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REVIEW ARTICLE

# Microbial ubiquinones: multiple roles in respiration, gene regulation and oxidative stress management

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## Overview

Spectacular advances have been made in recent years in our understanding of the structure and function of the membrane-bound protein complexes in respiratory and photosynthetic electron-transport chains. Perhaps as a result of this focus of attention, there is a temptation to regard quinones as a passive pool of redox carriers whose only function is to shuttle reducing equivalents between more 'interesting' protein complexes. However, it is now clear that quinones play additional vital roles in management of oxidative stress and, apparently, gene regulation. The purpose of this review is to give an overview of the different roles quinones fulfil in the prokaryotic cell with emphasis on *Escherichia coli*, although recent advances in yeast are also covered. The ubiquinone biosynthetic pathway has now been elucidated in both prokaryotes and lower eukaryotes, and a comparison of the numerous enzymes involved in these pathways is included. The literature cited is necessarily selective, with an emphasis on recent work; reviews have been used to cover early work.

## Quinone structure and distribution

Quinones are widely distributed in nature. They are best known as lipid-soluble components of membrane-bound electron-transport chains, but, in animal cells, ubiquinone is found not only in the inner mitochondrial membrane but also in endoplasmic reticulum, the Golgi apparatus, lysosomes, peroxisomes and the plasma membrane. This distribution strongly suggests roles other than in respiratory electron transport.

Bacterial respiratory quinones can be divided into two groups. The first, subject of this review, comprises a benzoquinone termed ubiquinone or coenzyme Q. Both names are recognized by the IUPAC Commission for Nomenclature, 'coenzyme Q' (CoQ) being more widely used in biomedical and clinical research. The abbreviation for ubiquinone used here is either UQ or UQ-*n*, where *n* refers to the number of isoprenoid units in the side chain. The second group contains the naphthoquinones menaquinone (vitamin $K_2$; MK or MK-*n*) and demethylmenaquinone (DMK or DMK-*n*). Animal cells synthesize only UQ, but MK is obtained from the diet. In prokaryotes, MK plays an additional role in the anaerobic biosynthesis of pyrimidines (Gibson & Cox, 1973). The quinone structure has isoprenoid side chains of various length depending on the species. Humans and tobacco plants have quinones with 10-unit isoprenoid side chains, whereas rodents, *E. coli* and yeast (*Saccharomyces cerevisiae*) mainly have 9-, 8- and 6-unit side-chains, respectively. The chemical structures of UQ-8 and MK-8 are shown in Fig. 1.

Isoprenoid quinones have been used as taxonomic tools due to the wide structural variation within different taxonomic groups and the relative ease with which they can be isolated and characterized (for a review, see Collins & Jones, 1981). Most Gram-positive bacteria and anaerobic Gram-negative bacteria contain only MK, whereas the majority of strictly aerobic Gram-negative bacteria contain exclusively UQ. Both types of isoprenoid quinones are found only in facultatively anaerobic Gram-negative bacteria. In *E. coli*, UQ-8, MK-8 and DMK-8 are the major quinones found, but smaller amounts of UQ-1 to UQ-7, MK-6, MK-7, MK-9 and DMK-7 have also been reported (Collins & Jones, 1981).

Bacterial quinones sometimes contain a varying degree of saturated or hydrogenated polyprenyl side-chains: archaea contain mainly MK, which is often dihydrogenated. Other forms of quinones, such as chlorobiumquinone, α-tocopherolquinone, rhodoquinone, epoxyubiquinone and sulphur-containing caldariellaquinone, have also been detected in various bacteria (Collins & Jones, 1981).

Phylloquinone (vitamin $K_1$), a naphthoquinone with a largely saturated side chain (Fig. 1), is found mainly in

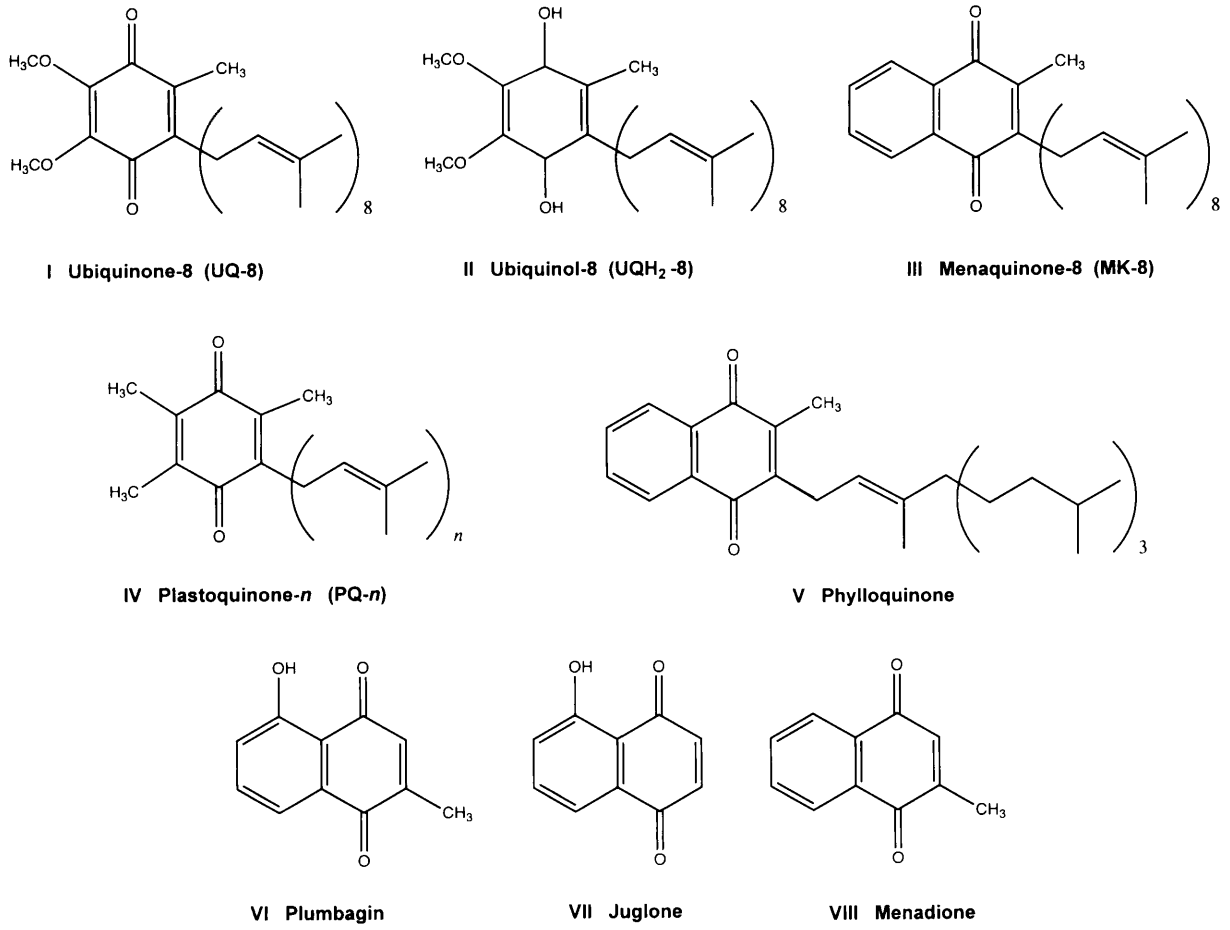


***Fig. 1.*** Chemical structures of quinones.

green plants and plays an important function in blood coagulation. Plastoquinone (PQ-*n*; Fig. 1) is a benzoquinone, which acts as a primary electron carrier in the photosynthetic tissues of higher plants as well as in cyanobacteria. Plumbagin and juglone are naphthoquinones, structural analogues of MK, which are excreted by plants of the *Plumbaginaceae* and walnut trees, respectively, to poison competitors. The lack of an isoprenoid side chain increases their aqueous solubility and they impose severe oxidative stress upon the cell by intercepting electrons from membrane-bound electron carriers and transferring them to molecular oxygen to produce superoxide. Plumbagin and juglone are commonly used to induce oxidative stress or as respiratory inhibitors in experimental studies. Reduced plumbagin is suitable as a substrate for *E. coli* anaerobic quinol oxidoreductases (Rothery *et al.*, 1998). Menadione (Fig. 1) is a commercially available synthetic analogue of MK, which is also often used as an electron donor in studies with quinone-dependent oxidoreductases, a redox mediator, or as a superoxide-generating agent.

## Chemical and physical properties of UQ

UQ was discovered independently by Morton in Liverpool, UK and Crane in the USA (reviewed by Morton, 1961; Crane, 1961); the latter proposed a role for UQ as a respiratory hydrogen (i.e. proton plus electron) carrier between NADH and succinate dehydrogenases and the cytochrome system. Reduction and oxidation of UQ involve two-electron transfers at the quinone nucleus associated with the addition or release of two single $H^+$ to form ubiquinol ($UQH_2$) and UQ, respectively (Fig. 1). These reactions are important for both linear electron transfer and transmembranous $H^+$ translocation. Removal or transfer of a single electron and $H^+$ gives the ubisemiquinone radical ($UQ^{\bullet -}$). This radical may be stabilized when bound to a protein enabling UQ to act as a transformer between one-electron and two-electron processes in respiratory chains. A highly stabilized $UQ^{\bullet -}$ is found associated with the $UQH_2$ oxidase, cytochrome *bo′* in *E. coli* (Ingledew *et al.*, 1995).

An important property of UQ is its hydrophobicity, which allows free movement between partner reductants and oxidants in the membrane. Lower homologues of UQ (e.g. UQ-1) have been widely used for biochemical assays *in vitro* in place of very lipophilic UQs with long side chains (e.g. Søballe & Poole, 1998). A broad spectrum of UQs (UQ-5 to UQ-10) have been shown to be biologically functional in a UQ-deficient strain of *S.*

*cerevisiae*, but the original UQ-6 species showed the highest activity (Okada *et al.*, 1998a).

## Biosynthesis of UQ

Key studies of the UQ biosynthetic pathway in *E. coli* were initiated by Gibson, Cox, Young and co-workers and as early as 1964 they provided the first evidence that UQ is derived from the shikimate pathway. Most of the information on UQ biosynthesis in *E. coli* has come from identification of intermediates accumulated by UQ-deficient mutant (*ubi*) strains (reviewed by Gibson, 1973; Gibson & Young, 1978; Cox & Downie, 1979); the only intermediate not purified in these studies was 2-polyprenyl-6-hydroxyphenol (Fig. 2). The fruit of these studies is a detailed description of the UQ biosynthetic pathway in *E. coli*, which has been reviewed in detail by Meganathan (1996). An overview of the pathway, including newer discoveries and insights, is described here.

The UQ biosynthetic enzymes may constitute a complex that is tightly bound to the membrane (Knoell, 1979). In the biosythetic pathway (Fig. 2) the nucleus is derived from chorismate (for a fascinating personal account, see Gibson, 1999), whereas the prenyl side chain is derived from prenyl diphosphate (prenyl $PP_i$) and the methyl groups are derived from *S*-adenosylmethionine. The biosynthesis of UQ under aerobic conditions requires oxygen, flavin-linked mono-oxygenases and NADH, whereas anaerobic UQ biosynthesis utilizes alternative hydroxylases and oxygen atoms derived from water (Alexander & Young, 1978; Meganathan, 1996). However, the reaction mechanisms of the anaerobic hydroxylases are unknown (Alexander & Young, 1978).

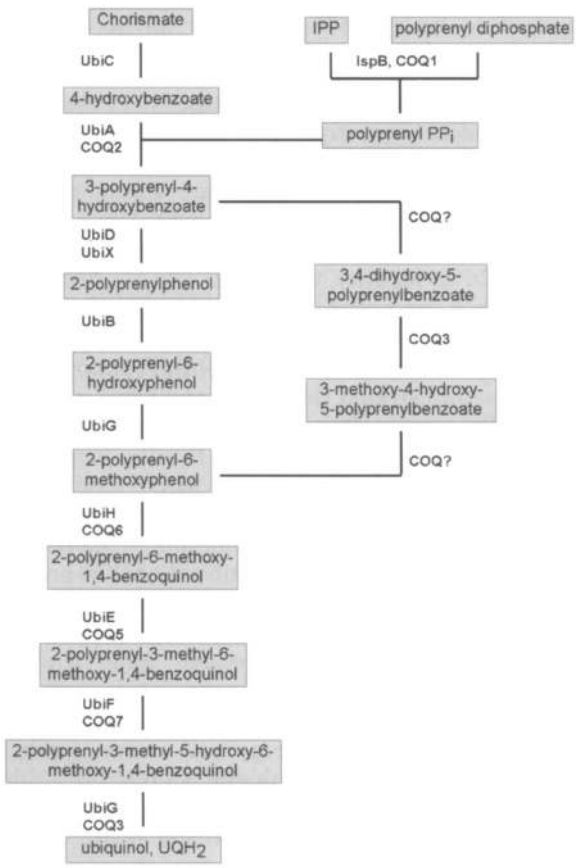


***Fig. 2.*** Pathway of UQ biosynthesis in *E. coli* (Ubi enzymes) and *S. cerevisiae* (COQ enzymes). The pathway is thought to diverge after the synthesis of 3-polyprenyl-4-hydroxybenzoate but converge from 2-polyprenyl-6-methoxyphenol to $UQH_2$. The polyprenyl side chain is octaprenyl in *E. coli* and hexaprenyl in *S. cerevisiae*.

The first defined step in UQ biosynthesis is catalysed by the *ubiC*-encoded enzyme chorismate lyase, which is the only soluble enzyme in the pathway. The side-chain precursor, polyprenyl $PP_i$, is derived by sequential condensation of isopentenyl diphosphate (IPP) with polyprenyl $PP_i$, a reaction catalysed by polyprenyl diphosphate synthase (Grünler *et al.*, 1994; Okada *et al.*, 1997). The length of the isoprenoid side chain of UQ is determined by the specific polyprenyl diphosphate synthase present, and not the specificity of the *p*-hydroxybenzoate polyprenyltransferase (UbiA) (Okada *et al.*, 1996a). In *E. coli*, the octaprenyl diphosphate synthase is encoded by the *ispB* gene (Fig. 2), which is essential for normal growth, as an *ispB* knockout mutant failed to grow unless the *ispB* gene or its homologue was supplied on a plasmid (Okada *et al.*, 1997).

UbiA in *E. coli* catalyses the prenylation of 4-hydroxybenzoate. Smaller amounts of UQs with various side chains are formed in *E. coli* because the prenyl transferase is relatively non-specific. The prenyl transferase incorporates geranyl-, farnesyl- and solanesyl $PP_i$ into 4-hydroxybenzoate (Melzer & Heide, 1994). The UbiA homologue COQ2 from *S. cerevisiae* also has limited substrate specificity; the *COQ2* gene has been expressed in an *E. coli ubiA* mutant and shown to result in increased production of UQ-8 (Suzuki *et al.*, 1994).

Formation of 2-polyprenylphenol is catalysed by the decarboxylase encoded by the *ubiD* gene. In *Salmonella typhimurium*, an alternative decarboxylase, encoded by *ubiX*, carries out this reaction (Howlett & Bar-tana, 1980). In *E. coli*, a homologous *ubiX* sequence has been found (Nonet *et al.*, 1987), which may encode decarboxylase activity in a *ubiD* mutant (Cox *et al.*, 1969).

The hydroxylation reactions are catalysed by the products of *ubiB*, *ubiH* and *ubiF* (Fig. 2). Mutants blocked in any of the hydroxylation reactions are capable of producing UQ under anaerobic conditions, due to the presence of three alternative hydroxylases (Alexander & Young, 1978). Strains grown under anaerobic conditions accumulate high amounts of 2-octaprenylphenol compared to aerobically grown cells. The biosynthetic apparatus charged with a pool of 2-octaprenylphenol is kept in a stand-by position in the anaerobic cell and effectively turned on upon availability of oxygen (Knoell, 1981).

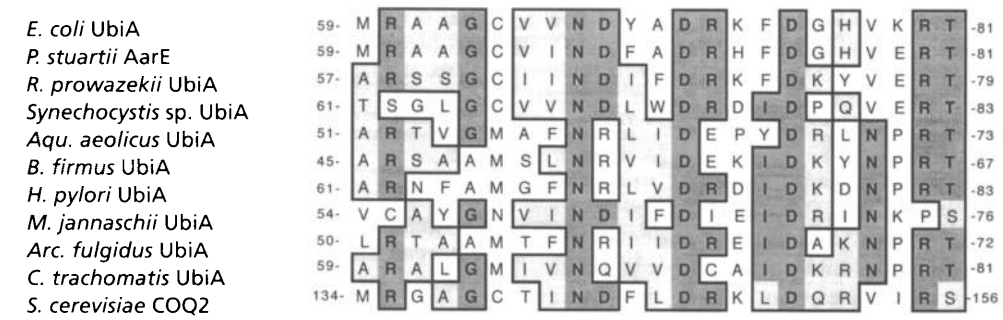


***Fig. 3.*** Alignment of the proposed binding site for allylic polyprenyl diphosphate of UbiA sequences encoding 4-hydroxybenzoate polyprenyltransferase. The *P. stuartii* AarE protein and *S. cerevisiae* COQ2 protein are proven homologues of *E. coli* UbiA (see text), whereas the remaining sequences are putative UbiA proteins. The amino acid numbers of the various sequences are indicated.

The methylation reactions, which alternate with the hydroxylation reactions, are catalysed by methyltransferases encoded by *ubiG* and *ubiE*, respectively. The UbiG methyltransferase consists of a homodimer (Wu *et al.*, 1992). The *ubiE* gene is also required for MK biosynthesis. After formation of chorismate, the MK biosynthetic pathway is independent of the UQ pathway (Meganathan, 1996), with the exception of the *C*-methylation step (Wissenbach *et al.*, 1992). In both systems, UbiE catalyses the insertion of a methyl group to a 1,4-quinoid ring system at position C-3, and both reactions occur at the membrane using *S*-adenosylmethionine as the methyl donor.

### *Mapping and identification of ubi genes*

In *E. coli*, the UQ biosynthetic genes (*ubi*) were mapped by Gibson and co-workers using interrupted-mating experiments and found to be scattered around the chromosomal map (Gibson, 1973). On the current linkage map, the *ubiB* (at 4024·1 kb), *ubiD* (not identified) and *ubiE* (at 4016·4 kb) genes form a cluster at about 86 min (Daniels *et al.*, 1992; Lee *et al.*, 1997), while *ubiF* (not identified), *ubiG*, *ubiH* and *ubiX* have been positioned at 15, 48 (2337·6 kb), 63 (3051·5 kb) and 50 min (2426·6 kb), respectively. The *ubiC* and *ubiA* genes are organized in a transcriptional unit (Wu *et al.*, 1993) at 91·5 min (4250·0 kb) on the *E. coli* chromosome (Heide *et al.*, 1993). The *ispB* gene is located at 69 min (3331·4 kb) on the linkage map (Asai *et al.*, 1994).

The *ubiCA* operon was identified simultaneously by several groups (Nichols & Green, 1992; Siebert *et al.*, 1992; Wu *et al.*, 1992). The UbiA protein, the polyprenyl transferase, is especially interesting as it is thought to catalyse a rate-limiting step in UQ biosynthesis, which determines the nature of UQ produced. In attempts to overproduce UQ by genetic engineering (Zhu *et al.*, 1995; see below), the expression of *ubiCA* and *ispB* proved to be of particular importance. The hydrophobicity profile of the *E. coli* UbiA polypeptide is characteristic of a membrane protein with five membrane-spanning hydrophobic regions (Wu *et al.*, 1993). *ubiA* sequences have been predicted in various strains of bacteria from throughout the prokaryotic kingdom. Fig. 3 is an alignment of predicted and identified UbiA amino acid sequences and shows the highly conserved aspartate-rich region, which has been proposed to be a binding site for allylic polyprenyl diphosphates (Ashby *et al.*, 1992).

The alignment contains UbiA predicted in the archaea *Methanococcus jannaschii* and the extreme halophile *Archaeoglobus fulgidus* (21% and 23% identity to *E. coli* UbiA, respectively), which are thought only to contain naphthoquinones and not UQ. UbiA was also predicted in the hyperthermophilic bacterium *Aquifex aeolicus* (26% identity to *E. coli* UbiA). *Providencia stuartii* UbiA homologue AarE shows a remarkable 68% identity to UbiA. Paradise *et al.* (1998) performed complementation experiments in both *P. stuartii* and *E. coli*, and demonstrated that AarE and UbiA are indeed functionally equivalent. Interestingly, predictions showed UbiA in the Gram-positive *Bacillus firmus* (21% identity to *E. coli* UbiA), but only MK-7 (and not UQ) was identified in this strain (Collins & Jones, 1981). Likewise, *Helicobacter pylori* contains predicted UbiA (22% identity to *E. coli* UbiA) but only MK-6 and MK-4 are found in this organism (Marcelli *et al.*, 1996). UbiA sequences are also predicted in the obligate intracellular parasites *Chlamydia trachomatis* and *Rickettsia prowazekii* (20% and 35% identity to *E. coli* UbiA, respectively), which apparently lack mechanisms for production of metabolic energy and cannot synthesize ATP. The *S. cerevisiae* homologue, COQ2 (32% identity to *E. coli* UbiA), contains an additional N-terminal mitochondrial leader sequence (Ashby *et al.*, 1992). In addition, it can be mentioned that the amino acid sequence of *E. coli* UbiA shows regions of similarity (overall 18·2% identity and 58·8% similarity; Nichols & Green, 1992) to farnesyltransferase, the product of the *cyoE* gene, in the *cyo* operon that encodes cytochrome *bo'* (Chepuri *et al.*, 1990). In particular, the putative allylic polyprenyl diphosphate binding domain

present in UbiA and COQ2 is conserved in a cytoplasmic loop of CyoE (Saiki *et al.*, 1992).

A hydrophobicity-profile analysis of the predicted UbiC polypeptide revealed a soluble protein. An ATP-binding motif was identified in the UbiC amino acid sequence (Wu *et al.*, 1993), but chorismate lyase activity does not appear to require ATP *in vitro* (Nichols & Green, 1992). *ubiB* was first discovered as a gene encoding flavin reductase (Spyrou *et al.*, 1991) and later identified (Daniels *et al.*, 1992). The UbiG (Wu *et al.*, 1992) and UbiE (Lee *et al.*, 1997) amino acid sequences both contain an *S*-adenosylmethionine-binding motif which is shared by some, but not all, methyltransferases. UbiE sequences have been predicted in *C. trachomatis*, *Mycobacterium tuberculosis*, *Cryptosporidium parvum*, *H. pylori*, *R. prowazekii*, *Arc. fulgidus* and a homologue has been identified in *S. cerevisiae* (COQ5). UbiG catalyses both O-methylation steps in the *E. coli* UQ pathway (Hsu *et al.*, 1996). UbiG sequences have been predicted in *Sal. typhimurium*, *Cry. parvum*, *Schizosaccharomyces pombe*, *R. prowazekii*, and a homologue identified in *S. cerevisiae* (COQ3). *ubiH* sequence predictions have been reported for *Synechocystis* sp. and *S. cerevisiae* (*COQ6*). Predicted *ubiX* genes have also been assigned in *B. firmus*, *Synechocystis* sp., *C. trachomatis* and *R. prowazekii*. Thus, it appears that UbiA sequences have been predicted in organisms that have no requirement for UQ biosynthesis: more work is needed to clarify whether these sequences have any physiological function.

### Comparison of UQ biosynthesis in E. coli and S. cerevisiae

Elucidation of the UQ biosynthetic pathway of *S. cerevisiae* has gained much attention recently and has benefited greatly from knowledge of the *E. coli* pathway and the availability of *ubi* mutants and cloned *ubi* genes. The mevalonate-dependent UQ biosynthetic pathway (Grünler *et al.*, 1994) is shared by yeast and other eukaryotes. A comparison of the biosynthetic pathways for UQ in *E. coli* and *S. cerevisiae* (Fig. 2) shows that they diverge after the assembly of 3-polyprenyl-4-hydroxybenzoate. This intermediate forms a predominant pool in *S. cerevisiae* and is present at fourfold higher abundance than the end product (Poon *et al.*, 1995). Prokaryotic UQ biosynthesis proceeds by decarboxylation of 3-polyprenyl-4-hydroxybenzoate followed by oxidation and O-methylation. In contrast, the eukaryotic pathway proceeds with oxidation of the common intermediate followed by O-methylation and decarboxylation. Hereafter, the pathway from 2-polyprenyl-6-methoxyphenol to UQ is thought to converge in the two organisms. UQ-deficient mutants of *S. cerevisiae* fail to grow on non-fermentable carbon sources and have been classified into eight complementation groups (*coq1–coq8*). The *COQ* genes were isolated by plasmid complementation of these *coq* mutant strains and tested for their ability to restore the UQ deficiency of *E. coli ubi* mutants.

The *COQ1* gene encodes the hexaprenyl diphosphate synthase (Ashby & Edwards, 1990). The homologous *E. coli* octaprenyl diphosphate synthase (IspB) has been expressed in a *S. cerevisiae coq1* mutant and the transformed yeast cells produced UQ-8, which was able to function in the electron-transfer chain of yeast mitochondria (Okada *et al.*, 1996a). Likewise, an *E. coli ispB* mutant harbouring *ispB* homologues from *Haemophilus influenzae* and *Synechocystis* produced UQ-7 and UQ-9, respectively (Okada *et al.*, 1997).

The *S. cerevisiae* *COQ2* gene encodes the 4-hydroxybenzoate hexaprenyl transferase (Ashby *et al.*, 1992); its gene product shows 31·4% identity and 67·9% similarity to *E. coli* UbiA (Suzuki *et al.*, 1994; Fig. 3) and the expression of *COQ2* complemented a *ubiA* strain. *COQ3* encodes the 3,4-dihydroxy-5-hexaprenylbenzoate methyltransferase, which shows 40% identity to the *E. coli ubiG* gene product. *ubiG* is able to restore respiration and UQ biosynthesis in a yeast *coq3* mutant by methylating the eukaryotic intermediate 3,4-dihydroxy-5-hexaprenylbenzoate (Hsu *et al.*, 1996).

The *COQ4* gene has been sequenced and is essential for UQ biosynthesis in yeast but its exact role is not yet known. COQ5 shows 44% identity to *E. coli* UbiE and both sequences contain motifs common to a wide variety of *S*-adenosylmethionine-dependent methyltransferases. The *ubiE* gene was able to rescue the respiratory deficiency of a yeast *coq5* mutant (Barkovich *et al.*, 1997).

The *COQ6* gene shows sequence similarity to *E. coli ubiH*. The *COQ7* gene showed no similarity to any of the known *E. coli ubi* sequences, but it is likely that it may correspond to the still-unidentified *ubiF* gene, as a *coq7* mutant was found to accumulate 2-hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinone (Marbois & Clarke, 1996). The *COQ7* gene was independently isolated as *CAT5*, a gene required for the release of gluconeogenic genes from glucose repression (Jonassen *et al.*, 1998).

### Regulation of E. coli UQ biosynthesis

In *E. coli*, the composition of the quinone pool is highly influenced by the degree of oxygen availability: aerobically grown *E. coli* cells contain significantly more UQ-8 than MK-8 and DMK-8, whereas in anaerobic cells the profile is reversed (Meganathan, 1996; Ingledew & Poole, 1984; Wissenbach *et al.*, 1990, 1992; Shestopalov *et al.*, 1997). The mechanism of this regulation is not yet known. Shestopalov *et al.* (1997) have presented evidence that these changes are unaffected by chloramphenicol, implicating post-translational regulation of quinone levels. Indeed, 2-octaprenylphenol accumulated anaerobically is converted by 'aerobic' monooxygenases to UQ-8 without enzyme synthesis (Knoell, 1981). The aeration-dependent changes of the quinone pool seemed to be unaffected by mutations in the transcriptional regulatory systems of Fnr, Arc and AppY (Shestopolov *et al.*, 1997).

There have been few attempts to study transcription of the UQ-biosynthesis genes. Suzuki *et al.* (1994) used plasmid-borne fusions and reported that Φ(*ubiA–lacZ*) expression was catabolite-repressed by glucose. Gibert *et al.* (1988) studied expression of multicopy Φ(*ubiG–lacZ*) fusions and likewise reported that the expression of *ubiG* was subject to catabolite repression. We have analysed the transcriptional regulation of the *ubiCA* operon using single-copy fusions to minimize artefacts from plasmid-borne fusions. The expression of a monolysogen Φ(*ubiC–lacZ*) operon fusion was higher during aerobic than anaerobic growth, and increased with the rate of oxygen supply. Glucose repressed expression, whereas various anaerobically utilized electron acceptors (nitrite, nitrate, fumarate) did not affect expression. The *ubiCA* operon appears not to be regulated in response to oxidative stress, but was negatively regulated by the transcriptional regulators Fnr (fumarate nitrate regulation) and IHF (integration host factor) (Søballe & Poole, 1997). It is possible that these regulators alter kinetics of changes in the quinone pool rather than the final levels of quinones.

Analysis of *ubiB* expression promises to be rewarding, since the product of the *ubiB* reaction cannot be readily isolated, whereas the substrate accumulates (Young *et al.*, 1973), which suggests that *ubiB* might be a rate-limiting enzyme.

### *Production of UQ by genetic engineering*

With the availability of *E. coli* and *S. cerevisiae* genes involved in UQ biosynthesis, an attempt to develop overproduction of UQ in *E. coli* by genetic engineering has been reported (Zhu *et al.*, 1995). In particular, strains containing a plasmid carrying either *ubiCA* or *COQ2* together with *ispB* and grown in the presence of 4-hydroxybenzoate produced higher UQ-8 amounts than did the wild-type. The same amount of UQ was achieved when all *E. coli ubi* genes available (*ubiCA*, *ubiB*, *ubiG*, *ubiH* and *ispB*) were introduced into *E. coli* (Zhu *et al.*, 1995), emphasizing the importance of *ubiCA* and *ispB*. Neither UQ-6 nor UQ-4 was produced in strains carrying *ubi* homologues from *S. cerevisiae* or *Erwinia herbicola* respectively, and *E. coli* genes were more effective in UQ-8 production than were the exogenous genes.

The introduction of the *E. coli ispB* gene into a *S. cerevisiae coq1* knockout mutant resulted in UQ-8 production (Okada *et al.*, 1996a), which is surprising as *E. coli* cells expressing *COQ1* do not produce UQ-6 (Zhu *et al.*, 1995). In contrast, the solanesyl-diphosphate synthase gene (*sdsA*) from *Rhodobacter capsulatus* has successfully been expressed in both *E. coli* and *S. cerevisiae*, resulting in the synthesis of UQ-6 without any noticeable effect on the growth of the cells (Okada *et al.*, 1996b). These results suggested the possibility of producing the clinically important UQ-10 in *S. cerevisiae* simply by expressing the *dps* gene encoding the decaprenyl-diphosphate synthase. The *DPS* gene has recently been cloned from *Schiz. pombe*. However, the gene could not be expressed in either *S. cerevisiae* or *E. coli*, suggesting that another yet unknown factor is required for yeast prenyl-diphosphate synthase activity (Suzuki *et al.*, 1997). Recently, the *ddsA* gene encoding decaprenyl diphosphate synthase from *Gluconobacter suboxydans* has been cloned and was able to produce UQ-10 in an *ispB* mutant of *E. coli* (Okada *et al.*, 1998b).

A recent attempt to develop a new process for bacterial production of UQ-10 was made by Yoshida *et al.* (1998), who found three strains of *Agrobacterium tumefaciens*, *Paracoccus denitrificans* and *Rhodobacter sphaeroides*, which are excellent natural producers of UQ-10. The productivity of two of these strains was improved by mutagenesis, and a green mutant of *Rho. sphaeroides* was obtained, which under limited aeration showed a remarkable multiple-layered inner membrane and produced 3·6 times more UQ-10 than did the parent strain (Yoshida *et al.*, 1998).

## A role for UQ in gene regulation?

The earliest indications of the possible involvement of UQ in gene regulation came from studies of *E. coli ubi* mutants. It was suggested that the absence of UQ in these stains resulted in changes in the electron-transfer chain 'similar to those existing in the absence of oxygen' (Azoulay *et al.*, 1978). A *ubi* mutant, which was isolated by screening for sensitivity to chlorate in aerobiosis, proved to be derepressed for a benzyl viologen dependent nitrate reductase, which is able to convert chlorate into chlorite, a highly toxic compound (Azoulay *et al.*, 1978; Giordano *et al.*, 1978). The pool size of MK has also been reported to be elevated fourfold under aerobic conditions in a *Ubi*$^-$ strain (Cox *et al.*, 1970).

A recent search has been performed in order to identify regulators of the *P. stuartii aac(2′)-Ia* gene encoding 2′-*N*-acetyltransferase, which is involved in the O-acetylation of peptidoglycan and capable of acetylating and inactivating certain aminoglycosides. This search led to the identification of a number of regulatory genes, two of which, *aarE* and *aarF*, turned out to be required for biosynthesis of UQ (Paradise *et al.*, 1998; Macinga *et al.*, 1998). A mutation in either *aarF* or *aarE*, which is a homologue of *ubiA* (Fig. 3), severely decreased *aac(2′)-Ia* mRNA accumulation. The role of *aarF* (*yigQR* in *E. coli*) in UQ biosynthesis remains to be discovered, but the *aarF* mutation caused an increase in gentamicin resistance and a severe defect in aerobic growth. Based on the data from the expression studies, a model was proposed in which reduced UQ serves as a signal to activate gene expression through an uncharacterized pathway (Macinga *et al.*, 1998). Transcription of *aac(2′)-Ia* is not inducible by aminoglycosides (Rather *et al.*, 1993) and therefore the decrease of expression cannot simply be explained by the decreased uptake of aminoglycosides, which have been reported in an *E. coli* UQ-deficient strain (Muir *et al.*, 1981). Another suggestion is that the decrease in *aac(2′)-Ia* gene expression results from decreased mRNA stability in a *Ubi*$^-$ strain

and reflects changes in the cellular level of ribonuclease (Paradise *et al.*, 1998).

In yeast, *coq* mutants show a defect in gluconeogenic gene activation, but this effect was attributed to a general defect in respiration (Jonassen *et al.*, 1998). Clearly, more information is needed in order to establish a direct correlation between UQ and gene regulation in microbes.

## Quinones in respiration

The prokaryotic respiratory system has a remarkably flexible design, which enables the cell to survive changes of growth conditions and environmental challenges. Most bacterial respiratory systems can be viewed as comprising several dehydrogenases that transfer reducing equivalents to a common pool of UQ and/or MK, which in turn is reoxidized by the activity of terminal oxidases with oxygen or an alternative oxidant (e.g. nitrate) as final electron acceptor (Fig. 4).

An extensive study of the respiratory function of UQ in *E. coli* was performed by Cox and co-workers using a *ubiB* mutant (Cox *et al.*, 1969, 1970). The *ubiB* mutant accumulated the intermediate 2-octaprenylphenol and showed a reduced aerobic growth yield and produced large quantities of lactic acid by glycolysis. Wallace & Young (1977a) studied the function of UQ and MK in *E. coli* and found that a double mutant (*ubiA menA*) showed a further decrease in aerobic growth rate compared to a single *ubiA* mutation, indicating that *menA* plays a significant role in aerobic metabolism when UQ is absent.

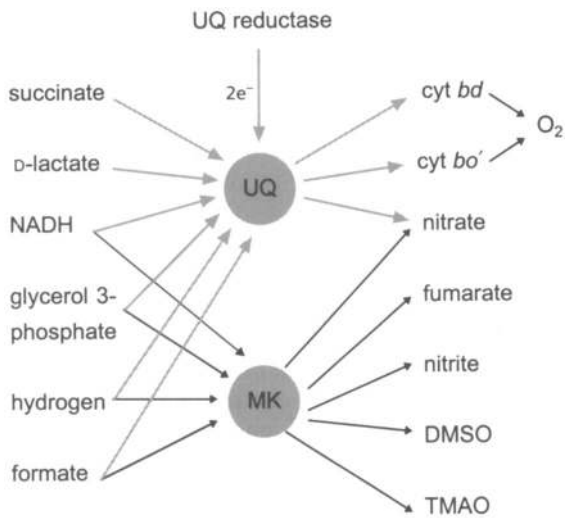


***Fig. 4.*** Selectivity of respiratory quinones in *E. coli*. UQ is obligatory in aerobic electron transfer to cytochrome *bo′* (cyt *bo′*) and cytochrome *bd* (cyt *bd*). Both UQ and MK can function in nitrate respiration but reduction of nitrite, fumarate, DMSO and TMAO is MK-dependent. UQ can accept electrons from NADH, succinate, D-lactate, glycerol 3-phosphate, hydrogen and formate, whereas MK mainly accepts electrons from NADH, glycerol 3-phosphate, hydrogen and formate. The possible two-electron reduction of UQ by UQ reductase (i.e. Qor; see text) is illustrated. The putative site for cytochrome(s) *b* upstream of the UQ pool (see text) is not shown.

Wallace & Young (1977b) also investigated the ability of UQ intermediates accumulating in *ubiE*, *ubiF* and *ubiG* mutants to function in respiration and found that the intermediate (2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinone, MQQ) accumulating in a *ubiF* mutant was able to support aerobic respiration with NADH, D-lactate and α-glycerophosphate, but not succinate.

A major problem in the interpretation of results obtained from studies of *ubi* mutants is that these mutants are often 'leaky' due to a partially functioning gene product, reversion or functional activity of certain UQ intermediates accumulated, such as MQQ. To obviate these difficulties arising from 'leaky' *ubi* alleles, a *ubiCA* knock-out mutant was constructed using a gene-replacement method (Søballe & Poole, 1998). The most striking phenotype of the *ubiCA* mutant was an inability to grow aerobically on succinate, showing that UQ cannot be functionally replaced by MK or DMK. Aerobic growth on limiting glucose and glycerol was diminished to 12–14% of wild-type levels, while anaerobic growth yields on nitrate and fumarate as electron acceptors were unaffected. Low oxygen-uptake rates (20–29% of wild-type levels) were demonstrated in membrane preparations using either NADH or lactate as substrates. However, these rates were greatly stimulated (three- to fivefold) by the addition of UQ-1 (Søballe & Poole, 1998).

The results of such quinone-function studies have led to the general conclusion that UQ functions as a redox mediator in aerobic and, to a lesser extent, in nitrate respiration, while MK functions mostly under other anaerobic conditions. Thus, only MK has been shown to function in anaerobic respiration involving fumarate, DMSO or trimethylamine-*N*-oxide (TMAO) as electron acceptors (Fig. 4). MK is also essential for formate-dependent nitrite reduction (Tyson *et al.*, 1997). UQ can accept electrons from NADH, succinate, D-lactate, glycerol 3-phosphate, hydrogen and formate via substrate-specific dehydrogenases, while MK is more selective and mainly accepts electrons from NADH, glycerol 3-phosphate, hydrogen and formate (Fig. 4; see Gennis & Stewart, 1996 for a review). In cells grown aerobically, the UQ concentration (about 200–300 nmol $g^{-1}$) is four to five times higher than the MK + DMK concentration. In contrast, under anaerobic conditions, the UQ levels are lowered 10-fold and constitute only one-third of the MK + DMK concentration (Wallace & Young, 1977a; Wissenbach *et al.*, 1992).

The selectivity of quinones for particular electron donors and acceptors could be due to differences in midpoint potential between $UQ/UQH_2$ ($E_{m,7}$, +113 mV) and $MK/MKH_2$ ($E_{m,7}$, −74 mV) (Gennis & Stewart, 1996). Thus, MK is more suitable for a respiratory chain with lower-potential electron ac-

ceptors such as fumarate, whereas UQ is well suited for oxygen and nitrate respiration. The midpoint potentials support the observations that MK is not suitable for the oxidation of succinate, and that $UQH_2$ is not suitable to donate electrons to fumarate. However, it has been reported that MK is an obligatory component of succinate respiration in *Bacillus subtilis*, in spite of its unfavourable redox potential (Lemma *et al.*, 1990). Recently, it has been proposed that the MK-dependent succinate dehydrogenase catalyses a reversed electron transport across the cytoplasmic membrane from the inner to the outer side, driven by proton potential (Schirawski & Unden, 1998).

In addition to relative mid-point potentials, the specificity for a particular quinone may be concerned with structural constraints of quinone-binding sites of dehydrogenases and oxidoreductases, or with compartmentalization of respiratory-chain reactions (Ingledew & Poole, 1984). It has been reported that NADH dehydrogenase I is essential for MK-dependent fumarate respiration and DMSO respiration, whereas NADH dehydrogenase II is more important in UQ-dependent aerobic respiration (Tran *et al.*, 1997).

The *ubiE* mutant AN70 is deficient in a specific methylase of the UQ biosynthetic pathway, which is also essential for the methylation of DMK, which results in MK. The *ubiE* mutant strain contained 1–3% of wild-type UQ levels, wild-type levels of DMK, but MK was completely missing (Wissenbach *et al.*, 1992). Using the *ubiE* mutant, the role of DMK in anaerobic respiration could be studied without the interference of MK. Growth and activity studies suggested that DMK was as efficient as MK in fumarate and TMAO respiration, but it was less efficient in DMSO respiration and nearly ineffective in nitrate respiration (Wissenbach *et al.*, 1992). Either UQ or MK, but not DMK, can transfer electrons to the periplasmic nitrate reductase (Tyson *et al.*, 1997).

A controversial aspect of UQ function in the *E. coli* aerobic respiratory chain has been the locations of UQ relative to cytochrome(s) *b*. The molar concentration of UQ in aerobically grown cells is 25 times that of cytochrome(s) *b* (Cox *et al.*, 1970).

The most commonly shown scheme for the sequence of carriers in the *E. coli* aerobic respiratory chain consists of a single UQ pool located immediately upstream of the oxidases and downstream of the respiratory dehydrogenases (Fig. 4). This scheme was last supported by Au *et al.* (1984), who investigated the role of UQ in the cytochrome *o*-terminated branch of the *E. coli* respiratory chain. However, these results conflict with earlier studies by Cox *et al.* (1970), who suggested the presence of a pool of cytochrome *upstream* of UQ. Such an organization was also supported by the experiments of Downie & Cox (1978) and Kita & Anraku (1981). A similar model has been proposed for *Proteus mirabilis*, an enterobacterium closely related to *E. coli*, grown anaerobically with nitrate (Van Wielink *et al.*, 1986). As mentioned earlier, disagreements in the reported *E. coli* electron-transfer sequences could be due to the use of 'leaky' *ubi* mutants. Therefore, in order to re-examine the role of *E. coli* UQ in aerobic and anaerobic respiration, we used a *ubiCA*-knockout mutant (Søballe & Poole, 1998). Continuous multi-wavelength spectrophotometry confirmed previous claims for enhanced cytochrome *b* reduction during respiration of a *ubi* mutant. Using the classical assumption that the electron-transfer components on the substrate side of an inhibitor become more reduced and those on the oxygen side become more oxidized, the data support a model in which UQ functions downstream of cytochrome(s) *b* (Søballe & Poole, 1998). The identity of the upstream cytochrome *b* is unknown but a likely candidate is cytochrome $b_{561}$, encoded by *cybB* (Murakami *et al.*, 1984). It is a low-potential dihaem cytochrome *b* ($E_m$, +2 mV) of unknown function (Murakami *et al.*, 1986) but might function upstream of UQ ($E_m$, +113 mV). This cytochrome is reducible by NADH and D-lactate. A knockout *cyb* mutation decreased NADH oxidase activity by 24% and L-lactate by 18%, compared to a wild-type (Yamato *et al.*, 1988).

Another explanation of the increased cytochrome *b* levels found in *ubi* mutants could be the presence of an intramolecular UQ tightly bound within a respiratory component receiving electrons from a low-spin haem. It has recently been reported that a novel high-affinity binding site ($Q_H$), distinct from the low affinity $UQH_2$ oxidation site ($Q_L$), has been identified in the *E. coli* cytochrome *bo'*-type $UQH_2$ oxidase. It is proposed that $Q_H$ mediates intramolecular electron transfer from $UQH_2$ in the $Q_L$ site to low-spin haem *b* as a one-electron transfer gate producing a highly stabilized $UQ^{\bullet-}$ radical (Sato-Watanabe *et al.*, 1995). In the absence of bound UQ at $Q_H$, it is possible that the low-spin cytochrome *b* cannot be fully oxidized, resulting in a higher level of cytochrome *b* reduction as reported in aerobically grown *ubi* mutants by Cox *et al.* (1970), Downie & Cox (1978), Kita & Anraku (1981) and Søballe & Poole (1998).

Tight binding of the $UQ^{\bullet-}$ radical confers kinetic and thermodynamic stabilization and prevents destructive side reactions. The reduction of dioxygen to water requires four electrons obtained from oxidation of two quinols to UQ. Cytochrome *bo'*-type terminal oxidase possesses only three metal centres, which suggests that the bound semiquinone, which remains after reduction of all three metal centres, provides the fourth electron (Ingledew *et al.*, 1995). Likewise, participation of a specifically bound MK has also been suggested in *E. coli* fumarate reductase and nitrate reductase (Brito *et al.*, 1995; Magalon *et al.*, 1997).

### *Pleiotropic phenotypes of E. coli ubi mutants*

The isolation of *E. coli* mutants defective in UQ biosynthesis has depended on the inability of such strains to grow on non-fermentable substrates, such as malate and succinate, while retaining their ability to grow on glucose (Cox & Downie, 1979). However, such

**Table 1.** Pleiotropic phenotypes of *E. coli ubi* mutants

| Mutation | Phenotype | Reference |
|---|---|---|
| *ubi*, *ubiCA*::Km | Unable to utilize succinate as sole carbon source | Cox & Downie (1979), Søballe & Poole (1998) |
| *ubiA* | Non-motile aerobically due to lack of flagella, but motile anaerobically | Bar-tana *et al.* (1977), Hertz & Bar-tana (1977) |
| *ubiA*, *ubiB* | Sensitive to chlorate | Azoulay *et al.* (1978), Giordano *et al.* (1978) |
| *ubiCA*::Km | Reduced production of siderophores | Cook *et al.* (1998) |
| *ubiD*, *ubiX* | Sensitive to thiols | Zeng *et al.* (1998) |
| *ubiF* | Partially resistant to streptomycin, resistant to gentamicin | Muir *et al.* (1981, 1985) |
| *ubiF* | Resistant to phleomycin and bleomycin, sensitive to $H_2O_2$, $\gamma$-irradiation and methyl methanesulfonate, resistant to heat | Collis & Grigg (1989) |
| *ubiH* | Sensitive to illumination with visible light under aerobic conditions, sensitive to paraquat | Nakahigashi *et al.* (1992) |

**Table 2.** Putative and identified UbiA sequences

| Micro-organism | Gene product | Identity to *E. coli* UbiA (%) | Reference/accession no. |
|---|---|---|---|
| *Escherichia coli* | UbiA | 100 | Nicholls & Green (1992), Siebert *et al.* (1992), Wu *et al.* (1992) |
| *Providencia stuartii* | AarE | 68 | AF036909 |
| *Rickettsia prowazekii* | UbiA | 35 | AJ234272 |
| *Synechocystis* sp. | UbiA | 33 | D64006 |
| *Aquifex aeolicus* | UbiA | 26 | AE000671 |
| *Bacillus firmus* | UbiA | 21 | U61168 |
| *Helicobacter pylori* | UbiA | 22 | AE000636 |
| *Methanococcus jannaschii* | UbiA | 21 | U67483 |
| *Archaeoglobus fulgidus* | UbiA | 23 | AE000954 |
| *Chlamydia trachomatis* | UbiA | 20 | AE001295 |
| *Saccharomyces cerevisiae* | COQ2 | 32 | Ashby *et al.* (1992) |

mutants show additional pleiotropic phenotypes, including resistance to heat and various antibiotics, and sensitivity to chlorate, thiols, visible light and oxidative stress induced by $H_2O_2$, paraquat, $\gamma$-irradiation or methyl methanesulfonate (for a summary and references see Table 1). These phenotypes can partly be explained by reduced uptake of antibiotics in *ubi* strains and by the proposed role of UQ in the defence against oxidative stress (see below).

We recently reported that a *ubiCA* mutant produces a significantly reduced level of iron-chelating siderophore (enterochelin) (Cook *et al.*, 1998). Moreover, a *ubiA* mutant was found to be non-motile aerobically due to the lack of flagella, but motile anaerobically, whereas a *ubiA menA* double mutant was non-motile under both conditions (Bar-tana *et al.*, 1977; Hertz & Bar-tana, 1977). This clearly suggests that a functional electron-transport system is obligatory for flagellar formation.

## Pro- and antioxidant properties of UQ

### Generation of superoxide and peroxide

Active oxygen species, such as the superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide ($H_2O_2$), have attracted much attention as agents that cause oxidative damage. Longevity of mammalian species has been inversely correlated with the rate of mitochondrial $O_2^{\cdot-}$ production (Lass *et al.*, 1997). *E. coli* strains lacking

superoxide dismutases (SODs) accumulate high levels of endogenous $O_2^{\cdot-}$ and critical biosynthetic enzymes become inactivated and the strains lack branched-chain, aromatic and sulphur-containing amino acids (Imlay & Fridovich, 1992).

The respiratory electron-transport chain is a significant source of $O_2^{\cdot-}$ production during exposure to hyperoxia *in vivo* (Gonzalez-Flecha & Demple, 1995) and it has been questioned whether $O_2^{\cdot-}$ arises from interaction between $UQ^{\cdot-}$ and molecular oxygen. However, $UQ^{\cdot-}$ is relatively stable only when protein-bound and the UQ pool in the lipid bilayer is therefore unlikely to be a major source of reactive oxygen species (Lenaz, 1998). UQ-binding sites of proteins are constructed to allow only two-electron transfer and thus prevent $O_2^{\cdot-}$ formation by direct interaction between $UQ^{\cdot-}$ and oxygen. In contrast, it is likely that the unpaired electron of $UQ^{\cdot-}$ serves to aid in the dismutation of $O_2^{\cdot-}$ radicals instead of participating in free radical formation (see next section). Moreover, in aerobically grown *E. coli* cells, most $O_2^{\cdot-}$ was found to be produced through autoxidation of anaerobic fumarate reductase, whereas the quinones did not form any detectable superoxide (Imlay, 1995).

In order for UQ to function as an antioxidant (see next section) it must be maintained in its reduced state, as $UQH_2$. $UQH_2$ can be attacked by $O_2^{\cdot-}$ to generate $UQ^{\cdot-}$ (1), which in turn reforms $O_2^{\cdot-}$ on reaction with oxygen (2). Therefore, at least *in vitro*, a chain reaction occurs, the overall product of which is $H_2O_2$ (3) (Nakayama *et al.*, 1997):

$$UQH_2 + O_2^{\cdot-} \rightarrow UQ^{\cdot-} + H_2O_2 \quad (1)$$

$$UQ^{\cdot-} + O_2 \rightarrow UQ + O_2^{\cdot-} \quad (2)$$

$$UQH_2 + O_2 \rightarrow UQ + H_2O_2 \quad (3)$$

The preventative role of SOD in the $UQH_2$-autoxidation process is uncertain. The presence of SOD has been shown to protect $UQH_2$ against oxidation *in vitro* by lowering the $O_2^{\cdot-}$ concentration (Nakayama *et al.*, 1997). However, *in vivo* the correlation between $UQH_2$ and SOD was tested using yeast *sod* mutants and these mutants contained the same amount of $UQH_2$ per cell as wild-type cells, which suggests that the autoxidation of $UQH_2$ is independent of SOD (Schultz *et al.*, 1996).

### $UQH_2$ as scavenger of oxygen radicals

In recent years, mammalian UQ has acquired increasing attention as an antioxidant. It is well-established that $UQH_2$ prevents initiation and propagation of lipid peroxidation in biological membranes and in serum low-density lipoprotein (LDL). The antioxidant activity of $UQH_2$ *in vivo* has been studied using a yeast UQ-deficient strain harbouring a deletion in the *COQ3* gene (Do *et al.*, 1996). The mutant was hypersensitive to the autoxidation products of unsaturated fatty acids (lipid peroxides and peroxyl radicals) and this could be prevented by the presence of the *COQ3* gene on a single-copy plasmid (Do *et al.*, 1996). In yeast, ascorbate is known to scavenge free radicals at the apoplast and it has been demonstrated, by the use of a *coq3* mutant, that extracellular stabilization of ascorbate is partly mediated by a UQ-dependent electron transport system in the plasma membrane (Santos-Ocaña *et al.*, 1998). Mitochondrial $UQH_2$ protects not only against lipid peroxidation but also against modification of integral-membrane proteins and DNA oxidation and strand breaks (Ernster & Dallner, 1995). Recently, it has been suggested that $UQH_2$ is able to scavenge nitric oxide radicals ($NO^{\cdot}$) *in vitro* (Packer *et al.*, 1998), but the biological significance of this reaction is not yet known.

The antioxidant properties of UQ are dependent on its presence in high concentrations and on its being in the reduced state; indeed the $UQH_2$/UQ ratio may indicate oxidative stress *in vivo* (Lagendijk *et al.*, 1996). In organelles other than mitochondria, it has been questioned how UQ becomes reduced. Recently it has been demonstrated that the cytosolic enzyme DT-diaphorase [NAD(P)H:(quinone acceptor) oxidoreductase] generates and maintains the reduced, antioxidant form of UQ by catalysing the unusual obligatory two-electron reduction of quinones and thus protects against cytotoxic and carcinogenic effects (Landi *et al.*, 1997).

The reduction levels of UQ in *E. coli* membrane preparations with added NADH under aerobic conditions were reported to be 55% (Cox *et al.*, 1970). *E. coli* possesses a soluble quinone oxidoreductase (Qor) complexed with NADPH (Thorn *et al.*, 1995). The crystal structure of Qor has been determined and shows strong structural homology to that of horse-liver alcohol dehydrogenase (Thorn *et al.*, 1995). Its physiological function has not been elucidated but it is tempting to speculate that Qor may carry out an activity similar to DT-diaphorase and maintain UQ in its reduced state and thereby promote its antioxidant function.

In plants, reduced plastoquinone found in chloroplast membranes exerts an antioxidant activity similar to that shown for UQ (Hundal *et al.*, 1995).

### Clinical use of UQ-10

Over the last 20 years, UQ-10 has been widely and successfully used as an orally administered prophylax and therapy for a great variety of diseases. UQ deficiency can be reversed by supplementation with UQ-10, which has no known toxicity or side effects.

$UQ/UQH_2$ is the only lipid-soluble antioxidant that can be synthesized by mammalian cells; the other lipid-soluble antioxidants (e.g. vitamins C, E and β-carotene) must be derived from the diet. The antioxidant activity of $UQH_2$ is independent of vitamin E (α-tocopherol), but $UQH_2$ potentiates the effect of vitamin E by regenerating the vitamin from its oxidized form (Stoyanovsky *et al.*, 1995).

Recently, UQ-10 has gained much attention, mainly because of its usefulness in the treatment of heart

disease. Human LDL is efficiently protected against lipid peroxidation by $UQH_2$-10, thereby delaying the onset of atherosclerosis. A significantly higher LDL/UQ ratio has been reported in patients suffering from ischaemic heart disease. Supplementation of the diet with UQ-10 increases the levels of $UQH_2$-10 within LDL, which results in an increased level of resistance to initiation of lipid peroxidation (Mohr *et al.*, 1992). Degenerative diseases and ageing may arise from a decreased ability to maintain adequate $UQH_2$-10 levels.

There is no information available on the use of UQ-10 for prevention of illness. This is an extremely important question, which to date does not have an answer.

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