Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1713 (2005) 129 – 137
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Coenzyme Q distribution in HL-60 human cells depends on the endomembrane system

Daniel J.M. Fernández-Ayala, Gloria Brea-Calvo, Guillermo López-Lluch, Plácido Navas*

Centro Andaluz de Biología del Desarrollo (CABD), Universidad Pablo de Olavide, Carretera de Utrera, Km. 1, 41013 Sevilla, Spain

Received 11 April 2005; received in revised form 12 May 2005; accepted 31 May 2005
Available online 15 June 2005

Abstract

Coenzyme Q (Q) is an essential factor in the mitochondrial electron chain but also exerts important antioxidant functions in the rest of cell membranes of aerobic organisms. However, the mechanisms of distribution of Q among cell membranes are largely unclear. The aim of the present work is to study the mechanisms of distribution of endogenous Q_{10} and exogenous Q_9 among cell membranes in human HL-60 cells. Endogenous Q_{10} synthesized using the radiolabelled precursor [^{14}C]-pHB was first detected in mitochondria, and it was later incorporated into mitochondria-associated membranes and endoplasmic reticulum (ER). Plasma membrane was the last location to incorporate [^{14}C]-Q_{10}. Brefeldin A prevented Q_{10} incorporation in plasma membrane. Exogenous Q_9 was preferably accumulated into the endo-lysosomal fraction but a significant amount was distributed among other cell membranes also depending on the brefeldin-A-sensitive endomembrane system. Our results indicate that mitochondria are the first location for new synthesized Q. Exogenous Q is mainly incorporated into an endo-lysosomal fraction, which is then rapidly incorporated to cell membranes mainly to MAM and mitochondria. We also demonstrate that both endogenous and dietary Q is distributed among endomembranes and plasma membrane by the brefeldin A-sensitive endo-exocytic pathway.

Keywords: Coenzyme Q; CoQ uptake; Endomembrane; CoQ distribution

1. Introduction

Coenzyme Q (Q) is an essential component of the mitochondrial electron transfer chain [1]. Q is also present in plasma membrane (PM), endomembranes and in serum lipoproteins [2,3], where it acts as a key component in the antioxidant machinery [4]. Q is the only lipophilic antioxidant synthesized in all aerobic organisms [5], and participates in adaptive responses of cells against oxidative stress [6]. Moreover, Q is an important factor in development and aging [7–10].

The presence of Q in extramitochondrial membranes suggests the existence of membrane-linked mechanisms of synthesis and/or distribution. Mitochondria contain the majority of enzymes required for the final phases of Q biosynthesis [11,12]. However, extramitochondrial Q synthesis has been also proposed [5] because initial enzymatic activities of the Q biosynthesis pathway have been reported in both endoplasmic reticulum (ER) and Golgi apparatus [3,5,13] and even peroxisomes [14,15]. Nevertheless, the presence of mitochondrial targeting signals in CoQ genes and the products of these genes in mitochondria [11,12,16,17], but not in ER or Golgi apparatus, indicates that biosynthesis of Q must be finished in mitochondria from where it is transported to other membranes in the cell.

Under non-pathological processes, the capability of tissues to synthesize Q decreases during aging [18,19]. Q deficiency is also involved in aging-related degenerative diseases such as Alzheimer's, Parkinson's and Huntington's, [20–24] and also in heritable neurological disorders such as

Abbreviations: BFA, brefeldin A; Q, coenzyme Q; ubiquinone; COX, cytochrome *c* oxidase; ECD, electrochemical detector; ER, endoplasmic reticulum; ETC, electron transport chain; MAM, mitochondria-associated membrane; pHB, *para*-hidroxibenzoic acid; PM, plasma membrane; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate

* Corresponding author. Tel.: +34 95 434 9385; fax: +34 95 434 9376.
E-mail address: pnavas@upo.es (P. Navas).

0005-2736/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2005.05.010

cerebellar ataxia [25]. Symptoms of these disorders have been partially recovered by Q supplementation. Then, therapies based in Q administration have been recently taken into consideration but the bioavailability of dietary Q in mammals is clearly unknown [5].

Several studies in mammals demonstrate that Q-supplemented diets induce an increase in its functional levels in cell membranes [26–29], but differences in the capability of tissues for Q uptake have been demonstrated in rodents [30–33]. However, very little information about the mechanism of distribution of Q in human cells and among their membranes is available. We have used human cells in culture that are able to assimilate exogenous Q and to incorporate it into mitochondrial membranes, where it can restore the mitochondrial function [34,35] and also the antioxidant function in extramitochondrial membranes [36]. We consider that Q uses the endo-exocytic pathway to distribute among cell membranes moving from and to mitochondria through mitochondria-associated membranes (MAMs). Using radioactive labelling experiments, we show in this work that mitochondria are the first location for endogenous Q. On the other hand, exogenous Q is incorporated to an endo-lysosomal fraction and also rapidly to mitochondria through MAMs. In both cases, Q is then distributed among membranes through the brefeldin A-sensitive endomembrane system, reaching plasma membrane as the last location.

2. Materials and methods

2.1. Cell culture

The human promyelocytic HL-60 cell line [37] was grown in RPMI-1640 medium (BioWhittaker, UK) supplemented with 10% foetal calf serum (FCS) (Linus, Spain), 2 mM glutamine (Sigma, Spain) and antibiotic/antimycotic solution (Sigma, Spain), at 37 °C in a humidified atmosphere of 5% CO_2 and 95% of air. Cell viability was determined by trypan blue exclusion method. Q_{10} and Q_9 (Sigma, Spain) were directly dissolved in serum and added to serum-free culture media at a final concentration of 2 μM Q and 10% FCS. Brefeldin A (BFA) (Sigma, Spain) was dissolved in pure ethanol and added to culture media at 0.1 μg/ml. When corresponded, controls were cultured in the presence of the same concentration of the vehicle used for treatments.

2.2. Cell fractionation

Cells were recovered by centrifugation at 500×*g* for 5 min at 4 °C and washed twice with ice-cold PBS. Mitochondria and mitochondria-associated membranes (MAM) purification was performed as previously described [35]. Briefly, cells were lysed with a glass homogeniser in 9 volumes of lysis buffer (0.32 M sucrose in 2 mM HEPES–KOH (pH 7.4), 0.15 mM $MgCl_2$, 10 mM KCl, 0.5 mM EGTA (Panreac, Spain), 20 μM cytochalasin B and 10 μg/ml protease inhibitor cocktail (Sigma, Spain). Homogenate was then centrifuged at 1000×*g* for 5 min and the pellet containing nuclei and cell debris was discarded. Supernatant was centrifuged twice, first at 8000×*g* and the new supernatant once more at 10,000×*g* for 15 min at 4 °C. Both pellets were pooled to obtain the crude mitochondrial fraction (P_{10}). This fraction was further centrifuged at 95,000×*g* for 90 min on a discontinuous sucrose gradient performed in 12.5 ml bucket tubes (SW41Ti Beckman rotor) by successively layering 2 ml each of 1.6, 1.4, 1.2, 1.0 and 0.8 M sucrose in 2 mM HEPES–KOH buffer (pH 7.4). After centrifugation, highly purified mitochondria were recovered at the 1.2/1.4 M sucrose layer (fraction 3) (Fig. 1). The layer observed between 0.8 M and 1.0 M sucrose solutions (fraction 1) was considered as mitochondria-associated membranes (MAM) accordingly to the literature [38]. Membranes that did not enter into sucrose gradient were recovered at the 0.32 M/0.8 M sucrose layer (fraction 0). Fraction 4 was considered as mitoplasts and broken mitochondria. Fraction 2 was considered as a mixed fraction and it was not used in this study. Sucrose excess in collected fractions was removed by its dilution with a solution of lysis buffer without sucrose, and centrifugation at 100,000×*g* for 30 min for fractions 0 and 1, and at 12,500×*g* for 30 min for fraction 3. Membranes were further resuspended in lysis buffer containing 0.32 M sucrose and stored at −80 °C.

ER purification was performed from supernatant resulting after isolation of crude mitochondrial fraction as

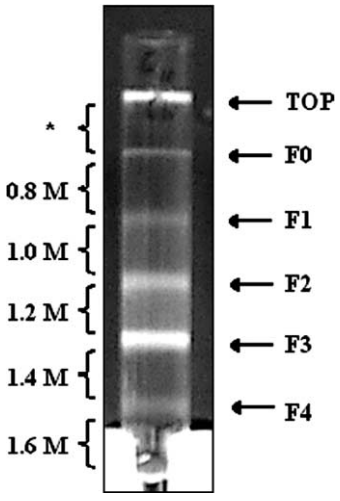

Fig. 1. Sucrose gradient purification of MAM and mitochondria. Cell homogenates were processes as described in Materials and methods. Centrifugation of crude mitochondrial fraction (P10) in a sucrose gradient permitted to obtain five different fractions. Distribution of fractions present in crude mitochondria in sucrose gradient was as follows: F0: very low density fraction (endo-lysosomal fraction); F1: MAM; F2: ER and PM contaminants; F3: mitochondria; F4: mitoplasts. Image representative of four different experiments.

previously described [39]. Briefly, mitochondria-free microsomes obtained after centrifugation at 100,000×*g* (P_{100}) were fractionated by centrifugation at 100,000×*g* for 90 min at 4 °C on a discontinuous sucrose gradient performed in 12.5 ml bucket tubes (SW41Ti Beckman rotor) by layering 4 ml each of 38% and 30% sucrose in 2 mM HEPES–KOH buffer (pH 7.4). ER-enriched fractions were recovered from the 30/38% sucrose layer, the 38% sucrose phase and the bottom of the tube. Gradient fractions were proceeded as indicated above for faction 1.

To isolate plasma membrane (PM), microsomes obtained from cellular homogenates after centrifugation at 40,000×*g* at 4 °C were isolated by the two-phase partition method as described previously [40]. Purified plasma membrane-enriched fractions were resuspended in pure distilled water and stored at −80 °C.

Purity of fractions were determined by Western blotting by using the following specific antibodies against cell membranes: mouse monoclonal anti-cytochrome *c* oxidase (1:1000) (RDI, USA, RDI-A6403); mouse monoclonal anti-Na^+/K^+ ATPase (1:500) (ABR, USA, MA3-929); mouse monoclonal anti-golgin 97 (1:200) (Molecular Probes, USA, A-21270); goat anti-ribophorin I (1:1000) (Santa Cruz Biotechnology, USA, sc-12164); rabbit anti-calnexin (1:1000) (StressGen Biotechnologies, Canada, SPA-865). Protein was determined by the Bradford's method [41].

2.3. Western blotting

Whole cell homogenate and purified fractions were electrophoresed in 10% acrylamide PAGE-SDS. Proteins were transferred to Immobilon membranes (Amersham Pharmacia Biotech, Spain) and further incubated with Tris Buffer Saline (TBS) pH 7.4 in the presence of 0.05% Tween-20 (TTBS) and 5% non-fat milk (TTBSL). Membrane specific proteins were probed by using primary specific antibodies as above indicated for at least 2 h at RT or overnight at 4 °C in TTBSL. After three washes with TTBS, membranes were probed with an HRP-labelled secondary anti-mouse, rabbit or goat antibodies for 2 h at RT in TTBSL. After washing again in TTBS and TBS, immunolabelled proteins were detected by using a chemiluminescence method (Biorad, USA).

2.4. Synthesis of [^{14}C]-pHB

Synthesis of [^{14}C]-pHB was performed from L-[U-^{14}C]-Tyrosine radioactively labelled in all its carbons as previously described [42]. Briefly, L-[U-^{14}C]-Tyrosine (250 μCi) (Amersham Pharmacia Biotech, Spain) was dried under vacuum, resuspended in 17.5 μl of 10 M KOH and 25 μl of 10 M NaOH (Panreac, Spain), and finally lyophilised. Dry residue was then heated at 270 °C for 5 min to force the chemical synthesis of [^{14}C]-*para*-hydroxibenzoate ([^{14}C]-pHB) by decarboxylation of L-[U-^{14}C]-Tyrosine. Bases were further neutralized with 400 μl 25% H_2SO_4 (Panreac, Spain), and [^{14}C]-pHB was extracted three times with ethyl-acetate (Panreac, Spain) followed by two washed with pure distilled water. Ethyl-acetate was then evaporated and [^{14}C]-pHB reconstituted with pure ethanol. Purity of [^{14}C]-pHB was determined by TLC (Merck, Spain) using a 2-propanol/water/amonium hydroxide (8:1:1) mobile phase. The concentration of [^{14}C]-pHB added to culture media was optimised at 4.5 nM.

2.5. Quantification of Q

Cells and membranes were disrupted in a final volume of 1 ml with 1% SDS. Then, 2 ml of a mixture of ethanol/2-propanol (95:5) was added and mixed vigorously. Q was recovered from SDS-alcoholic phase by extraction with 5 ml of hexane. Extraction was repeated twice and hexane phases were combined. Hexane was evaporated by using a Rotavapor (Buchi, Switzerland), and the dry residue resuspended in HPLC-grade ethanol. This procedure recovered near of 95% of total Q as determined by the use of internal standards such as decyl-ubiquinone.

Q was analysed by using a 15×0.46 cm C18 Kromasil 100 column (Scharlab, Spain) kept at 40 °C,

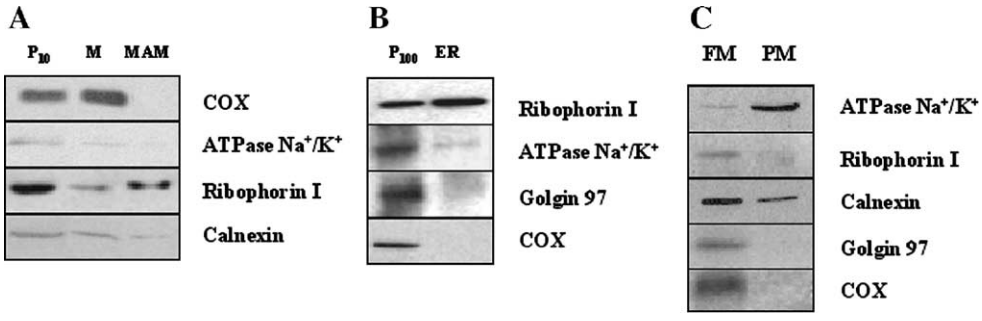

Fig. 2. Membrane purity determination. Purification procedures of mitochondria and MAM (A), endoplasmic reticulum (B) and plasma membrane (C) were performed as described in Materials and methods. Proteins (10 μg) were electrophoresed by SDS-PAGE and membrane purification markers determined by using specific antibodies. P_{10}, crude mitochondrial fraction; M, mitochondria; MAM, mitochondria associated membranes; P_{100}, mitochondria-free microsomal fraction; ER, endoplasmic reticulum; FM, microsomal fraction; PM, plasma membrane. Blots representative of at least two different experiments.

connected to a HPLC (Beckman-Coulter, USA) associated with an System Gold® UV-detector monitoring at 275 nm. Both a radio-flow detector LB 509 with a solid cell YG 150 μl-U4D (Berthold Technologies, Germany), and an electrochemical detector Coulochem III (ESA, USA) with a guard cell 5020 at +500 mV and an analytical cell 5010 with channel one at −500 mV and channel two at +500 mV were also used. The mobile phase was methanol/2-propanol (65:35) plus 70 mM sodium perchlorate (Panreac, Spain) at a constant flow of 1 ml per min.

2.6. Statistical analysis

Data represent the average ± the Standard Deviation (S.D.) from three different experiments performed in

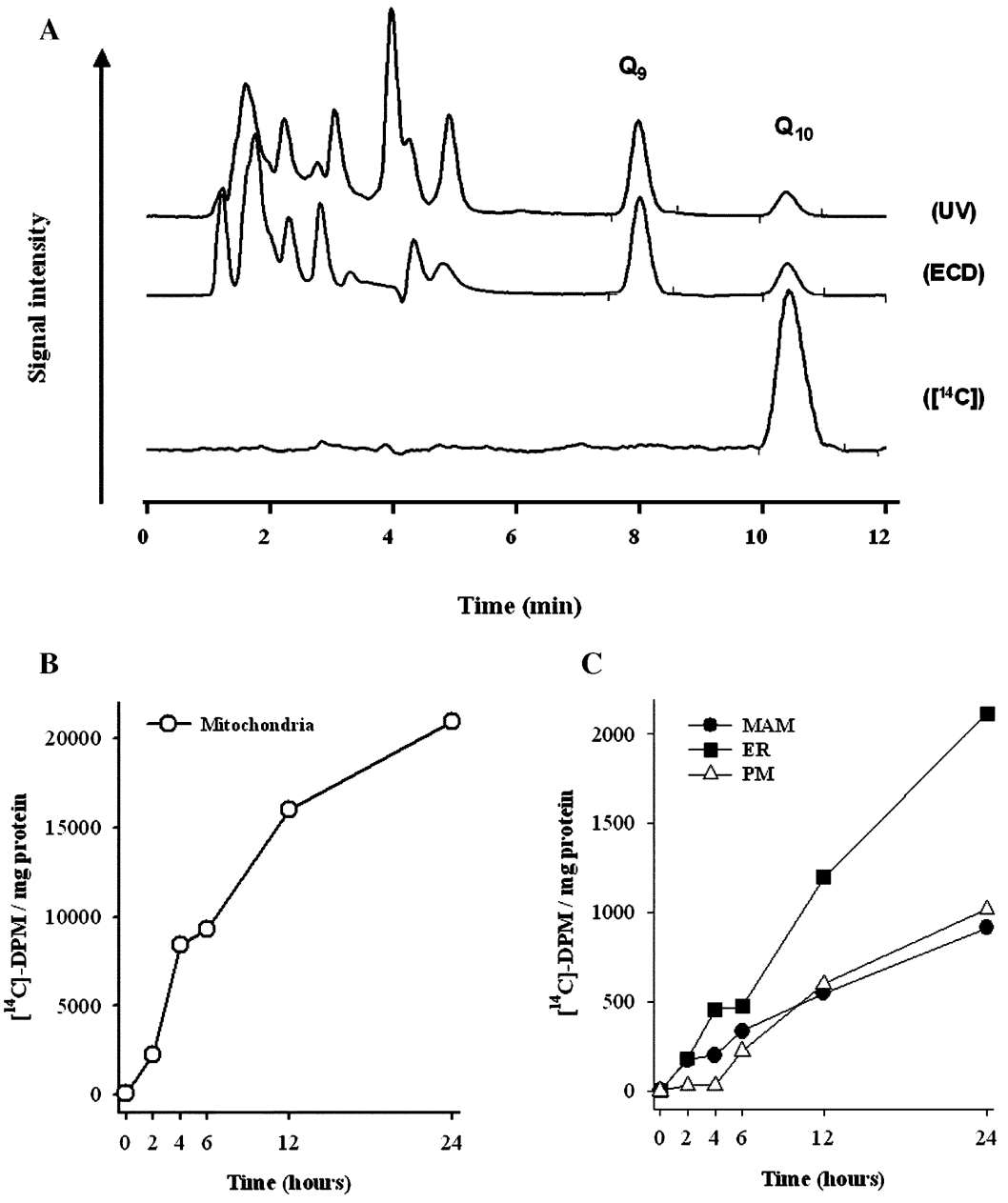

Fig. 3. Q synthesis and distribution in HL-60 cell membranes. (A) Q_{10} labelling with [^{14}C]-pHB. HL-60 cells were cultured in presence of 4.5 nM [^{14}C]-pHB for 24 h and their lipids extracted as describes below. Extracted lipids were separated by HPLC and detected by absorbance at 275 nm (UV). In parallel, lipids were also detected by their redox capability (ECD). Radioactivity of extracted lipids was also determined by a radio-flow detector as determined in Materials and methods ([^{14}C]). All the radioactivity of lipid extracts coeluted with Q_{10}. (B) HL-60 cells were grown in presence of 4.5 nM [^{14}C]-pHB. Aliquots were taken along time and membranes purified as described in Materials and methods. Radioactivity associated to mitochondrial Q_{10} was determined as above indicated and expressed as [^{14}C]-DPM/mg protein. (C) Radioactivity associated to Q_{10} in ER, MAM and PM membranes. In any case, data represent the mean of three different experiments performed in duplicate, S.D. was less than 10% in each incubation time and membrane and are not shown to improve clarity.

duplicate. Significant differences were assessed using the Student's *t* test.

3. Results

3.1. Purity of membrane fractions

Purity of membrane-enriched fractions isolated from HL-60 cells was assessed by Western blotting using antibodies against specific proteins considered as markers of the different organelles. Mitochondria-enriched fraction (M) showed the highest signal for the mitochondrial marker COX. Negligible amounts of both plasma membrane and ER were found in the mitochondria enriched fraction (Fig. 2A). MAM-enriched fractions only contained ER proteins but not PM and M markers (Fig. 2A). The presence of Golgin 97, a marker of Golgi apparatus was not detected in none of these fractions (data not shown).

ER-enriched fractions showed a high level of ER-markers, whereas mitochondria, plasma membrane and Golgi apparatus markers were almost absent (Fig. 2B). Fractions enriched in plasma membrane (PM) showed a clear concentration of sodium pump enzyme and an slight contamination of the ER marker calnexin, whereas mitochondria and Golgi apparatus markers were undetectable (Fig. 2C).

3.2. Mobilization of endogenous coenzyme Q_{10}

To study endogenous Q_{10} mobilization inside the cell, HL-60 cells were cultured with [^{14}C]-pHB, the specific precursor of Q biosynthesis [42]. The radioactivity was only detected in Q_{10} and not in any other hexane-extracted compounds (Fig. 3A) and this labelled quinone was used to study its distribution in HL-60 cells.

Mitochondria contained high levels of [^{14}C]-Q_{10} as early as 2 h after [^{14}C]-pHB addition (Fig. 3B), and showed a high rate of incorporation along 2 days of incubation. In the other membranes, [^{14}C]-Q_{10} was also detected but at lower than a 10% of mitochondrial levels (Fig. 3C). The presence of [^{14}C]-Q_{10} into ER showed a similar time pattern than in mitochondria. MAM incorporated detectable levels of [^{14}C]-Q_{10} from the beginning of the incubation but in a lower rate than ER. Detectable levels of [^{14}C]-Q_{10} in PM were only found after 4 h of incubation and also showed a lower rate of incorporation when compared with ER and mitochondria.

To determine if Q_{10} distribution depended on the endomembrane system dynamics, we treated HL-60 cells previously incubated with [^{14}C]-pHB with brefeldin A (BFA), a potent drug that inhibits this pathway [43]. BFA incubation produced a significant accumulation of [^{14}C]-Q_{10} in ER and a decrease of about 20–30% in both mitochondria and MAM (Fig. 4A). A higher decrease was observed in PM reaching only a 20% of control (Fig. 4A). This effect was more intense when BFA was added at the same time than the [^{14}C]-Q_{10} precursor (Fig. 4B). In this case, the presence of [^{14}C]-Q_{10} in PM was almost absent, whereas the levels in mitochondria, MAM and ER decreased to half of controls (Fig. 4B).

3.3. Distribution of exogenous Q_9

Q_9 isoform was used to study the distribution of Q among membranes as exogenous source. Exogenous sup-

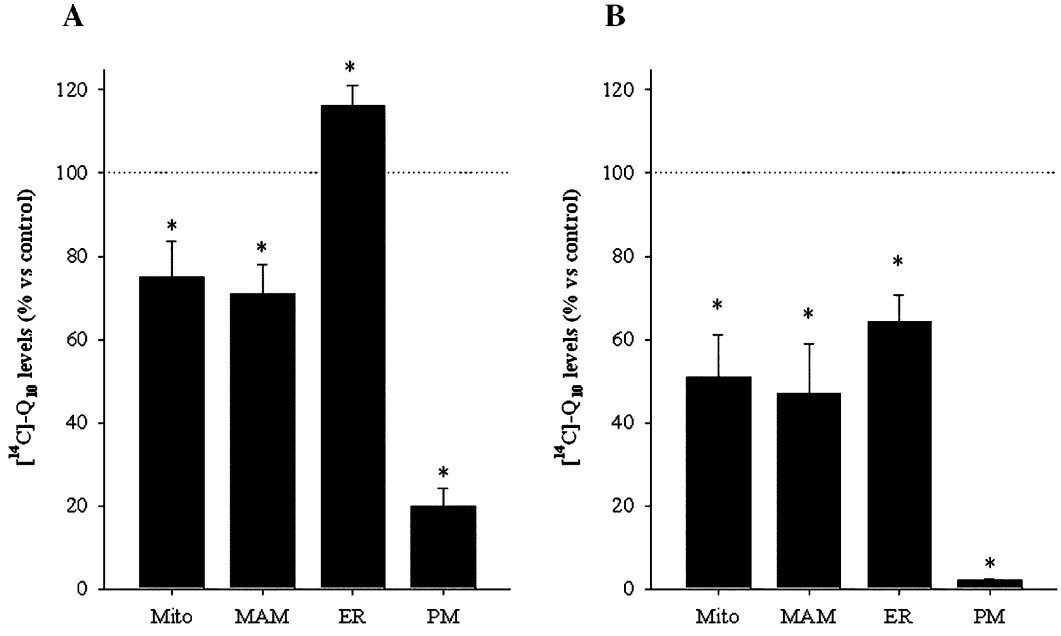

Fig. 4. Effect of BFA on the biosynthesis of Q_{10} in HL-60 cells. HL-60 cells were cultured with 4.5 nM [^{14}C]-pHB and [^{14}C]-Q_{10} in membranes determined after 24 h as indicated in Materials and methods. (A) BFA (0.1 μg/ml) was added 12 h after starting incubation with [^{14}C]-pHB. (B) BFA (0.1 μg/ml) was added just prior [^{14}C]-pHB addition. Data represent the percentage of [^{14}C]-Q_{10} found in each membrane vs. control. *Significant differences vs. control, $P<0.05$.

plemented Q_9 mainly accumulated in the crude mitochondrial fraction but, when subfractionation of crude mitochondria was performed, exogenous Q mainly incorporated in a very low density fraction that cosedimented with crude mitochondria but that did not enter in the sucrose gradient (fraction 0) (Fig. 1). This fraction can be identified as the light-density endo-lysosomal fraction, which is equivalent to that described to accumulate exogenous Q in rat liver [44]. Independently of the accumulation of Q_9 in this fraction, significant amounts were found in cellular endomembranes (Fig. 5A). Q_9 incorporated very early into both MAM and mitochondria. Incorporation of Q_9 into mitochondria increased in the course of the incubation, whereas Q_9 was stabilized in MAM after 6 h of treatment. On the other hand, both ER and PM showed a significant incorporation of exogenous Q_9 only after 6 h of treatment. Thus, a time-dependent incorporation of Q_9 in HL-60 cell membranes can be established from Fig. 5A, where PM was reached later than the other membranes.

To study if the endomembrane system through Golgi apparatus was also involved in the distribution of exogenous Q, we determined the presence of Q_9 in HL-60 cell membranes that were incubated for 12 h with both BFA and exogenous Q_9 (Fig. 5B). The presence of BFA induced a high increase of Q_9 concentration in the endo-lysosomal fraction (fraction 0) of HL-60 cells, whereas all other membranes showed a significant decrease of Q_9 levels in about 80% in PM, 40% in ER, and 20% in MAM and mitochondria.

4. Discussion

Q is present in all cell membranes of aerobic organisms. The highest levels are found in mitochondria, where it is an essential component of the mitochondrial electron transport chain, but also in endomembranes and plasma membrane [5]. In the other cell membranes, Q functions as antioxidant by itself of regenerating other antioxidants such as α-tocopherol and ascorbate [6]. The amount of Q in membranes is regulated by oxidative stress and aging [45,46], and dietary supplementation [30]. Then, a mechanism must exists to regulate the amount and distribution of Q in membranes of eukaryotic cells. This distribution is not apparently due to the presence of Q biosynthesis enzymes in all membranes of the cell because the enzymes involved in the last steps of the quinonic ring modifications are located in the matrix surface of the inner mitochondrial membrane [11,12,16,17]. However, some other enzymes involved in Q biosynthesis have been also located in the ER–Golgi system [3,5,13,47] and even in peroxisomes [15]. In any case, as Q is present in all the subcellular compartments, Q must be distributed from mitochondria, where its biosynthesis is finalized, to the other membranes including plasma membrane. However, little information about the mechanisms of distribution of Q among cell membranes is currently available. Then, we try to clarify the pathway(s) of distribution of both, endogenous and exogenous Q, in human cell membranes.

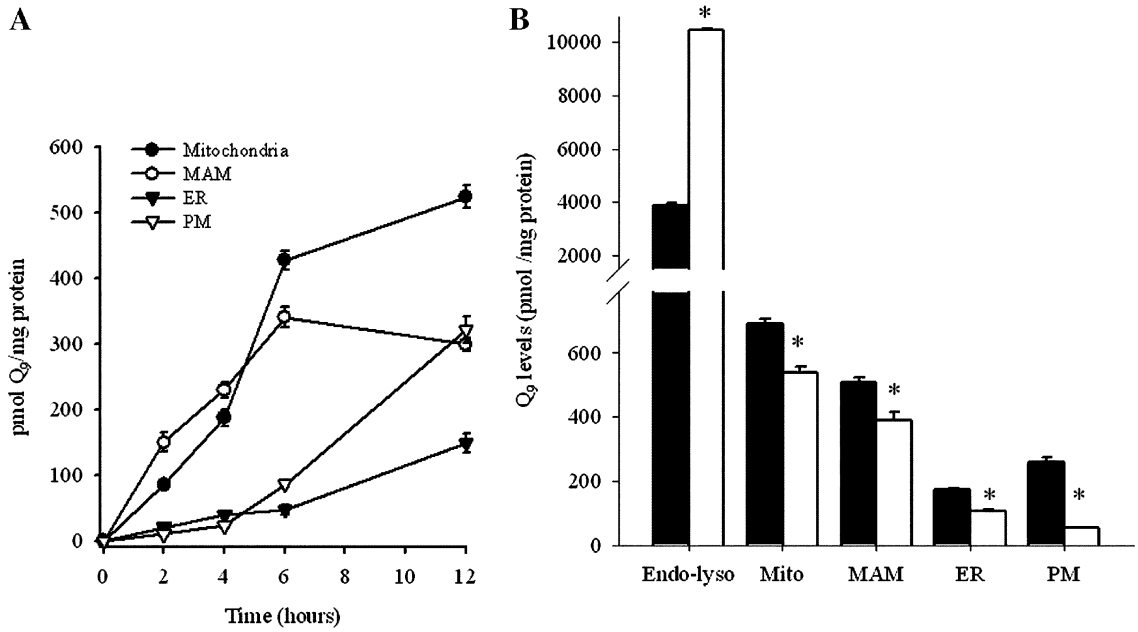

Fig. 5. Incorporation of exogenous Q_9 into HL-60 cell membranes. (A) HL-60 cells were cultured in presence of 2 μM Q_9 during 12 h. Aliquots were taken along indicated times and membranes purified as described in Materials and methods. Lipids were extracted, separated by HPLC and detected by using absorbance at 275 nm and ECD. The incorporation of Q_9 into mitochondria, MAM, ER and PM along time is indicated as pmol Q_9/mg protein. Data are indicated as pmol Q_9/mg protein and represent the mean of three different experiments performed in duplicate. (B) HL-60 cells were cultured for 12 h with 0.1 mg/ml BFA in the presence of 2 μM Q_9. After incubation, cells were recovered and membranes isolated as indicated in Materials and methods. Data represent the Q_9 levels as pmol/mg proteins present in each membrane in both control and BFA treatments. Date represent the mean ± S.D. from three different experiments performed in duplicate. *Significant differences vs. control, $P < 0.05$.

Our results indicate that the last steps of Q synthesis in HL-60 cells occur into mitochondria as measured by the incorporation of [^{14}C]-pHB into Q_{10} and after its biosynthesis it can reach ER and later PM. This apparent transition among membranes was demonstrated by the decrease of incorporation of [^{14}C]-Q_{10} in PM by the disruption of the secretory pathway with BFA, and its accumulation in ER. This drug is a potent compound that specifically inhibits the formation of vesicles involved in the forward movement form ER to Golgi apparatus [48,49]. Thus, the endomembrane system dynamics appear to be involved in the transport of newly synthesized Q_{10} to PM.

However, BFA also seems to produce the inhibition of Q_{10} synthesis in mitochondria when is added at the same time that the Q precursor, [^{14}C]-pHB. The product of the *coq-1* gene involved in the isoprenoid side chain biosynthesis is mainly located in ER [5]. Also, the coq-2 product that catalyses the transfer of side chain to the quinonic precursor [50] is located in mitochondria but also has been found in the Golgi apparatus, ER and peroxisomes [13,15,51–53]. Thus, it is reasonable to think that the disruption of the ER–Golgi apparatus transition would inhibit Q_{10} synthesis by preventing the transport of the precursors of Q synthesis from ER–Golgi to mitochondria. On the other hand, the accumulation of [^{14}C]-Q_{10} in ER found when cells are incubated with BFA after [^{14}C]-Q_{10} was already synthesized can be explained by the disruption of ER–Golgi apparatus transit by BFA, and the accumulation of [^{14}C]-Q_{10}-enriched vesicles [3]. The incorporation of Q_{10} to ER and the secretory pathway from mitochondria seems to occur through MAM, which also rapidly incorporated [^{14}C]-Q_{10}. MAMs are a membrane fraction that exert as bridge between ER and mitochondria facilitating interorganelle transport of lipids by membrane contact [54,55].

On the other hand, in studies carried out in rats, dietary Q is absorbed by the gut and transported to blood and to different tissues although with a different uptake rate by mammalian organs [32,56,57]. However, little is known about the mechanisms of distribution of exogenous Q into cell membranes. The incorporation of exogenous Q_9 in our experiments indicates that it highly accumulates into a very light lyso-endosomal fraction, but significant amounts are also incorporated into MAM, mitochondria and ER, and further in PM. In agreement with these results, it has been indicated that exogenous Q is incorporated by cells but only a small amount is transported up to mitochondria [58], which interacts with the components of the respiratory chain indicating its functional incorporation to the inner mitochondrial membrane [35]. Thus, we have found that exogenous Q_9 is effectively incorporated into mitochondria but also into other cell membranes. Incorporation and distribution of exogenous Q also required the integrity of a functional ER–Golgi apparatus communication because BFA induced a significant higher accumulation in the endo-lysosomal light fraction and a significant decrease in membranes and mitochondria. Taken together, our results indicate that the brefeldin A-sensitive pathway is essential for both endogenous and exogenous Q distribution among cell membranes, especially to PM. It seems that the transit of Q between mitochondria and the ER–Golgi system uses MAMs from which it rapidly can move to or from mitochondria.

It is currently increasing the number of neuromuscular disorders caused by a heritable primary Q deficiency [25,59,60], which partially responds to Q_{10} supplementation. Our results provide new clues to increase the knowledge of the mechanism of Q incorporation and distribution into cell membranes, which would help to understand the efficiency of dietary Q on cell uptake and recovering in processes linked to Q deficiency.

Acknowledgements

This work has been partially supported by the Spanish Dirección General de Investigación, Ciencia y Tecnología grant number BMC2002-01640. Daniel J.M. Fernández-Ayala was a recipient of a PDI fellowship of the Junta de Andalucía.

References

[1] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Clarendon Press, London, 1989.
[2] T. Takahashi, T. Okamoto, K. Mori, H. Sayo, T. Kishi, Distribution of ubiquinone and ubiquinol homologues in rat tissues and subcellular fractions, Lipids 28 (1993) 803–809.
[3] 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.
[4] J. Villalba, G. López-Lluch, C. Santos-Ocaña, J. Rodríguez-Aguilera, P. Navas, Extramitochondrial functions of coenzyme Q, in: V.E. Kagan, P.J. Quinn (Eds.), Coenzyme Q: Molecular Mechanisms in Health and Disease, CRC Press, Boca Raton, USA, 2000, pp. 83–98.
[5] M. Turunen, J. Olsson, G. Dallner, Metabolism and function of coenzyme Q, Biochim. Biophys. Acta (BBA), Biomembr. 1660 (2004) 171–199.
[6] J.M. Villalba, A. Arroyo, F. Navarro, J.C. Rodríguez-Aguilera, P. Navas, Insights in cellular protection against oxidative stress: the necessity for extramitochondrial coenzyme Q, in: M. Ebadi, J. Marwah, R.K. Chopra (Eds.), Mitochondrial Ubiquinone (Coenzyme Q): Biochemical, Functional, Medical and Therapeutic Aspects in Human Health and Diseases, Prominent Press, Scottsdale, USA, 2001, pp. 90–120.
[7] F. Levavasseur, H. Miyadera, J. Sirois, M.L. Tremblay, K. Kita, E. Shoubridge, S. Hekimi, Ubiquinone is necessary for mouse embryonic development but is not essential for mitochondrial respiration, J. Biol. Chem. 276 (2001) 46160–46164.
[8] P.L. Larsen, C.F. Clarke, Extension of life-span in *Caenorhabditis elegans* by a diet lacking coenzyme Q, Science 295 (2002) 120–123.
[9] C. Asencio, J.C. Rodríguez-Aguilera, M. Ruíz-Ferrer, J. Vela, P. Navas, Silencing of ubiquinone biosynthesis genes extends life span in *Caenorhabditis elegans*, FASEB J. 17 (2003) 1135–1137.
[10] N. Ishii, N. Senoo-Matsuda, K. Miyake, K. Yasuda, T. Ishii, P.S. Hartman, S. Furukawa, Coenzyme Q10 can prolong *C. elegans*

lifespan by lowering oxidative stress, Mech. Ageing Dev. 125 (2004) 41–46.
[11] T. Jonassen, C.F. Clarke, Genetic analysis of Coenzyme Q biosynthesis, in: V.E. Kagan, P.J. Quinn (Eds.), Coenzyme Q: Molecular Mechanisms in Health and Disease, CRC Press, Boca Raton, USA, 2000, pp. 185–208.
[12] N. Jiang, F. Levavasseur, B. McCright, E.A. Shoubridge, S. Hekimi, Mouse CLK-1 is imported into mitochondria by an unusual process that requires a leader sequence but no membrane potential, J. Biol. Chem. 276 (2001) 29218–29225.
[13] 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.
[14] W.J. Kovacs, L.M. Olivier, S.K. Krisans, Central role of peroxisomes in isoprenoid biosynthesis, Prog. Lipid Res. 41 (2002) 369–391.
[15] 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.
[16] G.I. Belogrudov, P.T. Lee, T. Jonassen, A.Y. Hsu, P. Gin, C.F. Clarke, Yeast COQ4 encodes a mitochondrial protein required for coenzyme Q synthesis, Arch. Biochem. Biophys. 392 (2001) 48–58.
[17] S.W. Baba, G.I. Belogrudov, J.C. Lee, P.T. Lee, J. Strahan, J.N. Shepherd, C.F. Clarke, Yeast Coq5 C-methyltransferase is required for stability of other polypeptides involved in coenzyme Q biosynthesis, J. Biol. Chem. 279 (2004) 10052–10059.
[18] A. Kalen, E.L. Appelkvist, G. Dallner, Age-related changes in the lipid compositions of rat and human tissues, Lipids 24 (1989) 579–584.
[19] 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 ageing rat, Mech. Ageing Dev. 78 (1995) 173–187.
[20] C.W. Shults, Coenzyme Q10 in neurodegenerative diseases, Curr. Med. Chem. 10 (2003) 1917–1921.
[21] M. Ebadi, P. Govitrapong, S. Sharma, D. Muralikrishnan, S. Shavali, L. Pellett, R. Schafer, C. Albano, J. Eken, Ubiquinone (coenzyme q10) and mitochondria in oxidative stress of Parkinson's disease, Biol. Signals Recept. 10 (2001) 224–253.
[22] M.F. Beal, Coenzyme Q10 as a possible treatment for neurodegenerative diseases, Free Radical Res. 36 (2002) 455–460.
[23] R.J. Ferrante, O.A. Andreassen, A. Dedeoglu, K.L. Ferrante, B.G. Jenkins, S.M. Hersch, M.F. Beal, Therapeutic effects of coenzyme Q10 and remacemide in transgenic mouse models of Huntington's disease, J. Neurosci. 22 (2002) 1592–1599.
[24] C. Lamperti, A. Naini, M. Hirano, D.C. De Vivo, E. Bertini, S. Servidei, M. Valeriani, D. Lynch, B. Banwell, M. Berg, T. Dubrovsky, C. Chiriboga, C. Angelini, E. Pegoraro, S. DiMauro, Cerebellar ataxia and coenzyme Q10 deficiency, Neurology 60 (2003) 1206–1208.
[25] C.M. Quinzii, A.G. Kattah, A. Naini, H.O. Akman, V.K. Mootha, S. DiMauro, M. Hirano, Coenzyme Q deficiency and cerebellar ataxia associated with an aprataxin mutation, Neurology 64 (2005) 539–541.
[26] A. Lass, L. Kwong, R.S. Sohal, Mitochondrial coenzyme Q content and aging, BioFactors 9 (1999) 199–205.
[27] A. Lass, R.S. Sohal, Comparisons of coenzyme Q bound to mitochondrial membrane proteins among different mammalian species, Free Radical Biol. Med. 27 (1999) 220–226.
[28] 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.
[29] C. Gómez-Díaz, M.I. Burón, F.J. Alcaín, R. González-Ojeda, J.A. González-Reyes, R.I. Bello, M.D. Herman, P. Navas, J.M. Villalba, Effect of dietary coenzyme Q and fatty acids on the antioxidant status of rat tissues, Protoplasma 221 (2003) 11–17.
[30] A. Lass, M.J. Forster, R.S. Sohal, Effects of coenzyme Q10 and alpha-tocopherol administration on their tissue levels in the mouse: elevation of mitochondrial alpha-tocopherol by coenzyme Q10, Free Radical Biol. Med. 26 (1999) 1375–1382.
[31] 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.
[32] L.K. Kwong, S. Kamzalov, I. Rebrin, A.-C.V. Bayne, C.K. Jana, P. Morris, M.J. Forster, R.S. Sohal, Effects of coenzyme Q10 administration on its tissue concentrations, mitochondrial oxidant generation, and oxidative stress in the rat, Free Radical Biol. Med. 33 (2002) 627–638.
[33] R.T. Matthews, L. Yang, S. Browne, M. Baik, M.F. Beal, Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 8892–8897.
[34] C. Santos-Ocaña, 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.
[35] D.J.M. Fernández-Ayala, G. López-Lluch, M. Garcia-Valdes, A. Arroyo, P. Navas, Specificity of coenzyme Q10 for a balanced function of respiratory chain and endogenous ubiquinone biosynthesis in human cells, Biochim. Biophys. Acta, Bioenerg. 1706 (2005) 174–183.
[36] M.P. Barroso, C. Gómez-Díaz, J.M. Villalba, M.I. Burón, G. López-Lluch, P. Navas, Plasma membrane ubiquinone controls ceramide production and prevents cell death induced by serum withdrawal, J. Bioenerg. Biomembr. 29 (1997) 259–267.
[37] S.J. Collins, R.C. Gallo, R.E. Gallagher, Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture, Nature 270 (1977) 347–349.
[38] J.E. Vance, Phospholipid synthesis in a membrane fraction associated with mitochondria, J. Biol. Chem. 265 (1990) 7248–7256.
[39] J. Vidugiriene, A.K. Menon, Early lipid intermediates in glycosylphosphatidylinositol anchor assembly are synthesized in the ER and located in the cytoplasmic leaflet of the ER membrane bilayer, J. Cell. Biol. 121 (1993) 987–996.
[40] P. Navas, D.D. Nowack, D.J. Morré, Isolation of purified plasma membranes from cultured cells and hepatomas by two-phase partition and preparative free-flow electrophoresis, Cancer Res. 49 (1989) 2147–2156.
[41] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.
[42] W.W. Poon, B.N. Marbois, K.F. Faull, C.F. Clarke, 3-Hexaprenyl-4-hydroxybenzoic acid forms a predominant intermediate pool in ubiquinone biosynthesis in *Saccharomyces cerevisiae*, Arch. Biochem. Biophys. 320 (1995) 305–314.
[43] Y. Misumi, K. Miki, A. Takatsuki, G. Tamura, Y. Ikehara, Novel blockade by brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes, J. Biol. Chem. 261 (1986) 11398–11403.
[44] M. Bentinger, G. Dallner, T. Chojnacki, E. Swiezewska, Distribution and breakdown of labeled coenzyme Q10 in rat, Free Radical Biol. Med. 34 (2003) 563–575.
[45] 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.
[46] R. De Cabo, R. Cabello, M. Rios, G. López-Lluch, D.K. Ingram, M.A. Lane, P. Navas, Calorie restriction attenuates age-related alterations in the plasma membrane antioxidant system in rat liver, Exp. Gerontol. 39 (2004) 297–304.
[47] 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.
[48] M. Sata, J.G. Donaldson, J. Moss, M. Vaughan, Brefeldin A-inhibited guanine nucleotide-exchange activity of Sec7 domain from yeast Sec7

with yeast and mammalian ADP ribosylation factors, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 4204–4208.
[49] N. Morinaga, R. Adamik, J. Moss, M. Vaughan, Brefeldin A inhibited activity of the sec7 domain of p200, a mammalian guanine nucleotide-exchange protein for ADP-ribosylation factors, J. Biol. Chem. 274 (1999) 17417–17423.
[50] M. Forsgren, A. Attersand, S. Lake, J. Grunler, E. Swiezewska, G. Dallner, I. Climent, Isolation and functional expression of human COQ2, a gene encoding a polyprenyl transferase involved in the synthesis of CoQ, Biochem. J. 382 (2004) 519–526.
[51] 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.
[52] 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.
[53] 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.
[54] G. Achleitner, B. Gaigg, A. Krasser, E. Kainersdorfer, S.D. Kohlwein, A. Perktold, G. Zellnig, G. Daum, Association between the endoplasmic reticulum and mitochondria of yeast facilitates interorganelle transport of phospholipids through membrane contact, Eur. J. Biochem. 264 (1999) 545–553.
[55] M. Wanke, G. Dallner, E. Swiezewska, Subcellular localization of plastoquinone and ubiquinone synthesis in spinach cells, Biochim. Biophys. Acta 1463 (2000) 188–194.
[56] 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.
[57] C. Weber, Dietary intake and absorption of coenzyme Q, Coenzyme Q: molecular mechanisms in health and disease, in: V.E. Kagan, P.J. Quinn (Eds.), Coenzyme Q: Molecular Mechanisms in Health and Disease, CRC Press, Boca Raton, USA, 2000, pp. 209–215.
[58] G. Dallner, P.J. Sindelar, Regulation of ubiquinone metabolism, Free Radical Biol. Med. 29 (2000) 285–294.
[59] C. Sobreira, M. Hirano, S. Shanske, R.K. Keller, R.G. Haller, E. Davidson, F.M. Santorelli, A.F. Miranda, E. Bonilla, D.S. Mojon, A.A. Barreira, M.P. King, S. DiMauro, Mitochondrial encephalomyopathy with coenzyme Q10 deficiency, Neurology 48 (1997) 1238–1243.
[60] A. Rotig, 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.