Prof. Giovanna Lippe, Department of Medicine, University of Udine, Piazzale Kolbe, I-33100 Udine, Italy
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Place and date of birth: Padova (Italy), July 15, 1957
Citizenship Italian
Marital Status Married, two sons

Degreee: in Biological Sciences, University of Padova, Italy, (1981) cum laude

Positions:

1982	State exam to become licensed Biologist (National Board), University of Padova, Italy
1984-1988	PhD student in "Molecular and Cellular Biology and Pathology" at the University of Padova
1986	EMBO short term fellow, University of Oxford, UK
1988	PhD degree presenting a thesis untitled "Regulatory mechanisms of mitochondrial ATPsynthase".
1991	Researcher of Biochemistry, University of Udine, Department of Biomedical Sciences and Technologies
2001	Associate Professor of Biochemistry, University of Udine, Department of Biomedical Sciences and Technologies
2008-2021	Associate Professor of Biochemistry, University of Udine, Department of Agricultural, Food, Environmental and Animal Sciences
July, 2020	National Scientific Qualification to function as Full Professor in Biochemistry (MIUR competition sector 05/E1 - General Biochemistry)
April, 2021	Associate Professor of Biochemistry, University of Udine, Department of Medicine

Scientific interests

Her research mainly concerned the modulation of the F-ATP synthase complex under different patho- physiological conditions in cellular and animal models.

In the first years of her career, a key topic has been the regulatory function of the natural inhibitor IF1 that binds to the catalytic F1 sector of F-ATP synthase. She defined the kinetic properties and the main effectors of IF1 binding to the mammalian complex by using a labelled IF1 and demonstrated the beneficial down-regulation of F-ATP synthase by IF1 binding under stress conditions in *in vitro* and *in vivo* experimental models. She analysed the potential role of IF1 in the structural stabilization of F-ATP synthase dimers in isolated mitochondria and, more recently, in H9c2 cardiomyoblasts, demonstrating that IF1 can promote, together with the FO subunit e, the stabilization of partially destabilized dimers/oligomers (Bisetto et al., *Biochim Biophys Acta* 1827, 807-16, 2013). The essential role of subunit e in the F-ATP synthase self-association has been further demonstrated by *in situ* limited proteolysis, native electrophoresis and mass spectrometry (Bisetto et al., *J Bioenerg Biomembr*. 40, 257-67, 2008). Finally, the ability of IF1 to modulate the ectopic form of F-ATP synthase has been defined in rat liver (Giorgio et al., *J Bioenerg Biomembr* 42, 117-23, 2010; Rai et al., *J Bioenerg Biomembr* 45, 569-79, 2013).

In the last years her experience in F-ATP synthase has enabled her to actively participate to the seminal discovery that this enzyme complex forms the permeability transition pore (PTP) under conditions of high matrix Ca^{2+} and oxidative stress (Bernardi et al., *FEBS J* 2021; Lippe et al., *Oxid Med Cell Longev* 8743257, 2019; Bernardi et al., *Circ Res* 116,1850-62, 2015; Bernardi et al., *Physiol Rev* 95, 1111-55, 2015). PTP is a conserved high-conductance channel located in the inner mitochondrial membrane that allows diffusion of solutes up to about 1,500 Da and whose long-lasting opening may represent a point of no return in cell commitment to death. The PTP molecular nature was still a matter of conjectures, and an essential step for its identification was her finding that the mitochondrial matrix protein Cyclophilin D (CyPD), a well-known PTP inducer, interacts with the F-ATP synthase OSCP subunit, which is located on top of the catalytic F1 sector, ensuring the structural and functional coupling between FO and F1 (Giorgio et al., *J Biol Chem* 284, 33982-8, 2009). Direct evidence that PTP forms from F-ATP synthase was then obtained by electrophysiology studies, which demonstrated that ATP synthase dimers from mitochondria of mammals, *Drosophila melanogaster* and yeast incorporated in planar bilayers triggered oxidant- and Ca^{2+}-dependent currents with features indistinguishable from those of the PTP (Giorgio et al., *PNAS* 110, 5887-92, 2013; von Stockum et al., *J Biol Chem* 290, 4537-44, 2015; *J Biol Chem* 289, 15980-5, 2014).
Further evidence that the PTP originates from F-ATP synthase has been obtained through genetic manipulation of the enzyme by deletion or site-directed mutagenesis (Giorgio et al., *EMBO Rep* 18, 1065-76, 2017; Antoniel et al., *EMBO Rep* 19, 257-68, 2018; Carraro et al., *Cell Physiol Biochem* 50,1840-1855, 2018; Guo L. et al., *J Biol Chem* 294, 10987-97, 2019; Galber C. et al, *Cell Rep* 35(6), 109111, 2021).

She has been also involved in the identification of marker proteins in food quality by proteomic approaches and in studies on the bioaccessibility of bioactive compounds.

She is a member of the Italian Society of Biomembranes and Bioenergetics, of which she was the executive secretary from 2005 to 2009 and from 2014 to 2018.

Bibliometric indications

As of March, 2023 Prof. Lippe has published 90 peer-reviewed articles (total citations 4588) with an H index of 32 and i10-index of 63 (Google Scholar). Her most quoted experimental paper [Dimers of mitochondrial ATP synthase form the permeability transition pore. V. Giorgio, S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, G.D. Glick, V. Petronilli, M. Zoratti, I. Szabó, **G. Lippe**, P. Bernardi. *Proc.Natl. Acad. Sci. U S A.* 110, 5887-92, 2013] has 867 citations; her most quoted review [The Mitochondrial Permeability Transition Pore: Channel Formation by F-ATP Synthase, Integration in Signal Transduction, and Role in Pathophysiology. P. Bernardi, A. Rasola, M. Forte, **G**. **Lippe** *Physiol Rev* 95, 1111-55, 2015] has 466 citations. Her "top ten" list gathers 2498 citations.

Editorial activities

Topic Editor: *Frontiers in Physiology*, Structure and Function of F- and V-ATPases, 2019

Ad hoc reviewer: *Ann N Y Acad Sci; Biochim Biophys Acta; Biocheml Biophys Res Commun; Biochem J; Br J Pharmacol; Cell Death Differ; Cell Metab; Cell Physiol Biochem; FASEB J; FEBS J; Food Chem; Food Rev Int; Int. Food Res; Front Physiol; Front Oncol; Hum Mol Genet*; *Innov Food Sci Emerg Technol; Int J Food Sci Nutr; J Alzheimer's Dis; J Agric Food Chem; J Bioenerg Biomembr; J Biol Chem; J Cell Mol Med ;* J. *Neurosci. Res; Mol Psychiatry; Oxid Med Cell Longev. Sci Rep.*

Meetings as organizer

Riunione Annuale del Gruppo Italiano di Biomembrane e Bioenergetica, Udine, 14-16 giugno 2009
Annual Meeting of the Italian Group of Biomembranes and Bioenergetics, Udine, June 18-20, 2015

Funding

Ministry for the University and Scientific Research, Italy: Regione Autonoma Friuli Venezia Giulia, Italy.

Lectures at Meetings

1. 8th European Bioenergetics Conference, Valencia, Spain 1994
2. 13th European Bioenergetic Conference, Pisa, Italy 2004
3. 47° Congresso Nazionale della Società Italiana di Biochimica, Riccione, Italy, 2005
4. 16th European Bioenergetic Conference, Warsaw, Poland 2010
5. II Indo-Italian Workshop on *Industrial and pharmaceutical Biotechnology*, Madurai, India, 2010
6. 9th BRSI Convention & International Conference on *Industrial Biotechnology*, Patiala, India 2012
7. Meeting *Industrial Pharmaceutical Biotechnology*, New Delhi, India 2014
8. 11th MiP *Conference* on Mitochondrial Physiology, Luční Bouda, Czech Republic, 2015
9. Annual Meeting of Young Researchers in Physiology**,** Magnano in Riviera, Udine, Italy, 2016

Invited seminars

1. Service de Bioénergétique & CNRS-URA, CEA Saclay, France (host Francis Haraux, 2005)
2. Università degli Studi di Padova, Italy (host Paolo Bernardi, 2006)
3. Instituto de Investigação e Inovação em Saúde, Universidade do Porto (host Clara Ferreira Pereira, 2017)

14th June, 2023

Giovanna Lippe

Scientific interests

Her research mainly concerned the modulation of FOF1 ATP synthase under different patho-physiological conditions in cellular and animal models.

A first topic has been the regulatory function of the natural inhibitor IF1 of ATP synthase. She defined the kinetic properties and the main modulators of IF1 binding to the mammalian complex by using a labelled IF1 and demonstrated the beneficial down-regulation of ATP synthase by IF1 binding under stress conditions in *in vitro* and *in vivo* experimental models, such as in biopsy samples from goat hearts subjected to anoxia and from anaesthetized open-chest goats subjected to ischemic preconditioning. She analysed the potential role of IF1 in the structural stabilization of the ATP synthase dimers. By physical depletion of IF1 from beef heart mitochondrial membrane preparations and analysis of the ATP synthase aggregation states by Blue Native Gel Electrophoresis (BN-PAGE) she demonstrated that the dimer formation can occur independently from the IF1 binding. On the other hand, analysing H9c2 cardiomyoblasts committed to cardiomyocyte differentiation she found that IF1 can promote the stabilization of partially destabilized dimers/oligomers containing sub-stoichiometric amount of the FO subunit e, which contributes to the dimerization and oligomerization interfaces. Indeed, she had elucidated the essential role of the subunit e in the self-association of ATP synthase in bovine heart mitochondria by *in situ* limited proteolysis, BN-PAGE and mass spectrometry (LC-MS/MS). Moreover, she proposed that the phosphorylation status of a Tyr residue in the γ subunit of F1 might also take part in the dimer stabilization.

Another topic of her research concerns the study of mechanisms underlying the oxidative inactivation of ATP synthase. She demonstrated that the enzyme inactivation can be mediated by Fe ions bound to the catalytic F1 sector through site-directed generation of highly reactive oxygen species and characterized the structural properties of Fe ions binding sites by biochemical approaches and by Electron Paramagnetic Resonance.

In the last years her experience in ATP synthase has enabled her to actively participate to the seminal discovery that F-ATP synthase dimers form the permeability transition pore (PTP) under conditions of high matrix Ca^{2+} and oxidative stress (Bernardi P et al., *FEBS J* 2021; Lippe G et al., *Oxid. Med. Cell. Longev*. 2019; Bernardi P. et al., *Circ. Res*. 2015; Bernardi P. et al., *Physiol. Rev* 2015). PTP is a conserved high-conductance channel located in the inner mitochondrial membrane that allows diffusion of solutes up to about 1,500 Da and whose long-lasting opening may represent a point of no return in cell commitment to death. The PTP molecular nature was still a matter of conjectures, and an essential step for its identification was her finding that the mitochondrial matrix protein Cyclophilin D (CyPD), a well-known PTP inducer, interacts with the OSCP subunit of ATP synthase, which is located on top of the catalytic F1 sector, ensuring the structural and functional coupling between FO and F1. Conversely, the PTP inhibitor CsA displaces CyPD from OSCP, resulting in enzyme activation (Giorgio V et al., *J Biol Chem* 2009). Direct evidence that PTP forms from ATP synthase was then obtained by electrophysiology studies, which demonstrated that ATP synthase dimers incorporated in planar bilayers triggered oxidant- and Ca^{2+}-dependent currents with features indistinguishable from those of the PTP (Giorgio V et al., *PNAS* 110, 5887-92, 2013). Further evidence has been more recently obtained through genetic manipulation of the enzyme by deletion or site-directed mutagenesis, briefly described below.
Given that PTP opening strictly requires Ca2+, an obvious candidate for C2+ binding site on F-ATP synthase was the nucleotide binding site on the catalytic beta subunits located in the F1 sector, where the physiological Mg2+ can be replaced by Ca2+ able to support the ATP hydrolysis. Consistently, partial T163S substitution in HeLa cells prevented Ca2+-ATP hydrolysis, and this change in relative affinity matched decreased sensitivity of the PTP to Ca2+, which was paralleled by resistance of cells

to death stimuli and to decreased apoptosis in zebrafish *in vivo* (Giorgio V. et al., *EMBO Rep.* 18, 1065-76, 2017).
Perhaps the most striking feature of the PTP is its strong inhibition by matrix H+ mediated by reversible protonation of diethylpyrocarbonate-reactive His residues accessible from the matrix side. When the H112Q and H112Y mutations of the F-ATP synthase OSCP subunit were performed in HeLa cell – which did not affect enzyme assembly and function – a complete prevention of the inhibitory effects of acidic pH both in PTP-dependent swelling measurements in mitochondria and in single channel patch-clamp recordings in mitoplasts was observed, demonstrating that H112 of OSCP is the unique, diethylpyrocarbonate-reactive His residue responsible for the PTP block by H+ (Antoniel M et al., *EMBO Rep.* 19, 257-68, 2018).
Experiments in yeast allowed to confirm that the PTP originates from F-ATP synthase and that e, g and b subunits create a domain within the membrane that is critical for the generation of the high-conductance channel (Carraro et al *J Biol Chem* 2014; Carraro et al., Cell Physiol Biochem 2018;50:1840-1855). More precisely, individual and simultaneous amino acid substitutions in subunit e and g demonstrated that the electrostatic interaction between R8 of subunit e and E83 of subunit g is important for generation of the full-conductance PTP from F-ATP synthase (Guo L. et al., *J Biol Chem* 294, 10987-97, 2019). As the f subunit is localized at the base of the ATP synthase peripheral stalk, the possibility that it may be involved in regulation of channel formation has been tested. Indeed, by downregulation of the f subunit in HeLa cells through RNA interference, a decrease in PTP size was observed, whereas its re-expression in f subunit knockdown cells rescues the PTP (Galber C. et al, *Cell Rep* 35(6), 109111, 2021).

and that mutant strains lacking the e and g F-ATP synthase subunits display a striking resistance to PTP opening