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 K2; 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 chlorobi umquinone, α-tocopherolquinone, rhodoquinone, epoxynubiquinone and sulphur-containing caldariella-quinone, have also been detected in various bacteria (Collins & Jones, 1981). Phylloquinone (vitamin K2), a naphthoquinone with a largely saturated side chain (Fig. 1), is found mainly in...
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 \textit{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; Cranc, 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\(^-\)). 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\(^-\) is found associated with the UQH\(_2\) oxidase, cytochrome bo\(^-\) in \textit{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 \textit{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 \textit{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

![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.*](image-url)
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 methylation step (Wissenbach systems, LJbiE catalyses the insertion of a methyl group (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 40241 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 (4520-0 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 an allylic polyprenyl diphosphates (Ashby et al., 1992).

The alignment contains *ubiA* predicted in the archaea *Methanococccus 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 hyperthermophlic 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 *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-adenosylmethylionine-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 Synecocystis sp. and S. cerevisiae (COQ6). Predicted ubiX genes have also been assigned in B. firmus, Synecocystis 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 (Grüner 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 *Synecocystis* 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.-adenosylmethylionine-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-identified *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 CAT3, 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; Ingledey & Poole, 1984; Wissenbach et al., 1990, 1992; Shetstopalov et al., 1997). The mechanism of this regulation is not yet known. Shetstopalov 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 to '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 (Shetstopalov 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 $\Phi(\text{ubiA-lacZ})$ expression was catabolite-repressed by glucose. Gibert al. (1998) studied expression of multicopy $\Phi(\text{ubiG-lacZ})$ fusions and likewise reported that the expression of $\text{ubiG}$ was subject to catabolite repression. We have analysed the transcriptional regulation of the $\text{ubiCA}$ operon using single-copy fusions to minimize artefacts from plasmid-borne fusions. The expression of a monolysogen $\Phi(\text{ubiC-lacZ})$ 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 $\text{ubiCA}$ 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) (Soballe & 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 $\text{ubiB}$ expression promises to be rewarding, since the product of the $\text{ubiB}$ reaction cannot be readily isolated, whereas the substrate accumulates (Young et al., 1973), which suggests that $\text{ubiB}$ 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 $\text{ubiCA}$ or COQ2 together with $\text{ispB}$ 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 $E.\ coli$ $\text{ubi}$ genes available ($\text{ubiCA, ubiB, ubiG, ubiH}$ and $\text{ispB}$) were introduced into $E.\ coli$ (Zhu et al., 1995), emphasizing the importance of $\text{ubiCA}$ and $\text{ispB}$. Neither UQ-6 nor UQ-4 was produced in strains carrying $\text{ubi}$ homologues from $S.\ cerevisiae$ or $Erwinia\ herbicola$ respectively, and $E.\ coli$ genes were more effective in UQ production than were the exogenous genes.

The introduction of the $E.\ coli$ $\text{ispB}$ 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 ($\text{sdsA}$) 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 $\text{dps}$ gene encoding the deca-prenyl-diphosphate synthase. The $\text{DPS}$ 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 $\text{ddsA}$ gene encoding decaprenyl diphosphate synthase from Gluconobacter suboxydans has been cloned and was able to produce UQ-10 in an $\text{ispB}$ 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 strains resulted in changes in the electron-transfer chain similar to those existing in the absence of oxygen (Azoulay et al., 1978). A $\text{ubi}$ 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 $\text{ubi}^-$ strain (Cox et al., 1970).

A recent search has been performed in order to identify regulators of the $P.\ stuartii\ aac(2')-la$ gene encoding $2'-N$-acetyltansferase, 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, $\text{aarE}$ and $\text{aarF}$, turned out to be required for biosynthesis of UQ (Paradise et al., 1998; Macinga et al., 1998). A mutation in either $\text{aarF}$ or $\text{aarE}$, which is a homologue of $\text{ubiA}$ (Fig. 3), severely decreased $\text{aac(2')-la}$ mRNA accumulation. The role of $\text{aarF}$ ($\text{yigQR}$ in $E.\ coli$) in UQ biosynthesis remains to be discovered, but the $\text{aarF}$ 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 $\text{aac(2')-la}$ 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 $\text{aac(2')-la}$ gene expression results from decreased mRNA stability in a $\text{Ubi}^-$ 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.

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 z-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 (Seballe & 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 (Seballe & 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/UQH₂ (E₅₀, 298K +113 mV) and MK/MKHSO₄ (E₅₀, 298K −74 mV) (Gennis & Stewart, 1996). Thus, MK is more suitable for a respiratory chain with lower-potential electron ac-

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**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.
Dehydrogenase I is more important in UQ-dependent aerobic respiration (Tran 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., 1997). 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 an ubiCA-knockout mutant (Soballe & 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 (Soballe & Poole, 1998). The identity of the upstream cytochrome b is unknown but a likely candidate is cytochrome b561, encoded by cybB (Murakami et al., 1984). It is a low-potential dihaem cytochrome b (Em,b +2 mV) of unknown function (Murakami et al., 1986) but might function upstream of UQ (Em,b +13 mV). This cytochrome is reducible by NADH and L-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 (QH1), distinct from the low affinity UQH2 oxidation site (QH2), has been identified in the E. coli cytochrome bo-type UQH2 oxidase. It is proposed that QH1 mediates intramolecular electron transfer from UQH2 in the Q1 site to low-spin haem b as a one-electron transfer gate producing a highly stabilized UQ+ radical (Sato-Watanabe et al., 1995). In the absence of bound UQ at QH, 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 Soballe & Poole (1998).

Tight binding of the UQ+ 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
mutants show additional pleiotropic phenotypes, including resistance to heat and various antibiotics, and sensitivity to chlorate, thiols, visible light and oxidative stress induced by $H_{2}O_{2}$, paraquat, γ-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 ($O_{2}^{-}$) and hydrogen peroxide ($H_{2}O_{2}$), have attracted much attention as agents that cause oxidative damage. Longevity of mammalian species has been inversely correlated with the rate of mitochondrial $O_{2}^{-}$ production (Lass et al., 1997). *E. coli* strains lacking
superoxide dismutases (SODs) accumulate high levels of endogenous $O_2^-$ 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 $O_2^-$ production during exposure to hyperoxia in vivo (Gonzalez-Flecha & Demple, 1995) and it has been questioned whether $O_2^-$ arises from interaction between UQ$^+$ and molecular oxygen. However, UQ$^+$ 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 $O_2^-$ formation by direct interaction between UQ$^+$ and oxygen. In contrast, it is likely that the unpaired electron of UQ$^+$ serves to aid in the dismutation of $O_2^-$ radicals instead of participating in free radical formation (see next section). Moreover, in aerobically grown E. coli cells, most $O_2^-$ 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 $O_2^-$ to generate UQ$^+$ (1), which in turn reforms $O_2^-$ 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):

$$UQH_2 + O_2^- \rightarrow UQ^+ + H_2O_2$$
(1)

$$UQ^+ + O_2 \rightarrow UQ + O_2^-$$
(2)

$$UQH_2 + O_2 \rightarrow UQ + H_2O_2$$
(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 $O_2^-$ 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') 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 prophylaxis 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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