and sequencing of the rat Coq7 gene and the mapping of mouse Coq7 to chromosome 7, Arch. Biochem. Biophys. 330 (1996) 285–289.
[161] J.J. Ewbank, T.M. Barnes, B. Lakowski, M. Lussier, H. Bussey, S. Hekimi, Structural and functional conservation of the *Caenorhabditis elegans* timing gene clk-1, Science 275 (1997) 980–983.
[162] T. Jonassen, P.L. Larsen, C.F. Clarke, A dietary source of coenzyme Q is essential for growth of long-lived *Caenorhabditis elegans* clk-1 mutants, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 421–426.
[163] N. Jiang, F. Levavasseur, B. McCright, E.A. Shoubridge, S. Hekimi, Mouse CLK-1 is imported into mitochondria by an unusual process that requires leader sequence but no membrane potential, J. Biol. Chem. 276 (2001) 29218–29225.
[164] A. Wong, P. Boutis, S. Hekimi, Mutations in the clk-1 gene of *Caenorhabditis elegans* affect developmental and behavioral timing, Genetics 139 (1995) 1247–1259.

[165] P. Gin, A.Y. Hsu, S.C. Rothman, T. Jonassen, P.T. Lee, A. Tzagoloff, C.F. Clarke, The *Saccharomyces cerevisiae* COQ6 gene encodes a mitochondrial flavin-dependent monooxygenase required for coenzyme Q biosynthesis, J. Biol. Chem. 278 (2003) 25308–25316.
[166] T.Q. Do, A.Y. Hsu, T. Jonassen, P.T. Lee, C.F. Clarke, A defect in coenzyme Q biosynthesis is responsible for the respiratory deficiency in *Saccharomyces cerevisiae* abc1 mutants, J. Biol. Chem. 276 (2001) 18161–18168.
[167] W.W. Poon, D.E. Davis, H.T. Ha, T. Jonassen, P.N. Rather, C.F. Clarke, Identification of *Escherichia coli* ubiB, a gene required for the first monooxygenase step in ubiquinone biosynthesis, J. Bacteriol. 182 (2000) 5139–5146.
[168] A.Y. Hsu, T.Q. Do, P.T. Lee, C.F. Clarke, Genetic evidence for a multi-subunit complex in the *O*-methyltransferase steps of coenzyme Q biosynthesis, Biochim. Biophys. Acta 1484 (2000) 287–297.
[169] T. Yamamoto, S. Shimizu, H. Sugawara, K. Momose, H. Rudney, Identification of regulatory sites in the biosynthesis of ubiquinone in the perfused rat heart, Arch. Biochem. Biophys. 269 (1989) 86–92.
[170] T. Yamamoto, H. Sugawara, S. Shimizu, K. Momose, Possible existence of an intermediate pool of ubiquinone in rat heart mitochondria, Int. J. Biochem. 22 (1990) 89–91.
[171] P.G. Elmberger, A. Kalen, U.T. Brunk, G. Dallner, Discharge of newly-synthesized dolichol and ubiquinone with lipoproteins to rat liver perfusate and to the bile, Lipids 24 (1989) 919–930.
[172] D. Wüstner, A. Herrmann, M. Hao, F.R. Maxfield, Rapid nonvesicular transport of sterol between the plasma membrane domains of polarized hepatic cells, J. Biol. Chem. 277 (2002) 30325–30336.
[173] M. Wanke, G. Dallner, E. Swiezewska, Subcellular localization of plastoquinone and ubiquinone synthesis in spinach cells, Biochim. Biophys. Acta 1463 (2000) 188–194.
[174] M. Wanke, E. Swiezewska, G. Dallner, Cell-free sorting of plastoquinone and ubiquinone in spinach cell, Plant Physiol. Biochem. 39 (2001) 467–472.
[175] C. Santos-Ocana, T.Q. Do, S. Padilla, P. Navas, C.F. Clarke, Uptake of exogenous coenzyme Q and transport to mitochondria is required for bc1 complex stability in yeast coq mutants, J. Biol. Chem. 277 (2002) 10973–10981.
[176] S. Osowska-Rogers, E. Swiezewska, B. Andersson, G. Dallner, The endoplasmic reticulumn–Golgi system is a major site of plastoquinone synthesis in spinach leaves, Biochem. Biophys. Res. Commun. 205 (1994) 714–721.
[177] M. Tekle, M. Bentinger, T. Nordman, E.L. Appelkvist, T. Chojnacki, J.M. Olsson, Ubiquinone biosynthesis in rat liver peroxisomes, Biochem. Biophys. Res. Commun. 291 (2002) 1128–1133.
[178] S.L. Thompson, R. Burrows, R.J. Laub, S.K. Krisans, Cholesterol synthesis in rat liver peroxisomes. Conversion of mevalonic acid to cholesterol, J. Biol. Chem. 262 (1987) 17420–17425.
[179] E.L. Appelkvist, A. Kalen, Biosynthesis of dolichol by rat liver peroxisomes, Eur. J. Biochem. 185 (1989) 503–509.
[180] J. Grünler, I. Parmryd, Subcellular distribution of farnesyl protein transferase in rat liver, FEBS Lett. 455 (1999) 233–237.
[181] H. van den Bosch, R.B. Schutgens, R.J. Wanders, J.M. Tager, Biochemistry of peroxisomes, Annu. Rev. Biochem. 61 (1992) 157–197.
[182] H. Schägger, K. Pfeiffer, Supercomplexes in the respiratory chains of yeast and mammalian mitochondria, EMBO J. 19 (2000) 1777–1783.
[183] G. Lenaz, A critical appraisal of the mitochondrial coenzyme Q pool, FEBS Lett. 509 (2001) 151–155.
[184] C. Asencio, J.C. Rodríguez-Aguilera, M. Ruiz-Ferrer, J. Vela, P. Navas, Silencing of ubiquinone biosynthesis genes extends life span in *Caenorhabditis elegans*, FASEB J. 17 (2003) 1135–1137.
[185] J.R. Faust, M.S. Brown, J.L. Goldstein, Synthesis of delta 2-isopentenyl tRNA from mevalonate in cultured human fibroblasts, J. Biol. Chem. 255 (1980) 6546–6548.
[186] Y. Reiss, M.S. Brown, J.L. Goldstein, Divalent cation and prenyl pyrophosphate specificities of the protein farnesyltransferase from rat brain, a zinc metalloenzyme, J. Biol. Chem. 267 (1992) 6403–6408.
[187] R.A. Willis, K. Folkers, J.L. Tucker, C.Q. Ye, L.J. Xia, H. Tamagawa, Lovastatin decreases coenzyme Q levels in rats, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 8928–8930.
[188] P.G. Elmberger, A. Kalen, E. Lund, E. Reihner, M. Eriksson, L. Berglund, B. Angelin, G. Dallner, Effects of pravastatin and cholestyramine on products of the mevalonate pathway in familial hypercholesterolemia, J. Lipid Res. 32 (1991) 935–940.
[189] J. Ericsson, A. Thelin, T. Chojnacki, G. Dallner, Substrate specificity of cis-prenyltransferase in rat liver microsomes, J. Biol. Chem. 267 (1992) 19730–19735.
[190] G. Ghirlanda, A. Oradei, A. Manto, S. Lippa, L. Uccioli, A.V. Greco, G.P. Littarru, Evidence of plasma CoQ10-lowering effect by HMG-CoA reductase inhibitors: a double-blind, placebo-controlled study, J. Clin. Pharmacol. 33 (1993) 226–229.
[191] S.A. Mortensen, A. Leth, E. Agner, M. Rohde, Dose-related decrease of serum coenzyme Q10 during treatment with HMG-CoA reductase inhibitors, Mol. Aspects Med. 18 (1997) 137–144.
[192] P. Löw, M. Andersson, C. Edlund, G. Dallner, Effects of mevinolin treatment on tissue dolichol and ubiquinone levels in the rat, Biochim. Biophys. Acta 1165 (1992) 102–109.
[193] A. Thelin, E. Peterson, J.L. Hutson, A.D. McCarthy, J. Ericsson, G. Dallner, Effect of squalestatin 1 on the biosynthesis of the mevalonate pathway lipids, Biochim. Biophys. Acta 1215 (1994) 245–249.
[194] R.K. Keller, Squalene synthase inhibition alters metabolism of nonsterols in rat liver, Biochim. Biophys. Acta 1303 (1996) 169–179.
[195] K. Machida, T. Tanaka, Farnesol-induced generation of reactive oxygen species dependent on mitochondrial transmembrane potential hyperpolarization mediated by F(0)F(1)-ATPase in yeast, FEBS Lett. 462 (1999) 108–112.
[196] K. Machida, T. Tanaka, Y. Yano, S. Otani, M. Taniguchi, Farnesol-induced growth inhibition in *Saccharomyces cerevisiae* by a cell cycle mechanism, Microbiology 145 (1999) 293–299.
[197] C.C. Correll, L. Ng, P.A. Edwards, Identification of farnesol as the non-sterol derivative of mevalonic acid required for the accelerated degradation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, J. Biol. Chem. 269 (1994) 17390–17393.
[198] H. Morimoto, I. Imada, G. Goto, Photooxidation of ubiquinone-7, Liebigs Ann. Chem. 735 (1970) 65–71.
[199] M. Turunen, E. Swiezewska, T. Chojnacki, P. Sindelar, G. Dallner, Regulatory aspects of coenzyme Q metabolism, Free Radic. Res. 36 (2002) 437–443.
[200] T. Ramasarma, Studies on ubiquinone, J. Sci. Ind. Res. 27 (1968) 147–164.
[201] H.N. Aithal, V.C. Joshi, T. Ramasarma, Effect of cold exposure on the metabolism of ubiquinone, Biochim. Biophys. Acta 162 (1968) 66–72.
[202] V.C. Joshi, J. Jayaraman, T. Ramasarma, Tissue concentrations of coenzyme Q, ubichromenol and tocopherol in relation to protein status in the rat, Biochem. J. 88 (1963) 25–31.
[203] K. Gohil, L. Rothfuss, J. Lang, L. Packer, Effect of exercise training on tissue vitamin E and ubiquinone content, J. Appl. Physiol. 63 (1987) 1638–1641.
[204] J.L. Quiles, J.R. Huertas, M. Manas, M. Battino, M. Cassinello, G.P. Littarru, G. Lenaz, F.J. Mataix, Peroxidative extent and coenzyme Q levels in the rat: influence of physical training and dietary fats, Mol. Aspects Med. 15 (1994) 89–95.
[205] M. Svensson, C. Malm, M. Tonkonogi, B. Ekblom, B. Sjödin, K. Sahlin, Effect of Q10 supplementation on tissue Q10 levels and adenine nucleotide catabolism during high-intensity exercise, Int. J. Sport Nutr. 9 (1999) 166–180.
[206] A.K. Sohlenius-Sternbeck, E.L. Appelkvist, J.W. DePierre, Effects of vitamin A deficiency on selected xenobiotic-metabolizing enzymes and defenses against oxidative stress in mouse liver, Biochem. Pharmacol. 59 (2000) 377–383.
[207] S. Vadhanavikit, H.E. Ganther, Decreased ubiquinone levels in tissues of rats deficient in selenium, Biochem. Biophys. Res. Commun. 190 (1993) 921–926.

[208] F. Navarro, P. Navas, J.R. Burgess, R.I. Bello, R. De Cabo, A. Arroyo, J.M. Villalba, Vitamin E and selenium deficiency induces expression of the ubiquinone- dependent antioxidant system at the plasma membrane, FASEB J. 12 (1998) 1665–1673.
[209] Y. Zhang, M. Turunen, E.L. Appelkvist, Restricted uptake of dietary coenzyme Q is in contrast to the unrestricted uptake of alpha-tocopherol into rat organs and cells, J. Nutr. 126 (1996) 2089–2097.
[210] W.H. Ibrahim, H.N. Bhagavan, R.K. Chopra, C.K. Chow, Dietary coenzyme Q10 and vitamin E alter the status of these compounds in rat tissues and mitochondria, J. Nutr. 130 (2000) 2343–2348.
[211] A. Lass, M.J. Forster, R.S. Sohal, Effects of coenzyme Q10 and α-tocopherol administration on their tissue levels in the mouse: elevation of mitochondrial a-tocopherol by coenzyme Q10, Free Radic. Biol. Med. 26 (1999) 1375–1382.
[212] F. Åberg, E.L. Appelkvist, A. Bröijersen, M. Eriksson, B. Angelin, P. Hjemdahl, G. Dallner, Gemfibrozil-induced decrease in serum ubiquinone and alpha- and gamma-tocopherol levels in men with combined hyperlipidaemia, Eur. J. Clin. Invest. 28 (1998) 235–242.
[213] Z. Guan, M. Söderberg, P. Sindelar, S.B. Prusiner, K. Kristensson, G. Dallner, Lipid composition in scrapie-infected mouse brain: prion infection increases the levels of dolichyl phosphate and ubiquinone, J. Neurochem. 66 (1996) 277–285.
[214] H. Shi, N. Noguchi, E. Niki, Dynamics of antioxidant action of ubiquinol: a reappraisal, Biofactors 9 (1999) 141–148.
[215] T. Takahashi, T. Yamaguchi, M. Shitashige, M. Okamoto, T. Kishi, Reduction of ubiquinone in membrane lipids by rat liver cytosol and its involvement in the cellular defence system against lipid peroxidation, Biochem. J. 309 (1995) 883–890.
[216] T. Kishi, T. Takahashi, A. Usui, N. Hashizume, T. Okamoto, Cytosolic NADPH-UQ reductase, the enzyme responsible for cellular ubiquinone redox cycle as an endogenous antioxidant in the rat liver, Biofactors 9 (1999) 189–197.
[217] R.E. Beyer, J. Segura-Aguilar, S. Di Bernardo, M. Cavazzoni, R. Fato, D. Fiorentini, M.C. Galli, M. Setti, L. Landi, G. Lenaz, The role of DT-diaphorase in the maintenance of the reduced antioxidant form of coenzyme Q in membrane systems, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 2528–2532.
[218] L. Landi, D. Fiorentini, M.C. Galli, J. Segura-Aguilar, R.E. Beyer, DT-Diaphorase maintains the reduced state of ubiquinones in lipid vesicles thereby promoting their antioxidant function, Free Radic. Biol. Med. 22 (1997) 329–335.
[219] C. Lind, E. Cadenas, P. Hochstein, L. Ernster, DT-diaphorase: purification, properties, and function, Methods Enzymol. 186 (1990) 287–301.
[220] J.M. Olsson, L. Xia, L.C. Eriksson, M. Björnstedt, Ubiquinone is reduced by lipoamide dehydrogenase and this reaction is potently stimulated by zinc, FEBS Lett. 448 (1999) 190–192.
[221] L. Xia, M. Björnstedt, T. Nordman, L.C. Eriksson, J.M. Olsson, Reduction of ubiquinone by lipoamide dehydrogenase. An antioxidant regenerating pathway, Eur. J. Biochem. 268 (2001) 1486–1490.
[222] L. Xia, T. Nordman, J.M. Olsson, A. Damdimopoulos, L. Björkhem-Bergman, I. Nalvarte, L.C. Eriksson, E.S. Arner, G. Spyrou, M. Björnstedt, The mammalian cytosolic selenoenzyme thioredoxin reductase reduces ubiquinone. A novel mechanism for defense against oxidative stress, J. Biol. Chem. 278 (2003) 2141–2146.
[223] B.D. Burleigh, C.H. Williams, The isolation and primary structure of a peptide containing the oxidation–reduction active cystine of *Escherichia coli* lipoamide dehydrogenase, J. Biol. Chem. 247 (1972) 2077–2082.
[224] S. Ronchi, C.H. Williams, The isolation and primary structure of a peptide containing the oxidation–reduction active cystine of *Escherichia coli* thioredoxin reductase, J. Biol. Chem. 247 (1972) 2083–2086.
[225] G. Krohne-Ehrich, R.H. Schirmer, R. Untucht-Grau, Glutathione reductase from human erythrocytes. Isolation of the enzyme and sequence analysis of the redox-active peptide, Eur. J. Biochem. 80 (1977) 65–71.
[226] T. Hayakawa, T. Kanzaki, T. Kitamura, Y. Fukuyoshi, Y. Sakurai, K. Koike, T. Suematsu, M. Koike, Mammalian alpha-keto acid dehydrogenase complexes: V. Resolution and reconstitution studies of the pig heart pyruvate dehydrogenase complex, J. Biol. Chem. 244 (1969) 3660–3670.
[227] A.V. Kozlov, L. Gille, K. Staniek, H. Nohl, Dihydrolipoic acid maintains ubiquinone in the antioxidant active form by two-electron reduction of ubiquinone and one-electron reduction of ubisemiquinone, Arch. Biochem. Biophys. 363 (1999) 148–154.
[228] L. Zhong, E.S. Arner, A. Holmgren, Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5854–5859.
[229] Y. Zhang, F. Åberg, E.L. Appelkvist, G. Dallner, L. Ernster, Uptake of dietary coenzyme Q supplement is limited in rats, J. Nutr. 125 (1995) 446–453.
[230] H. Yoshida, M. Yusin, I. Ren, J. Kuhlenkamp, T. Hirano, A. Stolz, N. Kaplowitz, Identification, purification, and immunochemical characterization of a tocopherol-binding protein in rat liver cytosol, J. Lipid Res. 33 (1992) 343–350.
[231] L.K. Kwong, S. Kamzalov, I. Rebrin, A.C. Bayne, C.K. Jana, P. Morris, M.J. Forster, R.S. Sohal, Effects of coenzyme Q(10) administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat, Free Radic. Biol. Med. 33 (2002) 627–638.
[232] R. Matthews, L. Yang, S. Brown, M. Baik, F. Beal, Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 8892–8897.
[233] M. Turunen, E.L. Appelkvist, P. Sindelar, G. Dallner, Blood concentration of coenzyme Q(10) increases in rats when esterified forms are administered, J. Nutr. 129 (1999) 2113–2118.
[234] G.W. Burton, K.U. Ingold, D.O. Foster, S.C. Cheng, A. Webb, L. Hughes, E. Lusztyk, Comparison of free α-tocopherol and α-tocopheryl acetate as sources of vitamin E in rats and humans, Lipids 23 (1988) 834–840.
[235] P.G. Elmberger, A. Kalen, E.L. Appelkvist, G. Dallner, In vitro and in vivo synthesis of dolichol and other main mevalonate products in various organs of the rat, Eur. J. Biochem. 168 (1987) 1–11.
[236] A. Rötig, E.L. Appelkvist, V. Geromel, D. Chretien, N. Kadhom, P. Edery, M. Lebideau, G. Dallner, A. Munnich, L. Ernster, P. Rustin, Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q10 deficiency, Lancet 356 (2000) 391–395.
[237] M. Nobuyoshi, T. Saito, H. Takahira, Y. Yamano, T. Kanazawa, Levels of coenzyme Q10 in biopsies of left ventricular muscle and influence of administration of coenzyme Q10, in: K. Folkers, Y. Yamamura (Eds.), Biomedical and Clinical Aspects of Coenzyme Q, vol. 4, Elsevier, Amsterdam, 1984, pp. 221–229.
[238] N. Kitamura, A. Yamaguchi, M. Otaki, O. Sawatani, T. Minoji, H. Tamura, M. Atobe, Myocardial tissue level of coenzyme Q10 in patients with cardiac failure, in: K. Folkers, Y. Yamamura (Eds.), Biomedical and Clinical Aspects of Coenzyme Q, vol. 4, Elsevier, Amsterdam, 1984, pp. 243–262.
[239] K. Folkers, S. Vadahanavikit, S.A. Mortensen, Biochemical rationale and myocardial tissue data on the effective therapy of cardiomyopathy with coenzyme Q10, Proc. Natl. Acad. Sci. U. S. A. 82 (1985) 901–904.
[240] M. Turunen, G. Dallner, Elevation of ubiquinone content by peroxisomal inducers in rat liver during aging, Chem. Biol. Interact. 116 (1998) 79–91.
[241] G. Lenaz, R. Fato, C. Castelluccio, M.L. Genova, C. Bovina, E. Estornell, V. Valls, F. Pallotti, G.P. Castelli, The function of coenzyme Q in mitochondria, Clin. Investig. 71 (1993) 66–70.

[242] A. Thelin, S. Schedin, G. Dallner, Half-life of ubiquinone-9 in rat tissues, FEBS Lett. 313 (1992) 118–120.
[243] M. Andersson, P.G. Elmberger, C. Edlund, K. Kristensson, G. Dallner, Rates of cholesterol, ubiquinone, dolichol and dolichyl-P biosynthesis in rat brain slices, FEBS Lett. 269 (1990) 15–18.
[244] M. Wanke, E. Swiezewska, G. Dallner, Half-life of ubiquinone and plastoquinone in spinach cells, Plant Sci. 154 (2000) 183–187.
[245] I. Imada, M. Watanabe, N. Matsumoto, H. Morimoto, Metabolism of ubiquinone-7, Biochemistry 9 (1970) 2870–2878.
[246] T. Nakamura, T. Ohno, K. Hamamura, T. Sato, Metabolism of coenzyme Q10: biliary and urinary excretion study in guinea pigs, Biofactors 9 (1999) 111–119.
[247] B. Burchell, M.W. Coughtrie, UDP-glucuronosyltransferases, Pharmacol. Ther. 43 (1989) 261–289.
[248] J.W. DePierre, A.K. Sohlenius, Y. Cai, A.M. Eriksson, K. Andersson, C. Sundberg, Peroxisome proliferation in response to xenobiotics, Biochem. Soc. Trans. 23 (1995) 425–429.
[249] J.K. Reddy, R. Chu, Peroxisome proliferator-induced pleiotropic responses: pursuit of a phenomenon, Ann. N.Y. Acad. Sci. 804 (1996) 176–201.
[250] P. Bentley, I. Calder, C. Elcombe, P. Grasso, D. Stringer, H.J. Wiegand, Hepatic peroxisome proliferation in rodents and its significance for humans, Food Chem. Toxicol. 31 (1993) 857–907.
[251] F. Åberg, Y. Zhang, E.L. Appelkvist, G. Dallner, Effects of clofibrate, phthalates and probucol on ubiquinone levels, Chem. Biol. Interact. 91 (1994) 1–14.
[252] F. Åberg, Y. Zhang, H. Teclebrhan, E.L. Appelkvist, G. Dallner, Increases in tissue levels of ubiquinone in association with peroxisome proliferation, Chem. Biol. Interact. 99 (1996) 205–218.
[253] M. Turunen, J.M. Peters, F.J. Gonzalez, S. Schedin, G. Dallner, Influence of peroxisome proliferator-activated receptor alpha on ubiquinone biosynthesis, J. Mol. Biol. 297 (2000) 607–614.
[254] M. Bentinger, M. Turunen, X.X. Zhang, Y.J. Wan, G. Dallner, Involvement of retinoid X receptor alpha in coenzyme Q metabolism, J. Mol. Biol. 326 (2003) 795–803.
[255] P. Barnett, H.F. Tabak, E.H. Hettema, Nuclear receptors arose from pre-existing protein modules during evolution, Trends Biochem. Sci. 25 (2000) 227–228.
[256] S. Khorasanizadeh, F. Rastinejad, Nuclear-receptor interactions on DNA-response elements, Trends Biochem. Sci. 26 (2001) 384–390.
[257] I. Issemann, S. Green, Activation of a member of the steroid hormone receptor superfamiliy by peroxisome proliferators, Nature 347 (1990) 645–650.
[258] H. Gronemeyer, Control of transcription activation by steroid hormone receptors, FASEB J. 6 (1992) 2524–2529.
[259] W. Wahli, O. Braissant, B. Desvergne, Peroxisome proliferator activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more, Chem. Biol. 2 (1995) 261–266.
[260] B.I. Frohnert, T.Y. Hui, D.A. Bernlohr, Identification of a functional peroxisome proliferator-responsive element in the murine fatty acid transport protein gene, J. Biol. Chem. 274 (1999) 3970–3977.
[261] M.C. Hunt, Y.-Z. Ynag, G. Eggertsen, C.M. Carnheim, M. Gåfvels, C. Einarsson, S.E. Alexson, The peroxisome proliferator-activated receptor α (PPARα) regulates bile acid synthesis, J. Biol. Chem. 275 (2000) 28947–28953.
[262] V.C. Yu, C. Delsert, B. Andersen, J.M. Holloway, O.V. Devary, A.M. Naar, S.Y. Kim, J.M. Boutin, C.K. Glass, M.G. Rosenfeld, RXR beta: a coregulator that enhances binding of retinoic acid, thyroid hormone and vitamin D receptors to their cognate response elements, Cell 67 (1991) 1251–1266.
[263] S.A. Kliewer, K. Umensono, D.J. Mangelsdorf, R.M. Evans, Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signaling, Nature 355 (1992) 446–449.
[264] S.A. Kliever, K. Umensono, D.J. Noonan, R.A. Heyman, R.M. Evans, Convergence of 9-cis retionic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors, Nature 358 (1992) 771–774.
[265] B.M. Forman, K. Umesono, J. Chen, R.M. Evans, Unique response pathways are established by allosteric interactions among nuclear hormone receptors, Cell 81 (1995) 541–550.
[266] K. Umesono, K.K. Murakami, C.C. Thompson, R.M. Evans, Direct repeats as selective response elements for the thyroid hormone, retinoic acid and vitamin D receptors, Cell 65 (1991) 1255–1266.
[267] A.J. Hörlein, A.M. Näär, T. Heinzel, J. Torchia, B. Gloss, R. Kurokawa, A. Ryan, Y. Kamei, M. Söderström, C.K. Glass, M.G. Rosenfeld, Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor, Nature 377 (1995) 397–404.
[268] S.S. Lee, T. Pineau, J. Drago, E.J. Lee, J.W. Owens, D.L. Kroetz, P.M. Fernandez-Salguero, H. Westphal, F.J. Gonzalez, Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators, Mol. Cell. Biol. 15 (1995) 3012–3022.
[269] T. Lemberger, O. Braissant, C. Juge-Aubry, H. Keller, R. Saladin, B. Staels, J. Auwerx, A.G. Burger, C.A. Meier, W. Wahli, PPAR tissue distribution and interactions with other hormone-signaling pathways, Ann. N.Y. Acad. Sci., U. S. A. 804 (1996) 231–251.
[270] Y.J. Wan, D. An, Y. Cai, J.J. Repa, T. Hung-Po Chen, M. Flores, C. Postic, M.A. Magnuson, J. Chen, K.R. Chien, S. French, D.J. Mangelsdorf, H.M. Sucov, Hepatocyte-specific mutation establishes retinoid X receptor alpha as a heterodimeric integrator of multiple physiological processes in the liver, Mol. Cell. Biol. 20 (2000) 4436–4444.
[271] Y.-J.Y. Wan, Y. Cai, W. Lungo, P. Fu, J. Locker, S. French, H.M. Sucov, Peroxisome proliferator-activated receptor α-mediated pathways are altered in hepatocyte-specific retinoid X receptor α-deficient mice, J. Biol. Chem. 275 (2000) 28285–28290.
[272] P.L. Larsen, C.F. Clarke, Extension of life-span in *Caenorhabditis elegans* by a diet lacking coenzyme Q, Science 295 (2002) 120–123.
[273] K. Lönnrot, T. Metsa-Ketela, H. Alho, The role of coenzyme Q-10 in aging: a follow-up study on life-long oral supplementation Q-10 in rats, Gerontology 41 (1995) 109–120.
[274] K. Lönnrot, P. Holm, A. Lagerstedt, H. Huhtala, H. Alho, The effects of lifelong ubiquinone Q10 supplementation on the Q9 and Q10 tissue concentrations and life span of male rats and mice, Biochem. Mol. Biol. Int. 44 (1998) 727–737.
[275] A. Kalen, E.L. Appelkvist, G. Dallner, Age-related changes in the lipid compositions of rat and human tissues, Lipids 24 (1989) 579–584.
[276] M. Battino, A. Gorini, R.F. Villa, M.L. Genova, C. Bovina, S. Sassi, G.P. Littarru, G. Lenaz, Coenzyme Q content in synaptic and non-synaptic mitochondria from different brain regions in the rat, Mech. Ageing Dev. 78 (1995) 173–187.
[277] M.L. Genova, C. Castelluccio, R. Fato, G. Parenti Castelli, M. Merlo Pinch, G. Formiggini, C. Bovina, M. Marchetti, G. Lenaz, Major changes in complex I activity in mitochondria from aged rats may not be detected by direct assay of NADH:-coenzyme Q reductase, Biochem. J. 311 (1995) 105–109.
[278] A. Lass, L. Kwong, R.S. Sohal, Mitochondrial coenzyme Q content and aging, Biofactors 9 (1999) 199–205.
[279] K. Folkers, G.P. Littarru, R. Nakamura, J. Scholler, Survey and new clinical studies on coenzyme Q in human muscular dystrophy, Int. J. Vitam. Nutr. Res. 42 (1972) 139–163.
[280] K. Folkers, Heart failure is a dominant deficiency of coenzyme Q10 and challenges for future clinical research on CoQ10, Clin. Invest. 71 (1993) 51–54.
[281] S. Ogasahara, A.G. Engel, D. Frens, D. Mack, Muscle coenzyme Q deficiency in familial mitochondrial encephalomyopathy, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 2379–2382.
[282] U. Manzoli, E. Rossi, G.P. Littarru, A. Frustaci, S. Lippa, A. Oradei,

V. Aureli, Coenzyme Q10 in dilated cardiomyopathy, Int. J. Tissue React. 12 (1990) 173–178.
[283] P.H. Langsjoen, A.M. Langsjoen, Overview of the use of CoQ10 in cardiovascular disease, Biofactors 9 (1999) 273–284.
[284] J.M. Olsson, L.C. Eriksson, G. Dallner, Lipid compositions of intracellular membranes isolated from rat liver nodules in Wistar rats, Cancer Res. 51 (1991) 3774–3780.
[285] J.M. Olsson, S. Schedin, H. Teclebrhan, L.C. Eriksson, G. Dallner, Enzymes of the mevalonate pathway in rat liver nodules induced by 2-acetylaminofluorene treatment, Carcinogenesis 16 (1995) 599–605.
[286] W. Dröge, Free radicals in the physiological control of cell function, Physiol. Rev. 82 (2002) 47–95.
[287] I. Eggens, P.G. Elmberger, P. Löw, Polyisoprenoid, cholesterol and ubiquinone levels in human hepatocellular carcinomas, Br. J. Exp. Pathol. 70 (1989) 83–92.
[288] B. Barbiroli, C. Frassineti, P. Martinelli, S. Iotti, R. Lodi, P. Cortelli, P. Montagna, Coenzyme Q10 improves mitochondrial respiration in patients with mitochondrial cytopathies. An in vivo study on brain and skeletal muscle by phosphorous magnetic resonance spectroscopy, Cell. Mol. Biol. 43 (1997) 741–749.
[289] W.J. Koroshetz, B.G. Jenkins, B.R. Rosen, M.F. Beal, Energy metabolism defects in Huntington's disease and effects of coenzyme Q10, Ann. Neurol. 41 (1997) 160–165.
[290] C.W. Shults, R.H. Haas, D. Passov, M.F. Beal, Coenzyme Q10 levels correlate with the activities of complexes I and II/III in mitochondria from parkinsonian and nonparkinsonian subjects, Ann. Neurol. 42 (1997) 261–264.
[291] M.F. Beal, R.F. Matthews, Coenzyme Q10 in the central nervous system and its potential usefulness in the treatment of neurodegenerative diseases, Mol. Aspects Med. 18 (1997) 169–179.
[292] C.W. Shults, D. Oakes, K. Kieburtz, M.F. Beal, R. Haas, S. Plumb, J.L. Juncos, J. Nutt, I. Shoulson, J. Carter, K. Kompoliti, J.S. Perlmutter, S. Reich, M. Stern, R.L. Watts, R. Kurlan, E. Molho, M. Harrison, M. Lew, Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline, Arch. Neurol. 59 (2002) 1541–1550.
[293] M. Söderberg, C. Edlund, I. Alafuzoff, K. Kristensson, G. Dallner, Lipid composition in different regions of the brain in Alzheimer's disease/senile dementia of Alzheimer's type, J. Neurochem. 59 (1992) 1646–1653.
[294] A. Contestabile, Oxidative stress in neurodegeneration: mechanisms and therapeutic perspectives, Curr. Top. Med. Chem. 1 (2001) 553–568.
[295] S.B. Prusiner, Prions, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 13363–13383.
[296] S. Schedin, P. Pentchev, G. Dallner, Reduced cholesterol accumulation and improved deficient peroxisomal functions in a murine model of Niemann–Pick type C disease upon treatment with peroxisomal proliferators, Biochem. Pharmacol. 56 (1998) 1195–1199.
[297] C.M. Palmeira, D.L. Santos, R. Seica, A.J. Moreno, M.S. Santos, Enhanced mitochondrial testicular antioxidant capacity in Goto–Kakizaki diabetic rats: role of coenzyme Q, Am. J. Physiol., Cell Physiol. 281 (2001) 1023–1028.
[298] J. Grünler, Compartmentalization of lipid metabolism in rat liver, PhD thesis. Stockholm University, 2000.
[299] I. Inoue, S. Goto, K. Mizotani, T. Awata, T. Mastunaga, S. Kawai, T. Nakajima, S. Hokari, T. Komoda, S. Katayama, Lipophilic HMG-CoA reductase inhibitor has an anti-inflammatory effect: reduction of mRNA levels for interleukin-1beta, interleukin-6, cyclooxygenase-2, and p22phox by regulation of peroxisome proliferator-activated receptor alpha (PPARalpha) in primary endothelial cells, Life Sci. 67 (2000) 863–876.
[300] P.A. Walravens, C. Greene, F.E. Freeman, Lovastatin, isoprenes, and myopathy, Lancet 2 (1989) 1097–1098.
[301] J. Ericsson, G. Dallner, Distribution, biosynthesis, and function of mevalonate pathway lipids, Subcell. Biochem. 21 (1993) 229–272.
[302] S. Yamashita, Y. Yamamoto, Simultaneous detection of ubiquinol and ubiquinone in human plasma as a marker of oxidative stress, Anal. Biochem. 250 (1997) 66–73.
[303] Y. Yamamoto, K. Wakabayashi, E. Nikki, M. Nagao, Comparison of plasma levels of lipid hydroperoxides and antioxidants in hyperlipidemic Nagase analbuminemic rats, Sprague–Dawley rats, and humans, Biochem. Biophys. Res. Commun. 189 (1992) 518–523.
[304] Y. Yamamoto, S. Yamashita, A. Fujisawa, S. Kokura, T. Yoshikawa, Oxidative stress in patients with hepatitis, cirrhosis, and hepatoma evaluated by plasma antioxidants, Biochem. Biophys. Res. Commun. 247 (1998) 166–170.
[305] M.N. Ashby, P.A. Edwards, Elucidation of the deficiency in two yeast coenzyme Q mutants: characterization of the structural gene encoding hexaprenyl pyrophosphate synthetase, J. Biol. Chem. 265 (1990) 13157–13164.
[306] K. Suzuki, K. Okada, Y. Kamiya, X.F. Zhu, T. Nakagawa, M. Kawamukai, H. Matsuda, Analysis of the decaprenyl diphosphate synthase (dps) gene in fission yeast suggests a role of ubiquinone as an antioxidant, J. Biochem. (Tokyo) 121 (1997) 496–505.
[307] K. Asai, S. Fujisaki, Y. Nishimura, T. Nishino, K. Okada, T. Nakagawa, M. Kawamukai, H. Matsuda, The identification of *Escherichia coli* ispB (cel) gene encoding the octaprenyl diphosphate synthase, Biochem. Biophys. Res. Commun. 202 (1994) 340–345.
[308] N. Uchida, K. Suzuki, R. Saiki, T. Kainou, K. Tanaka, H. Matsuda, M. Kawamukai, Phenotypes of fission yeast defective in ubiquinone production due to disruption of the gene for *p*-hydroxybenzoate polyprenyl diphosphate transferase, J. Bacteriol. 182 (2000) 6933–6939.
[309] M. Siebert, A. Bechthold, M. Melzer, U. May, U. Berger, G. Schroder, J. Schroder, K. Severin, L. Heide, Ubiquinone biosynthesis. Cloning of the genes coding for chorismate pyruvate-lyase and 4-hydroxybenzoate octaprenyl transferase from *Escherichia coli*, FEBS Lett. 307 (1992) 347–350.
[310] I. Bousquet, G. Dujardin, P.P. Slonimski, ABC1, a novel yeast nuclear gene has a dual function in mitochondria: it suppresses a cytochrome *b* mRNA translation defect and is essential for the electron transfer in the bc 1 complex, EMBO J. 10 (1991) 2023–2031.
[311] G.B. Cox, I.G. Young, L.M. McCann, F. Gibson, Biosynthesis of ubiquinone in *Escherichia coli* K-12: location of genes affecting the metabolism of 3-octaprenyl-4-hydroxybenzoic acid and 2- octaprenylphenol, J. Bacteriol. 99 (1969) 450–458.
[312] H. Zeng, I. Snavely, P. Zamorano, G.T. Javor, Low ubiquinone content in *Escherichia coli* causes thiol hypersensitivity, J. Bacteriol. 180 (1998) 3681–3685.
[313] K. Nakahigashi, K. Miyamoto, K. Nishimura, H. Inokuchi, Isolation and characterization of a light-sensitive mutant of *Escherichia coli* K-12 with a mutation in a gene that is required for the biosynthesis of ubiquinone, J. Bacteriol. 174 (1992) 7352–7359.
[314] R.J. Barkovich, A. Shtanko, J.A. Shepherd, P.T. Lee, D.C. Myles, A. Tzagoloff, C.F. Clarke, Characterization of the COQ5 gene from *Saccharomyces cerevisiae*. Evidence for a C-methyltransferase in ubiquinone biosynthesis, J. Biol. Chem. 272 (1997) 9182–9188.
[315] P.T. Lee, A.Y. Hsu, H.T. Ha, C.F. Clarke, A C-methyltransferase involved in both ubiquinone and menaquinone biosynthesis: isolation and identification of the *Escherichia coli* ubiE gene, J. Bacteriol. 179 (1997) 1748–1754.
[316] O. Kwon, A. Kotsakis, R. Meganathan, Ubiquinone (coenzyme Q) biosynthesis in *Escherichia coli*: identification of the ubiF gene, FEMS Microbiol. Lett. 186 (2000) 157–161.
[317] D.M. Morré, G. Lenaz, D.J. Morré, Surface oxidase and oxidative stress propagation in aging, J. Exp. Biol. 203 (2000) 1513–1521.