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QUARTERLY

Review

Ubiquinone. Biosynthesis of quinone ring and its isoprenoid side chain. Intracellular localization✪

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Ubiquinone, known as coenzyme Q, was shown to be the part of the metabolic pathways by Crane *et al.* in 1957. Its function as a component of the mitochondrial respiratory chain is well established. However, ubiquinone has recently attracted increasing attention with regard to its function, in the reduced form, as an antioxidant. In ubiquinone synthesis the *para*-hydroxybenzoate ring (which is the derivative of tyrosine or phenylalanine) is condensed with a hydrophobic polyisoprenoid side chain, whose length varies from 6 to 10 isoprene units depending on the organism. *para*-Hydroxybenzoate (PHB) polyprenyltransferase that catalyzes the condensation of PHB with polyprenyl diphosphate has a broad substrate specificity. Most of the genes encoding (*all-E*)-prenyltransferases which synthesize polyisoprenoid chains, have been cloned. Their structure is either homo- or heterodimeric. Genes that encode prenyltransferases catalysing the transfer of the isoprenoid chain to *para*-hydroxybenzoate were also cloned in bacteria and yeast. To form ubiquinone, prenylated PHB undergoes several modifications such as hydroxylations, O-methylations, methylations and decarboxylation. In eukaryotes ubiquinones were found in the inner mitochondrial membrane and in other membranes such as the endoplasmic reticulum, Golgi vesicles, lysosomes and peroxisomes. Still, the subcellular site of their biosynthesis remains unclear. Considering the diversity of functions of ubiquinones, and their multistep biosynthesis, identification of factors regulating their cellular level remains an elusive task.

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Abbreviations: PHB, *para*-hydroxybenzoate (4-hydroxybenzoate); HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate, GPP, geranyl diphosphate; FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; HexPP, hexaprenyl diphosphate; HepPP, heptaprenyl diphosphate.

Ubiquinone is a component of the mitochondrial respiratory chain [1], participating in electron transport in NADH-coenzyme Q reductase (complex I), succinate coenzyme Q reductase (complex II) and the cytochrome system. Folkers and his group [2] determined the structure of the quinone moiety which was found identical to that described by Morton and his team [3], and suggested the name "ubiquinone" referring to the ubiquitous occurrence of this compound in various tissues. The name ubiquinone (coenzyme Q) was officially accepted in 1975 by the IUPAC-IUB Commission on Biochemical Nomenclature.

The growing interest in ubiquinones is fully justified. In addition to participating in the respiratory chain, they are involved in the redox processes taking place in cytoplasmic as well as Golgi system membranes. In reduced form, as antioxidants, they efficiently protect membrane phospholipids and lipoproteins from lipid peroxidation, as well as membrane proteins and DNA from free radical-induced oxidative damage [4–6]. In plants similar roles are played by plastoquinones [7]. Some vitamins (E, K) are quinone derivatives.

The development of molecular biology and recombinant DNA technologies, the discovery of restriction enzymes and construction of vectors made new approaches possible to investigate ubiquinones. They allowed the identification and cloning of genes encoding enzymes participating in modifications of the ring moiety of the ubiquinone molecule; methyltransferases: *UBIG, UBIE* from bacteria and *COQ3* from yeast; hydroxylases *UBIB, UBIH* from bacteria and *COQ6* from yeast. Genes encoding prenyltransferases catalyzing the transfer of the isoprenoid chain to *para*-hydroxybenzoate in bacteria (*UBIA*) and yeast (*COQ2*) have also been cloned. Practically all the bacterial genes responsible for the synthesis of hexa- up to decaprenyl diphosphate synthases forming the ubiquinone polyisoprenoid side chain have been cloned. These results will allow identification of the respective human genes, characterization of their structure, level and site of expression which is of vital importance considering the functions of ubiquinones.

SYNTHESIS OF QUINONE MOIETY

The benzene moiety is derived mainly from tyrosine (in some cases from phenylalanine) converted to *para*-hydroxybenzoate [8, 9] (Fig. 1) which in turn is condensed with *all-E* polyisoprenoid diphosphate. A number of subsequent modifications of the ring are required for the completion of ubiquinone [10] (Fig. 2).

Modifications of *para*-hydroxybenzoate (4-OH-benzoate) condensed with a polyisoprenoid side chain start with C-hydroxylation, followed by O-methylation and decarboxylation. Two additional C-hydroxylations and one O-methylation are necessary for the final synthesis of ubiquinone. The first data on methylation and O-methylation of the benzene ring come from the elegant chemical work of Olson *et al.* [11]. The above sequence of events has been established in a bacterial system [12]. However, the results obtained by Kang *et al.* [13] suggest that in an animal system decarboxylation may occur prior to the first methylation [13]. Genes *UBIG* and *COQ3* encode an *S*-adenosylmethionine *O*-methyl transferase involved in O-methylation in bacteria and yeast, respectively (Fig. 2) [14, 15]. The *UBIH* gene encodes a mono-oxygenase thought to contain flavin adenine nucleotide that catalyses the conversion of the 6-octaprenyl-2-methoxyphenol to 6-octaprenyl-2-methoxy-1,4-benzoquinone [16]. Recently a novel gene *ohb1* encoding a reversible *para*-hydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum* was cloned and characterized [17]. Its amino-acid sequence shows 57% identity and 74% similarity to that of hypothetical proteins deduced from open reading frames in genomes from bacteria and archaea, suggesting the possible existence of a novel gene family. These genes could have encoded an ancient type of decarboxylase, which

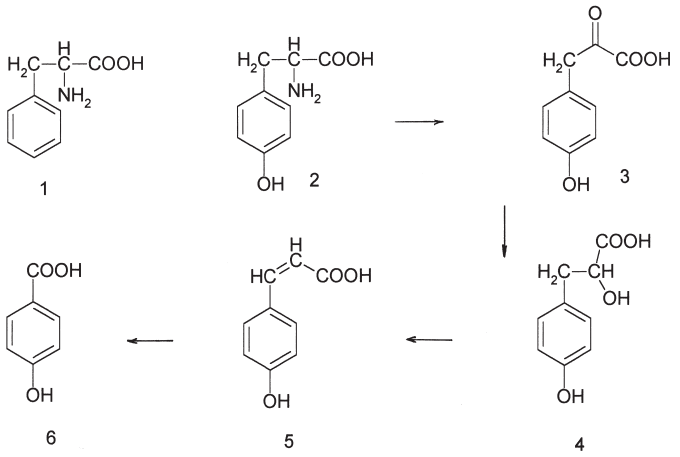

Figure 1. Multistep biosynthesis of *para*-hydroxybenzoate.

1, Phenylalanine or 2, tyrosine amino-acid precursors; 3, *para*-hydroxyphenylpyruvate; 4, *para*-hydroxyphenyllactate; 5, *para*-hydroxycinnamate; 6, *para*-hydroxybenzoate (4-hydroxybenzoate) [50].

was replaced during evolution by a more efficient one [17].

Not all the genes that encode enzymes participating in ubiquinone biosynthesis in higher eukaryotic cells have been cloned. It seems however, that they are similar to the yeast genes. Indeed, a rat cDNA homologue to *UBIG* and *COQ3* has been cloned by functional complementation of a yeast *coq3* mutant with a rat testis cDNA library [18]. In plants the biosynthesis of ubiquinones starting from isopentenyl diphosphate and *para*-hydroxybenzoate also requires a similar set of reactions i.e. hydroxylation, decarboxylation, O-methylation and methylation but neither the proteins nor the corresponding genes have been characterized so far [19].

SYNTHESIS OF THE POLYPRENYL SIDE CHAIN

The polyprenyl (isoprenoid) side chain of ubiquinone is synthesized from acetyl-CoA through a sequence of reaction named the mevalonic acid pathway, leading to the formation of farnesyl diphosphate (FPP) (Fig. 3).

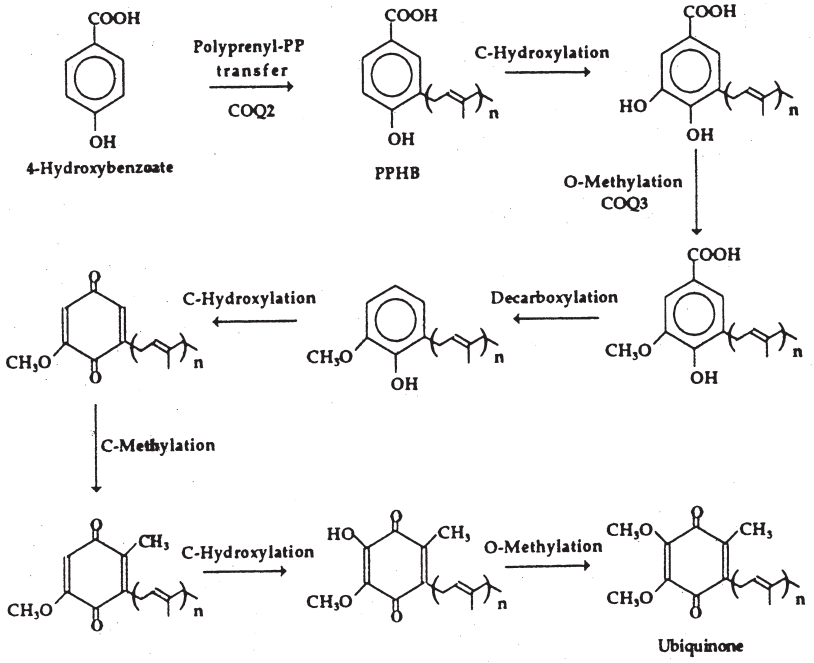

Figure 2. The pathway of ubiquinone biosynthesis from 4-hydroxybenzoate [76].

PPHB, polyprenyl *para*-hydroxybenzoate; *COQ2, Saccharomyces cerevisiae* gene encoding prenyltransferase catalysing the transfer of the polyisoprenoid chain to *para*-hydroxybenzoate; *COQ3, S. cerevisiae* gene encoding an *S*-adenosylmethionine *O*-methyl transferase.

The study of the isoprenoid biosynthesis was brought to an enzymatical level by the discovery made independently by the groups of

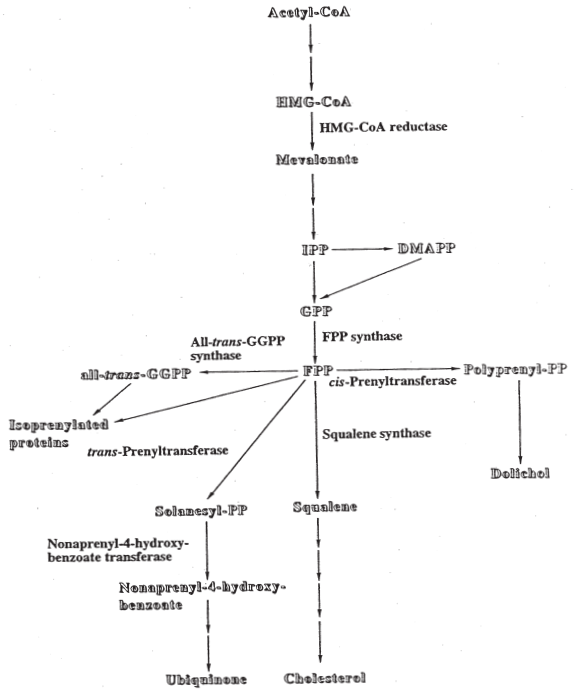

Figure 3. The mevalonic acid pathway.

Lynen and Bloch in 1958 [20, 21] of isopentenyl diphosphate (IPP) as the active isoprene unit.

It is worth mentioning that Rohmer *et al.* [22] discovered a novel biosynthesis pathway of IPP in which mevalonate is not involved. On the basis of labeling patterns of bacterial hopanoids and ubiquinones derived from ^{13}C-labeled metabolites of glycolysis as well as from [^{13}C]acetate, they proved that glyceraldehyde 3-phosphate occupies the branch point of the glycolysis leading to the non-mevalonate pathway synthesizing IPP [22, 23].

The discovery of IPP isomerase, which converts IPP to dimethylallyl diphosphate (DMAPP) [24], as well as the prenyltransferase that catalyses the head-to-tail condensations of IPP with DMAPP and with geranyl diphosphate (GPP), to form (*E,E*)-farnesyl diphosphate (FPP) (Fig. 4) has led to the recognition that a tremendous number of isoprenoid compounds exists in Nature. The chain length of prenyl diphosphates varies ranging from geraniol (C_{10}) to natural rubber whose carbon chain length extends to several millions. To date 16 prenyltransferases (enzymes that catalyze the transfer of prenyl groups to an acceptor which is usually IPP, but may also be an aromatic compound, a protein etc) with different catalytic functions have been characterized.

In contrast to dolichols composed of 14 to 23 isoprene units in yeast and man, the length of the side chain of ubiquinone varies to a much more limited extent. For instance, *Escherichia coli* produces mainly ubiquinones having 8 isoprene units, *Saccharomyces cerevisiae,* 6 isoprene units. Eukaryotic cells contain ubiquinones with 6–10 isoprene residues but in every species only one chain length dominates.

Organisms constitutively contain at least one of the short-chain prenyl diphosphate synthases FPP (geranyl*trans*transferase, EC 2.5.1.10) or GGPP (farnesyl*trans*transferase, EC 2.5.1.30) for the production of prenyl diphosphates which act as the priming substrates for other groups of prenyltransferases.

The short-chain prenyl diphosphates are also biosynthetic precursors of various isoprenoids including steroids, carotenoids and prenylated proteins. It is noteworthy that

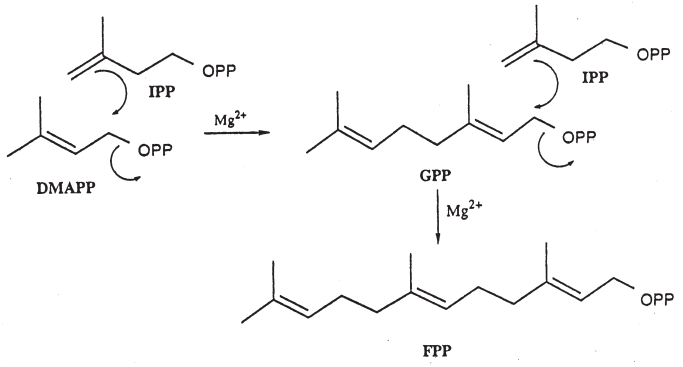

Figure 4. The two-step reaction catalyzed by farnesyl diphosphate (FPP) synthase.

IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate.

GGPP (geranylgeranyl diphosphate) synthase occurs not only in bacteria and plants but also in mammals.

FPP (Fig. 5) and GGPP synthases both have a homodimeric structure in which the subunits are tightly bound to each other [25].

In spite of the fact that all eukaryotic cells probably contain *E*-prenyltransferase activity, to date only a few investigations have been concerned with the characterization of this enzyme in animal cells. On the other hand, bacteria are good sources of prenyltransferases because they produce ubiquinones and menaquinones with polyprenyl side chains of chain lengths varying in a species-specific manner [26].

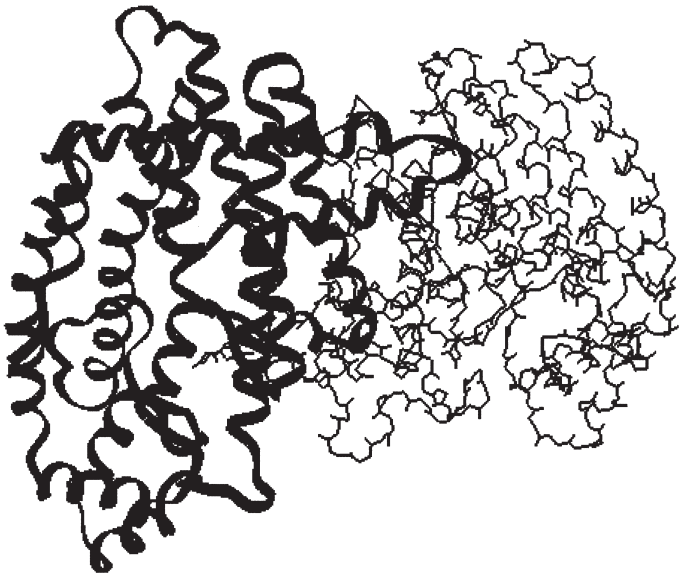

Figure 5. Yeast farnesyl diphosphate synthase homodimer model.

The model was constructed according to the avian crystal structure [25] by D. Płochocka from Bioinformatic Dept. of Inst. Biochem. Biophys. PAS, Warszawa. One monomer is in ribbon representation, the other is shown as a backbone.

Hexaprenyl diphosphate synthases

It has been found that the quinone side chain consisting of 6 isoprene units is synthesized by hexaprenyl diphosphate (HexPP) synthase [EC 2.5.1.30] which has a novel subunit system (Fig. 6). The enzyme catalyzes the synthesis of (*all-E*)-HexPP by adding three molecules of IPP to FPP, but it can not catalyze the steps of synthesis of GPP or FPP from DMAPP and IPP [27], FPP is supplied by FPP synthase which occurs in all bacteria. HexPP synthase has been cloned from *Micrococcus luteus* B-P 26, and sequenced [28]. Within a 2130 bp DNA which expresses HexPP synthase in *E. coli* cells, there are three consecutive open reading frames (ORF1 – 143 aa, ORF2 – 246 aa and ORF3 – 325 aa). It has been established that the two dissociable components A and B of HexPP synthase are products of ORF1 and ORF3, respectively[1]. These genes were similarly located in a newly identified gene cluster participating in menaquinone biosynthesis [30].

Ashby & Edwards [31] have isolated from a plasmid containing a wild-copy genomic DNA fragment a gene (*Coq1*) that is able to complement the yeast mutant in coenzyme Q biosynthesis and restore its HexPP synthase activity. This enzyme which has conserved regions characteristic of prenyltransferases, seems to correspond to the larger protein component of the two heterodimeric components of bacterial HexPP synthase. However, it is not known whether the yeast protein acts as HexPP synthase by itself or in association with another gene product similar to the smaller protein component.

[1]Koike-Takeshita, A., Koyama, T. & Ogura, K. (submitted for publication).

Heptaprenyl diphosphate synthases

Heptaprenyl diphosphate (HepPP) synthase [EC 2.5.1.30] has been found in *Bacillus subtilis* which produces exclusively menaquinone with a side chain of 7 isoprene units [32]. It catalyzes the synthesis of (*all-E*) HepPP by adding four molecules of IPP to FPP but like HexPP synthase it does not synthesize $C_5 \rightarrow C_{10} \rightarrow C_{15}$ compounds.

On the basis of the highly conserved amino-acid sequences of prenyltransferases two genes encoding two protein components of HepPP synthase from *Bacillus stearothermophilus* were identified [33]. One of the proteins constituting an enzyme system for the synthesis of HepPP has 323 amino-acid residues and shows 32% sequence similarity to the FPP synthase from the same bacterium [34]. This protein designated as component II′ has highly conserved regions typical of prenyltransferases. The other protein (component I′) is composed of 220 amino acids and has no such similarity nor has any similar protein been found within the protein entries in data bases. Therefore, it seems likely that component II′ carries the active sites for substrate binding and catalysis, while component I′ plays an auxiliary role.

A protein database search for amino-acid sequences similar to that of component II′ of the HepPP synthase from *B. stearothermophilus* [33] yielded the GerC3 protein of *B. subtilis* which is encoded in a cluster of three ORFs, *gerC1, gerC2* and *gerC3*. Two of the *gerC* products, GerC1 and GerC3, correspond to the two dissociable components I′ and II′ which constitute the HepPP synthase from *B. stearothermophilus* [35].

Octaprenyl diphosphate synthases

Bacterial (*all-E*) octaprenyl diphosphate synthase (forming an 8 isoprene residue polyprenyl chain) has been found and partially purified from *E. coli* [36]. This enzyme is a homodimeric protein that is functionally active by itself. Analysis of genes in the *E. coli* chromosome performed by Choi *et al.* [37] revealed an open-reading frame that showed significant similarity to the *ispA* gene which encodes FPP synthase of *E. coli* [38]. Jeong *et al.* [39] determined the entire sequence of this open reading frame and found a high similarity of the gene product to the HexPP synthase of *S. cerevisiae* [31] and the GGPP synthases of various organisms. Asai *et al.* [40] identified the *ispB* (cel) gene encoding the octaprenyl diphosphate synthase in *E. coli*. The deduced amino-acid sequence of this enzyme also shows the presence of the seven conserved regions typical of prenyltransferases [34].

Okada *et al.* [41] proved that *E. coli* octaprenyl diphosphate synthase (IspB) having a mitochondrial import signal is expressed in *S. cerevisiae*. Yeast cells produced ubiquinone 8 in addition to the originally existing ubiquinone 6. When *IspB* was expressed in a *S. cerevisiae COQ1* defective strain, *IspB* complemented the defect of growth on a non-fermentable carbon source.

Nonaprenyl and decaprenyl diphosphate synthases

(*all-E*) Nonaprenyl (solanesyl) diphosphate synthase (SPP) [EC 2.5.1.11] was isolated from *M. luteus* [42] as an enzyme catalyzing chain elongation from GPP up to C_{45}–PP. The enzyme is a homodimeric protein [43]. However, it requires a protein factor to maintain efficient catalytic turnover. This factor is a high molecular mass component of a soluble fraction of the same bacterium. Possibly it acts by removing from the active site the hydrophobic products which otherwise would inhibit the reaction.

Decaprenyl diphosphate synthase has been found and partially purified from *Paracoccus denitrificans* [44]. It is also a homodimeric protein utilizing GPP as substrate. Suzuki *et al.* [45] and Takahashi *et al.*[2] have cloned the

[2]Takahashi, S., Koyama, T., Nishino, T., (submitted for publication).

gene encoding the decaprenyl diphosphate synthase of the fission yeast *Schizosaccharomyces pombe* and *P. denitrificans,* respectively.

Both nona- and decaprenyl diphosphate synthases are present in plants. They have been characterized in spinach leaves [47]. As does the bacterial enzyme, they utilize GPP as substrate.

In most rat tissues, ubiquinone contains 9 isoprene residues but in brain, spleen and intestine, one-third of the ubiquinone has a decaprenyl side-chain [48–50].

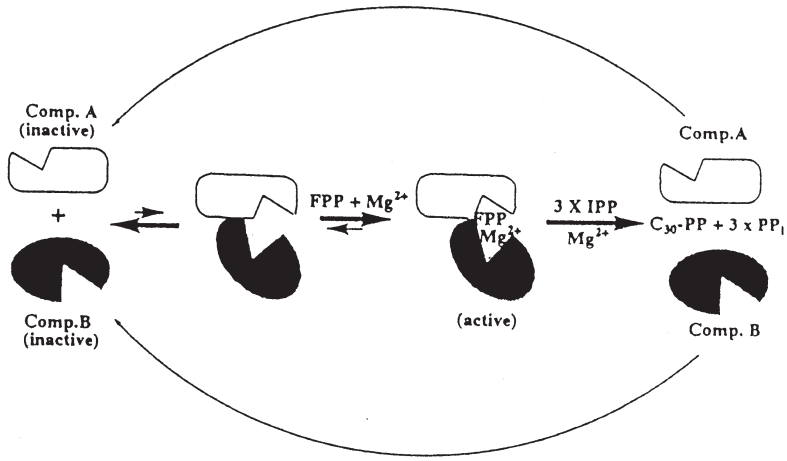

Figure 6. The dynamic formation and dissociation of the ternary complex of the heteromeric components of hexaprenyl diphosphate synthase.

The enzyme catalyzes the synthesis of (*all-E*) hexaprenyl diphosphate by adding three molecules of isopentenyl diphosphate (IPP) to farnesyl diphosphate (FPP) [27].

In all human tissues the predominant side chain of ubiquinones is decaprenol and only small proportion (2–7%) of nonaprenol is present.

TRANSFER OF POLYPRENYL DIPHOSPHATES TO *para*-HYDROXYBENZOATE

The *E. coli* gene *UBIA* encoding prenyltransferase catalysing the transfer of the isoprenoid side chain to *para*-hydroxybenzoate has been isolated [51]. The *UBIA* of *E. coli* and *COQ2* of *S. cerevisiae* genes have been analyzed and cloned [52–54]. Lutke-Brinkhaus *et al.* [55] have described PHB-polyprenyltransferase activity in plant mitochondria. The enzyme has a very broad substrate specificity.

LOCALIZATION OF QUINONE SYNTHESIS

Knowledge about the intracellular localization of the ubiquinone synthesizing enzymes is scant. They are believed to be membrane-bound or membrane associated [56]. It is thought that ubiquinone synthesis in cells of higher eukaryotes reflects the yeast pathway.

In plant cells, the precise localization of this biosynthetic pathway is a matter of controversy. Lutke-Brinkhaus *et al.* [55] and Lutke-Brinkhaus and Kleinig [57] found that plant mitochondria purified from potato tubers catalyze several reactions in the biosynthesis of ubiquinones. In contrast, Świeżewska *et al.* [47] found nonaprenyl-4-hydroxybenzoate and nonaprenyl-2-methylquinol transferase activities in microsomal and Golgi preparations, and proposed therefore, that ubiquinone biosynthesis occurs in the endoplasmic reticulum-Golgi system, followed by a selective transfer of ubiquinone to the mitochondria.

In animal systems the enzymes of the mevalonic acid pathway which supply polyprenyl side chain have a multilocal intracellular distribution. In addition to mitochondria [49] they are present in the endoplasmic reticulum [58], peroxisomes [59], Golgi system, lysosomes, plasma mem-

brane [60] and cytosol [61]. According to Dallner and his group [62] ubiquinone synthesis begins in the endoplasmic reticulum and is completed in the Golgi system, after which ubiquinone becomes distributed to other cellular organelles.

REGULATION OF UBIQUINONE SYNTHESIS

The factors influencing the regulation of ubiquinone cellular level are numerous. In view of the complexity of their function, structure and localization of synthesis, ubiquinones might be the subject of another review article. Only a few general points will be mentioned here.

Ubiquinone levels are regulated through the mevalonate pathway. Moreover, there is an intricate interplay among the three major biosynthetic products of mevalonate metabolism, i.e. ubiquinone, dolichol and sterol (Fig. 3) [63, 64].

In *E. coli* the composition of the ubiquinone pool is highly influenced by the degree of oxygen availability: aerobically grown *E. coli* cells contain significantly more ubiquinone 8. The mechanism of this regulation is not yet shown [65]. In yeast under glucose derepression the level of ubiquinone 6 synthesized increases.

Much less is known about the physiological regulation of ubiquinone level. No data is available concerning plants. In animal systems it has been observed that the ubiquinone level increases upon various forms of oxidative stress (physical exercise, cold adaptation, thyroid hormone treatment) and decreases with age. Relatively little is known about the mechanism involved in biodegradation of ubiquinone. The turnover rate of ubiquinone in various tissues is rather similar, ranging from 50 to 125 h [66]. This is in contrast to cholesterol and dolichol for which the turnover rates are several orders of magnitude higher in the liver than in the brain [67].

The decrease in ubiquinone content with increasing age is consistent with the free radical theory of aging [68], as reflected by an inverse correlation between longevity and peroxide-producing potential in mammalian tissues [69]. This decrease may also account for the age-related increase in the extent of oxidative damage to proteins [70] and DNA [71], the latter especially to mitochondrial DNA [72, 73]. Increase of ubiquinone concentration was found in neurodegenerative conditions in the brain, such as in Alzheimer's disease [74] and prion disease in mice [75]. It is not yet clear whether these diseases are related to an alteration in the bioenergetic capacity and/or in the antioxidant status of the tissues concerned.

REFERENCES

1. Crane, F.L., Hatefi,Y., Lester, R.L. & Widmer, C. (1957) Isolation of a quinone from beef heart mitochondria. *Biochim. Biophys. Acta* **25,** 220–221.

2. Wolf, D.E., Hoffman, C.H., Trenner, N.R., Arison, B.H., Shunk, C.H., Linn, B.O., McPherson, J.F. & Folkers, K. (1958) Coenzyme Q. Structure studies on the coenzyme Q group. *J. Am. Chem. Soc.* **80,** 4752–4758.

3. Festenstein, G.N., Heaton, F.W., Lowe, J.S. & Morton, R.A. (1955) A constituent of the unsaponificable portion of the animal tissue lipids (λ_{max} 272 mμ). *Biochem. J.* **59,** 558–566.

4. Genova, M.L., Bonacorsi, E., D'Aurelio, M., Formiggini, G., Narudo, B., Cuccomarino, S., Turi, P., Pich, M., Lenaz, G. & Bovina, C. (1999) Protective effect of exogenous coenzyme Q in rats subjected to partial hepatic ischemia and reperfusion. *Biofactors* **9,** 345–349

5. Miksovska, J., Schiffer, M., Hanson, D. & Sebban, K. (1999) Proton uptake by bacterial reaction centers: The protein complex re-

sponds in a similar manner to the reduction of either quinone acceptor. *Proc. Natl. Acad. Sci. U.S.A.* **96,** 14348–14353.

6. Gillman, I., Clark, T., N. & Manderville, R.A. (1999) Oxidation of ochratoxin A by an Fe-porphyrin system: Model for enzymatic activation of DNA cleavage. *Chem. Res. Toxicol.* **12,** 1066–1076.

7. Hundal, T., Forsmark, P., Ernster, L. & Andersson, B. (1992) Antioxidant activity of reduced plastoquinone in thylacoid membranes during strong illumination. *EBEC Short Reports* **7,** 6–8.

8. Aiyar, A. & Olson, R. (1964) Biosynthesis of the benzoquinone ring of coenzyme Q_9 in the rat. *Federation Proc.* **23,** 425.

9. Olson, R. (1965) Anabolism of the coenzyme Q family and their biological activities. *Federation Proc.* **24,** 85–92.

10. Parson, W. & Rudney, H. (1965) The biosynthesis of ubiquinone and rhodoquinone from *p*-hydroxybenzoate and *p*-hydroxybenzaldehyde in *Rhodospirillum rubrum. J. Biol. Chem.* **240,** 1855–1863.

11. Olson, R., Dialameh, G. & Bentley, R. (1961) *Ciba Fundation Symposium on Quinones in Electron Transport* (Welstenholme, G. & O'Connor, C., eds.) London.

12. Ramasarma, T. (1985) in *Coenzyme Q – Biochemistry, Bioenergetics and Clinical Applications of Ubiquinone* (Lenaz, G., ed.) pp. 67–81, Wiley, New York.

13. Kang, D., Takeshigate, K., Isobe, R. & Minakami, S. (1991) Evidence that the decarboxylation reaction occurs before the first methylation in ubiquinone biosynthesis in rat liver mitochondria. *Eur. J. Biochem.* **198,** 599–605.

14. Wu, G., Wiliams, H., Zamanian, M., Gibson, F. & Poole, R. (1992) 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,** 2101–2111.

15. Clarke, C., Wiliams, W. & Teruya, J. (1991) Ubiquinone biosynthesis in *Saccharomyces cerevisiae.* Isolation and sequence of coq3, the 3,4–dihydroxy-5-hexaprenylbenzoate methyltransferase gene. *J. Biol. Chem.* **266,** 16366–16644.

16. Nakahigashi, K., Miyamoto, K., Nishimura, K. & Inokuchi, H. (1992) 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,** 7352–7359.

17. Huang, J., He, Z. & Wiegel, J. (1999) Cloning, characterization, and expression of a novel gene encoding a reversible 4-hydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum. J. Bacteriol.* **181,** 5119–5122.

18. Marbois, B., Hsu, A., Pillai, R., Colicelli, J. & Clarke, C. (1994) Cloning of a rat cDNA encoding dihydroxypolyprenylbenzoate methyltransferase by functional complementation of a *Saccharomyces cerevisiae* mutant deficient in ubiquinone biosynthesis. *Gene* **138,** 213–217.

19. Avelange-Macherel, M.-H. & Joyard, J. (1998) Cloning and functional expression of *AtCOQ3,* the Arabidopsis homologue of the yeast *COQ3* gene, encoding a methyltransferase from plant mitochondria involved in ubiquinone biosynthesis. *Plant J.* **14,** 203–213.

20. Lynen, F., Eggerer, H., Henning, U. & Kessel, I. (1958) *Angew. Chem.* **70,** 738–742.

21. Chaykin, S., Law, J., Philips, A., Tchen, T. & Bloch, T. (1958) Phosphorylated intermediates in the synthesis of squalene. *Proc. Natl. Acad. Sci. U.S.A.* **44,** 998–1004.

22. Rohmer, M., Knani, M., Simonin, P., Sutter, B. & Sahm, H. (1993) Isoprenoid biosynthesis in bacteria: A novel pathway for the early

steps leading to isopentenyl diphosphate. *Biochem. J.* **295,** 517–524.

23. Rohmer, M., Seeman, M., Horbach, S., Bringer-Mayer, S. & Sahm, H. (1996) Isopentenol pyrophosphate isomerase. *J. Am. Chem. Soc.* **118,** 2564–2566.

24. Agranoff, B., Eggerer, H., Henning, U. & Lynen, F. (1959) Isopentenol pyrophosphate isomerase. *J. Am. Chem. Soc.* **81,** 1254–1255.

25. Tarshis, L., Proteau, P., Kellogg, B., Sacchettini, J. & Poulter, C. (1996) Regulation of product chain length by isoprenyl diphosphate synthases. *Proc. Natl. Acad. Sci. U.S.A.* **93,** 15018–15023.

26. Jeffries, L., Cawthorn, M., Harris, M., Diplock, A., Green, J. & Price, S. (1967) Distribution of menaquinones in aerobic Micrococcaceae. *Nature* **215,** 257–259.

27. Fujii, H., Koyama, T. & Ogura, K. (1982) Hexaprenyl pyrophosphate synthetase from *Micrococcus luteus B-P 26*. *J. Biol. Chem.* **257,** 14610–14612.

28. Shimizu, N., Koyama, T. & Ogura, K. (1998) Molecular cloning, expression, and characterization of the two genes encoding the two essential protein components of *Micrococcus luteus B-P 26* hexaprenyl diphosphate synthase. *J. Bacteriol.* **180,** 1578–1581.

29. Reference in the text as footnote [1].

30. Koike-Takeshita, A., Koyama, T. & Ogura, K. (1997) Identification of a novel gene cluster participating in menaquinone (Vitamin K_2) biosynthesis. *J. Biol. Chem.* **272,** 12380–12383.

31. Ashby, M. & Edwards, P. (1990) Elucidation of deficiency in two yeast coenzyme Q mutants. *J. Biol. Chem.* **265,** 13157–13164.

32. Takahashi, I., Ogura, K. & Seto, S. (1980) Heptaprenyl pyrophosphate synthetase from *Bacillus subtilis J. Biol. Chem.* **255,** 4539–4543.

33. Koike-Takeshita, A., Koyama, T., Obata, S. & Ogura, K. (1995) Molecular cloning and nucleotide sequence of the genes for two essential proteins constituting a novel enzyme for heptaprenyl diphosphate synthesis. *J. Biol. Chem.* **270,** 18396–18400.

34. Koyama, T., Obata, S., Osabe, M., Takeshita, A., Yokoyama, K., Uchida, M., Nishino, T. & Ogura, K. (1993) Thermostable farnesyl diphosphate synthase of *Bacillus stearothermophilus*: Molecular cloning, sequence determination, overproduction, and purification. *J. Biochem.* **113,** 355–363.

35. Zang, Y.-W., Koyama, T. & Ogura, K. (1997) Two cistrons of the *gerC* operon of *Bacillus subtilis* encode the two subunits of heptaprenyl diphosphate synthase. *J. Bacteriol.* **179,** 1417–1419.

36. Fujisaki, S., Nishino, T. & Katsuki, H. (1986) Isoprenoid synthesis in *Escherichia coli*. Separation and partial purification of four enzymes involved in the synthesis. *J. Biochem.* **99,** 1327–1337.

37. Choi, Y., Nishida, T., Kawamukai, M., Utsumi, R., Sakai, H. & Kamono, T. (1989) Cloning and sequencing of an *Escherichia coli* gene nlp, highly homologous to the ner genes of bacteriophages Mu and D108. *J. Bacteriol.* **171,** 5222–5225.

38. Fujisaki, S., Hara, H., Nishimura, Y., Horiuchi, K. & Nishino, T. (1990) Cloning and nucleotide sequence of the ispA gene responsible for farnesyl diphosphate synthase activity in *Escherichia coli. J. Biochem.* **108,** 995–1000.

39. Jeong, J., Kitakawa, M. & Isono, K. (1993) Cloning and nucleotide sequencing of the genes, *rpIU* and *rpmA*, for ribosomal proteins L21 and L27 of *Escherichia coli*. *DNA Sequence* **4,** 59–67.

40. Asai, K., Fujisaki, S., Nishimura, Y., Nishino, T., Okada, K., Nakagawa, T., Kawamukai, M. & Matsuda, H. (1994) The identification of *Escherichia coli ispB* (cel) gene encoding the

octaprenyl diphosphate synthase. *Biochem. Biophys. Res. Commun.* **202,** 340–345.

41. Okada, K., Suzuki, K., Kamiya, Y., Zhu, X., Fujisaki, S., Nishimura, Y., Nishino, T., Nakagawa, T., Kawamukai, M. & Matsuda, H. (1996) Polyprenyl diphosphate synthase essentially defines the length of the side chain of ubiquinone. *Biochim. Biophys. Acta* **1302,** 217–223.

42. Sagami, H., Ogura, K. & Seto, S. (1977) Solanesyl pyrophosphate synthase from *Micrococcus lysodeikticus*. *Biochemistry* **16,** 4616–4622.

43. Ohnuma, S., Koyama, T. & Ogura, K. (1991) Purification of solanesyl-diphosphate synthase from *Micrococcus luteus*. *J. Biol. Chem.* **266,** 23706–23713.

44. Ishi, K., Sagami, H. & Ogura, K. (1985) Decaprenyl pyrophosphate synthetase from *Paracoccus denitrificans*. *Biochim. Biophys. Acta* **835,** 291–297.

45. Suzuki, K., Okada, K., Kamiya, Y., Zhu, X., Nakagawa, T., Kawamukai, M. & Matsuda, H. (1997) Analysis of the decaprenyl diphosphate synthase (dps) gene in fission yeast suggests a role of ubiquinone as an antioxidant. *J. Biochem.* **121,** 496–505.

46. Reference in the text as footnote [2].

47. Świeżewska, E., Dallner, G., Andersson, B. & Ernster, L. (1993) Biosynthesis of ubiquinone and plastoquinone in the endoplasmic reticulum-Golgi membranes of spinach leaves. *J. Biol. Chem.* **268,** 1494–1499.

48. Teclebrahn, H., Olsson, J., Swiezewska, E. & Dallner, G. (1993) Biosynthesis of the side chain of ubiquinone: *Trans*-prenyltransferase in rat liver microsomes. *J. Biol. Chem.* **268,** 23081–23086.

49. Runquist, M., Ericsson, J., Thelin, A., Chojnacki, T. & Dallner, G. (1994) Isoprenoid biosynthesis in rat liver mitochondria. Studies on farnesyl pyrophosphate synthase and *trans*-prenyltransferase. *J. Biol. Chem.* **269,** 5804–5809.

50. Drabikowska, A. (1969) Biosynthesis of ubiquinone in animal tissues. *Post. Biochem.* **15,** 397–410 (in Polish).

51. Wu, G., Wiliams, H., Gibson, F. & Poole, K. (1993) Mutants of *Escherichia coli* affected in respiration: The cloning and nucleotide sequence of *ubiA*, encoding the membrane-bound *p*-hydroxybenzoate : octaprenyltransferase. *J. Gen. Microbiol.* **139,** 1795–1805.

52. Ashby, M., Kutsunai, S., Ackerman, S., Tzagoloff, A. & Edwards, P. (1992) COQ2 is a candidate for the structural gene encoding *para*-hydroxybenzoate : polyprenyltransferase. *J. Biol. Chem.* **267,** 4128–4136.

53. Siebert, M., Bechthold, A., Melzer, M., May, U., Berger, U., Schroder, G., Schroder, J., Severin, K. & Heide, L. (1992) Cloning of the genes coding for chorismate pyruvate-lyase and 4-hydroxybenzoate octaprenyl transferase from *Escherichia coli*. *FEBS Lett.* **307,** 347–350.

54. Melzer, M. & Heide, L. (1994) Characterization of polyprenyldiphosphate : 4-hydroxybenzoate polyprenyltransferase from *Escherichia coli*. *Biochim. Biophys. Acta* **1212,** 93–102.

55. Lutke-Brinkhaus, F., Liedvogel, B. & Kleinig, H. (1984) On the biosynthesis of ubiquinones in plant mitochondria. *Eur. J. Biochem.* **141,** 537–541.

56. Leppik, R., Stroobant, P., Shineberg, B., Young, I. & Gibson, F. (1976) Membrane-associated reactions in ubiquinone biosynthesis. *Biochim. Biophys. Acta* **428,** 146–156.

57. Lutke-Brinkhaus, F. & Kleinig, H. (1987) Ubiquinone biosynthesis in plant mitochondria. *Methods Enzymol.* **148,** 486–490.

58. Ericsson, J., Runquist, M., Thelin, A., Anderson, M., Chojnacki, T. & Dallner (1993) Distri-

bution of prenyltransferases in rat tissues. *J. Biol. Chem.* **268,** 832–838.

59. Aboushadi, N. & Krisans, S. (1998) Analysis of isoprenoid biosynthesis in peroxisomal-deficient Pex2 CHO cell lines. *J. Lipid Res.* **39,** 1781–1791.

60. Kalen, A., Norling, B., Appelkvist, E.-L. & Dallner, G. (1987) Ubiquinone biosynthesis by the microsomal fraction from rat liver. *Biochim. Biophys. Acta* **926,** 70–78.

61. Engfelt, W., Shackelford, E., Aboushadi, N., Jessani, N., Masuda, K., Paton, V., Keller, G. & Krisans, K. (1997) The characterization of UT-2 cells. The induction of peroxisomal 3-hydroxy-3-methylglutaryl coenzyme A. *J. Biol. Chem.* **272,** 24579–24587.

62. Elmberger, G., Kalen, A., Brunk, U. & Dallner, G. (1989) Discharge of newly synthesized dolichol and ubiquinone with lipoproteins to rat liver perfusate and to the bile. *Lipids* **24,** 919–930.

63. Szkopińska, A., Świeżewska, E. & Karst, F. (2000) The regulation of activity of main mevalonic acid pathway enzymes: Farnesyl diphosphate synthase, 3-hydroxy-3-methylglutaryl-CoA reductase and squalene synthase in yeast *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* **267,** 473–477.

64. Grabowska, D., Karst, F. & Szkopińska, A. (1998) Effect of squalene synthase gene disruption on synthesis of polyprenols in *Saccharomyces cerevisiae*. *FEBS Lett.* **434,** 406–408.

65. Meganathan, A. (1996) Biosynthesis of the isoprenoid quinones, menaquinone (vitamin K_2) and ubiquinone (coenzymeQ); in *Escherichia coli and Salmonella: Cell. Mol. Biol.* (Neidhardt F.C., *et al.)* pp. 642–656, American Society for Microbiology, Washington.

66. Thelin, A., Schedin, S. & Dallner, G. (1992) Half-life of ubiquinone-9 in rat tissues. *FEBS Lett.* **313,** 118–120.

67. Andersson, M., Elmberger, P., Edlund, C. Kristensson, K. & Dallner, G. (1990) Rates of cholesterol, ubiquinone, dolichol, and dolichyl-P biosynthesis in rat brain slices. *FEBS Lett.* **269,** 15–18.

68. Harman, D. (1988) Free radicals in aging. *Mol. Cell. Biochem.* **84,** 55–61.

69. Cutler, R. (1985) Peroxide-producing potential of tissues: Inverse correlation with longevity of mammalian species. *Proc. Natl. Acad. Sci. U.S.A.* **82,** 4798–4802.

70. Stadtman, E. & Oliver, C. (1991) Metal-catalyzed oxidation of proteins. Physiological consequences. *J. Biol. Chem.* **266,** 2005–2008.

71. Linnane, A., Marzuki, S., Ozawa, T. & Tanaka, M. (1989) Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases. *Lancet* **25,** 642–645.

72. Ozawa, T. (1997) Genetic and functional changes in mitochondria associated with aging. *Physiol. Rev.* **77,** 425–464.

73. Lenaz, G., Cavazzoni, M., Genova, M., D'Aurelio, M., Merlo, M., Palloti, F., Formiggini, G., Marchetti, M., Parenti, G. & Bovina, C. (1998) Oxidative stress, antioxidant defences in aging. *BioFactors* **8,** 195–204.

74. Soderberg, M., Edlund, K., Kristensson, K. & Dallner, G. (1990) Lipid compositions of different regions of the human brain during aging. *J. Neurochem.* **54,** 415–423.

75. Ericsson, J. & Dallner, G. (1993) in *Subcellular Biochemistry* (Borgese, N. & Harris, J., eds.) vol. 21, pp. 229–272, Plenum, New York.

76. Grunler, J., Ericsson, J. & Dallner, G. (1994) Branch-point reactions in the biosynthesis of cholesterol, dolichol, ubiquinone and prenylated proteins. *Biochim. Biophys. Acta* **1212,** 259–277.