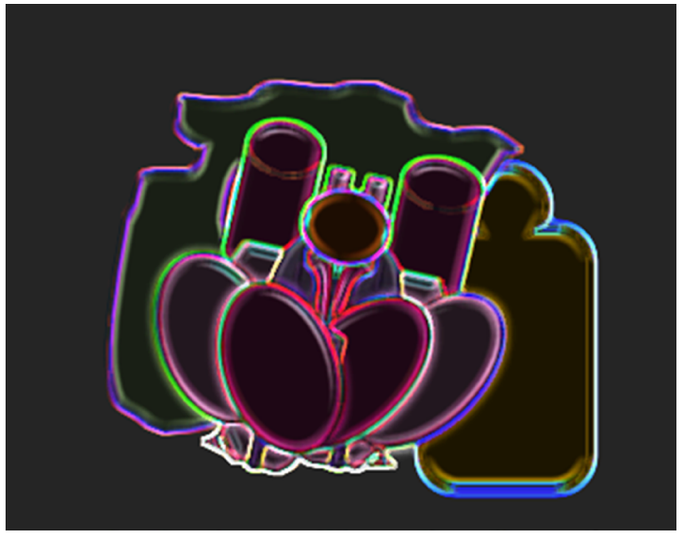
Effect of experimental complex III deficiency on respiratory chain assembly and function

Davoudi, Mina

2014



Schematic illustration of a supercomplex consisting of three respiratory chain complexes I, III and IV.

Print by Media-Tryck | Lund University 2014

MINA DAVOUDI Effect of experimental complex III deficiency on respiratory chain assembly and function

Series 2014:125

Lund University
Faculty of Medicine
Department of Clinical Sciences
Doctoral Dissertation Series 2014:125
ISBN 978-917619-054-8
ISSN 1652-8220

9789176190548

LUND UNIVERSITY
Faculty of Medicine

Effect of experimental complex III deficiency on respiratory chain assembly and function

MINA DAVOUDI
DEPARTMENT OF CLINICAL SCIENCES | FACULTY OF MEDICINE | LUND UNIVERSITY | 2014

Effect of experimental complex III deficiency on respiratory chain assembly and function

Mina Davoudi

LUND
UNIVERSITY

DOCTORAL DISSERTATION
With the permission of the Faculty of Medicine at Lund University, this thesis will be defended on 31 October 2014 at 13.00 in the Segerfalk Lecture Hall, Wallenberg Neuroscience Center, Lund, Sweden

Faculty opponent:

Docent Alexander Kastaniotis

Academy of Finland Research Group Leader
Biocenter Oulu and Department of Biochemistry
University of Oulu, Finland

Organization LUND UNIVERSITY	Document name DOCTORAL DISSERTATION
	Date of issue
Author(s): Mina Davoudi	Sponsoring organization

Title and subtitle: Effect of experimental complex III deficiency on respiratory chain assembly and function

Abstract

The assembly of respiratory chain complexes in the mitochondrial inner membrane requires specific factors. Respiratory chain complex III (CIII) is a dimer consisting of two monomers, each with eleven subunits. To date, seven assembly factors for CIII are known, of which BCS1L incorporates the Rieske iron-sulfur protein (RISP) in the last stage of the assembly. The most severe CIII deficiency, due to a mutation in *BCS1L* (homozygous c.232A>G), is GRACILE syndrome (Growth Restriction, Aminoaciduria, Cholestasis, Iron accumulation, Lactic acidosis, and Early death, MIM 603358).

To clarify the mechanisms of BCS1L-related disorders, especially possible changes in supercomplex formation, the specific aims of this thesis were to investigate CIII assembly and supercomplexes in a mouse model harboring the *Bcs1l* mutation c.232A>G. In homozygotes, the mutation results in a progressive CIII deficiency mimicking the human syndrome. To elucidate the role of the RISP subunit, wild-type mice were exposed to CIII inhibition with myxothiazol administration.

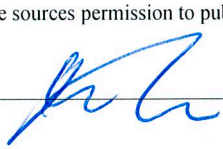
The result showed that complex I can interact with pre-complex III and form a supercomplex in the absence of mature holo-CIII. When RISP was inhibited in CIII by myxothiazol, supercomplex formation was not affected. The supercomplex assembly factor I (*Scafi*) is required for inclusion of complex IV in supercomplexes. Liver metabolomics of the progressive CIII deficiency in homozygous mice showed a starvation-like situation and signs of oxidative stress at the end stage of the disease.

In conclusion, supercomplex formation is a dynamic process that in the case of mutations in BCS1L or supercomplex assembly factor I is modified to incorporate the pre-complex of CIII and an increased amount of complex I to maintain respiratory chain function.

Key words: mitochondria, respiratory chain, supercomplex, assembly factors, BCS1L, GRACILE, SCAFI

Classification system and/or index terms (if any)

Supplementary bibliographical information	Language: English
ISSN and key title: 1652–8220	ISBN :978–917619–054–8
Recipient's notes / Number of pages 115	Price
Security classification	

Signature  Date 24 September 2014

Effect of experimental complex III deficiency on respiratory chain assembly and function

Mina Davoudi

LUND
UNIVERSITY

Faculty of Medicine,
Department of Clinical Sciences, Lund,
Pediatrics
Lund University, Sweden
2014

Front cover picture: Mitochondrion modified by Mina Davoudi

Back cover picture: Supercomplex

Faculty of Medicine, Department of Clinical Sciences, Lund
Pediatrics
ISBN 1652–8220
ISSN 978–917619–054–8
Lund University, Faculty of Medicine Doctoral Dissertation Series 2014:125

Printed in Sweden by Media-Tryck, Lund University
Lund 2014

In memory of Sara, Keshwar, Shamsi and Safiyeh, those who gave me the courage for life!

Contents

List of papers

I. **Davoudi M**, Kotarsky H, Hansson E, Fellman V. Complex I function and supercomplex formation are preserved in liver mitochondria despite progressive complex III deficiency. *PLoS ONE* 2014; 9:e86767.

II. **Davoudi M**, Kotarsky H, Hansson E, Fellman V. Supercomplex formation modified by SCAFI assembly factor and pre-complex III in different mouse strains with a homozygous *Bcs1l* mutation. In manuscript.

III. **Davoudi M**, Kallijärvi J, Marjavaara S, Kotarsky H, Hansson E, Levéen P, Fellman V. A mouse model of mitochondrial complex III dysfunction induced by myxothiazol. *Biochemical & Biophysical Research Communication* 2014; 446: 1079–1084.

IV. Kotarsky H, Keller M, **Davoudi M**, Levéen P, Karikoski R, Enot D, Fellman V. Metabolite profiles reveal energy failure and impaired beta-oxidation in liver of mice with complex III deficiency due to a BCS1L mutation. *PLoS One* 2012; **7**: e41156.

Abbreviations

ADP	adenosine diphosphate
ATP	adenosine triphosphate
BNGE	blue native gel electrophoresis
CI	complex I
CII	complex II
CIII	complex III
CIV	complex IV
DNP	dinitrophenylhydrazone
DNPH	2,4-dinitrophenylhydrazine
FAD^+	flavin adenine dinucleotide
mtDNA	mitochondrial DNA
MIM	mitochondrial inner membrane
MOM	mitochondrial outer membrane
NAD^+	nicotinamide adenine dinucleotide
nDNA	nuclear DNA
RC	respiratory chain
ROS	reactive oxygen species
SCAFI	supercomplex assembly factor I
TIM	translocase of inner membrane
TOM	translocase of outer membrane

Abstract

The assembly of respiratory chain complexes in the mitochondrial inner membrane requires specific factors. Once assembled to form a mature functional complex, complex III (CIII) is a dimer consisting of two monomers, each with eleven subunits. To date, seven assembly factors for CIII are known, of which BCS1L incorporates the Rieske iron-sulfur protein (RISP) in the last stage of the assembly. The most severe CIII deficiency, due to a mutation in *BCS1L* (homozygous c.232A>G), is GRACILE syndrome (growth restriction, aminoaciduria, cholestasis, iron accumulation, lactic acidosis, and early death, MIM 603358).

To clarify the mechanisms of BCS1L-related disorders, especially possible changes in supercomplex formation, the specific aims of this thesis were to investigate CIII assembly and supercomplexes in a mouse model harboring the *Bcs1l* mutation c.232A>G. In homozygotes, the mutation results in a progressive CIII deficiency mimicking the human syndrome. To elucidate the role of the RISP subunit, wild type mice were exposed to CIII inhibition with myxothiazol administration.

The result showed that complex I can interact with pre-complex III and form a supercomplex in the absence of mature holo-CIII. When RISP was inhibited in CIII by myxothiazol, supercomplex formation was not affected. The supercomplex assembly factor I (*Scafi*) is required for inclusion of complex IV in supercomplexes. Liver metabolomics of the progressive CIII deficiency in homozygous mice showed a starvation-like situation and signs of oxidative stress at the end stage of the disease.

In conclusion, supercomplex formation is a dynamic process that in the case of mutations in BCS1L or supercomplex assembly factor I is modified to incorporate the pre-complex of CIII and an increased amount of complex I to maintain respiratory chain function.

Background

Mitochondria

Mitochondria are responsible for cellular energy production. They were discovered in the nineteenth century by Carl Benda who named them after their appearance in a microscope. The term mitochondrion is derived from the Greek *mitos* for "thread" and *chondros* for "granule" (1-3). Mitochondria are thought to be the result of an early symbiosis between aerobic bacteria and anaerobic eukaryotic cells 1–2 billion years ago (4-9). During evolution, the aerobic bacteria increasingly adjusted more and more to their host, and subsequently many of the endosymbiont's genes were transferred to the nucleus (8). However, mitochondria still contain a ring-shaped DNA (mitochondrial DNA, mtDNA) that is translated and transcribed by specific enzymes in the mitochondria.

Mitochondria are located in the cell cytosol where they play a key role in the production of adenosine triphosphate (ATP) by oxidative phosphorylation (OXPHOS). In addition, mitochondria take part in many other functions of mammalian cells including β-oxidation of fatty acids, the Krebs cycle, amino acid metabolism (10), heme biogenesis, detoxification of reactive oxygen species (ROS) (11), and start the chain reaction during apoptosis, or necrosis (12, 13). The number of mitochondria in different cell types varies according to the cells' function and morphology (14). Due to the critical role of mitochondria in cellular energy production and metabolism, deficiencies and dysfunctions in the organelle lead to severe diseases in humans (15).

Structure

Mitochondria are double-membrane organelles approximately 0.5–1 μm in diameter and about 7 μm long. The mitochondrial outer membrane and inner membrane are both phospholipid bilayers with different important proteins embedded (3, 16).

The permeability of the outer membrane allows transport of small nutrient molecules, such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), as well as ions, and water, whereas pre-proteins are imported from the cytosol into mitochondria via the translocase of the outer membrane (TOM).

The inner membrane has a complex structure consisting of a lipid bilayer and protein complexes enclosing the central matrix, which is a protein-rich compartment within mitochondria. The mitochondrial inner membrane consists of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin (17). Due to its high cardiolipin content, the inner membrane is less permeable to molecules and ions than the outer membrane. The transfer of molecules and proteins through the inner membrane requires several specific transfer proteins. The respiratory chain (RC) complexes and supercomplexes are embedded in the inner membrane.

The inner membrane forms cristae -invaginations into the matrix- thereby increasing its surface area, and consequently enhancing the function of RC (12, 18, 19) (Figure 1).

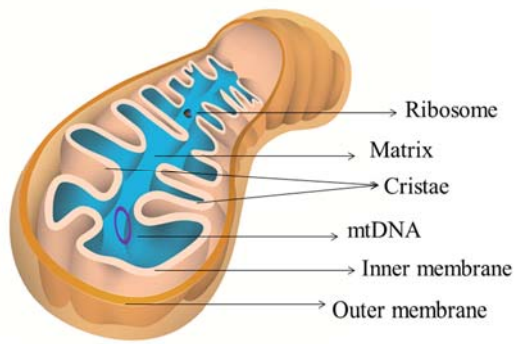


Figure 1. Schematic diagram of a mitochondrion
The mitochondrion consists of outer and inner membranes, an inter-membrane space, cristae, and matrix. Respiratory chain complexes and supercomplexes are embedded in the inner membrane surface, whereas ribosomes and mtDNA are found in the matrix.

Function

Mitochondria produce most of the cellular energy carrier ATP by oxidative phosphorylation (20-22). Nutritional molecules are processed in the cytosol, and fragments of sugars, fat, and proteins are transported into the mitochondrial matrix. There they are further processed to acetyl-CoA by pyruvate dehydrogenase or beta-oxidation. Acetyl-CoA enters the Krebs cycle (also called the citric acid cycle or tricarboxylic acid cycle), a cyclic metabolic pathway with both catabolic

and anabolic functions. In the catabolic pathway, the electron carriers, nicotinamide adenine dinucleotide (NAD^+), and flavin adenine dinucleotide (FAD^+) are loaded with electrons (see Figure 2). As an anabolic function, the Krebs cycle provides important precursors for gluconeogenesis, lipogenesis, amino acid, and porphyrin synthesis (23, 24). NADH and FADH generated in the Krebs cycle transfer their electrons to electron acceptors in RC complexes. The electrons pass through a series of redox reactions along the mitochondrial inner membrane, during which they release energy that is used to pump protons across the inner membrane, creating a pH gradient and an electrochemical potential. The proton gradient provides the proton motive force for production of ATP by F_1F_{0-} ATP synthase using ADP and P_i (20) (Figure 2).

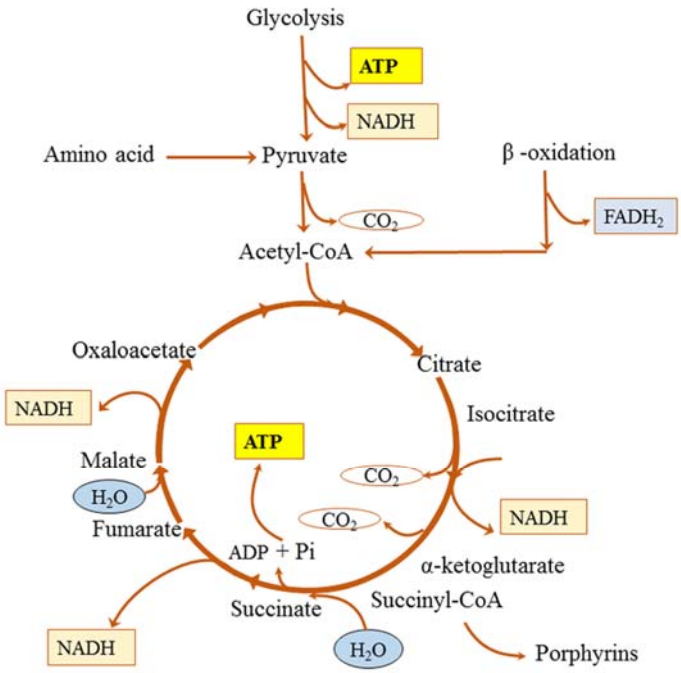


Figure 2. The Krebs cycle
In the Krebs cycle, the catabolic pathway converges at the production of acetyl-CoA. The cyclic pathway generates reducing equivalents (NADH) that are subsequently used as electron donors in the respiratory chain.

The respiratory chain and the OXPHOS system

The respiratory chain (RC) consists of four protein complexes (CI, CII, CIII, CIV) and electron carriers. Electrons are fed into the RC at CI and CII, protons pumped at CI, CIII, and CIV. CIV uses oxygen as an electron acceptor and catalyzes electron transfer to molecular oxygen, thereby forming water molecules (Figure 3).

RC complexes are multiprotein combinations of several subunits, and specific factors are needed for correct assembly, stability, and activity of the holoenzyme (25).

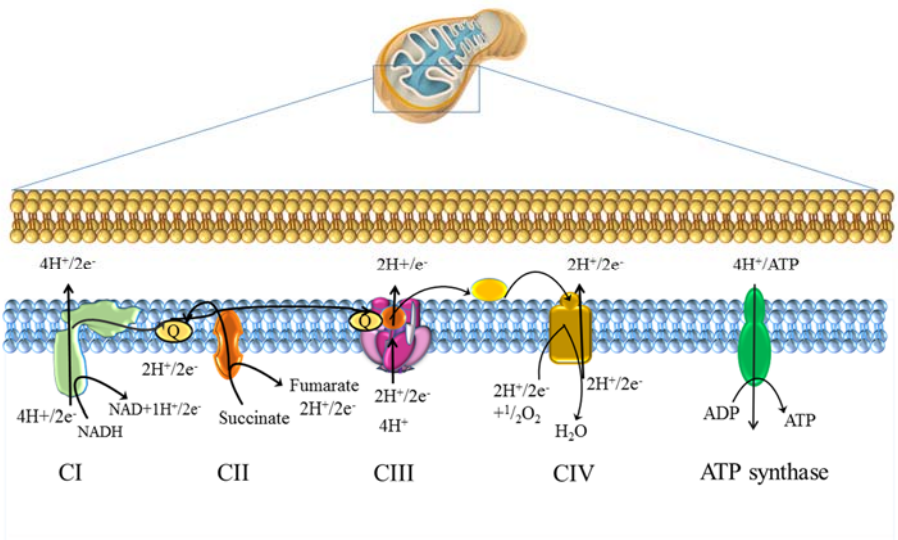


Figure 3. The OXPHOS system
Schematic illustration of the respiratory chain, describing electron flow and proton pumping through the complexes CI, CIII, and CIV. The established proton gradient drives ATP synthesis through the ATP synthase.

The organization of the RC components (protein complexes and electron carriers) has been intensively studied in recent years, and our understanding has evolved with time. The earlier "fluid state model" was based on the concept that single complexes moved freely within the mitochondrial inner membrane lipid layer. It was suggested that the electron transfer takes place by random, transient collisions between the individual RC complexes there (26-28).

New studies and new experimental techniques have challenged the fluid state model with the "solid state" model, first proposed as early as 1955 by Chance (29). This model suggests a stable interaction between the RC complexes and formation of new multiproteins, so-called supercomplexes (or respirasomes) (30).

These supercomplexes (SC) are composed of individual RC complexes associated with one another in different combinations, usually as CI/CIII and CIV, or CIII with CIV (31)**.**

The "dynamic aggregate" or "plasticity" model is the most recent model of the RC (32). The plasticity model suggests that one subset of complexes moves freely while another can be associated with SC formations of different combinations. Recent studies have showed the presence of free RC complexes and supercomplexes at the same time (33) (Figure 4).

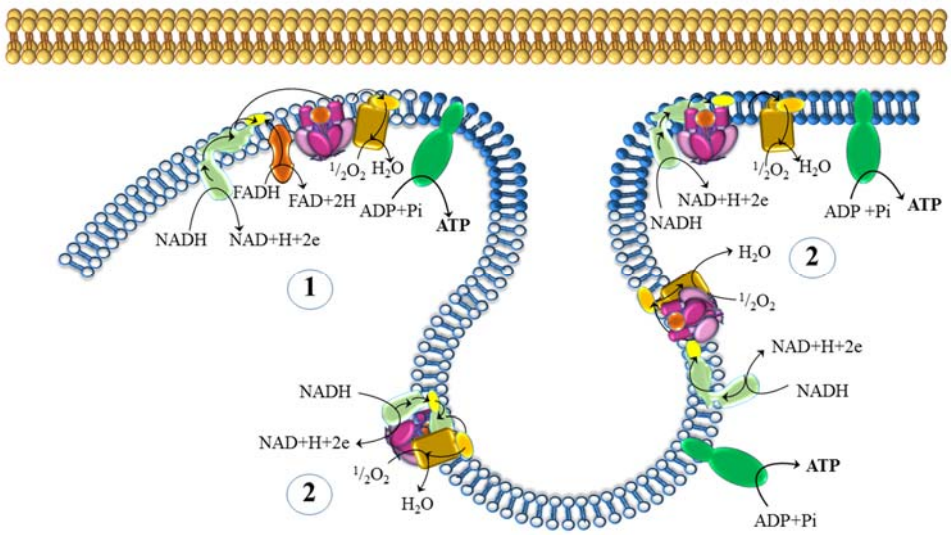


Figure 4. The dynamic aggregate model
RC supercomplex formation in the fluid model (1) and solid model (2).

Complex I

NADH: ubiquinone oxidoreductase; complex I (CI) (EC 1.6.5.3) (34), is the largest RC enzyme complex. CI accepts two high potential electrons from NADH. These electrons pass a series of iron-sulfur centers before they reduce ubiquinone to ubiquinol. The flow of electrons within CI is coupled to the pumping of four protons across the inner membrane. CI consists of forty-four subunits (34-36), of which fourteen (the catalytic core) are highly conserved between species. The other thirty subunits developed during evolution and are involved in the assembly, and stabilization of CI, and regulation of CI activity (36). It has been proposed that the stability and activity of CI are dependent on CIII and CIV (37, 38). One investigation of human and mouse fibroblasts showed that in the absence of CIII, CI is assembled, but is unstable (37). In addition, CI stability has been suggested

to depend on the concentration of ROS, since increased levels of ROS caused destabilization of CI in RISP knockout cells (39). On the other hand, CI has an important role in the stabilization and activity of other RC complexes such as CIV (40) and supercomplexes (41).

Mutations of nDNA- or mtDNA-encoded CI subunits, or of assembly factors for CI, are the main cause of mitochondrial disorder in humans: for example, they cause Leber's hereditary optic neuropathy and Leigh disease (42, 43).

Complex II

The smallest complex in RC is succinate dehydrogenase (SDH), succinate coenzyme Q reductase (SQR) (EC 1.3.5.1), or complex II (CII). CII consists of four subunits (SDH1, SDH2, SDH3, SDH4), which are all encoded by nDNA. CII is localized on the matrix side of the inner membrane and does not pump protons (44). CII has a dual function, as it is a complex of RC and acts as an enzyme in part of the Krebs cycle. NADH produced by the Krebs cycle binds to the CII and releases two electrons, which are transferred to ubiquinone and then to CIII (27, 45). Mutations in CII subunits are uncommon causes of mitochondrial deficiency, and may present as Leigh syndrome, encephalopathy, optic atrophy or neuroendocrine tumors such as familial paraganglioma syndrome (44, 46). In addition, CII has been implicated as an apoptosis sensor (44).

Complex III

Ubiquinol-cytochrome c reductase (EC 1.10. 2.2.) or complex III (CIII) is a dimeric multifunctional inner membrane complex. CIII catalyzes electron transfer from ubiquinol carrying two electrons (CoQH2) to the one electron carrier cytochrome c via the Q-cycle of CIII. Electron transfer is coupled to the pumping of protons across the inner mitochondrial membrane (47, 48). In mammals, the monomer CIII consists of eleven subunits, assembled into a symmetric homodimer molecule ($CIII_2$) with a molecular mass of 500 kDa (49). Ten subunits are encoded by nDNA (two core subunits, Core 1 and Core 2), Rieske iron sulfur protein (RISP), and six other subunits, as well as cytochrome c. Only one subunit, cytochrome b (cyt b), is encoded by mtDNA (47). The nomenclature of CIII subunits in humans is presented in Table 1.

RISP is one of the two catalytic subunits of CIII. The uptake of this subunit into mitochondria and its assembly into CIII has mainly been studied in yeast. The precursor of RISP is synthesized in the cytosol and brought into the mitochondrial matrix by TOM and translocase of inner membrane (TIM). In the matrix, RISP is processed and the iron-sulfur cluster is attached. The mature RISP protein is then

incorporated into a dimer of pre-complex III in the mitochondrial inner membrane by the assembly factor BCS1L. This process is ATP-dependent and an essential step of the CIII assembly (50, 51). Another chaperone, LYRM 7 has been suggested to participate in the final RISP insertion step into dimeric Pre-CIII (52) (Figure 7). A functional and fully assembled $CIII_2$ is essential for stabilization of CI in mammalian cells and other organisms (37, 53, 54). CIII deficiencies so far reported have been caused by mutations in the mtDNA-encoded cyt b, and the nDNA-encoded assembly factors BCS1L and TTC19.

Table 1. The nomenclature of CIII subunits in humans and mice.

Human		Mouse
Protein	Gene	Gene
Ubiquinol-cytochrome c reductase core protein I (Core 1)	*UQCRC1*	*Uqcrc1*
Ubiquinol-cytochrome c reductase core protein I (Core 2)	*UQCRC2*	*Uqcrc2*
Mitochondrially-encoded cytochrome b (Cytochrome b, cyt b)	*MT-CYB*	*mt-Cyb*
Subunit VII (UQCR7)	*UQCRQ*	*Uqcrq*
Ubiquinol-cytochrome c reductase binding protein	*UQCRB*	*Uqcrb*
Cytochrome c–1(CYC1)	*UQCR4*	*Uqcr4*
Subunit VIII (UQCR 8) acidic hinge	*UQCRH*	*Uqcrh*
Ubiquinol-cytochrome c reductase, subunit X	*UQCR10*	*Uqcr10*
Ubiquinol-cytochrome c reductase, subunit XI	*UQCR11*	*Uqcr11*
Subunit IX pre-sequence of RISP	*UQCRFS1*	*Uqcrfs1*
Rieske iron-sulfur protein (RISP)	*UQCRFS1*	*Uqcrfs1*

Complex IV

Cytochrome c oxidase (EC 1.9.3.1 COX) or complex IV (CIV) is the terminal complex in RC. CIV catalyzes the electron transfer from reduced cyt c to molecular oxygen. Mammalian CIV consists of thirteen subunits encoded by both nDNA and mtDNA. The subunit COX3 and the catalytic core of CIV, (COX1, COX2) are encoded by mtDNA (55). The other ten subunits (COX4, COX5A, COX5B, COX6A, COX6B, COX6C, COX7A, COX7B, COX7C, and COX8) are encoded by nDNA and surround the catalytic core. According to several studies, the subunits encoded by nDNA play a regulatory and stabilizing role for the catalytic core (25, 56, 57). In humans, tissue-specific isoforms of CIV subunits have been reported. Thus COX6A and COX7A have a heart and a liver isoform (58). Examples of CIV-related disorders are severe cardiomyopathy and encephalocardiomyopathy. Mutations in three subunits of CIV encoded by mtDNA have been related to human diseases (25, 59).

ATP synthase/complex V

Mitochondrial ATP synthase (ATP synthase, EC 6.3.14, F_1F_o-ATP), also called complex V (CV), uses the proton gradient established in the intermembrane space to catalyze the binding of inorganic phosphate (Pi) to ADP in the mitochondrial matrix, thereby generating ATP. ATP synthase in mammals is a multi-subunit protein complex with two functional units, F_1 and F_o. The F_o part of ATP synthase is composed of eight subunits (a, b, c, d, e, f, g, F6, A6L), which form a proton channel. F_1 consists of five subunits (three α, three β, and a central stalk consisting of γ, δ, and ε) (60) and is the catalytic domain of ATP synthase, directed at the mitochondrial matrix (61). ATP synthase subunits e and g are required for dimerization and oligomerization of the enzyme, which is important for cristae morphology (62).

Electron carriers

The two mobile electron carriers, ubiquinone (Coenzyme Q, CoQ) and cytochrome c (cyt c) serve as links between the complexes in the respiratory chain. Ubiquinone is a quinone molecule which is soluble in lipids, and hence in membranes. It transfers electrons from CI to CIII. Cytochrome c, a heme containing polypeptide, transfers electrons from CIII to CIV.

Supercomplexes

Definition and functional importance

Supercomplexes (SC) were first proposed as existing in a "solid state" within the RC, forming an assembly of flavins and cytochromes surrounded by a protein matrix in the mitochondrial inner membrane (29, 63).

Newer evidence of RC superorganization in mammalian mitochondrial membranes was found using blue native gel electrophoresis (BNGE) (30, 32, 64) and electron microscopy (65). The activity of isolated SC was determined with a Clark-type oxygen electrode, demonstrating the functionality of supercomplexes (32). Recent investigations suggest that individual RC complexes interact with each other in supercomplexes under physiological conditions (66).

The three RC complexes involved in proton transfer across the inner membrane (CI, CIII, and CIV) are more represented in the SC organization than CII, which is often found as a single complex in monomer form (67). Schägger et al. suggested that all monomeric CI is connected to the CIII dimer and variable copies of CIV (as $CI_n/CIII_2$ and $CI/CIII_2/CIV$) (68). In these supercomplexes, a small amount of CII was also detected (32).

The organization of SC in RC may be required for stability and assembly of CI, since several studies have shown that CI was unstable in the absence of CIII (37, 69, 70) and CIV (38, 40). It has also been suggested that supercomplexes limit the production of reactive oxygen species (ROS) (71).

Recently, a dynamic arrangement of SC has been suggested to provide greater flexibility and efficiency of the RC complexes (33, 72).

Reactive oxygen species

Reactive oxygen species (ROS) are chemical agents with the potential to cause oxidative deterioration of DNA, proteins, and lipids. ROS include the superoxide anion (O_2^-), the hydroxyl radical (OH^*), and hydrogen peroxide (H_2O_2), all of which are highly reactive. ROS are released as by-products of RC reactions, and because of their high reactivity they are responsible for oxidative damage, in addition to acting as signaling molecules. There is evidence of an association between ROS and the development of cancer and neurodegenerative diseases. An intracellular increase in production of ROS has been reported in some patients with mutation in *BCS1L* (73, 74).

The generation of ROS leading to lipid peroxidation, induces the reactive aldehydic derivatives such as malondialdehyde, which affects the hepatocytes.

In mitochondrial dysfunction, increased ROS production has been proposed, resulting in apoptosis necrosis, inflammation, fibrosis, genomic polymorphism, and generation of cytokines (75) (Figure 5).

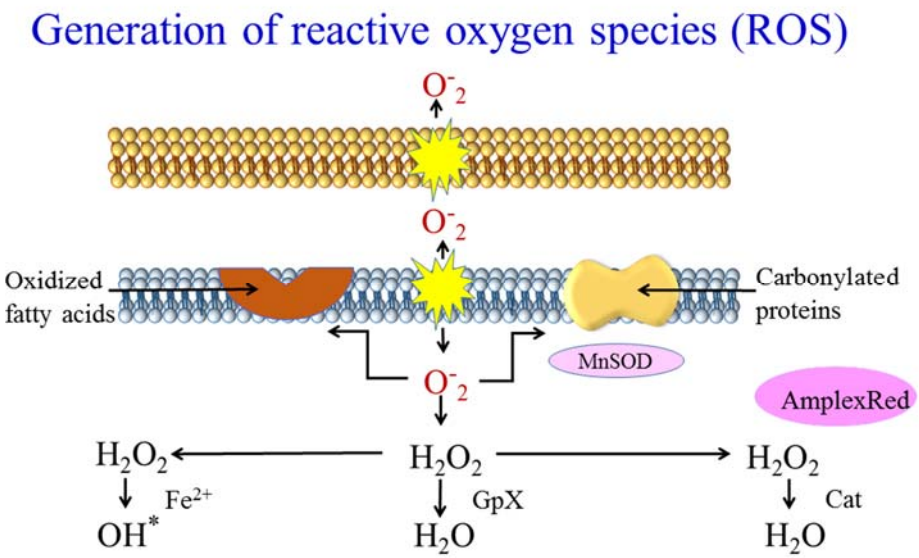


Figure 5. Mitochondrial ROS production
The respiratory chain is the source of reactive oxygen species in mitochondria. The antioxidant defense protecting mitochondria and tissues against ROS include manganese superoxide dismutase (MnSoD), catalase (Cat), and glutathione peroxidase (GpX). In the absence of an antioxidant defence, ROS can modify proteins, lipids, and DNA. ROS can be assessed with Amplex Red and oxidation measured as carbonylated proteins.

Assembly factors for respiratory chain complexes

Assembly of RC multi-protein complexes requires auxiliary factors for the individual complexes and SC to become functional. Those proteins do not participate in enzymatic activity. However, in the case of an absent or mutant auxiliary protein, the complex is not assembled and is therefore dysfunctional (43). Known assembly factors for RC complexes are shown in Table 2.

Table 2. Assembly factors for mitochondrial complexes I–V

Complex	Assembly Factor	Important for assembly of subunit
CI	C20ORF7	Q module intermate, early membrane arm ND1(43, 76-78)
	NUBPL	Periferal arm intermediate, early membrane armNDUFA9
	NDUFAF3	≈400 kDa intermediate, peripheral arm intermediate
	NDUFAF4	≈400 kDa intermediate, peripheral arm intermediate
	NDUFAF1	≈460 kDaintermediate ND2, ND3,ND6, NDUFB6
	ACAD9	≈460 kDa intermediateND2, ND3,ND6, NDUFB6
	C80RF38	Intermediate 460 kDa?
	FOXRED1	Intermediate 830 kDa-HoloCI?
	NDUFAF2	≈830 kDa intermediate, NDUFA1, 2, 10, 13
	NUBPL	Holocomplex I, NDUFV1, 2, 3, NDUFS1, 4, 6, NDUFA12
CII	SDHAF1	Insertion/retention iron-sulfur cluster within (43)
		the protein backbone of CII
	ADHAF2	Incorporation of FAD into SDHA
CIII	HCCS	Synthesis of cyt *c1* and cyt *c* (43)
	TTC19	Early assembly & interact with fully assembled CIII (79, 80)
	PTCD2	Maturation and stabilization of cytochrome *b* mRNA
	UQCC1	Participation in Cytochrome b biogenesis
	UQCC2	Participation in Cytochrome b biogenesis (81)
	BCS1L	Incorporation of Rieske iron-sulfur protein into the CIII (82)
	LYRM7	Maturation and stabilization of Rieske iron-sulfur protein (52)
CIV	SURF1	Formation of early subcomplex COX (43)
	SCO1	Incorporation of copper atoms into the catalytic sites
	SCO2	Incorporation of copper atoms into the catalytic sites
	COX10	Heme A synthesis
	COX15	Heme A synthesis
	COX11	Biosynthesis of heme A
	COX17	Copper recruitment
	COX19	Copper translocation to mitochondria
	LPPRC	RNA metabolism, regulation of mtDNA genes
	TACO1	COX subunit I translation
	C20ORF64	Early step of COX assembly
CV	ATPAF1	F1 assembly factor, for assembly of α+β (43)
	ATPAF2	F1 assembly factor, for assembly of α+β
	TMEM70	F1 assembly factor, for F1 interaction with Fo subunits

Complex III assembly factors

The assembly of CIII (Figure 6) requires factors that catalyze synthesis, binding, and the incorporation of different subunits. To date, seven different assembly factors for CIII have been identified (Table 2). The assembly of CIII starts with the formation of three different subassemblies, (I) UQCR10, CYC1 and UQCRH, (II) UQCRQ, UQCRB and MTCYB, and (III) UQUCRC2 with UQURCR1. These subassemblies are subsequently collected to form a pre-complex III in dimer form, after which UQCR11 and UQCRFS1 are incorporated into the pre-complex (25, 83, 84).

The assembly of CIII requires fully assembled subunits such as cytochrome c1 and cytochrome b. Holocytochrome c synthase (HCCS) catalyzes the binding of heme moieties to apocytochromes c and c1. Ubiquinol-cytochrome c reductase complex assembly factor 1 and 2 (UQCC1/UQCC2) are needed for assembly of the mtDNA-encoded cytochrome b subunit (81). Pentatricopeptide repeat domain protein 2 (PTCD2) is involved in maturation of cytochrome b (85). Thereafter, Tetratricopeptide repeats 19 (TTC19) connects cytochrome c with Core 1 and Core 2 to form an early CIII subassembly (80). It has been suggested that LYR-motif containing protein 7 (LYRM7) stabilizes RISP in the mitochondrial matrix before its insertion into pre-CIII (52, 86). Ubiquinol-cytochrome c reductase synthesis-like (BCS1L) inserts the catalytic subunit RISP into the pre-CIII dimer.

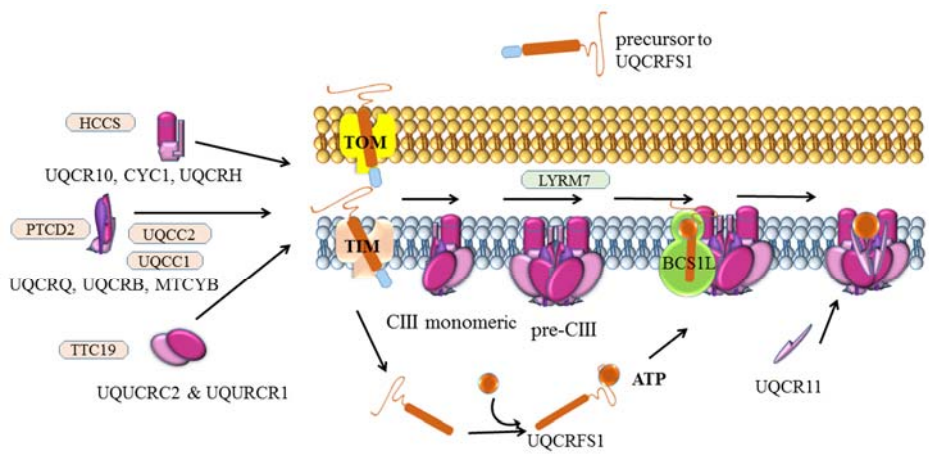


Figure 6. CIII assembly
UQCR10, CYC1, UQCRH + UQCRQ, UQCRB, MTCYB + UQUCRC2 & UQURCR1 assembles to a pre-CIII. A precursor of UQCRFS1 is transported to the matrix via TOM and TIM, then UQCR11 and a mature form of UQCRFS1 are incorporated in the pre-CIII to form a dimer holo-CIII.

BCS1L

The nDNA-encoded *BCS1L* gene is localized to Chromosome 2 and consists of eight exons encoding a protein, which in humans has 419 amino acids. BCS1L (ubiquinol-cytochrome c reductase synthesis-like) has homologues in all eukaryotes, shares 50% identity with yeast Bcs1 and 94% with mouse BCS1L proteins, and belongs to the AAA-ATPase protein family (87). In the BCS1L protein three defined regions can be identified: the N-terminal import sequence, the BCS1L specific region, and the AAA-ATPase region (Figure 7).

The yeast protein Bcs1 is imported into the mitochondrial inner membrane via TOM and TIM translocases (88). The finalization of CIII assembly in yeast was studied by Wagener et al. (89). The precursor of yeast Rieske iron-sulfur protein (Rip) is imported into mitochondria and processed to release the mature sized apo-protein, to which the iron-sulfur cluster is subsequently added. After folding of the C-terminus, Bcs1 recognizes Rip, the iron-sulfur-cluster domain is translocated across the inner membrane, and Rip is released to the pre-CIII complex. Release of Rip is ATP-dependent (89).

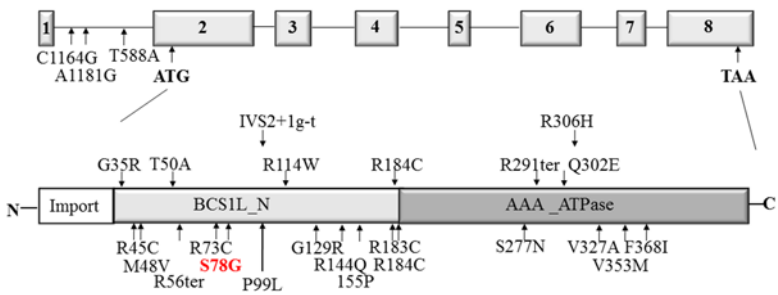


Figure 7. BCS1L gene and protein with mutations indicated.
The gene has eight exons, the corresponding protein sequence with mutations indicated as amino acid exchange. The GRACILE mutation is indicated in red. The illustration is adapted from (90).

BCS1L disorders

Several *BCS1L* mutations have been identified during the last decade (Figure 7) and are now a common etiology for CIII disease (91). GRACILE syndrome, (Fellman disease, MIM 603358), a neonatal disorder, is named after its characteristics: fetal growth restriction, aminoaciduria, cholestasis, iron overload, lactic acidosis, and early death (92, 93). It is caused by a homozygous missense mutation in the second exon of the gene (c.232A>G). The resulting amino acid exchange (p.S78G) leads to an unstable protein that has diminished assembly capacity and thus the incorporation of RISP into the pre-complex of CIII is decreased.

The GRACILE mutation is prevalent in the Finnish population with a frequency of about 1:50,000 live births (94). Several other mutations in *BCS1L* have been found in other populations causing similar disorders presenting in the neonatal period, for example the homozygous mutation p99L resulting in a GRACILE-like disease in Turkey (95), and several combined heterozygous mutations in Spain (96, 97). Common phenotypes due to *BCS1L* mutations include liver disease and proximal tubulopathy, but also encephalopathy, psychiatric disorder, as well as the least severe disorder, Björnstad syndrome which includes congenital deafness and brittle hair but is otherwise compatible with normal life (73, 98, 99) (Table 3).

Table 3. Mitochondrial CIII disorder due to *BCS1L* mutations

Organ system involvement	Disorder	Number of cases	Phenotype	Country
Mainly visceral	GRACILE MIM#603358	>34	Growth restriction, aminoaciduria, cholestasis, iron overload, severe lactic acidosis and early death	Finland(92) Sweden (100)
Exclusively neurological		1 1	Lactic acidosis, failure to thrive, encephalopathy, hypotonia, deafness	Italy Morocco (96)
Visceraland neurological GRACILE-like		>6 >3 >2 3	Lactic acidosis, aepatopathy, Hypotonia, Iron overload +/- tubulopathy	Turkey (101) Spain(97, 102) New Zealand(103) British (104)
Neuro-psychiatric		>2		Saudi Arabia(98)
Skin and ear	Björnstad MIM#262000		Congenital neurosensory hearing loss and distorted hair	(105) (73)
Muscle weakness		1	Hypotonia, progressive visual impairment and exercise fatigue	(97, 106)

Supercomplex assembly factor I

Recent investigations have suggested that the previous fluid model of RC complexes should be replaced with a SC model, where the SC facilitate interactions between the individual complexes. The structure and function of supermolecules have been studied with various techniques. The presence of cardiolipin (107-109) and specific proteins, or "assembly factors", are required for assembly and stabilization of SC (33, 110). Investigations in yeast and mammalian mitochondria have revealed important SC assembly factors needed for their formation (111-113) (33, 110). Recent studies have indicated that SC assembly factor I (SCAFI, Cox7a2l, COX7RP) is crucial for the association of CIV into SC formation with CI and CIII (33).

The present investigation

General objectives

The objectives of this study were

- To analyze the effect of progressive CIII deficiency on respiratory chain complexes and their function in a GRACILE mouse model
- To investigate the supercomplex assembly factor, SCAFI
- To study the effects of biochemical CIII inhibition in mice
- To study the effects of progressive CIII deficiency on liver metabolism

Specific aims

1. To investigate if supercomplex formation is impaired when CIII assembly is increasingly deficient with increasing age in *Bcs1l*$^{G/G}$ homozygous mice
2. To assess if the short variant of SCAFI affects SC formation and survival in *Bcs1l*$^{G/G}$ mice with CIII assembly deficiency
3. To assess if inhibition of RISP with myxothiazol mimics the CIII dysfunction in *Bcs1l*$^{G/G}$ homozygous mice and if it affects SC formation
4. To assess metabolic changes and ROS involvement in the GRACILE mouse model

Materials and methods

Materials

Animals

Transgenic mice with the mutation c.232A>G in the *Bcs1l* gene were studied. The gene construct was introduced into 129/Sv embryonic stem cells by standard gene targeting techniques (114). Mice homozygous for the mutation (*Bcs1l*$^{G/G}$) with mixed genetic background (129/svEvTaC x C57BL/6) as well as backcrossed to congenic C57Bl/6 background were included in the studies. Wild-type (*Bcs1l*$^{A/A}$) or heterozygous (*Bcs1l*$^{A/G}$) mice from the same litter were used as controls (Table 4).

Mixed-background mice (129Sv x C57Bl/6) were used in papers II and IV, congenic C57Bl/6 mice were used in papers I and II, and wild-type C57BL/J6 in Paper III. Mice used in papers I, II, and IV were maintained on rodent diet/Labfor R34, Lactamin, and in Paper III they were maintained with Harlan 2918 Teklad Global 18% protein Rodent diet with water available *ad libitum* in a vivarium with a 12 hour light/dark cycle. Mice were sacrificed by cervical dislocation and the liver tissue sampled for isolation of mitochondria, deep-freezing, and histology.

Table 4 Mouse strains used in the various papers

Paper	Number of Mouse	Background	Genotype
I	46	C57Bl/6 x C57Bl/6	*Bcs1l*$^{A/A}$, *Bcs1l*$^{A/G}$, *Bcs1l*$^{G/G}$
II	26	C57Bl/6 x C57Bl/6 129/Sv x C57Bl/6	*Bcs1l*$^{A/A}$, *Bcs1l*$^{A/G}$, *Bcs1l*$^{G/G}$ *Bcs1l*$^{A/A}$, *Bcs1l*$^{A/G}$, *Bcs1l*$^{G/G}$
III	24	C57Bl/J6 x 57Bl/J6	Wild type
IV	54	129/Sv x C57Bl/6	*Bcs1l*$^{A/A}$,*Bcs1l*$^{A/G}$ *Bcs1l*$^{G/G}$

Myxothiazol

Myxothiazol is a class I inhibitor of CIII directed at the ubiquinol oxidation sites targeting the proximal domain of Qo (115). Myxothiazol was administered intraperitoneally to induce CIII inhibition *in vivo* (Paper III). The extract from mycobacteria was received from Professor Rolf Muller of the Helmholtz Centre for Infection Research, Braunschweig, Germany.

Methods

Isolation of liver mitochondria

Mouse liver tissue was homogenized in an ice-cold homogenization buffer. Mitochondria were subsequently purified by sequential centrifugation including a density gradient in 19% Percoll (116, 117). The concentration of mitochondrial protein was measured via its absorbance at 280 nm using a NanoDrop. The isolated mitochondria were used immediately for high resolution respirometry or stored at –80 °C for determination of CIII activity or purification of RC complexes/SC.

Extraction of respiratory chain complexes and supercomplexes

Frozen mitochondria pellets were re-suspended in phosphate buffered saline (PBS) supplemented with complete mini protease inhibitor. Subsequently, mitochondrial membrane proteins were extracted by incubation with 0.8% digitonin, mixed with the sample, and stored at –80 °C for further use.

Blue native gel electrophoresis

RC complexes and supercomplexes were separated by blue native gel electrophoresis (BNGE) on a 4–16% Bis-Tris gel according the method of Schagger et al. (118). After electrophoresis the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot^{TM}Dry blotting system. Blocking of the membranes was followed by detection of RC complexes and supercomplexes using antibodies for individual complex subunits, followed by incubation with an HRP-coupled secondary antibody. Antibody binding was visualized by chemiluminiscence.

2D BNGE/SDS-PAGE followed by Western blot

To investigate supercomplex composition, a 2-dimensional (2D) electrophoresis technique was used. First, supercomplexes were separated on BNGE in the first dimension. Subsequently, the BNGE was cut into lanes, denaturized, and run in a second dimension (SDS-PAGE) to separate individual RC complex subunits (118). Subunits were identified by specific antibodies in Western blot.

Determination of respiratory chain oxygen consumption by high resolution respirometry

Mitochondrial respiration was measured by high resolution respirometry, which is based on kinetic determination of oxygen consumption by polarographic oxygen sensors in a small amount of mitochondria suspension (119). Freshly isolated liver mitochondria were re-suspended in MIR 05 buffer and loaded in the chamber of an Oroboros oxygraph–2K analyzer (Oroboros Instruments, Innsbruck, Austria). Oxygen consumption was determined in the mitochondria using the substrate-uncoupler-inhibitor titration (SUIT) protocol at 37 °C (119). Substrates and inhibitors to each individual RC complex were added stepwise to the mitochondrial suspension in the chamber. The data were analyzed with DatLab 4 software (Oroboros Instruments, Innsbruck, Austria).

Determination of CIII activity

Complex III activity was measured as the electron flux from decylubiquinol through complex III to cytochrome c. Reduction of cytochrome c was determined spectrophotometrically by measuring the absorbance at 550 nm (120). Complex III activities were calculated as the increase of absorbance per second from the linear part of the curve during the first minute of reaction, and corrected for non-enzymatic reduction of cytochrome c by subtracting the absorbance values of antimycin-treated mitochondria for each sample. Relative complex III activity for each *Bcs1l*$^{G/G}$ sample was expressed as a percentage of control activity.

Determination of carbonyl groups as biomarkers for oxidative stress

Reactive oxygen species (ROS) may cause protein oxidation, which can be detected by measuring carbonyl groups. The presence of carbonylated proteins in mitochondria was analyzed with an Elisa method. Mitochondria were incubated with 2,4-dinitrophenylhydrazine (DNPH), which interacts with carbonyl groups to form a stable dinitrophenylhydrazone (DNP). DNP formation was detected by an anti-DNP antibody, followed by incubation with a secondary antibody and substrate O-phenylenediamine, and absorbance at 490 nm was measured with a spectrophotometer (121).

RNA preparation and quantitative PCR

RNA was extracted from frozen liver tissue using a QIAGEN, RNA extraction kit. RNA quantity and quality were determined by NanoDrop and gel, respectively, before reverse transcription into cDNA using Taqman® reverse transcription reagents from Applied Biosystems. The resulting cDNA was analyzed in quantitative PCR using Taqman® gene expression assays.

Ethics statement

Animal experiments were performed according to Swedish national guidelines with the approval of the Lund regional research ethics committee (Permits M170–06, M158–08, 31–8265/08; M253–08, M274–10, 31–85/08; M253–08, M245–11), and the approval of the Research Animal Ethics Committee of Southern Finland (ESAVI–2010–07284/Ym–23).

Results

Here only a summary of the main results of each paper is given. For a detailed discussion, please see the individual papers.

Paper I

Complex I function and supercomplex formation are preserved in liver mitochondria despite progressive complex III deficiency

This study focuses on the effect of CIII deficiency on RC complex and supercomplex assembly.

Localization of RISP in the liver cells

A comparison of samples from liver tissue homogenate, cytosol, isolated mitochondria, and mitochondrial membrane using SDS-PAGE revealed the presence of RISP in homogenate, isolated mitochondria, and mitochondria membranes. The assay showed a reduced RISP content in homozygous (*Bcs1*$^{G/G}$) mice in all three preparations compared to wild-type animals.

RISP and CIII$_2$ formation at different ages in Bcs1l$^{G/G}$

The BNGE showed that the RISP content in young homozygotes was comparable to the controls, but the RISP incorporation into CIII$_2$ reduced progressively after P16. In the final stage of the disease, RISP was almost lacking, but this did not affect the other RC complexes.

Supercomplex organization and formation in homozygous Bcs1l$^{G/G}$ *mice*

In absence of RISP, a pre-complex structure replaced holo-CIII$_2$ in supercomplex composition with CI, while the amount of free CI and free pre-CIII$_2$ were increased compared to the controls.

With 2D-BNGE, we found that both mature and pre-complex CIII$_2$ participate in SC formation with CI.

The function of RC in homozygous mice liver mitochondria

Respirometry showed that in the presence of CI substrates (malate, pyruvate) and ADP, the liver mitochondria of homozygous mice had a higher oxygen consumption than the control mitochondria, but after addition of CII substrate (succinate), oxygen consumption was decreased. The maximal capacity electron flux in RC, assessed after addition of FCCP, was lower in mutant than in control mice. After adding TMPD as substrate for CIV, oxygen consumption was similar in mutants and controls.

Gene expression in homozygous and control animals

We assessed the expression of the mRNA level of *Bcs1l*, CIII subunits *Uqcrc1*, *Uqcrc2*, and *Uqcrcrfs1*, CI subunits *Ndufa9* and *Ndufv1*, CII *Sdhb* and CIV *Cox5* in sick homozygous mice and controls by qPCR. CI and CII subunits were significantly increased in homozygous mice.

Paper II

Supercomplex formation modified by SCAFI assembly factor and pre-complex III in different mouse strains with a homozygous Bcs1l mutation

As the commonly used mouse strains 129sv and C57Bl/6 have different *Scafi* alleles (33) we aimed to analyze supercomplex formation in liver mitochondria of *Bcs1l*$^{G/G}$ mice with different genetic backgrounds: mixed 129sv x C57Bl/6, and congenic C57Bl/6, respectively.

Assessment of Scafi allele in different mouse strains

Scafi allele assessment (short/long allele) showed that the 129/sv strain was homozygous for long *Scafi* (intact allele/intact allele) while homozygous mice with mixed background were heterozygous for *Scafi* (intact allele/deleted allele), and homozygotes of congenic C57Bl/6 strain were homozygous for the short *Scafi* allele (deleted allele/deleted allele).

Composition of RC complex and supercomplexes in different mouse strains

BNGE and 2D-BNGE analyses showed that SC containing CI/$CIII_2$ occurred in all strains, but CIV was only present in SC (CI/$CIII_2$/CIV and $CIII_2$/CIV) in mice of the 129/sv strain and in homozygotes with mixed genetic background. Further, in homozygotes of mixed background, pre-$CIII_2$ was combined with CI and CI/CIV, but only with CI in congenic mutants.

We found no association between the CIV participation in supercomplexes and life span in *Bcs1l*$^{G/G}$. Thus the severity of disease was not dependent on the *Scafi* allele.

Paper III

A mouse model of mitochondrial complex III dysfunction induced by myxothiazol

Here we induced an isolated CIII deficiency in order to elucidate possible differences in liver mitochondria between a decreased amount of RISP (GRACILE mouse model) and inhibited RISP function in CIII.

Mouse phenotype

Myxothiazol dosage was designed to cause sufficient CIII inhibition without causing deterioration in the mice. No abnormal behavior or health problems were observed in the animals.

Decreased CIII activity by myxothiazol

Myxothiazol administration induced a reversible CIII inhibition in liver mitochondria 2 hours after the injection. The activity of CIII decreased to 50% of that of sham-treated animals.

Liver phenotype and histology of the liver tissue

Liver histology from the myxothiazol-exposed mice showed only slight changes compared to the control animals.

RC complex and supercomplex formation

BNGE and 2D-BNGE assays showed that RC complex and SC assembly were unaffected by the inhibition of CIII with myxothiazol. The incorporation of RISP into CIII was similar to that in control animals.

Hepatoxicity and inflammation in mice exposed to myxothiazol

We analyzed the expression of genes related to inflammation and hepatoxicity, as well as CIII subunits with qPCR in mice exposed to myxotiazol for 74 hours. No significant changes were found in the gene expression.

Paper IV

Metabolite profiles reveal energy failure and impaired beta-oxidation in liver of mice with complex III deficiency due to a Bcs1l mutation

In this paper we investigated the impact of progressive CIII deficiency on the metabolic function of mouse livers.

Age dependent effects on metabolites and oxidative stress in homozygous mice

The metabolite profiles were similar in young homozygous and wild type animals. With increasing age, and at onset of the disease, the metabolite profile changed, showing increased level of the long chain acylcarnitines, AMP, bile acids, amino acids, and biogenic amines. A starvation-like condition appeared in the metabolites with energy depletion and decreased levels of glycogen and glucose, impaired beta-oxidation, and reduced ATP production at the end stage of the disease.

Metabolites indicating oxidative stress, such as methionine-sulfoxide and prostaglandins, were found at the end stage of the disease, but not at the disease onset. Further analyses of reactive oxygen species (hydrogen peroxide assessed with Amplex Red) did not show differences between homozygotes and litter mate wild type animals. In the young homozygotes no increased antioxidative defense was found; however, in sick mutant animals, manganese superoxide dismutase and catalase were down-regulated and glutathione peroxidase 3 was up-regulated.

Discussion

Over the last decade an increasing number of assembly factors for RC complexes and SC have been identified, and thus a variety of mitochondrial disorders have been found which are caused by mutations in these factors (81). After the initial reports on *BCS1L* mutations (94, 101), many new mutations have been found in the gene, leading to variable phenotypes. The typical Finnish mutation (c.232A>G) with genotype-phenotype consistency is located in the N-terminal import auxiliary sequence of the gene and causes the most severe form of the disorder (92). Several other mutations in adjacent gene regions cause CIII deficiency, suggesting that this part of the protein is crucial for BCS1L stability or function. BCS1L belongs to the AAA family of proteins, in which a common AAA motif enables ATP binding and hydrolysis for remodeling of substrate proteins (122). AAA proteases usually form hexameric complexes, which have also been detected for BCS1L (73, 123). Further, Hinson et al. suggest that in patient lymphocytes with mutations causing severe CIII deficiency, BCS1L lacks ATP binding or hydrolyzing capacity, thereby preventing the formation of higher molecular weight BCS1L complexes (73). In GRACILE patient fibroblasts, decreased levels of BCS1L were found in mitochondria, and levels of hexamers and the high molecular weight complex were slightly reduced, without, however, having an impact on RISP incorporation into CIII (124). In contrast, in GRACILE patient liver tissue, BCS1L and RISP levels were diminished, suggesting that there is a tissue-specific effect of BCS1L on CIII assembly. One tissue-specific feature is energy production and consumption. Compared with fibroblasts, hepatocytes have a considerably more intensive metabolism because they are the metabolic hub in mammals. A recent study by Ostojic et al. suggests that the energetic state of mitochondria modulates CIII synthesis through the ATP-dependent activity of Bcs1 in yeast (125). Thus, since liver is consuming a great deal of energy to provide other tissues with fuel, ATP levels in liver could fall to a level that no longer supports efficient CIII assembly, thereby starting a vicious circle. Therefore, to study CIII deficiency due to BCS1L mutations, the liver is the organ of choice.

In our mouse model of GRACILE syndrome, we see a progressive lack of RISP incorporation and concomitant CIII deficiency in several organs, but the main deterioration occurs in the liver (114). Interestingly, despite diminished levels of BCS1L from birth in *Bcs1l*$^{G/G}$ mice, RISP is incorporated into CIII until postnatal day fourteen, implying the presence of an additional CIII assembly factor in mice.

Whether BCS1L has another function in addition to being an assembly factor is unclear, but other AAA-family proteins, such as the mitochondrial LON protease, do have multiple functions. LON participates in quality control of mitochondrial proteins, regulates RC activity and synthesis, and influences mtDNA replication and transcription (126).

Many other AAA proteins have homologues in prokaryotes, whereas BCS1L homologues are only present in eukaryotes, implying co-evolution of BCS1L with mitochondria (50).

Experimental models are needed to elucidate the functions of proteins and disease mechanisms caused by their mutations. With the exception of CI, which is absent in yeast, many mtDNA- and nDNA-encoded mitochondrial genes are conserved between yeast and humans. The relative ease with which yeast cells may be manipulated, the presence of common metabolic pathways, and the ability to survive on fermentable carbon sources, make yeast a valuable model for analysis of human mutations (127). Thus, yeast has frequently been used to verify the impact of patient BCS1l mutations (94, 101, 128). Instead of yeast, patient fibroblasts can be used to investigate how mutations affect RC. However, as mentioned above, the effect of certain mutations might be tissue-dependent, thus requiring an analysis of the target tissue. Although yeast and fibroblasts can be used to demonstrate the impact of a mutation on respiratory chain activity, they cannot be used to discover how a particular mutation affects the multi-organ system of the human body. Therefore, transgenic mouse models for the study of nDNA-encoded mitochondrial proteins have been developed (129, 130). Due to the conserved nature of mitochondrial proteins, global knockouts are often embryonically lethal (131) and conditional knockouts do not always result in similar phenotypes (39). In addition, genetic differences between different mouse strains need to be taken into account (132) when assessing the phenotype, as well as the effects of environmental factors (133).

Two transgenic mouse models assessing CIII function are currently available:, a conditional knockout of the RISP protein in neurons (39); and the GRACILE mouse model with the human c.232A>G mutation introduced into mouse *Bcs1l*. There are no known human mutations in the human RISP gene (*UQCRFS1*), indicating that RISP deficiency is incompatible with life. Mice with conditional neuronal *Risp* knockout developed as controls until a sudden death after three months of age. Their brains, however, displayed extensive oxidative stress, especially in the piriform cortex (39). The introduction of the GRACILE mutation into mouse *Bcs1l* resulted in growth restriction, progressive liver disorder, and early death in homozygous $Bcs1l^{G/G}$ mice, a phenotype corresponding to the human disease (114).

In this thesis, the GRACILE mouse model was used to assess the molecular pathology of the GRACILE syndrome with investigations on mitochondrial SC

formation and composition in relation to the progressive CIII deficiency (Paper I and Paper II). Furthermore, the impact of progressive CIII deficiency on mouse liver metabolism was analyzed (Paper III).

In fibroblasts, deficient CIII assembly may influence CI stability and SC formation (69). In a recent study in human cancer cells, CI and respirasome assembly were studied after inhibition of mitochondrial translation (41). This study suggested that in human cells, a CI assembly intermediate acts as a scaffold for supercomplex assembly by accepting individual CIII and CIV subunits. The start of SC formation requires assembly of CIII and CIV to a certain threshold, thereafter leaving free CIII and CIV subunits to interact with the CI assembly intermediate. In contrast, in GRACILE mice, CI assembly and function, as well as supercomplex formation, were preserved (Paper I), despite deficient CIII assembly. In addition, pre-$CIII_2$ was found in supercomplexes of homozygous *Bcs1l*$^{G/G}$ mice where it probably compensated for the lack of $CIII_2$, thus preserving RC functionality. The formation of pre-$CIII_2$/CI SC was also observed in RISP knockout cells (39). In addition, these authors demonstrated that CI and SC assembly are sensitive to reactive oxygen species (ROS). Observed differences in CI stability and SC assembly between models might thus be ascribed to different oxygen and ROS levels in cell culture as compared to tissue.

The assembly of supercomplexes has recently been shown to be dependent on the presence of the SC assembly factor *Scafi*. In several mouse strains, including the commonly used strain C57Bl/6, the *Scafi* gene has a six nucleotide deletion, that results in unstable Scafi protein and a lack of CIV in SC (33). In contrast, the 129sv strain has full-length *Scafi*, detectable Scafi protein, and SC containing CIV. The authors further suggest that the presence of *Scafi* allows simultaneous oxidation of multiple substrates at optimum rates.

In Paper II we investigated the correlation between Scafi, supercomplex formation, and life span in *Bcs1l*$^{G/G}$ mice of mixed, 129/sv x C57BL/6, and congenic C57BL/6 background. In line with the publication of Lapuente-Brun, *Bcs1l*$^{G/G}$ mice of mixed background were heterozygous for the long *Scafi* gene and had CIV in supercomplexes, whereas *Bcs1l*$^{G/G}$ mice of C57BL/6 were homozygous for the short *Scafi* gene and concomitantly lacked CIV in SC. However, there was no correlation between *Scafi* gene alleles and increased survival in *Bcs1l*$^{G/G}$ mice of mixed background. Thus, the CIII deficiency in *Bcs1l*$^{G/G}$ mice could not be compensated for by the expression of intact Scafi and changed SC formation. This could be due to the limited amount of $CIII_2$ in *Bcs1l*$^{G/G}$. In mitochondria from mouse livers, independent of *Scafi* genotype, as well as several human cell lines, most of the CIV is not associated with SC in BNGE (33, 41). This could be due to methodological reasons—if there were a weak interaction between SC and CIV it might easily be disturbed during SC extraction. However, digitonin is a rather mild detergent, allowing the extraction of SC with or without CIV.

Bcs1l$^{G/G}$ mice present with CIII deficiency and a fatal liver disease. It is not known if the liver disease is caused by decreased CIII function or by an as yet unknown function of Bcs1l. Therefore, we induced CIII inhibition in C57BL/6 mice by injecting the CIII inhibitor myxothiazol. Myxothiazol binds to the Q_0 site of CIII dimers, thus preventing electron transfer from ubiquinol to cytochrome b and RISP. In liver mitochondria of myxothiazol-exposed mice, CIII activity was decreased to 50%. The remaining CIII activity seemed to be sufficient to maintain normal RC function as no behavioral changes and only slight pathological signs of tissue injury were observed. In addition, similar SC assembly was found in myxothiazol-exposed and untreated animals.

RC activity is tightly coupled to the metabolism in cells and mammalian organs, forming a network in which the liver plays a central role. Mitochondrial dysfunction due to CIII deficiency thus influences liver metabolism. Therefore, we investigated changes in the liver metabolome in *Bcs1l*$^{G/G}$ mice of different ages (Paper IV). Parallel with changes in SC formation (Paper I) we found changes in metabolomics. At disease onset at postnatal day P24, only slight metabolic changes, indicating impaired glucose turnover and beta-oxidation, were detected. The increased AMP level suggests that ATP production was decreased. Thus, the metabolic changes were small; most prominent were signs of energy deprivation. In sick animals, energy deprivation became even more pronounced with decreased carbohydrates, increased AMP and acylcarnitines. Oxidative stress is thought to be an important factor in development of steatosis (134). In *Bcs1l*$^{G/G}$ mice, however, oxidative stress markers were only slightly increased in the end stage of the disease. It thus remains unclear whether CIII and CI in our homozygous mouse produced slightly increased amounts of ROS, which could be responsible for changes in signaling pathways. The death mechanism in the homozygous mice is not fully clarified, but energy deprivation and hypoglycemia seem to be crucial factors.

In summary, in this thesis we studied the relation of RC complexes in presence of progressive CIII deficiency and a CIII inhibitor. The CIII deficient mouse model can be used to study alternative aspects of BCS1l in RC function and the development of new interventions. Acute inhibition of CIII by biochemical administration *in vivo* improves the possibility for investigations of mitochondrial dysfunctions.

Conclusions

To conclude:

We found that in complex III deficiency due to lack of Bcs1l, pre-CIII replaces CIII in SC formation. The CI can interact with pre-CIII and form SC in the absence of mature holo-CIII. Thus, a functional RC is preserved in the homozygous mice of the GRACILE model until, over time, a certain threshold is achieved, when RC capacity is impaired and a vicious cycle develops.

The long variant of supercomplex assembly factor I (SCAFI) is required for inclusion of CIV in SC formation, but seems not to be crucial for the functionality of RC in our mouse model. In the homozygotes, the absence of CIV in SC was not associated with a shorter life span or a more severe disorder.

Myxothiazol-exposed wild-type mice displayed an acute reversible CIII inhibition, which did not affect SC formation and other RC complexes. Administering this CIII inhibitor can be used to study further interactions between the respiratory chain complexes, for example in dysfunction of CI and CIV.

Progressive CIII deficiency clearly affected the liver metabolome at the end stage of the disease, suggesting energy deprivation and slight oxidative stress. The role of oxidative stress in the rapid development of steatosis and cirrhosis in our mouse model seems to be minimal.

Future perspectives

- The mouse model is useful for studying RC dysfunction and its relationship to the development of steatosis/cirrhosis, which is a major concern in diabetes and metabolic syndrome.
- The model has a disease-free interval and rapid deterioration, which makes it suitable for interventional studies. Several new pathways for influencing mitochondrial biogenesis and improving fuel availability have been proposed. Therapeutic benefits should be evident in the model within a few weeks observation.
- Interventions to improve liver function in the model and thus increase life span will provide the possibility for detailed study of other organs, which in the short-lived homozygotes seem to be unaffected. In patients, involvement of CIII dysfunction caused by BCS1L mutations, especially in the central nervous system, heart, and muscles, needs to be assessed if survival is to be extended.
- A translational approach enables further studies of beneficial interventions in clinical trials, which are urgently needed for mitochondrial disorders in humans.

Populärvetenskaplig sammanfattning

Kroppens förmåga att omvandla matens energi till energi som kan användas i organen sker i små organeller som finns i alla kroppens celler, mitokondrierna. Avvikelser i mitokondrierna förorsakar därför ofta svåra sjukdomar. GRACILE syndrom är en medfödd mitokondriell sjukdom, som leder till svår tillväxthämning, problem med ämnesomsättningen och tidig död. Det finns ingen behandling för dessa barn.

Sjukdomen beror på en mutation i *BCS1L* genen. Detta leder till ett förändrat BCS1L protein, som inte längre kan bygga ihop komplex III i andningskedjan.

Andningskedjan finns i mitokondrierna och har till uppgift att skapa cellernas energi, ATP. Andningskedjan består av fyra stora proteinkomplex. Om BCS1L proteinet inte kan bygga ihop komplex III blir andningskedjan mindre effektiv och kan inte längre producera tillräckligt ATP.

För att förstå komplex III bättre och på sikt kunna hjälpa patienter med mitokondriesjukdom p.g.a. minskad komplex III funktion har vi skapat en musmodell där vi kan undersöka hur komplexen och deras samspel i andningskedjan påverkas av en mutation i BCS1L.

Mutation i motsvarande gen i möss leder till snarlik sjukdomsbild som de nyfödda barnen har. Genom att isolera mitokondrier och membranproteiner från muslever analyserade vi proteinsammansättningen i sjuka möss och jämförde med kontroller. Resultatet visade att mutationen leder till att ett bristfälligt ihop byggt komplex III kan delta i formation av sammansatta komplex, så kallade superkomplex, och på så sätt kompensera för bristen. Vi studerade två musstammar med olika superkomplex och visade att variationen i superkomplexstrukturen inte hade betydelse för mössens överlevnad.

Våra studier visade att andningskedjans komplex kan bilda fungerande kombinationer på ett dynamiskt sätt om komplex III har en avvikande struktur och på så sätt upprätthålla andningskedjans funktion åtminstone en tid.

چکیده فارسی

یاخته ها ی (سلولهای) موجودات زنده هوازی، شامل اندامکهایی به نام راکیزه (میتوکندری) می باشند که انرژی حاصل از مواد غذایی را(قندها، اسیدهای چرب و...) با کمک فسفورولاسیون اکسیداتیو در زنجیره تنفسی تبدیل به انرزی شیمیایی به شکل ملکولهای "آدنوسین تری فسفات" مخفف به (ای. تی. پی) می کند.
جهش ژنتیکی یا اختلال در ملکولهای" دی ان ای" مربوط به پروتینهای زنجیره تنفسی باعث اخلال در این سیستم تنفسی شده و به نوبه خود روی روند و عملکرد این سیستم تاثیر میگذارد. این اختلالات باعث بروز بیماریهای ژنتیکی مرتبط با میتوکندری و سیستم زنجیره تنفسی می شود.
"سندرم گراسیال" معروف به سندروم" فلمن" یا "سندروم متابولیکی نوزادان فنلاندی" یکی از این بیماری هاست. نوزدان متولد با این دگرگونی ژنتیکی نشانه های بارزی مثل،عقب ماندگی در رشد، وجوداسیدهای امینه در ادرار، جمع شدن آهن درسلولهای بافت کبدی، لاکتوز اسیدی و مرگ زودرس دارند. این بیماری موروثی، اتوزومال مغلوب میباشد.
دگرگونی در ژن " بی سی اس وان ال " عامل این بیماری است. با دگرگونی در کد ژنتیکی، عملکرد این پروتیین که ساختار کمپلکس۳ در دیواره داخلی میتوکندری میباشد مختل میشود. کمپلکس ۳ یکی از چهارمجموعه پروتیینی مخصوص زنجیره تنفسی واقع بر دیواره داخلی میتوکندری میباشد که نقش کلیدی در گذار الکترونی در زنجیره تنفسی دارد. با اختلال در ساختار پروتئینی کمپلکس ۳ عملا انتقال الکترونها تحت تاثیر قرار گرفته و تولید(ای. تی .پی) مختل میشود.
ما برای مطالعه و تحقیق روی زنجیره تنفسی و پیدا کردن روش درمان برای بیماریهای ناشی از اختلال میتوکندریایی و کمپلکس۳، یک دگرگونی ژنتیکی شبیه به بیماری گراسیال در موش بوجود اوردیم که بعنوان مدل این بیماری مورد استفاده ما قرار گرفته است. نتیجه این تاثیر در کد ژنتیکی، نزدیکی زیادی با نمونه انسانی آن دارد.
مطالعه و تحقیق روی میتوکندری و جداسازی غشای داخلی انها، با استفاده از روشهای مختلف جداسازی ما را در شناخت بیشتر این اندامک کمک کرده است. با استفاده از بافت کبدی موشها برای جداسازی میتوکندری، غشای داخلی وپروئتینهای زنجیره تنفسی آن در مقایسه با نمونه های موشهای سالم شناخت بیشتری از مکانیسم این سیستم و روش بیماری پیدا کردیم.
تفاوت درتشکیل مجموعه های بزرگتر پروتئینی، معروف به "سوپر کمپلکس" درزنجیره تنفسی دو نوع از موشهای ازمایشگاهی یکی دیگر ازمطالعات ما در این تحقیق بود. این پژوهش نشان داد که موشهای ما فقط یک نوع از این سوپر کمپلکسها را در سیستم زنجیره تنفسی خود دارند.
نتیجه عمده تحقیقات ما این بود که در صورت کمبودیا نارسایی در پروتئینها یا سوپر کمپلکس ها ی زنجیره تنفسی عملکرد انها توسط دیگر پروتئینهای این زنجیره به طور محدودی جایگزین و جبران میشود.

Acknowledgments

I want to express my deepest gratitude to all the people who helped make this thesis possible:

My supervisors Vineta Fellman and Heike Kotarsky—thank you for giving me the opportunity to do the research, and for introducing me to the mitochondrial world. Thanks too, for all your support, guidance, and supervision during these years.

I am grateful to my colleague and roommate Eva Hansson (more than 1,000,000 times) for your patience, your help, your kind comments—and your "hard" ones.

Thank you Nika for all the laughs and good advice in the lab, and good luck with whatever you choose to do. Also, thank you Jay and Praveen for your hospitality and your company during my days in Helsinki.

I would like to extend my gratitude to all my collaborators, especially Per Levéen, Jukka Kallijärvi, and Sanna Marjavaara.

Special thanks go to Saori M, Eleonor Å, Albana, Sarah P, and Eskil E, for their friendly support and all the great days in Copenhagen.

I am grateful to all my colleagues at BMC B14 for their endless support and kindness. Mattias C, Ingbritt G, Inga-Maria F, Maria A, Maria B, Monica H, Ulla J, Silla R, Magnus L, Bo Å, Mathias M, Praveen P, Gopinatt K, Ravi B, Ramesh T, Ann-Charlotte S, Andreas S, Milo A, Arne E, Heiko H, Sined H, Suado A, Malgorzata B, Oonagh S, Sara D, Zara D, Swati S, Anita B, Pia A, Marcus R, Maria M, Martina K, Azadeh S, Christina NK, and Rosa A, thank you all.

Work at BMC C14 has been an incredible pleasure for me. Thank you Diana Karpman for your time in answering my questions whenever I asked. Also, Ann-Charlotte K, Anne-lie S, Ida A, Roland S, Milan C, Sebastian L, Johan R, Maria M, Robin K, Zivile B, Kalle, Ann-Sofi, Irene L, Zuzana K, Tina C, Åsa N, Lena W.R, Lena E, Vera C, Zahra M, Stefan H, Julia W, Jill S, Maria H, Britt T, Bahram M, Åsa H, and Eva F at the Pediatrics Office.

Special thanks to Karin Berger, Madeleine Durbeej-Hjalt, Annete Welin, and Ulla-Britt Andersson for all your support and kind words.

Thank you all my friends outside the lab. Scarlett, Hosni, for the "fika paus", Admira and Amer, Radmila my lovely friend in NY, Alireza A for your help with

the illustrations of the mitochondrion, and Mohammad T for your unceasing interest in my never-ending Ph.D. project.

I particularly appreciate my friends. My dearest Sousan, Enayat, Shirin, Omid, dearest Nahid, Abazar, Nima, Lovely Sara (I miss you so much), Maonochehr, Anoush, Babak, Vian, Said, Artin, Nadreh, Reza, Niki, Daniel, Mehrnoush, Reza, whose empathy for humanity, and love, always make my day. I am so happy to have you around me.

My family, Iran (Shamsi), Shirin, Shahla, Shahin, Hamid, Naznoosh, Said, Mehrnoush, Mehrnaz, Mehrangiz (Mina), Soheyla, Sahar, Salman, Amir, Parniyan, Shayan, Sharif, Hasti, Aria, Anisa, and my lovely parents, Abdol-Rashid and Gohar for your love and encouragement in all these years and all things I have done.

Heartfelt thanks to my Rashid (Abdoullah) for his patience and love, my lovely Behrang and Bahhar, who will forever be the best things in my life. You three are my stars. I love you.

References

1. Ernster, L., and Schatz, G. (1981) Mitochondria: a historical review. *J Cell Biol* **91**, 227s-255s
2. Benard, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T., and Rossignol, R. (2007) Mitochondrial bioenergetics and structural network organization. *J Cell Sci* **120**, 838-848
3. Zick, M., Rabl, R., and Reichert, A. S. (2009) Cristae formation-linking ultrastructure and function of mitochondria. *Biochim Biophys Acta* **1793**, 5-19
4. Lopez-Garcia, P., and Moreira, D. (1999) Metabolic symbiosis at the origin of eukaryotes. *Trends Biochem Sci* **24**, 88-93
5. Andersson, S. G., and Kurland, C. G. (1999) Origins of mitochondria and hydrogenosomes. *Curr Opin Microbiol* **2**, 535-541
6. Gray, M. W., Burger, G., and Lang, B. F. (2001) The origin and early evolution of mitochondria. *Genome Biol* **2**, REVIEWS1018
7. Dyall, S. D., Brown, M. T., and Johnson, P. J. (2004) Ancient invasions: from endosymbionts to organelles. *Science* **304**, 253-257
8. Wallace, D. C. (2007) Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. *Annu Rev Biochem* **76**, 781-821
9. Zimmer, C. (2009) Origins. On the origin of eukaryotes. *Science* **325**, 666-668
10. Finkel, T. (2012) Signal transduction by mitochondrial oxidants. *J Biol Chem* **287**, 4434-4440
11. Pan, Y. (2011) Mitochondria, reactive oxygen species, and chronological aging: a message from yeast. *Exp Gerontol* **46**, 847-852
12. Perkins, G. A., and Frey, T. G. (2000) Recent structural insight into mitochondria gained by microscopy. *Micron* **31**, 97-111
13. Jiang, X., and Wang, X. (2004) Cytochrome C-mediated apoptosis. *Annu Rev Biochem* **73**, 87-106
14. Fernandez-Vizarra, E., Enriquez, J. A., Perez-Martos, A., Montoya, J., and Fernandez-Silva, P. (2011) Tissue-specific differences in mitochondrial activity and biogenesis. *Mitochondrion* **11**, 207-213
15. Nunnari, J., and Suomalainen, A. (2012) Mitochondria: in sickness and in health. *Cell* **148**, 1145-1159
16. Bohnert, M., Wenz, L. S., Zerbes, R. M., Horvath, S. E., Stroud, D. A., von der Malsburg, K., Muller, J. M., Oeljeklaus, S., Perschil, I., Warscheid, B., Chacinska, A., Veenhuis, M., van der Klei, I. J., Daum, G., Wiedemann, N., Becker, T., Pfanner, N., and van der Laan, M. (2012) Role of mitochondrial inner membrane organizing system in protein biogenesis of the mitochondrial outer membrane. *Mol Biol Cell* **23**, 3948-3956

17. Gohil, V. M., and Greenberg, M. L. (2009) Mitochondrial membrane biogenesis: phospholipids and proteins go hand in hand. *J Cell Biol* **184**, 469-472
18. Frey, T. G., and Mannella, C. A. (2000) The internal structure of mitochondria. *Trends Biochem Sci* **25**, 319-324
19. Wilkens, V., Kohl, W., and Busch, K. (2013) Restricted diffusion of OXPHOS complexes in dynamic mitochondria delays their exchange between cristae and engenders a transitory mosaic distribution. *J Cell Sci* **126**, 103-116
20. Kadenbach, B. (2012) Introduction to mitochondrial oxidative phosphorylation. *Adv Exp Med Biol* **748**, 1-11
21. Saraste, M. (1999) Oxidative phosphorylation at the fin de siecle. *Science* **283**, 1488-1493
22. Lill, R., and Muhlenhoff, U. (2005) Iron-sulfur-protein biogenesis in eukaryotes. *Trends Biochem Sci* **30**, 133-141
23. Krebs, H. A., and Johnson, W. A. (1937) Metabolism of ketonic acids in animal tissues. *Biochem J* **31**, 645-660
24. Hartong, D. T., Dange, M., McGee, T. L., Berson, E. L., Dryja, T. P., and Colman, R. F. (2008) Insights from retinitis pigmentosa into the roles of isocitrate dehydrogenases in the Krebs cycle. *Nat Genet* **40**, 1230-1234
25. Fernandez-Vizarra, E., Tiranti, V., and Zeviani, M. (2009) Assembly of the oxidative phosphorylation system in humans: what we have learned by studying its defects. *Biochim Biophys Acta* **1793**, 200-211
26. Hackenbrock, C. R., Chazotte, B., and Gupte, S. S. (1986) The random collision model and a critical assessment of diffusion and collision in mitochondrial electron transport. *J Bioenerg Biomembr* **18**, 331-368
27. Lenaz, G., and Genova, M. L. (2009) Structural and functional organization of the mitochondrial respiratory chain: a dynamic super-assembly. *Int J Biochem Cell Biol* **41**, 1750-1772
28. Dudkina, N. V., Kouril, R., Peters, K., Braun, H. P., and Boekema, E. J. (2010) Structure and function of mitochondrial supercomplexes. *Biochim Biophys Acta* **1797**, 664-670
29. Chance, B., and Williams, G. R. (1955) A method for the localization of sites for oxidative phosphorylation. *Nature* **176**, 250-254
30. Schagger, H., and Pfeiffer, K. (2000) Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. *EMBO J* **19**, 1777-1783
31. Vartak, R., Porras, C. A., and Bai, Y. (2013) Respiratory supercomplexes: structure, function and assembly. *Protein & cell* **4**, 582-590
32. Acin-Perez, R., Fernandez-Silva, P., Peleato, M. L., Perez-Martos, A., and Enriquez, J. A. (2008) Respiratory active mitochondrial supercomplexes. *Mol Cell* **32**, 529-539
33. Lapuente-Brun, E., Moreno-Loshuertos, R., Acin-Perez, R., Latorre-Pellicer, A., Colas, C., Balsa, E., Perales-Clemente, E., Quiros, P. M., Calvo, E., Rodriguez-Hernandez, M. A., Navas, P., Cruz, R., Carracedo, A., Lopez-Otin, C., Perez-Martos, A., Fernandez-Silva, P., Fernandez-Vizarra, E., and Enriquez, J. A. (2013) Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. *Science* **340**, 1567-1570
34. Hirst, J. (2013) Mitochondrial complex I. *Annu Rev Biochem* **82**, 551-575

35. Balsa, E., Marco, R., Perales-Clemente, E., Szklarczyk, R., Calvo, E., Landazuri, M. O., and Enriquez, J. A. (2012) NDUFA4 is a subunit of complex IV of the mammalian electron transport chain. *Cell Metab* **16**, 378-386
36. Rak, M., and Rustin, P. (2014) Supernumerary subunits NDUFA3, NDUFA5 and NDUFA12 are required for the formation of the extramembrane arm of human mitochondrial complex I. *FEBS Lett* **588**, 1832-1838
37. Acin-Perez, R., Bayona-Bafaluy, M. P., Fernandez-Silva, P., Moreno-Loshuertos, R., Perez-Martos, A., Bruno, C., Moraes, C. T., and Enriquez, J. A. (2004) Respiratory complex III is required to maintain complex I in mammalian mitochondria. *Mol Cell* **13**, 805-815
38. Diaz, F., Fukui, H., Garcia, S., and Moraes, C. T. (2006) Cytochrome c oxidase is required for the assembly/stability of respiratory complex I in mouse fibroblasts. *Mol Cell Biol* **26**, 4872-4881
39. Diaz, F., Enriquez, J. A., and Moraes, C. T. (2012) Cells lacking Rieske iron-sulfur protein have a reactive oxygen species-associated decrease in respiratory complexes I and IV. *Mol Cell Biol* **32**, 415-429
40. Li, Y., D'Aurelio, M., Deng, J. H., Park, J. S., Manfredi, G., Hu, P., Lu, J., and Bai, Y. (2007) An assembled complex IV maintains the stability and activity of complex I in mammalian mitochondria. *J biol chem* **282**, 17557-17562
41. Moreno-Lastres, D., Fontanesi, F., Garcia-Consuegra, I., Martin, M. A., Arenas, J., Barrientos, A., and Ugalde, C. (2012) Mitochondrial complex I plays an essential role in human respirasome assembly. *Cell Metab* **15**, 324-335
42. Lazarou, M., Thorburn, D. R., Ryan, M. T., and McKenzie, M. (2009) Assembly of mitochondrial complex I and defects in disease. *Biochim Biophys Acta* **1793**, 78-88
43. Ghezzi, D., and Zeviani, M. (2012) Assembly factors of human mitochondrial respiratory chain complexes: physiology and pathophysiology. *Adv Exp Med Biol* **748**, 65-106
44. Grimm, S. (2013) Respiratory chain complex II as general sensor for apoptosis. *Biochim Biophys Acta* **1827**, 565-572
45. Ackrell, B. A. (2002) Cytopathies involving mitochondrial complex II. *Mol Aspects Med* **23**, 369-384
46. Rustin, P., and Rotig, A. (2002) Inborn errors of complex II--unusual human mitochondrial diseases. *Biochim Biophys Acta* **1553**, 117-122
47. Iwata, S., Lee, J. W., Okada, K., Lee, J. K., Iwata, M., Rasmussen, B., Link, T. A., Ramaswamy, S., and Jap, B. K. (1998) Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex. *Science* **281**, 64-71
48. Xia, D., Esser, L., Tang, W. K., Zhou, F., Zhou, Y., Yu, L., and Yu, C. A. (2013) Structural analysis of cytochrome bc1 complexes: implications to the mechanism of function. *Biochim Biophys Acta* **1827**, 1278-1294
49. Schagger, H., Brandt, U., Gencic, S., and von Jagow, G. (1995) Ubiquinol-cytochrome-c reductase from human and bovine mitochondria. *Methods Enzymol* **260**, 82-96
50. Wagener, N., and Neupert, W. (2012) Bcs1, a AAA protein of the mitochondria with a role in the biogenesis of the respiratory chain. *J Struct Biol* **179**, 121-125
51. Smith, P. M., Fox, J. L., and Winge, D. R. (2012) Biogenesis of the cytochrome bc(1) complex and role of assembly factors. *Biochim Biophys Acta* **1817**, 276-286

52. Sanchez, E., Lobo, T., Fox, J. L., Zeviani, M., Winge, D. R., and Fernandez-Vizarra, E. (2013) LYRM7/MZM1L is a UQCRFS1 chaperone involved in the last steps of mitochondrial Complex III assembly in human cells. *Biochim Biophys Acta* **1827**, 285-293
53. Calvaruso, M. A., Willems, P., van den Brand, M., Valsecchi, F., Kruse, S., Palmiter, R., Smeitink, J., and Nijtmans, L. (2012) Mitochondrial complex III stabilizes complex I in the absence of NDUFS4 to provide partial activity. *Hum Mol Genet* **21**, 115-120
54. Suthammarak, W., Morgan, P. G., and Sedensky, M. M. (2010) Mutations in mitochondrial complex III uniquely affect complex I in Caenorhabditis elegans. *J Biol Chem* **285**, 40724-40731
55. Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzawa-Itoh, K., Nakashima, R., Yaono, R., and Yoshikawa, S. (1996) The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 A. *Science* **272**, 1136-1144
56. Arnold, S., Lee, I., Kim, M., Song, E., Linder, D., Lottspeich, F., and Kadenbach, B. (1997) The subunit structure of cytochrome-c oxidase from tuna heart and liver. *Eur J Biochem* **248**, 99-103
57. Hornig-Do, H. T., Tatsuta, T., Buckermann, A., Bust, M., Kollberg, G., Rotig, A., Hellmich, M., Nijtmans, L., and Wiesner, R. J. (2012) Nonsense mutations in the COX1 subunit impair the stability of respiratory chain complexes rather than their assembly. *EMBO J* **31**, 1293-1307
58. Grossman, L. I., and Lomax, M. I. (1997) Nuclear genes for cytochrome c oxidase. *Biochim Biophys Acta* **1352**, 174-192
59. Kovarova, N., Cizkova Vrbacka, A., Pecina, P., Stranecky, V., Pronicka, E., Kmoch, S., and Houstek, J. (2012) Adaptation of respiratory chain biogenesis to cytochrome c oxidase deficiency caused by SURF1 gene mutations. *Biochim Biophys Acta* **1822**, 1114-1124
60. Rees, D. M., Leslie, A. G., and Walker, J. E. (2009) The structure of the membrane extrinsic region of bovine ATP synthase. *Proc Natl Acad Sci U S A* **106**, 21597-21601
61. Boyer, P. D. (1997) The ATP synthase--a splendid molecular machine. *Annu Rev Biochem* **66**, 717-749
62. Paumard, P., Vaillier, J., Coulary, B., Schaeffer, J., Soubannier, V., Mueller, D. M., Brethes, D., di Rago, J. P., and Velours, J. (2002) The ATP synthase is involved in generating mitochondrial cristae morphology. *EMBO J* **21**, 221-230
63. Fowler, L. R., and Richardson, S. H. (1963) Studies on the electron transfer system. L. On the mechanism of reconstitution of the mitochondrial electron transfer system. *J Biol Chem* **238**, 456-463
64. Cruciat, C. M., Brunner, S., Baumann, F., Neupert, W., and Stuart, R. A. (2000) The cytochrome bc1 and cytochrome c oxidase complexes associate to form a single supracomplex in yeast mitochondria. *J Biol Chem* **275**, 18093-18098
65. Schafer, E., Seelert, H., Reifschneider, N. H., Krause, F., Dencher, N. A., and Vonck, J. (2006) Architecture of active mammalian respiratory chain supercomplexes. *J Biol Chem* **281**, 15370-15375

66. Lenaz, G., Baracca, A., Barbero, G., Bergamini, C., Dalmonte, M. E., Del Sole, M., Faccioli, M., Falasca, A., Fato, R., Genova, M. L., Sgarbi, G., and Solaini, G. (2010) Mitochondrial respiratory chain super-complex I-III in physiology and pathology. *Biochim Biophys Acta* **1797**, 633-640
67. Genova, M. L., and Lenaz, G. (2013) A critical appraisal of the role of respiratory supercomplexes in mitochondria. *Biol Chem* **394**, 631-639
68. Schagger, H., and Pfeiffer, K. (2001) The ratio of oxidative phosphorylation complexes I-V in bovine heart mitochondria and the composition of respiratory chain supercomplexes. *J Biol Chem* **276**, 37861-37867
69. Schagger, H., de Coo, R., Bauer, M. F., Hofmann, S., Godinot, C., and Brandt, U. (2004) Significance of respirasomes for the assembly/stability of human respiratory chain complex I. *J. Biol. Chem.* **279**, 36349-36353
70. D'Aurelio, M., Gajewski, C. D., Lenaz, G., and Manfredi, G. (2006) Respiratory chain supercomplexes set the threshold for respiration defects in human mtDNA mutant cybrids. *Hum Mol Genet* **15**, 2157-2169
71. Gomez, L. A., Monette, J. S., Chavez, J. D., Maier, C. S., and Hagen, T. M. (2009) Supercomplexes of the mitochondrial electron transport chain decline in the aging rat heart. *Arch Biochem Biophys* **490**, 30-35
72. Althoff, T., Mills, D. J., Popot, J. L., and Kuhlbrandt, W. (2011) Arrangement of electron transport chain components in bovine mitochondrial supercomplex I(1)III(2)IV(1). *EMBO J* **30**, 4652-4664
73. Hinson, J. T., Fantin, V. R., Schonberger, J., Breivik, N., Siem, G., McDonough, B., Sharma, P., Keogh, I., Godinho, R., Santos, F., Esparza, A., Nicolau, Y., Selvaag, E., Cohen, B. H., Hoppel, C. L., Tranebjaerg, L., Eavey, R. D., Seidman, J. G., and Seidman, C. E. (2007) Missense mutations in the BCS1L gene as a cause of the Bjornstad syndrome. *N Engl J Med* **356**, 809-819
74. Moran, M., Marin-Buera, L., Gil-Borlado, M. C., Rivera, H., Blazquez, A., Seneca, S., Vazquez-Lopez, M., Arenas, J., Martin, M. A., and Ugalde, C. (2010) Cellular pathophysiological consequences of BCS1L mutations in mitochondrial complex III enzyme deficiency. *Hum Mutat* **31**, 930-941
75. Begriche, K., Igoudjil, A., Pessayre, D., and Fromenty, B. (2006) Mitochondrial dysfunction in NASH: causes, consequences and possible means to prevent it. *Mitochondrion* **6**, 1-28
76. Sugiana, C., Pagliarini, D. J., McKenzie, M., Kirby, D. M., Salemi, R., Abu-Amero, K. K., Dahl, H. H., Hutchison, W. M., Vascotto, K. A., Smith, S. M., Newbold, R. F., Christodoulou, J., Calvo, S., Mootha, V. K., Ryan, M. T., and Thorburn, D. R. (2008) Mutation of C20orf7 disrupts complex I assembly and causes lethal neonatal mitochondrial disease. *Am J Hum Genet* **83**, 468-478
77. Sheftel, A. D., Stehling, O., Pierik, A. J., Netz, D. J., Kerscher, S., Elsasser, H. P., Wittig, I., Balk, J., Brandt, U., and Lill, R. (2009) Human ind1, an iron-sulfur cluster assembly factor for respiratory complex I. *Mol Cell Biol* **29**, 6059-6073
78. Calvo, S. E., Tucker, E. J., Compton, A. G., Kirby, D. M., Crawford, G., Burtt, N. P., Rivas, M., Guiducci, C., Bruno, D. L., Goldberger, O. A., Redman, M. C., Wiltshire, E., Wilson, C. J., Altshuler, D., Gabriel, S. B., Daly, M. J., Thorburn, D. R., and Mootha, V. K. (2010) High-throughput, pooled sequencing identifies mutations in NUBPL and FOXRED1 in human complex I deficiency. *Nat Genet* **42**, 851-858

79. Nogueira, C., Barros, J., Sa, M. J., Azevedo, L., Taipa, R., Torraco, A., Meschini, M. C., Verrigni, D., Nesti, C., Rizza, T., Teixeira, J., Carrozzo, R., Pires, M. M., Vilarinho, L., and Santorelli, F. M. (2013) Novel TTC19 mutation in a family with severe psychiatric manifestations and complex III deficiency. *Neurogenetics* **14**, 153-160
80. Ghezzi, D., Arzuffi, P., Zordan, M., Da Re, C., Lamperti, C., Benna, C., D'Adamo, P., Diodato, D., Costa, R., Mariotti, C., Uziel, G., Smiderle, C., and Zeviani, M. (2011) Mutations in TTC19 cause mitochondrial complex III deficiency and neurological impairment in humans and flies. *Nat Genet* **43**, 259-263
81. Tucker, E. J., Wanschers, B. F., Szklarczyk, R., Mountford, H. S., Wijeyeratne, X. W., van den Brand, M. A., Leenders, A. M., Rodenburg, R. J., Reljic, B., Compton, A. G., Frazier, A. E., Bruno, D. L., Christodoulou, J., Endo, H., Ryan, M. T., Nijtmans, L. G., Huynen, M. A., and Thorburn, D. R. (2013) Mutations in the UQCC1-interacting protein, UQCC2, cause human complex III deficiency associated with perturbed cytochrome b protein expression. *PLoS Genet* **9**, e1004034
82. Nobrega, F. G., Nobrega, M. P., and Tzagoloff, A. (1992) BCS1, a novel gene required for the expression of functional Rieske iron-sulfur protein in Saccharomyces cerevisiae. *EMBO J* **11**, 3821-3829
83. Zara, V., Conte, L., and Trumpower, B. L. (2007) Identification and characterization of cytochrome bc(1) subcomplexes in mitochondria from yeast with single and double deletions of genes encoding cytochrome bc(1) subunits. *Febs J* **274**, 4526-4539
84. Zara, V., Conte, L., and Trumpower, B. L. (2009) Biogenesis of the yeast cytochrome bc1 complex. *Biochim Biophys Acta* **1793**, 89-96
85. Xu, F., Ackerley, C., Maj, M. C., Addis, J. B., Levandovskiy, V., Lee, J., Mackay, N., Cameron, J. M., and Robinson, B. H. (2008) Disruption of a mitochondrial RNA-binding protein gene results in decreased cytochrome b expression and a marked reduction in ubiquinol-cytochrome c reductase activity in mouse heart mitochondria. *Biochem J* **416**, 15-26
86. Bernard, D. G., Gabilly, S. T., Dujardin, G., Merchant, S., and Hamel, P. P. (2003) Overlapping specificities of the mitochondrial cytochrome c and c1 heme lyases. *J Biol Chem* **278**, 49732-49742
87. Conte, L., and Zara, V. (2011) The Rieske Iron-Sulfur Protein: Import and Assembly into the Cytochrome bc(1) Complex of Yeast Mitochondria. *Bioinorg Chem Appl* **2011**, 363941
88. Folsch, H., Guiard, B., Neupert, W., and Stuart, R. A. (1996) Internal targeting signal of the BCS1 protein: a novel mechanism of import into mitochondria. *EMBO J* **15**, 479-487
89. Wagener, N., Ackermann, M., Funes, S., and Neupert, W. (2011) A pathway of protein translocation in mitochondria mediated by the AAA-ATPase Bcs1. *Mol Cell* **44**, 191-202
90. Diaz, F., Kotarsky, H., Fellman, V., and Moraes, C. T. (2011) Mitochondrial disorders caused by mutations in respiratory chain assembly factors. *Semin Fetal Neonatal Med* **16**, 197-204

91. Fellman, V., and Kotarsky, H. (2011) Mitochondrial hepatopathies in the newborn period. *Semin. Fetal. Neonatal. Med.* **16**, 222-228
92. Fellman, V., Rapola, J., Pihko, H., Varilo, T., and Raivio, K. O. (1998) Iron-overload disease in infants involving fetal growth retardation, lactic acidosis, liver haemosiderosis, and aminoaciduria. *Lancet* **351**, 490-493
93. Fellman, V., Lemmela, S., Sajantila, A., Pihko, H., and Jarvela, I. (2008) Screening of BCS1L mutations in severe neonatal disorders suspicious for mitochondrial cause. *J. Hum. Genet.* **53**, 554-558
94. Visapaa, I., Fellman, V., Vesa, J., Dasvarma, A., Hutton, J. L., Kumar, V., Payne, G. S., Makarow, M., Van Coster, R., Taylor, R. W., Turnbull, D. M., Suomalainen, A., and Peltonen, L. (2002) GRACILE syndrome, a lethal metabolic disorder with iron overload, is caused by a point mutation in BCS1L. *Am J Hum Genet* **71**, 863-876
95. Ezgu, F., Senaca, S., Gunduz, M., Turner, L., Hasanoglu, A., Tiras, U., Unsal, R., and Bakkaloglu, S. A. (2013) Severe renal tubulopathy in a newborn due to BCS1L gene mutation: Effects of different treatment modalities on the clinical course. *Gene* **528**, 364-366
96. Fernandez-Vizarra, E., Bugiani, M., Goffrini, P., Carrara, F., Farina, L., Procopio, E., Donati, A., Uziel, G., Ferrero, I., and Zeviani, M. (2007) Impaired complex III assembly associated with BCS1L gene mutations in isolated mitochondrial encephalopathy. *Hum Mol Genet* **16**, 1241-1252
97. De Meirleir, L., Seneca, S., Damis, E., Sepulchre, B., Hoorens, A., Gerlo, E., Garcia Silva, M. T., Hernandez, E. M., Lissens, W., and Van Coster, R. (2003) Clinical and diagnostic characteristics of complex III deficiency due to mutations in the BCS1L gene. *Am J Med Genet A* **121A**, 126-131
98. Al-Owain, M., Colak, D., Albakheet, A., Al-Younes, B., Al-Humaidi, Z., Al-Sayed, M., Al-Hindi, H., Al-Sugair, A., Al-Muhaideb, A., Rahbeeni, Z., Al-Sehli, A., Al-Fadhli, F., Ozand, P. T., Taylor, R. W., and Kaya, N. (2013) Clinical and biochemical features associated with BCS1L mutation. *J Inherit Metab Dis* **36**, 813-820
99. Lin, A., Devlin, G., Lee, M., and Kerr, A. J. (2014) Performance of the GRACE scores in a New Zealand acute coronary syndrome cohort. *Heart*
100. Fellman, V., Visapaa, I., Vujic, M., Wennerholm, U. B., and Peltonen, L. (2002) Antenatal diagnosis of hereditary fetal growth retardation with aminoaciduria, cholestasis, iron overload, and lactic acidosis in the newborn infant. *Acta Obstet Gynecol Scand* **81**, 398-402
101. de Lonlay, P., Valnot, I., Barrientos, A., Gorbatyuk, M., Tzagoloff, A., Taanman, J. W., Benayoun, E., Chretien, D., Kadhom, N., Lombes, A., de Baulny, H. O., Niaudet, P., Munnich, A., Rustin, P., and Rotig, A. (2001) A mutant mitochondrial respiratory chain assembly protein causes complex III deficiency in patients with tubulopathy, encephalopathy and liver failure. *Nat Genet* **29**, 57-60
102. Blazquez, A., Gil-Borlado, M. C., Moran, M., Verdu, A., Cazorla-Calleja, M. R., Martin, M. A., Arenas, J., and Ugalde, C. (2009) Infantile mitochondrial encephalomyopathy with unusual phenotype caused by a novel BCS1L mutation in an isolated complex III-deficient patient. *Neuromuscul Disord* **19**, 143-146

103. Lynn, A. M., King, R. I., Mackay, R. J., Florkowski, C. M., and Wilson, C. J. (2012) BCS1L gene mutation presenting with GRACILE-like syndrome and complex III deficiency. *Ann Clin Biochem* **49**, 201-203
104. Meunier, B., Fisher, N., Ransac, S., Mazat, J. P., and Brasseur, G. (2013) Respiratory complex III dysfunction in humans and the use of yeast as a model organism to study mitochondrial myopathy and associated diseases. *Biochim Biophys Acta* **1827**, 1346-1361
105. Yanagishita, T., Sugiura, K., Kawamoto, Y., Ito, K., Marubashi, Y., Taguchi, N., Akiyama, M., and Watanabe, D. (2014) A case of Bjornstad syndrome caused by novel compound heterozygous mutations in the BCS1L gene. *Br J Dermatol***170**, 970-973
106. Tuppen, H. A., Fehmi, J., Czermin, B., Goffrini, P., Meloni, F., Ferrero, I., He, L., Blakely, E. L., McFarland, R., Horvath, R., Turnbull, D. M., and Taylor, R. W. (2010) Long-term survival of neonatal mitochondrial complex III deficiency associated with a novel BCS1L gene mutation. *Mol Genet Metab* **100**, 345-348
107. McKenzie, M., Lazarou, M., Thorburn, D. R., and Ryan, M. T. (2006) Mitochondrial respiratory chain supercomplexes are destabilized in Barth Syndrome patients. *J Mol Biol* **361**, 462-469
108. Mileykovskaya, E., and Dowhan, W. (2014) Cardiolipin-dependent formation of mitochondrial respiratory supercomplexes. *Chem Phys Lipids* **179**, 42-48
109. Pfeiffer, K., Gohil, V., Stuart, R. A., Hunte, C., Brandt, U., Greenberg, M. L., and Schagger, H. (2003) Cardiolipin stabilizes respiratory chain supercomplexes. *J Biol Chem* **278**, 52873-52880
110. Ikeda, K., Shiba, S., Horie-Inoue, K., Shimokata, K., and Inoue, S. (2013) A stabilizing factor for mitochondrial respiratory supercomplex assembly regulates energy metabolism in muscle. *Nat Commun* **4**, 2147
111. Chen, Y. C., Taylor, E. B., Dephoure, N., Heo, J. M., Tonhato, A., Papandreou, I., Nath, N., Denko, N. C., Gygi, S. P., and Rutter, J. (2012) Identification of a protein mediating respiratory supercomplex stability. *Cell Metab* **15**, 348-360
112. Strogolova, V., Furness, A., Robb-McGrath, M., Garlich, J., and Stuart, R. A. (2012) Rcf1 and Rcf2, Members of the Hypoxia-Induced Gene 1 Protein Family, Are Critical Components of the Mitochondrial Cytochrome bc1-Cytochrome c Oxidase Supercomplex. *Mol. Cell. Biol.* **32**, 1363-1373
113. Vukotic, M., Oeljeklaus, S., Wiese, S., Vogtle, F. N., Meisinger, C., Meyer, H. E., Zieseniss, A., Katschinski, D. M., Jans, D. C., Jakobs, S., Warscheid, B., Rehling, P., and Deckers, M. (2012) Rcf1 mediates cytochrome oxidase assembly and respirasome formation, revealing heterogeneity of the enzyme complex. *Cell Metab.* **15**, 336-347
114. Leveen, P., Kotarsky, H., Morgelin, M., Karikoski, R., Elmer, E., and Fellman, V. (2011) The GRACILE mutation introduced into Bcs1l causes postnatal complex III deficiency: a viable mouse model for mitochondrial hepatopathy. *Hepatology* **53**, 437-447
115. Esser, L., Quinn, B., Li, Y. F., Zhang, M., Elberry, M., Yu, L., Yu, C. A., and Xia, D. (2004) Crystallographic studies of quinol oxidation site inhibitors: a modified classification of inhibitors for the cytochrome bc(1) complex. *J. Mol. Biol.* **341**, 281-302

116. Hansson, M. J., Persson, T., Friberg, H., Keep, M. F., Rees, A., Wieloch, T., and Elmer, E. (2003) Powerful cyclosporin inhibition of calcium-induced permeability transition in brain mitochondria. *Brain Res* **960**, 99-111
117. Mansson, R., Hansson, M. J., Morota, S., Uchino, H., Ekdahl, C. T., and Elmer, E. (2007) Re-evaluation of mitochondrial permeability transition as a primary neuroprotective target of minocycline. *Neurobiol Dis* **25**, 198-205
118. Schagger, H., Cramer, W. A., and von Jagow, G. (1994) Analysis of molecular masses and oligomeric states of protein complexes by blue native electrophoresis and isolation of membrane protein complexes by two-dimensional native electrophoresis. *Anal Biochem* **217**, 220-230
119. Gnaiger, E. (2009) Capacity of oxidative phosphorylation in human skeletal muscle: new perspectives of mitochondrial physiology. *Int J Biochem Cell Biol* **41**, 1837-1845
120. Reed, J. S., and Ragan, C. I. (1987) The effect of rate limitation by cytochrome c on the redox state of the ubiquinone pool in reconstituted NADH: cytochrome c reductase. *Biochem J* **247**, 657-662
121. Buss, H., Chan, T. P., Sluis, K. B., Domigan, N. M., and Winterbourn, C. C. (1997) Protein carbonyl measurement by a sensitive ELISA method. *Free Radic Biol Med* **23**, 361-366
122. Snider, J., and Houry, W. A. (2008) AAA+ proteins: diversity in function, similarity in structure. *Biochem Soc Trans* **36**, 72-77
123. Gerdes, F., Tatsuta, T., and Langer, T. (2012) Mitochondrial AAA proteases--towards a molecular understanding of membrane-bound proteolytic machines. *Biochim Biophys Acta* **1823**, 49-55
124. Kotarsky, H., Karikoski, R., Morgelin, M., Marjavaara, S., Bergman, P., Zhang, D. L., Smet, J., van Coster, R., and Fellman, V. (2010) Characterization of complex III deficiency and liver dysfunction in GRACILE syndrome caused by a BCS1L mutation. *Mitochondrion* **10**, 497-509
125. Ostojic, J., Panozzo, C., Lasserre, J. P., Nouet, C., Courtin, F., Blancard, C., di Rago, J. P., and Dujardin, G. (2013) The energetic state of mitochondria modulates complex III biogenesis through the ATP-dependent activity of Bcs1. *Cell Metab* **18**, 567-577
126. Venkatesh, S., Lee, J., Singh, K., Lee, I., and Suzuki, C. K. (2012) Multitasking in the mitochondrion by the ATP-dependent Lon protease. *Biochim Biophys Acta* **1823**, 56-66
127. Baile, M. G., and Claypool, S. M. (2013) The power of yeast to model diseases of the powerhouse of the cell. *Front Biosci (Landmark Ed)* **18**, 241-278
128. Nouet, C., Truan, G., Mathieu, L., and Dujardin, G. (2009) Functional analysis of yeast bcs1 mutants highlights the role of Bcs1p-specific amino acids in the AAA domain. *J Mol Biol* **388**, 252-261
129. Wallace, D. C., and Fan, W. (2009) The pathophysiology of mitochondrial disease as modeled in the mouse. *Genes Dev* **23**, 1714-1736
130. Dogan, S. A., and Trifunovic, A. (2011) Modelling mitochondrial dysfunction in mice. *Physiol Rres* **60 Suppl 1**, S61-70
131. Farrar, G. J., Chadderton, N., Kenna, P. F., and Millington-Ward, S. (2013) Mitochondrial disorders: aetiologies, models systems, and candidate therapies. *Trends Genet* **29**, 488-497

132. Keane, T. M., Goodstadt, L., Danecek, P., White, M. A., Wong, K., Yalcin, B., Heger, A., Agam, A., Slater, G., Goodson, M., Furlotte, N. A., Eskin, E., Nellaker, C., Whitley, H., Cleak, J., Janowitz, D., Hernandez-Pliego, P., Edwards, A., Belgard, T. G., Oliver, P. L., McIntyre, R. E., Bhomra, A., Nicod, J., Gan, X., Yuan, W., van der Weyden, L., Steward, C. A., Bala, S., Stalker, J., Mott, R., Durbin, R., Jackson, I. J., Czechanski, A., Guerra-Assuncao, J. A., Donahue, L. R., Reinholdt, L. G., Payseur, B. A., Ponting, C. P., Birney, E., Flint, J., and Adams, D. J. (2011) Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature* **477**, 289-294
133. (2009) Troublesome variability in mouse studies. *Nat Neurosci* **12**, 1075
134. Rolo, A. P., Teodoro, J. S., and Palmeira, C. M. (2012) Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free Radic Biol Med* **52**, 59-69