Project 2: Multi-protein complex for ubiquinone biosynthesis: biochemical and structural studies

Group leader: Murielle Lombard

Permanent staff involved in the project (01/10/20):

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Ubiquinone, also called coenzyme Q (CoQ or Q), is a lipid molecule that is part of the bacterial plasmatic membrane where it works as an antioxidant and as an electron carrier in the respiratory channel. Ubiquinone is constituted by a redox-active benzoquinone ring substituted by a polyprenyl chain of 8 units in *Escherichia coli* (Q8), 9 units in the yeast *Saccaharomyces cerevisiae* (Q9) and 10 units in human (Q10) (Fig. 1). Ubiquinone has a crucial role in the functioning of the respiratory channel and in human, primary deficiencies in CoQ as well as mutations affecting the genes of its biosynthesis are associated with diverse severe pathologies (myopathies, renal diseases, cerebellar ataxia, infantile-onset encephalopathy, etc).

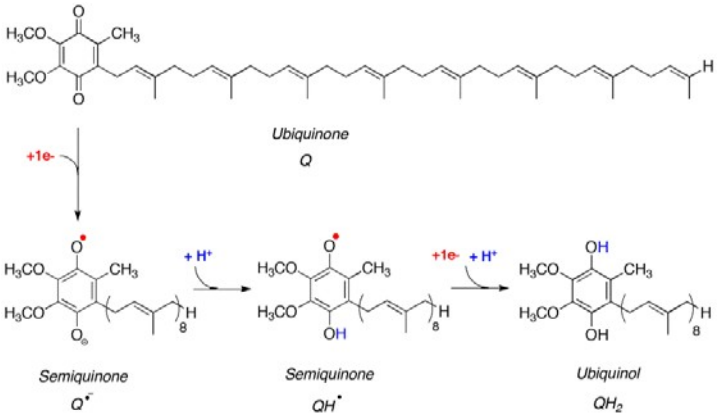

Fig. 1: Structure of ubiquinone and its different redox states

CoQ biosynthesis requires several chemical modifications of its aromatic ring, including one reaction of prenylation, one reaction of decarboxylation, three reactions of hydroxylation, and three reactions of methylation (Fig. 2). In *Escherichia coli*, 9 proteins have been identified as involved in the ubiquinone biosynthetic pathway (UbiA-UbiH, UbiX). Very recently, genetic studies conducted by our collaborators (*Fabien Pierrel*, CEA- Grenoble and *Frédéric Barras*, Marseille) allowed the identification of 3 new proteins: Vis C (UbiI), YigP (UbiJ), and YqiC (UbiK), all of them involved in the ubiquinone biosynthetic pathway. This pathway is very conserved among species, from prokaryotes to eukaryotes.

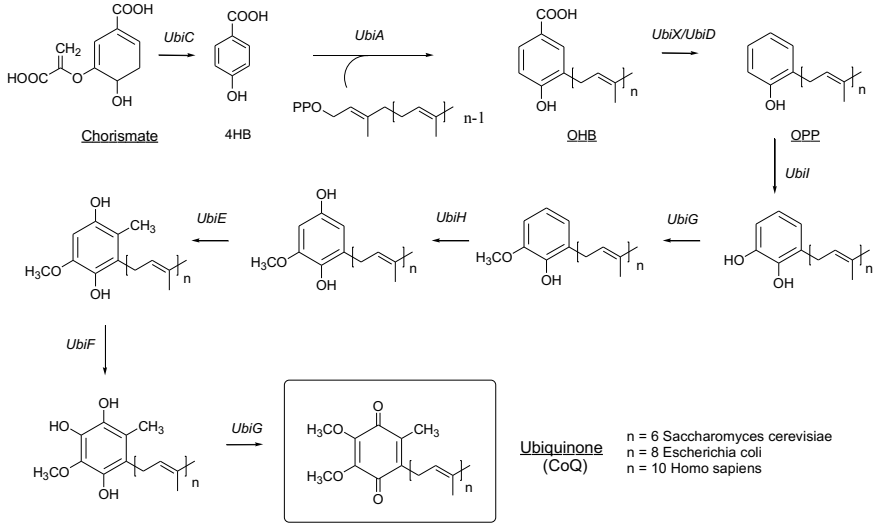

Fig. 2: Proposed ubiquinone biosynthetic pathway in *E.coli*

Moreover, these proteins seem to form a large multi protein assembly, as this is the case with Coq proteins, the eukaryotic homologues of the bacterial Ubi proteins involved in Q9 biosynthesis in the yeast Saccharomyces cerevisiae (Fig. 3). This high molecular complex is probably membrane- anchored.

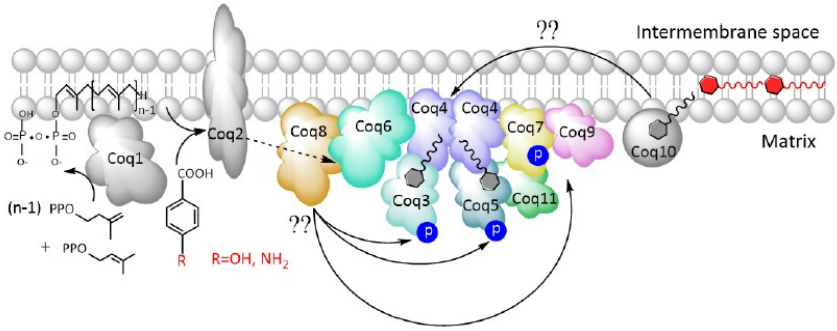

Fig. 3: Proposed model of the biosynthetic CoQ complex in the yeast *S. cerevisae* (Clarke CF, J Biol Chem, 2014).

Despite the biological importance of ubiquinone in the electron transport system and the involvement of the Ubi proteins in the pathologies described above, the function of each Ubi protein is not entirely known. And a number of uncertainties remain about the identification of the protein(s) responsible for each biosynthetic step, as well as the order of the reactions. Moreover, limited information is available regarding the substrate recognition, specificity, reaction mechanisms as well as the three-dimensional structure of these Ubi enzymes. This is partly because the protein themselves have a tendency to form high molecular weight oligomers and the native substrates of these proteins, the membrane-associated hydrophobic poly-isoprenyl aromatic compounds, are not commercially available and need to be synthetized. Furthermore, they are water- insoluble, and this impedes *in vitro* enzyme activity assays as well as determination of the crystal structures of the proteins in complex with the substrates. Moreover, obtaining detailed physico-chemical and structural data on purified proteins that are part of a complex *in vivo* can prove difficult.

We are interested in studying the structure and function of the ensemble of Ubi proteins involved in CoQ biosynthesis in Escherichia coli. We aim to precisely determine the biological role of each Ubi protein or enzyme constituting the Ubi multi-protein complex. The in vitro biochemical characterization of Ubi proteins, coupled to the determination of their 3D-structure, will lead to an understanding of their function and mechanism at the molecular level. We also aim to determine their mode of organization in a high weight molecular complex, in terms of supermolecular structure (exact composition of the protein complex, size, stoichiometry and cellular localization) and their hydrophobic anchor to the membrane (collaboration *F.Pierrel*, UMR5163, Laboratoire Adaptation et Pathogénie des Micro-organismes, UJF Grenoble). Our main goal is to have a deeper knowledge of the E. coli ubiquinone biosynthesis pathway, at the fundamental level. We use a combination of multidisciplinary approaches: biochemistry, molecular biophysics (pressure-perturbation spectroscopy, fluorescence resonance energy transfer, circular dichroïsm, laser flash photolysis, *Djemel Hamdane*) structural biology (X-ray crystallography, *Ludovic Pecqueur*) and organic synthesis (*Philippe Simon*).

We have also initiated the synthesis of ubiquinone biosynthetic analogues, with shorter chains having 3 terpenic units (farnesyl) instead of 8 (Ubiquinone UQ8).

Biochemical and structural study of the flavoproteins UbiI from *E. coli* and Coq6 *Saccharomyces cerevisiae*

We have studied **UbiI**, the enzyme that catalyzes the C5 hydroxylation of 2-octaprenyl phenol (OPP) in the fourth step of ubiquinone biosynthesis. UbiI shares 30 % sequence identity with UbiF and UbiH, two other proteins from the ubiquinone biosynthetic pathway that both catalyze reactions of hydroxylation (C1 hydroxylation for UbiH and C6 for UbiF). UbiI, UbiF and UbiH all are Flavin dependent mono-oxygenases. We purified an unstable form of UbiI that was prone to aggregation. After mild proteolysis, we obtained a soluble and truncated form that was lacking its C-terminal domain. This truncated form of UbiI successfully crystallized and we determined its **crystal structure** (2 Å resolution). It shares high structural similarity with other flavin-dependent monooxygenases, like para-hydroxy benzoate hydroxylase pHBH (Fig. 4). This is the first report of the structure of one Ubi monooxygenase and the first step towards elucidating the active site geometries of these enzymes.

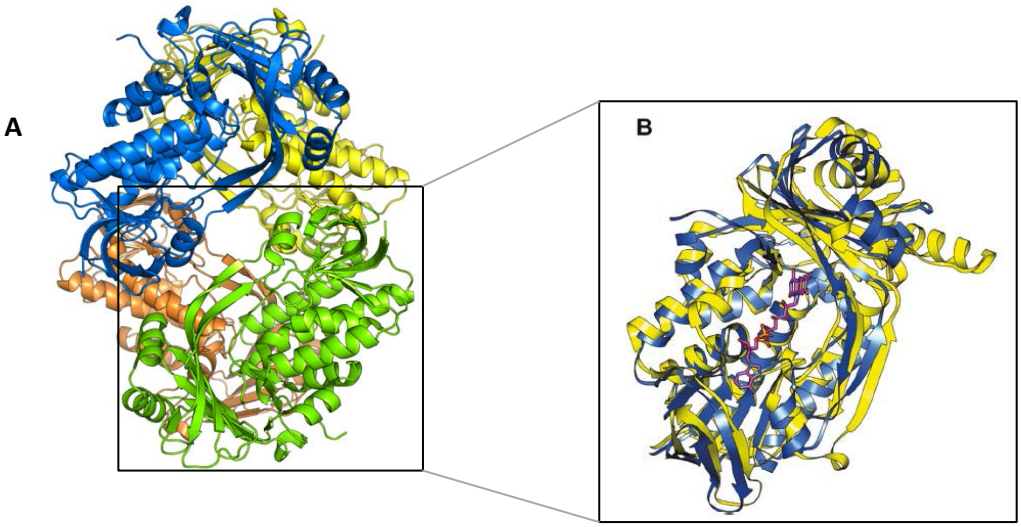

Fig. 4: A- X-ray Three-dimensional structure of an apo and truncated UbiI from *E.coli* (code PDB 4K22); B- superimposition of UbiI (blue) with PHBH (yellow), code PDB 1PBE (Hajj Chehade, 2014).

With the aim of a better understanding of Coq6, we generated a model by homology modelling. Molecular docking and molecular dynamics (MD) simulation studies have been performed to explore the putative binding modes of substrate in Coq6. This study allowed us to show the existence of three putative cavities for the substrate access to the active site (Fig. 5).

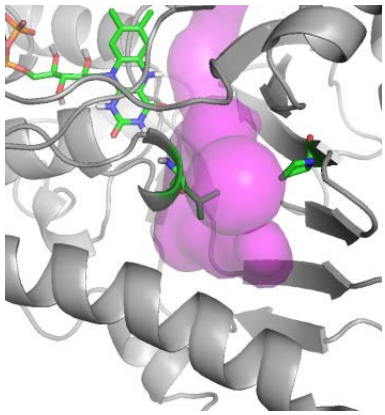

Fig. 5: Proposed model of the active site of Coq6 (FAD in green), with a putative substrate acces tunnel in purple (Caver).

Biochemical and structural study of two scaffold proteins UbiJ and UbiK from *E.coli*

We also started to study **UbiJ** and **UbiK** which are archetypal "new" Ubi proteins. They were not listed among the "Ubi" proteins before our recent studies and they have no predicted enzymatic activity. Previous studies have shown that **UbiK** from S. typhimurium was shown to promote liposome aggregation *in vitro*, suggesting a membrane fusogenic activity. UbiK shares 31 % sequence identity with Brick1, a precursor for the assembly of the Wave cytoskeleton complex, a multi-protein complex that promotes actin polymerization. This raises the possibility of **UbiK** acting as a **scaffold** protein, playing an important role for the formation, the structuration and the stability of the complex. We have purified **UbiJ** and **UbiK** and we have shown that they form a complex and that they would act as accessory factors of ubiquinone biosynthesis. We have shown that UbiJ binds palmitoleic acid, a natural fatty acid in *E.coli* but it also binds ubiquinone.

We recently obtained the crystal structure of the SCP2 domain of UbiJ (1.7 Å resolution), a dimer with a α/β-fold consisting of 5 α-helices and 5 β-strands forming a hydrophobic open cavity where ubiquinone intermediates could bind (Fig.6).

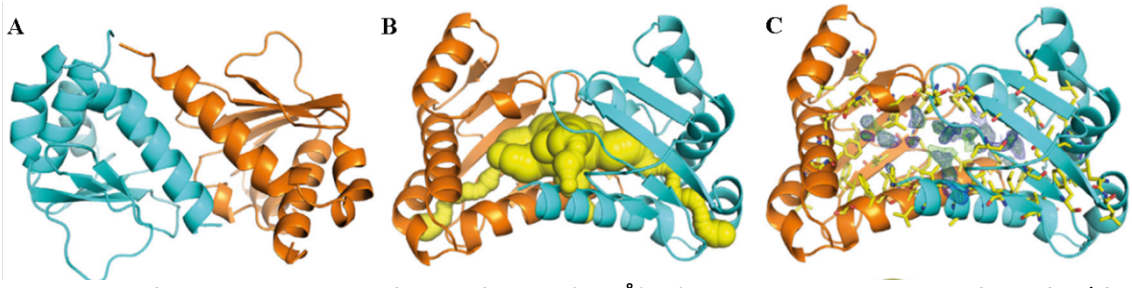

Fig. 6: Structure of the SCP2 domain of UbiJ of E. coli (1.7 Å). A) UbiJ-SCP2 dimer with a fold of α / β type. B) The yellow spheres represent the hydrophobic cavity within the UbiJ dimer. C) Model of the UbiJ-SCP2 cavity with hydrophobic residues bordering the cavity highlighted in yellow and showing an unmodelled electron density suggesting an ubiquinone intermediates lipid binding site.

Demonstration of a mega multi-protein complex of 1.2M Da of 7 Ubi proteins

With our collaborators, we have demonstrated that the UbiJ protein is a central protein for the stabilization of the Ubi mega complex which is in the cytosolic fraction. This megacomplex comprises 7 proteins including 5 enzymes (UbiE, UbiF, UbiG, UbiH, UbiI) and 2 scaffold proteins (UbiK and UbiJ). This complex is found in the cytosolic and non-membrane fraction. We have also shown that synthetic intermediates, such as OPP and DMQ8, accumulate in this complex, whereas little UQ8 is in this complex.

Thus, we can propose a model for the Ubi complex in which the UQ8 biosynthesis intermediates that are upstream of the OPP are not associated with the membrane lipids but with UbiJ at the level of the cytosol in the large Ubi complex (Fig. 6). In this model, the extrusion of OPP from the plasma membrane could be mediated by the UbiB protein. Indeed, recently Coq8, the eukaryotic homolog UbiB, has been postulated as being able to couple the hydrolysis of ATP to the extrusion of the polar heads of UQ8 intermediates out of the membrane. It is interesting to note that UbiB contains a transmembrane segment and a soluble part and that this protein is not part of the Ubi complex.

The results obtained make it possible to establish clearly the existence of a stable metabolon for the biosynthesis of ubiquinone (Fig. 7).

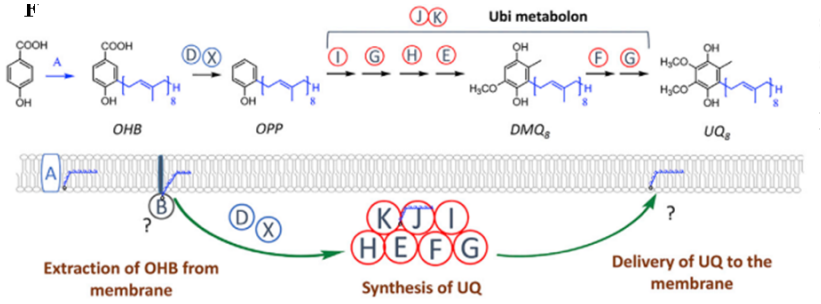

Fig. 7: UQ8 biosynthetic pathway and proposed model of physical organization of Ubi proteins and prenyl intermediates of UQ8 with respect to the inner membrane. UbiB facilitates the extraction of HBOT from the membrane and the OHB is then decarboxylated into OPP by UbiD-X. OPP is bound by UbiJ in the Ubi complex and the metabolite formed by the seven Ubi proteins encircled in red synthesizes UQ8, which is finally delivered to the membrane, where it performs its physiological functions.

Biochemical and structural studies of two Fe-S proteins involved in anaerobic ubquinone biosynthesis in *E.coli*: UbiU and UbiV

We have shown that the dependent and oxygen-independent ubiquinone biosynthetic pathways differ only in three hydroxylation steps, and identified three genes: ubiT, ubiU and ubiV, which are essential for the biosynthesis of ubiquinone in the absence of oxygen in E. coli.

The expression, purification and spectroscopic characterization of UbiV and the UbiU-UbiV heterodimeric complex clearly showed that each protein contained a [4Fe-4S] center. UbiU and UbiV form a heterodimeric complex, suggesting that these two proteins work together (Fig. 8).

The role of Fe-S centers in UbiU and UbiV is unknown at this stage. Our current working hypothesis is that they would act as electron transfer chains between the substrate (the biosynthetic intermediates of ubiquinone to be hydroxylated) and an unidentified electron acceptor required for substrate activation. Together, our results identify UbiU and UbiV as prototypes of a new class of oxygen-independent hydroxylases and open a new door for the study of oxygen-independent hydroxylation reactions.

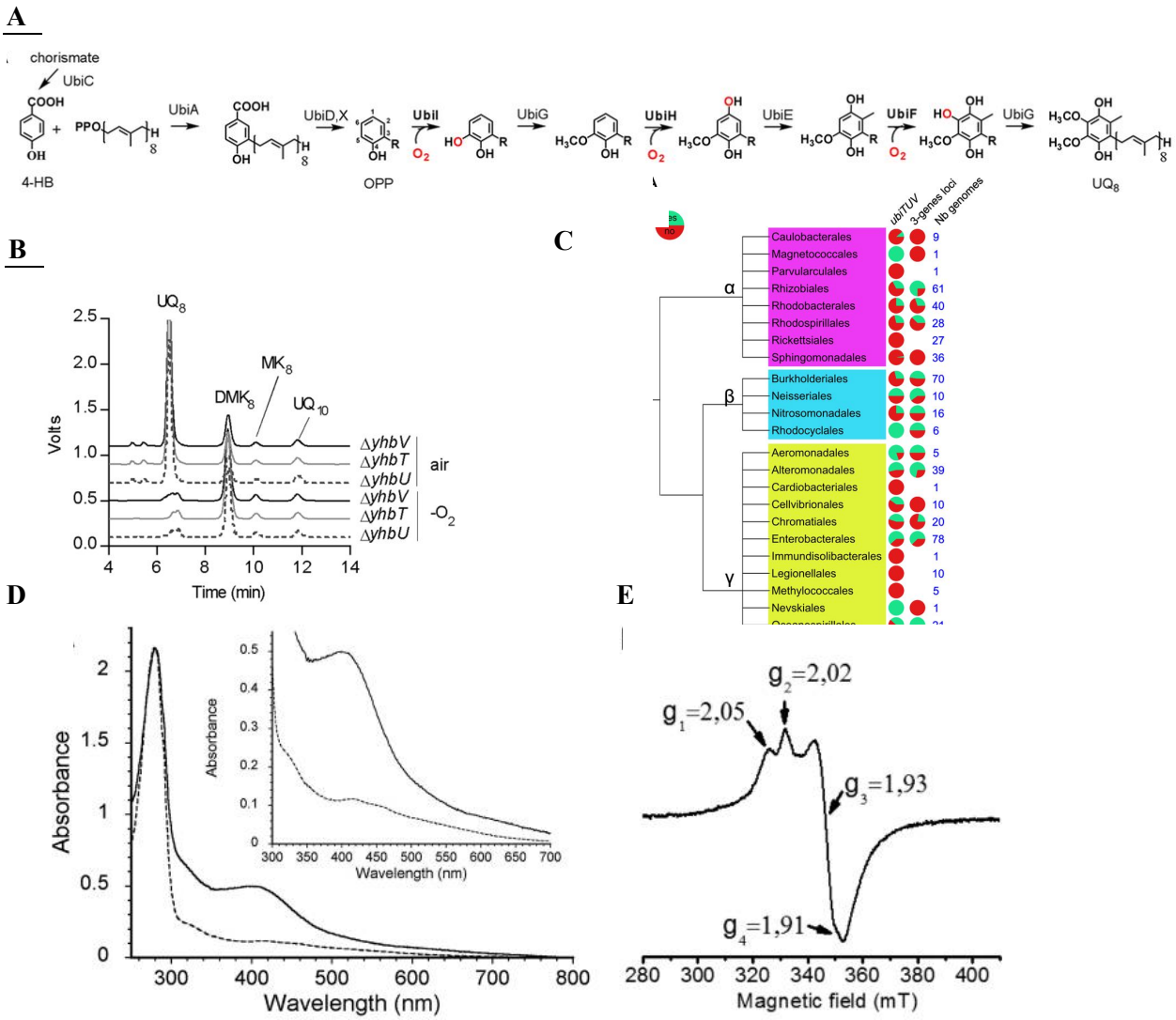

Fig. 8 : A) Pathways of aerobic and anaerobic biosynthesis of ubiquinone dependent. The UbiU proteins UbiV and UbiT intervene in place of the flavin dependent aerobic hydroxylases UbiI, UbiF and UbiH (noted in red here). B) HPLC-ECD analysis of lipid extracts of strains whose ubiU, ubiV and ubiT genes were deleted and grown in air or anaerobically. C) Occurrence of ubiU, V and T genes in 600 genomes of proteobacteria. D) UV-visible absorption spectrum of the UbiU-UbiV complex of E. coli. E) EPR spectrum of the UbiU-UbiV complex of E. coli.

Lab expertises:

- Cell culture : bacteria (*E. coli*)
- Molecular biology and biochemistry: cloning, expression of prokaryotic or eukaryotic recombinant proteins, directed mutagenesis, protein purification
- Enzymology : steady-state and pre-steady state (stopped-flow)
- Optical spectroscopy (UV-visible, fluorescence, CD)
- Characterization of metabolites by mass spectrometry
- Protein Crystallography
- Organic synthesis

Collaborations

- Fabien Pierrel, Laboratoire Adaptation et Pathogénie des Microorganismes, UMR 5163, CNRS, UJF-Grenoble (molecular genetics of the yeast, quinone measurements)
- Frédéric Barras, Laboratoire de Chimie Bactérienne, Microbiolgy Department, Institut Pasteur, Paris (molecular genetics of *E.coli*)

Publications

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