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The power of yeast to model diseases of the powerhouse of the cell

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1. ABSTRACT

Mitochondria participate in a variety of cellular functions. As such, mitochondrial diseases exhibit numerous clinical phenotypes. Because mitochondrial functions are highly conserved between humans and *Saccharomyces cerevisiae*, yeast are an excellent model to study mitochondrial disease, providing insight into both physiological and pathophysiological processes.

2. INTRODUCTION

While the mitochondrion is best known for its ability to synthesize ATP through oxidative phosphorylation (OXPHOS), it additionally participates in a number of fundamental cellular processes, including ion regulation, heme and Fe/S cluster synthesis, lipid metabolism, signal transduction, and programmed cell death.

Mitochondrial function requires the coordination of both the nuclear and mitochondrial genomes. Of the estimated 1000-1500 mitochondrial proteins in humans (approximately half of which are ubiquitously expressed in all tissues (1)), only 13 are encoded by the mitochondrial genome (mtDNA), all of which are subunits of the respiratory chain. In addition, 2 rRNAs and all 22 tRNAs are encoded by mtDNA. Mitochondria in *Saccharomyces cerevisiae* (herein referred to as "yeast") contain roughly 1000 proteins per mitochondrion (2, 3). Except for respiratory complex I subunits, which are absent in yeast, and Var1p, a constituent of the small ribosomal subunit which is present in yeast (4), all mtDNA genes are conserved between yeast and humans.

Mitochondrial diseases affect at least 1 in 5000 (5), and as a result of their participation in numerous cellular processes, present with a range of clinical phenotypes.

Depending on which genome is affected, mitochondrial disease can follow maternal or mendelian inheritance (6-9). Because mtDNA only contains a few genes, the majority of pediatric cases of mitochondrial disease involve defects originating in the nuclear genome (7). This allows these diseases to be studied with all of the classic advantages of the yeast system such as the ability to combine relatively easy genetic manipulations with robust biochemical approaches that collectively facilitate detailed mechanistic studies. In addition, yeast provides the added bonus of being amenable to suppressor screens and both large scale genetic and pharmacologic screens (for examples, see (10-13)).

Yeast also provide advantages specific to studying mitochondrial diseases, perhaps the most important of which is the ability to survive on fermentable carbon sources in the absence of mitochondrial function. Therefore, pathogenic mutations that lead to mitochondrial dysfunction are able to be maintained in yeast, so long as a fermentable carbon source is available. Furthermore, the growth phenotype provides a simple method of assessing mitochondrial function; when grown in media containing only non-fermentable carbon sources such as glycerol, ethanol, or lactate, strains exhibiting mitochondrial dysfunction are unable to grow.

A large number of diseases are associated with mtDNA defects, which are caused by point mutations, rearrangements, and/or deletions (14, 15). The utility of using yeast to model these diseases is exemplified by the unique ability to directly transform yeast mtDNA(16-18). In yeast, biolistic transformation of a *rho*^{0} strain (a strain completely lacking mtDNA) is able to generate the desired mutant strain relatively easily. This allows a defined mutation that may be identified in unrelated human patients to be studied in the context of one host nuclear genetic background. Of course, this capability additionally allows distinct mtDNA mutations in the same gene or different genes to be studied, compared and contrasted in the same genetic background.

In higher organisms, a limited number of mtDNA mutants have been described, most of which have been generated through indirect manipulation of mtDNA (19-21), although efficient complementation of a mtDNA deletion by targeting RNA to mitochondria has recently been described in mammalian tissue culture (22).

The difficulty in generating mutations in mammalian cells is compounded by their ability to harbor heteroplasmic mtDNA genomes. A single cell contains hundreds to thousands of individual mtDNA genomes. Normally all mtDNA copies are the same (termed "homoplasmy"), but when detrimental mutations are present, both wild type and mutant mtDNA genomes are present within the cell (termed "heteroplasmy") (23, 24). Unlike mammalian cells, yeast become homoplasmic within a few generations (25, 26). In mammals, a mutation in mtDNA may be present in a few copies, but not result in a clinical phenotype because the remaining wild type mtDNA is able to complement the defect. It is not until the mutant mtDNA reaches a minimum critical number that dysfunction is evident. This phenomenon, known as the threshold effect, is often ascribed to the progressive and varied onset of mitochondrial diseases and pleiotropic phenotypes (24, 25). While this aspect cannot be modeled well in yeast, the absence of additional mutants or a subpopulation of wild type genes make yeast useful in studying pathogenic mtDNA mutants in isolation.

2.1. Yeast strains

A number of genetically distinct laboratory strains have been used to study mitochondrial functions. Some commonly used strains include S288c, W303, and D273. A more comprehensive list of yeast strains used in mitochondrial studies can be found in (27).

Strain S288c carries numerous mutations that affect mitochondrial function. The *MIP1* gene, which encodes for the mitochondrial DNA polymerase, contains an Ala to Thr substitution at the highly conserved residue 661 (termed the *MIP1*[S] allele). This increases the mutability of mtDNA, especially at elevated temperature (27, 28). S288c also contains a Ty1 element inserted into the 3' region of *HAP1* (29). Hap1p is a transcription factor that induces the expression of multiple oxygen-inducible genes, including some that are incorporated into respiratory complexes (30). The mutation greatly reduces the ability of Hap1p to induce genes under the control of its upstream activation sequence (UAS), most notably *CYC1*, the gene encoding the mobile electron carrier, cytochrome *c* (29). Additionally, when the derivative of S288c, BY, was analyzed, it contained mutations in *SAL1* and *CAT5* (31). *SAL1* encodes the mitochondrial Mg-ATP/P_i exchanger, and *CAT5* encodes a protein required for ubiquinone (CoQ) biosynthesis. Thus, the S288c strain and its derivatives contain multiple mutations that result in less than optimal mitochondrial function. Despite this, S288c does have advantages; in addition to numerous auxotrophic markers, its genome has been completely sequenced, and the yeast knock-out collection was generated using the S288c derivative strains BY4741/2 (32).

W303 and its descendents do not contain mutations in *HAP1* (29), *SAL1*, or *CAT5*, leading to higher respiration rates than S288c. However, these strains do contain the *MIP1*[S] allele, increasing mtDNA mutability (28). Additionally, the presence of the *ade2* allele in combination with *MIP1*[S] can further increase mtDNA point mutability (27).

The D273 strain, commonly used due to its high respiratory rate, contains the wild type *MIP1* allele (28), although auxotrophic markers are limited, and the lack of the 2-micron episome prevents multi-copy plasmid transformation.

Therefore, the genetic background is an important consideration when using yeast to study mitochondrial processes. Wild type strains with decreased mitochondrial function may make the assessment of mutants with partial function more difficult. On the other hand, these strains may exacerbate certain phenotypes, making it easier to screen for suppressors or genetic interactions.

3. TRANSFER RNAS

The production of RNAs and proteins from mtDNA requires transcription and translation just like for the nuclear genome. For the most part, these processes are governed by genes encoded within the nuclear genome. Still, all 22 tRNAs required for mitochondrial protein synthesis are encoded by the mitochondrial genome. Mutations have been documented in each tRNA gene, and result in clinical phenotypes ranging from severe, such as MERFF (Myoclonic Epilepsy with Ragged Red Fibers), MELAS (Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke), and Leigh syndrome, to relatively mild, such as diabetes and PEO (Progressive External Ophthalmoplegia; reviewed in (33), (14)). Worth considering, mutations in a single tRNA can impact the production of every mitochondrially encoded protein as long as their transcript contains a codon recognized by the mutant tRNA. Thus, patients harboring tRNA mutations often present with deficiencies in multiple/all of the OXPHOS complexes that incorporate mitochondrially encoded subunits (Complex I, III, IV, and V).

The ability to transform yeast mtDNA, and the conservation between yeast and human mitochondrial tRNAs, in particular $tRNA^{Leu}$, allows pathogenic tRNA mutations to be modeled in yeast (34-37). The conservation between yeast and human tRNAs is further demonstrated by the ability to rescue tRNA mutations in mammalian cells by transfection with wild type yeast tRNAs (38). Pathogenic mutants in yeast cause defective respiration, abnormal mitochondrial morphology, and mtDNA instability (35). Interestingly, the severity of the clinical phenotype correlates with the yeast phenotype. For instance, when a pathogenic mutation in $tRNA^{Leu\ (UUR)}$ causing MELAS is modeled in yeast, a C to T mutation at nucleotide 26 (n.C26T), the strain shows severe growth defects in glycerol-containing media and an inability to respire. However, when a different mutation in $tRNA^{Leu\ (UUR)}$, causing mitochondrial myopathy and chronic PEO, is modeled in yeast (n.T20C), growth on glycerol and respiration is still defective compared to wild type, but not entirely absent (34).

Further validating yeast as a model, the molecular consequences of pathogenic mutations at conserved sites are the same in yeast as in humans. The n.A14G mutation in $tRNA^{Leu\ (UUR)}$, equivalent to a mutation causing MELAS, displays an acylation defect, consistent with *in vitro* results of the mutant human tRNA (36, 39). Additionally, mammalian orthologs of yeast tRNA mutation genetic suppressors are also able to alleviate the phenotypes of mammalian cells harboring the mutations (35, 40-42).

Potentially, the yeast model of tRNA mutations can provide a definitive assay to distinguish between pathogenic mutations and neutral polymorphisms. Multiple different scoring systems have been described to predict if a patient's mutation will be pathogenic or silent (33, 43, 44) with varying degrees of success. The difficulty in this decision is due, in part, to differing degrees of heteroplasmy in different tissues of a patient, an inability to always survey all relevant tissues for degree of heteroplasmy (e.g. those impacted by a mutation), the fact that exceptions to the rules established by the scoring systems are known to exist, and the inability to follow the segregation of the mutation with the disease in small families (33). The presence or absence of a respiratory growth defect in a yeast strain with the equivalent mutation provides higher confidence in a mutation's ultimate classification as pathogenic or neutral.

4. MITOCHONDRIAL DNA STABILITY

4.1. DNA polymerase

The mitochondrial genome is replicated by DNA polymerase gamma. The holoenzyme forms a heterotrimer (45), and is comprised of a catalytic subunit encoded by the nuclear gene *POLG*, and two accessory subunits encoded by the nuclear gene *POLG2*. The yeast ortholog, Mip1p, shares 43% identity with POLG (46, 47). However, in contrast to mammalian POLG, Mip1p is not thought to bind any accessory subunits. The linker region that binds the accessory subunits is poorly conserved between Mip1p and POLG. In contrast, the identity in the polymerase and exonuclease domains of Mip1p and POLG approaches 65%.

Mutations in *POLG* cause PEO, Alpers' syndrome, and ataxia-neuropathy syndrome (48). All three diseases are caused by increases in mtDNA depletion, large scale deletions, and/or point mutations that result in impaired mitochondrial function and ultimately cell death. Over 150 disease associated mutations in *POLG* have been documented, and are compiled at the Human DNA Polymerase Gamma Mutation Database (http://tools.niehs.nih.gov/polg/).

In yeast, the mechanistic defect of mutant Mip1p is relatively easy to resolve. Defects in mtDNA replication that cause depletion or large scale deletions result in a

cytoplasmic *petite* phenotype. *Petite* colonies can be identified by their inability to form large colonies on fermentable media (*petite* colonies are unable to survive on non-fermentable carbon sources), and the frequency of *petite* colony formation is easy to ascertain in *MIP1* mutants. Defects in mtDNA replication can result in *rho*^{0} or *rho*$^-$ (partial loss of mtDNA, but inability to respire) phenotypes. This can be further resolved by crossing the *petite* strain with *mit*$^-$ strains that harbor point mutations in mitochondrially encoded subunits of respiratory complexes III *(cob*) and IV *(cox2* or *cox3*). *Rho*^{0} colonies will be unable to complement the *mit*$^-$ strains, while the *petite* phenotype will be rescued after crossing *rho*$^-$ and *mit*$^-$ strains as long as the lesion in the *rho*$^-$ strain is not the same as the *mit*$^-$ strain. Alternatively, Southern blotting with a mtDNA specific probe or DAPI staining can indicate the presence of mtDNA. *MIP1* mutations that increase the frequency of point mutations are commonly assessed by the strain's ability to grow in the presence of erythromycin. Erythromycin inhibits mitochondrial protein synthesis, but point mutations in the mitochondrial 21S rRNA gene confer resistance. Therefore, *MIP1* mutations that result in an increased frequency of erythromycin resistance indicate an increase in point mutability (49).

The ability to model pathogenic *POLG* mutations in Mip1p has many advantages. Many patients carry multiple pathogenic mutations, both in *cis* and *trans*, as well as non-pathogenic single nucleotide polymorphisms (SNPs). In addition, limited family histories of patients make the mode of inheritance difficult to resolve. By modeling individual mutations in *MIP1*, disease-causing mutations can be distinguished from harmless SNPs. Once a pathogenic mutation is positively identified, dominant or recessive mechanisms can be determined by expressing the mutant in either haploid delta*mip1* or diploid cells containing one copy of wild type *MIP1*. For example, modeling the pathogenic human mutation G1076V (all missense mutations discussed in this review list the normal amino acid first followed by the amino acid position in the human or yeast protein and finally the identity of the mutant residue) in a haploid delta*mip1* yeast strain results in 100% *petite* colony formation, indicating that the mutation causes Mip1p dysfunction. When the same mutant Mip1p is heteroallelically expressed with wild type Mip1p, the frequency of *petite* colony formation is reduced to 1.7%; thus the G1076V functions as a recessive mutation. In contrast, the dominant pathogenic R853Q mutation results in 88% and 100% *petite* colony formation frequency when modeled in a diploid strain containing one wild type Mip1p and a haploid delta*mip1* strain, respectively (50).

Finally, yeast models allow for screens of potentially therapeutic suppressors. For instance, growth of Mip1p mutants on media containing reactive oxygen species (ROS) scavengers partially suppresses mtDNA damage (51), indicating that ROS damage contributes to mtDNA instability. ROS alone are able to induce mtDNA damage and depletions (52). Mutations in mtDNA caused by Mip1p mutants may cause respiratory complex dysfunction, which leads to the production of more ROS, creating a detrimental positive feedback loop.

A subset of POLG mutations occur at residues important for nucleotide binding and selectivity. When these mutants are modeled in Mip1p, the *petite* phenotype is suppressed by overexpression of Rnr1p, the yeast ribonucleotide reductase; or deletion of Sml1p, an Rnr1p inhibitor. In both cases, the mitochondrial dNTP pool is increased allowing suppression of this class of Mip1p mutant (51).

The large number of modeled mutations allow for a generalization of the conclusions, which in turn will aid in predicting the effect of novel mutations. Most POLG mutations are found in the polymerase domain. Recessive polymerase domain mutants could cause the polypeptide to be unstable, or unable to bind mtDNA. Dominant polymerase domain mutants could have defects in processivity, whereby they bind DNA but are unable to replicate it, and as a result inhibit the wild type Mip1p either directly or indirectly. Some dominant mutations resulting in low processivity also display an increase in point mutability. This is thought to occur by increasing the exposure of single stranded DNA, thereby increasing the susceptibility of the template to mutagenic lesions, such as those generated by ROS. For example, the Mip1p mutants T654A and R656Q increase both *petite* colony formation frequency and point mutagenesis (50). Most mutations in the exonuclease domain are less pathogenic, and result in increased point mutability rather than mtDNA depletion, with a few notable exceptions (53). To date, no disease-associated POLG mutation has demonstrated a total lack of exonuclease activity. In fact, the mtDNA mutation frequency when pathogenic mutations are modeled in the exonuclease domain is only 12% of that observed when the exonuclease domain is absent altogether (50).

Similar to the modeled tRNA mutants, a good correlation has been noted between the severity of disease and the degree of mtDNA maintenance. That is, mutations in patients with an earlier age of onset and more severe clinical phenotypes display higher frequencies of *petite* colony formation in the yeast model (53-55).

4.2. Adenine nucleotide translocase

The adenine nucleotide translocase (ANT) is the most abundant protein in the inner membrane and mediates the 1:1 exchange of ATP and ADP across the membrane, representing the terminal step in oxidative phosphorylation. The directionality of nucleotide transport by ANT is not inherent however, and productive transport (ATP_{out}, ADP_{in}) is driven by the membrane potential. Thus, under experimental conditions where the membrane potential is altered, ANT can preferentially undergo non-productive transport (ADP exchanged for ADP, or ATP exchanged for ATP) or counter-productive transport (ATP_{in}, ADP_{out}) (56). ANTs have long been thought to function as homo-dimers (57-59), although this has recently been challenged and a monomeric functional unit proposed (60-62).

There are four ANT isoforms in humans (63-65). ANT1 is expressed primarily in heart and skeletal muscle (66), and is 50% similar to the yeast ortholog, Aac2p (for ADP/ATP carrier) (67). Although three AAC isoforms are present in yeast, only Aac2p is required for respiratory growth (68).

Six ANT1 mutations have been described in patients, five of which present with autosomal dominant PEO and mtDNA depletion (69-74). The other ANT1 mutant, A123D, is recessive, and causes hypertrophic cardiomyopathy, mild myopathy with exercise intolerance, lactic acidosis, and large-scale mtDNA deletion in skeletal muscle (74). Worth briefly considering is that the phenotypes associated with ANT1 mutations are surprisingly mild given the critical role of ANT in OXPHOS. The most likely explanation for this is the existence of multiple ANT isoforms with overlapping functions and expression patterns. Five of the six pathogenic ANT1 mutations occur in regions highly conserved between the yeast and human proteins.

When the conserved pathogenic mutations are modeled in yeast Aac2p and then expressed in a strain lacking endogenous Aac2p, the mutant strains display reduced growth on respiratory media (71, 74, 75), and reduced cytochrome *b* and aa_3 spectra, indicating a reduction in assembled respiratory complexes III (cytochrome *b*) and IV (cytochrome aa_3). Interestingly, when the M114P, A128P, or A137D Aac2p mutants (equivalent to L98P, A114P, and A123D ANT1 mutants) are introduced into a delta*aac1*delta*aac2* strain, cytochromes *b* and aa_3 are reduced more than the null mutant alone (74, 75). Consistent with the reduced cytochrome spectra, the A128P, M114P, and S303M (equivalent to the V289M ANT1 mutation) mutants show a decrease in complex IV (COX) activity and respiration (75). It is currently unknown if the decrease in cytochrome content and respiratory complex activity is a direct result of mtDNA instability, or some other effect caused by the Aac2p mutations.

While the A137D Aac2p mutant exhibits no nucleotide transport activity (74), the A128P, M114P, and S303M mutants display altered transport kinetics such that ATP was preferred over ADP (75). One potential consequence of ATP/ATP homoexchange over productive heteroexchange is a resultant decrease in the intramitochondrial ADP concentration. This could explain the decrease in respiration efficiency, since respiratory complexes IV and V can be allosterically regulated by ADP (76, 77). This may also affect the dNTP pool, preventing accurate mtDNA replication (71).

The A128P, M114P and S303M Aac2p mutants, when expressed in heteroallelic strains together with wild type Aac2p, exhibited normal oxidative growth phenotypes, indicating that these phenotypes are recessive. However, the cytochrome *b* and aa_3 profiles are reduced when Aac2p harbor the A128P or M114P mutations and mtDNA mutability increases for A128P and S303M Aac2p mutants, demonstrating dominant phenotypes. Interestingly, mtDNA mutability is not affected in M114P and the cytochrome spectra is reduced in S303M, indicating that these phenotypes may arise through independent mechanisms (75).

Consistent with a gain of function dominant phenotype, A106D (equivalent to the A90D ANT1 mutation), A128P, M114P, and A137D Aac2p mutants result in an increased frequency of *petite* colony formation when overexpressed. As the number of A137D alleles increases, the total amount of mtDNA, as well as the mtDNA-encoded Cox2p, decreases. When these mutants are expressed (or overexpressed) in a diploid strain with a wild type copy of *AAC2*, in the presence of the ionophore carbonyl cyanide m-chlorophenyl hydrazone (CCCP) or absence of Yme1p (both are conditions which decrease the mitochondrial membrane potential), the cells are not viable on glucose media. The authors suggest that overexpression of the pathogenic Aac2p mutants induces an unregulated ion channel, decreasing the membrane potential, although this was not directly tested and may instead be the result of altered nucleotide transport modes (75). The observed decrease in mitochondrial membrane potential for Aac2p mutants, in combination with the presence of CCCP or absence of Yme1p, may reduce the membrane potential below the threshold required for viability (78). Consistent with this, respiratory control ratio values, a quantitative measure of respiratory coupling, are decreased in the heteroallelic Aac2p mutant strains (78). Strikingly, when the mutant A128P Aac2p is overexpressed, the mitochondrial membrane potential is lower than wild type Aac2p in the presence of CCCP (79). The decrease in membrane potential that results from pathogenic Aac2p mutations would likely decrease the import of nuclear encoded proteins into the mitochondrial matrix, including those involved in mtDNA maintenance and replication. However, the contribution of a reduced membrane potential caused by ANT1 mutants to the associated human disease has yet to be determined. Worth considering is that yeast with no Aac2p function are *petite* negative (80), that is, loss of Aac2p function in rho^0 yeast is lethal. Rho^0 yeast lack functional respiratory complexes capable of generating a membrane potential. The mitochondrial membrane potential is required for the import of the vast majority of mitochondrial proteins encoded in the nucleus, and thus for viability. In rho^0 yeast, a minimal membrane potential sufficient for mitochondrial biogenesis is maintained by Aac2p by the electrogenic exchange of ATP into and ADP out of the mitochondrial matrix (81). Therefore, mutations that affect Aac2p transport activity would cause the mitochondria to become especially sensitive to additional mtDNA defects.

4.3. Stress inducible yeast MPV17 homolog

MPV17 was first identified in a transgenic mouse containing a proviral insertion that disrupted its expression, causing severe renal failure (82). Originally thought to be localized to peroxisomes (83), the yeast ortholog, Sym1p (stress-inducible yeast MPV17) was shown to reside in the mitochondrial inner membrane (84). Delta*sym1* yeast are unable to grow on ethanol at elevated temperature. This

phenotype can be rescued with human MPV17, indicating functional conservation between the yeast and human protein (84).

While the function of MPV17 is still unknown, patients with *MPV17* mutations exhibit mtDNA depletion in the liver, which leads to organ failure. Phenotypes can range from severe hepatopathy causing early death, to moderate liver dysfunction, and can be accompanied by polyneuropathy, leukodystrophy, progressive spino-cerebellar ataxia, hypoparathyroidism, tubulopathy, intestinal dysmotility, and pigmentary retinopathy (85-89).

Of the seven *MPV17* mutations described in patients, three are missense mutations. Two families carried homozygous mutations R50Q and N116K; and the third carried a heterozygous R50W mutation with a 25 base pair deletion in the other *MPV17* allele, resulting in a predicted truncated protein (85). When the pathogenic mutations are introduced into Sym1p, neither N172K (equivalent to the N116K MPV17 mutant) nor R51W (= R50W MPV17 mutant) is able to rescue the delta*sym1* phenotype, whereas R51Q (= R50Q MPV17 mutant) can partially rescue delta*sym1*. All three mutants display an increase in *petite* colony formation. The *petite* frequency of R51W and N172K is similar to delta*sym1*, whereas the *petite* frequency of R51Q is approximately 50% of delta*sym1*. Southern blot analyses indicate that all three mutants contain large scale mtDNA rearrangements, consistent with the phenotype of patients and the *mpv17*$^{-/-}$ mouse (90).

Although both the yeast model and patient phenotypes indicate that *SYM1*/*MPV17* mutations result in mtDNA depletion, the link between Sym1p function and mtDNA maintenance remains enigmatic. *SYM1* was first identified as being upregulated during heat shock, and in playing a role in ethanol metabolism or tolerance (84). One study demonstrated that at elevated temperature, delta*sym1* yeast are unable to grow on ethanol but grow like wild type strains on glycerol, ethanol/glycerol, acetate, and to a lesser extent acetaldehyde, leading the authors to conclude that the absence of *SYM1* does not result in a general respiratory defect (84). However, a later study showed that delta*sym1* growth is reduced on ethanol, glycerol, or acetate, as well as lactate or 0.1% glucose (91). The reason behind these discrepant results is not immediately clear as both studies employed the same parental yeast strain.

Delta*sym1* yeast also display mitochondrial swelling, flattened cristae, and the accumulation of electron dense particles, consistent with the phenotype of the *mpv17*$^{-/-}$ mouse (90), implying that Sym1p function is necessary to maintain normal mitochondrial morphology (91). However, it has been noted that the absence of mtDNA causes altered mitochondrial morphology (92) and that mutations causing altered mitochondrial morphology eventually lead to mtDNA loss ((93, 94), reviewed in (95)). The causality between mtDNA depletion and altered mitochondrial morphology in the absence of Sym1p function has yet to be established.

The delta*sym1* phenotype is exacerbated in the absence of *ODC1*, *YMC1*, or either citrate carrier *CIT1* or *CIT2* (91). Ymc1p and Odc1p are mitochondrial carriers that transport tricarboxylic acid cycle (TCA) intermediates (96, 97). When TCA enzyme activity is analyzed, succinate dehydrogenase (SDH) activity is reduced by approximately 60% in the delta*sym1* yeast (91). Interestingly, a fraction of MPV17 patients also exhibit SDH defects in the liver (85, 88, 89).

The delta*sym1* phenotype can be suppressed by overexpression of Ymc1p and Odc1p; or by supplementing the media with glutamate, glutamine, aspartate, or asparagine, amino acids that can be metabolized and enter the TCA cycle as alpha-ketoglutarate or oxaloacetate (91). This indicates that the delta*sym1* phenotype can be alleviated by increasing the pool of TCA cycle intermediates. Ymc1p overexpression reduces the frequency of *petite* colony formation below that of the wild type strain, while Odc1p overexpression reduces *petite* colony formation to approximately 53% of delta*sym1*. Interestingly, *petite* colony formation is also decreased when Odc2p is overexpressed, but Odc2p is unable to rescue the growth phenotype, indicating that the delta*sym1* growth defect and mtDNA depletion are caused independently (91).

Consistent with the link between Sym1p and the TCA cycle is the observation that delta*sym1* yeast have reduced glycogen storage (91). Glycogen synthesis requires gluconeogenesis, which is regulated by the flux of TCA cycle intermediates. Defects in glycogen storage may explain the hypoglycemic crises suffered by patients with *MPV17* mutations (85, 98).

Despite the information provided by the yeast model, more work must be done to elucidate the molecular function of MPV17/Sym1p, and how mutations lead to mtDNA depletion. Unfortunately, the abundance of TCA cycle intermediates in delta*sym1* yeast has not been reported. Such metabolomic data may help to distinguish between Sym1p being directly involved in the regulation of the TCA cycle, and thus its absence resulting in altered metabolite levels; or in glycogen synthesis, where increasing the level of TCA intermediates above the normal range is sufficient to suppress the mutation. Additionally, the pathogenic missense mutations were only shown to affect growth on ethanol at elevated temperature and *petite* colony formation. It will be interesting to learn if the yeast strains harboring mutant Sym1p mimic the other phenotypes of the delta*sym1* strain.

5. MITOCHONDRIAL PROTEIN IMPORT

While mitochondria contain between 1000 and 1500 different proteins (1-3, 99), only about 1% are encoded by mtDNA. Therefore, most mitochondrial proteins must not only be targeted to mitochondria, but also sorted to the correct submitochondrial compartment (for a comprehensive review, see (100)). After synthesis on cytosolic ribosomes, virtually all preproteins are recognized

by the Translocase of the Outer Membrane (TOM) complex, which facilitates movement across the outer membrane (OM). OM beta-barrel proteins are inserted by the Sorting and Assembly Machinery (SAM) complex, while proteins with alpha-helical transmembrane domains require Mim1p for their insertion into the membrane (101, 102). Insertion into the inner membrane (IM) or translocation into the matrix invariantly requires the proton-motive force generated by the electron transport chain. Polytopic IM proteins with internal sorting signals are inserted into the IM by the Translocase of the Inner Membrane (TIM) 22 complex. Notably, external ATP is not needed for this process. Substrates of the TIM22 translocon include members of the mitochondrial carrier family as well as Tim23p and Tim22p of the TIM23 and TIM22 translocons, respectively. The TIM23 complex utilizes the mitochondrial membrane potential +/- ATP to deliver precursors containing N-terminal targeting signals to three distinct compartments: the matrix, the IM, and the intermembrane space (IMS). Import into the matrix is distinguished from release into the IM or IMS by additionally requiring ATP to fuel the Presequence translocase-Associated Motor, or PAM complex. For those precursors that contain a hydrophobic "stop-transfer" signal, the N-terminal targeting sequence is removed by the matrix processing peptidase followed by either the lateral release into the IM or, subsequent to further processing by additional proteases that remove the "stop-transfer" signal, release into the IMS.

5.1. Disulfide relay system

Another pathway for the import of soluble IMS resident proteins is the recently discovered disulfide relay system (reviewed in (100, 103-106)). Substrates of the disulfide relay system are characterized by their small size, lack of N-terminal targeting signal, and conserved cysteine residues. The cysteine residues are required for disulfide bond formation between the substrate and import machinery, and for proper protein folding. The IMS-resident receptor, oxidized Mia40p, forms a disulfide intermediate with incoming preproteins. Protein substrates are then released from Mia40p, leaving it in a reduced state. The FAD-dependent sulfhydryl oxidase Erv1p, re-oxidizes Mia40p, allowing another substrate to bind. Finally, Erv1p reduces cytochrome *c* allowing the re-oxidized Erv1p to participate in another round of import. Thus, this pathway mediates the proper folding of soluble proteins that contain disulfide bonds, indicating that the IMS is not contiguous with the cytosol with regards to its redox chemistry as previously thought. The proper folding of substrates of this pathway is postulated to prevent their retrograde translocation through the TOM complex back into the cytosol via a folding trap mechanism. Lastly, it is worth mentioning that reduced cytochrome *c* donates its electron to complex IV and finally O_2. As such, the disulfide relay system contributes, albeit to a minor extent, to the ultimate generation of the membrane potential across the IM.

The human homolog to *ERV1* is *GFER*. Recently, a Moroccan family presenting with congenital cataracts, progressive muscular hypotonia, sensorineural hearing loss, and developmental delay was identified with a mutation in GFER, R194H (107). Ultrastructural analyses of patient skeletal muscles demonstrated altered mitochondrial morphology, with thickened, electron dense cristae, and a subset of mitochondria with abnormal IMS morphology. Multiple mtDNA deletions were present.

When the pathogenic R194H mutation is modeled in yeast Erv1p (R182H), growth is normal on fermentative and respiratory media. However, Erv1p R182H does not support growth at elevated temperature. Cytochrome aa_3, but not *b* or *c*, is reduced, and cytochrome oxidase (COX or complex IV) activity is correspondingly reduced by 45%, suggesting that complex IV subunits or assembly factors are not efficiently imported. The frequency of *petite* colony formation in the mutant also doubled compared to the wild type controls (107).

Confirmed and predicted substrates of the disulfide relay system include several COX assembly factors (104, 105), which help to explain the specific cytochrome aa_3 reduction and decreased COX activity. Other substrates include Mdm35p and small Tim proteins which may cause abnormal mitochondrial morphology and mtDNA destabilization either directly or indirectly when they are not imported properly. Thus, the phenotypes observed in the patients likely result from the defective import of substrates of the disulfide relay system which was first identified and subsequently molecularly characterized in yeast (108-124).

5.2. Small Tim proteins

The small Tim proteins (in yeast Tim8p, Tim9p, Tim10p, Tim12p, and Tim13p) are structurally related IMS proteins which assemble into soluble hetero-oligomeric complexes (except for Tim12p), and contain twin CXXXC motifs that are required for import and folding (all of the small Tims). The small Tim proteins are canonical substrates of the aforementioned disulfide relay system. The assembled hexameric small TIM complexes (125) function to deliver hydrophobic polytopic membrane proteins from the TOM complex across the aqueous IMS to the TIM22 translocon which integrates such proteins into the IM (126-131). Tim9p assembles with Tim10p and Tim8p assembles with Tim13p. The Tim9/Tim10 complex is the major chaperone for substrates of the TIM22 pathway across the IMS. In addition, a separate pool of Tim9p and Tim10p, together with Tim12p, are stably associated with the TIM22 translocon (128). While both Tim9p and Tim10p are essential for yeast viability, yeast can survive in the absence of Tim8p and/or Tim 13p. To date, the only known substrate of the Tim8/Tim13 complex is the pore-forming subunit of the TIM23 translocon, Tim23p (129, 132, 133). Tim8p, and its human homolog, deafness dystonia peptide 1 (DDP1), were identified by sequence similarity to the other small Tim proteins in yeast (134). Functional conservation is demonstrated by delta*tim8*delta*tim13* yeast that co-express DDP1 and human TIM13. Mitochondria isolated from this strain are able to import both human and yeast Tim23p (135, 136).

Mutations in the human deafness dystonia peptide 1, *DDP1*, result in Mohr-Tranebjaerg syndrome. Patients present with sensorineural hearing loss, dystonia, mental retardation, and blindness. Most of the documented mutations result in a truncated protein due to frameshift mutations or premature stop codons (137-140)**,** with the exception of one intronic mutation predicted to alter splicing (141) and two missense mutations, C66W (142) and M1I (143). All of the mutations result in a complete loss of DDP1 function.

TIM8 is not essential for viability (134), but the delta*tim8*delta*tim13* strain is not viable at low temperature (133), providing a simple assay to test for Tim8p function in pathogenic mutants (144). The Tim8/Tim13 complex is especially required when the mitochondrial membrane potential is low (133), presumably to retain imported peptides in the IMS and prevent retrograde translocation back to the cytoplasm.

The pathogenic DDP1 mutation C66W occurs within the CXXXC motif. When this mutation is modeled in Tim8p, it cannot form a complex with Tim13p, despite proper mitochondrial localization (135, 144). In addition to an inability to fold properly, the mutation may also prevent proper handling by the disulfide relay system, thus providing another possible molecular explanation for the loss of function of this DDP1 mutation.

6. MITOCHONDRIAL DYNAMICS

Mitochondria are not static organelles; instead they form an elongated network that continually fuses, divides, and moves throughout the cell. Mitochondrial fusion allows mixing of proteins and mtDNA between individual mitochondrion, minimizing the effect of ROS-mediated mutations and allowing gene complementation (reviewed in (145, 146)). Mitochondrial fission, on the other hand, is important for segregation of dysfunctional mitochondria which will be eliminated by mitophagy (147), and plays a role in apoptosis (reviewed in (148)). Disruptions in the balance of these two opposing processes lead to mitochondrial dysfunction.

6.1. Fusion

6.1.1. Outer membrane fusion

In mammals, fusion of the outer membrane requires two genes: *MFN1* and *MFN2*. MFN1 and MFN2 are GTPases that tether adjacent membranes (149, 150) and mediate fusion. MFN1 and MFN2 can form homo- or hetero-oligomeric complexes via their heptad repeat region (150). Null mutations in either MFN1 or MFN2 are embryonic lethal in mice (151). However, only when both MFNs are disrupted in mouse embryonic fibroblasts (MEFs) is fusion completely abolished resulting in reduced growth, mitochondrial membrane potential, and respiration (151). Null mutations in only one MFN in MEFs results in greatly reduced mitochondrial fusion, but no major cellular dysfunction (151). As a result, distinct functions for each MFN have been postulated (149, 152). When Charcot-Marie-Tooth disease type 2A mutant MFN2 is expressed in MEFs, wild type MFN1 but not wild type MFN2 is able to rescue fusion (153), highlighting the functional importance of MFN1/MFN2 hetero-oligomers. Yeast contain only one MFN homolog, Fzo1p. Studies in yeast have been instrumental in determining the mechanism of mitochondrial outer membrane fusion (154, 155).

Mutations in MFN2 result in the autosomal dominant Charcot-Marie-Tooth disease type 2A (CMT2A) (156). Patients with *MFN2* mutations present with axonal degeneration of peripheral nerves and muscle weakness, although the age of onset and severity is variable (reviewed in (157)). Approximately 60 mutations in *MFN2* have been found in patients with CMT2A (158), most of which occur in or near the GTPase domain. Since wild type MFN1 can complement mutated MFN2 (153), it is suggested that tissues with lower MFN1 expression are more sensitive to MFN2 mutations.

Modeling CMT2A mutations in yeast Fzo1p have had mixed results. For example, the pathogenic mutant I213T, when modeled in Fzo1p, results in a complete loss of function. Mitochondria are fragmented and aggregated, and no GTPase activity is detected (159). However, when the CMT2A mutation T105M is modeled in yeast, mitochondria remain tubular, but with slightly altered morphology and poor distribution. Mitochondria retain the ability to fuse, and no respiratory growth defect is observed (159). In contrast, the MFN2 T105M mutant results in no mitochondrial fusion when expressed in *mfn1*$^{-/-}$ *mfn2*$^{-/-}$ MEFs (153). Similarly, no fusion defect is observed when the pathogenic CMT2A mutation L734V is modeled in yeast. In addition, the dominant negative phenotype of MFN2 mutations in CMT2A is not recapitulated in the yeast model (159). The inability of the yeast model to fully recapitulate the phenotype of patient mutations may reveal fundamental differences in OM fusion between yeast and mammals.

6.1.2. Inner membrane fusion

Fusion of the IM is mediated by OPA1 in mammals, and by its ortholog Mgm1p in yeast (160). Mgm1p is a dynamin-related GTPase that self assembles and tethers adjacent inner membranes, bringing them into close physical proximity for fusion (161). Like other dynamin family proteins, OPA1/Mgm1p contain a conserved GTPase domain, a middle domain, and an assembly or GTPase effector domain (GED) (162). The middle and GED domains are thought to promote self-assembly into spiral-like structures that stimulate GTPase activity (163). Similar to Fzo1p mutations, mitochondria in *mgm1* deficient yeast are unable to fuse (164, 165), leading to fragmented mitochondria. However, the fragmentation phenotype can be rescued by disrupting the mitochondrial fission gene, *dnm1* (164, 166).

There are multiple OPA1 isoforms that are generated as a result of alternative splicing and limited proteolysis by the *m*-AAA and *i*-AAA proteases (167-171). The proper balance of the numerous OPA1 isoforms is critical for IM fusion. This is illustrated by the fact that

OPA1 is subject to an inducible cleavage event, mediated by the metalloprotease OMA1, which is stimulated upon dissipation of the mitochondrial membrane potential and results in the selective removal of the long OPA1 isoform, thus preventing IM fusion (168, 172, 173). Dissipation of the membrane potential signals mitochondrial dysfunction and prevents fusion of damaged mitochondria with healthy mitochondria. In yeast, only two Mgm1p isoforms are present, a long (l-Mgm1p) and short (s-Mgm1p) isoform, which are present in roughly equal amounts. The short isoform is generated through cleavage by the rhomboid protease, Pcp1p (174, 175). Both isoforms are required for competent fusion and mitochondrial morphology maintenance (174). l-Mgm1p is anchored to the IM through its N-terminal domain, while s-Mgm1p is thought to be a soluble IMS protein. During fusion both isoforms associate to form heteromeric complexes, either in the same or in an adjacent membrane, to facilitate IM fusion (176, 177).

OPA1 is mutated in optic atrophy type 1, a dominant optic neuropathy. Patients present with progressive loss of visual acuity, optic nerve pallor, and abnormalities in color vision. These symptoms are suggested to be the result of retinal ganglion cell degeneration, which leads to optic nerve atrophy (178). Patients also exhibit altered mitochondrial morphology (160). Over 200 *OPA1* mutations have been documented (179), most of which are predicted to lead to OPA1 loss of function, either by truncation or mutations in the GTPase domain that render OPA1 catalytically inactive. Additionally, some patient cells harboring *OPA1* mutations exhibit increased OPA1 processing, leading to extensive mitochondrial fragmentation (168), a phenotype that highlights the importance of a proper balance of OPA1 isoforms for IM fusion.

Mgm1p mutations in the GTPase or GED domains are unable to rescue delta*mgm1* yeast (164, 166). Expression of mutant Mgm1p in the presence of wild type Mgm1p retains the fusion defect, which is exacerbated as the copy number of the mutant Mgm1p increases (166). This potentially provides a mechanism for the dominant inheritance of optic atrophy type 1. Any mutation that disrupts the function of the GTPase domain would assemble into oligomers with wild type Mgm1p and prevent proper mitochondrial fusion. Truncation mutants may exhibit dominant inheritance through haploinsufficiency, since the middle and GED domains required for oligomerization are located on the C-terminus (162, 166).

In yeast lacking *mgm1*, ATP synthase assembly is disrupted due to a decreased abundance of Tim11p/subunit e (180), which is required for ATP synthase multimerization (181). Additionally, cristae formation is altered, and cytochrome *c* levels are reduced (180). As oligomers of the ATP synthase are required to maintain normal cristae morphology (182-184), the reduced abundance of subunit e in the absence of Mgm1p may contribute to the altered cristae morphology. The loss of cytochrome *c*, which is also seen in *OPA1* mutants, could lead to increased levels of apoptosis, providing another potential mechanism of pathogenesis. Another possibility is that mitochondrial fusion enables protein complementation and mtDNA repair, consistent with the fact that loss of fusion leads to mtDNA depletion (185).

Defects in the mitochondrial fusion machinery also affect mitochondrial movements (186), which may help explain why neurons, which require mitochondria to traverse large distances from cell body to synapse, are most affected in mitochondrial fusion diseases (185, 187).

6.2. Fission

Mitochondrial fission is mediated by another dynamin-related protein, DRP1 in humans (188), and its homolog Dnm1p in yeast (189). Dnm1p is a GTPase that localizes to sites of mitochondrial fission (190), oligomerizes (191) and constricts membranes in a similar manner to dynamin (192). Interestingly, one difference between dynamin spirals and Dnm1p spirals is their size. The oligomeric Dnm1p spirals are larger than those formed by dynamin to accommodate the larger and more variable size of mitochondria compared to endocytic vesicles (192, 193). In the absence of DRP1/Dnm1p, mitochondria form elongated, highly branched networks due to continuing and unopposed mitochondrial fusion (189, 190).

In contrast to the numerous pathogenic mutations in proteins involved with mitochondrial fusion, to date only one pathogenic mitochondrial fission mutant has been reported. The patient showed abnormal brain development, abnormal gyral pattern and dysmyelination, optic atrophy, hypotonia, and elevated lactate levels. The patient died at 37 days. Despite the elevated lactate levels, which indicate respiratory chain dysfunction, histochemical analysis did not indicate a respiratory defect (194). A point mutation, A395D, was found in the middle domain of DRP1.

Middle domain mutants in Dnm1p had been previously shown to prevent oligomerization and consequently decrease GTP hydrolysis (195, 196). As such, the pathogenic DRP1 mutation as well as other middle domain mutations were shown by yeast two-hybrid to self-assemble into tetramers, the basic building block of DRP1/Dnm1p, but fail to form higher order oligomers. *In vitro* assays demonstrate that these mutants have decreased GTPase activity (197), consistent with studies in yeast that show an increase in GTP hydrolysis as Dnm1p assembles into higher order oligomers (193).

In delta*dnm1* yeast, mitochondria are still inherited by daughter cells normally and retain membrane potential, respiratory growth capacity, and proper protein import, despite displaying abnormal morphology (189). It is unclear how a mutation that does not appear to cause major mitochondrial dysfunction in yeast is able to translate into such a devastating phenotype in humans, although one possibility is through impaired mitophagy.

Mitophagy is the removal of mitochondria via macroautophagy, and facilitates both the reduction of

mitochondrial numbers during decreased metabolic demand and the targeted removal of dysfunctional mitochondria (198). The stark difference in phenotype between the *DRP1* mutant patient and delta*dnm1* yeast could be the result of high mitochondrial demand in neurons and lower demand for yeast grown in glucose-containing media.

7. MITOCHONDRIAL PROTEASES

The mitochondrial inner membrane possesses two metalloproteases that belong to the AAA (ATPase Associated with various cellular Activities) family of P-loop ATPases. The *i*-AAA protease is a homo-oligomeric complex that protrudes into the IMS, whereas the *m*-AAA protease faces the matrix. The *m*-AAA protease in yeast is strictly hetero-oligomeric, composed of the subunits Yta10p and Yta12p. In contrast, the mammalian *m*-AAA protease can assemble as hetero-oligomers and depending on the subunit, additionally into homo-oligomers (199). These proteases degrade non-assembled or misfolded subunits of respiratory complexes, and process premature peptides to their mature forms (for review, see (200)). As such, these proteases are critical components of the quality control apparatus of the mitochondrial IM.

The *m*-AAA protease in humans is encoded by *SPG7* (*PARAPLEGIN*) and *AFG3L2* (mice contain a 3rd gene, *AFG3L1* that is a pseudogene in humans). The yeast homologs are *YTA10* and *YTA12*. Expression of AFG3L2 and SPG7 in a delta*yta10*delta*yta12* yeast strain is able to restore protease activity demonstrating both functional conservation and that yeast substrates are recognized by the mammalian proteins. Intersubunit complementation is also possible; an *m*-AAA protease consisting of a Yta10p with a mutated ATPase domain and a Yta12p harboring a mutated protease domain retains proteolytic activity (201, 202). Among the substrates of the *m*-AAA protease are MrpL32p, a nuclear-encoded mitochondrial ribosome subunit; and Cox1p and Cobp, mitochondrially encoded subunits of respiratory complexes IV and III, respectively. In the absence of *m*-AAA protease function OXPHOS is impaired due to unassembled respiratory complexes (203-205), a consequence of impaired ribosome assembly due to the defect in MrpL32p processing and biogenesis (206). This provides an easy screening method in the yeast model; if a mutation in an *m*-AAA protease subunit ablates function, the complemented strain will be unable to grow on non-fermentative carbon sources.

Hereditary spastic paraplegia (HSP) is a group of heterogeneous neurodegenerative disorders characterized by progressive weakness and spasticity of the lower limbs (207, 208). One cause of HSP is a non-functional SPG7. Patients exhibit degeneration of axons, but not neuronal cell bodies, in corticospinal tracts, dorsal columns, and spinocerebellar fibers. Skeletal muscle biopsies show ragged red fibers (indicative of mitochondrial proliferation) and COX deficiency (209). The yeast model has been used to distinguish pathogenic mutations from silent polymorphisms in patient gene sequences. By co-expressing the mutated SPG7 gene with a non-functional AFG3L2 in a delta*yta10*delta*yta12* yeast strain, impairment of proteolytic activity can be easily inferred by the strain's ability to grow on respiratory substrates (202).

The other subunit of the *m*-AAA protease, AFG3L2, has recently also been implicated in human disease. AFG3L2 is one of the 28 known loci that cause autosomal dominant spinocerebellar ataxias; neurological disorders characterized by imbalance, progressive gait and limb ataxia, and dysarthria that results from Purkinje cell degeneration (210, 211). Like pathogenic SPG7 mutations, AFG3L2 mutations disrupt *m*-AAA proteolytic activity and cause a defect in COX activity. Unlike SPG7, AFG3L2 is able to form functional homo-oligomers. Thus, whereas in the absence of SPG7, functional AFG3L2 homo-oligomers persist, in the absence of AFG3L2, there is no functional *m*-AAA protease. This might explain the differences in the severity and pattern of inheritance that result from mutations in SPG7 versus AFG3L2. Six missense mutations have been described; four in the protease domain, one in the ATPase domain, and one in a region outside any conserved domain. When the AFG3L2 mutations are expressed in delta*yta10*delta*yta12* yeast, the five that occur in conserved regions fail to rescue respiratory growth or proteolytic activity (212). In the patients, no defect is observed in skeletal muscle. The authors' suggest that the neuron-specific phenotype may be due to differences in homo- versus hetero-oligomeric subunit composition in the affected cell types which may result from tissue specific differences in the relative expression of each subunit (199).

8. RESPIRATORY COMPLEXES

Respiratory complexes are multisubunit assemblies which, except for Complex II, incorporate proteins encoded by both the mitochondrial and nuclear genomes. Thus the biogenesis of the OXPHOS system is a complicated and highly regulated process that involves chaperones and assembly factors that facilitate complex assembly as well as the insertion of cofactors (FAD, Fe/S, hemes, and Cu) required for electron transport (reviewed in (213)). Given this complicated process, not surprisingly, mutations associated with disease reside within both genomes and target not only subunits of the OXPHOS complexes, but also proteins required for their proper assembly. Again, yeast lacks respiratory complex I and is thus not a suitable model to study this abundant class of mitochondrial disease. However, yeast has proven invaluable in characterizing mutant proteins required for the proper function of the other OXPHOS components (Figure 1), as detailed below.

8.1. Complex II

8.1.1. Complex II subunits

Succinate dehydrogenase (SDH) is simultaneously involved in two metabolic processes: in the TCA cycle, it oxidizes succinate to fumarate; and in the electron transport chain, it transfers electrons from succinate to coenzyme Q (CoQ) (Figure 2A). In mammals, SDH is composed of 4 nuclear encoded subunits, SDHA-D, which are homologous to yeast Sdh1-4p. SDHA/Sdh1p is a

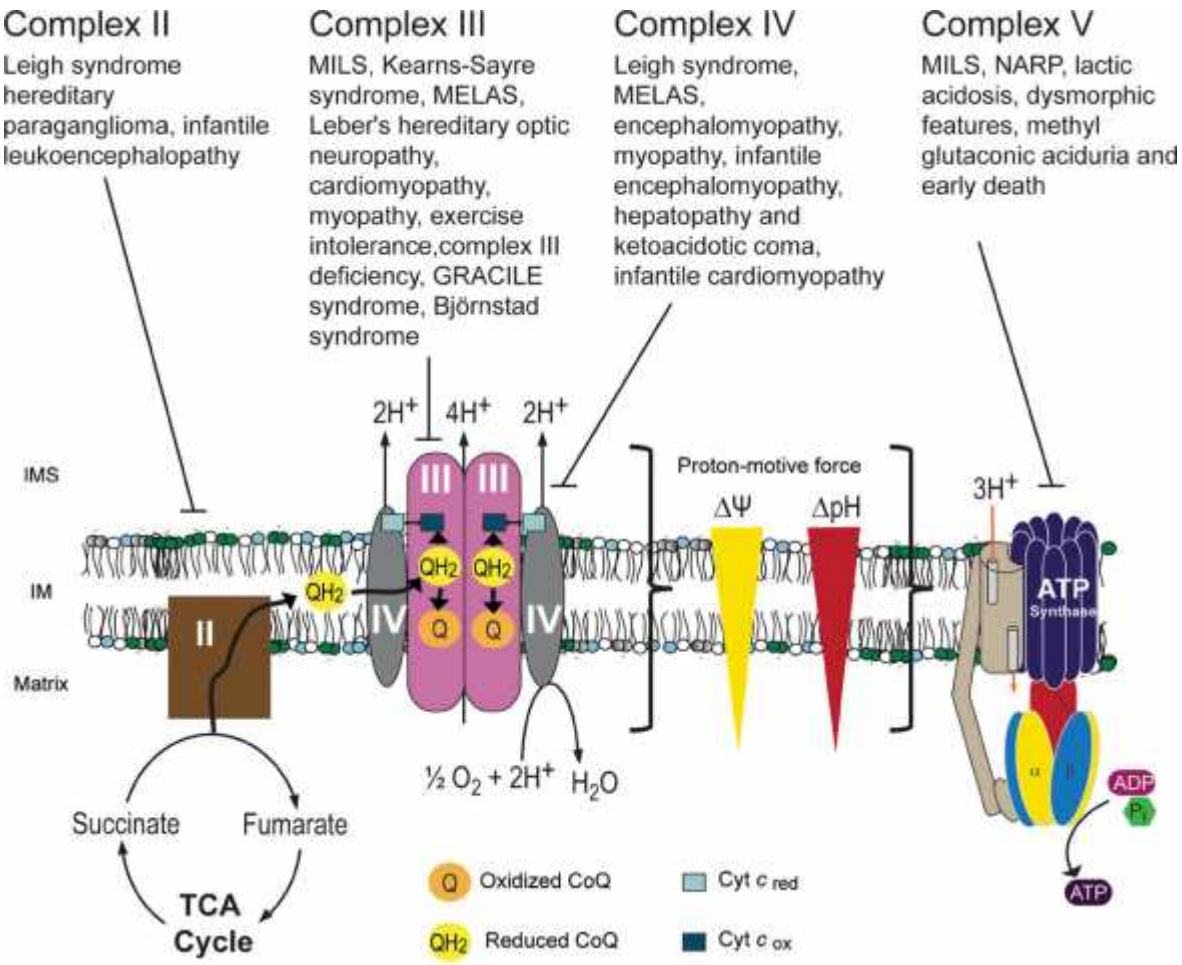

Figure 1. Summary of OXPHOS in yeast and the multitude of patient phenotypes associated with pathogenic lesions that impact each individual complex. Complex II (*brown*), also referred to as SDH, is a physical link between the TCA cycle and the electron transport chain. Electrons derived from the oxidation of succinate to fumarate by Complex II ultimately reduce CoQ (QH_2). QH_2 engages one of two CoQ binding sites within Complex III (*pink*) where its two electrons reach a fork in the road. One electron ultimately reduces cytochrome *c* while the other electron is delivered to oxidized CoQ (Q) in the 2nd CoQ binding site within Complex III producing a reactive semiquinone. The semiquinone is fully reduced to QH_2 upon the release of a second wave of electrons from another QH_2 completing the Q cycle. The release of electrons by QH_2 is coupled to the discharge of H^+ into the IMS. Reduced cytochrome *c* donates its single electron to Complex IV (*grey*) which couples the reduction of O_2 to H_2O with the vectorial transport of H^+ into the IMS. Complexes III and IV assemble in supercomplexes that increase the efficiency of the electron transport chain by substrate channeling. The proton-motive force established by Complex III and IV is harnessed by the Complex V (aka the ATP synthase) to produce ATP. The numerous phenotypes observed in patients with mutations associated with each individual OXPHOS complex is indicated.

flavoprotein that, with the Fe/S protein SDHB/Sdh2p, forms the catalytic core of SDH. SDHC/Sdh3p and SDHD/Sdh4p anchor the catalytic core to the matrix side of the IM and are the site of CoQ reduction (reviewed in (214, 215)).

Seven mutations in SDHA have been shown to cause Leigh syndrome (216-221). Leigh syndrome is a genetically heterogenous disease that results from a deficiency in ATP production (222). It is a progressive neurodegenerative disorder characterized by focal, bilateral spongiform lesions of the central nervous system, and presents as intellectual retardation, nystagmus, optic atrophy, opthalmoparesis, ataxia, dystonia, and abnormal breathing rhythm (222).

When the first SDHA mutation in Leigh syndrome patients was identified, the mutation was introduced into yeast Sdh1p. Compared to wild type, the mutant Sdh1p has a 50% decrease in SDH activity, providing evidence that the detected *SDHA* mutation was a *bona fide* pathogenic mutant, and that the symptoms were not the cause of some other, undetected mutation (216). It should be noted that this was the first defined

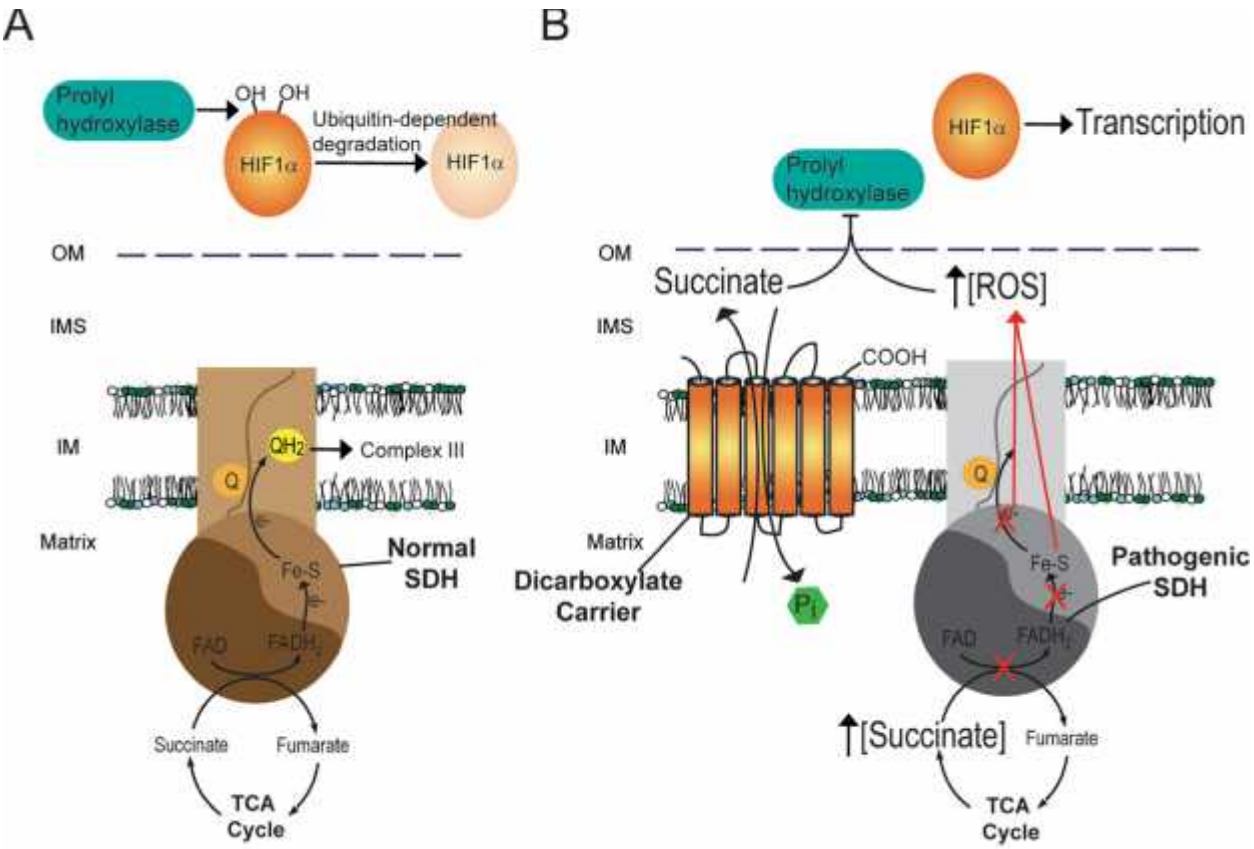

Figure 2. Two non-mutually exclusive models of how mutations in SDH promote tumorigenesis. (A) SDH is a nexus between the TCA cycle and the electron transport chain. In the TCA cycle, it oxidizes succinate to fumarate. The two released hydrogens in turn reduce the FAD cofactor associated with SDHA/Sdh1p. Electrons are transferred from $FADH_2$ to a series of three Fe/S clusters in SDHB/Sdh2p and ultimately to oxidized CoQ (Q) generating reduced CoQ (QH_2) which donates its electrons to Complex III. In the cytosol, HIF-1 alpha is rapidly degraded subsequent to its prolyl hydroxylation (degradation indicated by faded coloring). (B) Mutations in SDH result in an accumulation of succinate which is transported into the cytosol by the dicarboxylate carrier in exchange for P_i. If the pathogenic mutation occurs in a subunit other than SDHA/Sdh1p, the flow of electrons is uncoupled from the reduction of CoQ and ROS are generated. ROS and succinate can both inhibit HIF-1 alpha prolyl hydroxylases leading to HIF-1 alpha stabilization. HIF-1 alpha induces the expression of genes involved in angiogenesis, proliferation, cell survival, and glycolysis; pathways that would benefit a tumor.

mitochondrial disease-associated mutation of a nuclear encoded mitochondrial protein.

Mutations in SDH also cause autosomal dominant inheritable forms of cancer, such as head and neck paragangliomas, pheochromocytomas (217), and renal cell cancer (223). SDH acts as a classic tumor suppressor, where loss of function leads to tumorigenesis. The wild type allele is often deleted in patient tumors. This loss of heterozygosity completely abolishes SDH activity because the only remaining allele is non-functional (224, 225). Over 300 unique mutations have been found that cause paraganglioma/pheochromocytomas, spanning all four SDH subunits (217).

While the mechanism of how SDHA mutations cause Leigh syndrome is straightforward, how SDH mutations lead to tumorigenesis is less clear. Two non-mutually exclusive hypotheses have emerged (Figure 2B). In the ROS hypothesis, it is proposed that mutations in SDHB-D cause SDH defects, allowing for the uncoupled flow of electrons from succinate to oxygen or water (226, 227). Essentially, electrons enter complex II by virtue of the SDH activity of SDHA but they are unable to reduce CoQ. The generated ROS then can either mutagenize nuclear proto-oncogenes and/or tumor suppressors, or instead stabilize hypoxia inducible factor-1 alpha (HIF-1 alpha). HIF-1 alpha induces the expression of genes involved in angiogenesis, proliferation, cell survival, and glycolysis; pathways that would benefit a tumor (228).

The recent identification of a pathogenic SDHA mutation causing paraganglioma while inhibiting SDH activity (no succinate oxidation and thus electrons do not

enter complex II; (229)) argues against ROS generation as the only tumorigenic mechanism. In yeast harboring pathogenic mutations in Sdh2p, Sdh3p, or Sdh4p, increased ROS production is observed and the mutant yeast are hypersensitive to oxidative stress (227, 230). However, there is conflicting evidence as to whether the ROS generated in the mutants is sufficient to cause mtDNA damage (231, 232).

The second mechanism involves the accumulation of succinate. With impaired/absent SDH activity, succinate builds up in the matrix, and is transported into the cytoplasm by the dicarboxylate carrier (227, 232). Cytosolic succinate can then act as an inhibitor of alpha-ketoglutarate-dependent enzymes including prolyl hydroxylases that normally facilitate the rapid ubiquitin-dependent removal of HIF-1 alpha (233). Succinate-mediated inhibition of HIF-1 alpha prolyl hydroxylases thus stabilizes the normally transient HIF-1 alpha resulting in the activation of numerous tumor-promoting pathways (228). As both ROS and succinate can impinge at the level of HIF-1 alpha stabilization by inhibiting the HIF-1 alpha prolyl hydroxylases, both models are likely to contribute to tumorigenesis associated with *SDH* mutations.

8.1.2. Complex II assembly factors

Two SDH assembly factors have recently been identified. Sdh5p was identified in yeast as a soluble protein located in the matrix that is required for SDH activity but is not a stable component of complex II. In the absence of Sdh5p, Sdh1p is not flavinated, and therefore non-functional. In the human ortholog, hSDH5/SDHAF2 (SDH Assembly Factor 2), a G78R mutation was found in a Dutch paraganglioma lineage that co-segregated with the disease (234). As predicted based on the yeast studies, SDHA flavination is reduced by 95% in these patients. Growth on respiratory media of delta*sdh5* yeast can be rescued by wild type human SDHAF2, but not when the pathogenic mutant SDHAF2 is expressed.

Two mutations have been described in a second SDH assembly factor, SDHAF 1, in patients with infantile leukoencephalopathy (235-237). The yeast ortholog was deleted, revealing an inability to grow on respiratory media. When the pathogenic mutations G57R and R55P were modeled in the yeast gene (as G63R and R61P), SDH activity is decreased to 30-40% of wild type and respiratory growth inhibited. However, COX enzymatic activity, respiration, and cytochrome spectra show no abnormalities, indicating that the shortcoming is due to SDH deficiency and not a generalized respiratory defect (237). Thus, SDHAF1 functions as an evolutionarily conserved assembly factor, although further studies are necessary to determine its precise molecular function.

8.2. Complex III

8.2.1. Complex III subunits

The cytochrome bc_1 complex (complex III) transfers electrons from CoQ to cytochrome *c*. In mammals, Complex III consists of 11 subunits, whereas in yeast complex III only contains 10 subunits. Three catalytic subunits are responsible for electron transfer: cytochrome *b* (Cobp; the only subunit encoded in the mitochondrial genome), cytochrome c_1 (Cyt1p), and the Rieske Fe/S protein (Rip1p) (238, 239).

There are currently 33 reported COB mutations (14) that result in a variety of symptoms, including cardiomyopathy, myopathy, exercise intolerance, Leber's hereditary optic neuropathy, Kearns-Sayre syndrome, MELAS, and maternally inherited Leigh syndrome (14, 240). In addition, non-pathogenic polymorphisms have been described (241).

Modeling patient mutations in yeast has not only helped to distinguish between pathogenic and silent mutations, but also facilitated elucidation of the mechanism of complex III dysfunction. For example, when the pathogenic mutants K319P, G33S, S152P, and G291D are modeled in yeast, there is a decrease in steady state abundance of complex III subunits, implying that the mutations introduce structural changes into Cobp that either destabilize the enzyme, or prevent its assembly (242, 243). On the other hand, the pathogenic mutants E272D, E272Q, and Y279C all decrease the binding affinity of quinol (reduced CoQ; (243, 244)), and G167E alters the interaction between cytochrome *b* and Rip1p, hindering Rip1p movement and decreasing the catalytic rate of complex III (245).

8.2.2. Complex III Assembly Factors

BCS1 was first identified from a yeast mutant screen as being involved in the assembly of Rip1p (246). It resides in the inner membrane, and is an AAA protein family member. Required at a late stage in complex III biogenesis, Bcs1p binds to a complex III subcomplex in an ATP-dependent manner, and facilitates the incorporation of Rip1p (247).

Mutations in *BCS1L*, the human ortholog of yeast *BCS1*, result in a variety of phenotypes: complex III deficiency (248-251), which is manifested as neonatal tubulopathy, encephalopathy, and liver failure; GRACILE syndrome (252), characterized by growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death; and Björnstad syndrome (253), which presents as sensorineural hearing loss and pili torti.

In most studies of pathogenic BCS1L mutations, the human gene is expressed in a delta*bcs1* strain, and the ability to grow on respiratory media assessed. Wild type human BCS1L provides a partial rescue of the delta*bcs1* yeast growth defect (248), suggesting that despite the high similarity between the yeast and human proteins, subtle differences exist. Still, the growth assay provides a means to determine the severity of a pathogenic mutation. For instance, the R155P mutation causing complex III deficiency results in a complete loss of respiratory growth, while the S277N mutation retains some growth, albeit severely reduced when compared to wild type BCS1L. However, corresponding differences in the respiratory complex enzymatic activity in patients harboring these

particular mutations is not observed (248). When a BCS1L mutant associated with GRACILE syndrome is placed in a delta*bcs1* yeast strain, the respiratory growth defect is not rescued indicating a loss of function. However, curiously, biopsies of the patient's tissue revealed no defect in complex III function (252).

A BCS1L mutation associated with complex III deficiency, R45C, results in severe complex III deficiency in patient tissue (249), but surprisingly, when the mutation is modeled at the equivalent residue in the yeast Bcs1p, no growth abnormality or complex III defect is observed (254). The observed difference between the human patient and the yeast model likely reflects subtle differences in mitochondrial targeting in yeast and mammals. The two other BCS1L mutations from the same study, K192 and F401I, displayed the expected phenotype of no respiratory growth and stable complex III subcomplexes at the expense of fully assembled complex III (254).

8.3. Complex IV

8.3.1. Complex IV subunits

Cytochrome *c* oxidase (COX or complex IV) transfers electrons from cytochrome *c* to O_2. It consists of 3 mitochondrially encoded subunits, Cox1p, Cox2p, and Cox3p. Cox1p and Cox2p contain the heme groups (*a* and a_3) and copper centers (Cu_A and Cu_B) required for electron transfer. Cox3p may provide a structural role in stabilizing the catalytic core, or play a role in proton pumping (reviewed in (255)). The additional subunits (10 in mammals, 8 in yeast) stabilize the catalytic core, but are nonetheless essential for COX function, since the disruption of most subunits abolishes COX assembly (256).

COX deficiency is one of the most common respiratory chain defects in humans. Mutations in the mitochondrially encoded subunits present heterogeneous clinical phenotypes, including Leigh syndrome, MELAS, encephalomyopathy, myopathy, motor neuron-like degeneration, myoglobinuria, and Sideroblastic anemia (256, 257).

When the I280T COX1 mutant is modeled in yeast, respiratory growth is decreased, and COX activity is reduced to 50% of wild type. Although no decrease in overall COX content (as determined by cytochrome *a* spectra) is detected, the authors noted a subpopulation of the enzyme that is unable to bind cyanide or carbon monoxide (258), two inhibitors of COX. Potentially, the mutation affects assembly or decreases the stability of the holoenzyme, creating a minor population of misassembled or disassembled complexes. Alternatively, the mutation may increase the probability that Cox1p will misfold, resulting in a non-reactive binuclear center (the site of O_2 reduction in COX). Another COX1 mutant, M273T, also shows decreased respiratory growth and COX activity, while retaining wild type levels of cytochrome *a*. Unlike I280T, the M273T mutant displays an increased reaction rate of carbon monoxide with COX (258). Since these mutations occur near the conserved K channel, which provides access of H^+ from the matrix to the binuclear center (259), the rate of electron transfer from heme *a* to the binuclear center may be reduced, decreasing O_2 consumption (258).

Interestingly, the G317S COX1 mutant, which was detected in a patient presenting with COX deficiency and lactic acidosis, shows no COX defect when modeled in yeast, despite occurring at a conserved residue. Respiratory growth, COX activity, and cytochrome *a* content in the mutant is indistinguishable from wild type strains (258). Additional studies using patient-derived cells determined that the COX defect was of nuclear origin (260). Additionally, mutations L196I and A223S were identified in patients, but the yeast models display no COX defects (261). These mutations are now considered silent polymorphisms (14). These examples highlight the power of yeast models to distinguish between pathogenic mutations and neutral polymorphisms.

When the pathogenic deltaF102-F106 microdeletion is modeled in yeast Cox3p, respiratory growth is absent, and no COX activity is detected (258), consistent with the results seen in patient-derived tissue culture (262). A respiratory growth defect is also seen in a mutation resulting in a premature stop codon at residue 249 of Cox3p (261). This is in contrast to identified patient mutations G78S and A200T which display no defect when modeled in yeast Cox3p (261), and have also been reclassified as silent polymorphisms (14).

To date, only one mutation has been described in a nuclear-encoded COX subunit, COX6B1. Wild type COX6 is able to complement delta*cox12* yeast (yeast Cox12p is the ortholog of human COX6). Delta*cox12* yeast expressing mutant COX6 grow at a slower rate than wild type on respiratory media. Given that delta*cox12* yeast are unable to grow on respiratory media, these results indicate that the mutant protein still retains some function. Consistent with this, while oxygen consumption is 25% lower in the mutant than in wild type (263), there is no oxygen consumption in the delta*cox12* strain. Interestingly, Cox12p is one of the few COX subunits that are not absolutely required for COX activity once the enzyme is fully assembled (264).

8.3.2. Complex IV assembly factors

Over 20 additional proteins are required for the proper assembly of COX in yeast (reviewed in (255)). The human orthologs of many have been identified (265-267). In yeast, COX assembly begins with the translation of *COX1* which is enhanced by the Cox1p translational activator, Mss51p (268). In addition to functioning as a Cox1p translational enhancer, Mss51p additionally functions as a chaperone, which together with Cox14p and Coa3p (Cytochrome Oxidase Assembly), binds and stabilizes unassembled Cox1p (256, 269, 270). Worth mentioning, Mss51p in complex with unfolded Cox1p is unable to further stimulate Cox1p translation. Thus, in the absence of productive COX assembly, Mss51p is titrated out resulting in a drop in Cox1p production. Coa1p association then releases Mss51p and Cox14p and

promotes further Cox1p maturation (271, 272). The next step is believed to involve Cox11p and Shy1p which are required for the addition of the Cu_B cofactor to Cox1p, and Coa2p, Cox10p, and Cox15p, the latter two generating the heme *a* that is uniquely utilized by COX (273-276). Cofactor loaded Cox1p is further stabilized by two nuclear encoded subunits, Cox6p and Cox5p (277). The addition of Cu_A to Cox2p involves the Cu chaperone Cox17p, which traffics between the cytosol and IMS, and the copper binding protein Sco1p (and SCO2 in humans) (13, 278, 279). Steps downstream of this are at present ill-defined as are the exact mechanisms for the attachment of the numerous COX cofactors.

The yeast gene *SHY1*, and its human ortholog *SURF1*, is required for full COX activity (280), although its precise function remains incompletely defined. Mutations in *SURF1* cause Leigh syndrome (281-283) via COX deficiency. Surprisingly, patients' harboring SURF1 mutations still retain 10-30% normal COX activity. This indicates that either SURF1 is not absolutely required for COX assembly or that partially redundant activities exist.

When Leigh syndrome SURF1 missense mutations are modeled in Shy1p, mutants F249T and Y344D exhibit wild type levels of COX activity (284), although a cold sensitive phenotype on respiratory media is observed for the Y344D Shy1p mutant (285). The Y334D Shy1p mutant results in a destabilization of newly synthesized Cox1p, and an uncoupling of Cox1p translation from assembly (285). In contrast, when G137 is mutated to either Glu or Arg, both of which are pathogenic mutations, Shy1p is imported efficiently into mitochondria, but is degraded rapidly, resulting in much lower steady state levels of the mutant Shy1p (285). The G137E mutant was also used to screen for suppressors, which identified the previously known assembly factors Coa2p and Cox10p, as well as the novel assembly factor Coa4p (284).

Yeast *SCO1* and *SCO2* were first identified as high copy suppressors of the COX assembly factor Cox17p (13), a soluble protein that transfers Cu to the IM protein Sco1p (279, 286). Sco1p is able to bind Cu via the CXXXC motif (287, 288) and physically associate with Cox2p, although this association is not dependent on either Cu or conserved Cu binding residues (289). These observations have led to two models for Sco1p mediated Cu delivery to COX. Sco1p could directly transfer Cu from Cox17p to Cox2p (13). Or instead, Sco1p could reduce cysteine residues in Cox2p which is necessary for Cu binding (290). Despite homology with Sco1p, delta*sco2* yeast exhibit no COX deficiency and Sco2p overexpression cannot rescue a yeast *sco1* null mutant (13).

Humans also contain *SCO1* and *SCO2*; however sequence analysis shows that both human *SCO* genes display similar divergence from both yeast *SCO* genes, suggesting that gene duplication occurred independently in both yeast and humans, producing two sets of paralogous genes (291). Unlike yeast, both human SCO proteins are required for viability, and have non-overlapping functions in COX assembly (292).

The non-overlapping functions of human *SCO* genes is further supported by their clinical pathologies. Mutations in *SCO1* cause hepatopathy and ketoacidotic coma (293), while mutations in *SCO2* are associated with infantile cardiomyopathy (291, 294).

Human SCO1 is not able to rescue delta*sco1* yeast. However, chimeric yeast/human SCO1, consisting of an N-terminal yeast Sco1p and C-terminal human SCO1, is able to rescue delta*sco1* yeast (295). When the pathogenic mutation P174L is modeled in the chimeric protein (with the mutation occurring in the C-terminal human half of the protein), the mutant SCO1 is non-functional (296). The mutation occurs immediately adjacent to the CXXXC motif required for Cu binding (288), implying that the mutation affects the Cu binding ability of the mutant SCO1, although this still needs to be directly tested.

Yeast Sco1p has also been used to model human *SCO2* mutations. The mutation E155K in yeast Sco1p, matching the pathogenic E140K mutation in human SCO2, is able to rescue COX activity in yeast, although the steady state expression of the mutant Sco1p is slightly reduced (297). This is surprising since E140 is located only 3 amino acids away from the CXXXC motif and was predicted to negatively impact Cu binding of the mutant protein, similar to the pathogenic P174L SCO1 mutant. As such, this may illustrate subtle differences between the structure and/or function of yeast and human SCO proteins.

The S240F mutation, modeling the pathogenic human SCO2 mutation S225F, results in a dramatic COX defect (297). As with a delta*sco1* strain, Cox2p expression in the mutant is abolished, likely indicating that the mutant is unable to deliver Cu to Cox2p, which is then degraded. Interestingly, cytochrome aa_3 analysis suggests that COX may partially assemble, despite the complete absence of Cox2p, possibly demonstrating that the Sco1p polypeptide plays a role in stabilizing COX subassemblies independent of its role in metallating Cox2p (297).

Finally, it is worth mentioning that basic work on the biogenesis of COX in yeast has guided efforts to identify the basis of human COX deficiencies. One approach that has been successfully employed is to attempt to rescue COX-deficient patient-derived cells by overexpressing various human orthologs of COX assembly factors identified and characterized first in yeast. Such efforts have identified mutations in the heme A biosynthetic genes, *COX10* and *COX15*, in patients suffering from the entire range of phenotypes associated with COX deficiency or an early onset fatal cardiomyopathy, respectively (298, 299). Thus, in addition to defining the function of wild type genes and determining whether mutations are pathogenic or silent, work in yeast has guided efforts to identify pathogenic lesions in patients without prior molecular diagnoses.

8.4. Complex V

8.4.1. Complex V subunits

The mitochondrial ATP synthase utilizes the proton gradient generated by the other respiratory complexes to condense ATP from ADP and P_i (Figure 1). The ATP synthase consists of two functional units. F_1 faces the matrix and is hydrophilic. It consists of 5 subunits (3 alpha, 3 beta, gamma, delta, and epsilon), and is the section that generates ATP (or hydrolyzes it when not attached to the F_0). F_0 is hydrophobic and embedded in the inner membrane. It consists of 8 different subunits and mediates proton translocation through the oligomeric ring of c subunits (Atp9p in yeast). Only three subunits are encoded by the mitochondrial genome, Atp6p, Atp8p, and Atp9p, all of which are components of the F_0 (300).

Mutations in *ATP6* present with a range of phenotypes, from severe infantile maternally inherited Leigh syndrome (MILS), to adult onset neuropathy, ataxia, and retinitis pigmentosa (NARP) (301). Pathogenic mutations have been modeled in yeast to better understand the mechanism of ATP synthase dysfunction. The L183R Atp6p mutant decreases ATP synthesis by 90%, and decreases COX content by 95%. Despite the functional defect, ATP synthase assembles properly, indicating that the defect is catalytic in nature (302). When the same residue was mutated to Pro, ATP synthesis is only reduced by 40-50%, and COX abundance is decreased. BN-PAGE analysis showed an increased abundance of ATP synthase subcomplexes, indicating that the stability of the ATP synthase is reduced when this mutation is present (303).

Two pathogenic mutations at Leu 247 of Atp6p were also modeled. The L247R mutation causes complete ATP synthase disassembly, with a 95% decrease in ATP synthesis. The defect represents a complete loss of function, as it mimics the phenotype of delta*atp6* yeast (304). The L247P phenotype, on the other hand, is much less severe. ATP production is only decreased by 50%, and the complex displays increased instability (305). As with other yeast models, the authors note that the severity of the defect when each mutation is modeled in yeast correlates well to the severity of symptoms in patients.

8.4.2. Complex V assembly factors

Of the ATP synthase assembly factors identified in yeast ((306) and reviewed in (307)), only Atp11p and Atp12p have functional homologs identified in humans (308). Studies in yeast have shown that Atp11p and Atp12p bind selectively to newly imported beta and alpha subunits of F_1, respectively (309), acting as chaperones to facilitate the assembly of the alpha/beta hexamer of F_1. In the absence of either Atp11p or Atp12p, F_1alpha and F_1beta form large insoluble aggregates in the mitochondrial matrix (306).

A missense mutation, W94R, in human ATP12 has been reported in a patient presenting with lactic acidosis, dysmorphic features, and methyl glutaconic aciduria. The patient showed a severe defect in ATP synthase activity, as well as less severe defects in other respiratory complexes (310). Although many structure/function studies have been performed with Atp12p in yeast (reviewed in (311)), the molecular mechanism of the pathogenic mutant remains unknown, since it occurs outside of the functional domain, and point mutations at the homologous residue have not been reported.

9. IRON HOMEOSTASIS

Friedreich ataxia is a progressive autosomal recessive neurodegenerative disorder, causing ataxia in all four limbs, dysarthria, sensory loss, and muscle weakness. Most patients also present with skeletal deformities and cardiomyopathy (312). The disease-causing gene was identified as *FRATAXIN* (*FXN*), and it was noted that most pathogenic mutations were the result of GAA repeat expansions in the first intron (313). While most patients are homozygous for the expansion, others harbor the expansion in one allele and missense mutations in the other (312). Both forms of the *FXN* are thought to be loss of function mutations.

At the time of its identification, nothing was known about the function of FXN, other than it contained a putative N-terminal mitochondrial localization sequence. Homologs were identified in yeast and in *C.elegans* (313). Interestingly, the yeast homolog, *YFH1*, was identified as a high copy suppressor of the *bm-8* mutant, which is unable to grow on iron-limited media (314). Yfh1p was shown to be localized to mitochondria (314, 315), and disruption of the gene causes a *petite* phenotype associated with mtDNA instability (314-316). Pathogenic point mutations from patients were modeled in Yfh1p, and display a severe defect in respiratory capacity and mtDNA stability (315).

That Yfh1p was identified as a suppressor of *bm-8* implicated it in iron homeostasis. Indeed, studies in yeast have shown that in the absence of Yfh1p function, iron accumulates in mitochondria (314) at the expense of cytosolic iron (317). Importantly, iron accumulation is also seen in patients (318). Further demonstrating functional conservation between Yfh1p and human FXN, iron accumulation is rescued in delta*yfh1* yeast expressing the human *FXN* gene (319). When the pathogenic mutation G130V, associated with a mild clinical presentation, is generated in FXN and expressed in delta*yfh1* yeast, mutant FXN is properly imported into mitochondria but destabilized compared to wild type FXN. Surprisingly, despite the decreased abundance, this mutation is still sufficient to rescue the delta*yfh1* phenotype. In contrast, when a mutant FXN with a more severe clinical phenotype, W173G, is expressed in delta*yfh1* yeast, both FXN processing and stability are decreased, and the mutation is unable to rescue delta*yfh1*. These studies not only provide separate mechanisms for FXN dysfunction, but also show a good correlation between clinical severity and the extent of the phenotype in yeast.

Studies in yeast have also helped to elucidate the molecular function of Yfh1p/FXN. At low levels of

mitochondrial iron, Yfh1p exists as a monomer. However, at increased iron concentrations Yfh1p forms higher-order oligomers capable of sequestering up to 75 iron atoms per Yfh1p monomer (320-322) and, in-so-doing, preventing iron toxicity. This may provide a pathogenic mechanism for Friedreich ataxia. Without sequestration of iron, ROS can be generated and damage mtDNA, which is consistent with the *petite* phenotype of the delta*yfh1* strain.

However, Yfh1p was also shown to serve as an iron chaperone, keeping iron in a bioavailable form (322). Consistent with this, Yfh1p has been shown to physically interact with a subunit of SDH (323), which contains Fe/S centers; and Isu1p (324), the scaffold protein in the Fe/S cluster-assembly complex. The importance of Yfh1p/FXN in Fe/S cluster biosynthesis is seen in the lack of SDH and aconitase activity in both delta*yfh1* yeast strains (325) and Friedreich ataxia patients (326). Notably, Fe/S containing enzymes such as aconitase (an enzyme of the TCA cycle) and Sdh2p are highly susceptible to ROS mediated attack and inactivation, and FXN has been suggested to directly function as a chaperone that is capable of aconitase reactivation (327). Thus, FXN's role as an iron chaperone provides another possible mechanism for the respiratory defect seen in delta*yfh1* yeast (315) and patients suffering from Friedreich ataxia (326).

10. CARDIOLIPIN BIOSYNTHESIS

The lipid cardiolipin (CL) is enriched in mitochondrial membranes, especially the inner membrane, its site of synthesis (328). CL is an unusual lipid consisting of two phosphatidyl moieties bridged by glycerol. As such, CL contains four fatty acyl chains (Figure 3A) (329). When cardiolipin synthase forms CL from phosphatidylglycerol and cytidine diphosphate-diacylglycerol, the result is immature CL characterized by saturated acyl chains of variable length (Figure 3B) (330). Immature CL then undergoes a remodeling process in which the acyl chains are replaced with more unsaturated fatty acyl chains, forming mature CL, which displays a high degree of molecular symmetry (329). The remodeling process is initiated by a phospholipase (Cld1p in yeast), which removes an acyl chain, forming monolysocardiolipin (MLCL) (331). The MLCL transacylase, TAFAZZIN (Taz1p in yeast), then takes an acyl chain from another phospholipid (preferentially phosphatidylcholine or phosphatidylethanolamine) and adds it to MLCL thus regenerating CL (332). Through a series of such reactions, immature CL is converted to mature CL. Worth noting, the final form of CL in different tissues/organisms is not the same. However, within a given tissue, the molecular form of CL with respect to its acyl chain composition is highly homogenous ((333), for a review of CL metabolism, see (334)).

CL is associated with proteins involved in a diverse array of functions, including protein import (335-338); mitochondrial fusion (176); metabolite translocation (339-351); and OXPHOS (352-359). In fact, CL is bound to all the major OXPHOS components: respiratory complexes I, III, IV, and V, cytochrome *c*, ANT, and the phosphate carrier. Despite this, yeast lacking cardiolipin synthase (delta*crd1*) and thus CL, retain the ability to respire, although respiration is significantly diminished at elevated temperature (360, 361). Thus, while CL is not absolutely required for OXPHOS, it increases both OXPHOS efficiency and the dynamic range of conditions at which OXPHOS can function. Consistent with this, CL is required for the formation and/or stability of respiratory supercomplexes (362-364), large, multi-complex assemblies that in yeast consist of two complex IIIs and one or two complex IVs (365-367), and Aac2p-containing complexes, including an interaction between Aac2p and the respiratory supercomplex (368). Collectively, these higher order assemblies are thought to increase OXPHOS efficiency by substrate channeling.

Mutations in *TAFAZZIN* result in Barth syndrome (BTHS), an X-linked disease that presents as cardiac and skeletal myopathy, delayed growth until puberty, and cyclic neutropenia (330, 369). In addition, general respiratory chain deficiencies are observed (369-372), perhaps reflecting destabilized respiratory supercomplexes (373). In BTHS patients, CL levels are decreased and the acyl chain composition is notably abnormal (374). In addition, MLCL, the intermediate in the CL remodeling pathway, accumulates in the heart, skeletal muscle, lymphocytes, and lymphoblasts of BTHS patients (375).

Delta*taz1* yeast accumulate MLCL with a concurrent decrease in CL, which displays an immature acyl chain composition (376-380). In addition, yeast lacking Taz1p function display a growth defect on respiratory media at 37°C (378, 380). Consistent with BTHS patients, respiratory supercomplex assembly/stability has been reported in some (381), but not all (382) delta*taz1* yeast strains. Although 12 splice variants of human TAZ1 have been identified (383), only the splice variant lacking exon 5 is able to rescue delta*taz1* yeast (380, 384). Yeast Taz1p has been shown to assemble in distinct high molecular weight complexes with Aac2p and ATP synthase, as well as smaller complexes consisting of presently unidentified Taz1p binding partners (382). These former associations provide a direct link between Taz1p and the OXPHOS machinery that might explain some of the respiratory abnormalities observed in BTHS patients in addition to the documented defects in the levels and/or form of CL.

Of the 21 BTHS missense mutations that occur in regions of Taz1p conserved between humans and yeast, 18 are unable to rescue delta*taz1* yeast based on the significant accumulation of MLCL relative to delta*taz1* expressing wild type Taz1p (377). Of the four modeled BTHS mutations occurring in the interfacial membrane anchor, three mutations (V223D, V224R, and I226P) result in mislocalization of the mutant Taz1p to the mitochondrial matrix, while the G230R Taz1p mutant forms aberrant higher-order complexes (376).

Four other yeast BTHS mutants, A88R/E, S140R, and L148H, display reduced steady state expression when modeled in yeast Taz1p. These mutants are degraded by

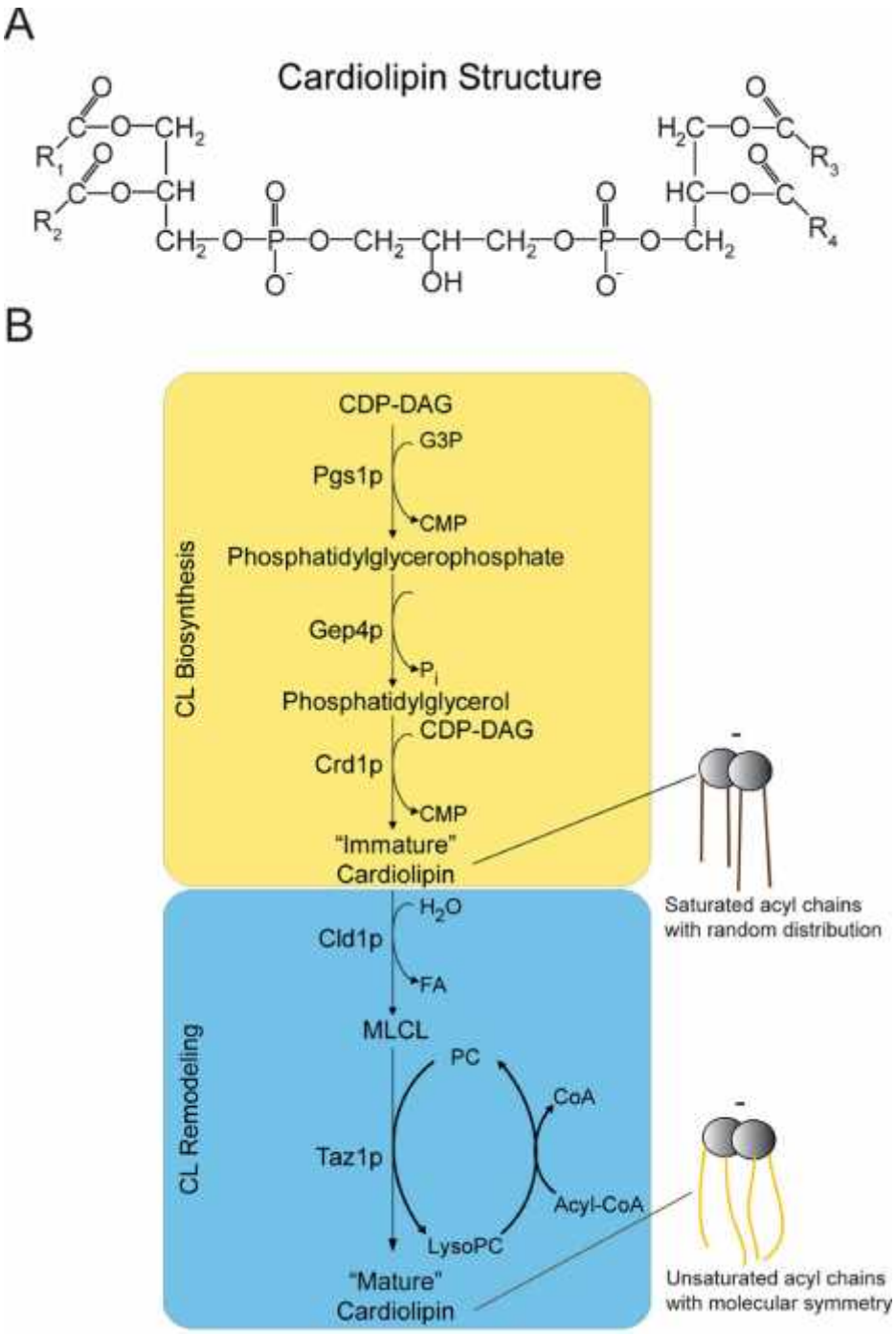

Figure 3. CL structure and schematic showing CL assembly and remodeling pathways in mitochondria. (A) Structure of CL. The position of the four attached acyl chains is indicated (R_1 thru R_4). (B) CL biosynthetic (*boxed* in *yellow*) and remodeling (*boxed* in *blue*) pathways. Pgs1p catalyzes the first and committed step in CL biosynthesis producing phosphatidylglycerophosphate from CDP-DAG and G3P (385). The recently identified phosphatidylglycerophosphate phosphatase, Gep4 (386), generates phosphatidylglycerol, the precursor of "immature" CL. Finally, "immature" CL is produced by Crd1p from phosphatidylglycerol and CDP-DAG. The acyl chains of "immature" CL are remodeled by the sequential action of the CL deacylase, Cld1p, and MLCL transacylase, Taz1p. The difference in acyl chain composition between "immature" and "mature" CL is illustrated on the right. CDP-DAG, cytidine diphosphatediphosphate-diacylglycerol; CMP, cytodine monophosphate; G3P, glycerol-3-phosphate; FA, fatty acid; PC, phosphatidylcholine; lyso-PC, lyso- phosphatidylcholine.

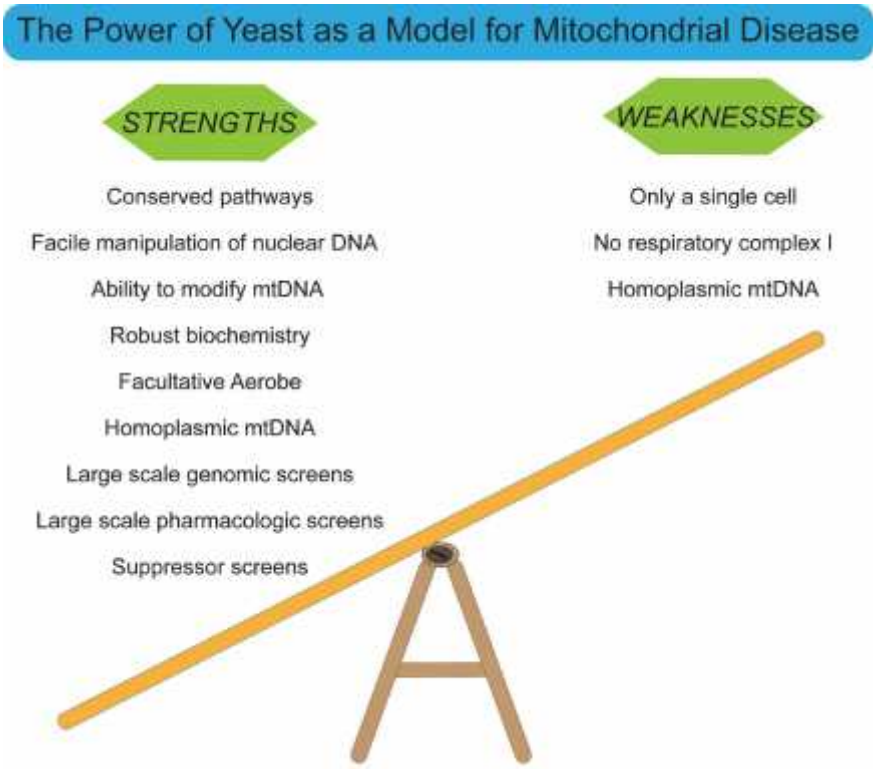

Figure 4. The power of yeast as a model for human mitochondrial disease. When the weaknesses of the yeast model (unicellular, absence of respiratory complex I, and homoplasmic mtDNA) are directly compared to its strengths (high degree of conservation of basic mitochondrial processes, easy genetics, robust biochemistry, ability to survive in the absence of a functional OXPHOS system, homoplasmic mtDNA, and capacity for large scale genetic, pharmacologic, and suppressor screens) with respect to modeling human mitochondrial disease, the value of yeast is clearly evident.

the *i*-AAA protease, and in the absence of the *i*-AAA protease, their steady state expression is returned to normal levels. Surprisingly, the activity of two mutants, A88E and L148H, is restored in the absence of the *i*-AAA protease. However, complexes containing these mutants are unstable, and rapidly disassemble (377). Potentially, a therapeutic strategy could be developed for this subset of BTHS patients that involves stabilizing the mutant Taz1p complexes.

Although the yeast model of BTHS mutations has provided possible pathogenic mechanisms for Taz1p loss of function, it is unclear if these mechanisms are conserved in patients. Unfortunately, robust biochemical characterization in higher systems is hampered by the lack of antibodies against mammalian TAZ1 isoforms. As such, yeast is currently the best model to understand the molecular mechanisms causing BTHS.

11. PERSPECTIVES

The yeast model is not without its limitations. Diseases affecting complex I represent a significant portion of respiratory chain defects and cannot be modeled in *S.cerevisiae*. That yeast quickly become homoplasmic also prevents a critical dissection of the threshold effect. Finally, models in multicellular organisms are required to understand the tissue specific phenotypes exhibited by many mitochondrial diseases.

Despite this, the conservation of mitochondrial function between yeast and humans make yeast an invaluable model for elucidating basic mitochondrial processes, as well as providing a better understanding of mitochondrial disease (Figure 4). The ability to efficiently generate mutations, even within mtDNA, has provided a greater understanding of the molecular mechanisms underlying disease (Table 1), which will ultimately enhance the ability to design specific therapeutic treatments. The yeast model has also proven to unambiguously distinguish between pathogenic mutations and silent polymorphisms which is critical for appropriate genetic counseling. Finally, basic research using yeast and focused on questions of fundamental importance concerning mitochondrial biogenesis and physiology provide logical candidates for assorted mitochondrial diseases which has already, and will continue, to streamline the important task of identifying the pathogenic lesions that cause mitochondrial disease. As such, the contribution of the

Table 1. Genes involved in mitochondrial disease and their yeast orthologs

Mitochondrial function	Human gene	Yeast gene	Molecular function	Molecular consequences of dysfunction	Clinical manifestations[1]
mtDNA	*POLG*	*MIP1*	mtDNA polymerase	mtDNA depletion, increased mtDNA mutability	PEO, Alpers Syndrome, ataxia-neuropathy syndrome
	ANT1	*AAC2*	ATP/ADP carrier	mtDNA depletion, abnormal ATP/ADP transport, loss of membrane potential	autosomal dominant PEO, hypertrophic cardiomyopathy, myopathy, exercise intolernace, lactic acidosis
	MPV17	*SYM1*	unknown	mtDNA depletion, abnormal mitochondrial morphology, ethanol intolerance	moderate to severe liver dysfunction
Protein import	*GFER*	*ERV1*	part of the disulfide relay system. Oxidizes Mia40p, donates electrons to cytochrome *c*.	respiratory defect at elevated temperature, COX activity decreased, mtDNA deletions	hypotonia, hearing loss, congenital cataract, developmental delay
	DDP1	*TIM8*	IMS chaperone of Tim23p precursor	reduced abundance of import substrates	Mohr-Tranebjaerg syndrome
Dynamics	*MFN1, MFN2*	*FZO1*	GTPase mediating OM fusion	fragmented or aggregated mitochondria, respiratory defect	Charcot-Marie-Tooth disease type 2A
	OPA1	*MGM1*	dynamin-related GTPase mediating IM fusion	fragmented or aggregated mitochondria, respiratory defect	dominant optic neuropathy
	DRP1	*DNM1*	dynamin-related GTPase mediating mitochondrial fission	elongated, tubular mitochondrial network	abnormal brain development, optic atrophy, hypotonia, elevated lactate levels
Proteases	*AFG3L2*	*YTA10/12*	*m*-AAA metalloprotease involved in precursor processing and quality control	unprocessed protein precursors, respiratory defect	autosomal dominant spinocerebellar ataxia
	SPG7	*YTA10/12*	*m*-AAA metalloprotease involved in precursor processing and quality control	unprocessed protein precursors, respiratory defect	spastic paraplegia
Respiratory complex subunits	*SDHA*	*SDH1*	flavoprotein subunit of succinate dehydrogenase	loss of SDH activity	Leigh syndrome, paraganglioma
	SDHB	*SDH2*	Fe/S subunit of succinate dehydrogenase	loss of SDH activity	paragangliomas, pheochromocytomas
	SDHC	*SDH3*	membrane anchor subunit of succinate dehydrogenase	loss of SDH activity	paragangliomas, pheochromocytomas
	SDHD	*SDH4*	membrane anchor subunit of succinate dehydrogenase	loss of SDH activity	paragangliomas, pheochromocytomas
	CYTB	*COB*	catalytic subunit of complex III	no complex III activity	MILS, Kearns-Sayre syndrome, MELAS, Leber's hereditary optic neuropathy, cardiomyopathy, myopathy, exercise intolerance
	COX1	*COX1*	catalytic subunit of complex IV	COX deficiency	Leigh syndrome, MELAS, encephalomyopathy, myopathy
	COX2	*COX2*	catalytic subunit of complex IV	COX deficiency	Leigh syndrome, MELAS, encephalomyopathy, myopathy
	COX3	*COX3*	subunit of complex IV	COX deficiency	Leigh syndrome, MELAS, encephalomyopathy, myopathy
	COX6B	*COX12*	subunit of complex IV	COX deficiency	infantile encephalomyopathy
	ATP6	*ATP6*	subunit of ATP synthase	decreased ATP synthesis	MILS, NARP
Respiratory complex assembly factors	*SDHAF1*	*SDH6*	involved in SDH assembly	decrease in SDH activity	infantile leukoencephalopathy
	SDHAF2	*SDH5*	SDHA flavination	no SDH activity	paraganglioma
	BCS1L	*BCS1*	incorporates Rip1 into complex III	reduced complex III activity	complex III deficiency, GRACILE syndrome, Björnstad syndrome
	SURF1	*SHY1*	involved in complex IV assembly, may stabilize Cox1p	reduced complex IV activity	Leigh syndrome
	SCO1	*SCO1*	transfer of Cu or cysteine reduction in Cox2p	reduced complex IV activity	hepatopathy and ketoacidotic coma
	SCO2	*SCO1*	transfer of Cu or cysteine reduction in Cox2p	reduced complex IV activity	infantile cardiomyopathy
	ATP12	*ATP12*	assembly of F1 portion of ATP synthase	reduced ATP synthesis, reduced activity of other respiratory complexes	lactic acidosis, dysmorphic features, methyl glutaconic aciduria and early death
Iron regulation	*FXN*	*YFH1*	iron chaperone, formation of Fe-S clusters	mitochondrial iron accumulation, mtDNA instability	Friedreich ataxia
Lipid remodeling	*TAZ*	*TAZ1*	CL transacylase	decreased CL abundance, increased MLCL levels, respiratory defects	Barth syndrome

[1] A description of the clinical phenotypes associated with each disease can be found in the text and references therein

yeast model to the field of mitochondrial medicine is immeasurable.

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Abbreviations: AAA: ATPase associated with various cellular activities; AAC: ADP/ATP carrier; ANT: adenine nucleotide translocase; BTHS: Barth syndrome; CCCP: carbonyl cyanide m-chlorophenyl hydrazone; CL: cardiolipin; CMT2A: Charcot-Marie-Tooth disease type 2A; CoQ: coenzyme Q; COX: cytochrome c oxidase; FAD: flavin adenine dinucleotide; GED: GTPase effector domain; GRACILE: growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death; HIF-1 alpha: hypoxia inducible factor-1 alpha; HSP: hereditary spastic paraplegia; IM: mitochondrial inner membrane; IMS: inner membrane space; MEF: mouse embryonic fibroblast; MELAS: mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke; MERFF: myoclonic epilepsy with ragged red fibers; l-Mgm1p: long isoform of Mgm1p; s-Mgm1p: short isoform of Mgm1p; MILS: maternally inherited Leigh syndrome; MLCL: monolysocardiolipin; mtDNA: mitochondrial DNA; NARP: neuropathy, ataxia, and retinitis pigmentosa; OM: mitochondrial outer membrane; OXPHOS: oxidative phosphorylation; PAM: presequence translocase-associated motor; PEO: progressive external ophthalmoplegia; ROS: reactive oxygen species; SAM: sorting and assembly machinery; SDH: succinate dehydrogenase; SNP: single nucleotide polymorphism; TCA: tricarboxylic acid; TIM: translocase of the inner membrane; TOM: translocase of the outer membrane

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