

Power²: The power of yeast genetics applied to the powerhouse of the cell

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The budding yeast *Saccharomyces cerevisiae* has served as a remarkable model organism for numerous seminal discoveries in biology. This paradigm extends to the mitochondria, a central hub for cellular metabolism, where studies in yeast have helped to reinvigorate the field and launch an exciting new era in mitochondrial biology. Here we discuss a few recent examples in which yeast research has laid a foundation for our understanding of evolutionarily conserved mitochondrial processes and functions, from key factors and pathways involved in the assembly of oxidative phosphorylation (OXPHOS) complexes to metabolite transport, lipid metabolism, and interorganelle communication. We also highlight new areas of yeast mitochondrial biology that are likely to aid in our understanding of the mitochondrial etiology of disease in the future.

Yeast is a powerful resource for understanding mitochondrial biology

The yeast *S. cerevisiae* is among the most powerful model organisms with which to approach an understanding of human biology. Although the examples are numerous, one area that has become particularly prominent recently is our understanding of mitochondrial function. Mitochondria are well known as the powerhouse of the cell, providing most ATP synthesis. These organelles have a much broader reach, however, with impacts on metabolite synthesis and catabolism, intracellular signaling, and organellar communication. As a result, impaired mitochondrial function is associated with a staggering variety of chronic human diseases [1].

While human genetics and biochemical analyses of mammalian mitochondria have been valuable tools in our efforts to understand the basic biology of mitochondria and the mitochondrial etiology of disease, the ability of *S. cerevisiae* to model mitochondrial biology and disease has arguably been even more powerful [2]. Most mitochondrial processes are conserved across eukaryotes and researchers have taken full advantage of the power of yeast genetics to dissect several of these pathways over the past several decades. These studies have been greatly enhanced by the recent development of many yeast

genome-wide collections, including deletions of nonessential genes [3], collections containing titratable alleles of essential genes [4], sequence-verified plasmid libraries [5,6], and collections of epitope-tagged open reading frames [7]. The application of these resources, including in powerful high-throughput genetic interaction studies called epistasis miniarray profiles (E-MAPs) [8], has helped assign functions to many uncharacterized genes involved in numerous mitochondrial pathways. Many conserved mitochondrial processes have been characterized in yeast and extensively reviewed elsewhere, including mitochondrial import [9], mitochondrial dynamics [10] and quality control [11], and mitochondrion-derived signaling [12].

Glossary

Cardiolipin: a diphosphatidylglycerol lipid and an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition. Cardiolipin has multiple functions, from regulating structure to serving as proton trap for OXPHOS to apoptosis.

Endoplasmic reticulum (ER)–mitochondrion encounter structure (ERMES): a protein complex comprising four core components (Mmm1, Mdm10, Mdm34, and Mdm12) and one associated protein (Gem1) that physically tethers the ER and mitochondrial membranes at ER–mitochondrion contact sites.

Epistasis miniarray profiles (E-MAPs): genetic epistasis studies performed on a large scale that are often used to assign function to uncharacterized genes.

Mitochondrial calcium uniporter (MCU): a multiprotein complex that enables the mitochondrial import of Ca²⁺ ions across the mitochondrial inner membrane into the matrix.

Mitochondrial carrier family (MCF): a family of transmembrane transporters that facilitate most solute transport across the mitochondrial inner membrane.

Mitochondrial pyruvate carrier (MPC): a protein complex that facilitates transport of pyruvate across the mitochondrial inner membrane. While known and characterized since the 1960s, the proteins in this complex were identified only in 2012.

Mitochondrion-associated membranes (MAMs): MAMs are reversibly tethered to mitochondria and are part of the ER. They allow the import of some lipids into mitochondria and are involved in calcium homeostasis, mitochondrial function, autophagy, and apoptosis.

Mitoferrin: a member of the MCF that is responsible for the transport of iron ions across the mitochondrial inner membrane.

Oxidative phosphorylation (OXPHOS): a mitochondrial metabolic pathway that uses energy released by the oxidation of nutrients to produce ATP. During OXPHOS, electrons are transferred from electron donors to electron acceptors such as oxygen in redox reactions that eventually result in the synthesis of ATP.

Phosphatidic acid (PA): a vital cell lipid that acts as a biosynthetic precursor for the formation of all acylglycerol lipids in the cell.

Retgrade pathway: a signaling pathway that originates from dysfunctional mitochondria and ultimately activates a transcriptional program mediated by the transcription factors Rtg1 and Rtg3. The purpose of the pathway is to help replenish TCA cycle intermediates in low-functioning mitochondria.

Succinate dehydrogenase (SDH) or Complex II: a tetrameric complex in the mitochondrial inner membrane that catalyzes the oxidation of succinate to fumarate and the reduction of quinone as an entry point to the electron transport chain as part of OXPHOS.

Vacuole and mitochondria patch (vCLAMP): a protein complex comprising subunits Vps39 and Ypt7 that functionally tethers the vacuole and the mitochondria and is thought to play a role in lipid and metabolite exchange between the organelles.

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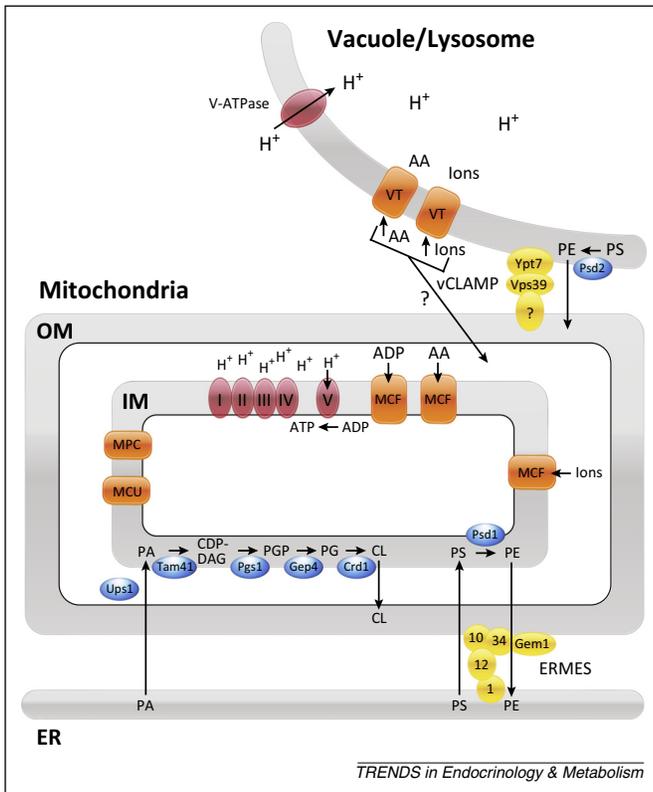


Figure 1. An overview of the four areas of mitochondrial biology highlighted in this review: assembly of the mitochondrial oxidative phosphorylation (OXPHOS) complexes (I–V) located in the inner mitochondrial membrane (red); import of metabolites into the mitochondrial matrix through transporters belonging to the mitochondrial carrier family (MCF), as well as additional transporters of interest to human disease (MPC, mitochondrial pyruvate carrier; MCU, mitochondrial calcium uniporter) (orange); interorganelle lipid transport between the endoplasmic reticulum (ER) and the mitochondria for synthesis of cardiolipin (CL) and phosphatidylethanolamine (PE) in the inner mitochondrial membrane [lipid enzymes in blue, the organelle-tethering ER–mitochondrion encounter structure (ERMES) in yellow]; and the physical and functional interaction between the vacuole/lysosome and mitochondria. The vacuole and mitochondria patch (vCLAMP) complex (yellow) tethers the vacuole and mitochondria and may be involved in the exchange of metabolites between these organelles [amino acids (AA), ions, and PE]. VT, vacuolar transporter.

Because the instances in which yeast has aided in the characterization of mitochondrial pathways are numerous, this review focuses on only a few recent examples where inquiries into the fundamental processes of yeast mitochondria have taught us how mitochondria work in higher organisms. The areas of focus include assembly of the OXPHOS machinery, mitochondrial metabolite transport and lipid metabolism, and interorganelle communication pathways involving the mitochondria. Although these areas are diverse, crosstalk between these pathways is prominent and ultimately critical for mitochondrial function, as illustrated in Figure 1. As we hope will become obvious, dissecting these pathways in yeast has had a great impact on our understanding of human diseases, including the discovery of many causal genes.

Assembly of mitochondrial OXPHOS complexes

Mitochondria are well known as the powerhouse of the cell, capturing energy in the form of ATP through OXPHOS (see [Glossary](#)), which couples electron transport in the inner mitochondrial membrane to the production of ATP. The five OXPHOS complexes include four large, multiprotein

complexes (complexes I–IV) that use the electrons from NADH and succinate to pump protons and establish the electrochemical potential across the mitochondrial inner membrane. The fifth complex, Complex V, harvests the energy of protons crossing back into the matrix for ATP production. These complexes contain between four and 46 different protein subunits, many of which are embedded in the membrane. Except for Complex II, each complex contains proteins that are translated in both the mitochondrial matrix and the cytosol. Each of the complexes that transfer electrons (I–IV) also hosts various redox-active cofactors (e.g., flavin–adenine dinucleotide, heme, iron–sulfur clusters) that have an inherent propensity to generate reactive oxygen species if not handled properly. These factors combine to make OXPHOS complex assembly a significant challenge for the cell [13,14].

One piece of evidence of the challenge presented by this assembly problem is the number of proteins that are specifically dedicated to ensuring that it occurs appropriately. The vast majority of these assembly factors were discovered and/or functionally characterized in yeast. The assembly of Complex IV (cytochrome *c* oxidase) is perhaps the most comprehensively studied of any of the complexes, based largely on the power of yeast genetics. As this has been reviewed extensively elsewhere [15–18], it is not addressed in this review. By contrast, a molecular understanding of the assembly process of Complex I has lagged behind for various reasons, including the fact that *S. cerevisiae* does not contain Complex I. Instead, other species have been used to study this assembly process and this has resulted in the identification of numerous assembly factors and the beginning of an understanding of their mechanisms of action [19]. However, yeast has been instrumental in our understanding of the assembly of complexes II and V.

Complex II

The past 5 years have witnessed a flurry of discoveries related to the assembly of Complex II, also called the succinate dehydrogenase (SDH) complex, which had previously been largely enigmatic. It is the simplest of the five OXPHOS complexes, containing only four proteins, all of which are encoded by the nuclear genome and translated in the cytosol. This relative simplicity might have led to the erroneous conclusion that Complex II assembles spontaneously, without the need for dedicated assembly factors. The discovery of four dedicated factors in the past few years has made it clear that even the simplest assembly problem in the electron transport chain is still complex [20]. Mutations in the genes encoding two of these four assembly factors of SDH – SDH5/SDHAF2 and SDHAF1 – have been shown to cause human disease: a familial tumor syndrome [21,22] or a rapidly progressing infantile neurodegenerative disease [21,22], respectively. In one case, this factor was discovered using yeast genetics and biochemistry; in the other, reconstitution studies in yeast demonstrated the role of the encoded protein in SDH assembly. Recently, two additional factors have been discovered using yeast, both of which appear to have evolutionarily conserved functions in SDH assembly [23,24] (reviewed in [25]).

Complex V

Complex V or ATP synthase is a fascinating machine comprising two separable multiprotein subcomplexes, F_0 and F_1 , the structure, function, and assembly of which have been recently reviewed [26]. F_0 is embedded in the mitochondrial inner membrane and F_1 is a soluble complex that attaches to the matrix face of F_0 . The relative rotation between F_1 and F_0 on proton translocation provides the energy for ATP synthesis [27–29]. Like the other OXPHOS complexes, Complex V poses a complicated assembly problem [30] and cells use dedicated assembly factors to prevent mistakes. The first two Complex V assembly factors, Atp11 and Atp12, were discovered in a yeast genetic screen and later shown to be required for proper assembly of the F_1 sector of ATP synthase [31–33]. These observations enabled the finding that human ATP11 and ATP12 also function in Complex V assembly [34] and that mutation of *ATP12* causes mitochondrial disease in humans [35,36]. A third factor, Fmc1, was discovered in yeast and appears to also be required for the assembly of F_1 , particularly at elevated temperatures [37]. Two additional factors, Atp10 and Atp23, have been discovered that act on F_0 , both of which appear to be specifically required for the normal assembly of the Atp6 protein into F_0 [38–41]. Recently, the discovery of the yeast INA complex containing Ina17 and Ina22, critical for the assembly of the F_1F_0 peripheral stalk, provided a new understanding of how the F_0 and F_1 sectors come together to form the intact ATP synthase [42]. Functional orthologs of these latter five factors remain to be discovered in mammals.

Mitochondrial metabolite transport

Being the site of many chemical reactions of particular importance for cellular metabolism, including those in pathways with steps both inside and outside the mitochondrial matrix, the transport of metabolites in and out of mitochondria is a critical feature of the physiology of this organelle. In general, the outer membrane does not provide a barrier to the transit of metabolites, although it does to most macromolecules. As a result, the regulated transport of metabolites across the mitochondrial inner membrane is of great importance and crucial in maintaining cellular bioenergetics and metabolic homeostasis. Several human diseases are caused by defects in mitochondrial inner membrane transport. Although such diseases are rare, they are typically very severe [43]. As is the case with many human diseases, the rare, severe, monogenic diseases affecting mitochondrial metabolite transport are likely to be instructive regarding the important, but more complex, roles of mitochondria in common polygenic diseases and those with a strong environmental component.

In many ways, metabolite transporters pose a more challenging set of problems to those intending to study them than do the enzymes that act on those metabolites. The genetics of metabolite transport is frequently complicated by complete or partial redundancy as well as by bypass mechanisms that obviate the necessity for a specific transport step [44]. Moreover, transporters do not perform any chemical modification and therefore *in vitro* assays of native function are possible only with a membrane-associated transporter embedded in an intact membrane. As a

result, classical biochemistry discovered most of the enzymes of the core metabolic pathways but our understanding of the transporters that perform equally important functions in these pathways lagged behind.

Mitochondrial carrier family (MCF)

While some molecules can pass through the mitochondrial inner membrane by passive diffusion, most, including charged and polar molecules, requires protein-provided assistance. In most cases, this facilitated transport is performed by members of a distinct protein family that appears to be almost completely dedicated to this function [45]. Members of this family, known as the MCF, are characterized by three repeated homologous sequences of roughly 100 residues, each containing two transmembrane domains [46]. Based on genome analysis by sequence conservation, it appears that most eukaryotic genomes encode 35–55 different members of the MCF family [47], with 35 members present in yeast [48,49].

A limited number of MCF transporters were purified and studied before the genomics era, but most were first identified based on the presence of their encoding gene in the yeast genome. Of course, this identification came with no information regarding their potential functions, particularly the substrates that they might transport. The Palmieri and Walker laboratories, their colleagues, and others undertook the ambitious task of identifying transport substrates for many of these uncharacterized proteins [50]. This successful effort directly led to the identification of functions for dozens of yeast MCF transporters, which has facilitated the identification of substrates for homologous mammalian proteins. We highlight a few of these transporters here.

Mitochondrial ADP/ATP carrier

The archetypal MCF member in yeast is Aac2, which is the most abundant yeast ADP/ATP carrier. These proteins catalyze the exchange of ATP produced in the matrix for cytosolic ADP, enabling continuous ATP production and export to the cytosol [51]. Mutations in the human *AAC2* homolog *ANT1* have been shown to cause mitochondrial DNA depletion syndrome and myopathy [52,53]. In addition, both gene and protein have been valuable models for deriving principles regarding the structure and function of the MCF protein family.

Mitochondrial dicarboxylate carrier (DIC)

The DIC is important for the anapleurotic filling of the tricarboxylic acid (TCA) cycle via the mitochondrial import of succinate and malate. The DIC was one of the first MCFs to be identified by Palmieri and colleagues using the powerful combination of biochemical reconstitution of purified protein and yeast genetics [54]. Both the yeast and bovine protein had been previously purified and studied biochemically, but the sequence of the protein and the encoding gene was unknown [55]. Based on the yeast protein sequence, the rat gene was cloned and was shown to have similar transport characteristics [56]. The dicarboxylate carrier has subsequently been shown to be important for glucose-stimulated insulin secretion in pancreatic β -cells [57].

Mitochondrial coenzyme A (CoA) importer

Mitochondrial CoA is required for the entry of carbon from pyruvate into the TCA cycle via synthesis of acetyl-CoA by pyruvate dehydrogenase. The first hints of the genetic basis of the mitochondrial CoA importer Leu5p came when the *leu5Δ* mutant, which is unable to synthesize leucine, was shown to fail to accumulate CoA in the mitochondria [58]. Two mammalian homologs were subsequently shown to perform a similar or identical function, one of which is the human Graves' disease protein [58] (which most likely is misnamed and has no role in Graves' disease), also called solute carrier family 25 member 16 (SLC25A16). The second is SLC25A42, which was convincingly shown, using *in vitro* reconstitution and transport assays as well as genetic complementation in yeast, to also function in mitochondrial CoA import [59]. Orthologs were recently characterized in plants [60]. To our knowledge, this transporter or defects in it have not yet been implicated in human disease, but the fundamental knowledge of the proteins that enable this critical transport step in metabolism and the genes that encode them is likely to become valuable in our understanding of human disease in the future.

Mitoferrin proteins

Iron has essential roles in many cellular compartments, including the mitochondria, which are the site of iron-sulfur cluster biogenesis. The mitoferrin proteins, which enable mitochondrial iron transport, were first discovered and characterized in yeast due to a relationship with the yeast homolog of the gene mutated in Friedreich's ataxia. They were shown to be involved in mitochondrial iron metabolism [61,62], but their function was unknown until work in *S. cerevisiae* demonstrated that Mrs3/Mrs4 function as mitochondrial iron transporters [63,64]. Mitoferrin is an example where the genes were known in other systems, including in zebrafish [65], before the elucidation of their function in any species. The power of yeast genetics and biochemistry enabled functional discoveries that may have been difficult in other, more complex systems.

Non-MCF transporters

While the systematic approach to determining the transport substrates of MCF proteins has proven to be of great value, it obviously is unable to assist in the discovery of transporters that are not members of this family. Two such examples have been discovered in the past few years. The mitochondrial calcium uniporter (MCU) is predominantly responsible for mitochondrial calcium uptake in mammalian cells. Although the *S. cerevisiae* model system lacks the MCU and did not aid in the discovery of this transporter, yeast has proven to be useful for the comparative phylogenetics approach that enabled the discovery of the members of the MCU complex [66–72].

Like the MCU, the transporter that enables pyruvate, the product of cytosolic glycolysis, to enter the mitochondria and fuel the TCA cycle had been observed and studied for decades before its recent molecular identification. Two groups simultaneously discovered a complex containing at least two mitochondrial pyruvate carrier (MPC) proteins (MPC1 and MPC2) that is necessary for mitochondrial

pyruvate uptake [73,74]. Mutations in *MPC1* were shown to cause a severe neuromuscular phenotype in humans [73]. Based on reconstitution experiments in bacteria, MPC1 and MPC2 appear to also be sufficient for pyruvate transport [74]. In this case, genetic and biochemical approaches using yeast provided the key data that enabled this discovery. Since this initial discovery, four other groups have confirmed the role of the MPC in mitochondrial pyruvate import, with major potential implications for human metabolism and metabolic disease [74–79]. While the structure of the MPC complex is unknown, the determination of the structure of distantly related sugar transporters suggests that it might function as a MPC1/MPC2 heterodimer [80].

Studying gene mutations

In addition to the utility of the yeast model system for discovering protein function, it also provides a facile mechanism for testing the consequences of mutations found in the genes encoding human transporters. The principle of human gene complementation of yeast deletion mutants has been used repeatedly in the study of mitochondrial transporters [50], including the discovery that mutations in the *MPC1* gene encoding one subunit of the MPC are loss-of-function mutations [73], as described above. The yeast system was also used to assess the functionality of mutations in the gene encoding the aspartate–glutamate carrier, which are tightly associated with a specific form of urea cycle deficiency [81]. A final example is the study of mutations in the gene encoding the ornithine transporter SLC25A15 found in patients with hyperornithinemia–hyperammonemia–homocitrullinuria syndrome [82]. This transporter was first found and characterized in yeast [83,84], with the human ortholog being discovered and characterized later based on homology [85]. This powerful capability of the yeast system in allele characterization will become increasingly valuable as genome-sequencing efforts are increased and as the remaining mitochondrial transporters are functionally annotated.

Mitochondrial lipid metabolism

Like metabolite transport and OXPHOS assembly, our understanding of mitochondrial lipid biology has relied heavily on yeast biology and genetics. Proper mitochondrial lipid composition is key to the efficient function of most mitochondrial processes. Several genes encoding proteins involved in mitochondrial lipid synthesis pathways were first identified for their secondary effects on other mitochondrial processes such as import [86] and fusion [87]. The lipid composition of the mitochondrial membrane is distinct from that of other cellular membranes. It is highly enriched in phosphatidylethanolamine (PE), phosphatidylcholine (PC), and cardiolipin (CL) and normally deficient in sterols and sphingolipids [88]. Mitochondria import lipids such as phosphatidylserine (PS) and phosphatidic acid (PA) as precursors for the synthesis of PE and CL within the mitochondria. Sequential enzymes that function in the synthesis of these lipids often reside in separate mitochondrial subcompartments or in another organelle entirely. Thus, intra- and interorganelle transport of lipids is required for both CL and PE synthesis

[89]. The enzymes and transport steps involved in these pathways are highly conserved from yeast to humans and here we highlight several recent examples in which the power of yeast has greatly enhanced our understanding of mitochondrial lipid metabolism.

Cardiolipin

Cardiolipin (CL) is the hallmark lipid of the mitochondria and most of the genes involved in this pathway were first discovered in yeast, including two enzymes and one lipid transport protein in the past few years alone. CL is a unique phospholipid in that it contains two phosphatidyl groups linked to a glycerol backbone. It is localized almost exclusively in mitochondria and associates with proteins in mitochondrial import, OXPHOS, and mitochondrial dynamics [90]. In addition to a core requirement for CL in basic mitochondrial processes, proper remodeling of CL's acyl chains in the outer mitochondrial membrane is also necessary for normal mitochondrial health. The last step of CL remodeling is performed by the conserved acyltransferase Tafazzin/Taz1 [91]. Mutations in the human *TAZ* gene impair acyl chain remodeling and lead to Barth syndrome, an X-linked disease characterized by cyclic neutropenia and skeletal and cardiac myopathies [92,93]. While the function of the *TAZ* gene was first identified in humans, studies on the yeast *TAZ1* have greatly accelerated research in this area, including providing potential pathogenic mechanisms for loss-of-function mutations in *TAZ*.

CL is synthesized in the mitochondria and starts with the formation of cytidine diphosphate–diacylglycerol (CDP–DAG) from PA. CDP–DAG is then converted to phosphatidylglycerolphosphate (PGP) by phosphatidylglycerolphosphate synthase 1 (*Pgs1*) [94], followed by dephosphorylation of PGP to phosphatidylglycerol (PG). Finally, cardiolipin synthase (*Crd1*) converts PG into CL through reaction with another CDP–DAG [95,96], followed by remodeling by *Cld1* and *Taz1*. The encoding genes *PGS1* and *CRD1* were discovered in yeast in 1998 [94–96] and paved the way for the identification of the orthologous mammalian genes several years later [97–99]. Remarkably, despite the fact that CL was first isolated in 1945 [100], the genes required for the formation of CDP–DAG from PA in the mitochondria, as well as the dephosphorylation of PGP to PG, were identified only recently.

PA is synthesized in the endoplasmic reticulum (ER) and is transported to the mitochondria for CL synthesis. It was initially thought that a small subset of CDP–DAG synthase (*Cds1*), an ER-localized enzyme that converts PA to CDP–DAG in the ER, must also be present in mitochondria for the conversion of PA to CDP–DAG [101,102]. However, Tamura *et al.* recently demonstrated that *Cds1* is exclusively ER localized and showed that the highly conserved mitochondrial protein Tam41 directly catalyzes the formation of CDP–DAG from PA in the mitochondrial inner membrane [103]. Tam41 was originally isolated as a protein required for the activity of the Tim23 inner membrane translocase [86] and was first implicated in CL biosynthesis in 2008 [104]. The human sequence homolog of *TAM41* is *TAMM41*, although its role in CL synthesis remains to be confirmed.

Similar to the identification of Tam41, the yeast phosphatase *GEP4*, which is responsible for the conversion of PGP to PG, was identified only recently by yeast genetics. Osman *et al.* first identified *GEP4* in a genome-wide genetic array for genes required in the absence of mitochondrial prohibitins [105]. Several CL-associated genes were present in the interaction set with prohibitins and this subsequently helped the authors identify *Gep4* as the long-sought PGP phosphatase [106]. Subsequently, an unrelated phosphatase [protein tyrosine phosphatase, mitochondrial 1 (PTPMT1)] was shown to catalyze the same reaction as *GEP4* in mice [107].

Intra- and interorganelle transport

In addition to the enzymatic steps of CL synthesis, yeast has also proven to be a resourceful system for the understanding of both intra- and interorganelle transport of lipids in the CL and PE synthesis pathways. The synthesis of CL starts from PA, which is generated in the ER and must make its way to the inner mitochondrial membrane for the first step of CL biosynthesis. Likewise, PE is generated from PS in the inner mitochondrial membrane by the conserved enzyme *Psd1*, and PS also must move from the ER to mitochondria [89]. The transfer of PA and PS to the mitochondria is predicted to occur at well-characterized contact sites between the ER and mitochondria called mitochondrion-associated membranes (MAMs), which have been extensively reviewed elsewhere [108].

A recent study in yeast uncovered the first protein complex potentially involved in lipid transport at these contact sites [109]. The ER–mitochondrion encounter structure (ERMES) comprises four core protein subunits [maintenance of mitochondrial morphology (*Mmm*) protein 1; mitochondrial distribution and morphology (*Mdm*) protein 10, 12 and 34; and the *Gem1* GTPase] that together function to tether the ER and mitochondrial membranes [110]. In support of a role for the ERMES complex in lipid transport, several members of the complex contain conserved synaptotagmin-like mitochondrial-lipid-binding protein (SMP) domains, which are potential lipid-binding motifs that may play a role in lipid transfer [111]. Additionally, Kornmann *et al.* showed that cells lacking ERMES components exhibited slower rates of PS-to-PC conversion, which requires ER–mitochondrion lipid transfer. However, mammals contain no obvious homologs of the ERMES complex and a recent study in yeast suggested that ERMES has no impact on PS-to-PE conversion rates [112]. Thus, the exact role of ERMES in mitochondrial lipid biosynthesis requires further clarification and it is likely that other, undiscovered systems work in conjunction with ERMES to mediate ER–mitochondrion lipid transfer.

After PA is moved to the mitochondrial outer membrane from the ER, it must be transported to the mitochondrial inner membrane for conversion to CDP–DAG in CL biosynthesis. An impressive collection of recent studies in yeast has identified the conserved protein *Ups1/PRELI* as a mediator of intramitochondrial PA transport. *Ups1* was initially described as a protein required for processing of the mitochondrial fusion protein *Mgm1* [87]. The role of *Ups1* in *Mgm1* processing was subsequently linked to its direct function in CL metabolism by two separate groups

[105,113]. Both Osman *et al.* and Tamura *et al.* showed that loss of Ups1 severely compromised CL biosynthesis [105,113] and Langer's group went on to elegantly demonstrate that Ups1 functions as the long-sought PA transfer protein, shuttling PA from the outer to the inner mitochondrial membrane in a CL-responsive manner [114]. The transfer activity of Ups1 also requires the conserved protein Mdm35 [115,116] and Langer's group has since demonstrated that the orthologs of Ups1 (PRELI) and Mdm35 (TRIAP1) function similarly in PA transport in mammals [117].

Mitochondrion–vacuole crosstalk

In classic textbook illustrations, mitochondria are depicted as lone-wolf organelles. This view of the mitochondria could not be further from reality, as it is now appreciated that they are part of an ever-expanding interconnected network of organelles within the cell. Recent studies have described both physical and functional interactions of mitochondria with several organelles and these interactions are critical for mitochondrial function [118]. As with the other areas of mitochondrial biology discussed here, studies in yeast are leading the way in this field.

Mitochondrial communication with other organelles

Many examples of mitochondrial communication with other organelles have been described in detail elsewhere. The classic example is the yeast retrograde response, in which deficits in mitochondrial function are communicated to the nucleus through the action of the retrograde regulation (RTG) transcription factors [119]. In this context, the functional status of the mitochondria is relayed to the cell's transcriptional machinery, which in turn regulates the cellular metabolic state. Since the discovery of the retrograde response in yeast, similar retrograde pathways have been described in other organisms, which have been reviewed elsewhere [120]. As we discussed in the lipid metabolism section, mitochondria also form physical and functional contacts with the ER that are important for lipid metabolism, calcium homeostasis, and mitochondrial dynamics [121]. More recent studies in yeast have identified a physical tether between mitochondria and the plasma membrane [122] and outlined a close association between mitochondria and peroxisomes [123]. Mitochondria and peroxisomes also share the same fission machinery [124] and recent studies in mammals have outlined a vesicle-mediated pathway for the delivery of mitochondrial proteins to peroxisomes [125].

The mitochondrion–vacuole relationship

An emerging topic in the arena of interorganelle crosstalk is the tight physical and functional association of mitochondria with the yeast vacuole. The vacuole is similar to the mammalian lysosome and it is becoming increasingly clear that this organelle plays key roles in cellular metabolism. The presence of a functional metabolic link between the mitochondria and vacuole is nothing new. During the initial characterization of genes important for vacuole function in the late 1980s and early 1990s, it was discovered that loss-of-function mutations in the vacuolar H⁺-ATPase (V-ATPase) prevent growth of yeast on non-fermentable carbon sources

[126]. The V-ATPase is an evolutionarily conserved protein complex that pumps protons into the lumen of the yeast vacuole and mammalian lysosome, acidifying these organelles and generating a proton gradient that is used for uptake and storage of metabolites within the vacuole [127]. Several more recent studies have confirmed the requirement for vacuole function in mitochondrial respiratory metabolism, and the collection of V-ATPase protein-coding genes has been described as the largest class of non-mitochondrially localized proteins required for mitochondrial function [128].

Despite the longstanding functional interaction between the mitochondria and vacuole, the underlying mechanism for this connection remains unclear. Vacuolar acidity is required for two main vacuole functions: degradation of proteins and metabolites within the vacuole lumen and storage of ions and amino acids [127]. It has been proposed that loss of V-ATPase activity compromises mitochondrial function through three potential avenues: (i) failure to properly turn over mitochondrial proteins through autophagy; (ii) increased production of oxidative stress; and (iii) mitochondrial overload from failed storage of ions and metabolites [128]. Consistent with the second hypothesis, loss of V-ATPase function leads to a dramatic increase in oxidative stress, potentially through disruption of normal iron metabolism [129,130]. An early study on V-ATPase function also demonstrated rescue of mitochondrial deficiencies in V-ATPase mutants with the addition of excess iron, supporting the idea that iron metabolism may play a role in mitochondrion–vacuole crosstalk [131]. A more recent study examining mitochondrial function in the context of yeast aging addressed the role of protein degradation and metabolite storage in mitochondrion–vacuole crosstalk. In this study, Hughes and Gottschling showed that age-induced mitochondrial dysfunction is driven by loss of vacuolar acidity and provided evidence against a role for protein degradation in this connection [132]. Instead, the authors demonstrated that the mitochondrion–vacuole relationship is likely to be governed by compromised vacuolar storage of neutral amino acids in the vacuole lumen. It remains to be determined how imbalances in the cellular distribution of neutral amino acids impact mitochondrial function and whether the same metabolic connection exists in mammals. Mitochondrion–vacuole crosstalk is clearly a complex process and additional studies are required to separate out the role of oxidative stress and amino acids in this process.

A recent and exciting development in the mitochondrion–vacuole relationship was the discovery of a physical association between the vacuole and mitochondria mediated by a tether called the vacuole and mitochondria patch (vCLAMP) [133,134]. The discovery of vCLAMP was independently reported by the Ungermann and Schuldiner groups using differing approaches. Ungermann and colleagues used a candidate gene approach to find genes that enhanced contact sites between vacuoles and mitochondria on overexpression, while the Schuldiner group identified vCLAMP in a microscopy-based screen looking for genes that impacted the amount of ERMES contacts per cell. Remarkably, both approaches led to the same gene, Vacuolar Protein Sorting 39 Homolog (*VPS39*), a well-characterized

subunit of the vacuole homotypic fusion and vacuole protein sorting (HOPS) machinery. The authors showed that Vps39, in conjunction with the Rab GTPase Ypt7, mediates the tethering of vacuoles to mitochondria. How the two proteins associate with mitochondria is currently unclear.

Much like the ERMES complex at the ER–mitochondrion interface, a comprehensive understanding of the function of vCLAMP remains to be elucidated. vCLAMP shares some functional overlap with the ERMES complex and it appears that these two complexes are regulated in an interconnected manner [133,134]. Elbaz-Alon *et al.* showed that vCLAMP is important for lipid exchange between the vacuole and the mitochondria and that mitochondrion–vacuole lipid exchange is likely to compensate for defects in ER–mitochondrion lipid exchange in ERMES mutants, which may provide an explanation for the apparent discrepancy reported for the role of ERMES in lipid transport discussed earlier. Excitingly, Elbaz-Alon *et al.* also find that metabolite transporters are potentially enriched near mitochondrion–vacuole contact sites [133,134]. Thus, it is easy to envision a role for vCLAMP beyond lipid metabolism and it will be interesting to see whether vCLAMP connections facilitate the amino acid-dependent mitochondrion–vacuole crosstalk described by Hughes and Gottschling [132]. Unlike the other sections of this review, mitochondrion–vacuole communication remains to be firmly established in mammals. However, we highlight this connection as an exciting area of recent discovery in yeast that we believe will ultimately also bear fruit in the mammalian system. Homologs of Vps39 are apparent in mammals [135] and it has already been demonstrated that mouse mitochondria are physically connected to lysosome-related organelles of pigment cells [136].

Concluding remarks and future perspectives

The four areas that we have highlighted provide a glimpse of the impact that fundamental studies in yeast have had on our understanding of mitochondrial biology. That

impact has extended far beyond the knowledge of protein function and detailed mechanisms, to include many examples where the discovery of human disease genes was directly enabled by observations made in yeast. Many outstanding questions remain in the field of mitochondrial biology and we have highlighted some of those that pertain to the four topics discussed here (Box 1). As we enter an era where demands for applicability to human health and disease are being made of scientific inquiry as never before, we must not forget that often the most efficient way to understand the biology of the complex human is to interrogate fundamental mechanisms in yeast.

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Box 1. Outstanding questions

- What additional factors are important for OXPHOS assembly?
- What are the causal genes in the remaining SDH assembly-related disease states without an assigned gene?
- What are the substrates of the orphan mitochondrial carrier proteins?
- Is there a high-order structural organization of transporters and their related enzymes within the mitochondrial interior?
- How are lipids distributed to the correct location within the mitochondria?
- What is the mechanism of lipid transport by the ERMES and vCLAMP complexes?
- What proteins play the role of the ERMES at ER–mitochondrion contact sites in mammals?
- Are there additional lipid transport proteins at ER–mitochondrion contact sites?
- How does loss of vacuole/lysosome acidity compromise mitochondrial function?
- Is the vCLAMP involved in amino acid-related mitochondrion–vacuole crosstalk?
- What metabolites are exchanged between the mitochondria and vacuole and what is the role of vCLAMP in this process?
- How is vCLAMP attached to the mitochondrial outer membrane?

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