Circulation Research

REVIEW

Mitochondrial Structure and Function in Human Heart Failure

Antentor Hinton, Jr,* Steven M. Claypool,* Kit Neikirk, Nanami Senoo, Celestine N. Wanjalla, Annet Kirabo, Clintoria R. Williams

ABSTRACT: Despite clinical and scientific advancements, heart failure is the major cause of morbidity and mortality worldwide. Both mitochondrial dysfunction and inflammation contribute to the development and progression of heart failure. Although inflammation is crucial to reparative healing following acute cardiomyocyte injury, chronic inflammation damages the heart, impairs function, and decreases cardiac output. Mitochondria, which comprise one third of cardiomyocyte volume, may prove a potential therapeutic target for heart failure. Known primarily for energy production, mitochondria are also involved in other processes including calcium homeostasis and the regulation of cellular apoptosis. Mitochondrial function is closely related to morphology, which alters through mitochondrial dynamics, thus ensuring that the energy needs of the cell are met. However, in heart failure, changes in substrate use lead to mitochondrial dysfunction and impaired myocyte function. This review discusses mitochondrial and cristae dynamics, including the role of the mitochondria contact site and cristae organizing system complex in mitochondrial ultrastructure changes. Additionally, this review covers the role of mitochondria-endoplasmic reticulum contact sites, mitochondrial communication via nanotunnels, and altered metabolite production during heart failure. We highlight these often-neglected factors and promising clinical mitochondrial targets for heart failure.

Key Words: cardiovascular diseases ■ heart failure ■ hypertension ■ mitochondria ■ myocardium

Cardiovascular diseases (CVDs), which affect blood vessels or the heart, are the leading cause of death globally,[1] accounting for 25% of US deaths.[2] Although CVD generally affects the left ventricle, the right ventricle plays a crucial role in cardiovascular diseases, including pulmonary hypertension.[3] Other forms of heart disease include valvular heart disease, cardiomyopathy, and arrhythmias. The most common form of heart disease is coronary artery disease, which results from the buildup of plaque in the arteries that supply blood to the heart. Coronary artery disease leads to chest pain, heart attacks, and heart failure (HF),[4–6] which is defined by the American Heart Association as a condition in which the heart pumps insufficient blood to meet the body's needs.[7] HF, which presents clinically with or without preserved ejection fraction[8] and is the result of cardiomyocyte injury, is characterized by the inability of the heart to fill and expel blood from the left ventricle effectively.[7,9] Injury to the myocardium can result from hypertension, diabetes, and coronary artery disease.[9] Various types of HF present with different symptoms that can include shortness of breath, fatigue, congestion, and peripheral edema (Figure 1).[9,10]

It is important to understand the molecular bases of the various types of HF. CVD increases with age,[11] as does mitochondrial dysfunction.[12] Many lines of evidence from animal models and human studies implicate mitochondrial DNA (mtDNA) heteroplasmy[13] and mitochondrial biogenesis impairment[14] occurring before and during early HF,[15] suggesting mitochondrial dysfunction is an early hallmark of HF and a potential therapeutic target in CVD.[16,17] Reviews that consider mitochondria for the treatment of HF[16–22] have focused on mitochondrial dynamics or function. The multifaceted regulation of

Correspondence to: Antentor Hinton, Jr, PhD, Department of Molecular Physiology and Biophysics, Vanderbilt University, 750 Robinson Research Bldg, 2200 Pierce Ave., Nashville, TN 37232. Email antentor.o.hinton.jr@vanderbilt.edu
*A. Hinton and S.M. Claypool contributed equally as co-first and co-senior authors.
For Sources of Funding and Disclosures, see page 389.

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Nonstandard Abbreviations and Acronyms

3D	3 dimensional
AMPK	AMP-activated protein kinase
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
Cdk1	cyclin-dependent kinase 1
CVD	cardiovascular disease
CypD	cyclophilin D
DRP1	dynamin-related protein 1
ER	endoplasmic reticulum
FIS1	fission 1 protein
GRP75	glucose-regulated protein 75
GSK-3β	glycogen synthase kinase 3β
HF	heart failure
HFpEF	heart failure with preserved ejection fraction
IMM	inner mitochondrial membrane
IP3R	inositol trisphosphate receptor
IPCR2	type 2 inositol 1,4,5-triphosphate receptor
MCU	Mitochondrial mCa^{2+} uniporter
MERC	mitochondria-endoplasmic reticulum contact
MFF	mitochondria fission factor
MFN1	mitofusin 1
MFN2	mitofusin 2
MICOS	mitochondria contact site and cristae organizing system
mtDNA	mitochondrial DNA
NF-κB	nuclear factor-κB
OMM	outer mitochondrial membrane
OPA1	optic atrophy 1
OxPhos	oxidative phosphorylation
PGAM5	phosphoglycerate mutase family member 5
ROS	reactive oxygen species
RyR2	type 2 ryanodine receptor/Ca^{2+} release channel
SEM	scanning electron microscopy
SERCA2a	sarco(endo)plasmic reticulum Ca^{2+} ATPase
SLC25	soluble carrier family 25
SR	sarcoplasmic reticulum
VDAC	voltage-dependent anion channel

mitochondrial structure and function[23] is poorly understood, especially in the context of age-dependent HF. Tools that allow for this understanding have only been developed and become more widely available within the last 10 years. For example, focus ion-beam scanning electron microscopy (SEM),[24] serial block face SEM,[25] and correlative light electron microscopy,[26] have all undergone significant improvements, which have allowed for 3-dimensional (3D) structures of organelles including mitochondria to be better interrogated in heart development[27] and failure.[28] 3D tomography has also been used to perform morphological analysis of cardiac mitochondria to provide greater insights into ultrastructural changes.[29] Concurrent advances in cryo-electron microscopy have enabled the resolution of the mitochondrial oxidative phosphorylation machinery, such as the mitochondrial supercomplex $I_1III_2IV_1$.[30] Outside of microscopy, proteome, and acetylome analyses have accelerated, which have allowed researchers to glean the functional role of specific acetylation sites.[31] The study of in vivo freshly excised hearts has used high-resolution respirometry to study respiratory function,[32] while mechanistic studies have continued to be guided by broader advancements such as those in CRISPR/Cas9.[33] Together, these technologies have continued to bolster our understanding of mitochondria.

Mitochondria are central to many of the signaling cascades involved in HF (reviewed by He et al[34]). Here, we highlight recent developments and important future directions in the field, including the potential role of mitochondrial dynamics and interactions in HF. We discuss the dynamic nature of mitochondria and cristae and the role of the mitochondria contact site and cristae organizing system (MICOS) complex in these dynamics. We also discuss mitochondrial communication via both mitochondria-endoplasmic reticulum contacts (MERCs) and nanotunnels, as well as HF-associated changes in metabolite production. By taking an interconnected view of mitochondria, we consider areas of further study that may offer greater insights into mitochondrial-mediated roles in HF.

RISK FACTORS OF HF

It is pertinent to study mitochondria in the context of HF as the burden of HF continues to grow and new therapies are necessary. Within the United States, the burden of HF continues to grow, with roughly 6.2 million American adults (ie, 20+ years old) having HF in the span of time from 2013 to 2016, representing approximately a 10% increase over the preceding 3-year period.[35] In 2012, this cumulative prevalence represents an estimated cost of over $30 billion.[35] Current projections expect this prevalence to only continue growing, nearly by 50%, to reach over 8 million American adults with HF by 2030.[35] The probability of developing HF during one's lifetime varies significantly by race and gender, with estimates ranging from 20% to 42% in men and 24% to 46% in women, highlighting higher risks in Black women, but overall exuberated risk in men.[35]

HF disproportionately affects racial minorities within the United States: Black people, who are affected by socioeconomic and social factors, such as John Henryism,[36,37] have higher rates of hypertension compared

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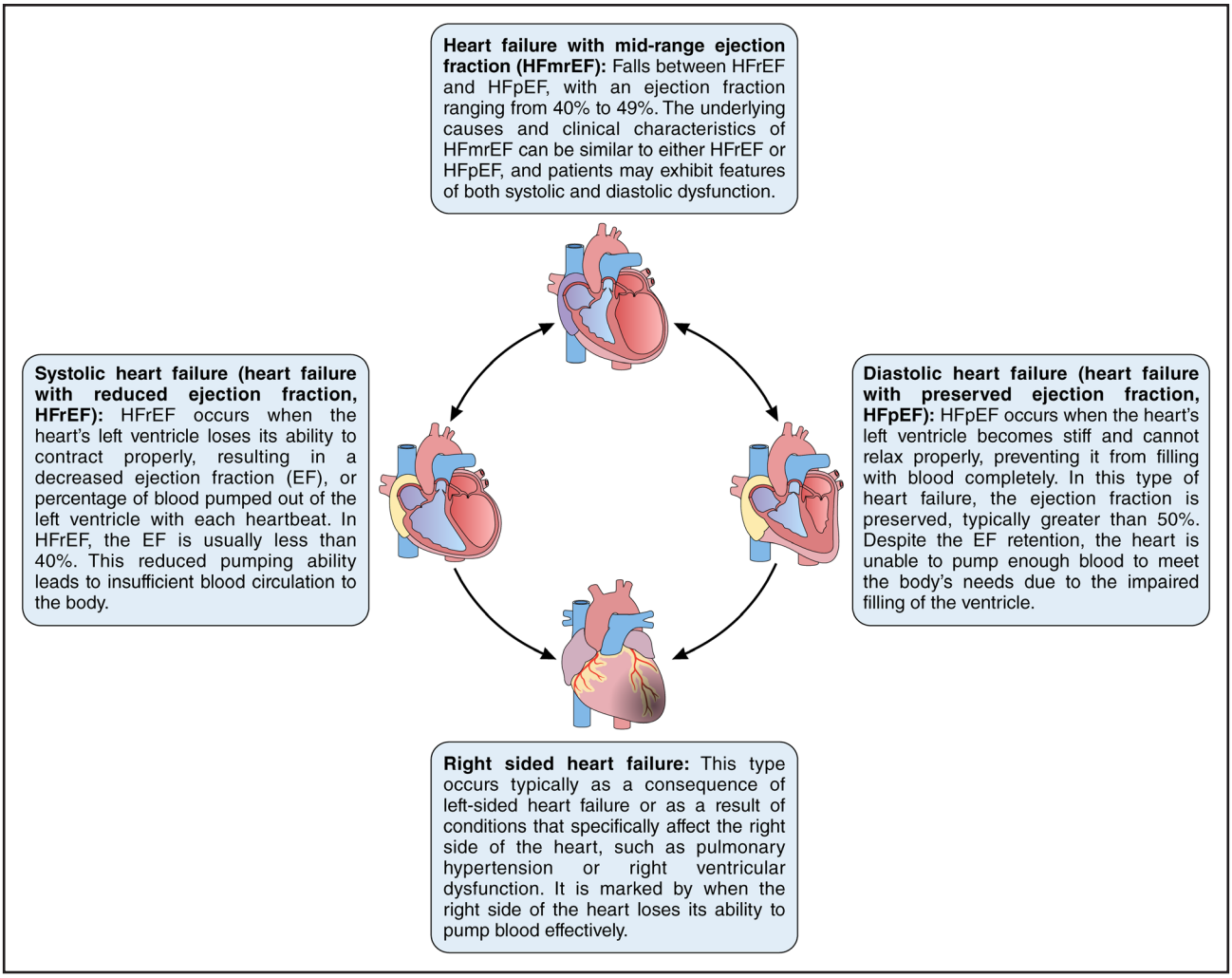

Figure 1. Overview of the main types of heart failure (HF).
This schematic illustration describes HF with reduced ejection fraction (HFrEF), HF with preserved ejection fraction (HFpEF), HF with mid-range ejection fraction (HFmrEF), right-sided HF, and congestive HF. It describes the affected side of the heart, the ejection fraction range, and key symptoms for each type. Understanding the various types of HF is critical for accurate diagnosis and treatment. Illustration credit: Sceyence Studios.

with other racial and ethnic groups. Moreover, as a result of disparities in health care access and socioeconomic factors, Black and Hispanic individuals are more likely to have both poor prognoses and outcomes in the context HF.[38] Race is a social construct; however, the cumulative effects of intergenerational stressors, hereditary factors, and exposome (ie, the totality of external environmental exposures one experiences throughout their life) factors may change mitochondrial function, leading to an increased risk of HF. Other risk factors for CVD include obesity, diabetes, smoking, a sedentary lifestyle, and poor diet, all of which can be correlated with lower socioeconomic status.[35] As a result, while non-Hispanic White women have an ≈1.9% prevalence of HF, this is more than double, at 3.9%, in Black women.[35] Although exposome factors have been implicated in affecting mitochondria, it remains unclear the full extent which these factors are involved in mitochondrial regulation.

Generally, men develop heart disease at younger ages than women.[11] Risk for the development of CVD in women increases significantly after menopause when they experience a decline in estrogen, a hormone with beneficial effects on blood vessels and lipid metabolism.[39–43] In addition to the protective effect of estrogens on blood pressure,[44] estrogens may also alter mitochondrial membrane permeability and microviscosity,[45,46] concurrently improving mitochondrial 3D shape.[47] In general, women with diabetes are at a higher risk for heart disease than men,[39–41] suggesting that the protective effects of estrogens premenopause are unable to overcome this risk factor. Yet, while the loss of estrogen in postmenopausal women blunts the sex-dependent difference, unlike many other CVDs, men typically are still at a higher risk of HF in old age.[35] This suggests that even in the absence of active estrogen, women have long-lasting cardioprotective effects against HF. However, other

factors could conceivably be at play beyond estrogen, and it is still too early to clearly explicate the impact of gender-affirming hormonal treatments on the risks associated with HF.

Age is the greatest risk factor for HF.[48] According to the 2013 to 2016 National Health and Nutrition Examination Survey, a small percent of the population from 20 to 30 years old have HF (ie, 0.3% for men and 0.2% for women).[35] However, this progressively increases with a more pronounced sex-dependent difference, especially at 60 to 79 years of age (ie, HF prevalence is 6.9% for men and 4.8% for women) and over 80 years old (ie, HF prevalence is 12.8% for men and 12.0% for women).[35] Aging may have a further confluence with race, as HF before 50 years of age is more common among Blacks, with Black men of 55 to 64 years old having an incidence of HF of 11.2 per 1000 person-years, which is much higher than White men of the same age (3.9 per 1000 person-years) and even among White men of 65 to 74 years old (11.0 per 1000 person-years).[35] Generally, however, with age these differences begin to narrow, while sex-dependent differences remain more pronounced. Hypertension, another risk factor for the development of HF, increases with age.[49] Thus, mechanisms associated with aging may provide a key to understanding and treating CVD, particularly the progressive loss of mitochondrial function.[50,51] Although there is some dispute concerning the mechanisms associated with aging and mitochondrial dysfunction,[52] the escalating decline of mitochondria with age and its potential role in HF has led to greater interest in mitochondria as a therapeutic target for HF.[52]

MITOCHONDRIAL FUNCTION IN THE HEART

In the 1960s, Mitchell introduced the chemiosmotic hypothesis, proposing a mechanism for ATP synthesis in biological systems, with energy transfer and ATP synthesis in mitochondria, coupled by a transmembrane electrochemical potential gradient.[53] We have now come to understand this mechanism as oxidative phosphorylation (OxPhos), the process through which mitochondria respond to the energetic needs of the body, with reactive oxygen species (ROS) as a deleterious byproduct. The heart needs large amounts of energy in the form of ATP to produce contractions strong enough to fulfill systemic demands,[54] but ATP storage is low. To overcome storage limitations, mitochondria, the primary producers of ATP,[54] comprise ≈30% to 40% of the cardiomyocyte volume. Every day, this large volume of mitochondria produces ≈90% of the ATP that the heart uses, generating 30 kg a day and nearly 880 000 kg across one's lifespan.[55] The other 60% volume of cardiomyocytes is typically occupied by myofibrils,[56] which can undergo changes in orientation and size in HF.[28]

ATP is generated by OxPhos machinery, which primarily consists of 5 complexes. Deficiency of Complex I—a 45 subunit complex that serves as an entry point for electrons through oxidization of NADH, —through inhibition of *Ndufs4* decreases respiration and accelerates HF.[57] Right-ventricular failure, conversely, is associated with increased levels of Complex II—a direct link between the TCA cycle and the electron transport chain that couples the oxidation of succinate to the reduction of coenzyme Q without pumping protons—which in turn increases oxidative stress, a byproduct of the electron transport chain.[58] In interfibrillar mitochondrial, aging decreases complex III activity—transfers electrons from reduced coenzyme Q to cytochrome *c* via a mechanism that is coupled to proton pumping—leading to ischemia-reperfusion injury due to tandem defects in electron flow and increased oxidant production.[59] In HF, unchanged cardiolipin levels in cardiac mitochondria concomitant with increased phosphorylation of complex IV—the terminal electron acceptor that moves electrons from cytochrome *c* to molecular oxygen while vectorially transporting protons into the intermembrane space—alters oxidative phosphorylation by either hindering the incorporation of complex IV into supercomplexes or reducing their stability.[60] Although the extent of OxPhos changes in HF goes beyond these specific examples, a key regulator of mitochondrial cardiomyopathies are mitochondrial mutations which can cause deficiencies through both mutations in the nuclear and mitochondrial genome (reviewed by El-Hattab and Scaglia[61]).

Mitochondrial DNA (mtDNA) plays a central role in the pathogenesis of human HF. A study from left ventricular tissue from end-stage HF has shown that in a failing heart, mtDNA content is decreased by >40%, with significantly reduced replication, which in turn impairs mitochondrial biogenesis.[15] Additionally, mitochondrial DNA depletion is an early sign in right-ventricular hypertrophy during the transition from hypertrophy to failure in patients with congenital heart disease.[14] The Atherosclerosis Risk in Communities, a broad study conducted over ≈30 years, showed an inverse relationship between the mtDNA copy number and the risk of incident HF.[62] This loss in mtDNA content is preceded by oxidative stress and results in decreased expression of mtDNA-encoded genes and respiratory chain complex enzymes.[63] Notably, this oxidative stress only affects mtDNA, not the nuclear genome,[63] potentially due to the lack of protective histones in mtDNA, which generally has less clearly defined epigenetic modification.[64] Still, this oxidative stress affecting mtDNA has been targeted through MitoQ antioxidants, which can in turn reduce oxidative stress.[65]

Mutations in mtDNA similarly may contribute to the pathogenesis of HF through the release of cytochrome *c*.[66] However, the cardiac involvement of mutations varies across cohorts and specific mutations (eg, mitochondrial myopathy, encephalopathy, lactic acidosis, and

stroke-like episodes, myoclonus epilepsy with ragged-red fibers, and Kearns-Sayre syndrome),[67] so it remains unclear if certain polymorphisms across certain genetic backgrounds may be responsible for the increased risk of HF in certain populations. Notably, Cao et al[68] showed that *Acsl6* is a genetic determinant of diastolic function, which may confer sex-dependent differences in HF. This involvement of mtDNA mutations in HF has raised mtDNA repair mechanisms as potential therapeutic targets (reviewed by Marín-García[69]). Nuclear genomics are equally important, as recent results have demonstrated that *IFIT3*, *XAF1*, *RSAD2*, and *MX1* are all mitochondrial-related genes that serve as biomarkers for HF.[70]

HETEROGENEITY IN HF

In addition to ATP production, mitochondria play a vital role in cellular metabolism, calcium (Ca^{2+}) homeostasis, lipid synthesis, and redox regulation in the myocardium.[54] Altered metabolism, a principal pathomechanism of human HF, and its relevance to the treatment of HF have been reviewed extensively.[22] This altered metabolism can confer heterogeneity in HF across regions and types of HF.

There are several types of HF (Figure 1), including HF with preserved ejection fraction (ie, diastolic HF) and HF with reduced ejection fraction (ie, systolic HF). Although over 50% of patients have diastolic HF, in both of these types of HF, mitochondrial function is central to the pathogenesis, with abnormal myocardial stiffness in diastolic HF and impaired energy production in systolic HF.[18,71] In HF with preserved ejection fraction, oxidative stress is a key regulator with increased proinflammatory and pro-fibrotic signaling, myocardial fibrosis, and altered Ca^{2+} handling.[72] Furthermore, in diastolic HF, impaired mitochondrial function and altered TCA cycle metabolites are significantly associated with a protein hyperacetylation pattern, which was effectively ameliorated by nicotinamide riboside supplementation.[73] In a mouse model of cardiac hypertrophy, reduced mitochondrial oxidative metabolism led to an energy deficit, potentially driving the progression from hypertrophy to systolic HF.[74] Yet, it remains unclear if mitochondrial function is directly correlated to ejection fraction. Knez et al[75] found a correlation between mtDNA content and ejection fraction in ion-ischemic HF. Another study found that cardiac phosphocreatine/β-ATP ratios, while inversely related to age, was not significantly linked to ejection fraction.[76] Thus, it remains an open area of research to understand whether distinct hallmarks of mitochondrial dysfunction are associated with specific types of HF and can serve as a biomarker of relative ejection fraction.

Cardiomyocytes have heterogeneous populations of mitochondria with discrete roles.[77,78] The organization of mitochondria within the heart include intermyofibrillar, subsarcolemmal, or perinuclear locations (Figure 2). Generally, HF may cause mitochondrial structure to change on the basis of these mitochondrial subpopulations.[79] Heterogeneity also arises on the basis of cardiac region. Interestingly, differences in the rates of aging of various tissues affect the function of the heart and other

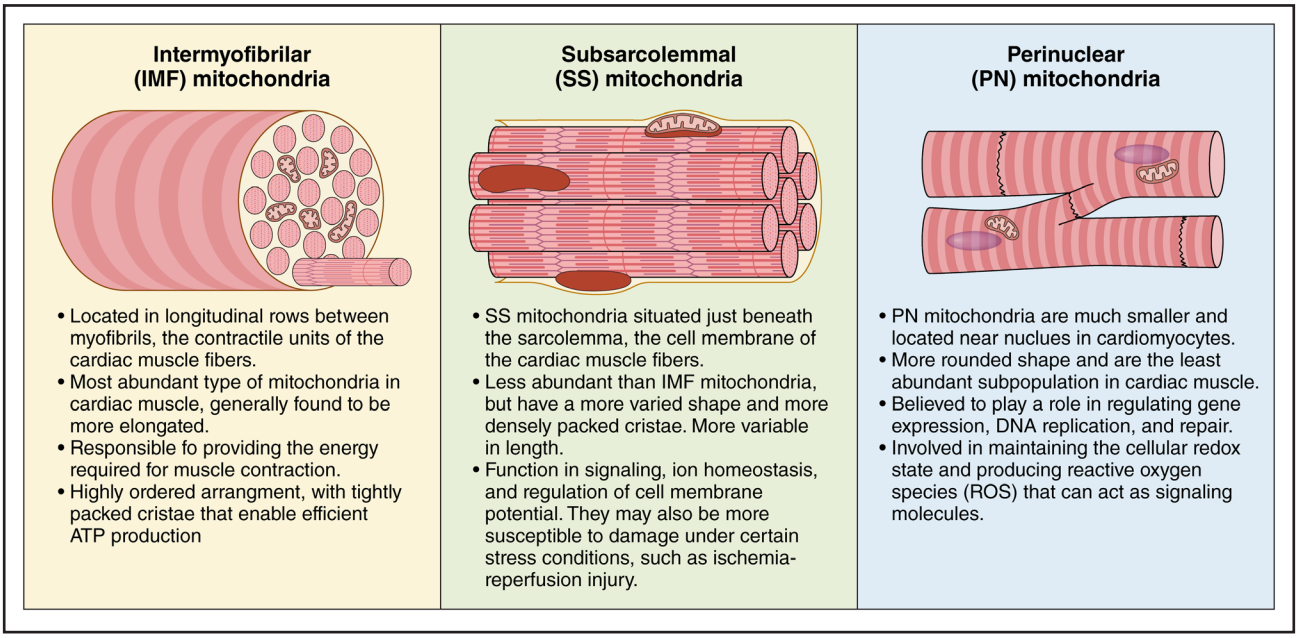

Figure 2. Distribution and characteristics of mitochondrial subpopulations in cardiac muscle fibers.
Mitochondria in cardiac muscle fibers include intermyofibrillar (IMF), subsarcolemmal (SSL), and perinuclear (PN) mitochondria. Each has a distinct relative abundance, location within the cardiac muscle cell, and structural features. Characterizing the differences among these mitochondrial subpopulations is crucial to understanding the impact of mitochondrial dysfunction in cardiac diseases and developing targeted therapies to improve cardiac function. Illustration credit: Sceyence Studios.

organs[79]; thus, mitochondria may exhibit different characteristics in HF tissues compared with other organs. Based on 3D analysis, murine cardiac tissue mitochondria display different phenotypes than their murine skeletal muscle counterparts during aging.[80] An earlier review focused on the role of mitochondria in regulating neonatal cardiomyocyte maturation, which may be due to the unique mitochondrial network that forms in cardiac tissue.[81] Because energy changes may differ by heart tissue subtype and by region-specific mitochondria, mitochondrial fusion, and fission proteins could be expressed differently in each region. For example, the endocardium (ie, the inner lining of the heart chambers), the myocardium (ie, the thick, muscular middle layer responsible for the heart's contraction), and the pericardium (ie, protective double-layered sac encasing the heart)[82] may functionally express different dynamics components that impact mitochondrial function and distribution. In particular, the elastic pericardium plays important roles in systolic function, hemodynamics, and ventricular function.[83] Conversely, endocardial smooth muscle cells may play a role in reducing left ventricular wall stress and systolic dysfunction.[84] Because we do not know mitochondrial structures across the entire geography of the heart and whether there exist correlations between a given structure and how that area pumps blood, we do not understand how the structural and functional relationships depend on unique signatures of aging in the various cardiac regions. Understanding these differences is crucial to developing targeted therapies for HF.

DYNAMIC NATURE OF MITOCHONDRIA

Mitochondrial dynamics refers to the coordinated, continuous cycles of fusion and fission[85–87] as mitochondria respond to the changing energy demands of the cell. Mitochondrial morphology is intricately linked to function[88]; thus, mitochondrial dynamics affect HF. Mitochondrial fusion pools mtDNA, preserving the mitochondrial genome, and contributes to the maintenance of mitochondrial OxPhos machinery.[88,89] Conversely, mitochondrial fission facilitates the elimination of damaged mitochondrial components through autophagy, also called mitophagy, regulates mitochondrial size and energy distribution, and modulates mitochondrial quality control.[85,90] A healthy mitochondrial network balances fusion and fission cycles,[88] and identifying the regulators of these cycles may provide therapeutic targets for HF.

A key hallmark of the failing heart is the dysregulation of mitochondrial dynamics. There is generally an increase in levels of fission-associated proteins (eg, DRP1 [dynamin-related protein 1]) and a decrease in levels of fusion-associated proteins (eg, MFN2 (mitofusin 2) and OPA1 [optic atrophy 1]).[91] The fusion of outer versus inner membranes of 2 mitochondria is mediated by different proteins. Fusion of the outer mitochondrial membrane (OMM) depends on conformational changes and GTP-dependent dimerization of 2 dynamin-like proteins, mitofusin 1 (MFN1) and MFN2, which are reduced across aging.[88,92,93] This is counterbalanced by fission, which is typically mediated by DRP1 . The absence of MFN1 or MFN2 leads to distinct types of fragmented mitochondria.[94] Cells lacking both MFN1 and MFN2 have severe cellular defects, including impaired growth, mitochondrial membrane potential heterogeneity, and reduced cellular respiration.[95] In HF, there is decreased expression of the mitochondrial dynamics regulator MFN2 (eg, in diabetic mice), leading to excessive mitochondrial fission that is linked to the development of HF in rats and humans with pulmonary arterial hypertension.[96] As previously reviewed, this results in a shift in the MFN2:DRP1 ratio toward pro-fission states in HF.[97] As discussed, beyond shaping mitochondria, these dynamics-related dysfunctions also alter the structure of cristae, in turn disrupting ATP production.[98] Although excessive fission drives decreased respiration, inhibition of this fission may in turn restore ATP production, improve cardiac fractional output, and reduce mitochondrial dysfunction, cumulatively protecting against HF.[99] Yet, the complete ablation of DRP1 causes dilated cardiomyopathy, since fission is necessary for the distribution of mtDNA nucleoids, protein-bound structures that contain active mtDNA.[100] Thus, there must be a careful balance of fusion and fission in the heart.

Fusion of the inner mitochondrial membrane (IMM) is facilitated by OPA1, a membrane-bound GTPase.[101] OPA1 is highly vulnerable to mutations, which can in turn increase ROS, reduce mtDNA content, and increase the risk of cardiomyopathy.[102] OPA1 is decreased in HF[102] likely by a posttranscriptional mechanism.[21] Fusion requires partial proteolytic cleavage of OPA1, yielding a soluble short form and a long transmembrane form.[103,104] Both forms of OPA1 also contribute to the formation of the MICOS complex that regulates the width of cristae.[105] An imbalance of mitochondrial fusion versus fission can lead to devastating alterations in mtDNA and trigger cellular apoptosis.[102] Proteolytic processing of OPA1 by 2 ATP-dependent metalloproteases, YME1L (YME1 Like 1 ATPase) and OMA1 (OMA1 zinc metallopeptidase), results in the inhibition of IMM fusion.[103,106,107] OPA1 also regulates cristae morphology and mitochondrial fragmentation.[80,102,108–111] Overexpression of OPA1 decreases mitochondrial fission, inhibiting autophagy.[108] Conversely, low OPA1 caused by its excessive processing by the overlapping stress-activated proteolytic activity of OMA1,[110] inhibits fusion, resulting in mitochondrial fragmentation[102,109] that leads to cell death and eventually heart disease.[110] Similarly, samples from human patients with HF show reduced *OPA1* expression, which correlates with mitochondrial fragmentation,[21,88] and a reduction of OPA1 increases the risk for cardiomyopathy due to decreased ATP production.[102]

Importantly, imbalanced OPA1 processing, leading to mitochondrial fragmentation, contributes to dilated cardiomyopathy and HF, while preventing mitochondrial fragmentation by deleting OMA1 protects against these cardiac issues.[112] OMA1 also protects against cardiomyopathy through the mitochondrial stress response, which protects against ferroptosis and associated lipid peroxidation–dependent impairments of OxPhos.[113] When activated by mitochondrial stress, OMA1 also cleaves DELE1 (DAP3 binding cell death enhancer 1), leading to heme-regulated inhibitor (HRI)-mediated eIF2α (eukaryotic initiation factor 2 alpha) phosphorylation and ATF4 (activating transcription factor 4) translation.[114] We have recently found that OPA1 action in forming contact sites occurs in an ATF4-dependent manner,[115] showing that OMA1 may further affect OPA1 downstream of DELE1 cleavage. Similarly, this also involves OMA1 as a novel regulator of the integrated stress response,[116] which may in turn effect dynamics through mitochondrial contact site formation with endoplasmic reticulum. Finally, OMA1 may also provide an alternative mode of fragmentation: in the context of a stress-inducing CHCHD10 mutation, OMA1 facilitates mitochondrial fragmentation alongside alterations in the electron transport chain (ETC).[117] However, there is little information on the differential expression of the isoforms of OPA1 in HF or whether changes in YME1L and OMA1 proteases contribute to alterations in mitochondrial structure and function in HF. The development of an immunoblotting technique that differentiates OPA1 isoforms[118] may help to determine the role of isoforms in heart disease, as certain isoforms, such as 1 and 7, may confer a protective phenotype.[107]

Mitochondrial fission occurs via 2 mechanisms. The first mechanism involves the recruitment of DRP1 to the OMM, where it binds to adaptor proteins and oligomerizes around mitochondria, driving membrane constriction.[119,120] However, as the diameter of helical DRP1 is insufficient to drive the initial constriction,[121] actin filament nucleators at mitochondria-ER Contact sites (MERCs) facilitate preconstriction of the mitochondrial membrane, which is required for DRP1 localization to the site of scission.[121] The second pathway involves the phosphorylation of DRP1, which occurs at serine (S)40, S44, S579, S585, S616, S637, S656, and S693.[122–124] Phosphorylation at S616, mediated by mitogen-activated protein kinases, Cdk1 (cyclin-dependent kinase 1), and Ca^{2+}/CaMKII (calmodulin-dependent protein kinase II),[125,126] is associated with increased DRP1 activity and mitochondrial fission. Similarly, phosphorylation at S693 is mediated by GSK-3β (glycogen synthase kinase 3β) and promotes DRP1 activation and mitochondrial fission. Interestingly, β-amyloid plaques increase GSK-3β activation, thus, promoting mitochondrial fission, a hallmark of Alzheimer disease.[127] DRPI, in response to pressure overload-induced cardiac hypertrophy, is translocated to mitochondria which coincides with the activation of mitochondrial autophagy; DRP1 haploinsufficiency exacerbates mitochondrial dysfunction and HF, presumably due to impaired turnover of dysfunctional mitochondria.[128]

Beyond these proteolytic processing units, a multitude of other proteins are emerging as regulators of fusion and fission. Stress-induced mitochondrial hyperfusion, which represents a compensatory mechanism to increase ATP, requires concurrent expression of SLP-2, MFN1, and long-isoforms of OPA1.[129] Mitochondrial elongation factors 1 and 2 (also known as MiD51 [mitochondrial elongation factor 1] and MiD49 [mitochondrial elongation factor 2]) recruit DRP1, with slightly distinct effects due to Mid49 having a stronger affinity toward binding Drp1 (dynamin-related protein 1/dynamin-1-like protein).[130] How the recruitment of DRP1 by MiD51 or MiD49 impacts mitochondrial dynamics is controversial. It was reported that MiD49- and MiD51-mediated recruitment of DRP1 to the mitochondrial surface inhibited DRP1's ability to initiate fission and caused mitochondrial elongation.[130] Contrarily, MiD51 and MiD49 were shown to recruit DRP1 to initiate fission in the absence of other fission receptors.[124] Although not studied in HF, in pulmonary arterial hypertension, MiD49 and MiD51 are upregulated leading to increased fission.[131] Another promoter of DRP1-recruitment, mitochondrial FIS1 (fission 1 protein), has been shown to drive to septic cardiomyopathy upon engagement with DRP1.[132] Notably, FIS1 displays a different type of fission than MFF (mitochondria fission factor)-mediated fission, which govern only midzone fission.[133] Thus, the roles of MFF, FIS1, MiD49, and MiD51 in HF must be clarified. Additionally, posttranslational modifications are important. Phosphoglycerate mutase family member 5 (PGAM5) is an emerging posttranslational modifier that dephosphorylates MFN2 to drive mitochondrial network formation.[134] In the context of cardiac ischemia-reperfusion injury, MFN2 and OPA1 levels are increased in cardiac-specific PGAM5 knockout mice alongside the retained phosphorylation of DRP1 at Ser-637.[135]

Activation of DRP1 by phosphorylation and the accompanying fission-induced mitochondrial fragmentation are linked to cardiac dysfunction and HF.[136] DRP1 can be phosphorylated at sites that include Ser-40, Ser-44, Ser-579, Ser-585, Ser-592, Ser-616, Ser-637, Ser-656, Ser-693, with particular attention paid to S637 and S656.[122] Phosphorylation at S637, mediated by a cAMP-dependent protein kinase, inhibits DRP1 activity and promotes mitochondrial fusion.[136] Dephosphorylation of S637 by calcineurin, a Ca^{2+}-dependent phosphatase, enhances DRP1 activity and mitochondrial fission.[136] In ischemia-reperfusion, dephosphorylation at S637 contributes to left ventricular dysfunction[137]; thus, calcineurin may play a role in the pathophysiology of HF. PGAM5 deletion can inhibit dephosphorylation at S637 additionally, despite inverse increases in fusion proteins, showing a slightly paradoxical role.[135] Similarly, phosphorylation at S656 by AMPK (AMP-activated protein kinase) activation inhibits

DRP1 activity and mitochondrial fission,[138] which serves as a potential mediator for endoplasmic reticulum (ER) stress. Interestingly, during HF, a shift from AMPKα2 to AMPKα1 expression correlates with the prevention of mitochondrial fragmentation.[139] Although different functions are associated with the various phosphorylation sites, HF-associated differences in phosphorylation sites are not well described. Additionally, as previously reviewed,[140] DRP1 can be SUMOylated, which provides a protective role in ischemia-reperfusion injury,[141] and S-palmitoylated, which is necessary for DRP1 mitochondrial translocation.[142] Thus, diverse DRP1 posttranslational modifications regulate mitochondrial dynamics in myriad ways. Further research is needed to understand the roles of site-specific DRP1 phosphorylation and dephosphorylation in the heart and whether pathways that promote or inhibit fission run in tandem during HF, modulating the fission responses in cardioprotective environments.

Although OPA1 and DRP1 are the main focus of studies on mitochondrial dynamics, modulating factors may be equally important. For example, it is not known whether the rates of FIS1 and MFF-mediated fission change in HF, which could explain how disruptions in the fusion-fission balance in HF[143] lead to bioenergetic dysfunctions (Figure 3). Equally, there are other, often-neglected regulators in mitochondrial dynamics which have important roles in HF pathology. Although not required for fission, deletion of Mitochondrial Fission Process 1 in the heart leads to dilated cardiomyopathy, mitochondrial defects, and increased sensitivity to cell death.[144] Additionally BNIP3, through its multifaceted molecular interactions including MICOS, alters metabolic pathways in HF with reduced ejection fraction.[145] In brain aging, BNIP3 has unresolved roles in healthy aging by modulating mitochondrial dynamics.[146] Additionally deficiency in Prohibitin 2, an important modulator of mitochondrial dynamics, causes impairment of fatty acid oxidation concomitant with HF progression.[147] Finally, overexpressing transmembrane protein 135 causes increased mitochondrial fragmentation and ER stress antecedent to cardiac abnormalities,[148] suggesting an alternative mechanism through which fission may be modulated in the absence of DRP1 (reviewed

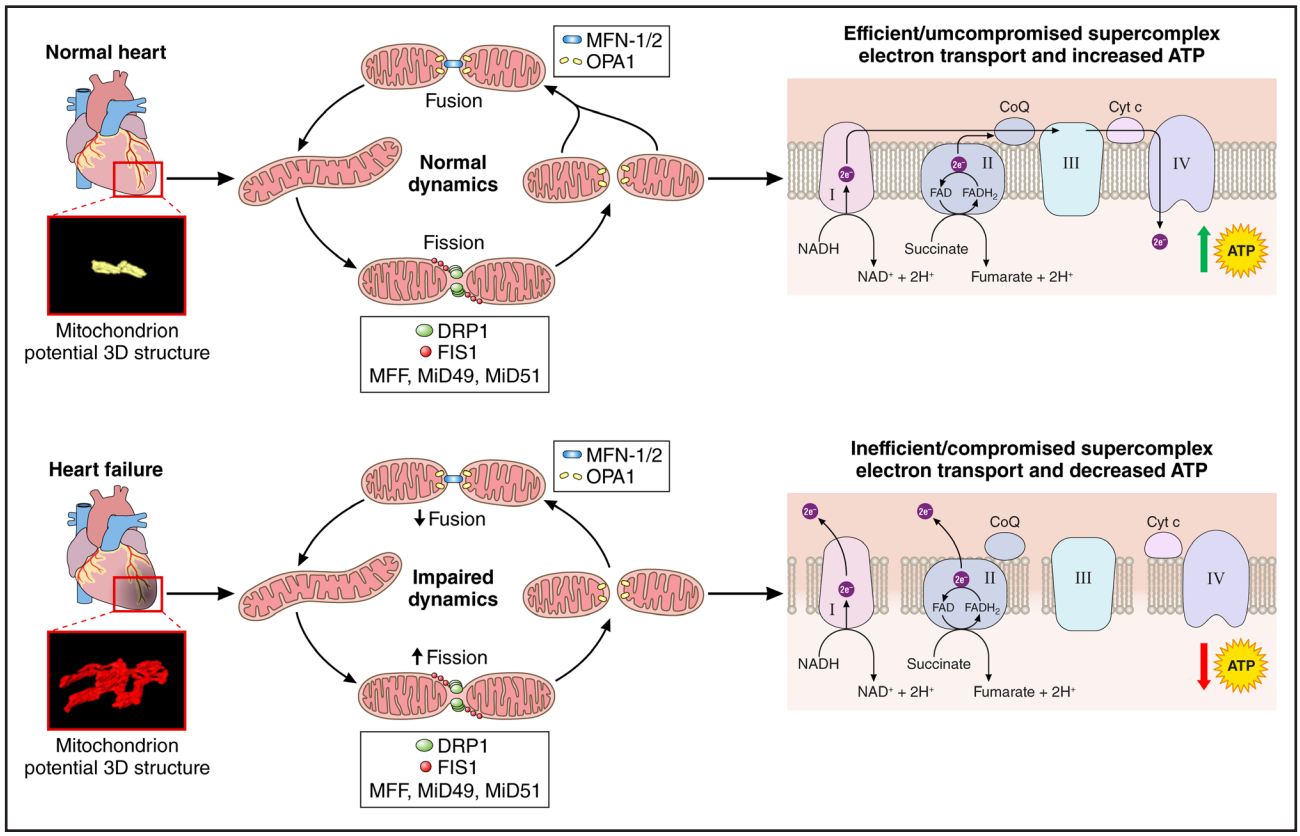

Figure 3. Biopsies from normal vs failing heart tissue.
The schematic illustrates the dysfunction in mitochondrial dynamics that disrupts ATP production. Although results have shown varied changes in structure and dynamics in heart failure (HF), the schema demonstrates commonly reported changes. From our previous 3-dimensional (3D) reconstruction, we found normal mitochondrial structure in healthy cardiac tissue, indicating proper fusion and fission dynamics; however, in HF, there are complex, altered mitochondrial structures, while other studies have also reported fragmentation. The dysregulation of key fusion and fission mitochondrial proteins, such as OPA1 (optic atrophy 1) and DRP1 (dynamin-related protein 1), in failing cardiac tissue results in disruption of the electron transport chain. Commonly this is an uptick in fission and decrease in fusion, but previous results show varied dynamic changes. Restoring phenotypically normal mitochondria may also restore ATP production and reduce cardiomyopathy. MiD4 indicates mitochondrial elongation factor 2; and MiD51, mitochondrial elongation factor 1. Illustration credit: Sceyence Studios.

by Beasley et al[149]). Many dynamics proteins are thus involved in the pathogenesis of HF, potentially modulating the relationship between HF progression, mitochondrial dynamics, and altered HF metabolism.

TARGETING MITOCHONDRIAL DYNAMICS

As extensively reviewed, targeting mitochondrial dynamics is highly promising across numerous pathologies (reviewed in Refs.18,150–152). To briefly summarize some therapies that modulate dynamics in the context of HF, long-term therapy with elamipretide (ie, Szeto-Schiller peptide SS-31 or Bendavia) has been shown to reverse HF-dependent changes in dynamics and biogenesis.[91] Elamipretide treatment significantly increases the long Opa1:short Opa1 isoform ratio compared with controls, resulting in a reduction of mitochondrial fragmentation in fibroblasts from patients with dilated cardiomyopathy with ataxia syndrome.[153] Although not directly modifying mitochondrial structure, pyrroloquinoline quinone can protect against HF by altering Ca^{2+} overload (see Disrupted Calcium Homeostasis and ROS Production in HF section) through increased biogenesis.[154] Mitochondrial-mimetic therapy, represented by the bioactive conjugate TPT encapsulated in nanomicelles, plays roles in effectively regulating mitochondrial dynamics.[155] In a rat model of HF, Neuregulin-1 was suggested to protect against mitochondrial dysfunction by maintaining its structural integrity.[156] Finally, while mdivi-1 is a standard DRP1 inhibitor, Drpitor1, and Drpitor1a inhibit fission with higher potency and exert cardioprotective properties in the context of cardiac ischemia-reperfusion injury.[87] As treatments continue to develop, there are several additional promising avenues to pursue.

As ROS accumulate during aging,[157] antioxidants may protect against HF. Antioxidants such as MitoQ[158] mitigate murine age-related aortic stiffness, a predictor of CVD. ROS pathways may be intrinsically linked to mitochondrial dynamics, as mutations in *Opa1* can lead to an increase in ROS.[159,160] HF is associated with increases in the cardiomyocyte oxidative stress state,[161] and an increase in ROS is accompanied by decreased antioxidant capacity in HF mitochondria.[162,163] Loss of DRP1 or downregulation of *Fis1* results in abnormally elongated mitochondria as fission is blunted,[124] but overexpression of *Fis1* induces cellular apoptosis, demonstrating the importance of mitochondrial dynamic homeostasis.[20] Maintenance of normal amounts of mitochondrial dynamic proteins and their associated machinery may inhibit ROS,[159,160] but FIS1 can also be modulated by ROS,[160] indicating their complex but poorly understood interdependence. Although FIS1 and MFF are independently predictive of fission pathways,[133] it is not known whether these pathways are involved in alternative ROS generation. Thus, it will be important to determine whether an imbalance in mitochondrial dynamics is a cause or effect of the damaged myocardium.[90] This may allow us to target ROS to mitigate HF through modulation of mitochondrial dynamics.

Potential therapeutic candidates that directly target mitochondrial dynamics include LARP7 (La ribonucleoprotein domain family member 7), a master regulator of the RNA polymerase II pausing pathway and the DNA damage response.[164] LARP7 is decreased in patients with HF and mitochondrial dynamics are impaired in LARP7 deficient cardiomyocytes.[164] Notably, the reintroduction of LARP7 may restore bioenergetics and associated dynamics. Other possible targets to improve mitochondrial dynamics include linoleic acid,[165] voltage-dependent anion channels,[166] and the transcription factor PRDM16 (PR-domain containing 16).[167] However, many pathways can alter mitochondrial dynamics. In noncardiac tissue, estrogen receptor-α has been linked to increases in blood pressure,[43] and treatment with 17β-estradiol restores mitochondrial dynamics.[46] To reveal the full complement of possible therapeutic targets to mitigate HF damage, it is essential to explicate mitochondrial dynamics changes in gene knockouts across a variety of model organisms.[168]

CRISTAE DYNAMICS AND THE MICOS COMPLEX IN HF

Mitochondria have 4 subcompartments separated into the OMM, the intermembrane space, the IMM, and the matrix.[121] The IMM is further separated into the inner boundary membrane that runs parallel to the OMM, and the IMM invaginations, called cristae,[121] that house the oxidative phosphorylation machinery.[86] High-resolution electron microscopy has shown that cristae, like mitochondria, undergo dynamic morphological cycles to accommodate the changing needs of the cell.[169,170] Cristae dynamics refers to the morphological changes of cristae in response to energetic, developmental, or physiological cues.[171] Altered cristae morphology can modulate oxidative phosphorylation efficiency by changing the spatial arrangement of respiratory chain components. The sites where the OMM, IMM, and cristae coalesce are cristae junctions, which are formed and maintained by the components of the MICOS complex.[121] The mammalian MICOS complex comprises 7 subunits,MIC60, MIC27, MIC26, MIC25, MIC19, MIC13, and MIC10 (reviewed by Kozjak-Pavlovic[172])—distributed in the MIC10 and MIC60 subcomplexes, named for their core components.[173,174] In addition to maintaining cristae morphology, cristae junctions serve as diffusion barriers for metabolites and proteins,[171] which suggests that they might also maintain the IMM pH gradient and limit the diffusion of ADP/ATP.[171]

The MIC10 and MIC60 subunits, which mediate the membrane bending that is needed to form cristae junctions, are the best-characterized MICOS complex components (reviewed by Anand et al[175]). The loss of MIC60 results in a loss of cristae architecture, resulting in the

formation of membrane stacks.[176] This is accompanied by a decrease in the expression and destabilization of all components of the MICOS complex. The loss of MIC10 results in aberrant cristae morphology and the components of the MIC10 subcomplex are reduced, while the MIC60 subcomplex remains largely unaffected.[171–174,177] MIC13, which bridges the MIC10 and MIC60 subcomplexes, is also a key MICOS subunit. Although MIC13 knockdown results in destabilization of the MIC10 subcomplex,[175,178] MIC60 subcomplex is largely stable in the absence of MIC13.[173,179,180] In addition, the MICOS complex interplays with cardiolipin, a signature phospholipid residing in the IMM (reviewed in Refs. 181, 182). Specifically, MIC27 binds to cardiolipin.[183] Double knockout of MIC26 and MIC27 reduces cardiolipin levels and destabilizes respiratory complexes.[180] These studies further highlight the crucial role of the MICOS complex in maintaining optimal mitochondrial respiratory function. The other MICOS subunits may also have roles unrelated to the cristae organization. For example, in addition to maintaining cristae morphology, MIC19 and MIC25 regulate key mitochondrial dynamic proteins, such as DRP1 and OPA1.[172,184,185] However, OPA1 also has nonfusion mitochondrial roles; not only do endogenous OPA1 and MIC60 interact, OPA1 deletion results in increased amounts of MIC60 in mouse embryonic fibroblasts.[186] OPA1 is epistatic to MIC60;[187] however, MIC60 also has a unique role in the nucleoid (ie, active mtDNA) distribution.[173,188] Future studies should determine the functional importance of the OPA1-MIC60 association and whether this interaction is affected by HF.

Because the MICOS complex is vital to the maintenance and integrity of cristae, it is important to understand how this complex changes in disease. The MICOS complex facilitates the folding of cristae, which increases their surface area to maximize cellular respiration.[185] The density and morphology of cristae depends on the energy demands of the cell.[189] Cardiomyocytes and skeletal muscle cells have more densely packed stacks of cristae than less energetically demanding tissues.[185] Mitochondrial and cristae degeneration correlate with impaired heart function in patients with mitochondrial cardiomyopathy arising due to an mtDNA mutation.[190] Furthermore, Hypertrophic Cardiomyopathy multi-omics profiling showed decompensation concomitantly with reduced cristae density.[191] Aberrant cristae morphology can affect the heart pluralistically in cardiomyocytes, dysregulated cristae led to declines in the mitochondrial network concomitant with the overproduction of mitochondrial ROS, resulting in increased myocardial size.[192] Thus, it is possible that cristae abnormalities in muscle cells, like in cardiomyocytes, increase disease severity and the risk of complications compared with similar abnormalities in less energetically demanding environments. However, it is unclear whether cristae density and morphology differ in different regions of cardiac tissue or whether cristae density can serve as a diagnostic predictor of cardiac dysfunction.

Cristae remodeling also controls other critical cellular processes; for example, during apoptosis, cristae junctions widen for the release of cytochrome *c* from the intercristal space.[171] In addition, human diseases, including diabetes and cardiomyopathy, are associated with altered expression of MICOS complex components.[171,185] Rare variants in *MIC19* and *MIC25* are associated with hypoplastic left heart syndrome.[193] Consistent with a causal role, depletion of Mic19 in the *Drosophila* heart compromises heart function, decreases ATP, and results in the accumulation of mitochondrial aggregates, suggestive of altered mitochondrial dynamics.[193] In mammals, knockout of either MIC10 or MIC60 results in a reduction in maximal mitochondrial respiration.[171,173,185] However, it is not known whether there are differences in MICOS complex expression across different types of HF (Figure 1) or whether only certain MICOS complex subunits are essential for stability in HF. However, mitochondrial dysfunction and decreases in ATP production are both hallmarks of the pathophysiology of HF[20]; thus, understanding the maintenance of cristae integrity will be vital in developing potential therapeutics for HF.[90]

A critical player in apoptosis is the Bcl-2 family member, Bid, which also regulates metabolism and DNA damage.[194] Following cleavage by caspase-8 during apoptosis, tBID facilitates the rearrangement of cristae and the permeability of the OMM.[194] The loss of BID leads to the loss of most cristae, with some aberrant cristae that remain. In *Bid*$^{-/-}$ mice, the induction of acute cardiac stress amplifies the disordered cristae phenotype and results in cardiac dysfunction.[194] Stressed *Bid*$^{-/-}$ mice have a lower threshold for developing HF from unmet energetic demands. Moreover, *Bid*$^{-/-}$ acute cardiac stress mice also have a reduced ejection fraction and an increased diameter of left ventricles.[194] Although tBID is not MICOS specific, there is a link between cristae and HF. Therefore, understanding the proteins involved in cristae dynamics may identify potential therapeutic targets for HF.

To better understand how HF affects bioenergetics, we need highly resolved cristae structures. There are 2-dimensional [195] and 3D reconstructions of cristae for quantitative evaluation, including volume. We have characterized cristae and mitochondrial 3D structural changes using a knockout of the MICOS complex subunits MIC19, MIC25, and MIC60 in fibroblasts,[80] which parallels age-related changes;[80,111,196] however, it is not clear whether these changes also exist in HF. We have also developed a method for quantifying and scoring 3D cristae structures.[196] This technique can provide measurements of cristae that can be correlated with biochemical and bioenergetic changes in the MICOS complex and mitochondria, respectively, that may occur in HF.[172]

Currently, volume microscopy techniques, including serial block-face SEM, focused ion-beam SEM, and automated tape-collecting ultramicrotome SEM are commonly used to measure 3D structures, as described and compared previously.[24,25,197–199] Emerging techniques such as correlative light electron microscopy[26] and cryo-soft x-ray tomography, which have been used for 3D analysis of cristae,[200] will continue to expand our understanding of cristae structure. However, there are few studies using these modern tools on HF samples.

UNDERSTANDING MITOCHONDRIAL SHAPES DURING HF

In HF, mitochondria become fragmented or swollen, are heterogenous, and lose cristae (reviewed by Daghistani et al[201]). We found that in aging mice, cardiac mitochondria adopt a more compact phenotype,[111] suggesting that mitochondrial changes in HF are independent of their age-related changes. Therefore, it is important to understand changes in mitochondrial structure and the functional implications of these changes in HF. In sheep HF, 3D rearrangement of the sarcoplasmic reticulum (SR) alters mitochondrial organization.[202] In our 3D structure studies of human HF mitochondria, we found that mitochondrial volume increases, as does mitochondrial complexity, with diverse phenotypes that include increased branching, mitochondrial donuts, and nanotunnels[28] with concomitant shifts in myofibril orientation and quantity.[28] Similarly, the 3D structure of intercalated discs, involved in communication across cardiac myocytes, is altered in HF.[203] However, the small sample size and the heterogeneity across and within patient samples in our study[28] underscores the need for further analysis of how HF across various models affects the frequency of mitochondrial phenotypes. Although this study analyzed intermyofibrillar mitochondria in scarred tissue, it is not clear whether HF affects mitochondrial structure equally across the heart and regions of cardiac tissue.

Mitochondria can adopt 8 distinct phenotypes that may be expressed concomitantly, including small or large, indicating relative size for ATP synthesis, and elongated or compact, indicating relative surface area for organelle interactions (reviewed Glancy et al[88]). Thus, it is important to view mitochondria holistically, considering the interplay between mtDNA mutations, structure, and bioenergetics that all play a role in HF.

While typically arising in liver, megamitochondria are adaptive responses to reduce intracellular ROS, yet if megamitochondria are overwhelmed by an excess of free radicals, an apoptotic signaling cascade occurs.[204] Unusually large megamitochondria may affect cellular energy metabolism by reducing ETC activity.[204] In a cardiomyopathy case study of a patient at risk for HF, SEM revealed abnormal mtDNA that resulted in pleomorphic megamitochondria in the myocardium;[205] however, this contrasts with several studies that found fragmented mitochondria in HF across several regions.[201] While some of this variation may be due to interregional variability, it also suggests that megamitochondria may represent a stress-adaptive mechanism in only certain circumstances. Ang II-induced cardiac damage has similarly been demonstrated to cause the formation of megamitochondria in vivo.[206] Similarly, knockout of Klf15, which plays an important cardioprotective role in myocardial growth and remodeling, causes an increase in megamitochondria formation in cardiomyocytes.[207] Cumulatively, while discussed more in depth in past reviews,[208] it is clear that the pluralistic formation mechanisms and roles of megamitochondria in HF remain poorly explicated.

Mitochondria undergo structural changes associated with aging,[88] such as donut-shaped mitochondria in monkey brain tissue, which are a hallmark of cognitive decline.[47] In our study of mitochondria in human HF, we observed donut-shaped mitochondria[28] but no megamitochondria. DRP1-associated fission factors, including MFF and FIS1, may be differentially expressed in various cellular conditions, such as in neurodegeneration where mitochondrial dynamics are dysregulated,[133,209] providing a possible path to selecting mitochondrial phenotypes. For example, it is possible that spatial distribution of these proteins is a determinant in phenotype formation. Similarly, alterations in fission associated with HF may alter mitochondrial phenotypes. HF modulation of brain function via changes in blood flow due to reduced cardiac efficiency has been studied,[210] but the brain-heart interface may also influence mitochondrial structure,[210] which remains understudied. The donut-shaped mitochondria that result from hypoxia-reoxygenation stress and related alterations in OPA1 levels[211] suggest at least 1 mechanism by which HF affects mitochondrial structure in distinct tissue regions.

Immobilized mitochondria develop nanotunnels that allow the transfer of materials between 2 mitochondria[212,213] and serve as a means of communication through thin double-membrane protrusions that involve both the IMM and OMM. Nanotunnels, which are associated with Ca^{2+} transfer, form in response to impaired homeostasis,[213–215] and nanotunnels increase in cells with genetic mitochondrial defects and perturbed Ca^{2+} signaling.[214] As nanotunnel formation depends on MICOS, OPA1, and F1/F0 ATP synthase,[212] and OPA1 prevents ROS formation, nanotunnels may help to maintain a balance between ROS production and elimination.[216] Nanotunnels may also be affected by fission dynamics, as it has previously been hypothesized that nanotunnels form as a result of incomplete fission.[213,217] For example, whereas both forms of fission depend on DRP1, peripheral fission and midzone fission depend on FIS1 and MFF, respectively.[133] However, it is unknown whether mitochondrial nanotunnels are a precursor or end result of either of these dual fates of mitochondrial fission. Finally,

the abundance of nanotunnels may increase with the mitochondrial stress that occurs in HF. However, neither nanotunnels nor other mitochondrial phenotypes have been rigorously characterized in HF.

FUTURE STUDY OF HF THROUGH IMAGING

Although biochemical and functional techniques have elucidated much of the pathophysiology of HF, imaging techniques such as transmission electron microscopy provide 2-dimensional micrographs of mitochondria[195,218] that reveal the structural causes or consequences of mitochondrial dysfunction. However, characterizing specialized mitochondrial structures, like MERCs and nanotunnels, requires newer techniques, such as 3D reconstruction, which provides quantitation of volume, branching of mitochondria, nanotunnels, and other features.[24,25,197,214] Confocal imaging can be used to assess the intracellular real-time movement of proteins that are associated with mitochondrial dynamics, such as estrogen.[219] Furthermore, single particle analysis and cryo-electron tomography can be performed together, providing a visualization of cristae alongside protein-protein binding sites.[201] These advanced imaging technologies can be used to characterize features of mitochondria that are not easily captured through traditional 2-dimensional

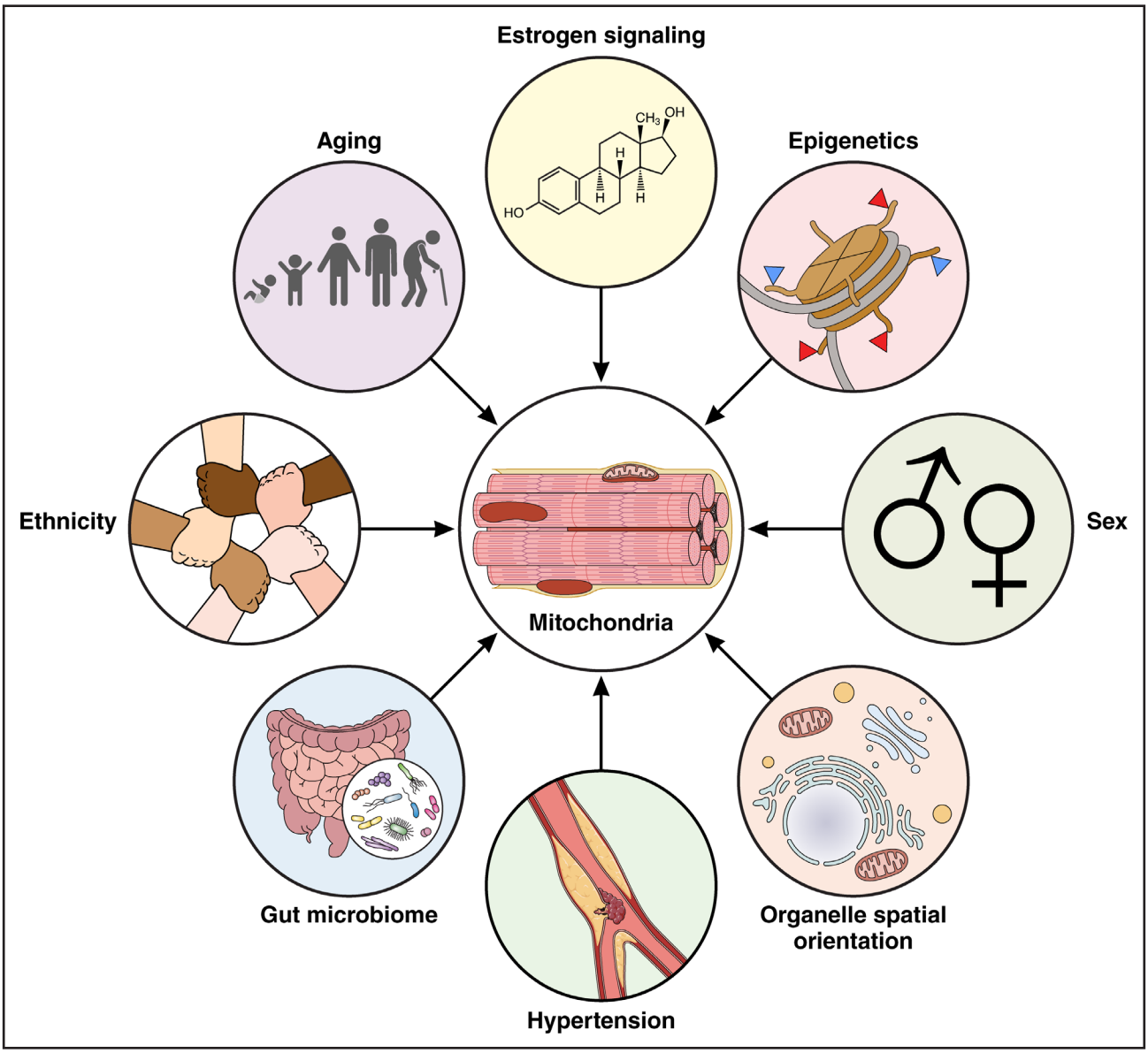

Figure 4. Diverse effectors of mitochondria in cardiac tissue.
Mitochondria in cardiac tissue sarcomeres are affected by aging, estrogen signaling, epigenetics, sex, relative organelle rearrangement (eg, mitochondria-endoplasmic reticulum contact sites), changes in pulse wave velocity and arterial stiffness, the gut microbiome, and potentially ethnicity. These are all avenues of research as they may confer risks for heart failure and should be researched for future studies. Illustration credit: Sceyence Studios.

histological analyses. Furthermore, these new methods use machine learning for faster analysis of various organelles in HF under many conditions. In particular, focused ion-beam SEM has high contrast, which makes it ideal for machine learning applications,[197] but new techniques must be developed for serial block-face SEM to expedite workflows. CDeep3M is a promising technology for isolating mitochondria from cardiac myocytes for serial block-face SEM.[220] Machine learning may be used in future studies to characterize other alterations in cardiac mitochondria.

Further characterization of mitochondrial 3D structure in human HF is needed to determine how aging and pathology affect mitochondrial morphology and function in a tissue-specific manner. Such studies would determine the frequency and distribution of various mitochondrial structures in different tissues, contributing to a mitochondrial connectome for human HF. For example, MitoCarta provides a systematic catalog of mitochondrial proteins and their functions.[221] However, it does not address the interplay between mitochondria and other cellular components, highlighting the need for a more integrated approach to studying mitochondria in HF. An effective connectome would consider not only mitochondria but also their relationships with other organelles, as well as the relative frequency of their morphology and spatial distribution, both across subregions (eg, intermyofibrillar and SS) as well as cardiac regions (eg, pericardium and myocardium). Additionally, such studies may further categorize other factors affecting mitochondria including sex, ethnicity, hormonal regulation, and aging (Figure 4), which may also contribute to tissue-dependent structure. For example, although recent studies have investigated the role of sex-based mitochondrial differences in sex-dependent HF development[68] and estrogen as a regulator of HF through mitochondria,[90] little is known of their effects on mitochondrial structure; however, past reviews have summarized pathologies related to mitochondria.[222,223] Despite earlier studies on the interplay between age-related pathologies, including HF, mitochondrial alterations, and epigenetic changes[64] or ethnicity,[224] more in-depth research is needed. Thus, to better understand the mechanisms that underlie the formation of these 3D structural phenotypes, we need better information on the localization of mitochondrial fusion and fission proteins by techniques such as mass spectroscopy imaging,[225] as well as changes in the distribution of active mtDNA (ie, nucleoids), which may be determined by ER contact sites,[226] across a diverse (both in sampling site and population) data set.

DISRUPTED CALCIUM HOMEOSTASIS AND ROS PRODUCTION IN HF

Abnormal Ca^{2+} signaling is a hallmark of HF initiation,[227] which is in turn linked to mitochondrial dysfunction.[228] In healthy hearts, Ca^{2+} enters cardiomyocytes primarily through L-type Ca^{2+} channels during each heartbeat, triggering a larger release of Ca^{2+} from the SR, yet, in HF this positive feedback loop is inhibited by a reduction in Ca^{2+} influx.[228] As reviewed by Gorski et al,[229] the cardiac sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA2a), which pumps Ca^{2+} back into the SR and is regulated by phospholamban, has reduced activity, resulting in a duality of insufficient systolic Ca^{2+} release and diastolic uptake.[229] Resultingly, SERCA2a and phospholamban have become key targets aimed at modulating HF Ca^{2+}, with gene-therapies increasing SERCA2a offering cardioprotective effects.[230] Impaired Ca^{2+} signaling may be further exacerbated by a dysregulated Ca^{2+}-calcineurin-Nuclear Factor of Activated T-cells (NFAT) pathway.[231] Notably, as previously reviewed, mitochondria, well-known to be intertwined with Ca^{2+} signaling, may contribute to the decreased Ca^{2+} availability for each contraction and impaired relaxation characteristic of HF, thus representing a valuable potential therapeutic target.[232]

Mitochondria form organelle-to-organelle contacts and engage in crosstalk with the cytoplasm for macromolecular synthesis, and dysregulation of this communication is linked to pathology.[233] Communication between the mitochondria and the ER depends on MERCs,[234] which facilitate the transfer of lipids, Ca^{2+}, and ROS between the ER and mitochondria.[234] MERCs are formed by interconnecting membrane protein complexes that mediate interactions between the mitochondria and ER without fusion.[92] The isolation of MERCs via biophysical protocols yields mitochondrial-associated membranes (reviewed by Giacomello and Pellegrini[235]). Although MERCs can vary in structure and composition, we are using the term MERCs, analogous to their biochemical fractional counterpart mitochondrial-associated membranes, as the most common term to refer to these exchange sites. MERCs are essential for transferring lipids and maintaining Ca^{2+} homeostasis,[236] and disturbances to MERCs result in perturbed lipid transport, the loss of Ca^{2+} homeostasis, and mitochondrial destruction.[237] MERCs regulate Ca^{2+} flow from the ER to the mitochondrion; when normal Ca^{2+} flow is altered, the increased mitochondrial Ca^{2+} leads to changes in mitochondrial function and morphology.[237] Moreover, an excessive mitochondrial Ca^{2+} load is a key contributor to myocardial hypertrophy and subsequent HF.[238] The accumulation of mitochondrial Ca^{2+} triggers a vicious cycle of self-destruction in cardiomyocytes. The increased Ca^{2+} load leads to the accumulation of ROS,[239] which damages the components of the respiratory chain and reduces ATP production (Figure 3). ROS accumulation also damages mtDNA and polyunsaturated membrane lipids.[240,241] This positive feedback loop triggers apoptosis, leading to cardiomyocyte death.

Several mitochondrial-associated proteins are involved in Ca^{2+} signaling. Overexpression of mitochondrial mCa^{2+}

uniporter (MCU) decreases oxidative stress and alleviates HF.[242] Conversely, chronic inhibition of the MCU in the myocardium results in increased oxygen consumption rates and impaired physiological intracellular Ca^{2+} homeostasis, yet, paradoxically, does not protect against ischemia-reperfusion injury despite reduced ROS and preserved mitochondrial membrane potential.[243] Specific study of this uniporter has further explicated that MICU1 (mitochondrial calcium uptake 1), a regulatory subunit of MCU, restricts cation flux in the absence of Ca^{2+}, suggesting that the Ca^{2+} binding status of MICU1's EF-hands may serve to influence MCU activity.[244] Although the role of MCU in the development of HF is promising, and it has been reviewed extensively,[245] scant research has been reported on the roles of other potential effectors of Ca^{2+} homeostasis in HF.

Oxidative metabolism that generates ATP requires Ca^{2+} signaling,[246] and Ca^{2+} transfer is hypothesized to occur across MERCs.[247] Mitofusin 2, a protein essential for MERC formation,[92] is reduced in HF in mice.[248] MERCs are composed of several proteins involved in the regulation of Ca^{2+} homeostasis: GRP75 (glucose-regulated protein 75), a chaperone protein, acts as a crucial linker between the IP3Rs (inositol trisphosphate receptors) on the ER and the voltage-dependent anion channel (VDAC) 1 on the outer mitochondrial membrane.[234,235] In vitro studies have shown that IP3R3 is responsible for Orai-mediated Ca^{2+} entry and store-operated Ca^{2+} entry through the formation of a complex with STIM1 (stromal interaction molecule 1) following an inositol trisphosphate signaling cascade in low Ca^{2+} conditions.[249] The role of IP3R (inositol 1,4,5-trisphosphate receptors) is further intriguing as it facilitates direct Ca^{2+} transfer to mitochondria, stimulating oxidative metabolism in states of limited MERC coupling.[250] However, VDACs' role in mitochondrial Ca^{2+} exchange, beyond forming Ca^{2+} permeable pores in the OMM, remains controversial (reviewed by Sander et al[251]). Furthermore, a tertiary and necessary component of this IP3R3-GRP75-VDAC1 complex is DJ-1 (protein deglycase DJ-1; Parkinsonism-associated deglycase), which is deficient in Parkinson disease.[252] Conversely, in neurological disorders, increased DJ-1 activity is associated with deficits in L-type voltage-gated Ca^{2+} channel activity,[253] showing that DJ-1 has poorly defined pluralistic roles in Ca^{2+} homeostasis. Translocase of the mitochondrial outer membrane 70, a subunit of the OM translocase that mediates the import of nuclear-encoded mitochondrial precursors, is downregulated in hypertrophic hearts.[254] Depletion of translocase of mitochondrial outer membrane 70 in neonatal rat ventricular myocytes results in decreased OPA1 import and elevated ROS; its forced expression is protective against pro-hypertrophic perturbations.[254] Furthermore, translocase of mitochondrial outer membrane 70 has emerged as a modulator of IP3R3-GRP75-VDAC1 through reinforcing ER-embedded IP3R3.[255] Despite, this defined multicomponent tether system, few studies have examined it in depth in HF. One study in cardiomyocytes showed that the mitochondrial chaperone, CypD (cyclophilin D), inhibits MERC Ca^{2+} transfer by interacting with the VDAC1/GRP75/IP3R1 complex, thereby protecting the cells from mitochondrial Ca^{2+} overload and subsequent death during hypoxia-reoxygenation injury.[256] Since CypD is a matrix chaperone, further investigation is necessary to see if this is only a functional interaction, or a structural interaction as well. Since Ca^{2+} overload is a key determinant in HF,[238] this suggests that, while a lack of tethering may contribute to Ca^{2+} deficiency, an overabundance of MERC tethering is involved in HF pathogenesis. Therefore, future analyses should focus on MERCs, which may establish a strategy to restore Ca^{2+} homeostasis through structural alterations that optimize these inter-organelle gap widths to modify their crosstalk.[257,258]

In the myocardium, the 2 main types of intracellular Ca^{2+} release channels are the RyR2 (type 2 ryanodine receptor)/Ca^{2+} release channel and the IPCR2 (type 2 inositol 1,4,5-triphosphate receptor).[238] In mouse models mimicking HF, RyR2 Ca^{2+} leak contributes to increased Ca^{2+} load and excessive ROS generation in cardiomyocytes.[238] Mitochondria from the HF mouse model are dysfunctional and have distorted morphologies, with a marked decrease in form factor, size, and aspect ratio, indicating that the fusion-to-fission ratio is low.[238] In addition, the oxidation and phosphorylation of RyR2 channels by protein kinase A results in Ca^{2+} leak from the SR, the ER of muscle cells.[238] In Alzheimer disease, Ca^{2+} release via RyR2 channels and ROS generation, leads to mitochondrial Ca^{2+} overload and fragmentation.[259] The RyR2 receptor modulation Ca^{2+} leak is also believed to contribute to the progression of HF,[238] but the full role of RyR2 in this mechanism has not yet been established. Distinctly, inhibition of DRP1 with a short-term inhibitor known as Drpitor1a, independent from its roles in fission, decreases RyR2 concomitantly with reduced ROS production.[260] Yet, this same study found that prolonged DRP1 deletion conversely leads to cardiomyopathy.[260] Another recent study has had similar findings, showing that in hyperglycemic conditions DRP1 inhibition, normalizes Ca^{2+} homeostasis through reducing RyR2-dependent Ca^{2+} leak.[261] Together, this suggests that beneficial targeting RyR2 may be achieved through short-term DRP1 inhibition.

The connection between MERCs and nanotunnels is unclear. MERCs, as a critical site of Ca^{2+} transfer,[262] may affect nanotunnel formation, which arises in conditions of impaired Ca^{2+} homeostasis.[213] Notably, nanotunnels can form in cardiomyocytes with leaky RyR2 Ca^{2+} release channels.[166,215] The role of Ca^{2+} signaling in HF is a critical avenue to explore, as reestablishing the balance of Ca^{2+} to ADP is important in restoring cardiac contractile function in HF.[263] It may be that when VDACs and other MERC proteins are unable to perform mitochondrial

Ca^{2+} uptake in overloaded conditions, such as those caused by RyR2 mutations, nanotunnels exist as a final compensatory mechanism. The involvement of DRP1 in modulating RyR2[260] further underscores the importance of further explicating how DRP1 and its associated fission factors may be related to the formation of nanotunnels. Nanotunnels may also be important in HF because they facilitate the exchange of metabolites and other essential molecules to optimize ATP production.[213,214] Thus, compromised nanotunnels may negatively affect energy metabolism in cardiomyocytes, thereby contributing to HF.

It is known that the ER can have different conformations, which should be better characterized in general,[264] as different types of ER influence MERC functionality. Yet, it is not known whether different types of MERCs are associated with HF; however, certain cellular conditions give rise to new mitochondrial contacts. For example, a wrappER is a rough ER that wraps around mitochondria and functions in lipid flux.[265] However, there may be additional MERC phenotypes in HF in response to changes in mitochondrial structure. It is possible that ER-associated protein degradation mechanisms result in the formation of certain MERC phenotypes, as opposed to general ER stress states. Furthermore, mitochondrial-ER synthetic linkers, previously reviewed by Csordás et al [234] may, as therapeutics, prevent the formation of nanotunnels or impairment of Ca^{2+} homeostasis. Synthetic linkers that tighten individual mitochondria-junctional SR contacts mitigate myocyte death and attenuate mitochondrial Ca^{2+} overload in ex vivo ischemia/reperfusion injury.[266] However, alterations in tethering may also negatively affect cardiac function,[266] so further research is needed on artificial tethers that modulate Ca^{2+} overload and increase mitochondrial connectivity.

ALTERED METABOLITE PRODUCTION

Mitochondrial metabolism responds to changing environments. For example, embryonic development is partially moderated by mitochondria, which serve as a pacemaker that slows overall metabolism.[267] Changes in cardiomyocyte metabolism underlie the progression of HF. At rest, the heart primarily uses fatty acid oxidation to produce energy,[268] with glucose providing the remaining ≈20%. As the heart fails, mitochondrial oxidation declines, resulting in an energy deficit.[268] The failing myocardium compensates for the energy loss by switching to glycolysis, and mitochondrial ATP production decreases with the oxidation of amino acids and glucose.[268] However, fatty acid oxidation changes are complex and depend on the type (with or without reduced ejection fraction) and severity of HF. The importance of mitochondria in maintaining redox homeostasis is evident as the loss of the manganese antioxidant superoxide dismutase in HF leads to the disruption of cristae and mitochondrial gross architecture.[269] The combined metabolic alterations in HF cause the already failing heart to become even less efficient.[268,270] In advanced stages of HF, circulating ketone bodies, which may provide fuel, reflect the severity of cardiac dysfunction[270,271] and are neurohormonal triggers for the activation of HF.[270] Thus, impaired mitochondrial function contributes to the development and progression of HF by affecting energy metabolism and the redox balance in cardiomyocytes. As in embryonic species-specific development, mitochondria may be the governing pacemakers for HF through their roles in balancing the redox state and metabolism. Thus, HF must be viewed through the dual lenses of metabolic changes concomitant with mitochondrial structural changes.

Central to metabolic dysregulation is NAD(+):NADH ratios. NAD(+):NADH regulates ATP production, but also pluralistically influences anaerobic and aerobic metabolism, posttranslational protein modification, and energy substrate preference, conferring alterations in cardiac efficiency in HF.[272] Loss of NAD^+ in aging or stress leads to altered metabolic status and increased disease susceptibility, which are intertwined with HF pathology.[272] Additionally, NAD+ homeostasis is modulated through autophagic flux in cardiomyocytes, with autophagy impairment causing loss of NAD^+ due to the induction of nicotinamide N-methyltransferase and a process regulated by the SQSTM1-NF-κB (nuclear factor-κB)-NNMT signaling pathway.[273] This study further shows that inhibition of nicotinamide N-methyltransferase restores NAD^+.[273] Notably, mitochondrial ETC complex I deficiency leads to an decreased NAD(+):NADH ratio, accelerating HF progression, which may also be reversed by NAD^+ redox balance.[31] Finally, another promising avenue to restore NAD^+:NADH is through manipulation using an engineered enzyme or tool, as performed by Patgiri et al,[274] Sharma et al,[275] and Titov et al. [276] For example, LOXCAT, an engineered enzyme that converts lactate to pyruvate, rectifies intracellular NAD(+):NADH imbalance, suggesting a novel approach for treating intracellular redox imbalances by targeting circulating metabolites.[274]

In addition to understanding how changes in metabolism affect the heart, we need a better understanding of the effects of these changes on organismal systems. Although ketones are important, we need to understand the interplay between amino acids and mitochondria,[277] in particular, whether amino acids modulate mitochondrial structure and mediate region-dependent mitochondrial changes. The integrated stress response is linked to amino acid metabolism via ATF4 regulation, which ameliorates amino acid starvation.[278] Defects in branched-chain amino acid catabolism increase the risk of HF[279]; thus, ATF4 may serve as a therapeutic target to limit oxidative stress and the associated ketogenesis caused by amino acid starvation.

Mitokines, communicable markers of mitochondrial stress, which include FGF21 and GDF15, are best known in skeletal muscle, where they provide inter-tissue

crosstalk in healthy aging (reviewed by Burtscher et al[280]) and are beneficial for preventing HF in aging (reviewed by Duan et al[281]). However, mitokines show differences in expression in type 2 diabetes, and GDF15 plasma levels are associated with a higher risk of HF.[282] One question concerning mitokines is whether HF sends mitokine signals to other tissues, leading to cachexia and other skeletal muscle diseases, which are common in HF.[283] If so, then mitochondria could be targeted to prevent the cross-organ dysregulation caused by HF. Similarly, more information on the role of mitokines in mitochondrial structure could improve our understanding of HF. For example, mitokine FGF21, which is associated with healthy aging in type 2 diabetes,[282] is secreted following *Opa1* deletion in skeletal muscle.[284] Although this effect is not known for HF, it suggests that changes in mitochondrial dynamics may affect mitokine signaling, which may participate in a bidirectional relationship.

Recent findings have demonstrated that there is altered myocardial metabolism in HF with preserved ejection fraction, particularly through reduced utilization of fatty acids and other alternative fuels like glucose, ketones, and branched-chain amino acids.[285] Additionally, expression of genes associated with lipolysis and oxidation are reduced in HFpEF myocardium, suggesting wider lipidomic changes that remain unclear.[285] This differential catabolism in HFpEF is evidenced by lower glucose, ketone, and branched-chain amino acid utilization compared with HF with reduced ejection fraction.[285] Yet it is unclear if mitochondrial changes are driving this altered energetic utilization and differential mitochondrial regulation may be responsible for differences in metabolism between HF types. Notably, recent studies in brown adipose tissue have shown that SLC25 (soluble carrier family 25) A44 determines active branched-chain amino acids catabolism.[286] More broadly, other mitochondrial SLC25 proteins may drive altered metabolism. SLC25A37 and SLC25A39, which are essential for mitochondrial glutathione import and iron uptake, respectively, have recently appreciated roles in supporting OxPhos.[287] In HF, SLC11A1, and SLC2A3 have been suggested as biomarkers for acute myocardial infarction.[288] In septic cardiomyopathy, HIF1A and SLC25A37 are differentially expressed iron metabolism-related genes in both the myocardium and blood monocytes of patients with sepsis.[289] Although the role of transporters in HF has been more comprehensively reviewed by Kumar et al,[290] together these findings suggest that soluble carrier transport family members may contribute to differences between HF types.

Although 70% of cardiac fuel sources are derived from fatty acids, HF can cause an uptick in nonoxidized fatty acid derivatives, leading to lipotoxicity.[291] In HF, the HIF1alpha-PPARgamma axis mediates metabolic changes, leading to increased glycolytic flux and decreased fatty acid utilization accompanied by lipid accumulation.[292] This lipid accumulation, often caused by increased ROS generation, leads to lipotoxicity and apoptosis, ultimately exacerbating HF.[291] In the context of mitochondria, mitochondrial very-long-chain acyl-CoA dehydrogenase, which is involved in the β-oxidation of very-long-chain fatty acids, is required for normal mitochondrial bioenergetics and associated cardiac heart rate regulation.[293] Additionally, lipotoxicity causes ROS generation, which in turn posttranslationally modifies mitochondrial fusion and fission proteins, causing dysfunctional dynamics.[160] This suggests that the switch from fatty acid utilization, and associated lipid accumulation, pluralistically targets mitochondria. Yet, advanced HF shows decreased myocardial lipid content and increased myocardial ketone utilization.[294] This suggests that as HF continues, the body has natural mechanisms to abrogate lipid accumulation. It is possible that treatments, such as sulforaphane, may accelerate this response by promoting lipid utilization through increasing mitochondrial biogenesis.[295] Additionally, a subpopulation of cells effectively collects and stores lipids in nonalcoholic fatty liver disease to reduce lipotoxicity,[296] yet it is unclear if this same cellular heterogeneity can be exploited in HF.

More broadly, we do not understand whether the formation of mitochondrial structural phenotypes leads to or is the result of changes in metabolite utilization and production. Do changes in ketone levels regulate mitochondrial shape and organization in different regions? For example, lipid flux may alter as failing hearts shift to ketone utilization,[294] leading to changes in MERCs (see previous section). Thus, mitochondrial structure and dynamics are interconnected with factors critical to HF pathology. Furthermore, we must target therapeutics instigated to explicate if mitochondria can reverse lipid accumulation. The utilization of new tools, such as MitoCarta 3.0, which offers a comprehensive catalog of mitochondrial proteins,[221] has provided increased insight into the specific proteins involved in mitochondrial structural changes and their relationship to altered metabolite utilization, thereby informing the development of targeted therapeutics for conditions like lipid accumulation reversal.

Although mitochondrial energetics are reduced across all types of HF, fatty acid oxidation is different in HFpEF versus in HF with reduced ejection fraction (reviewed by De Jong et al[297]). If different types of HF lead to different mitochondrial dynamics and energetic capacities, could mitochondria regulate the various forms of HF? If so, what are the implications for the treatments needed to restore a normal mitochondrial structural phenotype if metabolism is not changed consistently across all forms of HF? This suggests that mitochondria could be a target for individualized medicine that considers the specific type of HF. Furthermore, if mitochondria serve as determinants of ejection fraction, biopsies from patients with HF may provide new information on the factors that lead to phenotypic differences.

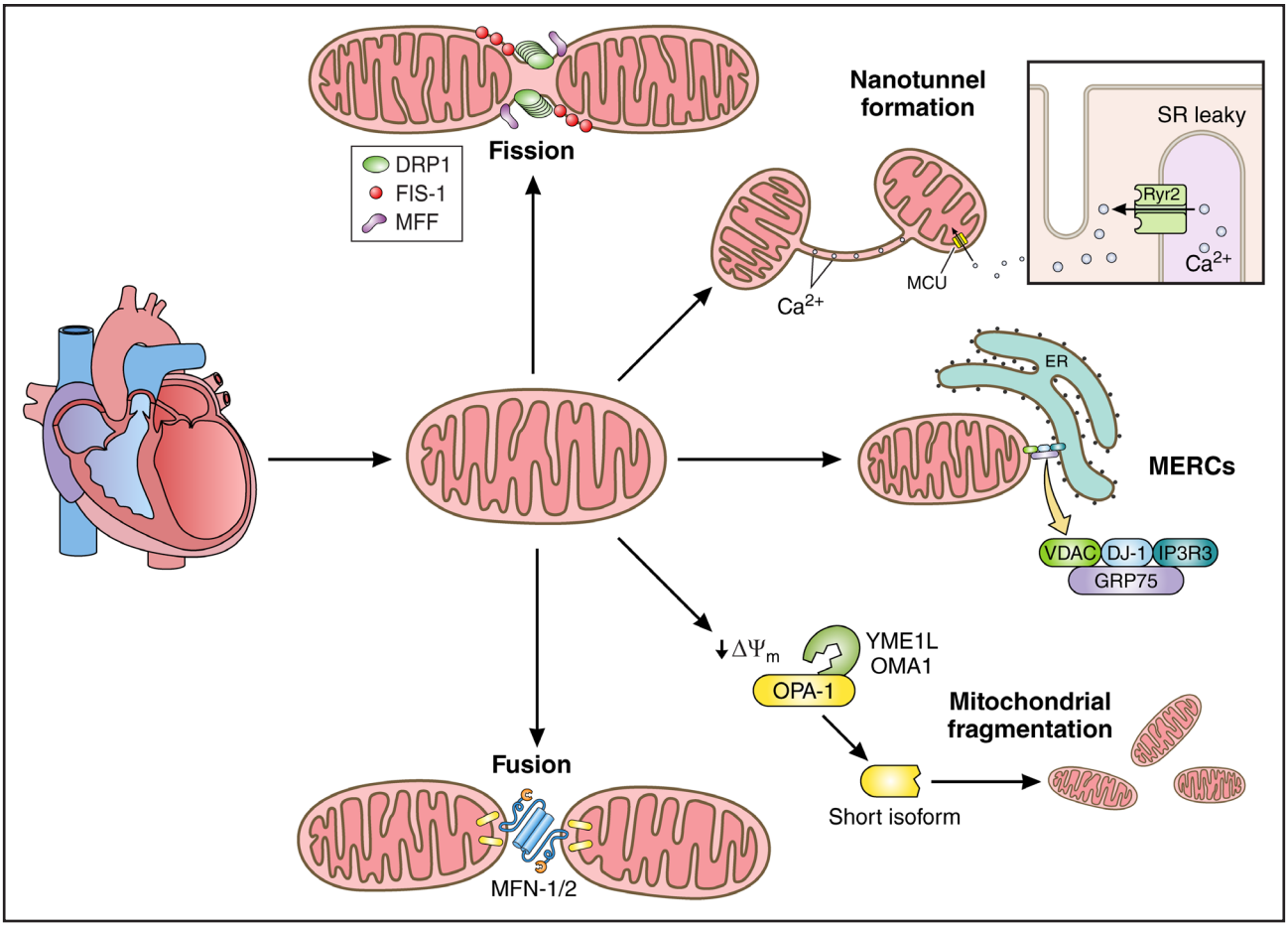

Figure 5. Normal vs pathological alterations of mitochondrial morphology.
Mitochondria are not static but change in response to stimuli to survive under stress. Fusion and fission result in changes in mitochondrial size; however, unregulated fission may result in fragmentation, whereas unregulated fusion may result in hyperbranching. Mitochondria may burst or be recycled via mitophagy. In addition to the typical spheres, mitochondria can assume many shapes and sizes, including megamitochondria. For transport of ions, including Ca^{2+}, mitochondria may form nanotunnels and mitochondria-endoplasmic reticulum contacts (MERCs). Understanding these mitochondrial states and developing therapies that induce or prevent them is an important avenue in heart failure (HF) research. DJ-1 indicates protein deglycase DJ-1; DRP1, dynamin-related protein 1; FIS1, fission 1 protein; GRP75, glucose-regulated protein 75; IP3R3, inositol trisphosphate receptor 3; MFF, mitochondria fission factor; Mfn1/2, mitofusin 1 or 2; OMA1, OMA1 zinc metallopeptidase; OPA1, optic atrophy 1; Ryr2, type 2 ryanodine receptor/Ca2+ release channel; SR, sarcoplasmic reticulum; S616, serine 616; S637, serine 637; S656, serine 656; S693, serine 693; VDAC, voltage-dependent anion channel; and YME1L, YME1 Like 1 ATPase. Illustration credit: Sceyence Studios.

CONCLUSIONS AND FUTURE PERSPECTIVES

Here, we have presented the current knowledge on the roles of mitochondrial and cristae dynamics, including the role of the MICOS complex in mitochondrial ultrastructure changes in the context of HF. Furthermore, we analyzed the roles of MERC sites, mitochondrial communication via nanotunnels, altered metabolite production, and external factors that affect mitochondrial structure in the failing heart. Each component provides a potential mitochondrial therapeutic target for mitigating cardiomyocyte death in HF. Given the complexity of the development and progression of human HF, effective measures will likely require a combination of treatments with distinct targets that require a better understanding of mitochondrial structure and dynamics and modulatory factors in HF. New insights into mitochondrial structural changes in HF and other factors of HF pathology that affect mitochondria (Figure 4) should provide a better understanding of the development of mitochondria in HF, allowing for individualized treatment regimens.

Mitochondria are indispensable for energy production, Ca^{2+} homeostasis, and other cellular processes. Aging is a major risk factor for HF and other cardiovascular diseases,[71] and aging mitochondria produce ROS, leading to mutations in mtDNA.[13] Because decreasing mutations in mtDNA can reduce mitochondrial dysfunction,[13] it is important to understand how mitochondria change during aging to reverse these changes. In advanced HF cases in which damaged mitochondria are beyond repair, autogenous mitochondrial transplantation might restore function.[298,299] Although autogenous mitochondrial transplantation reduces cardiomyocyte loss, it is not known whether these transplanted

mitochondria can establish MERCs and other important structures needed for long-term health.

In the future, as we examine age-related pathologies and their relation to HF, it is important to consider the mitochondrial role, both within the heart and outside of the heart. In particular, recent studies have illustrated the organ-dependent aging rates, with accelerated aging in the heart conferring increased risk of HF.[300] In practice, as reviewed by Xie et al,[301] this comes about, in part, via alterations in circulating metabolic signatures. However, equally important is understanding the role of mitochondria in these age-related signature alterations. Notably, different types of HF may come about, in part, due to these altered metabolic signatures. Additionally, recent findings suggest that HFpEF can cause skeletal muscle wasting through mitochondrial dysfunction, suggesting that accelerated aging of the heart may have systemic effects.[302] Yet, it is unclear if skeletal muscle mitochondrial structure and dynamics differ on the basis of ejection fraction, which would offer a potential biomarker for the different types of HF (Figure 1). This may offer insight into how mitochondria change in accelerated aging and pathology states, and the pluralistic roles mitochondria play in HF pathology (Figure 5).[303]

ARTICLE INFORMATION

Affiliations

Department of Molecular Physiology and Biophysics (A.H., K.N.) and Department of Medicine, Division of Clinical Pharmacology (C.N.W., A.K.), Vanderbilt University Medical Center, Nashville. Department of Physiology, Mitochondrial Phospholipid Research Center, Johns Hopkins University School of Medicine, Baltimore, Maryland (S.M.C., N.S.). Vanderbilt Center for Immunobiology (A.K.). Vanderbilt Institute for Infection, Immunology and Inflammation (A.K.). Vanderbilt Institute for Global Health (A.K.). Department of Neuroscience, Cell Biology and Physiology, Wright State University, Dayton, OH (C.R.W.).

Sources of Funding

The study was funded by United Negro College Fund (UNCF)/Bristol Myers Squibb E.E. Just Faculty Fund, Career Award at the Scientific Interface (Career Awards at the Scientific Interface [CASI] award) from Burroughs Welcome Fund (BWF) ID number 1021868.01, BWF ad hoc award, National Institutes of Health (NIH) Small Research Pilot Subaward to 5R25HL106365-12 from the NIH Programs to Increase Diversity among Individuals Engaged in Health-Related Research (PRIDE) Program, DK020593, Vanderbilt Diabetes and Research Training Center (DRTC) Alzheimer's Disease Pilot and Feasibility Program. The Chan Zuckerberg Initiative (CZI) Science Diversity Leadership grant number 2022-253529 from the Chan Zuckerberg Initiative DAF, an advised fund of the Silicon Valley Community Foundation (A. Hinton). A postdoctoral fellowship from the American Heart Association and the Barth Syndrome Foundation (award ID: 828058 to N. Senoo). NIH Grants R01HL147818 and R01HL144941 (A. Kirabo) and R01HL165729 and R01GM151746 (S.M. Claypool). Doris Duke CSDA 2021193 (CNW), K23 HL156759 (CNW), Burroughs Wellcome Fund 1021480 (CNW). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of the NIH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Disclosures

None.

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