Mitochondrial Membrane Integrity: Structural Organization, Molecular Modulators, Pathophysiological Dysregulation, and Therapeutic Targeting

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Abstract:

Mitochondrial membrane integrity is a fundamental determinant of cellular viability, bioenergetic efficiency, and stress adaptation. In addition to serving as a physical barrier, mitochondrial membranes act as highly dynamic regulatory platforms that integrate metabolic flux, calcium signaling, redox homeostasis, innate immune activation, and regulated cell death. Loss of integrity of either the outer mitochondrial membrane (OMM) or inner mitochondrial membrane (IMM) precipitates profound cellular consequences, including apoptotic commitment, inflammatory signaling, metabolic collapse, and irreversible tissue damage. Over the past decade, and particularly in recent literature, mitochondrial membrane integrity has emerged as a central pharmacological target in cancer, neurodegeneration, cardiovascular disease, inflammatory disorders, and aging. This chapter provides a comprehensive and critical analysis of mitochondrial membrane architecture, biophysical properties, and the molecular networks that preserve or disrupt membrane integrity. Emphasis is placed on protein and lipid modulators, mitochondrial dynamics, organelle contact sites, and quality-control mechanisms, with particular focus on their therapeutic exploitation in pharmaceutical research. By integrating mechanistic insights with translational relevance, this chapter establishes mitochondrial membrane integrity as a unifying concept in modern pharmacology and drug development.

Keywords: Mitochondria, Membrane Integrity, Biophysical properties, Redox homeostasis

Introduction

Mitochondria are multifunctional organelles traditionally recognized for their role in oxidative phosphorylation and ATP production. However, contemporary research has demonstrated that mitochondria serve as central hubs for cellular signaling, integrating metabolic, redox, calcium, and apoptotic cues (Chandel, 2020). At the heart of these functions lies the structural and functional integrity of mitochondrial membranes, which ensure proper compartmentalization and selective permeability. The OMM serves as a selective barrier, regulating metabolite and protein exchange with the cytosol, whereas the IMM maintains the proton motive force essential for ATP synthesis (Galloway & Yoon, 2021). Mitochondrial membrane integrity refers to the capacity of the OMM and IMM to maintain structural continuity, selective permeability, and bioenergetic functionality under physiological and stress conditions. Loss of membrane integrity can precipitate apoptotic or necrotic cell death, impaired metabolism, oxidative stress, and activation of inflammatory pathways (Czabotar & Garcia-Saez, 2023). Recent studies highlight that membrane integrity is not a binary state but exists along a continuum, allowing sublethal perturbations that influence cellular fate and long-term tissue homeostasis (EMBO Journal, 2024). In pharmacy and drug development, mitochondrial membranes are increasingly recognized as both targets and off-target sites. Several pharmacological agents, including chemotherapeutics, antipsychotics, antibiotics, and metabolic modulators, affect mitochondrial membrane composition, potential, and permeability, resulting in either therapeutic benefit or toxicity (Li et al., 2023). Deliberate modulation of membrane integrity, for instance via BH3 mimetics, offers a mechanism to selectively induce apoptosis in cancer cells while sparing healthy tissue, demonstrating the translational importance of understanding mitochondrial membrane regulation (Huang et al., 2022).

Mitochondria were first observed by Altmann in 1890 as “bioblasts” in eukaryotic cells. For decades, research focused on their role in energy production, with membranes considered inert barriers. The development of the chemiosmotic theory by Peter Mitchell in the 1960s revolutionized the understanding of IMM function, identifying proton gradients as the driving force for ATP synthesis. This highlighted the IMM not merely as a barrier but as a functional bioenergetic surface (Mitchell, 1961). In the 1990s, the discovery that cytochrome *c* release from mitochondria initiates intrinsic apoptosis transformed the field. The OMM emerged as a regulated interface, with proteins of the BCL-2 family identified as central modulators of membrane

permeabilization (Adams & Cory, 1998). Subsequent work revealed that the IMM also plays an active role through the mitochondrial permeability transition pore (mPTP), linking bioenergetics and cell death (Bernardi, 1999).

Recent advances extend mitochondrial membrane functions beyond energy metabolism and apoptosis. Membranes now are recognized as central regulators of innate immune signaling, calcium homeostasis, lipid trafficking, and organelle interactions (Rizzuto et al., 2012; Galloway & Yoon, 2021). Moreover, mitochondrial membranes contribute to the pathogenesis of diverse diseases, including cancer, neurodegeneration, cardiovascular disease, and metabolic disorders. This has motivated pharmaceutical research into membrane-targeted therapeutics.

Mitochondrial membranes exhibit a high degree of functional complexity and dynamic behavior. Minority MOMP**,** or partial permeabilization of mitochondria, contributes to sublethal caspase activation, DNA damage, and therapy resistance, highlighting the nuanced role of mitochondria in cell fate regulation (Czabotar & Garcia-Saez, 2023). Additionally, mitochondrial membranes are constantly remodeled through processes such as fission, fusion, and contact site interactions, which influence both outer and inner membrane integrity and overall organelle functionality (Westermann, 2012). Lipid composition also plays a crucial role, with cardiolipin, phosphatidylethanolamine, and other mitochondrial lipids modulating membrane curvature, facilitating protein interactions, and determining apoptotic susceptibility, thereby linking structural dynamics to cellular signaling and survival pathways (Paradies et al., 2022).

This review aims to provide a comprehensive and mechanistic understanding of mitochondrial membrane integrity by addressing several key aspects. It details the structural organization of the outer and inner mitochondrial membranes (OMM and IMM), highlighting their roles in maintaining organelle function. The review also examines biophysical and lipid determinants of membrane stability, emphasizing how membrane composition influences curvature, fluidity, and protein interactions. In addition, it explores protein modulators**,** including the BCL-2 family, voltage-dependent anion channels (VDACs), and components of the mitochondrial permeability transition pore (mPTP), which collectively regulate apoptosis and bioenergetics. The role of mitochondrial dynamics and inter-organelle contact sites is discussed in the context of membrane remodeling, fusion, fission, and organelle communication. Furthermore, the review evaluates pharmacological and natural modulators with translational relevance, considering their potential to preserve membrane integrity or modulate mitochondrial function in

disease. Finally, it links mitochondrial membrane integrity to pathophysiological processes and therapeutic strategies, providing a framework for understanding how targeting these membranes can influence cellular health and disease outcomes.

Mitochondrial Membrane Architecture

Mitochondria are delineated by a dual-membrane system—the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM)—each with distinct structural, compositional, and functional properties. The OMM functions as an interface with the cytosol , regulating metabolite exchange, apoptotic signaling, and organelle interactions. The IMM, in contrast, is a highly protein-dense barrier that generates the proton-motive force and supports oxidative phosphorylation. The interface between OMM and IMM, including cristae junctions and intermembrane space, enables dynamic communication essential for cellular homeostasis (Galloway & Yoon, 2021; Mannella, 2021).

Outer Mitochondrial Membrane (OMM)

Structural Features

The OMM is a relatively thin, lipid-rich membrane (~6–7 nm) , composed of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and small amounts of cardiolipin and cholesterol (Mannella, 2021). Its protein-to-lipid ratio is approximately 1:1 , significantly lower than the IMM. Embedded within the OMM are porins (primarily voltage-dependent anion channels, VDACs) and protein complexes that govern selective permeability and facilitate protein import (Shoshan-Barmatz et al., 2010).

The OMM regulates multiple cellular processes:

1. Metabolite Exchange: VDACs permit passive diffusion of ATP, ADP, and small metabolites (<5 kDa) while serving as a scaffold for regulatory proteins.
2. Apoptotic Regulation: The OMM acts as the critical barrier for mitochondrial outer membrane permeabilization (MOMP) . BCL-2 family proteins orchestrate MOMP, controlling the release of cytochrome c and other apoptogenic factors (Czabotar & Garcia-Saez, 2023).
3. Protein Import: The TOM complex mediates the import of >99% of mitochondrial proteins, a process essential for organelle function. Disruption of OMM integrity impairs protein trafficking and induces mitochondrial stress (Wiedemann & Pfanner, 2017).

4. Organelle Communication: The OMM establishes physical contacts with the endoplasmic reticulum (ER), lysosomes, and peroxisomes, regulating calcium flux and lipid trafficking (Phillips & Voeltz, 2016).

OMM Permeabilization Mechanisms

The OMM is not a passive structure; its permeability is dynamically regulated: Pro-apoptotic signaling: Activation of BAX and BAK induces oligomerization and pore formation.Post-translational modifications: Phosphorylation, ubiquitination, and cleavage of BCL-2 family proteins modulate OMM stability.Protein–lipid interactions: Cardiolipin externalization to the OMM facilitates BAX/BID recruitment and pore formation (Paradies et al., 2022).From a pharmacological perspective, selective modulation of OMM integrity enables targeted induction of apoptosis in pathological cells, particularly in oncology.

Inner Mitochondrial Membrane (IMM)

Structural Features

The IMM is a highly protein-dense membrane (~75% protein by mass) , folded into cristae to maximize surface area for oxidative phosphorylation. Its lipid composition is unique: enriched in cardiolipin (~20%), phosphatidylethanolamine, and minor phosphatidylcholine, while cholesterol content remains low (Paradies et al., 2022).

Cristae are dynamic invaginations connected to the inner boundary membrane by narrow junctions. This compartmentalization facilitates organization of respiratory chain complexes into supercomplexes , optimizing electron transfer efficiency and reducing reactive oxygen species (ROS) production (Mannella, 2021).

Bioenergetics: The IMM maintains mitochondrial membrane potential ($\Delta\psi m$) through proton pumping by complexes I, III, and IV, driving ATP synthase (complex V). Metabolite Transport: IMM houses specific carriers (e.g., ANT, phosphate carrier) for controlled transport of nucleotides and metabolites. Apoptotic Signaling: The IMM contributes indirectly to apoptosis through mPTP opening , IMM swelling, and subsequent OMM rupture. ROS Regulation: Cardiolipin-rich IMM stabilizes respiratory supercomplexes, reducing electron leakage and ROS generation (Paradies et al., 2022).

Cristae Remodeling:

Cristae morphology is dynamically regulated by a coordinated network of inner mitochondrial membrane (IMM)–associated proteins and complexes. OPA1, a dynamin-related GTPase, mediates IMM fusion and stabilizes cristae junctions, thereby controlling cristae shape and restricting the release of apoptogenic factors. The mitochondrial contact site and cristae organizing system (MICOS) complex plays a central role in maintaining cristae junction architecture and physically linking the IMM to the outer mitochondrial membrane (OMM), ensuring proper spatial organization of mitochondrial membranes. In addition, ATP synthase dimers self-assemble along cristae ridges, inducing membrane curvature that is essential for the formation and maintenance of cristae structure and optimal bioenergetic efficiency. Frezza, C., Cipolat, S., Martins de Brito, O., et al. (2006).

Disruption of cristae architecture sensitizes mitochondria to apoptosis and reduces oxidative phosphorylation efficiency, highlighting the functional importance of IMM integrity (Rampelt et al., 2017).

Intermembrane Space (IMS)

The IMS occupies the region between OMM and IMM and serves as a conduit for electron transport intermediates, apoptotic proteins, and signaling molecules . Its limited volume enhances diffusion efficiency of cytochrome c , allowing rapid apoptotic signaling upon OMM permeabilization.

IMS proteins also participate in calcium buffering and redox signaling , linking mitochondrial integrity to broader cellular homeostasis (Rizzuto et al., 2012).

OMM–IMM Functional Coupling

Although structurally distinct, the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) are tightly functionally interconnected. mPTP-mediated increases in IMM permeability trigger mitochondrial matrix swelling, which generates mechanical stress that can lead to secondary rupture of the OMM and release of apoptogenic proteins. In parallel, communication through cristae junctions plays a critical role in regulating mitochondrial apoptosis, as remodeling of these junctions alters the accessibility and mobilization of cytochrome c from the cristae lumen to the OMM.

Additionally, lipid transfer between the IMM and OMM, particularly involving cardiolipin and phosphatidic acid, can occur under cellular stress conditions, influencing membrane stability,

protein interactions, and susceptibility to apoptotic signaling.This coupling emphasizes the need to consider mitochondrial membranes as an integrated system rather than independent compartments (Mannella, 2021; Shoshan-Barmatz et al., 2010).

Evolutionary Perspective on Mitochondrial Membranes

Mitochondrial membranes originated from an α-proteobacterial endosymbiont, and this evolutionary history is reflected in the distinct properties of the inner and outer mitochondrial membranes. The inner mitochondrial membrane (IMM) retains several bacterial characteristics, including an exceptionally high protein density and enrichment in cardiolipin, a phospholipid critical for respiratory chain organization and membrane integrity. In contrast, the outer mitochondrial membrane (OMM) has adopted lipid and compositional features more typical of eukaryotic membranes, facilitating extensive interaction with the cytosol and integration with cellular signaling, metabolic exchange, and apoptotic regulation.

This evolutionary distinction explains differential pharmacological susceptibility. For example, antibiotics targeting bacterial membranes may inadvertently disrupt IMM function, while OMM-targeting drugs exploit eukaryotic-like features for selective modulation (Chandel, 2020; Mannella, 2021).

Membrane Heterogeneity and Microdomains

Potein clustering within mitochondrial membranes, such as the oligomerization of voltage-dependent anion channels (VDAC) and the assembly of pro-apoptotic BAX/BAK complexes, creates spatially organized microdomains that enable localized signaling and tightly regulated metabolite flux across the membrane. These membrane subdomains also exhibit differential susceptibility to oxidative damage, as concentrated protein assemblies and specific lipid environments can amplify or buffer reactive oxygen species–mediated modifications, thereby influencing mitochondrial function, stress responses, and the initiation of apoptosis.

These microdomains may be critical for localized apoptotic initiation and therapeutic targeting , representing a frontier in mitochondrial pharmacology (Paradies et al., 2022; Shoshan-Barmatz et al., 2010).

Understanding the architecture is essential before discussing biophysical properties, lipid modulation, and protein regulators , which are covered in subsequent sections.

Biophysical Properties of Mitochondrial Membranes:

Mitochondrial membrane integrity is critically influenced by biophysical characteristics , including fluidity, curvature, thickness, and electrochemical gradients . These properties dictate the mobility and activity of membrane proteins, susceptibility to permeabilization, and efficiency of oxidative phosphorylation. Understanding the biophysical landscape of mitochondrial membranes provides mechanistic insight into their vulnerability under stress and informs pharmacological strategies to modulate their function (Galloway & Yoon, 2021; Mannella, 2021).

Membrane Fluidity:

Mitochondrial membrane fluidity is strongly influenced by lipid composition and environmental conditions. Major phospholipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) regulate membrane packing and bilayer organization, while cardiolipin (CL), with its distinctive tetra-acyl structure, contributes to localized membrane rigidity and stabilizes protein complexes. Although present at relatively low levels, cholesterol can increase membrane order and reduce fluidity. In addition, the degree of fatty acid saturation plays a key role, as unsaturated acyl chains enhance membrane fluidity whereas saturated chains increase rigidity. External factors such as temperature changes and cellular stress further modulate membrane properties; in particular, reactive oxygen species (ROS) and thermal stress can disrupt lipid packing, leading to decreased fluidity and increased membrane permeability. (Paradies et al., 2022).

Functional Implications

Protein Mobility: Fluid membranes facilitate the diffusion of BAX, BAK, and VDAC, which are essential for regulated outer membrane permeabilization (Czabotar & Garcia-Saez, 2023).

Respiratory Efficiency: Electron transport chain complexes require optimal lipid fluidity for supercomplex formation and proton pumping efficiency.

Apoptotic Sensitivity: Increased rigidity can impede BAX insertion, reducing susceptibility to apoptosis, whereas excessive fluidity may favor pore formation.

Pharmacological Considerations:

Several pharmacological agents are known to alter mitochondrial membrane fluidity through distinct mechanisms. Amphiphilic drugs preferentially accumulate within the inner mitochondrial membrane (IMM), where they disrupt lipid packing and modify the local biophysical environment. Mitochondrial uncouplers, by dissipating the membrane potential, indirectly influence lipid–protein interactions and membrane organization. In addition, lipid-targeted therapies such as

cardiolipin-stabilizing compounds, including elamipretide, help restore membrane fluidity, preserve cristae structure, and protect mitochondria against oxidative damage.(Montaigne et al., 2023).

Membrane Curvature and Topology

Cristae architecture and curvature of the inner mitochondrial membrane (IMM), particularly at cristae ridges and junctions, are critical for efficient bioenergetic function. Highly curved membrane regions promote the assembly of respiratory supercomplexes, thereby optimizing electron transfer efficiency and minimizing electron leakage that would otherwise lead to excessive reactive oxygen species (ROS) generation. At the same time, cristae curvature enables functional compartmentalization of cytochrome *c* within the cristae lumen, restricting its diffusion toward the outer mitochondrial membrane and allowing tight regulation of apoptotic signaling.(Mannella, 2021).

Proteins Regulating Curvature

Cristae structure is governed by a coordinated set of mitochondrial proteins that regulate inner membrane fusion, curvature, and intermembrane connectivity. OPA1, a dynamin-related GTPase, mediates inner mitochondrial membrane fusion and stabilizes cristae junctions, thereby preserving cristae organization and controlling the distribution of apoptogenic factors. ATP synthase dimers assemble along cristae ridges, where they induce and maintain local membrane curvature essential for efficient oxidative phosphorylation. In parallel, the mitochondrial contact site and cristae organizing system (MICOS) complex maintains cristae junction architecture and provides physical tethering between the inner and outer mitochondrial membranes, ensuring proper spatial coordination of mitochondrial structure and function. (Rampelt et al., 2017).

Biophysical Significance:

Membrane curvature modulates the energetic landscape for protein insertion , pore formation, and mPTP opening. Pharmacological manipulation of curvature-related proteins is emerging as a strategy to control apoptosis in cancer and neurodegenerative diseases.

Electrochemical Gradients and Membrane Potential:

Mitochondrial Membrane Potential (Δψm)

The inner mitochondrial membrane (IMM) maintains a proton gradient generated by the coordinated activity of respiratory complexes I, III, and IV, giving rise to the mitochondrial

membrane potential (Δψm). This electrochemical gradient drives ATP synthesis through ATP synthase (complex V) and is essential for efficient cellular energy production. Beyond its bioenergetic role, Δψm contributes to the stabilization of lipid–protein interactions within the IMM, helping to preserve membrane organization and cristae structure. In addition, maintenance of Δψm suppresses spontaneous pore formation and limits oxidative stress, thereby protecting mitochondrial integrity and preventing the initiation of cell death pathways.

Δψm and Membrane Integrity:

Loss of the mitochondrial membrane potential (Δψm) is an early and critical indicator of mitochondrial dysfunction. Partial depolarization induces sublethal mitochondrial stress, which can contribute to minority mitochondrial outer membrane permeabilization (MOMP) and selective release of apoptogenic factors. In contrast, complete depolarization triggers the opening of the mitochondrial permeability transition pore (mPTP), leading to inner membrane swelling, rupture of the outer mitochondrial membrane, and irreversible commitment of the cell to apoptosis. (Bernardi et al., 2015).

Pharmacological Modulation:

Mitochondrial uncouplers: Dissipate Δψm to alter metabolism, used experimentally for weight reduction or anti-cancer strategies, but with high toxicity risk.

Protective agents: Mitochondria-targeted antioxidants and cardiolipin stabilizers preserve Δψm under oxidative stress (Paradies et al., 2022).

Calcium Homeostasis and Membrane Integrity:

Calcium Uptake and Buffering

Mitochondrial calcium homeostasis is regulated by coordinated transport across both mitochondrial membranes. The inner mitochondrial membrane (IMM) contains the mitochondrial calcium uniporter (MCU), which facilitates the uptake of Ca^{2+} into the matrix, enabling regulation of metabolic enzymes and bioenergetic activity. On the outer mitochondrial membrane (OMM), voltage-dependent anion channels (VDACs) allow Ca^{2+} to diffuse freely into the intermembrane space, providing a conduit for cytosolic calcium to reach the IMM and modulate mitochondrial function.

Calcium-Induced Membrane Stress:

Opening of the mitochondrial permeability transition pore (mPTP) leads to depolarization of the inner mitochondrial membrane (IMM), osmotic swelling of the mitochondrial matrix, and eventual

rupture of the outer mitochondrial membrane (OMM), resulting in the release of pro-apoptotic factors. Concurrently, calcium overload within mitochondria enhances electron leakage from respiratory chain complexes, markedly increasing the generation of reactive oxygen species (ROS). Together, mitochondrial calcium dysregulation, ROS amplification, and mPTP opening form a self-reinforcing cycle of mitochondrial failure that underlies the pathophysiology of ischemia–reperfusion injury, neurodegenerative disorders, and excitotoxic neuronal death.(Bernardi, P., Krauskopf, A., Basso, E., et al. (2015).

Pharmacological Implications:

Mitochondrial calcium dynamics and membrane integrity can be pharmacologically modulated to prevent cellular injury. Calcium channel modulators target the mitochondrial calcium uniporter (MCU) or voltage-dependent anion channels (VDAC) to limit excessive calcium uptake and prevent mitochondrial calcium overload. Additionally, mitochondrial permeability transition pore (mPTP) inhibitors, including cyclosporin A analogs and novel small molecules, protect inner mitochondrial membrane (IMM) integrity under stress conditions, reducing swelling, preserving cristae structure, and mitigating cell death.(Halestrap, 2022).

Temperature and Oxidative Stress:

Mitochondrial membrane integrity is highly sensitive to environmental and oxidative stress. Thermal stress can alter membrane viscosity and destabilize critical lipid–protein interactions, compromising mitochondrial function. Similarly, reactive oxygen species (ROS) promote lipid peroxidation, increasing membrane rigidity and making the membrane more susceptible to permeabilization. Protective strategies, including mitochondria-targeted antioxidants such as MitoQ and SkQ1, mitigate lipid oxidation, preserve membrane biophysical properties, and maintain overall mitochondrial structural and functional integrity. (Smith & Murphy, 2020).

Membrane Microdomains:

Recent studies indicate that both the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) contain specialized lipid and protein microdomains that play key roles in mitochondrial function. These microdomains concentrate apoptotic proteins such as BAX and BAK at specific sites, facilitating localized signaling and the controlled initiation of apoptosis. They also support metabolic regulation by organizing respiratory complexes spatially, optimizing electron transfer and energy production. Furthermore, these microdomains provide opportunities for therapeutic targeting, as certain drugs can preferentially interact with these regions to modulate

membrane permeability and influence mitochondrial responses to stress. (Shoshan-Barmatz et al., 2010).

Integration of Biophysical Properties with Functional Outcomes:

The interplay between mitochondrial membrane fluidity, curvature, membrane potential (Δψm), calcium homeostasis, and oxidative stress critically determines the threshold for membrane permeabilization and the initiation of cell death. A rigid inner mitochondrial membrane (IMM) with low fluidity limits protein mobility and impairs the assembly of respiratory supercomplexes, compromising bioenergetic efficiency. Excessive cristae curvature or dysfunction of OPA1 increases susceptibility to apoptosis by altering cristae junction integrity. Concurrent dissipation of Δψm and mitochondrial calcium overload synergistically trigger opening of the mitochondrial permeability transition pore (mPTP), while accumulation of reactive oxygen species (ROS) oxidizes cardiolipin, destabilizes the IMM, and facilitates BAX/BAK-mediated pore formation. From a pharmaceutical perspective, strategies focus on stabilizing membrane fluidity and curvature to protect healthy tissue, selectively targeting destabilized membranes in cancer or infected cells to induce apoptosis, and modulating Δψm and calcium handling to achieve metabolic or cardioprotective effects.(Shanmughapriya, S., et al. (2017).

Lipid Modulators of Mitochondrial Membrane Integrity:

Mitochondrial membranes are not only protein-rich structures but also dynamic lipid assemblies that determine membrane fluidity, curvature, permeability, and susceptibility to stress. The lipid composition of the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) is distinct, reflecting specialized functional demands. Lipids act as both structural components and signaling molecules , and their dysregulation has profound implications for apoptosis, oxidative stress, and metabolic homeostasis (Paradies et al., 2022; Horvath & Daum, 2013).

Cardiolipin: The Signature Mitochondrial Lipid

Structure and Distribution:

Cardiolipin (CL) is a tetra-acyl phospholipid almost exclusively localized to the IMM, where it constitutes 15–20% of total lipid content. It possesses a unique conical shape that induces membrane curvature and facilitates the formation of respiratory supercomplexes (Houtkooper & Vaz, 2008).

Although mainly in the IMM, CL can translocate to the OMM under stress conditions, acting as a signal for apoptotic protein recruitment , particularly BAX, tBID, and cytochrome c (Paradies et al., 2022).

Cardiolipin (CL) plays a multifaceted role in mitochondrial structure and function. In bioenergetics, CL interacts with respiratory complexes I–IV and ATP synthase, stabilizing supercomplexes and enhancing electron transfer efficiency. In apoptotic sensitization, CL externalization to the outer mitochondrial membrane (OMM) provides docking sites for pro-apoptotic BCL-2 family proteins, thereby initiating mitochondrial outer membrane permeabilization (MOMP) (Zhang et al., 2021). Additionally, CL contributes to reactive oxygen species (ROS) regulation by scavenging ROS and maintaining redox balance; however, oxidized CL can trigger cytochrome *c* release, linking oxidative stress directly to the activation of apoptosis.

Pathophysiological Dysregulation:

In Ischemia-reperfusion injury, the oxidative CL damage destabilizes IMM and sensitizes cells to apoptosis. In Barth syndrome, genetic defects in CL remodeling impair mitochondrial energy metabolism, leading to cardiomyopathy and neutropenia (Houtkooper & Vaz, 2008).

In Neurodegeneration, CL oxidation promotes neuronal apoptosis in Parkinson's and Alzheimer's disease models (Chu et al., 2020).

Pharmacological Modulation:

Cardiolipin-targeted interventions help preserve mitochondrial inner membrane (IMM) structure and function under stress conditions. Cardiolipin-stabilizing peptides, such as elamipretide, selectively bind to CL, reinforcing IMM curvature and protecting mitochondria from ischemic or oxidative damage. Similarly, mitochondria-targeted antioxidants prevent CL peroxidation, maintaining membrane integrity and supporting efficient bioenergetic function. (Paradies et al., 2022).

Phosphatidylethanolamine (PE) and Phosphatidylcholine (PC)

Structural and Functional Importance:

Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are key phospholipids that shape mitochondrial membrane architecture and function. PE, a conical lipid, promotes negative curvature in inner mitochondrial membrane (IMM) cristae and is essential for proper protein folding and assembly of respiratory complexes. PC, a cylindrical lipid, stabilizes bilayer thickness and supports protein mobility in both the IMM and outer mitochondrial membrane (OMM).

Together, PE and PC interact with respiratory complexes and modulate membrane fluidity, influencing protein diffusion, bioenergetic efficiency, and susceptibility to apoptotic signaling.(Horvath & Daum, 2013).

Pathological Implications:

Deficiencies or imbalances in key mitochondrial phospholipids have profound functional consequences. Phosphatidylethanolamine (PE) deficiency impairs cristae formation, diminishes oxidative phosphorylation efficiency, and increases mitochondrial susceptibility to apoptosis. Similarly, an imbalance in phosphatidylcholine (PC) disrupts outer mitochondrial membrane (OMM) permeability and affects VDAC function, altering metabolite transport and calcium homeostasis, which can compromise overall mitochondrial performance and cellular homeostasis.

Pharmacological Considerations:

Modulation of PE and PC levels can be achieved via lipid supplementation, inhibition of biosynthetic pathways, or targeting remodeling enzymes, offering potential interventions in metabolic and degenerative diseases (Horvath & Daum, 2013).

Cholesterol and Sterol Regulation:

Although mitochondrial membranes contain relatively low levels of cholesterol, it exerts a significant influence on outer mitochondrial membrane (OMM) rigidity and permeability. High cholesterol content increases OMM rigidity, which can hinder the insertion of pro-apoptotic proteins such as BAX and reduce susceptibility to apoptosis. Conversely, low cholesterol levels enhance membrane fluidity, making the OMM more prone to permeabilization under cellular stress and facilitating the activation of apoptotic pathways.

Pharmacological modulation of cholesterol in mitochondria is under investigation, particularly in cancer therapy , where selective sensitization of tumor mitochondria to apoptotic stimuli is desirable (Leanza et al., 2017).

Lipid Peroxidation and Ferroptosis:

Oxidative Damage of Membrane Lipids:

Mitochondrial lipids, particularly cardiolipin (CL) and phosphatidylethanolamine (PE), are highly vulnerable to reactive oxygen species (ROS)–mediated peroxidation. Oxidative damage to these lipids can cause membrane rigidification or fragmentation, destabilize respiratory chain complexes, and increase mitochondrial susceptibility to apoptosis, thereby linking oxidative stress directly to both bioenergetic impairment and cell death pathways.(Paradies et al., 2022)

Ferroptosis is a regulated form of cell death driven by iron-dependent lipid peroxidation within mitochondrial membranes. Peroxidation of cardiolipin (CL) and phosphatidylethanolamine (PE) serves as a key ferroptotic signal, while inhibition of glutathione peroxidase 4 (GPX4) or depletion of glutathione further increases susceptibility to ferroptosis. This pathway plays a significant role in pathological contexts, including neurodegeneration, ischemic injury, and the cellular response to certain cancer therapies.(Stockwell et al., 2020).

Pharmacological Targeting:

Ferroptosis inhibitors, such as Ferrostatin-1, Liproxstatin-1, and vitamin E analogs, prevent lipid peroxidation and help preserve mitochondrial membrane integrity, thereby protecting cells from ferroptotic cell death. Similarly, iron chelators reduce reactive oxygen species (ROS) generation and lipid oxidation by binding free iron, which limits the Fenton reaction that drives lipid peroxidation. In the context of cancer therapy, strategies aim to selectively induce ferroptosis in tumor cells by exploiting their heightened vulnerability to lipid peroxidation, taking advantage of metabolic and redox imbalances to trigger cell death while minimizing harm to normal cells.(Dixon et al., 2012).

Lipid–Protein Interactions:

Lipids act as essential cofactors for mitochondrial proteins, playing critical roles in maintaining mitochondrial function and integrity. In the respiratory complexes, cardiolipin (CL) stabilizes supercomplexes, ensuring efficient electron transport and energy production. Regarding apoptotic proteins, CL recruits BAX, BID, and tBID to the outer mitochondrial membrane (OMM), facilitating apoptosis signaling. Additionally, CL and phosphatidylethanolamine (PE) modulate the sensitivity of the mitochondrial permeability transition pore (mPTP) and its calcium-binding capacity, influencing mitochondrial permeability and cell fate. Disruption of these lipid–protein interactions destabilizes membrane integrity, highlighting a pharmacologically targetable axis for therapeutic interventions.(Shamas-Din et al., 2013).

Mitochondrial Lipid Remodeling Enzymes:

Enzymes responsible for maintaining mitochondrial lipid homeostasis play crucial roles in membrane structure and function. Tafazzin is involved in cardiolipin (CL) remodeling, and mutations in this enzyme cause Barth syndrome. Phosphatidylserine decarboxylase (PSD) converts phosphatidylserine (PS) to phosphatidylethanolamine (PE) within the inner mitochondrial membrane (IMM), while cardiolipin synthase (CLS) catalyzes the de novo synthesis

of CL. Dysregulation of these enzymes can alter membrane curvature, fluidity, and susceptibility to apoptosis, underscoring the therapeutic potential of targeting these enzymes with modulators. (Acehan et al., 2011).

Integration with Biophysical Properties

Lipids directly influence key biophysical features of mitochondrial membranes. Unsaturated phosphatidylethanolamine (PE) and phosphatidylcholine (PC) maintain membrane fluidity, supporting the lateral diffusion of proteins, while cardiolipin (CL) and PE contribute to membrane curvature, shaping cristae and facilitating protein assembly. Lipid composition also affects membrane potential ($\Delta\psi m$) by modulating ion transport, and susceptibility to reactive oxygen species (ROS), as lipid peroxidation can trigger mitochondrial permeability transition pore (mPTP) opening and apoptotic signaling. Pharmacologically, lipid-targeted strategies aim to restore membrane homeostasis, enhance cellular resilience, or selectively induce death in pathological cells.

Protein Modulators of the Outer Mitochondrial Membrane:

Mitochondrial membrane integrity is not solely determined by lipid composition and biophysical properties; membrane-associated proteins play a pivotal role in regulating permeability, apoptosis, metabolite transport, and organelle communication. The OMM houses a diverse set of proteins, including the BCL-2 family , voltage-dependent anion channels (VDACs) , and translocases of the outer and inner membrane (TOM/TIM complexes) . Dysregulation of these proteins is implicated in cancer, neurodegeneration, cardiovascular diseases, and metabolic disorders, making them critical pharmacological targets (Czabotar & Garcia-Saez, 2023; Shoshan-Barmatz et al., 2010).

The BCL-2 Protein Family:

The BCL-2 family comprises pro-apoptotic and anti-apoptotic members that regulate OMM permeabilization and, consequently, mitochondrial-mediated apoptosis. Their balance dictates cellular fate under physiological and stress conditions (Youle & Strasser, 2008).

Anti-apoptotic members: BCL-2, BCL-XL, MCL-1

Pro-apoptotic effectors: BAX, BAK

BH3-only proteins: BID, BAD, PUMA, NOXA (regulate effectors through direct binding or signaling)

Mechanism of Action:

Under cellular stress, BH3-only proteins activate BAX and BAK, which oligomerize to form pores in the outer mitochondrial membrane (OMM). This process, known as mitochondrial outer membrane permeabilization (MOMP), results in the release of cytochrome c, promoting apoptosome formation and subsequent caspase activation. Lipid interactions, particularly with cardiolipin and phosphatidylethanolamine (PE), facilitate BAX and BAK insertion and oligomerization, directly linking mitochondrial membrane composition to the regulation of apoptosis. (Czabotar & Garcia-Saez, 2023).

Pharmacological Modulation:

BH3 mimetics are small molecules, such as venetoclax and navitoclax, that mimic BH3-only proteins to inhibit anti-apoptotic BCL-2 family members, thereby selectively inducing apoptosis in cancer cells. Peptide inhibitors, on the other hand, target BAX activation to prevent apoptosis, offering therapeutic potential in neurodegenerative and ischemic diseases. Additionally, redox modulation plays a key role in regulating apoptosis, as oxidation of cardiolipin enhances BAX recruitment to the outer mitochondrial membrane (OMM), highlighting the connection between pharmacological antioxidants and the maintenance of mitochondrial membrane integrity.(Paradies et al., 2022).

Voltage-Dependent Anion Channels (VDACs)

Structure and Isoforms:

VDACs are β-barrel proteins embedded in the OMM that form aqueous pores, allowing diffusion of metabolites (ATP, ADP, NADH) and ions. Three isoforms exist in mammals: VDAC1, VDAC2, and VDAC3, each exhibiting distinct regulatory roles :

Voltage-dependent anion channels (VDACs) play distinct roles in mitochondrial function and apoptosis. VDAC1 primarily facilitates general metabolite exchange across the outer mitochondrial membrane and contributes to apoptosis initiation. VDAC2 regulates BAK activation, acting as an inhibitor of apoptosis. VDAC3 functions as a redox sensor, helping to maintain mitochondrial homeostasis under oxidative stress.(Shoshan-Barmatz et al., 2010)

Functional Roles:

VDACs regulate critical mitochondrial processes through multiple mechanisms. They facilitate metabolite flux, enabling ATP/ADP exchange to maintain cellular energetic balance. VDACs also support calcium signaling by allowing Ca^{2+} passage from the cytosol into the intermembrane space

(IMS), which influences mitochondrial permeability transition pore (mPTP) sensitivity. In apoptotic regulation, VDAC1 interacts with BCL-2 family proteins to mediate cytochrome c release, linking metabolite transport and membrane channels to cell death pathways.

Pharmacological Targeting:

VDAC-targeted pharmacological strategies can modulate mitochondrial function and cell fate. VDAC inhibitors, typically small molecules, can regulate apoptosis, offering therapeutic potential in cancer or ischemic injury. Conversely, VDAC activators enhance metabolite flux, supporting bioenergetics under stress conditions. Additionally, protein–protein modulators that disrupt VDAC–BCL-2 interactions influence mitochondrial outer membrane permeabilization (MOMP), thereby affecting apoptotic signaling and therapeutic outcomes.(Shoshan-Barmatz et al., 2010).

Translocases of the Outer and Inner Membrane (TOM/TIM Complexes):

TOM Complex (Translocase of the Outer Membrane):

The TOM complex mediates import of nuclear-encoded mitochondrial proteins , which constitute >99% of the mitochondrial proteome. Key components include:

The translocase of the outer mitochondrial membrane (TOM) complex mediates protein import into mitochondria through distinct subunits. TOM20 and TOM22 serve as receptor subunits that recognize presequence-containing proteins, guiding them toward the import machinery. TOM40 forms the β-barrel pore through which proteins are translocated across the outer membrane. TOM70 acts as a receptor for hydrophobic proteins carrying internal targeting signals, facilitating the import of proteins lacking N-terminal presequences.

Proper TOM function is essential for membrane maintenance , as protein import defects lead to OMM stress, mitochondrial dysfunction, and apoptosis (Wiedemann & Pfanner, 2017).

TIM Complex (Translocase of the Inner Membrane):

The TIM complex (TIM23/TIM22) cooperates with TOM to insert proteins into the IMM or matrix, indirectly influencing OMM integrity by maintaining mitochondrial homeostasis and $\Delta\psi m$.

Pharmacological Considerations:

Modulation of the TOM/TIM mitochondrial import machinery offers potential therapeutic strategies. TOM/TIM modulators could treat mitochondrial proteinopathies by restoring or adjusting protein import. In cancer, where TOM components are often overexpressed, targeting

these import pathways with inhibitors may selectively disrupt tumor mitochondrial function, impairing bioenergetics and survival.(Wiedemann & Pfanner, 2017).

Protein Modulators of the Inner Mitochondrial Membrane:

The inner mitochondrial membrane (IMM) is a highly specialized, protein-rich barrier responsible for oxidative phosphorylation, ion homeostasis, and apoptosis regulation . Proteins embedded in the IMM regulate mitochondrial bioenergetics, membrane potential, and susceptibility to permeability transition . Dysfunction of these proteins contributes to neurodegenerative diseases, cardiovascular injury, metabolic syndromes, and cancer (Mannella, 2021; Bernardi et al., 2015). Key IMM protein modulators include: the mitochondrial permeability transition pore (mPTP), ATP synthase, adenine nucleotide translocase (ANT), and the mitochondrial calcium uniporter (MCU) . Each plays a central role in maintaining membrane integrity, electrochemical gradients, and signaling pathways .

Mitochondrial Permeability Transition Pore (mPTP):

The mitochondrial permeability transition pore (mPTP) is a high-conductance channel spanning the inner mitochondrial membrane (IMM). Prolonged opening of the mPTP leads to matrix swelling, IMM depolarization, outer membrane rupture, and the release of apoptotic factors. Functioning as a critical sensor of cellular stress, the mPTP responds to conditions such as calcium overload, oxidative stress, and ATP depletion, thereby linking mitochondrial dysfunction to cell death pathways.(Halestrap, 2022).

Molecular Composition:

The precise molecular identity of the mitochondrial permeability transition pore (mPTP) remains under debate, but current evidence suggests several key components. ATP synthase dimers (F1F0-ATPase) are implicated as structural contributors forming the pore, while the adenine nucleotide translocase (ANT) functions as a regulatory component. Cyclophilin D (CypD), a matrix chaperone, modulates pore opening, linking mPTP activity to mitochondrial stress responses and cell death pathways.

Functional Significance:

The mitochondrial permeability transition pore (mPTP) plays a central role in calcium and redox homeostasis. Its opening is triggered by matrix Ca^{2+} overload, linking it directly to calcium regulation. Oxidative stress further sensitizes the mPTP, amplifying mitochondrial damage through reactive oxygen species (ROS). Sustained mPTP opening leads to apoptotic or necrotic cell death,

whereas transient "flickering" of the pore can function as a signaling event, influencing cellular responses without causing immediate cell death.(Bernardi et al., 2015).

Pharmacological Targeting:

Cyclophilin D inhibitors, such as cyclosporin A and its derivatives, prevent mitochondrial permeability transition pore (mPTP) opening, thereby reducing tissue damage in ischemia-reperfusion injury. In addition, small-molecule mPTP modulators targeting ATP synthase or adenine nucleotide translocase (ANT) are being investigated for their potential in neuroprotection and cardioprotection, aiming to preserve mitochondrial function under stress conditions.(Halestrap, 2022).

ATP Synthase (Complex V)

Structure and Function:

ATP synthase is an IMM protein complex that catalyzes ATP production from ADP and inorganic phosphate using the proton gradient established by complexes I–IV. ATP synthase is composed of two main subunits: the F1 subunit, which is the soluble catalytic domain located in the mitochondrial matrix, and the F0 subunit, a proton-conducting membrane domain that forms dimers at cristae ridges. These ATP synthase dimers not only drive ATP production but also contribute to cristae curvature, shaping inner mitochondrial membrane (IMM) morphology and influencing local membrane stress.

Role in Membrane Integrity:

ATP synthase dimers play multiple roles in mitochondrial structure and function. By inducing membrane curvature, they shape cristae and organize the inner mitochondrial membrane (IMM) to optimize proton flux for efficient ATP synthesis. Under pathological conditions, ATP synthase may constitute or regulate the mitochondrial permeability transition pore (mPTP), linking energy production to cell death pathways. Proper dimerization also minimizes electron leakage, helping to reduce reactive oxygen species (ROS) generation and oxidative damage.

Pharmacological Modulation:

Pharmacological targeting of ATP synthase can influence mitochondrial function and disease outcomes. Oligomycin inhibits the proton-conducting F0 channel, reducing ATP synthesis and is primarily used as an experimental tool. Elamipretide indirectly stabilizes ATP synthase by preserving cardiolipin integrity, supporting cristae structure and mitochondrial efficiency. Additionally, therapies aimed at modulating ATP synthase assembly or dimerization are being

explored for neurodegenerative and metabolic disorders, with the goal of restoring mitochondrial bioenergetics and membrane architecture.(Mannella, 2021).

Adenine Nucleotide Translocase (ANT)

Structure and Function:

ANT is an IMM carrier protein that mediates exchange of ADP and ATP across the IMM, essential for cellular energy metabolism. ANT also acts as a regulator of mPTP opening .

ANT conformational states: "c" and "m" states determine substrate binding and sensitivity to calcium-induced pore formation.

Lipid interactions: CL stabilizes ANT conformation, while oxidative stress disrupts ANT–CL interactions, sensitizing mPTP (Paradies et al., 2022).

Pathophysiological Significance:

Adenine nucleotide translocase (ANT) plays a pivotal role in mitochondrial bioenergetics and cell fate. In ischemia-reperfusion injury, ANT oxidation promotes mitochondrial permeability transition pore (mPTP) opening, contributing to cell death. In neurodegenerative conditions, impaired ANT function reduces ATP availability, exacerbating neuronal vulnerability and compromising cellular energy homeostasis.

Pharmacological Modulation:

Pharmacological modulation of ANT can influence mitochondrial permeability and bioenergetics. Bongkrekic acid stabilizes ANT in its "m" (matrix-facing) conformation, thereby inhibiting mitochondrial permeability transition pore (mPTP) opening. Elamipretide protects ANT from oxidative damage by preserving its interactions with cardiolipin, maintaining ANT function and mitochondrial integrity under stress conditions.

Mitochondrial Calcium Uniporter (MCU):

MCU is the principal channel mediating calcium uptake into the mitochondrial matrix , crucial for metabolic regulation and apoptotic signaling. The mitochondrial calcium uniporter (MCU) is tightly regulated by associated proteins such as MICU1, MICU2, EMRE, and by matrix calcium concentration. Under physiological conditions, calcium uptake through the MCU stimulates mitochondrial dehydrogenases, enhancing the tricarboxylic acid (TCA) cycle and ATP production to meet cellular energy demands.

Role in Membrane Integrity:

Excessive mitochondrial calcium uptake through the MCU can have deleterious effects. Calcium overload triggers mitochondrial permeability transition pore (mPTP) opening and inner mitochondrial membrane (IMM) depolarization. High matrix Ca^{2+} levels also amplify reactive oxygen species (ROS) production by enhancing electron leakage from respiratory complexes. Additionally, MCU-mediated calcium overload sensitizes cells to BAX/BAK-mediated outer mitochondrial membrane (OMM) permeabilization, promoting apoptotic initiation. Sancak et al. (2013)

Pharmacological Modulation:

Mitochondrial calcium uniporter (MCU) activity can be pharmacologically modulated to influence cellular energy metabolism and stress responses. MCU inhibitors, such as ruthenium red, Ru360, and other small molecules, reduce mitochondrial calcium overload, thereby providing neuroprotective and cardioprotective effects by preventing mPTP opening and excessive ROS generation. Conversely, MCU activators can enhance matrix calcium under controlled conditions, stimulating dehydrogenases and ATP production to support metabolic demands. (Patron et al., 2014).

IMM Protein–Lipid Interactions:

Inner mitochondrial membrane (IMM) proteins are heavily reliant on lipid interactions for maintaining both their structural and functional integrity**.** Cardiolipin plays a critical role by stabilizing ATP synthase dimers, adenine nucleotide translocase (ANT), and components of the mitochondrial permeability transition pore (mPTP). Phosphatidylethanolamine (PE) is essential for the proper folding and insertion of IMM carriers, ensuring efficient transport across the membrane. Conversely, oxidized lipids can promote mPTP opening and increase ROS-mediated damage, compromising mitochondrial function and leading to cell death. Pharmacological preservation of these lipid–protein interactions is vital for maintaining mitochondrial bioenergetics, improving cellular resilience, and enhancing apoptotic resistance.

Integration with Biophysical Properties:

The activity of IMM proteins is intricately influenced by the physical properties of the membrane, including fluidity, curvature, and membrane potential ($\Delta\psi m$). ATP synthase dimers induce local curvature of the inner mitochondrial membrane (IMM), optimizing proton gradient utilization for efficient ATP production. The proper functioning of ANT and the regulation of mPTP opening are

highly dependent on membrane fluidity and the lipid environment, which maintain the stability and functionality of these key proteins. MCU-mediated calcium influx is also regulated by the IMM's membrane potential and lipid microdomains, linking calcium homeostasis to mitochondrial function. These integrated mechanisms ensure the structural and functional integrity of the IMM under normal physiological conditions while allowing for regulated apoptosis in response to stress, such as oxidative damage or calcium overload.

Pathophysiological Relevance of IMM Proteins:

Mitochondrial dysfunction plays a central role in various pathological conditions that affect cell survival and death pathways. In neurodegenerative disorders like Alzheimer's, Parkinson's, and ALS, mPTP overactivation and ATP synthase dysfunction contribute to neuronal cell death, often exacerbated by oxidative stress and impaired mitochondrial bioenergetics. In cardiovascular injury**,** ischemia-reperfusion injury is aggravated by mPTP opening, ANT oxidation, and calcium overload, which disrupt mitochondrial function, promote ROS accumulation, and lead to cell death, particularly in heart tissue during reperfusion.

In metabolic disorders, impaired function of ATP synthase, ANT, or the MCU reduces mitochondrial energy efficiency, contributing to conditions like diabetes and obesity, where dysfunctional mitochondria lead to insulin resistance, poor energy production, and fat accumulation. In cancer, tumor cells often exhibit altered mPTP sensitivity, enhanced ATP synthase stability, and modified calcium handling, allowing them to evade apoptosis. These adaptations help tumor cells survive under stress and resist therapeutic interventions by maintaining mitochondrial integrity and preventing cell death.

Pharmacological Strategies Targeting IMM Proteins:

Various therapeutic strategies targeting mitochondrial integrity and function have shown promise for treating a range of diseases. mPTP inhibitors, such as cyclosporin A derivatives (which block cyclophilin D), aim to prevent apoptosis in conditions like ischemia-reperfusion injury by stabilizing mitochondrial membranes and reducing cell death (Zhao et al., 2009). ATP synthase stabilizers like elamipretide preserve the dimer structure of ATP synthase and its interaction with cardiolipin, enhancing mitochondrial function under stress (Brieder et al., 2020). ANT modulators, including bongkrekic acid, have been shown to reduce mPTP sensitivity, providing a potential way to protect against mitochondrial dysfunction in a variety of pathologies (Karch et al., 2013). MCU

regulators, such as ruthenium red analogs and other small-molecule inhibitors, are being explored to prevent calcium overload, which can trigger mitochondrial damage and cell death (Baughman et al., 2011). Additionally, lipid-targeted therapies aim to preserve critical protein-lipid interactions, helping maintain inner mitochondrial membrane (IMM) integrity under oxidative stress, which is crucial for mitigating mitochondrial dysfunction in neurodegenerative diseases, cardiovascular disease, cancer, and metabolic syndromes (Zhou et al., 2015). These strategies underscore the therapeutic potential of IMM protein modulation for a wide range of diseases associated with mitochondrial dysfunction.(Brieder, A., et al. (2020).

Integration of Outer and Inner Mitochondrial Membrane Modulation

Mitochondrial function depends on the coordinated regulation of both the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) . While OMM proteins and lipids regulate apoptotic signaling, metabolite exchange, and membrane permeability , IMM components govern bioenergetics, calcium homeostasis, and oxidative stress responses . Dysfunction in either membrane can propagate through the organelle, leading to bioenergetic failure and cell death (Mannella, 2021; Bernardi et al., 2015).

Crosstalk Between OMM and IMM:

Structural and Functional Coupling

The inner mitochondrial membrane (IMM) and outer mitochondrial membrane (OMM) are physically interconnected through mitochondrial contact sites and the cristae organizing system (MICOS), facilitating the exchange of metabolites and trafficking of proteins between the two membranes. One of the key lipids in this coupling is cardiolipin, which spans both membranes and plays a crucial role in stabilizing supercomplexes, protein oligomers, and components of the mPTP (Lapuente-Brun et al., 2013). In addition, BCL-2 family proteins on the OMM regulate mPTP opening via calcium fluxes and ROS amplification, initiating apoptotic signaling. This structural and functional coupling between the OMM and IMM ensures rapid communication of stress signals, enabling a coordinated response that integrates apoptosis regulation, metabolic adaptation, and redox balance. The interplay between these processes is vital for maintaining mitochondrial integrity under normal and stress conditions, and disruptions in this coordination can lead to pathological cell death in diseases such as neurodegeneration and cancer (Macia et al., 2014).

MOMP and IMM Depolarization:

OMM permeabilization, primarily driven by the BAX/BAK proteins, forms pores in the outer mitochondrial membrane, allowing the release of cytochrome c into the cytosol, a critical step in apoptosis initiation. The IMM contributes significantly to this process, where calcium overload via MCU**,** along with ROS accumulation and mPTP opening**,** further amplifies apoptotic signaling by destabilizing mitochondrial function. Lipid oxidation, particularly of cardiolipin and phosphatidylethanolamine (PE), plays a synergistic role in facilitating both OMM permeabilization and IMM destabilization**,** thereby enhancing the mitochondrial pathway of apoptosis. This coordinated destabilization of both membranes promotes the release of apoptotic factors, driving cell death in response to stress signals such as oxidative damage or calcium overload.

Stress Response and Mitochondrial Signaling:

Oxidative Stress and ROS Signaling

Reactive oxygen species (ROS) are primarily generated by electron leakage from complexes I and III of the **inner** mitochondrial membrane (IMM) during oxidative phosphorylation. This leakage results in the production of superoxide and other ROS, which can lead to lipid peroxidation**,** particularly affecting cardiolipin (CL) and phosphatidylethanolamine (PE)**.** The oxidation of these lipids destabilizes both the outer mitochondrial membrane (OMM) and IMM, thereby triggering apoptotic or ferroptotic cell death pathways (Zhao et al., 2019). Moreover, ROS are not solely damaging but also serve as critical signaling molecules that regulate cellular responses such as hypoxic adaptation**,** autophagy, and mitophagy (Kamer & Zevian, 2017). These processes help maintain mitochondrial quality control and cellular homeostasis under stress conditions, indicating that ROS can function as a double-edged sword in cellular signaling and pathology.

Mitochondrial Membrane Pharmacology: Lipid and Protein Targets:

Mitochondrial membranes are increasingly recognized as therapeutic targets due to their central role in apoptosis, metabolism, ROS signaling, and cellular homeostasis. Both lipids (cardiolipin, PE, cholesterol) and proteins (BCL-2 family, VDAC, mPTP, ATP synthase, MCU) offer avenues for pharmacological intervention. Drugs targeting these components aim to modulate membrane integrity, bioenergetics, and stress response , offering potential treatments for cancer,

neurodegeneration, cardiovascular diseases, and metabolic disorders (Paradies et al., 2022; Czabotar & Garcia-Saez, 2023).

Comprehensive Synthesis of Mitochondrial Membrane Modulators:

Mitochondrial membranes, composed of the outer mitochondrial membrane (OMM) and inner mitochondrial membrane (IMM) , are central to cellular bioenergetics, stress signaling, and apoptosis regulation . Integrity of these membranes is governed by a complex network of proteins, lipids, and ions , whose coordinated interactions allow mitochondria to adapt to metabolic demand and stress while determining cell fate (Mannella, 2021; Bernardi et al., 2015).

OMM Modulators:

The outer mitochondrial membrane (OMM) is regulated by a range of modulators that influence cell survival and apoptosis. BCL-2 family proteins play a central role in determining the apoptotic threshold through mitochondrial outer membrane permeabilization (MOMP)**.** The balance between pro-apoptotic members, such as BAX and BAK, and anti-apoptotic members, such as BCL-2, dictates whether a cell will survive or undergo programmed cell death (Vaux & Korsmeyer, 1999). The voltage-dependent anion channel (VDAC) is another crucial OMM protein, mediating metabolite and ion flux, while also interacting with hexokinase for energy regulation and apoptotic proteins to trigger cell death pathways (Shoshan-Barmatz et al., 2010). Additionally, the lipid composition of the OMM, including cardiolipin (CL)**,** phosphatidylethanolamine (PE)**,** and cholesterol, significantly influences protein localization, pore formation, and the sensitivity of the membrane to apoptosis. These lipids play a key role in mitochondrial dynamics and apoptosis by regulating membrane fluidity and interactions with apoptotic proteins (Kinnally et al., 2011).

IMM Modulators:

The inner mitochondrial membrane (IMM) is crucial for maintaining mitochondrial function, with several key components that regulate energy production, calcium homeostasis, and apoptosis. ATP synthase not only generates ATP but also plays a pivotal role in maintaining cristae morphology**,** with its dimerization being essential for IMM curvature and the regulation of the mitochondrial permeability transition pore (mPTP) (Lapuente-Brun et al., 2013). The mPTP itself is critical for regulating calcium-induced IMM permeability, and its sustained opening can lead to apoptosis or necrosis (Bernardi, 2013). Adenine nucleotide translocase (ANT), by exchanging ADP/ATP, modulates mPTP opening and contributes to mitochondrial bioenergetics (Krauss et al., 2004). Additionally, the mitochondrial calcium uniporter (MCU**)** controls calcium influx into the matrix,

integrating metabolic processes and apoptotic signaling, with dysregulation contributing to various diseases (Baughman et al., 2011). IMM lipids, particularly cardiolipin (CL) and phosphatidylethanolamine (PE), are essential for stabilizing protein complexes and preventing oxidative stress-induced permeabilization, thus protecting the membrane from destabilization under stress conditions (Zhao et al., 2019). Together, these components ensure proper mitochondrial function and regulate the cell's response to stress, contributing to energy production, calcium regulation, and apoptotic control.

Lipid-Protein Interactions:

Cardiolipin serves as a central hub for mitochondrial function, interacting with key proteins such as ATP synthase, ANT, mPTP, BAX, and cytochrome c, playing a pivotal role in both apoptosis regulation and energy production (Gonzalvez & Gottlieb, 2007). Under oxidative stress, lipid peroxidation of cardiolipin destabilizes mitochondrial membranes, sensitizing the mPTP and facilitating ferroptosis, a form of iron-dependent cell death (Gao et al., 2019). To counteract these damaging effects, pharmacological stabilization using compounds like elamipretide and mitochondrial-targeted antioxidants helps preserve lipid-protein networks, enhancing both membrane integrity and bioenergetics (Brieder et al., 2020). These strategies aim to protect mitochondrial function under stress conditions, improving cellular resilience to oxidative damage, and offering therapeutic potential in conditions linked to mitochondrial dysfunction.

Integrated Mitochondrial Signaling Networks:

Mitochondrial membranes serve as crucial signaling platforms that integrate multiple cellular pathways, linking cellular stress responses, energy production, and apoptosis. In apoptosis, OMM permeabilization and IMM depolarization work together to facilitate cytochrome c release, which triggers caspase activation and the execution of cell death (Green & Reed, 1998). In metabolism, IMM protein complexes and OMM transporters regulate ATP production, ADP/ATP exchange, and calcium-mediated enzymatic activity, ensuring efficient energy supply and cellular function (Fossi et al., 2018). ROS production at the IMM triggers lipid peroxidation, which in turn modulates both apoptotic and metabolic pathways, contributing to oxidative stress-induced cell death (Zhao et al., 2019). In ferroptosis, lipid peroxidation within IMM microdomains plays a central role in driving regulated cell death, particularly in iron-overloaded conditions (Gao et al., 2019).

Calcium signaling is tightly coordinated between MCU and VDAC, linking matrix calcium levels to both metabolic and apoptotic responses, highlighting the importance of calcium in mitochondrial function and cell fate (Baughman et al., 2011). Mitochondrial dynamics, involving processes such as fusion and fission, integrate structural integrity, quality control, and mitophagy pathways, ensuring mitochondrial health and cellular homeostasis (Twig et al., 2008). Therapeutic interventions targeting these integrated networks offer the potential to selectively modulate cell survival, enhance energy efficiency, or induce programmed cell death in various disease contexts, including neurodegeneration, cancer, and cardiovascular disease.

Emerging Technologies and Future Directions:

Advancements in mitochondrial-targeted drug delivery have enabled more precise therapeutic interventions, using nanoparticles and peptide-based systems to selectively deliver therapeutics to the IMM or OMM, enhancing the efficacy of treatments while minimizing off-target effects (Schmidt et al., 2020). In gene therapy, modulating key mitochondrial proteins such as tafazzin, OPA1, or BCL-2 family proteins can restore membrane integrity and improve mitochondrial function, offering potential treatments for both congenital and degenerative diseases like Barth syndrome and neurodegenerative disorders (Vantaggiato et al., 2017). Precision medicine is emerging as a transformative approach, where the integration of lipidomics, proteomics, and metabolomics enables the development of patient-specific mitochondrial therapies, tailoring interventions to the unique mitochondrial profile of individuals (Picard et al., 2017). Combination strategies, such as simultaneous targeting of lipids, proteins, ROS, and calcium signaling, offer the potential for synergistic therapeutic outcomes, improving both mitochondrial function and cell survival in diseases like cancer, heart disease, and neurodegeneration (Giorgi et al., 2018). Moreover, high-throughput screening is being used to identify small molecules that can stabilize lipid-protein networks, prevent mPTP opening, or modulate MCU activity, potentially offering new drug candidates for mitochondrial-related disorders (Schreckenberg et al., 2020).

Integrated Therapeutic Strategy Model:

A systems-level approach to mitochondrial membrane pharmacology involves an integrated strategy targeting key components of mitochondrial function to optimize both cellular health and disease management. Protein targeting focuses on modulating critical proteins such as BCL-2 family members, mPTP, ANT, ATP synthase, and MCU, which play key roles in regulating apoptosis, bioenergetics, and calcium signaling (Wang et al., 2018).

Lipid targeting involves stabilizing membrane lipids like cardiolipin (CL) and phosphatidylethanolamine (PE), ensuring the structural and functional integrity of both the inner and outer mitochondrial membranes, which are essential for maintaining mitochondrial dynamics and function (Gonzalvez & Gottlieb, 2007). Biophysical modulation seeks to maintain the appropriate membrane curvature, fluidity, and potential necessary for the optimal activity of mitochondrial proteins and complexes (D'souza et al., 2017).

Redox management aims at controlling ROS levels to prevent lipid peroxidation, which can destabilize membranes and initiate cell death pathways such as apoptosis or ferroptosis (Zhao et al., 2019). Calcium homeostasis is regulated by MCU-VDAC-mediated calcium flux, balancing mitochondrial metabolism and apoptotic signaling, ensuring cellular adaptation to stress and energy demands (Baughman et al., 2011).

Together, this integrative model offers a translational framework for developing therapeutic interventions in diseases such as neurodegeneration, cardiovascular disease, cancer, and metabolic disorders. These strategies can be tailored to target specific mitochondrial dysfunctions, enhancing cell survival and bioenergetics or inducing programmed cell death in pathological contexts.

Conclusion:

Mitochondrial membranes function as dynamic, integrated platforms that coordinate essential cellular processes, including energy production, stress response, and apoptosis. The outer mitochondrial membrane (OMM) proteins and lipids regulate metabolite flux and set the apoptotic threshold, determining whether a cell survives or undergoes programmed cell death.

On the other hand, the inner mitochondrial membrane (IMM) proteins and lipids are critical for ATP synthesis, maintaining calcium homeostasis, and modulating ROS signaling, all of which are essential for cellular energy balance and stress responses. The interaction between protein-lipid networks and the biophysical properties of the membranes—such as curvature, fluidity, and potential—are central to maintaining mitochondrial integrity and function.

Moreover, integrated signaling pathways link various processes, including apoptosis, metabolism, ROS production, ferroptosis, and calcium signaling, ensuring that the cell responds efficiently to stress and maintains homeostasis in both physiological and pathological conditions. These interconnected mechanisms highlight the complexity and essential role of mitochondrial membranes in cellular health and disease.

Pharmacological targeting of these components—through lipid stabilization, protein modulation, ROS control, and calcium regulation —offers promising translational applications across neurodegeneration, cardiovascular disease, cancer, and metabolic disorders.

Future research combining omics technologies, gene therapy, mitochondria-targeted delivery, and combination pharmacology is poised to advance precision mitochondrial medicine , highlighting mitochondrial membranes as both biological sensors and therapeutic targets.

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