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Review

Dynamic death decisions: How mitochondrial dynamics shape cellular commitment to apoptosis and ferroptosis

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SUMMARY

The incorporation of mitochondria into early eukaryotes established organelle-based biochemistry and enabled metazoan development. Diverse mitochondrial biochemistry is essential for life, and its homeostatic control via mitochondrial dynamics supports organelle quality and function. Mitochondrial crosstalk with numerous regulated cell death (RCD) pathways controls the decision to die. In this review, we will focus on apoptosis and ferroptosis, two distinct forms of RCD that utilize divergent signaling to kill a targeted cell. We will highlight how proteins and processes involved in mitochondrial dynamics maintain biochemically diverse subcellular compartments to support apoptosis and ferroptosis machinery, as well as unite disparate RCD pathways through dual control of organelle biochemistry and the decision to die.

INTRODUCTION

Given the irreversible fate of a cell committed to die, this decision cannot be simple. Indeed, the past four decades of regulated cell death (RCD) literature support this notion and have defined a diverse repertoire of RCD pathways that respond to countless developmental cues, inherent signaling, and pharmacological stressors.[1,2] Mitochondrial biology is involved in several forms of RCD; most notable and well-researched is apoptosis, but roles in ferroptotic cell death have also developed in recent years (Figure 1).[3] For apoptosis, mitochondria establish and maintain a permissive environment to support pro-death signaling that leads to a loss of outer mitochondrial membrane (OMM) integrity and the subsequent release of intermembrane space (IMS) proteins. This activates the intracellular signal to die.[4] In contrast, the signal to die for ferroptosis is an iron-dependent accumulation of lipid peroxides within the plasma membrane, leading to its rupture, yet numerous mitochondrial functions influence lipid homeostasis and the extent of lipid peroxidation.[5] Taking the above into account, we postulate that the mitochondrial network broadly influences RCD signaling and commitment to death. Furthermore, mitochondrial dynamics, an evolutionarily conserved process of balancing mitochondrial shape with function, likely intersects with multiple RCD pathways to biochemically influence the decision to die.[6]

Here, we will discuss how proteins and processes captured within the mitochondrial dynamics machinery influence cellular sensitivity and the execution of apoptosis and ferroptosis—not as a switching mechanism between these pathways, but to uniquely support each RCD pathway. That being said, there are instances where a macromolecular perturbation (e.g., genomic stress) may be differentially sensed to activate either apoptosis or ferroptosis based on a cell's pre-existing stress response network (e.g., the p53 pathway).[7] Indeed, the concepts under consideration may also be applicable to RCD mechanisms beyond apoptosis and ferroptosis, and it is critical to keep in mind that much of what we know about RCD and mitochondrial dynamics has been characterized using immortalized and transformed cell lines that harbor oncogenic mutations, inactivated tumor suppressor pathways, altered metabolism, and no tissue microenvironment. Therefore, model systems should be taken into consideration before generalizing the biological concepts discussed here.

RCD PATHWAYS

As we introduce the mechanisms of apoptosis and ferroptosis, there is some often-undiscussed cell biology to highlight. Most of the RCD literature was built upon a concept of treating cultured cells with perturbagens (e.g., DNA-damaging agents and kinase inhibitors) that can activate cell death, yet most drugs are not pure inducers of one cellular response or RCD pathway. For example, the DNA-damaging agent, etoposide, commonly activates a series of cellular responses, including oxidation-reduction (REDOX) alterations, cell-cycle arrest, autophagy, and multiple RCD pathways, which may manifest differently in diverse genetic backgrounds.[8] These phenotypes may obscure the biochemical dissection between mechanism versus consequence to a particular cellular insult. Although we focus on apoptosis and ferroptosis in the context of mitochondrial

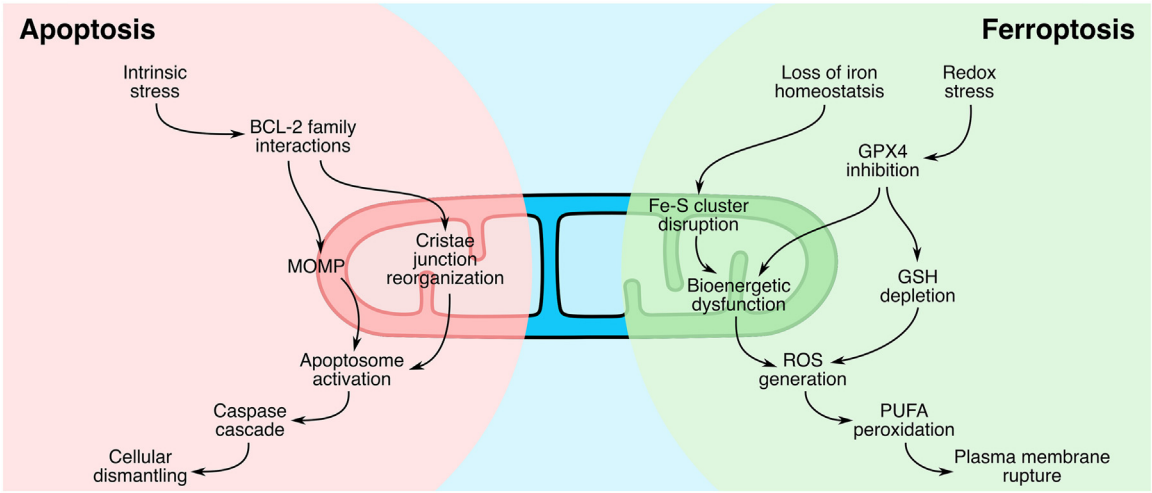

Figure 1. Role of mitochondria in apoptosis and ferroptosis

The intrinsic pathway of apoptosis is induced by macromolecular stress and ultimately activates the caspase cascade to cleave a myriad of cellular substrates and dismantle the cell. The primary regulators of this pathway are the BCL-2 family of proteins, which coordinate to regulate MOMP and the subsequent commitment to apoptosis. In ferroptosis, excess labile iron and REDOX stress results in aberrant PUFA peroxidation, ultimately culminating in plasma membrane dysfunction. PUFA peroxidation is caused by excessive ROS generation, which is promoted by dysfunction of mitochondrial homeostasis. Mitochondria serve as both a sink and source of pro-ferroptotic oxidants. Abbreviations: BCL-2, B cell lymphoma 2; Fe-S, iron-sulfur; GPX4, glutathione peroxidase 4; GSH, glutathione; MOMP, mitochondrial outer membrane permeabilization; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species.

dynamics, recent reviews on how mitochondrial biology collectively influences more diverse RCD pathways are available elsewhere.[3]

Apoptosis

The hallmark of this RCD is the activation of evolutionarily conserved cysteine-aspartate proteases called caspases, which can be activated by two distinct signaling pathways.[3,9] In the extrinsic pathway of apoptosis, the signal to die originates from outside the cell via extracellular ligands (e.g., CD95L/FasL) binding to death receptors (e.g., CD95/Fas), and this pathway is commonly observed in immune cells.[10] By contrast, in the intrinsic pathway of apoptosis, accumulation of macromolecular stress within the cell (e.g., DNA damage, endoplasmic reticulum [ER] stress, and metabolic vulnerabilities) leads to signal transduction mediated by the B cell lymphoma 2 (BCL-2) family of proteins, resulting in mitochondrial outer membrane permeabilization (MOMP) and the subsequent diffusion of pro-apoptogenic factors (e.g., cytochrome *c*) from the mitochondrial IMS into the cytosol.[11,12] As MOMP is traditionally viewed as the "point of no return" for the commitment to death, this process is also referred to as the mitochondrial pathway of apoptosis (Figure 1). Both the extrinsic and intrinsic pathways lead to rapid caspase activation, thereby generating the conserved phenotypes associated with apoptosis: phosphatidylserine (PS) exposure, cellular contraction, nuclear condensation, and cellular blebbing (Table 1).[9]

Our discussion will focus on the intrinsic pathway of apoptosis, which is regulated by the BCL-2 family of proteins at the OMM.[11,13,14] The BCL-2 family is comprised of almost twenty members that are divided into two functional classes: anti-apoptotic and pro-apoptotic. Anti-apoptotic BCL-2 proteins (e.g., BCL-2) are comprised of four BCL-2 homology domains (BH1–4), are generally integrated within the OMM, and block apoptosis by binding pro-apoptotic members; for example, BCL-2-antagonist killer 1 (BAK), BCL-2-associated X protein (BAX), and BCL-2-interacting mediator of cell death (BIM).[4,14,15] The effector proteins BAK and BAX homo-oligomerize into proteolipid pores within the OMM and are required to induce MOMP.[16,17] However, BAK/BAX require activation steps in order to undergo the necessary conformational changes that permit oligomerization, and this is commonly governed through transient interactions with "direct activator" BH3-only proteins (e.g., BIM).[14,18] Indeed, the majority of apoptotic responses are mediated through BIM-induced BAX activation, as a variety of cell stress pathways upregulate BIM function to induce BAX-mediated MOMP. Therefore, from the protein:protein interaction perspective, the BIM-BH3 domain is predominately responsible for BAX activation and the subsequent insertion, oligomerization, and pore formation within the OMM. As mitochondria are the targets of pro-apoptotic BCL-2 family signaling, we aim to explore how the mitochondrial dynamics machinery influences the cellular commitment to apoptosis.

Ferroptosis

This more recently described RCD pathway is characterized by severe membrane lipid peroxidation mediated by polyunsaturated fatty acid (PUFA)-targeted lipoxygenases (LOXs).[19] This form of non-apoptotic cell death was originally defined using Erastin, a sulfasalazine inhibitor of system x_c^-.[20] System x_c^- is an amino acid antiporter that assists glutamate export and cystine import into the cells and, upon inhibition, depletes the free radical scavenging tripeptide glutathione (GSH) and leads to excessive lipid peroxidation. Ferroptosis also requires the presence of labile iron (Fe^{2+}) to amplify membrane perturbations.[21] The process of lipid peroxidation requires free radicals generated by the Fenton reaction between Fe^{2+} and hydrogen peroxide (H_2O_2) in the initiation stage and a subsequent

Table 1. Reagents to study cell death and mitochondrial biology in high-throughput formats

Biology	Reporters (and their targeted hallmarks)	Measurement	Methods
Cell death			
Apoptosis	Annexin V (phosphatidylserine exposure); D-E-V-D peptides (caspase activation); YO-PRO-1 (selective PM permeability)	positivity	FC, MPFS, TC, THFM
Ferroptosis	BODIPY 581/591 C11, Liperfluo (lipid/PUFA peroxides)	intensity, ratiometric	FC, MPFS, FM
Necroptosis/non-specific	7-AAD, propidium iodide, SYTOX, YOYO-3 (PM permeability)	positivity	FC, MPFS, TC, THFM
Mitochondria			
Function	DiOC6, JC-1, MitoTracker Red, TMRE/TMRM (mitochondrial potential, $\Delta\Psi_m$)	intensity	FC, MPFS
Respiration	MitoSOX (ROS)	intensity, positivity	FC, MPFS
Metabolism	resazurin/alamarBlue (cellular REDOX)	intensity	FC, MPFS
Dynamics/network	MitoTracker Green, MitoView, NAO, rhodamine 123 (mitochondrial membranes/matrix)	shape, length	FM

Abbreviations: FC, flow cytometry; FM, fluorescence microscopy; MPFS, microplate fluorescence spectroscopy; NAO, nonyl acridine orange; PM, plasma membrane; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; TC, time-course assays; THFM, time-lapse high-throughput fluorescence microscopy.

peroxidation chain reaction sustained by iron.[22] Ultimately, the signal to die stems from the lipid peroxidation of PUFAs within intracellular membrane structures, usually starting at the ER and eventually eroding the integrity of the plasma membrane, causing dysregulated ion transport, osmotic swelling, and cellular rupture.[19,21,23] Within the laboratory, it is most common to measure lipid peroxide accumulation in live cells using chemical probes and general viability dyes to quantify cellular rupture (Table 1).

Ferroptosis is an RCD mechanism that is not directly induced by a defined transcriptional response or molecular signaling pathway; rather, cells succumb to the inability to prevent and/or repair lipid peroxidation (Figure 1). In the absence of stress, multiple cellular defense mechanisms antagonize induction of ferroptosis. For example, the glutathione peroxidase 4 (GPX4) enzyme utilizes GSH as a cofactor to scavenge lipid hydroperoxides to their corresponding alcohols, thereby preventing lipid peroxidation and ferroptosis.[24] Although the effects of cytosolic GPX4 are more predominant in ferroptosis, mitochondrial GPX4 may also mitigate ferroptosis.[25] Similarly, the oxidoreductase enzyme, ferroptosis suppressor protein 1 (FSP1), inhibits lipid peroxidation by regenerating the reduced form of lipid peroxyl scavenger, ubiquinol (coenzyme Q [CoQ]), using NAD(P)H as a cofactor.[26] Ferroptosis was originally described in Ras-driven cancer cells, and mitochondria appeared to be dispensable; however, more recent literature determined that mitochondria are required for ferroptosis in several model systems.[5,20,27–29] This discrepancy may be explained by a parallel involvement of lysosomes in maintaining iron homeostasis and reactive oxygen species (ROS) signaling.[30]

As mitochondria sequester most cellular ubiquinol/CoQ as an e^- carrier within the electron transport chain (ETC), they act as a buffering platform against ferroptosis. Intriguingly, the mitochondria-specific pyrimidine biosynthetic enzyme dihydroorotate dehydrogenase (DHODH) is another ubiquinol-/CoQ-regenerating agent with anti-ferroptotic properties.[31] Mitochondria are also sites of iron-sulfur (Fe-S) cluster biogenesis, a process that further scavenges the cellular Fe^{2+} pool.[32] Despite having multiple protective roles against ferroptosis, mitochondria may also contribute to ferroptosis when metabolism is altered under cystine starvation. The key mitochondria-derived negative regulators of ferroptosis are α-ketoglutarate (αKG) generated in the tricarboxylic acid (TCA) cycle and the soluble ROS generated through the ETC.[29,33,34] It is worth noting that an additional phenotype of ferroptosis is fragmented mitochondria with disordered, electron-dense cristae.[20,35] This morphological attribute of mitochondria, taken together with their metabolic regulation of ferroptosis, prompts us to question: how does the mitochondrial dynamics machinery mechanistically intersect with these processes to control cellular sensitivity to, and the execution of, ferroptosis?

FUNDAMENTALS OF MITOCHONDRIAL DYNAMICS

The characteristic shape of a single mitochondrion is recognizable to most scientists; however, this shape fails to capture the complexity and dynamic nature of the mitochondrial network in living cells. Individual mitochondria constantly undergo fusion and fission, which is necessary for the homogeneous distribution of lipids, proteins, and mitochondrial genomes and for supporting efficient mitochondrial metabolism, respiration, and overall homeostasis.[36] Mitochondrial dynamics are controlled by a number of highly conserved large guanosine triphosphatases (GTPases), transient interactions with the ER, and molecular motors via the cytoskeleton, which we will now explore (Figure 2).

Mitochondrial fusion

Mitochondrial fusion involves uniting two or more neighboring mitochondria by the sequential joining of the OMMs and inner

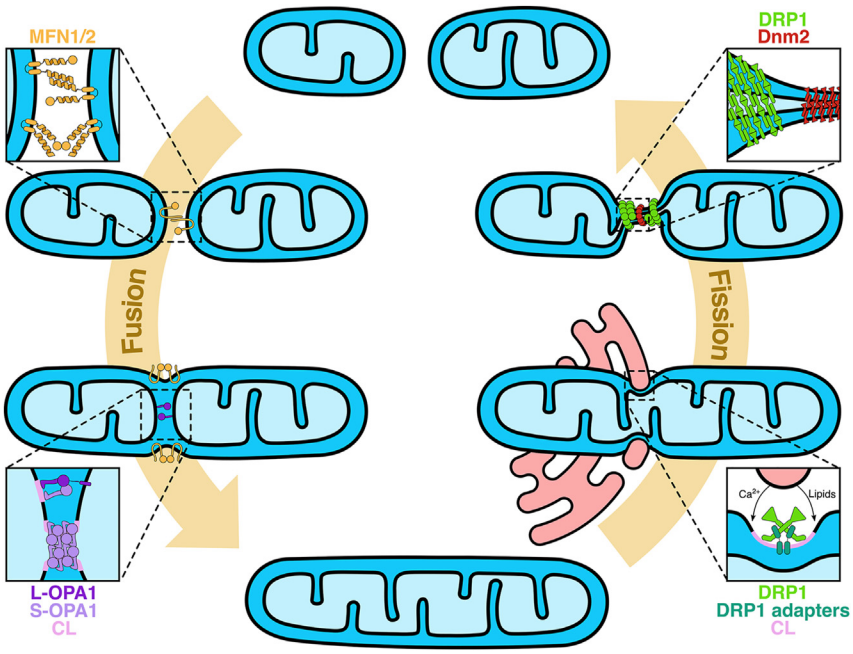

Figure 2. Mitochondrial dynamics are governed by several GTPases

Fusion of mitochondria is initiated by the OMM proteins MFN1/2, which dimerize via either the α-helical tails or GTPase domains to tether and join the OMMs. Subsequently, interactions between OPA1 and CL-enriched regions of the IMM serve to tether proximal IMMs. Further recruitment of S-OPA1 to CL-enriched membranes results in polymeric structure formation, membrane deformation, and IMM fusion. Fission of mitochondria is marked by mitochondria:ER contact sites that mediate transfer of calcium and lipid species necessary for membrane constriction. Following PTM-mediated activation in the cytosol, the pro-fusion large GTPase DRP1 is recruited to the OMM by adapter proteins and facilitated by interactions with CL. The GTPase activity of DRP1 results in formation of helical macrostructures that wrap around and constrict the OMM. Similarly, the small GTPase Dnm2 may be recruited to further pinch and complete the fission of mitochondria.

Abbreviations: CL, cardiolipin; Dnm2, dynamin2; DRP1, dynamin-related protein 1; ER, endoplasmic reticulum; GTP, guanine 5′-triphosphate; IMM, inner mitochondrial membrane; L-OPA1, long-optic atrophy 1; MFN1/2, mitofusin-1/2; OMM, outer mitochondrial membrane; PTM, post-translational modification; S-OPA1, short-optic atrophy 1.

mitochondrial membranes (IMMs). The process is orchestrated by three large GTPases: mitofusin-1 and -2 (MFN1/2), two homologous proteins sharing ∼80% amino acid sequence similarity, which induce fusion via homo- and heterodimeric interactions at the OMM, and optic atrophy protein 1 (OPA1), which uniquely functions at the IMM.[37,38] The mechanisms of OMM fusion are not fully understood, and currently there are two structural models describing MFN-mediated OMM tethering and fusion: one model suggests MFN interactions through the N-terminal GTPase domain, whereas the other model suggests interactions through the alpha-helical C-terminal domain.[39–41] Regarding the IMM, recent advanced bioimaging has revealed that OPA1 nucleation and polymerization create a lattice structure to facilitate IMM fusion.[42,43]

MFNs alone are insufficient to complete fusion, as they require the assistance of OMM-localized phospholipids and their related enzymes, and later we will revisit how each of these components contributes to cell death signaling. For example, MitoPLD, a member of the phospholipase D family, is anchored to the OMM and converts cardiolipin (CL) to phosphatidic acid (PA) on the opposing OMM.[44] CL is an anionic phospholipid species with four unsaturated acyl chains that are responsible for negative membrane curvature and interact with numerous resident IMM proteins (e.g., ETC components) to either functionalize or enhance their activities.[45,46] The conversion of CL to PA on the OMM promotes fusion through several complimentary mechanisms: PA-enriched membranes recruit nucleoside diphosphate kinase 3 (NME3/NDPK-C), which coordinates with MFNs and tethers mitochondria for fusion; PA binds and directly inhibits fission proteins (i.e., dynamin-related protein 1 [DRP1], discussed below); PA induces less spontaneous negative membrane curvature compared with CL; and reduction of OMM CL inhibits processes antagonistic to fusion (detailed in the following sections).[47–49] Given the potent fusogenic properties of PA, it is regulated by its conversion to, for example, diacylglycerol (DAG), the glycerolipid with the highest negative spontaneous curvature due to its small hydroxyl headgroup, which disrupts proteins binding to PA.[50–53] Furthermore, MFN2 is not exclusive to the mitochondrial network as 10%–15% is localized to the ER and supports ion, lipid, and protein transfer between the organelle networks.[54] The biochemical region allowing for these activities is referred to as mitochondria:ER contact sites (MERCS), which influences broad mitochondrial functions, including certain TCA cycle enzymes (e.g., isocitrate dehydrogenase and αKG dehydrogenase), thereby forming higher amounts of reducing equivalents and increasing the bioenergetic capacity of the organelle.[55] Additionally, MERCS designate sites of mitochondrial fusion through MFN localization, transfer of the fusogenic lipid PA and the MFN-interacting lipid PS, and localized lipid conversion.[56–58] Apart from MERCS, MFNs impact mtDNA maintenance and replication, as the *Mfn1*/*Mfn2* double knockout mouse failed to maintain mtDNA copy number and manifested distorted mitochondrial morphology.[59] Indeed, the mitochondrial genome encodes for 13 essential ETC subunits (and the translational machinery to support their matrix-localized expression), and mtDNA loss/mutations diminish oxidative phosphorylation (OXPHOS) and force metabolic adaptations.[60,61]

Finally, subsequent to successful MFN1/2-mediated OMM fusion, IMM fusion is engaged and facilitated by interactions between OPA1 and CL, which stimulate OPA1 GTPase activity and the joining of proximal IMM structures.[62,63] Interestingly, in a manner similarly observed with MFNs, OPA1 interacts with hexameric NME4/NDPK-D, which delivers GTP to OPA1 and facilitates fusion in membranes enriched with CL.[64,65] *Opa1* generates numerous isoforms, and OPA1 cleavage products impact not only IMM fusion but also cristae formation, with direct implications for mitochondrial membrane potential ($\Delta\Psi_m$).[66,67] Alternative splicing generates eight different isoforms of OPA1, which

may be further processed into either long (L-OPA1) or short OPA1 (S-OPA1).[66,68–70] Mitochondrial bioenergetics directly influence OPA1 processing, and, when $\Delta\Psi_m$ is ideal, L-OPA1:CL binding is encouraged; in regions of reduced CL levels, homodimeric L-OPA1 interactions facilitate cristae formation.[62]

Mitochondrial fission

DRP1 is the major GTPase that promotes mitochondrial fission, and its cytosolic localization, re-distribution to the OMM, and oligomerization into a scission complex controls its activity.[71,72] The localization and activity of DRP1 are regulated by a series of post-translational modifications (PTMs), including phosphorylation, ubiquitylation, SUMOylation, O-GlcNAcylation, and nitrosylation, and the impact of these PTMs is thoroughly described in recent reviews.[73] However, there are two major regulatory phospho-serine residues in DRP1, Ser616 and Ser637, which are activating and inhibiting, respectively.[74–76] Numerous cellular signaling pathways promote DRP1-Ser616 phosphorylation, allowing the cytosolic protein to translocate to the OMM and interact with receptors (e.g., Fis1, MFF, MID49, and MID51) that contribute to OMM localization, function, and specificity of membrane fission.[77,78] Indeed, activating Ser616 phosphorylation alone is insufficient to attain maximal DRP1 function, as the inhibitory Ser637 phosphorylation must be removed by phosphoglycerate mutase family member 5 (PGAM5).[79–81]

Interactions with membranes and lipids are essential to DRP1-dependent fission in a manner complementary to MFN1/2-dependent fusion, and, indeed, MERCS also designate sites for mitochondrial fission.[82] One mechanism by which MERCS designate and potentially initiate mitochondrial fission is by tethering and constricting mitochondrial diameter in a Ca^{2+}-dependent process aided by the cytoskeletal elements actin and myosin IIA.[83] The ER and mitochondrial membrane proteins, INF2 and Spire1C, respectively, also allow for biochemical crosstalk between the two organelles and support actin polymerization and myosin assembly.[84,85] Similar to OPA1, DRP1 interactions with CL stimulate GTPase activity, with further constriction of the mitochondria.[86,87] Interestingly, there is some evidence that CL may be enriched at ER contact sites, either due to MERCS-localized CL-modifying enzymes (e.g., ALCAT1) or CL transfer from the IMM at IMM:OMM contacts adjacent to MERCS.[88,89] Several reports also suggest that the small GTPase, dynamin 2 (DNM2), participates in DRP1-dependent mitochondrial fission.[90] Together, these protein:membrane platforms assist in DRP1 recruitment to the OMM, oligomerization, and organelle scission. Next, we will discuss how each of these components of the mitochondrial dynamics machinery intersects with apoptosis and ferroptosis.

MITOCHONDRIAL DYNAMICS AND APOPTOSIS

Mitochondrial dynamics support pro-apoptotic BCL-2 family function

When considering the relationships between the mitochondrial dynamics machinery, mitochondrial shape, and apoptosis, there are two main questions: (1) do these pathways communicate upstream of the decision to induce BAK/BAX-dependent MOMP and (2) do they influence the kinetics of apoptosis post-MOMP? For our discussion, we are not focusing on the myriad of perturbagens that activate the mitochondrial pathway of apoptosis because they all mechanistically converge by stabilizing a combination of "sensitizer" BH3-only proteins that inhibit anti-apoptotic BCL-2 proteins (e.g., BAD neutralizes BCL-2, BCL-xL, and BCL-w), along with direct activator BH3-only proteins (e.g., BIM) that promote intra-molecular conformational changes in BAK/BAX, leading to their activation and MOMP.[14,91] What we will focus on is the concept that mitochondria are not passive in the cellular decision to engage MOMP, but, rather, the mitochondrial dynamics machinery establishes a biochemical environment that is permissive for BAK/BAX-dependent MOMP and apoptosis to proceed efficiently.

The original evidence linking mitochondrial dynamics to apoptosis centered upon DRP1 and BAX co-localization at distinct sites on the OMM—referred to as "apoptotic foci"—and this was coincident to fission of the mitochondrial network. These apoptotic foci appear to preferentially form at constricted OMM:IMM junctions that permit the transfer of lipids (e.g., CL) between these two membranes, while also serving as a site for inter-organellar communication (e.g., ER and mitochondria) and ion homeostasis (e.g., Ca^{2+} uptake into mitochondria).[89,92,93] More recent mechanistic interrogations of BAX suggest that BH3-activated BAX monomers preferentially insert into the OMM at apoptotic foci, undergo dimerization, and eventually form high molecular weight BAX oligomers that fall into several categories: arcs, lines, and rings to promote MOMP.[94,95] The phenomenon of DRP1 and BAX co-localization may directly promote, and/or be the consequence of, a biochemical environment containing the necessary lipids and membrane curvature that are required for BAX and DRP1 function, and, indeed, this process is regulated in several manners.[96–98] First, DRP1 does not directly interact with the OMM but, instead, requires the OMM adaptor proteins (i.e., Fis1, MFF, MiD49, and MiD51), and these may provide recruitment specificity in different tissue types and bioenergetic/metabolic states.[99–101] Second, DRP1 dynamically exchanges between the cytosol and OMM, and mitochondrial-anchored protein ligase (MAPL)-dependent DRP1 SUMOylation trigged by apoptotic signaling causes OMM accumulation of DRP1, which is reported to occur at MERCS to support Ca^{2+} and lipid transfer, both of which likely regulate the biochemical environment for BAX activation.[102] Indeed, CL-rich membranes exhibit more curvature and become disordered in the presence of Ca^{2+}.[103] Third, DRP1 may harbor the ability to directly bind and facilitate BAX activation.[104]

Specific lipid environments have also been identified to directly control BAX activation and BH3-only protein function at the OMM, with evidence pointing toward several lipid classes. CL was the first mitochondrial lipid identified to be essential for BAX-dependent membrane permeabilization, and subsequent work identified that CL was specific for BH3-interacting domain death agonist (BID) and BAX cooperation.[17,105] Recent structural investigations revealed that membrane phospholipids bridge and support BAK core dimers, providing the first structural determination of lipid contributions to BAK pore formation.[106] Interestingly, it seems that this phenomenon does not occur in BAX pore formation due to sequence differences between BAK and BAX.[107] Moreover, recent data suggest that apoptotic cells

utilize unsaturated fatty acids of phosphatidylcholine and phosphatidylethanolamine to promote BAX-dependent MOMP, while saturated fatty acids render BAX inactive.[108] Likewise, sphingolipids have historically been associated with the mitochondrial pathway of apoptosis, as both inducers and required contributors to pro-apoptotic BCL-2 family function, namely BAK and BAX. Biochemical screens and structural analysis revealed that terminal sphingolipid products likely originating from the ER (e.g., 2-*trans*-hexadecenal) cause intra-molecular conformational changes associated with BAX activation.[109] Together, these lipids gain access to BAX due to proteinaceous tethers between the ER and mitochondrial network, which are the product of MFN1/2 interactions and/or DRP1 within apoptotic foci to juxtapose CL with BAX.[54,98]

Not only does the mitochondrial dynamics machinery regulate lipid and ion interactions, but also the overall mitochondrial membrane curvature and absolute number of individual mitochondria within a cell.[98,110] Comparisons between different-sized mitochondria revealed that small mitochondria with high membrane curvature are less receptive to BAX-dependent MOMP as the C-terminal α9 helix of BAX fails to integrate in small mitochondria, and this observation was corroborated by the use of biochemically defined OMM model systems.[111] Furthermore, cells with increased mitochondrial mass and/or mitochondrial number require relatively greater apoptotic stimulation to induce death, and we speculate this could be the result of a disrupted BAX:mitochondria ratio that is necessary to promote MOMP.[112,113] For example, a cell with one hundred molecules of activated BAX and ten mitochondria would be able to support approximately ten BAX molecules per mitochondrion, which is sufficient to induce pore formation. If mitochondrial fission is favored, the minimum number of activated BAX molecules necessary to form a pore per mitochondrion may not be achievable, potentially leading to apoptotic resistance despite fully functional stress-sensing and apoptotic machineries. Such fundamental control of MOMP via mitochondrial composition, shape, and number may be the evolutionary consequence of very few molecules of activated BAX being required to initiate MOMP and apoptosis; therefore, the mitochondrial network has adapted numerous mechanisms to govern the decision the die.[112] Additionally, there is growing evidence that, in instances of minority or incomplete MOMP, where cell death is not enacted, cells are subjected to genomic instability or inflammatory responses triggered by the release of mitochondrial genetic material from BAK/BAX megapores.[114–116] Occurrences of minority MOMP are influenced by the mitochondrial dynamics machinery, including cristae organization, and so it could be argued that the mitochondrial network can dictate commitment to apoptosis by titrating BAK/BAX pore formation and subsequent release of mitochondrial factors.[117,118] It is also worth noting that oncogenes (e.g., RAS^{G12V}) disrupt mitochondrial dynamics to favor DRP1-dependent fission, which is associated with apoptotic resistance similar to MFN1 removal or DRP1 gain of function.[75]

However, the above relationships between the mitochondrial dynamics machinery and cell death are not unopposed; for example, studies have uncoupled DRP1 and apoptosis. Comparing wild-type and DRP1 deficient cells revealed only a partial inhibition or slowing of cytochrome *c* release following MOMP, while other IMS proteins were released at similar rates. In both cell types, apoptosis still occurred, suggesting that the role of DRP1 in apoptosis may lean toward cristae remodeling to ensure complete cytochrome *c* release and not solely MOMP. As such, genetic ablation of *Drp1* in the mouse also results in embryonic lethality with marked apoptosis, demonstrating DRP1-independent execution of cell death.[119–121] Whereas MFN1 and MFN2 over-expression, which elongates mitochondria, also leads to decreased apoptotic sensitivity, MFN1/2 removal enhances apoptosis and, importantly, soluble BAX monomers drive mitochondrial fusion via interactions with MFN2.[117,122–124] How do we unite these observations in the context of the historical observations and reproduced requirements for the mitochondrial dynamics machinery in apoptosis?

It is worth considering that the historical data on DRP1:BAX co-localization, with co-incident mitochondrial fission, may represent only a single time point of the true biology. We have limited information on where in the mitochondrial network BAX monomers insert and undergo activation; therefore, it is not inconceivable that the DRP1:BAX co-localization data are the result of DRP1 and BAX homo-oligomers simply congregating together at apoptotic foci due to membrane constraints. Indeed, BAX pores are likely mobile within a single mitochondrion to ensure rapid and complete cytochrome *c* release, and there is evidence that BAX pores "jump" between membranes.[125] Also, DRP1 and MFN1/2 regulate numerous aspects of organelle dynamics, not limited to ER:mitochondria tethering, lipid composition, and ion signaling.[73,126] So, it is possible that their gain of function or loss of function results in an unidentified signal and/or stress that masks their homeostatic role to build a mitochondrial platform for productive BCL-2 family interactions and apoptosis. There are also additional proteins and interactions that warrant investigation to gain a clearer understanding. For example, we focused almost exclusively on BAX, but what about the other pro-apoptotic effector, BAK? There is evidence that MFN1 phosphorylation and sphingosine-1-phosphate promote BAK activation, yet the mechanisms are unclear.[109,127] Indeed, we predict that a conserved mitochondrial regulation of BAK and BAX is not likely as BAK is constitutively mitochondria localized with its α9 residing in the OMM.[128] This may potentially override a requirement for mitochondrial shape and apoptotic foci contributions to BAK-dependent MOMP. Finally, we cannot find any literature showing how mitochondrial dynamics controls anti-apoptotic proteins' localization or ability to bind BH3-only proteins, and, given the structural conservation between the globular BCL-2 members, this may be an important area worth exploring.

A mitochondrial dynamics GTPase encourages caspase activation

The above discussion focused on how the mitochondrial dynamics machinery establishes a platform for MOMP activation, but does the same cohort of proteins also impact on cell death signaling post MOMP? Indeed, an opportunity for post-MOMP regulation centers on the control of mitochondrial ultra-structural domains referred to as cristae junctions.[62] These domains cooperate with IMM resident proteins to support Complex V, while also ensuring that cytochrome *c* is sequestered proximally

to the ETC within cristae.[129] The sequestration of cytochrome *c* represents a unique challenge to a cell, as the redistribution of cytochrome *c* from mitochondria to the cytosol is essential to dimerize initiator caspase-9 via the apoptotic protease activating factor 1 (APAF-1) apoptosome.[130,131] Dimerized and activated caspase-9 then cleaves caspase-3, -6, and -7, and these executioner caspases are responsible for cellular dismantling and removal to minimize inflammation.[132,133] Yet, how does cytochrome *c* gain access to the cytosol from within cristae? Our current understanding suggests that a process referred to as cristae junction remodeling occurs post-MOMP, which opens these sub-organellar regions, allowing for cytochrome *c* to diffuse from cristae, through the IMS, and eventually into the cytosol.[134] This process is closely associated with the mitochondrial dynamics machinery—in particular, OPA1.[135,136]

As discussed earlier, OPA1 isoforms are divided into long and short, and each of these likely represents several species.[66–70] L-OPA1 is IMM anchored via an amino-terminal transmembrane (TM) domain, and S-OPA1 is processed to remove the TM domain and is often characterized as soluble.[67,69] L-OPA1 and S-OPA1 cooperate with the mitochondrial contact site and cristae organizing system (MICOS) and other proteins to form cristae junctions, which are the IMM regions that display a 90° inward bend linking the IMS to the cristae. These junctions are circumscribed by L-OPA1 and tightly sealed by S-OPA1.[137–139]

Mechanistic connections between OPA1 and apoptosis began with explorations of how IMS proteins (e.g., cytochrome *c*, SMAC, and endonuclease G [EndoG]) gain access to the cytosol to initiate caspase activation. SMAC and EndoG localize to the IMS, and, therefore, MOMP is sufficient to promote their complete release.[140] In contrast, the majority of cytochrome *c* localizes within cristae, so MOMP is insufficient to provide cytosolic access.[62] To circumvent this problem, cristae junctions reorganize to widen and reduce the diffusion barrier by altering L-OPA1 and/or S-OPA1 localization.[135,136,139,141,142] This appears to be stimulated downstream of BAK/BAX activation, per se, and DRP1-dependent membrane remodeling during apoptosis, but it can also be induced by BAK and BH3-only proteins (e.g., BID and BIM).[143–145] Indeed, OPA1 gain of function preserves cristae junction architecture and prevents apoptosis following certain cellular stresses, and unbalanced expression of either L-OPA1 and S-OPA1 alters cellular bioenergetics and apoptotic sensitivity.[146,147] One potential reason for the stepwise approach—MOMP, IMS protein release, cristae junction remodeling, and complete cytochrome *c* release—could involve a requirement for oxidation. Cytochrome *c* tightly binds CL, which is inhibited when either is oxidized.[143–145] It is tempting to hypothesize that partial IMS protein release reduces ETC efficiency, increases mitochondrial ROS generation, and leads to CL and/or cytochrome *c* oxidation to facilitate its complete release and caspase activation.[148] Interestingly, NME4/NDPK-D, which supplies OPA1 with GTP in healthy mitochondria, switches function upon loss of $\Delta\Psi_m$ and shuttles CL to the OMM; thus, this molecular switch links mitochondrial function, cristae reorganization, and CL exposure on the OMM.[149] Literature focused on metabolism and REDOX certainly supports these arguments, but a unified dataset in a single-model system that incorporates all these concepts has yet to be established.

MITOCHONDRIAL DYNAMICS AND FERROPTOSIS

The mechanisms of ferroptosis are more recently described than apoptosis, and the field's fundamental and translational knowledge continues to grow, with gradual acceptance of mitochondrial biology as an upstream critical regulator of cellular sensitivity to ferroptosis and the kinetics of execution. As described in the recent literature, mitochondrial control of ferroptosis centers on at least five pathways: (1) energy metabolism, (2) CoQ biosynthesis, (3) iron metabolism, (4) PUFA availability, and (5) mitochondrial stress signaling.[5] Our goal is to view these concepts through the perspective of the mitochondrial dynamics machinery and its influence on mitochondrial shape, which, for ease of discussion, we classify into either fused or fragmented. These five pathways, separately or in conjunction, participate in mitochondrial dynamics by transforming nutrient availability into mitochondrial shape changes. For example, increased energy requirements originating from nutrient starvation necessitate mitochondrial fusion to facilitate ETC/OXPHOS to fuel ATP synthesis.[150] On the contrary, excess nutrients promote mitochondrial fission, which may lead to enhanced ROS generation and macromolecular damage.[151] Therefore, balanced nutrient availability and subsequent mitochondrial dynamics likely protect against ferroptosis. Similarly, dysfunctional mitochondria result in aberrant mitochondrial dynamics, impaired REDOX, and cellular metabolism that decreases the reserves of reducing equivalents necessary to thwart ferroptosis.

First, we examine a relationship between oxidative stress, the mitochondrial dynamics machinery, and ferroptosis. Ras-selective lethal 3 (RSL3), a pharmacological inhibitor of the cytosolic and mitochondrial forms of GPX4, sensitizes cells to ferroptosis.[24,152] The primary function of GPX4 is to scavenge lipid peroxides and prevent oxidative damage, and RSL3-induced ferroptosis is blocked by MFN1-dependent mitochondrial fusion.[153] Likewise, mitochondrial fusion is responsive to changes in REDOX; for example, in response to oxidized GSH levels, MFN1 forms a disulfide bond, likely facilitating fusion.[41,154] Therefore, intracellular and pharmacological insults that promote oxidative stress may be mitigated by a fused mitochondrial network, potentially influencing a cell's sensitivity to ferroptosis.

The antioxidant CoQ mitigates lipid peroxidation to block ferroptosis when present at the plasma membrane. As mitochondria are sites of CoQ biosynthesis, we explore how mitochondrial dynamics might be involved in regulating cellular distribution of CoQ. The function of this conjugated dicarbonyl compound in mitochondria is to carry electrons from ComplexI/II to ComplexIII, and, therefore, it exists in either reduced or oxidized forms.[155] The reduced form ($CoQ_{10}H_2$) is lipid soluble and readily diffuses from mitochondria to other cellular locations (e.g., the ER, Golgi, and plasma membrane), preventing lipid peroxidation. In contrast, the non-lipid-soluble CoQ_{10} requires active transport out of the mitochondrial network. StAR-related lipid transfer protein 7 (STARD7) is localized in the IMS for CoQ biosynthesis, and, upon cleavage by Presenilins-associated rhomboid-like protein (PARL), STARD7 translocates to the cytosol, carrying CoQ_{10} to the plasma membrane and other organellar membranes to prevent lipid peroxidation.[156] STARD7 appears to also regulate

mitochondrial bioenergetics and dynamics through DRP1 and MFN1/2 but in an OPA1-independent manner.[157,158] Curiously, what links OPA1 into this pathway is that PARL also can cleave OPA1 to regulate cristae organization, bioenergetics, and cell death.[159] Furthermore, PARL activity is also reported to be necessary in maintaining efficient mitochondrial bioenergetics and CoQ_{10} levels to prevent ferroptosis in a developmental context.[160] Given the shared machinery linking CoQ biosynthesis and transport to PARL, we speculate that the mitochondrial GTPases also mechanistically intersect within this pathway to regulate CoQ pools and ferroptosis sensitivity.

As mitochondrial fragmentation is often associated with ferroptosis, and iron is positioned at the center of ferroptosis induction, we examined whether there is a connection between iron and fission machinery. Indeed, mitochondria are sites of Fe-S cluster formation and heme biosynthesis, as both are essential for ETC assembly, function, and efficiency. Mitochondria take up iron through voltage-dependent anion channel (VDAC) on the OMM and Mitoferrin1 at the IMM, while mitochondrial Ferritin shields excess iron within the matrix.[22] An increased labile iron pool is associated with increased mitochondrial recruitment of DRP1 through MFF phosphorylation by AMP-activated protein kinase α (AMPKα), and defects in Fe-S cluster biogenesis also sensitize to ferroptosis, both potentially happening co-incident to mitochondrial division.[32,161] Indeed, excess iron transport or inefficient iron incorporation into proteins creates a positive feedback loop, resulting in enhanced iron import into the cells, likely enhancing ferroptosis sensitivity.[162] Therefore, the fragmented mitochondrial network phenotype associated with ferroptosis possibly stems from disrupted cytosolic and mitochondrial iron homeostasis, yet the overall contribution to ferroptosis sensitivity and kinetics beyond decreased ETC efficiency and enhanced ROS remains largely underexplored. Indeed, the above speculation is supported in that quercetin, a ferroptosis inducer, alters both iron homeostasis and DRP1-dependent fission.[163]

Moving beyond iron, PUFA levels are regulated by mitochondrial fission and directly alter ferroptosis initiation and execution. For example, several reports describe that DRP1-mediated mitochondrial fission is a requirement for fatty acid metabolism within mitochondria and that fragmented mitochondrial networks are best equipped for fatty acid breakdown.[164,165] The Golgi resident small GTPase protein ADP ribosylation factor 1 (ARF1) contributes to the final steps of mitochondrial fission and appears to regulate mitochondrial import of fatty acids.[166,167] As a result, excess intracellular fatty acids stimulate extracellular signal-regulated kinase 1 (ERK1)-dependent DRP1-Ser616 phosphorylation, which enhances both their metabolism and β-catenin-dependent upregulation of the anti-ferroptotic enzyme GPX4.[168,169] From these observations, PUFA availability via fatty acid import and metabolism is regulated by DRP1-dependent mitochondrial division, with several pathways providing additional anti-ferroptotic influences. Likewise, in diverse cancer cell settings, mitochondrial division is enhanced by oncogenic signaling, which promotes fatty acid utilization, nuclear factor erythroid 2-related factor 2 (NRF2) stabilization, and system x_c^- scavenging to decrease ferroptosis sensitivity.[75,170] However, there are conflicting reports; for example, the pro-ferroptotic enzyme acyl-coenzyme A synthetase long-chain family member 4 (ACSL4) is localized at MERCS, a potential site of lipid peroxidation, which enhances both PUFA synthesis and DRP1-dependent mitochondrial division.[171–174] Perhaps the control of PUFA availability by a fragmented mitochondrial network attempts to protect against ferroptosis. However, as lipid peroxidation rates supersede the network's ability to defend, this results in execution of ferroptosis, with the consequence of a fragmented mitochondrial network.

Lastly, the integrated stress response (ISR) originating from mitochondria is a signaling pathway associated with ferroptosis, with direct links to the mitochondrial dynamics machinery. Early work demonstrated that OXPHOS-deficient tissues engage the ATF4-ISR to block ferroptosis through OMA1 activation and GPX4 accumulation.[175] Additionally, OMA1 zinc metallopeptidase (OMA1) activation also results in mitochondrial fragmentation through OPA1 cleavage, suggesting potential cross-talk between the ISR and dynamics machineries.[69] Most recently, genetic loss of *Opa1* was demonstrated to promote marked resistance to ferroptosis, with reconstitution experiments demonstrating that OPA1-mediated ferroptosis requires the GTPase domain but is fusion-independent.[176] The suggested mechanism is that the presence of OPA1 in wild-type cells allows for normal mitochondrial bioenergetics, lipid peroxidation, and suppression of ISR, which contrasts with OPA1 deficiency leading to ISR activation upon challenge with ferroptosis inducers. Additional literature also links ATF4 function to multiple anti-ferroptosis mechanisms, including system x_c^- expression and the NRF2 pathway.[177,178]

FUTURE PERSPECTIVES

Based on our discussions, we propose clear roles for mitochondrial dynamics in apoptosis and ferroptosis, but not for conserved reasons (Figure 3). For apoptosis, the mitochondrial dynamics machinery contributes to establishing a platform for MOMP along with promoting caspase activation to ensure rapid packaging and removal of the dying cell. In contrast, connections between mitochondrial dynamics and ferroptosis are related to maintaining a cytoplasmic environment that influences lipid homeostasis and peroxidation. These may not be mutually exclusive, as alterations in the platforms supporting pro-apoptotic BCL-2 family function (e.g., apoptotic foci, MERCS, and mitochondrial mass) likely impact on lipid homeostasis. Likewise, mitochondrial-dynamics-regulated mitochondrial metabolism, REDOX, iron homeostasis, and mitochondrial stress signaling certainly inform the detection and resolution of macromolecular stress that has the potential to lead to pro-apoptotic signaling.

Debates surrounding the involvement of mitochondrial dynamics in RCD likely exist for several reasons. One, most studies were performed in transformed murine fibroblast and human cancer cell lines, where numerous signaling pathways (e.g., metabolism, proliferation, and stress detection/signaling) are disrupted and do not likely represent the basal cellular state nor capture tissue-specific requirements. Second, genetic or RNAi-mediated reductions of the mitochondrial GTPases controlling dynamics likely select for unconventional (maybe undefined) mechanisms to support organelle homeostasis and may themselves elicit unanticipated stress. Third, initial

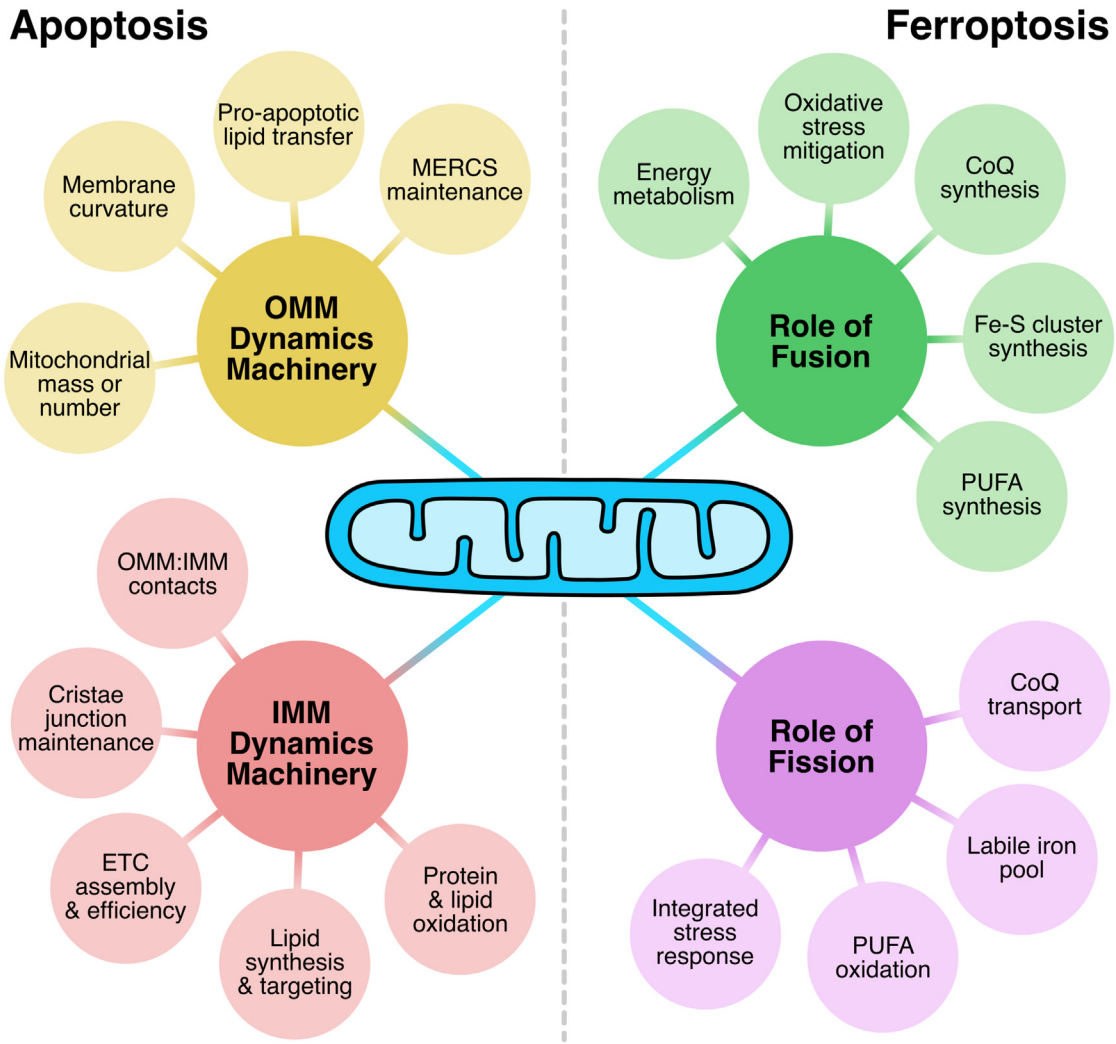

Figure 3. Mitochondrial dynamics regulate several aspects of apoptosis and ferroptosis signaling
In apoptosis, the mitochondrial dynamics machinery broadly modulates the cellular commitment to death by directly or coincidently controlling the environment for MOMP (an event involving the OMM) and the efficiency by which pro-apoptotic IMS proteins are released into the cytosol (an event involving the IMM). In contrast, ferroptotic outcomes are mediated by the metabolic/bioenergetic, biochemical, and macromolecular outputs of mitochondria, all of which are heavily influenced by fusion and fission of the mitochondrial network. Detailed coverage of these topics is provided in the text.
Abbreviations: CoQ, coenzyme Q; ETC, electron transport chain; Fe-S, iron-sulfur; IMM, inner mitochondrial membrane; MERCS, mitochondria-endoplasmic reticulum contact sites; OMM, outer mitochondrial membrane; PUFA, polyunsaturated fatty acid.

pharmacological tools to regulate the RCD and mitochondrial dynamics machineries display off-target effects, though more specific molecules have recently been developed.[117,179] Taking these into consideration, along with the advent of modern single-cell and live-cell time-lapse microscopy methodologies, increased kinetic resolution of mitochondrial network shape changes and contributions in RCD phenotypes may now be possible and are certainly worth re-evaluating, yet the technologies do require additional development (Box 1).

Finally, RCD signaling has evolved in a clever manner to ensure removal of targeted cells. Like we mentioned at the beginning of our discussion, a single stress often activates numerous RCD mechanisms; the same is becoming clear for apoptosis and ferroptosis, with mitochondrial dynamics potentially at the center. There may also be collateral regulation of the apoptosis and ferroptosis pathways. For example, in a situation when MOMP is incomplete, the partial release of cytochrome *c* causes activation of the ATF4-dependent ISR, leading to heightened sensitivity to RSL3-induced ferroptosis.[199] Similarly, there are descriptions of ferroptosis leading to activation of BH3-only proteins.[200] Additionally, it is likely that stresses may directly alter mitochondrial dynamics to unwittingly favor one RCD pathway over another. What dictates the "winning" cell death program likely depends on: (1) what signaling pathway is responding to the stress (e.g., the p53 pathway), (2) what RCD pathways are expressed in the cell/tissue under investigation, (3) the historical stress of the cell (e.g., how close to the death threshold does the cell already exist),

Box 1. Studying cell death and mitochondrial dynamics with live-cell time-lapse microscopy—Balancing throughput, sampling, signal, and resolution

How do we measure cell death? The cell death field has a long history of using microscopy to identify and measure cell death, going back to the original observations and coining of "apoptosis" in the late 1960s and early 1970s.[180] The cell death field embraced flow cytometry as a preferred method to detect cell death, which was relatively high-throughput, quantitative, and compatible with live or fixed cells, but was limited to endpoint observations due to sample collection. More recently, the advent of climate controlled, multi-well live-cell imagers (e.g., Cytation, Lionheart [BioTek/Agilent]; xCELLigence [Agilent]; IncuCyte [Sartorious]) has enabled researchers to conduct real-time kinetic cell death studies in high-throughput microplate formats, which are conducive to drug and genetic screens.[181,182] Could these imaging-based techniques permit simultaneous investigations into mitochondrial biology?

How do we detect cell death using live-cell time-lapse microscopy? Typically, traditional microscopy and cytometry approaches can utilize the same labeling reagents because both techniques apply reagents post-collection, immediately prior to measurement, and are commonly washed off or not detected outside of cells. In contrast, real-time live-cell approaches require cells to be cultured with labeling reagents—ideally in a "label-and-go" format—which necessitates that labels be non-perturbing to the cell and exhibit minimal signal prior to binding the intended substrate. An ideal cell death marker should be specific, easily detectable, relevant to the underlying cell death biology, and targetable without inducing cell stress or damage. Currently, only apoptosis, ferroptosis, and necroptosis have reporters that meet these requirements (see Table 1), though reagents can overlap between pathways, albeit with differing kinetics.[183–188] Other forms of RCD currently lack established biochemical hallmarks conducive to long-duration kinetic labeling and, therefore, cell-impermeant "viability" dyes remain the most common and widely applicable reagent for high-throughput real-time cell death studies.

How do we currently study mitochondrial biology? Several fluorescent reagents are commercially available to study many facets of mitochondrial biology (see Table 1). Experiments with these reagents are commonly conducted by flow cytometry, which provides reasonable throughput and is sensitive enough to detect changes in fluorescence intensity at the population and single-cell level.[189] Fluorescence spectroscopy microplate readers can also be used for these investigations, thereby providing increased throughput and accessibility but reducing resolution to whole-well populations and bulk data collection. The study of mitochondrial dynamics, however, requires imaging to visualize and characterize the mitochondrial network. The capabilities of automated multi-well live-cell imagers suggest that they could be used for high-throughput time-course studies of mitochondrial biology, and there is some evidence to support this.[190–192] However, more work is needed to fully assess the durability, longevity, photostability, and toxicity of these reagents in culture for experiments lasting several days with frequent imaging. Indeed, most dye-based labeling approaches are not compatible with long-duration live-cell assays and may perturb the organelles they target. In the meantime, imaging flow cytometers (e.g., FlowSight, ImageStream [Cytek]) combine throughput, sensitivity, and spatial insights to integrate investigations into cell death and mitochondrial biology within the same cell.

Can cell death and mitochondrial biology studies be multiplexed in live-cell time-lapse microscopy? The technology and software of these microscopy platforms are certainly capable of detecting and measuring mitochondria, and so it seems logical that both investigations could be combined in a single assay. One consideration is the difference in the timescale: changes to mitochondrial biology happen within minutes to hours, while cell death occurs in hours to days, and so the desired imaging interval is likely to be different. Another factor is the balance of throughput, sampling, and resolution. Cell death studies only require cellular resolution, and the lower magnification affords larger sampling of the population, which is rapidly assessed for binary labeling events (i.e., positivity). In contrast, mitochondrial studies require subcellular resolution, which reduces field of view, requires additional sampling for statistical power, and impacts throughput. Balancing the different needs of these investigations may require some compromises, but the rise of real-time live-cell imaging technology promises to provide new opportunities to study the intersection of mitochondrial biology and cell death with a throughput that was previously unattainable. Furthermore, as label-free, deep learning, and post hoc analytical workflows continue to be developed and adapted for more tailored investigations, researchers may be able to multiplex their live-cell time-lapse studies while minimizing compromises to throughput, sampling, and spatiotemporal resolution.[193–198]

and (4) what supportive cell biology could promote or inhibit those RCD pathways? Focusing on the latter question throughout our discussion, we presented how the mitochondrial dynamics machinery regulates the apoptosis and ferroptosis pathways to control the decision to die. As we continue to define the intersections between mitochondrial proteins, lipids, and metabolites in RCD pathways, along with advancing biological and pharmacological tools to explore mitochondrial dynamics, we predict that the roles of the mitochondrial dynamics machinery within the RCD pathways will provide deeper mechanistic insights into the fundamentals of biology, while also shaping the future of human health.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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