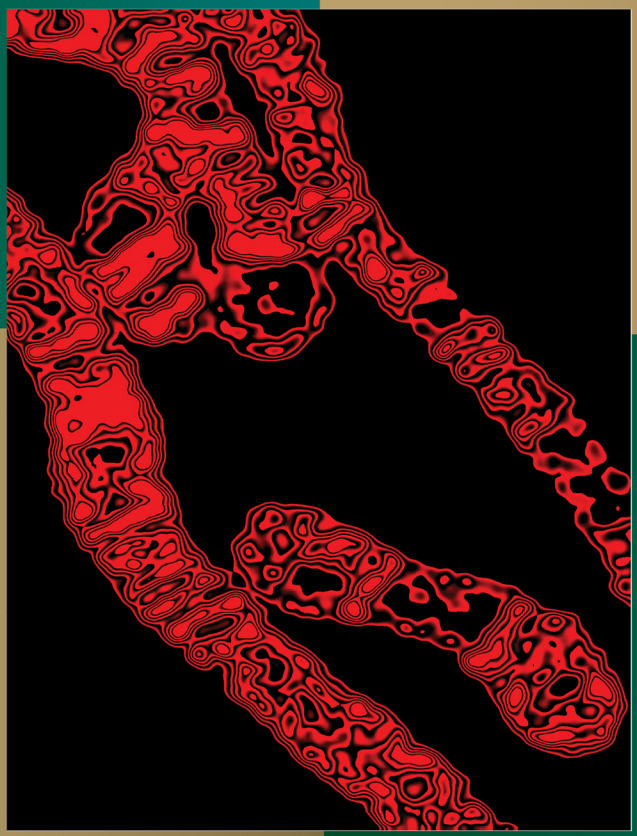
THE DYNAMIC NATURE OF MITOCHONDRIA

FROM ULTRASTRUCTURE TO HEALTH AND DISEASE



EDITED BY

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The Dynamic Nature of Mitochondria

… a novel and amazing view into mitochondrial research revealing the dynamic nature of this organelle and how this is linked to numerous fundamental biological processes …

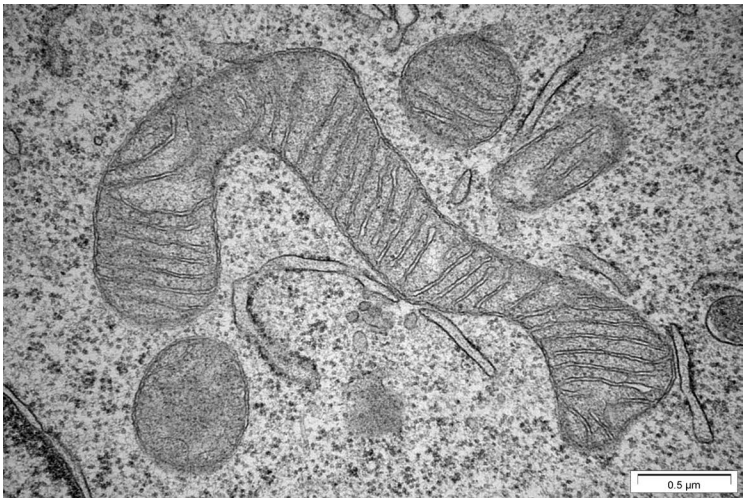

FIGURE 0.1 Electron micrograph of wild-type mitochondria in HEK cells. Tubular mitochondrial section with regular lamellar cristae is shown. Close association of mitochondria to the endoplasmatic reticulum is nicely visible at multiple regions. (Image with courtesy of Andrea Borchardt and Ruchika Anand.)

Mitochondrial research has exploded over the last ~150 years. This book gives an amazing view on a conceptual change in our understanding of mitochondrial biology. It becomes clear that mitochondria are extremely dynamic in nature, controlling life at multiple levels. Mitochondria rule energy conversion, adapt cells well to changing stress and nutrient conditions, and regulate many cellular processes including immunity. The dynamic nature of mitochondria occurs at an intramitochondrial level but also includes its ability to interact with other organelles and to modulate multiple signalling pathways. It is thus not surprising that alterations or inabilities to ensure this dynamic behaviour is linked to ageing and human diseases.

The following sections give an updated view on mitochondria:

- Mitochondrial ultrastructure: molecular mechanisms shaping the inner membrane
- Mitochondrial cristae and lipid dynamics: from super-resolution microscopy to lipid-OXPHOS interplay
- Mitochondrial control of cellular homeostasis: From redox signalling to interorganellar contact sites
- Mitochondria in health and disease: from mtDNA release to Complex I assembly
- Advanced methods in mitochondrial biology and metabolism research
- Integrative view on mitochondrial research and outlook

The field of mitochondrial research has always been full of surprises and has helped science to advance tremendously. It developed hand in hand with landmark developments in technology, such as super-resolution microscopy (nanoscopy), and is currently influencing an increasing number of scientific disciplines. There is still much ‘new’ to find out about this ‘old’ organelle, and I think that you can find interesting and also unexpected aspects of mitochondrial biology in this book. I hope the book will enhance your scientific curiosity and inspire your own research.

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The Dynamic Nature of Mitochondria

From Ultrastructure to Health and Disease

Edited by Andreas S. Reichert

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Designed cover image: Psychedelic art 'Mitochondria texting'. Original data obtained by Arun Kumar Kondadi showing mammalian mitochondria expressing MIC13-SNAP localized to cristae and crista junctions which was visualized by live-cell STED nanoscopy. Psychedelic modification of data image was created by Andreas S. Reichert.

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Preface

Mitochondrial research has expanded tremendously over the last ~150 years and plays fundamental roles in many scientific fields, including basic and applied research in biology, biochemistry, cell biology, physiology, toxicology, pharmacology, and medicine. Here we give a general overview on major advances along this time but we focus on a conceptual change in our understanding of mitochondrial biology, namely that mitochondria are extremely dynamic in nature at different levels. One level is that they constantly undergo dynamic cristae membrane remodelling within mitochondria and that lipids and proteins are highly dynamic moving between distinct membrane compartments. Another level is the dynamic reshaping of the mitochondrial network by fusion and fission events between mitochondria. Moreover, we know that mitochondria are in close contact to different organelles such as peroxisomes, lysosomes, and the endoplasmatic reticulum. It is amazing to see how mitochondria are well integrated and connected to nearly all non-mitochondrial processes in a cell and how they control a pleiotropy of functions that have previously been thought to be separated. Mitochondria rule energy conversion and adaptation to changing stress and nutrient conditions and harbour many essential other cellular functions including anabolic and catabolic functions as well as signalling functions. Thus, the dynamic nature of mitochondria which also includes its flexibility to interact with other organelles and pathways is essential for cells to adapt to changing conditions. It is thus not surprising that alterations or an inability to ensure this dynamic behaviour is linked to ageing and human diseases.

How mitochondrial ultrastructure is established is elegantly described in **Chapter 1** by **Patrick Horten** and **Heike Rampelt**, focusing on the intricate interplay of several players such as namely OPA1, the F_IF_O ATP synthase, and the MICOS complex. Another level of regulation of mitochondrial ultrastructure via proteolysis is very well covered in **Chapter 2** by **Gunjan Purohit** and **Oleh Khalimonchuk**. In which way the spatial distribution within the different subcompartments is established and how it can adapt to changing conditions or stresses is well summarized here. A focus is given to the proteolytic control of mitochondrial architecture by the inner membrane protease OMA1. Despite the fact that cristae are highly variable in structure, they were thought to be rather static for a long time. Now different studies using super-resolution nanoscopy in living cells led to an updated view on cristae being highly dynamic instead. These developments and the role of the MICOS complex is covered beautifully in **Chapter 3** by **Ruchika Anand** and **Arun Kondadi**. The role of lipids and its stable and dynamic interactions with protein complexes, in particular with Complex I of the electron transport chain, is very nicely reviewed in **Chapter 4** by **Nanami Senoo** and **Steven Claypool**. How and which lipids determine mitochondrial membrane dynamics and its many physiological functions is one of the underexplored aspects of mitochondrial research. **Chapter 5** by **Jan Riemer** and **Helmut Sies** gives us an excellent overview on the role of mitochondria in redox signalling, whereas **Chapter 6** by **Carla Lopes** and **Nuno Raimundo** give astonishing insights into the complex interplay of mitochondria and lysosomes, and how this is integrated in a diverse set of cellular functions. That mitochondria are signalling hubs raised major attention in recent years. In **Chapter 7** by **Andrea Irazoki** and **David Pla-Martín**, the authors give a wonderful view into the role of mitochondria in regulating the innate immune response. They discuss how mitochondria act as signalling platforms recruiting complexes that initiate signalling to the nucleus. Further, they summarize the different pathways of mtDNA release and how mtDNA acts as a mitochondrial damage-associated molecular pattern initiating inflammatory responses. Released mtDNA in the cytoplasm, inside endosomes, or secreted to the extracellular compartment acts as intra- or intercellular/paracrine signals of mitochondrial dysfunction. How Complex I of the electron transport chain harbouring 45 subunits assembles with the help of assembly factors and how impairments thereof lead to mitochondrial diseases is elaborated excellently in **Chapter 8** by **Luke Formosa**. Last but not least, in **Chapter 9** by

Odette Deen-Rozalen, Marius Nieke, Sara Shumka, Hannes Beyer, and Matias Zurbriggen, we get a detailed and amazing overview into state-of-the-art methods in optogenetics to study and manipulate mitochondrial functions and processes linked, including mitophagy and modulation of mitochondrial shape.

Certainly, this book can only cover a minor fraction of the set of mitochondrial functions but it gives important insights on the overall highly dynamic nature of mitochondria and its integration in a larger context. Moreover, it summarizes several technological advances in mitochondrial research leading to these views. In **Chapter 10**, beyond some personal views, I aim to give a synopsis on the field, its development over time, and its possible future. I further propose the '*Dyna*mic-*Tr*ap-and-Fl*ux*' ('DynaTrux') model giving a possible rational for the physiological function of mitochondrial cristae dynamics.

Editing this book was a true challenge and I fully underestimated the work associated with it. Still, it was also inspiring and I thank **Helmut Sies** and **Enrique Cadenas** for giving me the chance to do so. I am deeply grateful to all contributors of this book, who did a fantastic job in sharing their expertise and giving excellent insights into the topic. I hope that all readers find interesting and also unexpected aspects of mitochondrial biology in these chapters, and even more importantly that it inspires their own research and scientific curiosity – enjoy reading.

Andreas S. Reichert

Editor

Andreas S. Reichert has been a Professor and Director of the Institute of Biochemistry and Molecular Biology I at the Heinrich Heine University Düsseldorf, Germany since 2014. He's been a board member of the Biologisch-Medizinisches Forschungszentrum (BMFZ) at Heinrich Heine University Düsseldorf, Germany since 2016, and a speaker of BMFZ since 2021. Prof. Dr. Reichert received his Ph.D. under Prof. Dr. h.c. mult. Svante Pääbo at the Institute of Zoology, Ludwig-Maximilians-Universität München, Germany in 1999, and was a Post-Doc under Prof. Dr. h.c. mult. Svante Pääbo and Prof. Dr. Mario Mörl at the Max Planck Institute for Evolutionary Anthropology, in Leipzig, Germany from 1999–2000. After that he worked as a group leader in the group of Prof. Dr. h.c. mult. Walter Neupert at the Adolf Butenandt Institute for Physiological Chemistry, Ludwig Maximilians-Universität München, Germany from 2000–2007 before he operated as Professor and Head of Mitochondrial Biology at Goethe University Frankfurt am Main, Germany, from 2007 to 2014.

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Part I

Mitochondrial Ultrastructure

Molecular Mechanisms Shaping the Inner Membrane

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1 Roles of the F_1F_o-ATP Synthase and MICOS in Mitochondrial Membrane Organization

Patrick Horten and Heike Rampelt[*]

INTRODUCTION

The intricate architecture of the mitochondrial inner membrane is of fundamental importance for many aspects of mitochondrial physiology, including energy metabolism, protein and lipid biogenesis, and mitochondrial inheritance. In contrast to the planar outer membrane, the inner mitochondrial membrane is characterized by large membrane invaginations, the cristae, resulting in topologically and functionally distinct subcompartments of both the inner membrane and the intermembrane space (IMS). The lamellar or tubular cristae are connected to the smooth inner boundary membrane (IBM) by narrow membrane necks called crista junctions (Frey et al., 2002; Mannella, 2006; Zick et al., 2009; Pánek et al., 2020; Klecker & Westermann, 2021). Crista junctions impose restraints on diffusion between the subcompartments of the membrane and the IMS and thereby contribute to their functional specialization. Protein complexes are asymmetrically distributed between the subcompartments: the protein translocation and biogenesis components are localized in the IBM, whereas the oxidative phosphorylation (OXPHOS) complexes are embedded in the cristae membranes (Gilkerson et al., 2003; Vogel et al., 2006; Cogliati et al., 2016; Stoldt et al., 2018). Moreover, the particular architecture of cristae membranes enables optimal respiration and ATP synthesis, and rapidly adapts to metabolic changes and altered availability of substrates (Hackenbrock, 1966; Strauss et al., 2008; Davies et al., 2011; Colina-Tenorio et al., 2020).

Inner membrane topology and compartmentalization is determined by specialized protein machineries in synergy with phospholipids. While many open questions remain, especially regarding the dynamics and variability of the inner membrane, it is clear that the F_1F_O-ATP synthase and the mitochondrial contact site and cristae organizing system (MICOS) are central determinants of inner membrane architecture (Cogliati et al., 2016; Kühlbrandt, 2019; Colina-Tenorio et al., 2020; Eramo et al., 2020; Anand et al., 2021; Mukherjee et al., 2021). These two protein complexes preferentially localize to distinct submitochondrial regions and, among other functions, generate opposite types of membrane curvature that are required to shape cristae in a balanced manner. Dimers and dimer rows of the F_1F_O-ATP synthase are necessary to generate the positive curvature at crista rims, while MICOS stabilizes the negative curvature at crista junctions. Owing to advanced microscopy approaches including super-resolution microscopy, electron tomography and FIB-SEM (focused ion beam scanning electron microscopy) (Wolf et al., 2019; Jakobs et al., 2020; Kondadi et al., 2020; Ohta et al., 2021; Zabeo & Davies, 2022), we have seen considerable progress in the last few years in the study of membrane architecture, as well as of membrane dynamics and remodelling, in unprecedented structural detail. These studies have revealed general principles of cristae morphogenesis. In *Saccharomyces cerevisiae*, there appear to exist at least two parallel pathways for the biogenesis of tubular versus sheet-like cristae (Harner et al., 2016; Kojima et al., 2019; Klecker & Westermann, 2021). Both pathways depend on the function of MICOS as well as the ATP synthase (Harner et al., 2016). The biogenesis of tubular cristae is thought to rely on the

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progressive growth of inner membrane invaginations and, consequently, also depends on phospholipid transport (Kojima et al., 2019). In yeast, the biogenesis of sheet-like cristae additionally requires the action of the dynamin-like GTPase Mgm1 (Harner et al., 2016) which mediates inner membrane fusion and functions as a membrane tether in cristae architecture (Meeusen et al., 2006; Cogliati et al., 2016; Faelber et al., 2019; Gao & Hu, 2021). In contrast, the human Mgm1 ortholog OPA1 is not required for sheet-like cristae (Stephan et al., 2020), but rather stabilizes crista junctions as reported previously (Frezza et al., 2006; Ishihara et al., 2006; Glytsou et al., 2016; Gao & Hu, 2021; Yapa et al., 2021). Instead, it appears that in human cells the Mic10 subcomplex plays an important role in the biogenesis of lamellar cristae (Stephan et al., 2020).

Importantly, it is becoming apparent that cristae membranes are unexpectedly dynamic and heterogeneous. Not only do lamellar cristae disappear within minutes after inactivation of Mgm1 in yeast (Harner et al., 2016), but time-resolved fluorescence imaging has shown that cristae in mammalian mitochondria are highly mobile and appear to even fuse and divide rapidly (Hu et al., 2020; Kondadi et al., 2020). Contrary to the notion that the mitochondrial membrane potential equilibrates across the entire inner membrane, a recent study discovered that individual cristae constitute independent units with differing membrane potentials (Wolf et al., 2019). Moreover, human mitochondria efficiently remodel the existing aberrant inner membranes of MICOS mutants upon re-expression of the missing component (Stephan et al., 2020). In summary, cristae membranes display an astonishing degree of dynamism and malleability beyond the rapidly interconvertible metabolic states of orthodox versus condensed mitochondria that have been known for half a century (Hackenbrock, 1966). The F_1F_O-ATP synthase and MICOS, central determinants of cristae architecture, as well as their functional crosstalk, are key to a mechanistic understanding of mitochondrial cristae morphogenesis and dynamic behaviour.

THE F_1F_O-ATP SYNTHASE

The F_1F_O-ATP synthase, or Complex V of the OXPHOS machinery, synthesizes the vast majority of the cellular ATP, using as its energy source the proton gradient across the inner membrane that is generated by the complexes of the respiratory chain. The F_1F_O-ATP synthase functions like a turbine: translocation of protons across the inner membrane via the membrane-embedded F_O part results in rotation of its c-ring, and the torque is transmitted through the central stalk, driving conformational changes and ATP synthesis by the catalytic F_1 part (Allegretti et al., 2015; Nirody et al., 2020; Almendro-Vedia et al., 2021). However, the mitochondrial F_1F_O-ATP synthase performs an additional crucial function: it induces the strong membrane curvature of cristae rims and edges (Figure 1.1A) (Paumard et al., 2002; Strauss et al., 2008; Davies et al., 2012; Nirody et al., 2020). In contrast to bacterial and chloroplast ATP synthases that are monomeric and evenly distributed, ATP synthases from mitochondria form dimers where the monomers connect at a pronounced angle (almost 90° in the so-called type I dimers of animal and fungal ATP synthase), resulting in membrane bending (Daum et al., 2010; Kühlbrandt, 2019; Nirody et al., 2020). Moreover, *in vivo* dimers associate to oligomeric rows that can reach lengths of more than 1 µm and localize to the strongly curved rims of lamellar cristae (Davies et al., 2011; 2012; Blum et al., 2019; Kühlbrandt, 2019). Oligomerization and membrane bending are widely conserved features of mitochondrial ATP synthases even in organisms with distinct dimer structures or subunit composition such as ciliates, unicellular green algae and others (Blum et al., 2019; Mühleip et al., 2019; 2020; Flygaard et al., 2020; Nirody et al., 2020; Gahura et al., 2022). Mathematical modelling indicated that strongly curved membranes can accommodate a higher charge density, and accordingly cristae are thought to function as proton traps (Strauss et al., 2008). Importantly, the mitochondrial ATP synthase creates its own topological niche within cristae that enables it to function as a proton sink at cristae rims, optimally exploiting the pH gradient across the mitochondrial inner membrane (Strauss et al., 2008; Cogliati et al., 2016; Blum et al., 2019; Toth et al., 2020; Rieger et al., 2021). Moreover, the local membrane potential in cristae was found to be higher than that at the IBM, in a manner directly dependent on ATP synthase activity (Wolf et al., 2019).

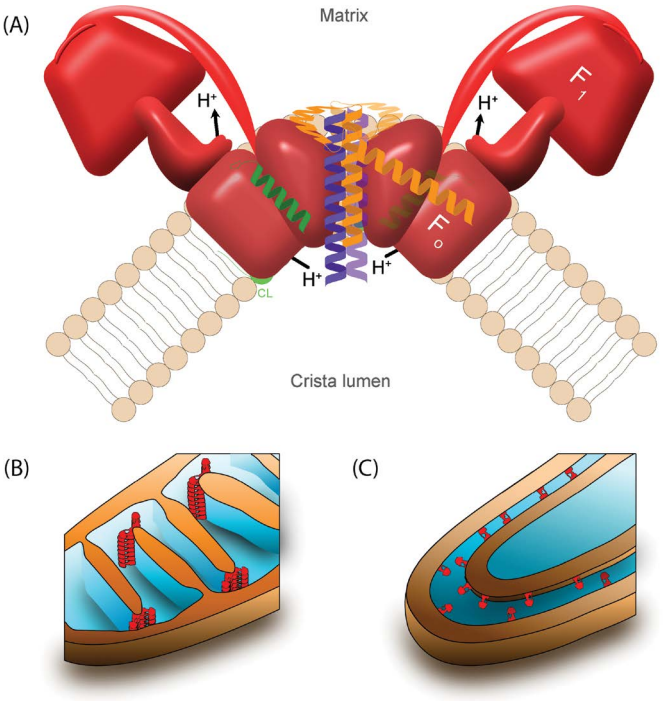

FIGURE 1.1 (A) The F_1F_O-ATP synthase of fungal and animal mitochondria forms dimers at an angle of almost 90° that induce the positive membrane curvature found at the edges and rims of cristae. Dimerization and membrane bending relies on the subunits Atp20 (subunit g, Orange) and Atp21 (subunit e, violet) that form a wedge domain. The subunit Atp19 (subunit k/DAPIT, dark green) additionally stabilizes dimers and dimer rows of the ATP synthase. Dimerization is not required for the catalytic action of the ATP synthase but supports optimal OXPHOS function via its role for cristae architecture. Cardiolipin (CL) stabilizes higher order assemblies of the ATP synthase. (B) In mitochondria from wild-type cells, ATP synthase dimers associate to oligomeric rows that localize to areas of strong inner membrane curvature, in particular to the strongly folded edges of cristae sheets. (C) Loss of ATP synthase dimerization results in aberrant inner membranes that are largely devoid of strong curvature and appear as balloons or onion skins.

Loss of ATP synthase dimerization and, consequently, of oligomerization results in aberrant inner membrane architecture: instead of cristae (Figure 1.1B), the inner membrane forms large balloon- or onion-like structures that can septate the entire mitochondrion and lack the strong membrane curvature seen at cristae rims (Figure 1.1C) (Paumard et al., 2002; Rabl et al., 2009; Davies et al., 2012). Due to the crucial role of ATP synthase higher order assemblies for cristae architecture, their disruption reduces the mitochondrial membrane potential and results in growth defects and human disease (Bornhövd et al., 2006; Habersetzer et al., 2013; Barca et al., 2018; Siegmund et al., 2018; Rampelt et al., 2022). Formation of the membrane bending ATP synthase dimer relies on the subunits Atp20 (subunit g) and Atp21 (subunit e) that form wedge-shaped assemblies bound to Atp4 (subunit b) on both sides of the dimer (Figure 1.1A) (Paumard et al., 2002; Arselin et al., 2004; Hahn et al., 2016; Guo et al., 2017; Pinke et al., 2020; Spikes et al., 2020). Atp20 and Atp21 interact within the wedge via conserved GxxxG motifs in their transmembrane segments (Arselin et al., 2003; Bustos & Velours, 2005; Gahura et al., 2022), but they also connect monomers from adjacent dimers (Arselin et al., 2003; Bustos & Velours, 2005; Pinke et al., 2020; Spikes et al., 2020). Additionally, Atp19 (subunit k, in mammals also known as DAPIT) engages in contacts with subunits from adjacent monomers, thereby stabilizing oligomers; in *S. cerevisiae*, it also stabilizes the oligomerization-competent dimer (Wagner et al., 2010; He et al., 2018; Pinke et al., 2020; Spikes et al., 2020). The monomer-monomer

contacts differ between mammalian and yeast mitochondrial ATP synthases, explaining the lower stability of the mammalian dimer (Guo et al., 2017; Pinke et al., 2020; Spikes et al., 2020). Mutation of human DAPIT causes the mitochondriopathy Leigh syndrome due to a pronounced destabilization of ATP synthase dimers and oligomers, resulting in cristae defects (Barca et al., 2018; Siegmund et al., 2018). The membrane-shaping function of the ATP synthase is also relevant in the context of ageing: the inner mitochondrial membrane becomes progressively disordered during ageing in various organisms and ultimately vesiculates (Daum et al., 2013; Brandt et al., 2017). In the ageing model organism *Podospora anserina*, these ultrastructural changes correlate with a loss of oligomeric and dimeric ATP synthase complexes (Brust et al., 2010; Daum et al., 2013).

Higher order assemblies of the ATP synthase are stabilized by the mitochondrial signature phospholipid cardiolipin. Cardiolipin has unique features: it comprises two phosphatidyl groups that harbour two negative charges and four acyl chains, and it has a conical shape in the membrane, resulting in a propensity to segregate to curved membrane regions (van den Brink-van der Laan et al., 2004; Osman et al., 2011; Schlame & Greenberg, 2016; Mårtensson et al., 2017; Almendro-Vedia et al., 2021). Moreover, cardiolipin is uniquely well-suited to alleviate packing stress in the membrane caused by protein complex formation and the extraordinarily high protein concentration of the inner mitochondrial membrane (Ren et al., 2014; Xu et al., 2019; 2021). Consequently, cardiolipin is required for the stability and full function not only of respiratory chain supercomplexes but also of metabolite carriers and protein translocases (Pfeiffer et al., 2003; Horvath & Daum, 2013; Mileykovskaya & Dowhan, 2014; Böttinger et al., 2015; Basu Ball et al., 2017; Mårtensson et al., 2017; Chatzispyrou et al., 2018; Hartley et al., 2019; Rathore et al., 2019). In humans, defects in cardiolipin remodelling that are associated with decreased cardiolipin levels (due to formation of monolyso-cardiolipin) cause Barth syndrome, another mitochondrial disease that presents with cardiomyopathy (Barth et al., 2004; Dudek et al., 2016; Ghosh et al., 2019). Importantly, decrease or lack of cardiolipin results in dissociation of ATP synthase dimers and oligomers (Acehan et al., 2011; Chatzispyrou et al., 2018). Remarkably, cardiolipin molecules specifically bind ATP synthase in different organisms, in particular as part of the Atp20/Atp21 wedge domain (Duncan et al., 2016; Mühleip et al., 2019; Spikes et al., 2020; Gahura et al., 2022).

The oligomeric state of the ATP synthase can also be regulated by protein-protein interactions involving additional factors. Under certain metabolic conditions, the activity of the ATP synthase is subject to regulation by inhibitory factor 1 (IF1) which, dependent on the pH, inserts in between the α and β subunits and is thought to block catalysis of the reverse reaction that would result in ATP hydrolysis (Cabezón et al., 2003; García-Aguilar & Cuezva, 2018; Gatto et al., 2022). Since IF1 forms dimers that link adjacent F_1 particles, binding of IF1 induces tetramerization of the ATP synthase (Gu et al., 2019; Pinke et al., 2020; Mendoza-Hoffmann et al., 2022). It remains unclear whether the structure of the IF1-stabilized ATP synthase tetramer corresponds to the arrangement of ATP synthase in the non-inhibited oligomeric rows. However, forcing ATP synthase tetramerization causes alterations in cristae ultrastructure (Campanella et al., 2008; Weissert et al., 2021), suggesting that IF1 regulates not only enzymatic activity but also the membrane deforming activity of the ATP synthase, with implications for mitochondrial bioenergetics and apoptotic resistance (Campanella et al., 2008; Faccenda et al., 2013; Rieger et al., 2021). In plant mitochondria whose monomeric ATP synthase does not have a membrane-shaping function, catalysis in the dark, which would result in ATP hydrolysis due to an inadequate energy status, is shut down by a different mechanism involving reversible disulfide bond formation in the γ subunit of the central stalk (Nalin & McCarty, 1984; Hahn et al., 2018; Kühlbrandt, 2019).

In a prime example of regulatory crosstalk between membrane-shaping machineries, Mic10, a core component of MICOS (see the next section), interacts with dimers and oligomers of the ATP synthase and stabilizes them (Eydt et al., 2017; Rampelt et al., 2017; Rampelt et al., 2022). Recent work has demonstrated that the interaction is conserved even in the evolutionarily very distant unicellular parasite *Trypanosoma brucei* (Cadena et al., 2021). This regulatory function of Mic10 is important for efficient metabolic adaptation and optimal mitochondrial physiology in yeast mitochondria

(Rampelt et al., 2022). This interaction may support the actions of the two machineries during cristae biogenesis (see section "Antagonism and crosstalk of MICOS and F_1F_O-ATP synthase").

THE MITOCHONDRIAL CONTACT SITE AND CRISTAE ORGANIZING SYSTEM

The cisternal or tubular cristae membranes are separated from the contiguous but topologically distinct inner boundary membrane (IBM) by relatively uniform membrane necks, the crista junctions. Crista junctions are circular or slit-like and typically 15–40 nm in diameter, depending on the species (Zick et al., 2009). These structures display a pronounced membrane curvature and require the widely conserved MICOS for their stability. MICOS is a heterooligomeric protein complex comprising at least six subunits in yeast and seven in human mitochondria that consists of two modules (Figure 1.2A) (Hoppins et al., 2011; von der Malsburg et al., 2011; Harner et al., 2011; Alkhaja et al., 2012; Pfanner et al., 2014; Guarani et al., 2015; Kozjak-Pavlovic, 2017; Colina-Tenorio et al., 2020; Anand et al., 2021; Mukherjee et al., 2021). The subunits are thought to be present in multiple copies, it is unclear however whether MICOS has a defined stoichiometry. Upon disruption of MICOS, crista junctions are lost, and the resulting cristae membranes are aberrant and lack a connection to the IBM (Figure 1.2B). Depending on the cell type and the subunit deletion, the inner membrane in MICOS-deficient mitochondria forms elongated membrane stacks or structures resembling a tube or onion; in some cases, inner membrane invaginations are mostly absent (Rabl et al., 2009; Darshi

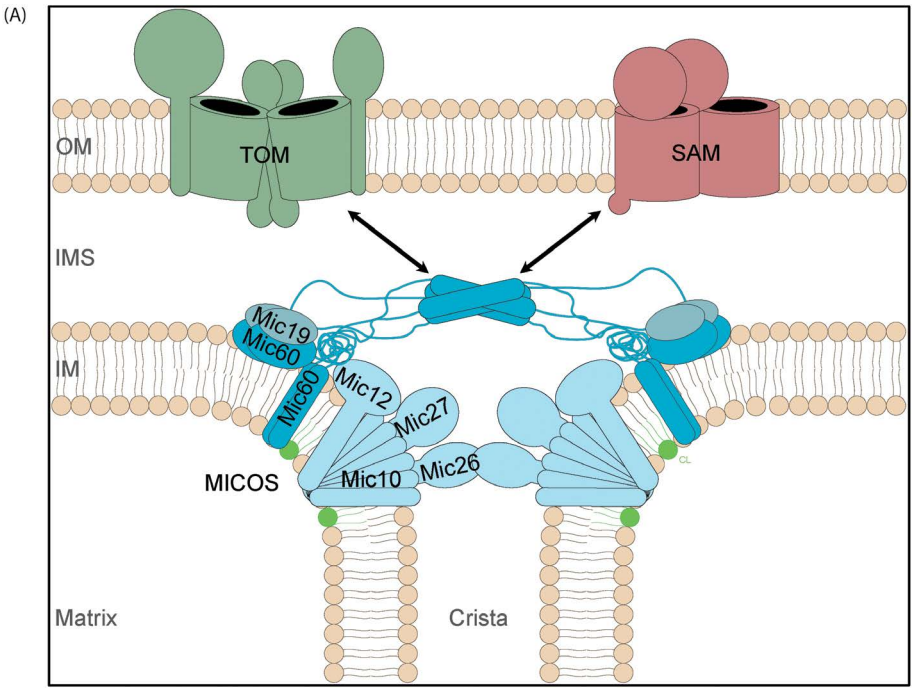

FIGURE 1.2 (A) MICOS (mitochondrial contact site and cristae organizing system) is a multimeric protein complex predominantly localized at crista junctions, the neck-like membrane structures that connect cristae to the inner boundary membrane. The MICOS subcomplex consisting of Mic60 and Mic19 forms contact sites between the inner and outer membrane (IM and OM) by interacting with the mitochondrial protein biogenesis machineries TOM (translocase of the outer mitochondrial membrane) and SAM (sorting and assembly machinery). In addition, the Mic60-Mic19 subcomplex has been suggested to form a dome over the crista junction. The Mic10 subcomplex that also includes Mic12 (QIL1 in mammals), Mic26 and Mic27 induces membrane curvature via oligomerization of Mic10 and influences the phospholipid environment of MICOS by binding cardiolipin (CL).

(Continued)

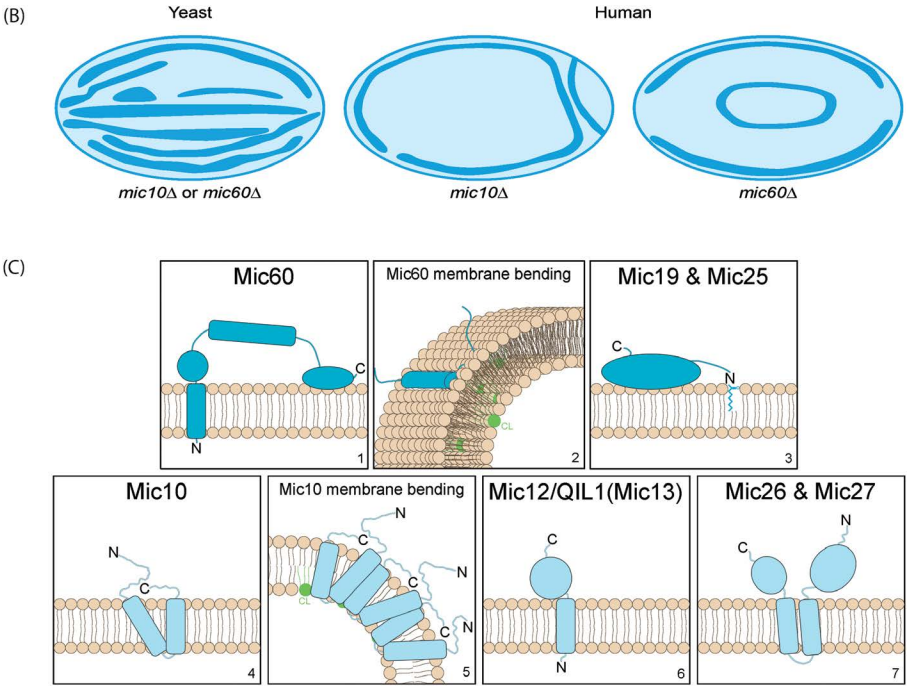

FIGURE 1.2 *(Continued)* (B) Mitochondrial inner membrane architecture in MICOS-deficient cells. The almost complete loss of crista junctions is a common feature for MICOS mutants, but the resulting cristae architecture differs somewhat between yeast and human cells. In yeast (left panel), strong MICOS mutations such as loss of Mic10 or Mic60 result in the accumulation of mostly parallel stacks of cristae membranes. Human mitochondria lacking Mic10 or Mic60 (middle and right panel) display a reduction of cristae membranes as well as aberrant membrane architecture. IMS/crista lumen shown in dark blue, matrix in light blue. (C) Topologies of MICOS subunits at the inner membrane and modes of membrane shaping. All MICOS subunits face the intermembrane space (IMS). The Mic60 subcomplex comprises Mic60 and Mic19, and in vertebrate cells, the Mic19 ortholog Mic25 (panels 1–3). Mic60 (panel 1) has an N-terminal transmembrane segment followed by several α-helical domains that form homomeric interactions or that bind the lipid bilayer (panel 2) or Mic19 (panel 3). Mic60 induces membrane bending by an amphipathic helix that asymmetrically displaces phospholipids from the IMS-facing monolayer of the inner membrane. Mic19 and Mic25 are anchored to the membrane by N-terminal myristoylation (panel 3) and interact with Mic60 via their CHCH (coiled-coil helix coiled-coil helix) domain. The Mic10 subcomplex comprises Mic10, Mic12 (QIL1/Mic13 in mammalian cells), Mic26 and Mic27 (panels 4–7). Mic10 has two transmembrane segments that form a hairpin (panel 4). This wedge-like shape together with its ability to oligomerize (panel 5) makes Mic10 a strong membrane-shaping factor. The phospholipid cardiolipin (CL) is important for Mic10 oligomer stability. Mic12/QIL1 (panel 6) bridges the Mic10 and Mic60 subcomplexes. Mic26 and Mic27 (panel 7) are related proteins that have regulatory functions for the Mic10 subcomplex.

et al., 2011; Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011; Alkhaja et al., 2012; Friedman et al., 2015; Guarani et al., 2015; Stephan et al., 2020). MICOS disruption results in a decreased membrane potential, impaired respiratory growth, lower efficiency of specific mitochondrial protein and cofactor biogenesis pathways, defects in mitochondrial dynamics and inheritance and impaired ability to adapt to metabolic changes (Rabl et al., 2009; Harner et al., 2011; Hoppins et al., 2011; von der Malsburg et al., 2011; Darshi et al., 2011; Alkhaja et al., 2012; Bohnert et al., 2012; Itoh et al., 2013; Ott et al., 2015; Guarani et al., 2015; Bohnert et al., 2015; Callegari et al., 2019; Anand et al., 2020; Dietz et al., 2021; Jakubke et al., 2021; Rampelt et al., 2022). Moreover, mutations in MICOS subunits can be causative for human mitochondriopathies: patient mutations

in QIL1 (the human ortholog of Mic12) and MIC26 cause severe mitochondrial diseases (see below; Guarani et al., 2016; Zeharia et al., 2016; Gödiker et al., 2018; Benincá et al., 2021; Peifer-Weiß et al., 2023). Mutations in the Mic60 mitochondrial targeting sequence with a dominant effect in reducing Mic60 levels have been identified in patients with Parkinson's disease (Tsai et al., 2018; Van Laar et al., 2019). In addition, alterations in the levels of MICOS subunits, in particular Mic60, have been implicated in the pathology of diabetes, epilepsy and other conditions both in patients and in disease models (Colina-Tenorio et al., 2020; Eramo et al., 2020; Mukherjee et al., 2021).

The two core components of MICOS, Mic60 and Mic10, are the evolutionarily oldest subunits. They exist in all major eukaryotic lineages and their presence correlates with that of inner membrane invaginations (Muñoz-Gómez et al., 2015; 2016; Huynen et al., 2016; Kaurov et al., 2018; Muñoz-Gómez et al., 2023). Mic60 homologs were identified even in a number of α-proteobacteria and thus predate the endosymbiotic event that gave rise to mitochondria (Muñoz-Gómez et al., 2015; Muñoz-Gómez et al., 2023). Since Mic10 and Mic60 both induce membrane curvature (see below), these results show that MICOS is an evolutionarily ancient membrane-shaping complex of the inner membrane. Interestingly, Mic10 and Mic60 form independent subcomplexes (von der Malsburg et al., 2011; Harner et al., 2011; Hoppins et al., 2011; Bohnert et al., 2015; Friedman et al., 2015; Guarani et al., 2015; Anand et al., 2016; Zerbes et al., 2016): the Mic60 subcomplex additionally includes its regulatory partner Mic19 (in metazoa also the closely related Mic25) (Xie et al., 2007; Rabl et al., 2009; Darshi et al., 2011; An et al., 2012; Ott et al., 2012; Ding et al., 2015; Tang et al., 2019). The Mic10 complex comprises Mic12 (yeast) or QIL1/Mic13 (animals) which connects the two subcomplexes, as well as the regulatory subunits Mic26 and Mic27 (Weber et al., 2013; Bohnert et al., 2015; Guarani et al., 2015; Koob et al., 2015; Zerbes et al., 2016; Rampelt et al., 2018; Urbach et al., 2021).

Mic60, also known as mitofilin, performs various functions in mitochondria that are non-redundant with those of other MICOS subunits. It is anchored in the inner membrane by an N-terminal transmembrane helix and exposes its bulk to the IMS, and it forms dimers as well as larger assemblies (Figure 1.2A) (Rabl et al., 2009; Körner et al., 2012; Zerbes et al., 2012; Hessenberger et al., 2017). Mic60, independently or together with its subcomplex partner Mic19, forms contact sites between the inner and outer mitochondrial membranes by interacting with protein translocases such as the translocase of the outer membrane (TOM) and the sorting and assembly machinery (SAM), as well as with other outer membrane proteins (Harner et al., 2011; von der Malsburg et al., 2011; Bohnert et al., 2012; Körner et al., 2012; Ott et al., 2012; Zerbes et al., 2012; Kaurov et al., 2018). In human cells, MICOS and the SAM complex form a stable higher order assembly, the mitochondrial intermembrane space bridging (MIB) complex (Xie et al., 2007; Darshi et al., 2011; Ott et al., 2012; 2015; Huynen et al., 2016; Sastri et al., 2017; Tang et al., 2019). These OM interactions of Mic60 directly facilitate the biogenesis of some mitochondrial proteins and phospholipids (Harner et al., 2011; von der Malsburg et al., 2011; Bohnert et al., 2012; Aaltonen et al., 2016; Kaurov et al., 2018; Eichenberger et al., 2019; Callegari et al., 2019). Since downregulation of mammalian SAM components results in MICOS destabilization as well as in a defective cristae architecture (Ott et al., 2012; 2015; Tang et al., 2019), the contact sites may also anchor crista junctions to the outer membrane and thereby stabilize them.

Interactions of Mic60 with OM proteins show differential dependence on its C-terminal mitofilin domain and the coiled-coil domain that includes a long extended α-helix (Figure 1.2C, panel 1) (Körner et al., 2012; Zerbes et al., 2012; Jumper et al., 2021). In the evolutionarily distant *T. brucei* whose MICOS complex is highly diverged, a mitofilin domain appears to be lacking in Mic60 but may be supplied in *trans* by trypanosome-specific MICOS subunits (Kaurov et al., 2018; Hashimi, 2019; Jumper et al., 2021). The coiled-coil domain mediates formation of a tetramer consisting of two antiparallel dimers (Figure 1.2A) (Bock-Bierbaum et al., 2022). The interactions of Mic60 are modulated by its MICOS subcomplex partner Mic19 via Mic19's CHCH (coiled-coil helix coiled-coil helix) domain, also known as a twin CX_9C domain. This domain, which is widespread among IMS proteins, is stabilized by conserved disulfide bonds, and proteins are imported by the mitochondrial import and assembly (MIA) pathway (Sakowska et al., 2015; Wiedemann & Pfanner, 2017; Habich et al., 2019). Association of Mic19 with the Mic60 mitofilin domain shifts Mic60