A new member of the family of di-iron carboxylate proteins: Coq7 (clk-1),
a membrane-bound hydroxylase involved in ubiquinone biosynthesis*

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Running Title: Coq7 is a di-iron carboxylate protein

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SUMMARY

Ubiquinone (UQ) is an essential cofactor for respiratory metabolism. In yeast, mutation of the COQ7 gene results in the absence of UQ biosynthesis and demonstrates a role for this gene in the step leading to the hydroxylation of 5-demethoxyubiquinone (DMQ). Intriguingly, the disruption of the corresponding gene in Caenorhabditis elegans, clk-1, results in a prolonged lifespan and a slowing of development. Due to the pleiotropic effect of this disruption, the small size of the protein, and the lack of obvious homology to other known hydroxylases, it has been suggested that Coq7 may be a regulatory or structural component in UQ biosynthesis, rather than acting as the hydroxylase per se. Here we identify Coq7 as belonging to a family of a di-iron containing oxidases/hydroxylases based on a conserved sequence motif for the iron ligands, supporting a direct function of Coq7 as a hydroxylase. We have cloned COQ7 from Pseudomonas aeruginosa and Thiobacillus ferrooxidans and show that indeed this gene complements an E. coli mutant which lacks an unrelated DMQ hydroxylase. Based on the similarities to other well-studied di-iron carboxylate proteins, we propose a structural model for Coq7 as an interfacial integral membrane protein, and discuss substrate binding and catalysis.

INTRODUCTION

Ubiquinone (UQ) is an essential electron carrier in the respiratory chain of all eukaryotes and most prokaryotes. The early steps in UQ biosynthesis differ between eukaryotes and prokaryotes but then converge for the last four steps (reviewed in (1,2)). These later steps utilize precursors that are identical apart from the length of the isoprenoid side chain, which varies according to organism. In yeast, COQ7 has been shown to be required for the hydroxylation of 5-demethoxyubiquinone (DMQ) to 5-hydroxyubiquinone at the penultimate step in the biosynthesis of UQ (3) (Fig. 1). UQ biosynthesis can be restored
in COQ7 null mutants of *Saccharomyces cerevisiae* by complementation with rat COQ7 (4), human COQ7 (5) or *Caenorhabditis elegans* clk-1 (6). Orthologues of Coq7 (clk-1) are widely found among eukaryotes (7), and have been identified in a few prokaryotes including *Rickettsia prowazekii* (8).

In *S. cerevisiae*, a point mutation within the COQ7 gene disrupts UQ synthesis resulting in a petite (non-respiring) phenotype and accumulation of the UQ precursor, DMQ (3). In contrast, a deletion in the COQ7 gene causes accumulation of an earlier compound in the biosynthetic pathway, suggesting a possible regulatory or structural role of COQ7 in UQ biosynthesis (2). Disruption of the homologous gene (clk-1) in the more complex organism, *C. elegans*, results in an extended lifespan, a slowdown of developmental processes, and a slowing of a number of rhythmic adult behaviors (6,9). Since there is no apparent effect upon mitochondrial respiration, this called into question whether clk-1 is involved in DMQ hydroxylation in *C. elegans*. Recent studies have addressed the disparity between the respiratory phenotypes in yeast and *C. elegans*. Jonassen et al. (10) have shown that the *C. elegans* clk-1 mutant does not synthesize UQ, but instead depends upon UQ from a dietary source (10). In addition, Miyadera et al. (11) have found an accumulation of DMQ in the mitochondrial membrane in the clk-1 mutant, and have shown that it is redox-active and may be able to compensate for some of the respiratory functions of UQ (11). These studies show that the striking pleiotropic effect of the clk-1 mutation is due to the absence of UQ biosynthesis.

However, the question still remains as to whether COQ7/clk-1 encodes the hydroxylase itself. The small size of this integral membrane protein (187 amino acids in *C. elegans*) and the lack of readily identifiable sequence homology to other known hydroxylases has led some workers to propose that COQ7 may instead encode a structural or regulatory component of the hydroxylation process (11).
In this paper we demonstrate that Coq7 does function as a DMQ hydroxylase by utilizing the fact that this step in *Escherichia coli* UQ biosynthesis is catalyzed by an evolutionarily unrelated protein, UbiF (12). We identify and clone *COQ7* from *Pseudomonas aeruginosa* and *Thiobacillus ferrooxidans* and show that it restores UQ biosynthesis in the *ubiF* mutant strain, *E. coli* JF496. In addition, we identify a conserved iron-binding motif that places the enzyme within the family of di-iron carboxylate proteins that includes soluble methane monoxygenase hydroxylase, two membrane-bound quinol oxidases, and a number of other enzymes. Structural modeling and comparison to these proteins provides insight into the nature of the active site as well as the mode of membrane association, and suggests a potential catalytic mechanism.

**EXPERIMENTAL PROCEDURES**

*Materials and Strains*—Plasmid pUNI-10, its host strain *E. coli* BW23474, and *E. coli* BL21/pQL123 for the expression of Cre recombinase (13) were gifts from Stephen Elledge (Baylor College of Medicine, Houston, TX, USA). Cre-recombinase was purified using glutathione-Sepharose beads (13). Genomic DNA of *P. aeruginosa* PAO1 was obtained from Bengt Wretlind and Kerstin Bergman (Huddinge University Hospital, Huddinge, Sweden) and of *T. ferrooxidans* from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). *E. coli* JF496 (14) was obtained from the *E. coli* Genetic Stock Center (CGSC, Yale University, New Haven, CT, USA). *E. coli* TOP10 and pBAD/ThioE were from Invitrogen (Carlsbad, CA, USA).

*Cloning and Plasmid Construction*—The *COQ7* gene was cloned into pUNI-10 by PCR amplification of both the gene and the vector followed by cotransformation of 2.5 µl of each PCR product into 50 µl of CaCl₂-competent *E. coli* BW23474, utilizing the endogenous recombinase activity of *E. coli* to recombine the fragments (15). Colonies were selected on
LB plates containing 40 µg/ml kanamycin. The following primers were used: pUNI-10, CATATGAATTCCAGATACTTC and TAAGGATCCGCGGCCGCA; P. aeruginosa COQ7, GTTATCTGGAATTCTATATGTCGCGACCACCCAC and GCGGCCGCGGATCCTTATCAGATGGGTAGGCTC; T. ferrooxidans COQ7, GTTATCTGGAATTCTATATGTCGCGACCACCCAC and GCGGCCGCGGATCCTTATCAGATGGGTAGGCTC. EcoRI and BamHI sites, present in pUNI-10, are shown underlined in the primer sequences. The resulting plasmids (pUNI-PaCOQ and pUNI-TfCOQ) were isolated and sequenced.

The two genes were cloned into pUNI-10 in order to take advantage of the versatility of the Univector in vitro recombination system for rapid subcloning into a variety of expression plasmids (13). The expression vector pHB-MALc2M is derived from a low-expressing mutant of pMALc2 (New England Biolabs, Beverly, MA, USA), pMALc2M, which was converted into a host expression vector by inserting a Cre-lox recombination site (loxP). Further details on the isolation and characterization of pMALc2M and the construction of pHB-MALc2M will be given elsewhere. pUNI-PaCOQ and pUNI-TfCOQ were recombined in vitro with the expression host vectors pHB-MALc2M and/or pBAD/ThioE using Cre recombinase (13), then transformed into E. coli TOP10. Recombinant colonies were selected on 40 µg/ml kanamycin. The resulting recombinant plasmid pPaCOQ-MALc2M encodes the maltose binding protein (MBP) fused to the N-terminus of Coq7 under an IPTG-inducible tac promoter. pPaCOQ-ThioE and pTfCOQ-ThioE each encodes a His-patch thioredoxin (TRX) fused to the N-terminus of Coq7 under an arabinose promoter. Recombinations were verified by restriction digest.

**Growth Conditions**—For complementation assays, expression plasmids or empty host vectors were freshly transformed into E. coli JF496 and grown at 37°C to an OD₆₀₀ of 0.5-0.8 in LB
with 60 µg/ml ampicillin (16). The cultures were diluted ca. 1x10^5 and 50 µl was plated on M9 agar plates containing 1 mM MgSO_4, 20 µM CaCl_2, 5 µM FeSO_4, 0.5 µg/ml thiamine, 0.12 % casamino acids, 40 µg/ml D-L-methionine, 100 µg/ml L-asparagine, 60 µg/ml ampicillin (16) and containing either 0.5 % glucose or 0.5 % succinate (12). Where expression was to be induced, 0.1 mM IPTG or arabinose was added as indicated. To analyze quinone content, *E. coli* JF496 with or without expression plasmid was grown at 37°C in LB with 0.5% glucose and harvested at an OD_600 of 0.5-1.0. Ampicillin (60 µg/ml) was added to the medium as required.

**Quinone extraction and Analysis** – 2 ml of *E. coli* suspension in 0.15 M NaCl (OD_600 of 4-6) was extracted with 18 ml of methanol and 12 ml of petroleum-ether (b.p. 40-60°C) (19). After phase separation, the upper phase was evaporated under nitrogen and the lipid residue was dissolved in 400 µl of methanol:ethanol (1:1) and reduced with 4 µl of 0.5 M NaBH_4 in 0.05 M NaOH. Samples were analysed by reverse phase HPLC (RESOLVE 5µ spherical C18 column, 3.9 mm x 15 cm, Waters, Millipore) coupled to an electrochemical detector (+0.70 V, oxidative mode). Solvent A (methanol:water, 9:1) and solvent B (ethanol:2-propanol, 95:5) both contained 0.2% LiClO_4. The system was run at 30% solvent B for 5 min followed by a linear increase of solvent B to 50% within 25 min, using a flow rate of 1 ml/min. When UQ8 and DMQ8 were collected for analysis by mass spectrometry, LiClO_4 was omitted and the absorption of the eluate was monitored at 210 nm. The collected quinones were dried under nitrogen and dissolved in methanol:hexane (19:1) for direct inlet introduction to the mass spectrometer (Quattro Ultima Micromass, Manchester, UK). The instrument was calibrated with CsI and the electrospray ionization spectra were detected in negative mode.
Sequence alignments and Modeling– Bacterioferritin from *E.coli* and Coq7 from *P. aeruginosa* (accession number: AAG04044) and a third sequence that is suggested to be a bacterioferritin in *P. aeruginosa* (accession number: AAG08265) were aligned using ClustalW (18) and then manually adjusted. The alignment was used to thread the *P. aeruginosa* Coq7 sequence (residues 46-60, 62-121, and 131-195) upon the coordinates of the bacterioferritin structure (19) using Swiss Pdb Viewer (20). DMQ was docked to the model manually.

RESULTS AND DISCUSSION

Identification of Coq7 as a di-iron carboxylate protein– A number of databases were searched for the di-iron protein motif \( E < X_{n1} > EXXH < X_{n2} > E < X_{n3} > EXXH \) (where “\( X \)” indicates any amino acid and “\( n \)” a variable number of residues). This yielded several groups of homologous proteins in addition to the subject of this study, the Coq7 homologues. Besides the annotated proteins, further searches uncovered many examples of sequences encoding protein homologous to Coq7 among the proteobacteria, including *P. aeruginosa*, and *T. ferrooxidans*. Partial sequences for Coq7 in the eukaryotic *Trypanosoma brucei* and the red alga *Porphyra yezoensis* were also found. An alignment of six representative Coq7 proteins is shown in Fig. 2. A total of fifteen amino acids are completely conserved, including the four Glu and two His that are predicted to be ligands to a di-iron center (marked with stars in Fig. 2.). Other members of the di-iron carboxylate protein family (reviewed in (21)), which all share this iron-binding motif, include the ribonucleotide reductase subunit R2 (RNR), the hydroxylase subunit of methane monooxygenase (MMO), toluene monooxygenase, the acyl carrier protein \( \Delta^9 \) fatty acid desaturase, rubrerythrin, bacterioferritin, the mitochondrial alternative oxidase (AOX) (22), and the plastid terminal (plastoquinol) oxidase (PTOX) (23). These enzymes catalyze diverse reactions, but among
them are found hydroxylases (MMO and toluene monooxygenase) as well as membrane-bound proteins with quinol binding sites (the AOX and the PTOX). Therefore, the presence of this di-iron motif in Coq7 strongly suggested to us that this protein would be a hydroxylase.

*Complementation of ubiF E. coli*—To demonstrate that Coq7 is capable of the hydroxylation that converts DMQ to 5-hydroxyubiquinone, we cloned the COQ7 gene from the proteobacteria *P. aeruginosa* and *T. ferrooxidans* and constructed N-terminal fusions with MBP and/or TRX. These plasmids were introduced into *E. coli* JF496, which lacks UbiF, a protein unrelated to Coq7, but which catalyzes the conversion of DMQ to 5-hydroxyUQ in *E. coli* (12) (Fig. 1). UbiF has strong homology to *E. coli* UbiE and yeast Coq6, both of which are involved in a previous hydroxylation step in UQ biosynthesis and are predicted from sequence motifs to be flavin-containing monooxygenases (2,12). As *ubiF* *E. coli* contains an anaerobic hydroxylase activity that can bypass the *ubiF* lesion in UQ biosynthesis (24), care was taken to maintain the cultures in an aerobic condition by using small volumes in shake flasks and harvesting the cells well before stationary phase.

The results of the complementation assay on agar plates are shown in Table 1. As expected, there was growth of all cells on the fermentable carbon source, glucose. When succinate replaced glucose, normal growth occurred only for those cells that contained the Coq7 fusion proteins; the empty host vectors expressing only MBP or TRX yielded barely visible colonies. Growth on succinate requires UQ as a substrate for succinate dehydrogenase; although DMQ is redox active and can substitute for UQ with NADH dehydrogenase and the bacterial UQ oxidases, it is not a good substrate for succinate dehydrogenase (11,25). Apparently a very low level of expression of Coq7 is required to complement the *ubiF* mutant, and uninduced expression was sufficient to fully rescue aerobic

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respiration with all three fusions, MBP-Coq7 and TRX-Coq7 \( (P. \text{ aeruginosa}) \), and TRX-Coq7 \( (T. \text{ ferrooxidans}) \).

**Identification of DMQ8 and UQ8** – In order to verify that the complementation of aerobic respiration was due to the synthesis of UQ, liquid cultures of \( \text{ubiF}^{-} \ E. \text{ coli} \) JF496 with and without the Coq7-encoding plasmid pPaCOQ-MALc2M were each harvested and extracted as described in Experimental Procedures. HPLC analysis with an electrochemical detector showed that the non-rescued \( \text{ubiF}^{-} \ E. \text{ coli} \) accumulates a large peak at 16.3 minutes (Fig 3A). On the other hand, \( \text{ubiF}^{-} \ E. \text{ coli} \) expressing the MBP-fusion of \( P. \text{ aeruginosa} \) Coq7 shows a loss of the 16.3 minute peak concomitant with the appearance of a peak at 18.2 minutes (Fig 3C). Upon mass spectral analysis, the former lipid exhibited a molecular ion peak at \( m/z \) 696.4 (Fig 3B) and could thus be identified as DMQ8 (theoretical mass \([C_{48}H_{72}O_{3}] = 696.55\)) as shown previously by Young et al. (26). The more hydrophobic HPLC peak at 18.2 minutes showed a molecular ion at \( m/z \) 726.4 (Fig3D) that corresponds to with the theoretical mass of UQ8 \([C_{49}H_{74}O_{4}] = 726.56\). In accord with the expectation that the endogenous \( E. \text{ coli} \) UbiG immediately methylates the 5-hydroxy-UQ product of Coq7 to UQ, the hydroxyl intermediate is not observed. Although the substrate for the \( P. \text{ aeruginosa} \) Coq7 would normally be DMQ9, Fig. 3 shows that this enzyme is able to utilize the DMQ8 provided by \( E. \text{ coli} \).

**A proposed structure of Coq7** – The high degree of structural conservation between the di-iron carboxylate proteins for which an X-ray crystallographic structure has been solved allows one to model even weakly homologous di-iron proteins with a reasonable level of confidence. Each of the known structures contains a four-helix bundle with particularly long \( \alpha \)-helices that are thought to stabilize the apoprotein prior to iron chelation. Six amino acids contribute to binding the di-iron center, with a glutamate located on the first and third helix,
and an EXXH motif located on the second and fourth helix. The spacing of these residues in Coq7 from \textit{P. aeruginosa} is $E < 29 > EXXH < 48 > E < 31 > EXXH$ (where the numbers in brackets indicate the intervening number of amino acids), which is consistent with a typical four-helix bundle di-iron binding motif (27).

A BLAST search of databases using the Coq7 sequence does not retrieve any of the other di-iron carboxylate proteins, as the sequence identity is much too low. However, manual alignment of sequences using the iron ligands as guides shows Coq7 to be most related to bacterioferritin, followed by ruberythrin, AOX and PTOX (in no readily-definable order). Although the function of bacterioferritin is not certain, its structure has been determined (19). We have modeled \textit{P. aeruginosa} Coq7 on this structure using an alignment of Coq7 with the \textit{E.coli} bacterioferritin and a probable bacterioferritin from \textit{P. aeruginosa} that shows some homology to both proteins. In the resulting structural model (Fig. 4), 14 of the 15 completely conserved residues in Coq7 cluster in a region surrounding the active site.

The insertions in the \textit{S. cerevisiae} Coq7 sequence (Fig. 2) fall in the loop regions of the model, as would be expected for conservation of the structure of the four-helix bundle.

Coq7 has been shown to be an integral membrane protein in the mitochondrial inner membrane (28), and, as expected, in our expression of the two bacterial enzymes the protein is associated with the membrane fraction (data not shown). Hydropathy plots have identified a region between helix 2 and 3 that is consistently hydrophobic through Coq7 sequences, and previously it has been suggested that this region forms a transmembrane helix (28). However, our structural model precludes this possibility, because two helices of the four-helix bundle (and their associated iron ligands) would fall on the opposite side of the membrane. Instead, we propose that Coq7 associates with the membrane as an interfacial integral membrane protein, similar to prostaglandin synthase (29) and squalene cyclase (30). In these proteins a “plateau” of hydrophobic residues interacts with one leaflet of the
membrane bilayer. A rim of positively charged amino acids around this plateau is thought to
interact with phospholipid head groups and stabilize the binding. The surface distribution of
nonpolar and charged amino acids in our model of Coq7 is consistent with this type of
membrane association (Fig. 4B). This mode of membrane-binding has also been proposed in
the di-iron carboxylate proteins AOX and PTOX (22,27).

The active site of the Coq7 model shows a typical di-iron center, with each Fe atom
chelated by one histidine and one terminal carboxylate ligand (Fig 4C). Two glutamates,
E94 and E178, bridge the Fe atoms. A cavity exists from the proposed membrane-binding
surface to the active site, which could allow access of DMQ to the Fe center from the lipid
phase. This would allow the DMQ substrate to be located in the vicinity of the open
coordination sites on the Fe where oxygen is bound and activated, as required for
hydroxylation. In this model DMQ is positioned similar to F208 in RNR. Mutation of this
Phe to a Tyr in RNR results in a suicide reaction in which this residue becomes hydroxylated
(31). Hydroxylation of the native Phe also occurs in RNR in the double mutant
Y221F/E238A, where the Y221 is the site of the long-lived Tyr radical (31).

Two point mutations have been identified in COQ7 that inhibit UQ biosynthesis in
their respective organisms. The point mutation E158K in C. elegans corresponds to E178 in
P. aeruginosa (6). This Glu is completely conserved and serves to bridge the two iron atoms
of the di-iron center. E178 is essential for the formation of a stable di-iron site and it would
be expected that changing this position to a positively charged amino acid would disrupt the
di-iron site and abolish activity. The S. cerevisiae mutation G104D corresponds to position
C66 in the P. aeruginosa sequence (4). C66 is located between the iron ligand E64 and the
completely conserved A67 in what we believe to be the DMQ-binding pocket. It is not
surprising that introducing a larger and negatively charged amino acid in this sensitive area of
the protein would affect the activity.
The similar coordination environment of Coq7 and the other di-iron containing oxidases/hydroxylases suggests that there will be mechanistic similarities in oxygen activation and substrate transformation. In the best-studied di-iron proteins, RNR and MMO, the reaction cycle has been characterized in detail and has been shown to involve high valent iron intermediates. The exact geometry of O₂ activation in these proteins is still an issue of debate, but in the most probable mechanisms, one oxygen atom of the lysed O₂ molecule ends up as an oxygen adduct bridging the two iron atoms (21,32). A reaction path involving a ferryl oxo species as the hydroxylating unit, as has been suggested for MMO, is an attractive scenario for Coq7. However, whereas MMO is unique in being able to activate primary hydrogen carbon bonds, the aromatic quinol substrate of Coq7 would be significantly easier to activate. Therefore, it cannot be excluded that a less reactive reaction intermediate (e.g. a peroxo species) catalyses the initial substrate activation and oxygen insertion step in Coq7.

We have established that Coq7/clk-1 functions as a hydroxylase in the biosynthesis of ubiquinone and have identified it as a di-iron carboxylate protein. In the future, the determination of a high-resolution structure of the enzyme and detection of catalytic intermediates will help to resolve the structure-function relationship of Coq7 and its mechanistic relationship to other di-iron proteins.

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REFERENCES


FOOTNOTES

1 Abbreviations: AOX, alternative oxidase; DMQ, 5-demethoxyubiquinone-8; MBP, maltose binding protein; MMO, methane monooxygenase; PTOX, plastid terminal oxidase; RNR, ribonucleotide reductase; TRX, His-patch thioredoxin; UQ, ubiquinone.

2 Berthold, D. A., Stenmark, P. and Nordlund, P. manuscript in preparation

FIGURE LEGENDS

Figure 1. The final two reactions in the biosynthesis of ubiquinone. The hydroxylation of demethoxyquinone to 5-hydroxyUQ requires Coq7 in eukaryotes and many eubacteria, but is catalyzed by the unrelated UbiF in E. coli. Coq3 and the homologous UbiG (E. coli) catalyze the methylation of 5-hydroxyUQ to UQ. The number of isoprenoid units (n) varies between species, with n=6 for S. cerevisiae, n=8 for E. coli and T. ferrooxidans (33), n=9 for P. aeruginosa (34), and n=10 for Homo sapiens.

Figure 2. Protein sequence comparison of homologues of Coq7/clk-1. The sequence of Coq7 from P. aeruginosa (P.aer, NCBI accession number: AAG04044), T. ferrooxidans (T.fer), Rickettsia prowazekii (R.pro, CAA14656), S. cerevisiae (S.cer, AAB36435) and H. sapiens (H.sap, NP_057222) and clk-1 from C. elegans (C.ele, AAG00035) were aligned using Clustal W (18). The T. ferrooxidans sequence was identified by a BLAST search of preliminary sequence data from The Institute for Genomic Research (http://www.tigr.org) using the R. prowazekii sequence. The positions of the predicted ligands to the di-iron center are indicated with stars. The positions of the
two mutations that affect UQ biosynthesis (E158K in \textit{C. elegans} and G104D in \textit{S. cerevisiae}) are shown with arrows. The numbers in the alignment correspond to the \textit{P. aeruginosa} sequence. Fully conserved residues are shaded black and partially conserved, gray.

Figure 3. Elution profiles and mass spectra of quinones from non-rescued \textit{ubiF} \textit{E. coli} and after rescue with MBP-fusion of \textit{P. aeruginosa} Coq7. Neutral lipid extracts from non-rescued (A) and rescued \textit{E. coli} (C) were analyzed by reverse phase HPLC coupled to an electrochemical detector. B. Mass spectral analysis of the major peak in chromatogram A exhibits a molecular ion (M') that corresponds to the theoretically calculated mass for DMQ8. D. The molecular ion for the major peak in chromatogram C corresponds to UQ8 and differs from the molecular ion of DMQ8 (B) by the molecular mass of a methoxy group ([CH$_2$O] = 30).

Figure 4. A structural model of the \textit{P. aeruginosa} Coq7. A. Side-view of the four-helix bundle of Coq7 shown inserted into the membrane phase (gray). The two Fe atoms of the di-iron center are shown in green, and DMQ (containing two isoprenoid units) is positioned in the proposed substrate binding site. B. Identical view of the space-filling model of Coq7 showing the hydrophobic patch predicted to be responsible for membrane-binding. Non-polar residues are colored gray, positively-charged residues are colored blue, negatively-charged residues are red, and uncharged polar residues are shown in yellow. C. Schematic view of the active site of Coq7, showing the four carboxylate and two histidine ligands to the di-iron center. Two conserved residues from helix 1, A67 and Y71, are shown as well as the proposed position of the DMQ substrate.
Table 1. Complementation in *E. coli* JF496 with Coq7 from *P. aeruginosa* and *T. ferrooxidans*. Cells were plated on M9 agar with the following additions as described in Experimental Procedures. IPTG (0.1 mM) was used to induce the MBP-containing plasmids and 0.1 mM arabinose the TRX-containing plasmids. Relative size of colonies after 36 hours of growth at 37°C are indicated by “+.” Pinpoint colonies that were barely visible are indicated by “0”.

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Figure 1

DMQ \xrightarrow{\text{Coq7 UbiF}} 5\text{-hydroxyUQ} \xrightarrow{\text{Coq3 UbiG}} UQ
Figure 3

A

DMQ

B

696.4

C

UQ8

D

726.4
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