

Meeting Report

Cardiolipin as key lipid of mitochondria in health and disease, Bari, Italy, September 17, 2013

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The idea of a Cardiolipin workshop in Italy came to the meeting organizers in June 2011, during the mini-sabbatical of Angela Corcelli in New York City in the Laboratory of Michael Schlame. They thought to take advantage of the presence of the 54th International Conference on the Bioscience of Lipids (ICBL) at Bari in 2013 to organize the Cardiolipin workshop as a satellite event. The web page of the Cardiolipin Meeting was kindly supported by the Euro Fed Lipid organization. About 60 scientists attended the meeting focused on the multiple roles of cardiolipin in mitochondria in physiological and pathological states in various organisms as well as in bacterial membranes. In addition to ICBL participants, many students and colleagues of the Universities of Bari and Lecce attended the meeting, increasing the number of total participants to about 100. As defects in cardiolipin metabolism may cause Barth syndrome, the meeting also presented an occasion to establish contacts between the nascent Italian Barth Syndrome Foundation and scientists actively involved in cardiolipin research.

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1 Brief state of art of cardiolipin research

Cardiolipin, a marker lipid of mitochondria, was discovered in 1947 by the chemist Mary Pangborn. It was the first phospholipid whose structure was elucidated, but its role in mitochondrial bioenergetics is still unclear.

Among anionic phospholipids, cardiolipin is unique because it has a dimeric structure with four acyl chains and two ionizable phosphate groups.

Cardiolipin is typically present in membranes that are able to generate an electrochemical potential for ATP synthesis and substrate transport.

Most if not all proteins participating in oxidative phosphorylation establish interactions with cardiolipin. Cardiolipin not only interacts with integral membrane proteins involved in oxidative phosphorylation and mitochondrial carriers but also with bacterial respiratory chain enzymes and with the reaction centers of photosynthetic bacteria and plants.

Cardiolipin analogues have been described even in archaea and some evidence for involvement in bioenergetic functions of these prokaryotes have been presented.

Cardiolipin turnover in mitochondrial, bacterial, and archaeal membranes can be very fast and produce quick changes in cardiolipin membrane levels and/or its redistribution within membranes.

Recent findings have shown that cardiolipin is associated with yeast vacuolar ATPase and represents a true membrane component of viruses where it is possibly involved in the mechanism of infection because it can be a specific cofactor of viral small GTPases.

The enzymes of cardiolipin biosynthesis and degradation have been described. Among these, tafazzin, a phospholipid-lysophospholipid transacylase that alters the molecular composition of cardiolipin, seems to play a critical role in mitochondrial bioenergetics and cristae morphology. Mutations in the gene encoding tafazzin, cause an increase in the level of monolysocardiolipin together with an alteration of the function and morphology of mitochondria. In humans, these mutations lead to a disease called Barth syndrome.

The interest in cardiolipin has been growing exponentially, since it has become clear that this phospholipid plays a fundamental role in the functioning of mitochondria in health and disease.

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2 The meeting

The ICBL satellite meeting was a one-day workshop starting in the morning of September 17 and ending 1 h before the opening of the main ICBL meeting. To give the opportunity to meet in a relaxed environment and to have informal discussions, a welcome dinner was organized for the participants of the cardiolipin meeting.

The meeting was briefly introduced by Peter Slotte, president of ICBL, and Sergio Papa, pioneer of mitochondrial studies in Bari and FEBS president.

Three main aspects of the emerging functional roles of cardiolipin have been considered in the sessions of the cardiolipin meeting: (i) the impact of cardiolipin on the performance of the respiratory chain, (ii) mitochondrial lipids and proteins as determinants of cristae morphology, and (iii) the role of cardiolipin in bacteria.

In the first talk of the morning, William Dowhan of the Houston Medical School in Texas, USA, presented results of a study investigating the role of cardiolipin in the reconstitution of respiratory supercomplexes from purified *Saccharomyces cerevisiae* complexes III and IV. He pointed out that the dependence on cardiolipin for supercomplex formation suggests that changes in cardiolipin levels resulting from changes in physiological conditions may control the equilibrium between individual respiratory complexes and supercomplexes in vivo.

Grant Hatch of the University of Manitoba, Canada, has focused his presentation on enzymes acting on monolysocardiolipin, reporting evidence that monolysocardiolipin acyltransferase-1 expression improves mitochondrial function in lymphoblasts.

Given the importance of CL in human health, underscored by the observation that perturbation of CL biosynthesis causes the severe genetic disorder Barth syndrome, Miriam Greenberg's research group at Wayne State University, Detroit, USA, carried out genome-wide expression profiling of the yeast CL mutant *crd1Δ* to understand the global cellular response to the loss of CL. She presented results showing that the loss of CL in this mutant leads to increased expression of iron uptake genes accompanied by elevated levels of mitochondrial iron and increased sensitivity to iron and hydrogen peroxidation.

The relevant conclusion of her study is that CL is required for Fe–S biogenesis and to maintain mitochondrial and cellular iron homeostasis.

Steven Claypool (Johns Hopkins University, Baltimore, USA) has critically examined the role of cardiolipin chains remodeling in yeast. He pointed out that it remains unclear how distinct molecular CL species might support different mitochondrial functions.

Andreas Reichert of Goethe University, Frankfurt, Germany and Luca Scorrano of the University of Padova, Italy have examined different mitochondrial systems involved in the membrane dynamics of cristae. Using complexome

profiling, Reichert and collaborators identified apolipoprotein O (APOO) and apolipoprotein O-like protein (APOOL) as putative components of the mitofilin/MINOS protein complex which was recently implicated in determining cristae morphology. APOOL is a mitochondrial membrane protein facing the intermembrane space that specifically binds to cardiolipin in vitro but not to the precursor lipid phosphatidylglycerol. Overexpression of APOOL led to fragmentation of mitochondria, a reduced basal oxygen consumption rate, and altered cristae morphology. Downregulation of APOOL impaired mitochondrial respiration and caused major alterations in cristae morphology. APOOL physically interacts with several subunits of the MINOS complex, namely mitofilin, MINOS1, and SAMM50. Findings suggest that APOOL is a cardiolipin-binding component of the mitofilin/MINOS protein complex determining cristae morphology in mammalian mitochondria.

Scorrano presented data obtained in the course of investigations aiming to clarify the relationship between respiratory function and mitochondrial ultrastructure and provided evidence that cristae shape determines the assembly and stability of RCS and hence mitochondrial respiratory efficiency. In addition, unpublished findings on the role of OPA protein and its interaction with cardiolipin were also shown in his presentation.

In the afternoon session entitled “Bacterial and mitochondrial cardiolipins face to face,” studies on the role of cardiolipin in bacteria and mitochondria from different organisms were described.

Recently, X-ray resolution structures of several respiratory complexes of *Escherichia coli* have identified the location of phospholipids within the complexes. Crystallographic data obtained by studying the quinol oxidizing nitrate reductase complex of *E. coli* were presented by Axel Magalon (CNRS, Marseille, France); cardiolipin binds specifically in the vicinity of heme and the quinol substrate site and this binding activates the respiratory complex by tuning the interaction with quinol substrate and it participates in supercomplex formation.

Peter Buetikofer (University of Bern, Switzerland) underlined the role of cardiolipin in the single mitochondrion present in the cell of the eukaryotic protozoan parasite *Trypanosoma brucei*, the causative agent of human African sleeping sickness. The location of enzymes involved in the biosynthesis of CL was investigated. The CL synthase of this organism is of bacterial type. The different effects of decreased expression of CL synthase in *T. brucei* procyclic and bloodstream forms were described as well. Remarkably, the research group in Bern was able to demonstrate that CL synthase and phosphatidylglycerophosphate synthase are part of a high molecular mass complex of about 700 kDa. The identity of possible interaction partners of the two enzymes is currently under investigation.

The role of phosphatidic acid as precursor of CL biosynthesis was described by Takashi Tatsuda of the

University of Cologne, Germany. In yeast, Ups1 is part of a membrane protein family located in the intermembrane space of mitochondria. This protein shuttles phosphatidic acid between the outer and the inner membrane of mitochondria. Lipid transfer occurs only after the dynamic assembly of Ups1 with Mdm35. It has been suggested that intramitochondrial lipid trafficking may involve a regulatory feedback mechanism that limits the accumulation of cardiolipin in mitochondria.

3 Posters

Twelve posters were presented. They covered the role of cardiolipin in yeast, cancer cells, bacteria, and viruses. Reports of laboratories analyzing samples of Barth patients were also presented (Ann Bowron from England and Amelia Morrone from Italy).

4 Perspectives in cardiolipin research

Data presented in Bari outline possible trends in cardiolipin research in the near future.

It remains unclear what the role of tafazzin is in cardiolipin turnover and what the significance of CL acyl chain remodeling is in mitochondria.

The precise location of tafazzin in mitochondria and its possible interaction with respiratory complexes and super-complexes is not yet known. Since tafazzin defects alter the mitochondrial cristae structure, the possible interdependence of the tafazzin function with that of other components of mitochondrial cristae, needs to be elucidated.

In the past, cardiolipin has been considered as a kind of side topic for masochistic scientists. The success of the cardiolipin workshop in Italy shows that things are changing and that cardiolipin is acquiring a main role in lipid and mitochondrial studies.

We are grateful to the ICBL scientific committee for giving us the opportunity to organize the cardiolipin workshop as satellite event of 54th ICBL. We are also grateful to Frank Amoneit of Eurofedlipid, who kindly offered his help to built and handle the web page of the workshop. The cardiolipin workshop was sustained by research funds of Angela Corcelli and by the supports of Barth Syndrome Foundation, Avanti Polar Lipids, and Bruker Daltonics. We thank all invited speakers who have personally covered travel expenses and therefore significantly contributed to lowering the budget of the meeting. Last but not least, we are grateful to Simona Lobasso for supporting us as local organizer and to the biology students Valentina Tanese and Ruggero Gorgoglione together with the post-docs Rita Vitale and Roberto Angelini for their help on site during the meeting.

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