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Review

Biosynthesis and physiology of coenzyme Q in bacteria ☆

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ABSTRACT

Ubiquinone, also called coenzyme Q, is a lipid subject to oxido-reduction cycles. It functions in the respiratory electron transport chain and plays a pivotal role in energy generating processes. In this review, we focus on the biosynthetic pathway and physiological role of ubiquinone in bacteria. We present the studies which, within a period of five decades, led to the identification and characterization of the genes named *ubi* and involved in ubiquinone production in *Escherichia coli*. When available, the structures of the corresponding enzymes are shown and their biological function is detailed. The phenotypes observed in mutants deficient in ubiquinone biosynthesis are presented, either in model bacteria or in pathogens. A particular attention is given to the role of ubiquinone in respiration, modulation of two-component activity and bacterial virulence. This article is part of a Special Issue entitled: 18th European Bioenergetic Conference.

1. Introduction

In most living organisms, catalytic reactions involved in cell energization generate electrons which are funneled to the quinone pool. Thereafter, the reduced quinones (quinols) serve as substrates for reduction of the terminal acceptors. In *Escherichia coli*, three kinds of quinones are involved in this process: ubiquinone, also known as coenzyme Q (Q), menaquinone (MK) and demethylmenaquinone (DMK) [1–3]. MK and DMK have low midpoint potentials ($E'° = -74$ mV and $+36$ mV, respectively, [4]) and are involved in anaerobic respiration while Q, which has a higher midpoint potential ($E'° = +100$ mV, [4]), is involved in aerobic respiration [5,6]. The *E. coli* genome contains gene clusters for three cytochrome oxidase enzymes: cytochrome *bo* oxidase (*cyoABCD*), cytochrome *bd*-I oxidase (*cydABX*) and cytochrome *bd*-II oxidase (*appCD*). The three enzymes function as the major terminal oxidases in the aerobic respiratory chain of *E. coli* catalyzing electron transfer from ubiquinol to oxygen [7].

Ubiquinone is a widespread redox-active lipid which consists of a conserved aromatic ring and a polyprenyl hydrophobic tail, with the number of isoprenyl units varying among species: six in *Saccharomyces cerevisiae*, eight in *E. coli* and ten in humans [8–10]. Therefore, *E. coli* ubiquinone is designated Q_8. Its biosynthesis is a highly conserved pathway, which involves a large number of genes, named *ubi*, that have been identified from genetic studies [11,12]. Q_8 is located in the bacterial plasma membrane and was described to be an essential element for aerobic respiratory growth, gene regulation, oxidative stress adaptation, and various processes depending upon proton motive force (PMF) [13–16]. In this review, we present the genes involved in the Q_8 biosynthetic pathway in bacteria, with a particular attention on those recently identified and on the remaining gaps in current knowledge. We focus on the enzymatic actors, i.e. those involved in the decoration of the aromatic ring leading to Q_8, as well as the accessory ones. A few structures of Ubi proteins are presented and the phenotypes associated with *ubi* mutations are described and discussed.

2. The Q_8 biosynthetic pathway in bacteria

The *ubi* genes have been extensively studied over a period of five decades since the pioneering work of Cox and Gibson [17]. Biosynthesis of Q_8 in *E. coli* requires nine *ubi* genes, most of them encoding enzymes that decorate the aromatic ring of the 4-hydroxybenzoate (4-HB) universal precursor (Fig. 1). Noticeably in *E. coli*, *ubi* genes are all scattered around the chromosome (Fig. 2A and B). It is important to stress that in a few cases, the mutation of genes required for Q_8 production led to the accumulation of 3-octaprenylphenol (OPP), an early intermediate of Q_8 biosynthetic pathway (Fig. 1 and Table 1). As a consequence, these

Abbreviations: SAM, S-Adenosylmethionine; Q/Q_8, Coenzyme Q/Q_8; DMK/DMK_8, Demethylmenaquinone; $DDMQ_8$, Demethyldemethoxy coenzyme Q_8; DMQ_8, Demethoxy coenzyme Q_8; DMSO, Dimethyl sulfoxide; FAD, Flavine adenine dinucleotide; FMN, Flavin mononucleotide; H_2O_2, Hydrogen peroxide; 3-HB, 3-Hydroxybenzoate; 4-HB, 4-Hydroxybenzoate; Fe–S, Iron–sulfur; MK/MK_8, Menaquinone; OHB, 3-Octaprenyl-4-hydroxybenzoate; 4-HP_8, 3-Octaprenyl-4-hydroxyphenol; OPP, 3-Octaprenylphenol; PHBH, Para-hydroxybenzoate hydroxylase; PMF, Proton motive force; O_2^-, Superoxide anion; TMAO, Trimethylamine N-oxide

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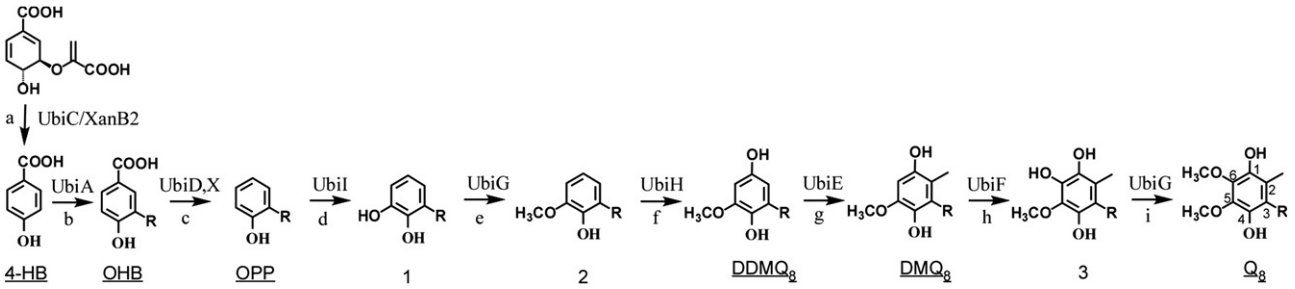

Fig. 1. Biosynthetic pathway of ubiquinone in *Escherichia coli*. The numbering of the aromatic carbon atoms is shown on coenzyme Q_8, and the octaprenyl tail is represented by R on C-3 of the different biosynthetic intermediates. The name of the enzymes catalyzing the reactions (each labeled with a lowercase letter) is indicated. Abbreviations used for 4-hydroxybenzoate (4-HB), 3-octaprenyl-4-hydroxybenzoate (OHB), 3-octaprenylphenol (OPP), coenzyme Q_8 (Q_8), C1-demethyl-C6-demethoxy-Q_8 (DDMQ8), and C6-demethoxy-Q8 (DMQ_8) are underlined. The XanB2 protein, present in some prokaryotes but not in *E. coli*, catalyzes the production of 4-HB from chorismate. The biosynthetic intermediates that accumulate in mutants affected in the different steps are listed in Table 1.

genetics studies sometimes failed at identifying precisely the biosynthetic step altered by the mutation.

In 1968, a screen based on the selection of mutants unable to grow on malate and examination of the quinone content of these strains grown on glucose led to the identification of *ubiA* as the first gene involved in Q_8 biosynthesis [18]. Poole and colleagues found the *ubiA* mutant to be unable to grow aerobically on non-fermentable substrates but able to grow anaerobically on glycerol with alternative electron acceptors such as fumarate [19]. The *ubiA* gene was predicted to encode a membrane-bound 4-hydroxybenzoate octaprenyltransferase. Within the same operon, *ubiA* lies downstream the *ubiC* gene (Fig. 2B). The UbiC protein catalyzes the first committed step in the biosynthesis of Q_8, the conversion of chorismate to 4-HB (Reaction a, Fig. 1) [20]. Interestingly, in *Xanthomonas campestris* (which does not contain any *ubiC* gene or homologue), the XanB2 protein was reported to convert chorismate into 3-HB or 4-HB using two distinct catalytic domains not related to UbiC (Reaction a, Fig. 1) [21].

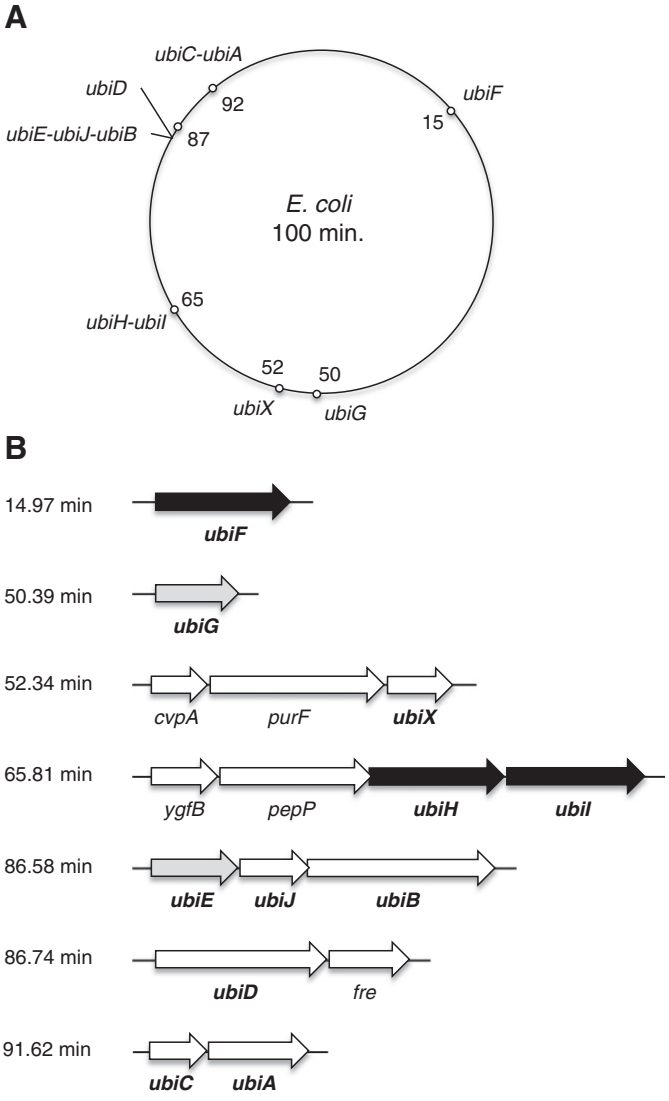

Fig. 2. Genetic organization and location of *ubi* genes on the *Escherichia coli* chromosome. A. Location of the eleven *ubi* genes on the 100 min map of the *E. coli* chromosome. B. Genetic organization of the *ubi* genes in *E. coli*. The location of each gene (or the first gene of the operon) is indicated on the left. The genes required for ubiquinone biosynthesis are indicated in bold. Genes encoding monooxygenases are symbolized by black arrows and genes encoding methyltransferases are symbolized by gray arrows.

Mutation in the *E. coli ubiG* gene yields strains unable to grow aerobically on nonfermentable substrates [22]. UbiG is one of the few Ubi enzymes that has been purified and assayed in vitro. Activity assays showed that UbiG is a S-adenosylmethionine (SAM)-dependent methyltransferase, which catalyzes the two O-methylation steps of Q_8 biosynthesis (Reactions e and i, Fig. 1) [23].

The *ubiE* gene encodes the second, also SAM-dependent, methyltransferase of the Q_8 biosynthetic pathway (Reaction g, Fig. 1) [24]. The *ubiE* mutant is deficient for growth on succinate and accumulates demethyldemethoxy-coenzyme Q_8 ($DDMQ_8$) and demethylmenaquinone (DMK_8) as predominant intermediates (Table 1), leading to the conclusion that *ubiE* is required for C-methylation in both ubiquinone and menaquinone synthesis [24].

An *E. coli ubiD* mutant accumulates 3-octaprenyl-4-hydroxybenzoate (OHB) (Reaction c, Fig. 1) [25,26]. It was shown that a partially purified membrane-bound UbiD protein was able to convert OHB into OPP, strongly suggesting UbiD involvement in the decarboxylation step of Q_8 biosynthesis [26]. However, a *ubiD* mutant retains the ability to produce about 25% of the wild-type levels of Q_8, consistent with the existence of a second decarboxylase [26]. Inactivation of the *ubiX* gene leads to low levels of Q_8, a reduced growth on succinate and accumulation of OHB (Table 1), suggesting a decarboxylase activity for UbiX [27]. A hypothesis is that UbiD and UbiX, which share no sequence similarity, function together during the decarboxylation of OHB (Reaction c, Fig. 1) [27]. Moreover, it might be worth noting that in *Salmonella enterica* serovar Paratyphi, *ubiX* and *ubiD* are organized as a single fusion gene *ubiX-ubiD* (SPA2778 gene). In *E. coli* O157:H7, in addition to UbiD and UbiX, a probable aromatic acid decarboxylase called Pad1 was identified but its role in Q_8 biosynthesis has not been investigated so far.

Hydroxylations represent three of the nine reactions required for Q_8 biosynthesis in *E. coli*. These reactions introduce hydroxyl groups at positions C-5, C-1, and C-6 of the aromatic ring of Q_8 (Reactions d, f and h, Fig. 1), and the genes encoding these enzymes were proposed to be *ubiB*, *ubiH*, and *ubiF*, respectively (Fig. 2A and B) [28]. *ubiH* was the first of these genes to be identified and, based on sequence comparison, was proposed to be a flavin-containing monooxygenase [29]. A *ubiF* mutant was shown to accumulate demethoxy coenzyme Q_8

Table 1
Accumulated intermediates from the Q_8 biosynthetic pathway in *Escherichia coli* mutants.

Gene affected by the mutation[a]	Deficient reaction	Accumulated Q_8-intermediates[b]	Reference
ubiB	Not known	OPP	[31]
ubiC	a	None[c]	[73]
ubiD	c	OHB[c]	[27]
ubiX	c	OHB[c]	[27]
ubiI	d	4-HP_8[c]	[32]
ubiG	e and i	OPP and compound 3 (Fig. 1)	[74]
ubiE	g	$DDMQ_8$	[24]
ubiF	h	DMQ_8	[30]
ubiA	b	No prenylated products formed	[19]
ubiJ	Not known	No intermediate characterized	[34]
ubiH	f	OPP, compounds 1 and 2 (Fig. 1)	[75]

[a] The mutations that affect the *ubi* genes reported in this table vary from point mutation to transposon insertion or complete deletion. See references for details.
[b] As detected by the techniques used in the reported studies. For example, OPP is adequately detected by using radiolabeled 4-HB but is not evidenced when using electrochemical detection coupled to HPLC.
[c] A low level of Q_8 is observed in these strains.

(DMQ_8) (Table 1, Fig. 1) indicating that UbiF, also related to flavin-containing monooxygenases, was responsible for introducing a hydroxyl group at C-6 of the aromatic ring [30]. UbiB was initially proposed to be involved in the C-5 hydroxylation because a *ubiB* mutant was shown to accumulate OPP and failed to produce Q_8 (Table 1) [25,31]. However, the UbiB protein does not contain any signature sequence for monooxygenases but rather shares identity with a large family of eukaryotic-type protein kinases [31]. Actually, we discovered that the C-5 hydroxylase was the product of the *visC* gene [32]. Accordingly, the *visC* gene, locating immediately downstream of *ubiH* within the same operon, was renamed *ubiI* (Fig. 2B). A *ubiI* mutant has a low level of Q_8 and accumulates 3-octaprenyl-4-hydroxyphenol (4-HP_8), an intermediate that results from a C5-hydroxylation defect (Table 1) [32]. In fact, 4-HP_8 is formed by hydroxylation of OPP on C1 by UbiH without reaction d and e taking place (Fig. 1). Therefore, the UbiH protein is able to perform the C1-hydroxylation in the absence of the methoxyl group on C5. The UbiI protein displays the typical domains of flavin-containing monooxygenases and shares 30 and 39% sequence identity with UbiH and UbiF, respectively [32]. Thus, we proposed to assign to UbiI the first hydroxylation step in Q_8 biosynthesis (Reaction d, Fig. 1) [32]. The role of the *ubiB* gene product remains to be investigated.

In *E. coli*, the *yigP* gene (called *ubiJ* in Fig. 2B) lies in between the *ubiE* and *ubiB* genes [31]. Recently, *yigP* was proposed to encode a small RNA of 252 nucleotides (referred to as *esrE*), which was proposed to be essential in *E. coli* [33]. We also characterized the *yigP* gene in *E. coli* and *Salmonella* and our results did not support these conclusions as *yigP* deletions were obtained in both bacteria. We found *yigP* to be required for Q_8 biosynthesis and we changed the name of *yigP* into *ubiJ* [34]. Regarding the small RNA issue, a "scrambled" *ubiJ* allele including the mutation of 30% of the nucleotides without changing the amino acid sequence restored Q_8 biosynthesis in *Salmonella ubiJ* mutant [34]. We therefore believe that the biological function of *ubiJ* is mediated by a protein. We also demonstrated that a *ubiJ* mutant was impaired for growth under aerobic conditions, but did not present any growth defect anaerobically, either with glucose or glycerol supplemented with different electron acceptors [34].

In conclusion, all enzymatic reactions necessary for Q_8 biosynthesis in aerobic conditions have been assigned to a Ubi protein (Fig. 1). However, the role of UbiB and UbiJ proteins remains to be established, as they are also essential for this process.

3. Structural analysis

The *E. coli* chorismate lyase UbiC structure was solved at 1.4 Å resolution [35,36]. This monomeric enzyme is composed of 164 amino acid residues with a molecular weight of 19 kDa [19]. The fold involves a 6-stranded antiparallel β-sheet with no spanning helices and novel connectivity [36]. Moreover, the 4-HB is completely sequestered from solvent in a largely hydrophobic environment behind two helix–turn–helix loops [36]. Ultrahigh resolution (1.0 Å) crystal structure of the *E. coli* UbiC product complex (PDB ID: 1TT8) reveals details of a substrate-sized internal cavity, also behind the flaps, near the product site (Fig. 3) [37]. A common feature of the UbiC structures is the presence of two internal-binding pockets connected by a short tunnel [37].

Structural information about the UbiX decarboxylase are available from studies carried out in *Pseudomonas aeruginosa* and *E. coli* O157:H7. The *P. aeruginosa* UbiX structure was determined to 1.5 Å resolution [38]. It shows that the enzyme assembles into a dodecamer, each subunit displaying a typical Rossmann fold, and contains one Flavin MonoNucleotide (FMN) at the interface between two subunits (PDB ID: 3ZQU) [38]. A paralog of UbiX named Pad1 (52% identity) was identified in *E. coli* O157:H7. Its three-dimensional structure has been determined and refined at 2.0 Å resolution (PDB ID: 1SBZ) (Fig. 4A) [39]. Each Pad1 monomer of the dodecameric assembly consists of a typical Rossmann fold and contains a non-covalently bound molecule of FMN (Fig. 4A) [39]. The FMN cofactor also lies at the interface between two monomers. As expected, the structures of UbiX from *P. aeruginosa* and Pad1 from *E. coli* O157:H7 are highly comparable as shown by the root-mean-square deviation of aligned Cα atoms in the range 1.1–1.5 Å (Fig. 4B).

Structural information for the UbiD decarboxylase derived from studies carried out in *P. aeruginosa* and *E. coli*. Two genes, PA0254 and PA5237, predicted to encode UbiD homologs arise in *P. aeruginosa* [40]. The three-dimensional structure of PA0254 has been determined in two different crystal forms to resolutions of 1.95 and 2.3 Å, respectively, showing a dimeric assembly (PDB ID: 4IP2) (Fig. 5A). Strikingly, the quaternary structure of *P. aeruginosa* PA0254 (25% identity with *E. coli* UbiD) differs from the hexameric organization of UbiD from *E. coli* (PDB ID: 2IDB, unpublished, Fig. 5B) and of PA5237 (76% identity with *E. coli* UbiD) [40]. Each subunit of PA0254, whose overall fold is

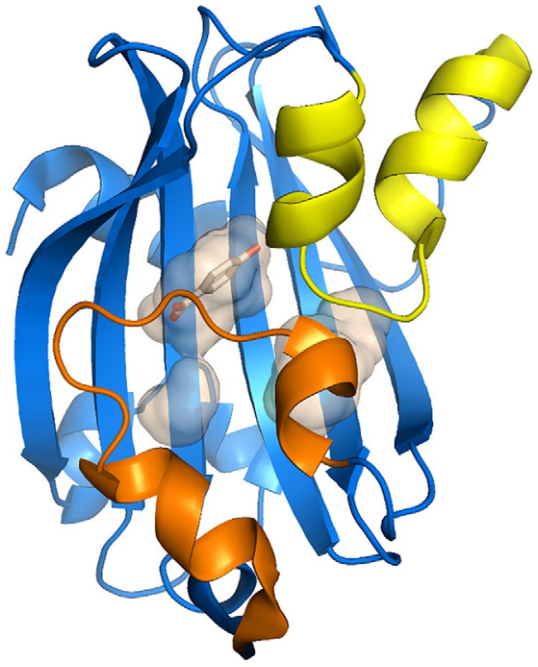

Fig. 3. Structure of the chorismate lyase UbiC from *Escherichia coli*. Crystal structure of the chorismate lyase UbiC from *E. coli* in complex with the 4-hydroxybenzoate, 1.0 Å resolution (PDB ID: 1TT8) [37]. The core of the protein fold is a 6-stranded antiparallel β-sheet, with two helix–turn–helix loops colored in yellow and orange. The two internal cavities are colored in gray. The 4-HB product is located in one of these cavities, behind the two flaps in a hydrophobic pocket.

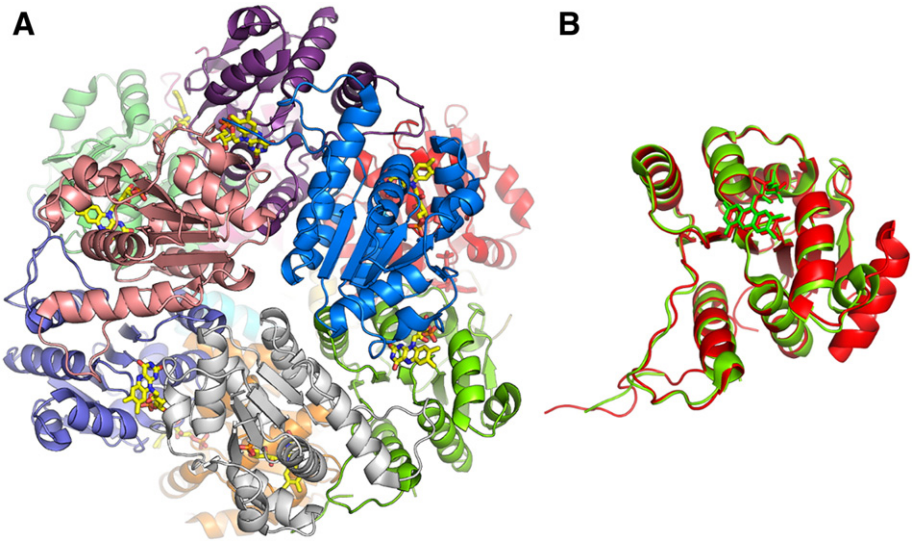

Fig. 4. Structures of the UbiX decarboxylase from *Pseudomonas aeruginosa* and the Pad1 paralog from *Escherichia coli* O157:H7. A. Crystal structure of the dodecameric Pad1 from *E. coli* O157:H7 complexed with a flavin mononucleotide (FMN), 2.0 Å resolution (PDB ID: 1SBZ) [39]. The view along the threefold axis shows the trimeric arrangement of Pad1. Each subunit is shown in a different color. The FMN cofactor is shown as yellow sticks and its binding site is located at the interface between two monomers. B. Superimposition of one subunit of the dodecameric Pad1 from *E. coli* O157:H7 (in green) with the corresponding subunit of the dodecameric UbiX from *Pseudomonas aeruginosa* (PDB ID 3ZQU) (in red). The flavin cofactors FMN of the two proteins are colored in green in UbiX and in red in Pad1.

similar to that of UbiD from *E. coli*, consists of three domains. The N-terminal part of the molecule is built up of two domains that pack tightly to each other and a C-terminal α/β domain which displays a topology characteristic for the UbiD protein family [40]. The middle domain shows significant structural similarity to the FMN-binding split barrel from a family of flavoproteins, including the NADH: FMN oxidoreductase from *Methylobacillus flagelates* (PDB ID: 3E4V), the flavin reductase from *Shewanella baltica* (PDB ID: 3HMZ), or the flavoredoxin from *Desulfovibrio vulgaris* (PDB ID: 2D5M). This middle domain also contains a metal binding site, with a magnesium ion coordinated by two histidine and glutamate residues, which are conserved in the corresponding metal binding site of some FMN binding proteins. However, no evidence for the incorporation of a flavin cofactor in PA0254 is available so far.

Recently, we determined the crystal structure of a truncated form of UbiI, involved in the aerobic C5-hydroxylation reaction (Fig. 6A) [32]. Only this form, lacking the 35C-terminal residues, and not the full-length protein, too prone to precipitate, could crystallize. Nevertheless, this structure provides some useful information, in particular as it shares many features with that of the canonical FAD-containing para-hydroxybenzoate hydroxylase (PHBH) [41]. In particular, comparison with PHBH reveals a FAD binding site in UbiI (Fig. 6B) which has been validated by site directed mutagenesis, thus supporting that UbiI and probably UbiH and UbiF are indeed FAD-containing monooxygenases [32].

As shown from this brief survey, it is obvious that a lot is still missing regarding the structural biology of Q_8 pathway: few proteins have been structurally characterized and rather incomplete information has been gained from those which have been crystallized. This opens a large field of research for the future.

4. Physiological role of Q_8

4.1. Interplay between Q_8 and redox sensing two-component systems

The Arc (anoxic redox control) two-component system of *E. coli* comprises the ArcB transmembrane sensor kinase and the cytosolic ArcA response regulator. It plays a major role in a transcriptional regulatory network that allows facultative anaerobic bacteria to sense various respiratory growth conditions [42]. Georgellis and colleagues showed that quinones act as direct negative signals that inhibit

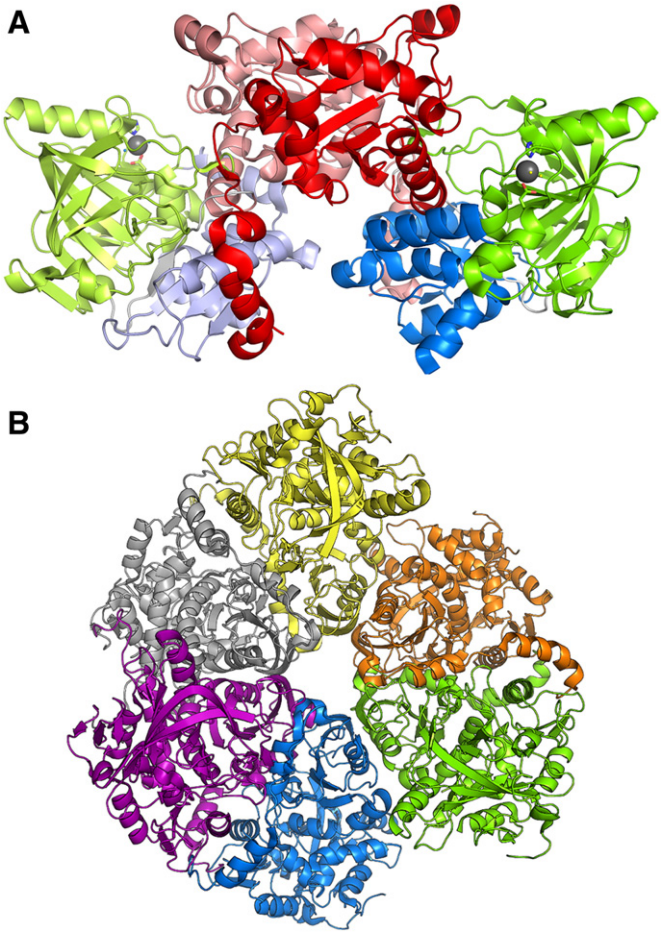

Fig. 5. Structures of the UbiD decarboxylases from *Pseudomonas aeruginosa* and *Escherichia coli*. A. Crystal structure of the dimeric PA0254 from *P. aeruginosa*, a putative aromatic acid decarboxylase bearing a C-terminal domain characteristic of the UbiD protein family, 1.95 Å resolution (PDB ID: 4IP2) [40]. The three domains of each subunit are shown in blue (N-terminal domain), green (middle domain) and red (C-terminal domain). The magnesium site is indicated by a black sphere in the middle domain. B. Crystal structure of the hexameric UbiD from *E. coli* (PDB ID: 2IDB). Each subunit is shown in a different color.

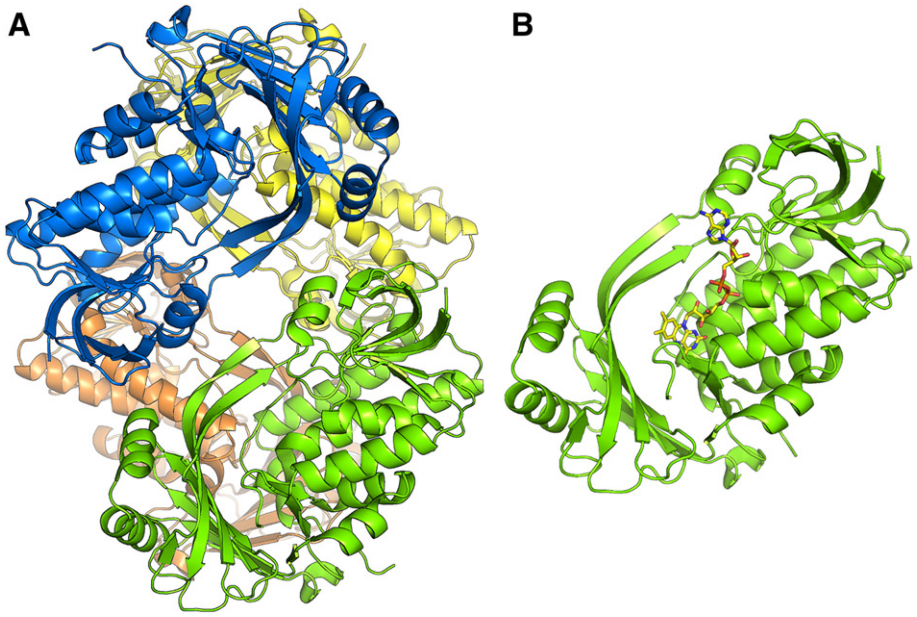

Fig. 6. Structure of the UbiI monooxygenase from *Escherichia coli*. A. Crystal structure of the C-terminal truncated form of *E. Coli* C5-hydroxylase UbiI involved in Q_8 biosynthesis, 2.0 Å resolution (PDB ID: 4 K22) [32]. B. Proposed molecular docking model of UbiI with FAD. The flavin cofactor is shown as yellow sticks.

autophosphorylation of ArcB during aerobiosis [43]. They demonstrated that the oxidative power provided by the Q_8 pool induced the formation of two intermolecular cysteine disulfide bonds within the cytosolic domains of an ArcB dimer, leading to the inactivation of ArcB kinase activity [43,44]. Bekker and colleagues provided evidence that additional regulation of ArcB kinase activity by the redox state of menaquinone (MK_8) was prevalent under microaerobic to anaerobic conditions [45]. They concluded that activation of the ArcBA system was controlled by both MK_8 and Q_8 pools [45]. Moreover, the extent of ArcA phosphorylation was shown to be modulated by the oxygen supply rate in an *ubiE* mutant, leading to the conclusion that the oxidized form of DMK_8 could inactivate ArcB [46]. However, the authors did not take in consideration the $DDMQ_8$ which also accumulates in a *ubiE* mutant [24,34] and could play a role in ArcB regulation in the *ubiE* mutant. A complementary study demonstrated that (D)MK_8 was required for activation of ArcB upon a shift to anoxic conditions [4]. The midpoint redox potential of the cysteines of ArcB was determined to be approximately −41 mV, which is in agreement with the proposed model in which the Q_8 pool can oxidize these cysteines under aerobic conditions and the disulfide bonds can be reduced by the (D)MK_8 pool upon a shift to anaerobic conditions [4].

Another example of redox regulation is illustrated by the RegBA two-component system of *Rhodobacter capsulatus*. RegB is a membrane-spanning sensor kinase that autophosphorylates and transfers the phosphoryl group to its cognate response regulator RegA [47]. The RegB/RegA system regulates the synthesis of numerous energy-generating and energy-utilizing systems [48]. Recently, RegB was shown to bind weakly both oxidized and reduced Q_8 with nearly equal affinity, although oxidized Q_8 alone inhibited kinase activity [49]. The current view is that interaction of RegB with both the reduced and oxidized forms of Q_8 allows the kinase to monitor and tune the cellular energy state [49].

In conclusion, in both *E. coli* and *R. capsulatus* models, and presumably many other bacteria, a connection between the pool, and the redox state, of Q_8 and two-component systems endows the cell with the capacity to modulate the expression of multiple genes in response to changes in redox conditions.

4.2. Q_8 and oxidative stress

The involvement of ubiquinone in oxidative stress resistance was first addressed in eukaryotes, where it was suggested that its reduced form, i.e. ubiquinol, was able to function as a lipid-soluble anti-oxidant [50]. Ubiquinol was shown to scavenge lipid peroxyl radicals and thereby prevents a chain reaction causing oxidative damage to polyunsaturated fatty acids of biological membranes, a process known as lipid peroxidation [51]. In yeast, the biosynthetic intermediate DMQ_6 was concluded to lack antioxidant activity because it failed to protect cells against oxidative stress generated by hydrogen peroxide (H_2O_2) or linolenic acid [52]. In prokaryotes, an exhaustive study aimed at investigating the role of Q_8 in resistance against oxidative stress was carried out by Søballe and Poole [53]. Using a *ubiCA* mutant deficient for Q_8 production, they observed an accumulation of superoxide ($O_2^{\bullet -}$) in the membranes of the mutant compared to wild-type membranes, reflecting the lack of superoxide-scavenging ubiquinol [53]. Moreover, intracellular H_2O_2 concentration was increased 1.8-fold in the *ubiCA* mutant, which was also found to be hypersensitive to an oxidative stress mediated by H_2O_2 [53]. Expression of *katG* gene, encoding a catalase, and intracellular catalase activity, were both increased in the *ubiCA* mutant [53]. These observations are consistent with the hypothesis that Q_8 limits $O_2^{\bullet -}$ and H_2O_2 accumulation in scavenging reactive oxygen species. However, given the multiplicity of catalases, peroxidases and superoxide dismutases present in most bacteria, the relative contribution of Q_8 to the overall anti-oxidative stress defenses system remains to be assessed.

4.3. Q_8 and respiration

The respiratory chain of *E. coli* contains many enzymes allowing the organism to transfer electrons to oxygen or to use alternative terminal acceptors such as nitrate, dimethyl sulfoxide (DMSO), trimethylamine N-oxide (TMAO) or fumarate [1,2,7]. Q_8 is generally depicted as the aerobic quinone since it is more abundant than MK_8 during aerobic growth, whereas MK_8 is essential for anaerobic respiration using fumarate, DMSO and TMAO as electron acceptors [13,14]. In this way, a *ubiCA* mutant showed severely diminished growth yields aerobically but not anaerobically using nitrate or fumarate as terminal electron acceptors [54]. Conversely, a *ubiE* mutant, which contains DMK_8 but no MK_8, was able to grow with fumarate, TMAO and DMSO, but not with nitrate as electron acceptor [55]. It was then concluded that DMK_8 (in addition to MK_8) could serve as a redox mediator in fumarate, TMAO and DMSO respiration, but not in nitrate respiration [55]. Moreover, it was

demonstrated that nitrate reductase A could accept electrons from both Q_8 and MK_8 pools, the coupling being more effective with ubiquinol than with menaquinol [56].

Recently, Bekker and colleagues reported that DMK_8 played a role not only in TMAO —, DMSO- and fumarate-dependent respiration, but also in oxidation of succinate [15]. They further concluded that all three quinol oxidases of *E. coli* accepted electrons from DMK_8 based on the residual aerobic respiration observed in a *ubiE* mutant [15]. As already mentioned, another hypothesis is that $DDMQ_8$ might provide electron towards the quinol oxidases in the *ubiE* mutant. In the future, it will be interesting to know whether these remaining uncertainties will challenge the classic view associating ubiquinone to aerobic growth and menaquinone to anaerobic growth.

4.4. Q_8 and antibiotics resistance

The proton-pumping NADH:ubiquinone oxidoreductase, also called complex I, catalyzes the electron transfer from NADH to Q_8 linked with a proton translocation from the negative inner to the positive outer side of the membrane [57]. Thus, a proton-motive (PMF) force is generated, which is utilized mainly for ATP synthesis. PMF is instrumental in allowing either import or export of antibiotics [58–60]. As a consequence, *ubi* mutations are likely to modify levels of resistance to antibiotics sustained by bacteria. In fact, resistance to low levels of aminoglycoside antibiotics has been used for long for the isolation of menaquinone-deficient mutants in *Bacillus subtilis* since the MK deficiency depresses the rate of accumulation of aminoglycosides, which accounts for the resistance phenotype [61]. Thereby, a phenotype of increased aminoglycoside resistance led to the identification of the *aarE* gene, the *ubiA* homologue in *Providencia stuartii* [62]. In *E. coli*, a *ubiF* mutation was associated with pleomycin resistance [63] while a *ubiD* mutation was found to be associated with decreased transport of streptomycin and gentamicin, and increased resistance to those antibiotics [64]. This mutant also exhibited increased resistance to several other aminoglycoside antibiotics, but not spectinomycin [64]. A series of *E. coli* mutants located in the vicinity of *ubiB* and *ubiD* and consistent with a disruption of Q_8 synthesis were found to present zwittermicin A resistance, a novel broad-spectrum antibiotics produced by *Bacillus cereus* [65]. Rationally, a *ubiD* mutant was also resistant to zwittermicin A [65]. All together, these studies highlight that connections between Q_8 production and antibiotics resistance occur in many bacteria.

4.5. Q_8 and bacterial virulence

In the *Caenorhabditis elegans* model, Clarke and colleagues revealed that nematodes fed diets of respiratory deficient *E. coli* lacking Q_8 lived significantly longer than worms fed the wild-type parental strain [66]. Moreover, these *E. coli* mutants were degraded in the early adult worm and did not accumulate in the intestinal tract [66]. These data led the authors to propose that bacterial respiration might act as a virulence factor, influencing the ability of bacteria to colonize – and subsequently harm – the animal host [66]. As a matter of fact, the role of respiration in host–pathogen interactions has recently been underlined in several studies, including those dedicated to *Shigella* pathogenicity [67] and more recently to *Salmonella* [34].

Indeed, early studies brought in light the requirement of a functional Q_8 biosynthesis pathway for the flagellation of *Salmonella* [68]. Interestingly, the increase in motility response occurred within a narrow range of the increase in Q_8 content [68]. More recently, we showed that *Salmonella ubiJ* mutant, a strain deficient for Q_8 production under aerobic conditions, was killed within macrophages [34]. When macrophages were infected with an anaerobic inoculum of the *ubiJ* mutant (in which Q_8 was still produced), the *ubiJ* mutant recovered its proliferation capacity, clearly establishing the requirement for Q_8 for efficient intracellular proliferation [34]. Several possibilities can be considered to connect Q_8 defects and virulence attenuation: (i) the necessity for the bacterium to use aerobic respiration – thus Q_8 – to grow intracellularly; (ii) the antioxidant role of Q_8 to avoid the oxidative burst produced by the host; (iii) the requirement of a PMF-dependent process for virulence. Thereby, our recent data combined to results from other groups highlighted the importance of Q_8 biosynthesis in the context of the host–pathogen interplay and revealed an unexpected link between Q_8 and bacterial virulence.

5. Conclusion and perspectives

In this review, we have presented the actors involved in Q_8 biosynthetic pathway. Phenotypic characterizations of various mutants coupled with the examination of Q_8 content led to the identification of genes involved in the decoration of the aromatic ring, from the 4-HB precursor to the final ubiquinone. When available, the three-dimensional structures of these enzymes were presented. Last, the role of Q_8 in diverse cellular processes was detailed: genetic regulation through the two-component systems ArcBA and RegBA, adaptation to oxidative stress, respiration, antibiotics resistance and bacterial virulence.

Whereas nine *ubi* genes were found to be directly involved in Q_8 biosynthesis, the biological function of *ubiB* and *ubiJ* gene products remain unknown. The involvement of UbiB in the C-5 hydroxylation step has to be definitively abandoned. Instead, UbiB might play a regulatory role through a kinase activity, even though it is still unknown if it displays such an activity and what substrate it acts on. Based on sequence similarities between *ubiJ* and genes involved in lipid metabolism, UbiJ could serve as a carrier of the isoprenoid hydrophobic tail prior the action of monooxygenases and methyltransferases. Alternatively, it might chaperone prenylated intermediates during the biosynthetic process.

Intriguingly, we found that the *ubiJ* mutant synthesizes almost wild-type levels of Q_8 under anaerobic growth conditions [34]. Actually, *ubiH*, *ubiF* and *ubiI* mutants also synthesize significant levels of Q_8 anaerobically [28,32]. The three latter genes encode flavin-dependent monooxygenases, which use molecular oxygen to catalyze their respective hydroxylation reactions. In consequence, these proteins will not participate in Q_8 biosynthesis under anaerobic conditions because of the absence of molecular oxygen. Therefore, alternative anaerobic hydroxylases must carry out the hydroxylation reactions in anaerobic conditions as proposed by Alexander and Young [28], but so far the identity of these proteins has remained elusive. The involvement of UbiJ only in aerobic Q_8 biosynthesis is more difficult to rationalize than that of the aerobic monooxygenases and may reflect a functional link with those enzymes.

It is important to note that with the exception of the *E. coli* methyl transferase UbiG [23], the partially purified membrane-bound UbiD [26] and the UbiA octaprenyltransferase [69], none of the other enzymes (the decarboxylases, the putative kinase and the hydroxylases) that participate to Q_8 biosynthesis have been assayed under in vitro conditions using pure proteins. As a consequence, most of the predicted activities associated with Ubi enzymes remain to be fully established by straight biochemical analyses. This is not surprising considering the difficulties to purify these proteins, their probable association to the membranes and their instability, as well as the difficulties to get access to the substrates, not commercially available.

Another issue is whether Ubi proteins may belong to a large multiprotein complex as suggested by an early report [70]. By a combination of sonication, gel filtration and equilibrium sedimentation with a sucrose gradient, the author purified a mega complex of at least 12 proteins, ranging from 40 to 80 kDa and exhibiting a molecular weight estimated to 2.10^6 Da. This complex had the capacity to process OPP into Q_8 in the presence of NADPH, SAM and O_2. It is intriguing that, in spite of this fascinating observation, no further studies concerning this bacterial complex have been reported then. The existence of a high-molecular weight complex has also been documented in *Saccharomyces cerevisiae*, mostly by genetic and partly by biochemical studies [71,72]. The complex may

actually consist of several subcomplexes likely associated with the mitochondrial inner membrane [72], but neither its complete composition nor its structural organization is known. In particular, the purification of this complex has not been reported. Thus, the question of the existence of a Q_8 biosynthetic complex in bacteria remains open and needs further investigation.

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