Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1772 (2007) 509–523
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbadis

Review

Mitochondrial protein import and human health and disease

James A. MacKenzie [a,*], R. Mark Payne [b]

[a] Department of Biological Sciences, 133 Piez Hall, State University of New York at Oswego, Oswego, NY 13126, USA
[b] Department of Pediatrics and Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Received 26 July 2006; received in revised form 6 December 2006; accepted 7 December 2006
Available online 9 December 2006

Abstract

The targeting and assembly of nuclear-encoded mitochondrial proteins are essential processes because the energy supply of humans is dependent upon the proper functioning of mitochondria. Defective import of mitochondrial proteins can arise from mutations in the targeting signals within precursor proteins, from mutations that disrupt the proper functioning of the import machinery, or from deficiencies in the chaperones involved in the proper folding and assembly of proteins once they are imported. Defects in these steps of import have been shown to lead to oxidative stress, neurodegenerative diseases, and metabolic disorders. In addition, protein import into mitochondria has been found to be a dynamically regulated process that varies in response to conditions such as oxidative stress, aging, drug treatment, and exercise. This review focuses on how mitochondrial protein import affects human health and disease.

Keywords: Mitochondria; Protein targeting; Protein translocation; Human health and disease

1. Introduction

Proteins act as hormones, receptors, enzymes, channels, and structural components within the cell, and although most are synthesized by the same set of cytosolic ribosomes, each protein has a specific subcellular location in which it resides and functions properly. Blobel and colleagues first postulated the "signal hypothesis", which predicted that proteins contain "zip codes" within their amino acid sequences that determine their subcellular location [1,2]. The importance of this hypothesis was supported by the finding that preproinsulin from two different fish species could be efficiently imported into dog pancreas microsomal vesicles, confirming that the system of protein targeting is evolutionarily conserved throughout nature [3]. In addition to specific targeting and compartmentalization within the cell, proteins also undergo post-translational modifications such as processing, folding, and oligomerization.

Errors in any of the steps that proteins must go through before they become fully active in their proper location can result in disease. While a great deal of attention has been focused on how abnormal targeting and folding of proteins cause human diseases (e.g., cystic fibrosis, hypercholesterolemia, Alzheimer's) [4], there has been little focus on how problems in mitochondrial protein import contribute to human health and disease. This review addresses how errors in the molecular mechanisms of import can lead to human disease, and also how mitochondrial protein import is variable and can be altered by various conditions. In order to appreciate the complexity of mitochondrial protein import and how it relates to disease, we begin with a brief overview of the import mechanisms.

2. Mitochondrial protein import

The targeting and import of mitochondrial proteins involve a series of receptors, import pores, and molecular machines located in the four intra-mitochondrial locations; the outer and inner mitochondrial membranes, the intermembrane space (IMS), and the matrix [5,6]. Most mitochondrial proteins are synthesized by free cytosolic ribosomes and are imported post-translationally (Fig. 1). All nuclearly-encoded mitochondrial proteins initially enter mitochondria via the protein translocase machinery of the outer membrane (TOM complex). From there, they are sorted to their final destination by the combined actions

* Corresponding author. Tel.: +1 315 312 3031; fax: +1 315 312 3059.
E-mail address: jmackenz@oswego.edu (J.A. MacKenzie).

0925-4439/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2006.12.002

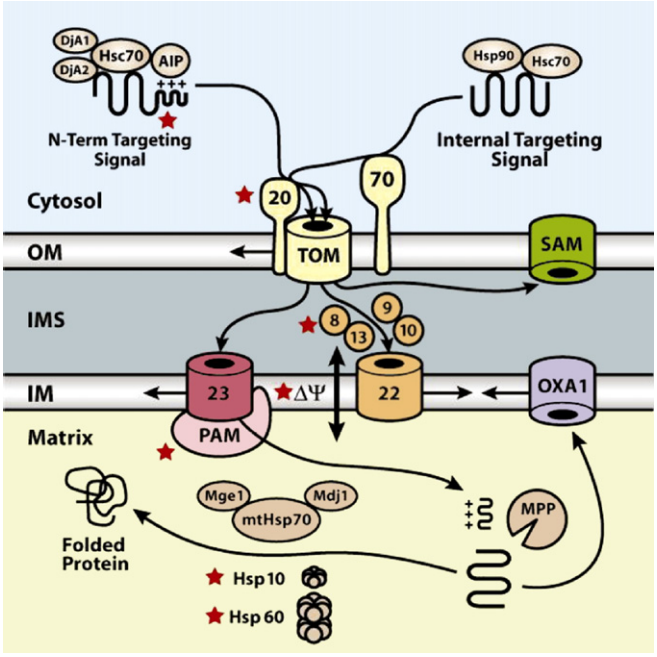

Fig. 1. Mitochondrial protein import pathways. Most mitochondrial proteins are predicted to be synthesized in their entirety in the cytosol and are then imported post-translationally. Proteins are maintained in an import-competent state by cytosolic chaperones and are specifically targeted to receptors at the mitochondrial surface. Proteins are then transferred from the receptors to the general import pore of the outer membrane, the TOM complex. Once across the outer membrane, proteins are sorted to one of the four intra-mitochondrial locations. Outer membrane proteins are inserted into the membrane either by the TOM or the sorting and assembly machinery (SAM) complex. Matrix proteins are sorted via the TIM23 complex and the presequence translocase-associated motor (PAM), while some single-pass inner membrane proteins are directly inserted into the membrane. Once matrix proteins cross the inner membrane, they are processed and folded via mitochondrial processing peptidase (MPP) and matrix chaperonins, respectively. Some single-pass inner membrane proteins are re-exported via OXA after being imported into the matrix. Polytopic inner membrane proteins are guided across the intermembrane space by the small Tim proteins (Tim9/10, Tim8/13) to the TIM22 complex where they are inserted into the inner membrane. The red stars denote import factors that either cause a disease state, or represent acquired changes.

of the translocation machinery and the targeting information encoded within the protein.

2.1. Targeting signals and cytosolic chaperones

Precursor proteins possessing a cleavable amino-terminal targeting signal (N-MTS) are destined for the IMS, the inner membrane, or the matrix. An N-MTS usually consists of about 20–30 amino acids, and although there is no sequence identity shared between N-MTSs, they all form amphiphilic α-helices that are enriched in basic, hydroxylated, and hydrophobic residues [7–9]. The helicity of N-MTS can also be interrupted by a central flexible linker region [10,11]. The physical interactions that occur between the N-MTS and the translocation machinery are not well understood, but data indicate that the basic and hydrophobic residues of the N-MTS are vital [12–14]. According to the 'acid-chain' hypothesis, the basic amino acids interact electrostatically with domains of the translocation machinery that have a high content of acidic amino acids [15,16]. The basic residues are not essential for import because their loss can be compensated for by the removal of the linker region, which results in increased helical stability and length [17–19]. The linker region and hydrophobic residues are believed to be important for early recognition by receptor components, whereas the basic residues are utilized in subsequent import steps [12–14].

Outer and inner membrane proteins contain internal targeting sequences that are not cleaved after import, and in contrast to N-MTSs, they are not well characterized. Outer membrane proteins either possess an anchoring sequence at the amino- or carboxy-terminus (single pass proteins), or have their targeting information encoded in structural elements that involve different regions of the protein (β-barrel proteins) [20]. Most of our knowledge of polytopic inner membrane protein targeting signals comes from studies of the ATP/ADP carrier protein (AAC). AAC belongs to a family of carrier proteins that have three distinct regions that are involved in import, each consisting of a pair of transmembrane domains (TMDs) connected by a matrix-exposed loop [21,22].

Newly synthesized proteins interact with cytoplasmic chaperones, which prevent their aggregation and keep them unfolded so they may be translocated through the import pores [23]. Which TOM receptor recognizes the chaperone–precursor protein complex depends upon the chaperone and the nature of the targeting sequence [24,25]. Hsc70 transfers precursor proteins to the Tom20/22 receptor complex, which preferentially binds to proteins containing an N-MTS [13,14,25,26]. Mammals contain additional cytosolic factors that act as co-chaperones with Hsc70. Arylhydrocarbon receptor interacting protein (AIP) binds to Hsc70, the N-MTS, and Tom20, and prevents substrate aggregation and enhances import [27]. The type I DnaJ homologs DjA1 and DjA2 enhance both the ATPase activity of Hsc70 and protein import [28,29]. Mammalian Hsp90, in conjunction with Hsc70, delivers proteins to Tom70 [30], which binds internal targeting sequences [24,31]. Proteins delivered to Tom70 are first passed to Tom20/22 [32,33], which then transfers proteins to the general import pore (GIP) [34].

2.2. Translocation across, or insertion into, the outer membrane

The GIP forms the central unit of the TOM and in yeast contains Tom40, Tom22, and the small Tom proteins (Tom5, Tom6, and Tom7) [34–36]. Mammals contain a similar complex [37–40], although Tom5 and Tom6 have yet to be identified. Tom40 forms the channel across the membrane and contains N-MTS binding sites that are spatially arranged from the *cis* to the *trans* side of the pore [16,41–43]. The translocation of proteins with internal targeting sequences is not well understood, but it appears as though the protein spans Tom40 as a hairpin-like structure that exposes the loop connecting two TMDs to the IMS [21]. After translocation across the outer membrane, the sorting pathways diverge to either one of the translocase of the inner membrane complexes (TIM23 or TIM22), or to the sorting and assembly machinery (SAM) complex.

Outer membrane proteins containing a single α-helical TMD are released directly into the membrane from the TOM complex; however, the mechanism for such a transfer is still unclear [44]. Outer membrane β-barrel proteins are inserted into the membrane via the SAM complex after they have been translocated across the outer membrane [45–47]. The yeast SAM complex is comprised of Sam50, Sam35, Sam37, and Mdm10 [48], and its importance is demonstrated by Sam50 being essential for yeast viability [46]. Very little is known about the role of the SAM complex in mammals and only Sam50 has been identified [49,50].

2.3. Translocation across the inner membrane and processing in the matrix

The TIM23 complex sorts precursor proteins with an N-MTS and consists of Tim17, Tim21, Tim23, and Tim50 [51]. Tim23 forms the channel across the inner membrane, and translocation across the membrane requires the inner membrane electrochemical potential ($\Delta\Psi$) and the presence of an N-MTS [52,53]. Precursor protein transfer from the TOM complex to TIM23 involves the interaction of the IMS domain of Tom22 with Tim23 and Tim50 [54–57]. Tim50 also promotes the closure of the Tim23 channel in the absence of precursor proteins, allowing for a tight regulation of the $\Delta\Psi$ [58]. Tim17 and Tim21 are involved in switching between a TIM23 complex that inserts proteins into the inner membrane and one that imports proteins into the matrix by either promoting TOM tethering or recruiting the presequence translocase-associated motor (PAM), respectively [59].

Once the N-MTS of matrix-bound proteins crosses the inner membrane, it interacts with the PAM, which is a dynamic complex of Tim44, Pam16, Pam17, Pam18, Mge1, Mdj2, and mtHsp70 that uses ATP to "pull" proteins across the inner membrane [60]. MtHsp70 drives protein translocation by interacting with the other PAM proteins and cycling between an ATP- and ADP-bound state, which alters the affinity of mtHsp70 for the precursor protein [61]. The required cycling of the ATP/ADP-bound states is regulated by the nucleotide exchange factor Mge1 [62]. Tim44 tethers mtHsp70 to the inner membrane by interacting with the ATPase and peptide-binding domains of mtHsp70, and the mtHsp70/Tim44 complex is disrupted upon precursor protein binding through conformational changes in mtHsp70 [63,64]. Pam16, Pam17, Pam18, and Mdj2 are involved in promoting mtHsp70/Tim44 complex formation, the association of that complex with TIM23, and the stimulation of mtHsp70 ATPase activity [65–70].

Once in the matrix, the N-MTS of most precursor proteins is removed by the mitochondrial processing peptidase (MPP) to form the mature protein [71]. MPP is a metalloendoprotease that processes precursor proteins as they are being folded and is only active in its heterodimeric form consisting of α-MPP and β-MPP [72–74]. Acidic residues of β-MPP determine the cleavage site through interaction with the Arg at the −2 position relative to the cleavage site [75,76]. Other precursor proteins are processed into their mature form via the sequential cleavage steps of MPP followed by the mitochondrial intermediate peptidase [77].

Matrix proteins are folded and assembled into complexes by the heat shock proteins mtHsp70, Hsp60, and Hsp10 [78]. MtHsp70 functions in one of two complexes; as part of the aforementioned PAM complex, and as part of a protein folding complex [79]. The mtHsp70 folding complex utilizes the co-chaperones Mge1 and Mdj1, which enhance the ATPase activity of mtHsp70, and hence its ATP/ADP cycling and protein folding functions [80,81]. Unlike Mge1, Mdj1 is not involved in the translocation of proteins across the inner membrane, but rather, it assists mtHsp70 in protein folding by enabling mtHsp70 to bind proteins in an ATP-dependent manner [80–83]. The Hsp60/Hsp10 folding complex uses ATP to fold proteins and prevent their aggregation [84–87]; however, little is known about the mechanism of action. Most of our knowledge is inferred from their bacterial homologs, the chaperonin GroEL and its co-chaperonin GroES [78,88]. GroEL and Hsp60 both consist of two heptameric toroidal rings [89,90]. Based on the GroEL/GroES system, Hsp10 coordinates the Hsp60 monomers and regulates the ATPase activity, and Hsp60 passively provides an environment that prevents aggregation, thus allowing folding to proceed [91,92]. Indeed, the two-ring structure of mammalian Hsp60 is promoted in the presence of Hsp10 and ATP [89]. New evidence however, suggests that the GroEL central cavity might actively participate in folding by altering folding rate, and for some proteins, even altering the folding mechanism [93].

2.4. Sorting to the intermembrane space

The localization of proteins with an N-MTS to the IMS is illustrated by the import of cytochrome b_1 and cytochrome b_2 [94,95]. These proteins are targeted to the TIM23 complex as noted above, but become arrested in the pore by an uncharged "sorting signal" that immediately follows the N-MTS ("stop-transfer" mechanism). Insertion of the N-MTS into the pore is driven by the $\Delta\Psi$ and import does not require mtHsp70 [96]. The N-MTS is cleaved off by MPP in the matrix and the mature protein is released into the IMS by proteolytic cleavage at the outer face of the inner membrane by the inner membrane peptidases [71,97]. IMS proteins containing internal targeting signals and zinc-finger motifs are translocated across the outer membrane and retained in the IMS either by binding to metal cofactors [98–101], or by forming disulfide bonds [102–106].

2.5. Insertion into the inner membrane

Single-pass inner membrane proteins contain an N-MTS and are inserted into the membrane by one of two mechanisms [107]. Some proteins have a hydrophobic "stop-transfer" signal that arrests the protein in the TIM23 complex, followed by the lateral release of the protein directly into the membrane [108,109]. Other precursor proteins are imported entirely into the matrix following the same steps and requirements as matrix proteins and are then exported from the matrix into the inner membrane [110–112]. The export process involves the inner

membrane protein Oxa1 [113,114], which is also required for the insertion of mitochondrially-encoded proteins into the inner membrane [115,116].

Polytopic inner membrane proteins are guided through the IMS by the small TIM proteins Tim8, Tim9, Tim10, Tim12, and Tim13 [117,118]. Two soluble 70-kDa complexes (Tim9/10 and Tim8/13) mediate the transfer of integral membrane proteins to the TIM22 complex [119]. Most polytopic inner membrane proteins are passed from Tom40 to Tim9/10, which then complexes with Tim12 to finally transfer the protein to TIM22 [98,120–123]. Other proteins cross the IMS with the help of Tim8/13 [124]. Protein insertion at the twin-pore TIM22 complex is coordinated by the $\Delta\Psi$ and the internal targeting sequence [117,125].

3. Diseases due to variation in mitochondrial targeting signals

It is clear that the targeting, import, and assembly of mitochondrial proteins involve a complex series of events which are dependent on the mitochondrial translocation machinery and the information encoded in the imported protein. As we discuss below, errors in these events can result in a protein not reaching its final destination, ultimately leading to a disease state in humans (Table 1). In addition to errors in the import process, mitochondrial protein import is a dynamic process that can be altered in response to various conditions (Table 2). Understanding the orchestration of how nuclearly-encoded proteins reach their final destination in mitochondria is essential to the understanding of mitochondrial function as a whole.

3.1. Pyruvate dehydrogenase deficiency

The first report of a defect in a mitochondrial targeting signal causing a human disease described a mutation in the N-MTS of one of the subunits of the mitochondrial matrix protein complex pyruvate dehydrogenase (PDH), that leads to PDH deficiency [126]. The PDH complex catalyzes the oxidative decarboxylation of pyruvate to acetyl CoA, and PDH deficiency is one of the most common causes of primary lactic acidosis in infants and children [127]. The most common cause of PDH deficiency is a defect in the X-linked PDH E1α subunit, which leads to significant structural abnormalities of the central nervous system, including microcephaly and cerebral atrophy [128]. The human E1α protein is synthesized in the cytosol with a 29-residue N-MTS that is removed in the matrix after import [129]. In two male siblings with X-linked PDH E1α deficiency, a base substitution of G to C at nucleotide 134 in the gene encoding PDH E1α was identified that resulted in an Arg→Pro substitution at codon 10 in the N-MTS [126]. Cultured skin fibroblasts from these patients had 28% PDH and 23% PDH E1 activity, and showed a decreased PDH E1α protein level. Mitochondria import assays using chimeric constructs with the normal and mutant PDH E1α N-MTS indicated that the mutation caused a decrease in import efficiency. It is unknown why the R10P mutation decreases import, but the simultaneous loss of a basic residue and the addition of a potential helix-breaking residue may seriously affect the functioning of the N-MTS. The hydrophobic moment of the N-MTS is significantly reduced in the R10P mutant due to the decrease in the number of basic residues on the hydrophilic face of the helix [126]. Also, the addition of the helix-breaking proline could destabilize the helix, which is important for aligning the basic and hydrophobic residues in the N-MTS for recognition by the import machinery [7,8].

Table 1
Abnormal mitochondrial protein import and human health and disease

Disease	Protein	References
Defects in mitochondria targeting signals		
Primary hyperoxaluria type 1	AGT	[131,132]
Pyruvate dehydrogenase deficiency	PDH E1α	[126,128]
Susceptibility to severe alcoholic liver disease	MnSOD	[147,148]
Defects in the translocation or processing machinery		
Atypical mitochondria disease involving multisystem failure	Hsp60	[173,174]
Dilated cardiomyopathy with ataxia	DNAJC19	[172]
Human Deafness Dystonia Syndrome	DDP1/Tim 8	[155,156, 158–164]
Spastic Paraplegia-13	Hsp60	[177,178]

Table 2
Acquired defects in mitochondrial protein import

Condition	Effect	References
Aging	Reduction in the import of mitochondrial proteins needed for DNA repair	[182]
Chronic muscle stimulation	Increased rate of import and increase in protein import machineries	[195,196]
Oxidative stress	Inhibits the import of precursor proteins and leads to their degradation	[183]
Thyroid hormone (T_3) treatment	Increased rate of import and increases in protein import machineries	[191–193]

3.2. Primary hyperoxaluria type 1

Alanine/glyoxylate aminotransferase 1 (AGT) deficiency can be caused by its mistargeting to mitochondria leading to primary hyperoxaluria type 1 (PH1) [130]. AGT catalyzes the conversion of glyoxylate to glycine in the peroxisomes of hepatocytes. Although most cases of PH1 are characterized by a lack of active AGT, approximately one third of patients have been described in whom AGT is present and active, but is mistargeted to mitochondria rather than peroxisomes [131,132]. These PH1 patients have at least one allele carrying two separate point mutations, each of which causes a single amino acid substitution (P11L and G170R) [132]. The P11L polymorphism produces a weak mitochondrial targeting sequence, that in conjunction with the G170R mutation, leads to the mistargeting of AGT to mitochondria [133,134]. The G170R mutation, or other mutations also associated with PH1, must accompany the P11L mutation for the disease phenotype to occur because they hinder the folding and dimerization of AGT [135,136]. Unlike protein import into peroxisomes, which

can import fully folded oligomeric proteins [137], mitochondria can only import proteins that are unfolded or loosely folded monomers [138,139]. Thus, the combination of a weak targeting signal and the unfolded state of AGT is sufficient for AGT to be mistargeted to mitochondria. The mistargeting of AGT disrupts peroxisomal function, leading to glyoxylate being oxidized to oxalate and the deposition of calcium oxalate in the kidney and urinary tract, eventually leading to kidney failure and the deposition of calcium oxalate in almost every organ and tissue in the body [140].

3.3. Severe alcoholic liver disease

Even if proteins are correctly targeted to mitochondria, they must also be properly imported and sorted to one of the four intra-mitochondrial locations. Manganese superoxide dismutase (MnSOD) is a nuclearly-encoded matrix protein that is targeted via a cleavable N-MTS [141]. Superoxide anions generated as a result of oxidative phosphorylation are converted to hydrogen peroxide by MnSOD, which can then be reduced to water by glutathione peroxidase (GPX) [142]. A genetic dimorphism for either Val or Ala at residue 16 exists in the N-MTS of MnSOD [143,144]. *In vitro* import studies indicate that while Val-type precursor proteins are properly targeted to mitochondria, they become partly arrested in the inner membrane [145]. As discussed below, this difference in import efficiency can lead to varying amounts of the enzyme and has been associated with numerous diseases. The Ala-precursor protein generates 30–40% more active, processed MnSOD homotetramer than the Val-precursor protein [145]. Additionally, expression and activity of the Ala-MnSOD variant is 4x greater than Val-MnSOD in transfected cells [146]. The translocation difficulty of Val-MnSOD has been proposed to be caused by a disruption of the α-helical configuration of the MTS by the presence of Val [144].

The presence of at least one Ala allele at the MnSOD locus is a risk factor for severe alcoholic liver disease (microvascular steatosis, alcoholic hepatitis, and cirrhosis), and increases the rates of hepatocellular carcinoma and death in cirrhotic patients [147,148]. Alcohol consumption increases reactive oxygen species in mitochondria, which are detoxified by MnSOD and GPX [149,150]. It is hypothesized that the increased import of Ala-MnSOD could lead to an imbalance between MnSOD and GPX resulting in a buildup of hydrogen peroxide, which can then form the extremely reactive hydroxyl radical in the presence of iron [147]. In fact, transfection with Ala-MnSOD causes a 60% reduction in superoxide anion levels compared to Val-MnSOD [146]. The increase in oxidative stress due to increased activity of the Ala-isoform of MnSOD and the subsequent increase in hydroxyl radicals could damage mitochondria, promote both apoptotic and necrotic cell death, and contribute to alcohol-induced liver diseases [151].

Differences in the Val and Ala genotypes have also been associated with nonfamilial idiopathic cardiomyopathy [152], diabetic nephropathy in patients with type II diabetes [153], and hereditary hemochromatosis in patients who have developed dilated or nondilated cardiomyopathy [154]. For reasons that are not yet clear, the Val allele appears to be associated with these disease states.

4. Diseases due to defects in the mitochondrial import and processing machinery

4.1. Human deafness dystonia syndrome

The X-linked neurodegenerative disorder Human Deafness Dystonia Syndrome (DDS, also known as Mohr–Tranebjaerg syndrome) is characterized by postlingual progressive sensorineural deafness, dystonia, spasticity, dysphagia, mental deterioration, paranoia, and cortical blindness and illustrates how a defect in the mitochondrial import machinery can lead to human disease [155]. DDS is caused by mutations in the IMS protein deafness dystonia peptide 1 (DDP1/Tim8), which is homologous to the fungal protein Tim8 [156,157]. In all, eight mutations have been described in the DDP1/Tim8 gene of patients with DDS, seven of which are either missense, deletion, frameshift, or stop mutations [155,156,158–164]. In mammalian mitochondria, DDP1/Tim8 is found in a complex with Tim13 [165]. The most studied of the DDP1/Tim8 mutations that causes DDS, and the one that helped elucidate the function of DDP1/Tim8, is in a cysteine of the 'twin Cx_3C' motif (C66W) [166]. $Tim8^{C66W}$ and Tim13 were not detectable in the cultured fibroblast cell lines from a DDS patient even though the mRNA levels of both were detected at levels of control cells. The mutation in yeast does not disrupt mRNA stability or translation, and has no effect on targeting or localization to the IMS [100], supporting the hypothesis that the absence of $Tim8^{C66W}$ is due to its inability to form a stable complex in the IMS [166].

The small Tim proteins of the IMS are characterized by a 'twin Cx_3C' motif [167], and are proposed to be retained in the IMS either by binding to metal cofactors [98–101], or by forming disulfide bonds [102–106]. The C66W mutation may cause the mutated protein to incorrectly fold and lose its ability to assemble with Tim13 because Zn^{2+} is required for a stably folded wild-type DDP1/Tim8, and $Tim8^{C66W}$ has impaired Zn^{2+} binding to the zinc finger motif [100]. The folding of various other small Tim proteins is also dependent on the coordination of Zn^{2+} ions by the cysteine residues of the zinc finger motif [98,99]. Another possibility is that the loss of the cysteine residue may prevent $Tim8^{C66W}$ from interacting with Mia40, thus preventing disulfide bond formation. Mia40 was first identified in yeast as an IMS protein that is crucial for the transport of the small Tim proteins across the outer membrane and their assembly into IMS complexes [102]. Along with Erv1, Mia40 functions to catalyze the import of proteins into the IMS by an oxidative folding mechanism [103–105]. Although a human Erv1 ortholog has yet to be shown to participate in the import of IMS proteins, human Mia40 has been identified as a component of the import pathway whose depletion affects the steady-state levels of twin Cx_3C-containing IMS proteins, including DDP1/Tim8 [168]. Evidence to date suggests that DDP1/Tim8 achieves a stably folded conformation in the IMS

via a combination of both mechanisms [100,168], as has been suggested for other proteins [102,167].

The complete absence of the DDP1/Tim8 protein was described in a DDS patient with a missense mutation (G→C transversion in nucleotide position 38) of the first exon [158]. The mutation resulted in a loss of the start codon (M1I) and complete lack of the protein, while mRNA levels were unaffected. Like the patient with the C66W mutation, this patient exhibited typical neurodegenerative symptoms associated with DDS, and although Tim13 mRNA levels were unaffected, Tim13 protein levels were significantly reduced. An intronic mutation at position −23 relative to the first nucleotide of exon 2 was identified in two male DDS patients and their unaffected mothers [159]. This is the only described case of an intronic mutation being associated with DDS and the authors suggested that the problem may have arisen from mRNA splicing alterations.

Different hypotheses have been proposed on how a dysfunctional DDP1/Tim8 can lead to DDS. The human DDP1/Tim8–Tim13 complex in the IMS is directly involved in the import of human Tim23 [165,166,169]. Based on this finding, it has been hypothesized that the mechanism underlying the severity of DDS is the impaired biogenesis of the TIM23 complex as a result of a dysfunctional Tim8. This would lead to severe pleiotropic mitochondrial dysfunction because the TIM23 complex is used for the import of all matrix proteins and some IMS and inner membrane proteins. The Ca^{2+}-responsive aspartate/glutamate carriers, aralar1 and citrin, are also chaperoned across the IMS by DDP1/Tim8–Tim13 to TIM22 for insertion into the inner membrane, suggesting that Tim8 defects could lead to decreased NADH levels due to a decrease in the aspartate/malate NADH shuttle [169]. Finally, the Tim8–Tim13 complex in yeast has been implicated in the import and assembly of the outer membrane β-barrel proteins Tom40 and porin [170,171]. If this finding is demonstrated in humans, it could further elucidate the consequences that a dysfunctional Tim8 can have on mitochondrial function.

4.2. Dilated cardiomyopathy with ataxia

A recent study has suggested that a defect in Pam18 (Tim14) might be involved in dilated cardiomyopathy with ataxia (DCMA) [172]. This novel autosomal recessive condition was identified in the Canadian Dariusleut Hutterite population and is characterized by early onset dilated cardiomyopathy, non-progressive cerebral ataxia, growth failure, and in some patients, testicular dysgenesis and 3-methylglutaconic aciduria. Using homozygosity mapping in 11 patients with DCMA, a novel gene was identified, DNAJC19, that shares similarity with fungal Pam18. Pam18 is an inner membrane protein in all eukaryotes that participates in the import of matrix proteins, possibly by enabling mtHsp70 to interact with Tim44 in a regulated manner [65–67]. All DCMA patients were found to be homozygous for a G→C transversion in the AG splice acceptor site of intron 3 of DNAJC19, while all unaffected siblings were not [172]. The mutation resulted in a loss of exon 4 in the mRNA transcript, and it was predicted that translation would be prematurely terminated in exon 5, leaving a protein that lacked the DNAJ domain. Pam18 stimulates the ATPase activity of mtHsp70 through the J domain and disruption of the J domain alone is sufficient to make fungal cells unviable [66,67]. It is not yet clear if DNAJC19 is the human ortholog of Pam18; but if it is, the DCMA phenotype might be the result of dysfunctional import of matrix proteins through the TIM23 complex.

4.3. Atypical mitochondrial disease involving multisystem failure

Hsp60, a mitochondrial matrix chaperone involved in the folding and assembly of mitochondrial proteins, is the only component of the mitochondrial folding and assembly machinery whose dysfunction has been implicated in causing human disease. Patients with an atypical mitochondria disease involving multisystem failure have been linked to a deficiency in Hsp60 [173,174]. Analysis of their mitochondria indicated that there was a decreased number of mitochondria per cell, and the ones that were present had ultrastructural abnormalities, were swollen, and were mainly localized around the nucleus [173–175]. The enzyme activities of oxidative phosphorylation, pyruvate metabolism, β-oxidation, and other mitochondrial metabolic pathways were markedly decreased in cultured fibroblasts [173,174], and the reduced levels were associated with decreased amounts of Hsp60 [175]. In contrast, the activity of enzymes localized in the outer membrane or in other cellular organelles were normal [173]. The low steady state amount of Hsp60 in the fibroblasts was caused by the decreased synthesis of the protein and not by its enhanced degradation or ability to oligomerize into its native 14-mer complex [176]. Although the authors suggested that the molecular basis of the disease was a primary defect in Hsp60 synthesis and/or processing, there has been no evidence presented to date to support this hypothesis.

4.4. Spastic paraplegia-13

A dysfunctional Hsp60 has also been implicated in causing spastic paraplegia-13 (SPG13), an autosomally dominant form of pure hereditary spastic paraplegia (HSP) [177,178]. HSP represents a clinically and genetically heterogeneous group of neurodegenerative disorders characterized by progressive weakness and spasticity of the lower limbs due to degeneration of corticospinal axons. Two affected members of a family with SPG13 were found to be heterozygous for a G→A variation at nucleotide 292, resulting in Val→Ile substitution at residue 72 in the mature Hsp60 protein [178]. The $Hsp60^{V72I}$ mutant protein is functionally impaired when expressed in *E. coli*. Taken together, the defects in Hsp60 illustrate how a disruption in the protein folding and assembly machinery in the mitochondrial matrix can lead to a decrease in the synthesis and maintenance of mitochondria.

5. Dynamics of protein import

Work utilizing animal models and mammalian cell culture has furthered our understanding of mitochondrial protein import

as a dynamic process and has raised intriguing questions about human health and disease. We now recognize that mitochondrial protein import responds to various conditions including oxidant stress, aging, and external stimuli.

5.1. Oxidant stress and import of DNA repair enzymes

The highly oxidative environment of mitochondria has lead to the hypothesis that mitochondrial DNA damage contributes to aging and disease [179], which is supported by mouse studies that show aging may be driven by apoptosis that is caused by an accumulation of mitochondrial DNA mutations [180]. While it has been proposed that the targeting of DNA repair enzymes to mitochondria could reduce oxidative stress and enhance mitochondrial DNA repair [181], there is an age-dependent reduction in the import of mitochondrial proteins needed for DNA repair [182]. Although the activity of the DNA repair enzyme 8-oxoguanine-DNA glycosylase (OGG1) is increased in mitochondria from older mice livers and pre-senescent primary human fibroblasts, a large fraction of the enzyme is stuck in the outer membrane in the precursor form where it would be unable to functionally interact with DNA [182]. Mitochondrial uracil-DNA glycosylase (UDG), another DNA repair enzyme, is also trapped in the outer membrane of hepatocyte mitochondria. Neither protein is found as an import intermediate in hepatocytes from young mice or in replicating fibroblasts, implicating that the impaired import of these enzymes is a result of aging.

It is not currently known why these proteins become stuck in the import machinery. Oxidative stress inhibits the import and processing of other matrix proteins *in vitro* and *in vivo* [183]. Thus, import may be affected because of the age-associated mitochondrial dysfunctions resulting from cumulative oxidative damage that decrease the mitochondrial inner membrane $\Delta\Psi$ and membrane fluidity, and alter expression of genes involved in mitochondrial bioenergetics [184]. Aging has been directly associated with an increase in mitochondrial inner membrane proton permeability in mitochondria from hepatocytes, lymphocytes, and cardiomyocytes [185–188]. Since experimentally dissipating the $\Delta\Psi$ causes precursor proteins to remain in the TOM complex [189,190], it is possible that the age-related decrease in $\Delta\Psi$ causes these DNA repair enzymes to become stuck in mid-translocation. It is unclear whether this phenomenon would be specific for OGG1 and UDG, or would apply to all matrix proteins. However, as discussed above with the import of MnSOD, small variations in MTSs can have an affect on the complete translocation of precursor proteins into the matrix [145]. It is likely that different proteins will have varying degrees of import problems as a result of a diminished $\Delta\Psi$.

5.2. Thyroid hormone treatment

Mitochondria derived from cardiac muscle of animals treated with thyroid hormone (T_3) have a 40–60% higher import rate of matrix proteins, and an associated increase in components of the import machinery (Tom20, mtHsp70, and Hsp60) [191]. T_3 treatment also causes an increase in cardiac Tim23 and Tim44 mRNA levels by 50%, and 55%, respectively [192]. The increase in mtHsp70 is due to both an increase in mRNA production and a higher import rate of the precursor mtHsp70 protein [193]. Although protein degradation and cytosolic factors are likely important in maintaining steady-state levels of mitochondrial proteins, they have little effect on mediating T_3-induced changes [191].

5.3. Chronic skeletal muscle stimulation

Chronic stimulation of skeletal muscle causes changes in mitochondrial protein import. Isolated mitochondria from muscles that have been chronically stimulated electrically, or by endurance training, have an increased import rate of matrix proteins as well as increases in the expression of components involved in import (Tom20, mtHsp70, Hsp60, and Hsp10) [194–196]. Chronic muscle contraction stimulates the synthesis and degradation of proteins that are directly involved in contraction and energy production, as well as promoting the biogenesis of new mitochondria [197,198]. The upregulation of these protein import components may be to ensure the proper import of proteins during organelle synthesis [194]. Protein import and the ATP producing capabilities of mitochondria are directly related [199], and exercise causes an increase in State III and the coupling ratio in skeletal muscle mitochondria [200]. It is possible that the change in import during chronic muscle stimulation is due to an increase in the respiratory state of the mitochondria. This is supported by the finding that chronic contractile activity does not affect the import of outer membrane proteins [195], which do not use the $\Delta\Psi$ for import [201–203].

6. Conclusions and perspectives

Proper mitochondrial functioning is dependent on the interactions of proteins from both the nuclear and mitochondrial genomes. As described above, the targeting, import, and processing of nuclearly-encoded mitochondrial proteins have a direct effect on human health and disease. However, there are undoubtedly more disease states that are caused by errors in mitochondrial protein import. For example, an error in the proteolytic maturation of a protein by either MPP or MIP could lead to a non-functional protein. Our understanding of import as a regulated series of events is also continuing to expand. It was recently shown that Mge1 acts as a thermosensor for the mtHsp70 system, as heat shock induces the conversion of Mge1 from the active dimeric form to an inactive monomeric form [204]. The inactive form is unable to regulate the mtHsp70 ATPase and chaperone cycle. This conversion between active and inactive forms would allow for a reversible inhibition of the import of newly synthesized proteins so as to decrease the amount of aggregation-prone proteins in the matrix during stress.

Although it is well established that mitochondrial proteins can be imported post-translationally, evidence suggests that some proteins may be targeted and imported co-translationally [23,205]. This alternate delivery system, if validated, would offer a new set of mechanisms that may be important in human

health and disease. For instance, the cytosolic chaperone nascent polypeptide-associated complex (NAC) in yeast interacts with nascent precursor proteins, promotes ribosome docking to the mitochondrial surface, and its disruption leads to defects in protein targeting to mitochondria [206–208]. Two important mechanisms must be present for co-translational targeting to occur, namely, translational inhibition of mitochondrial proteins, and the specific targeting of ribosomes translating mitochondrial proteins. Translational inhibition is important to allow for targeting to occur before the protein is fully translated. This event has recently been shown to occur *in vivo* in slime mold for some mitochondrial proteins [209]. While the mechanism for this translational inhibition is unknown, it is probable that cytosolic factors are involved in a manner similar to what the signal recognition particle does in the co-translational import of proteins to the endoplasmic reticulum [210]. Targeting ribosomes to mitochondria may also involve mechanisms susceptible to damage. Mammalian mitochondria bind ribosomes in a receptor-like manner that uses GTP hydrolysis to differentiate between ribosomes carrying mitochondrial proteins to ones that do not [211,212]. While evidence suggests that mitochondria contain a specific ribosome receptor and that there are GTP-binding protein(s) that participate in the recognition and binding of ribosomes carrying mitochondrial proteins, neither has been identified. Problems with NAC, or any of the hypothesized proteins that may participate in the translational pausing and ribosomal delivery of proteins to mitochondria, are some of the possible places that errors in co-translational targeting could lead to disease. This may be highly relevant, for example, during periods of high oxidant stress, such as ischemia–reperfusion injury (e.g., heart attacks, strokes), where multiple intracellular processes have been disrupted. In this situation, mitochondria may no longer be able to meet the metabolic demands of the cell, and cell death ensues. As more evidence for co-translational import is found, and more of the mechanisms are worked out, it is very possible that these events may also be found to contribute to overall mitochondrial function, and the subsequent health of humans.

Of the hundreds of mitochondrial proteins in mammals, thirteen are encoded by mitochondrial DNA and include components of complexes I, III, IV, and V of the oxidative phosphorylation system. Mitochondrially-encoded inner membrane proteins are exported via Oxa1-dependent and -independent pathways [113,115], which offer yet another set of targeting and insertion mechanisms where defects could lead to human disease. Recent evidence suggests that Wolf–Hirschhorn syndrome (WHS) may be caused, in part, by the lack of a nuclearly-encoded mitochondrial protein that is involved in the export of mitochondrially-encoded inner membrane proteins. WHS is a multigenic disease resulting from variably-sized deletions in the subtelomeric region of chromosome 4 that lead to a complex and variable phenotype of severe neurological and neuromuscular abnormalities [213]. In most cases of WHS there is a deletion of the Letm1 gene [214], which is an ortholog to the yeast inner membrane proteins Mdm38 and Ylh47 [215–217]. Mdm38 is involved in one of the Oxa1-independent export pathways in yeast, where it interacts with newly synthesized proteins and forms stable complexes with mitochondrial ribosomes [218]. Mdm38 is required for the efficient export of cytochrome *b* and Atp6 and to a lesser extent Cox1 and -2; and Δ*mdm38* yeast cells have reduced Complex III and IV levels, an accumulation of unassembled Atp6 of Complex V, and a significant reduction in the $\Delta\Psi$ [218]. Although the molecular function of Letm1 is still unknown, its functional similarity to Mdm38 is demonstrated in that it can partially rescue growth defects in *Δmdm38* yeast [215]. The role of Mdm38 in the biogenesis of the respiratory chain in yeast might explain how

Table 3
Components in protein import into fungal and human mitochondria

	Fungal	Human
Cytosolic chaperones	Hsp70	Hsc70 [219]
	Ydj1p	DjA1 [28], DjA2 [29]
		AIP [27]
		Hsp90 [30]
TOM	Tom40	Tom40 [50,220] [a]
	Tom22	Tom22 [39,221]
	Tom70	Tom70 [222] [a]
	Tom20	Tom20 [26,223]
	Tom7	Tom7 [37] [a]
	Tom6	
	Tom5	
SAM	Sam50	Sam50 [49,50]
	Sam35	
	Sam37	
	Mdm10	
Soluble IMS TIMs	Tim9	Tim9 [117,224]
	Tim10	Tim10 [117,224]
	Tim8	Tim8 [156,157]
	Tim13	Tim13 [165]
TIM22	Tim22	Tim22 [117,224]
	Tim12	Tim10b [117,224]
	Tim54	
	Tim18	
TIM23	Tim23	Tim23 [225] [a]
	Tim17	Tim17a [225,226] [a],
		Tim17b [225] [a]
	Tim50	Tim50 [227] [a]
	Tim21	
PAM	Tim44	Tim44 [225] [a]
	mtHsp70	mtHsp70 [228] [a]
	Mge1	mtGrpE [229] [a]
	Pam18	
	Pam16	
OXA	Oxa1	Oxa1 [114]
IMP	Imp1	Imp1 [230] [b]
	Imp2	Imp2 [231] [b]
	Som1	
Matrix	Mas1	β-MPP [232] [b]
	Mas2	α-MPP [233] [b]
	MIP	MIP [234]
	Hsp60	Hsp60 [86,235]
	Hsp10	Hsp10 [236]
	mtHsp70	mtHsp70 [228] [a]
	Mdj1	Tid [237,238]
	Mge1	mtGrpE [229] [a]

[a] The human protein has been identified and localizes to mitochondria; however, any role in protein import and processing is only inferred from its fungal and mammalian orthologs.
[b] Only the human DNA sequence has been identified.

the loss of Letm1 in WHS leads to phenotypes that are typically associated with mitochondrial diseases.

While the import of nuclearly-encoded mitochondrial proteins has been highly conserved throughout eukaryotic evolution, and it is useful to make inferences on mammalian import based on import in fungi, there are differences in both the machinery and the mechanisms. For example, Tom40 is inserted into the outer membrane via Sam50 in both yeast and humans, but the import intermediates that are formed differ in size and susceptibility to externally added protease [50]. Although there have been great strides in identifying and characterizing the human counterparts of the mitochondria import machinery (Table 3), much is still unknown. The precise roles of some of the identified human import components have not been completely elucidated and only the genes for others have been identified. Based on the known components in fungi, it is probable that there are many more human components that have yet to be identified. To further our understanding of how errors in the import process contribute to a disease state, the following should be addressed: (1) all of the components of the human import machinery need to be determined, (2) the human mitochondrial import apparatus needs to be fully characterized, and (3) those components of the import machinery that are likely to be compromised by inherited mutations, or may be damaged by acquired defects such as oxidant stress, need to be determined. Only when we have a complete understanding of the targeting and import process in humans, and how it changes to accommodate the specific needs and conditions of a cell, can attempts then be made to manipulate the system to achieve desirable outcomes.

Acknowledgments

We thank Prof. Charles Croll for his editorial comments. This work was supported in part by NIH grant R01 DK67763 (RMP).

References

[1] G. Blobel, B. Dobberstein, Transfer of proteins across membranes: I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma, J. Cell Biol. 67 (1975) 835–851.
[2] G. Blobel, B. Dobberstein, Transfer to proteins across membranes: II. Reconstitution of functional rough microsomes from heterologous components, J. Cell Biol. 67 (1975) 852–862.
[3] D. Shields, G. Blobel, Cell-free synthesis of fish preproinsulin, and processing by heterologous mammalian microsomal membranes, Proc. Natl. Acad. Sci. U. S. A. 74 (1977) 2059–2063.
[4] C. Castro-Fernandez, G. Maya-Nunez, P.M. Conn, Beyond the signal sequence: protein routing in health and disease, Endocr. Rev. 26 (2005) 479–503.
[5] N. Hoogenraad, L. Ward, M. Ryan, Import and assembly of proteins into mitochondria of mammalian cells, Biochim. Biophys. Acta 1592 (2002) 97–105.
[6] P. Dolezal, V. Likic, J. Tachezy, T. Lithgow, Evolution of the molecular machines for protein import into mitochondria, Science 313 (2006) 314–318.
[7] G. vonHeijne, Mitochondrial targeting sequences may form amphiphilic helices, EMBO J. 5 (1986) 1335–1342.
[8] D. Roise, S.J. Horvath, J.M. Tomich, J.H. Richards, G. Schatz, A chemically synthesized pre-sequence of an imported mitochondrial protein can form an amphiphilic helix and perturb natural and artificial phospholipid bilayers, EMBO J. 5 (1986) 1327–1334.
[9] D. Roise, G. Schatz, Mitochondrial presequences, J. Biol. Chem. 263 (1988) 4509–4511.
[10] G. von Heijne, Control of topology and mode of assembly of a polytopic membrane protein by positively charged residues, Nature 341 (1989) 456–458.
[11] C. Karslake, M.E. Piotto, Y.K. Pak, H. Weiner, D.G. Gorenstein, 2D NMR and structural model for a mitochondrial signal peptide bound to a micelle, Biochemistry 29 (1990) 9872–9878.
[12] E. Schleiff, T.S. Heard, H. Weiner, Positively charged residues, the helical conformation and the structural flexibility of the leader sequence of pALDH are important for recognition by hTom20, FEBS Lett. 461 (1999) 9–12.
[13] Y. Abe, T. Shodai, T. Muto, K. Mihara, H. Torii, S. Nishikawa, T. Endo, D. Kohda, Structural basis of presequence recognition by the mitochondrial protein import receptor Tom20, Cell 100 (2000) 551–560.
[14] T. Obita, T. Muto, T. Endo, D. Kohda, Peptide library approach with a disulfide tether to refine the tom20 recognition motif in mitochondrial presequences, J. Mol. Biol. 328 (2003) 495–504.
[15] G. Schatz, Just follow the acid chain, Nature 388 (1997) 121–122.
[16] T. Komiya, S. Rospert, C. Koehler, R. Looser, G. Schatz, K. Mihara, Interaction of mitochondrial targeting signals with acidic receptor domains along the protein import pathway: evidence for the 'acid chain' hypothesis, EMBO J. 17 (1998) 3886–3898.
[17] K. Thornton, Y. Wang, H. Weiner, D.G. Gorenstein, Import, processing, and two-dimensional NMR structure of a linker-deleted signal peptide of rat liver mitochondrial aldehyde dehydrogenase, J. Biol. Chem. 268 (1993) 19906–19914.
[18] P.K. Hammen, D.G. Gorenstein, H. Weiner, Structure of the signal sequences for two mitochondrial matrix proteins that are not proteolytically processed upon import, Biochemistry 33 (1994) 8610–8617.
[19] T.S. Heard, H. Weiner, A regional net charge and structural compensation model to explain how negatively charged amino acids can be accepted within a mitochondrial leader sequence, J. Biol. Chem. 273 (1998) 29389–29393.
[20] D. Rapaport, Finding the right organelle. Targeting signals in mitochondrial outer-membrane proteins, EMBO Rep. 4 (2003) 948–952.
[21] N. Wiedemann, N. Pfanner, M.T. Ryan, The three modules of ADP/ATP carrier cooperate in receptor recruitment and translocation into mitochondria, EMBO J. 20 (2001) 951–960.
[22] M. Endres, W. Neupert, M. Brunner, Transport of the ADP/ATP carrier of mitochondria from the TOM complex to the TIM22.54 complex, EMBO J. 18 (1999) 3214–3221.
[23] T. Beddoe, T. Lithgow, Delivery of nascent polypeptides to the mitochondrial surface, Biochim. Biophys. Acta 1592 (2002) 35–39.
[24] J. Brix, K. Dietmeier, N. Pfanner, Differential recognition of preproteins by the purified cytosolic domains of the mitochondrial import receptors Tom20, Tom22, and Tom70, J. Biol. Chem. 272 (1997) 20730–20735.
[25] T. Komiya, S. Rospert, G. Schatz, K. Mihara, Binding of mitochondrial precursor proteins to the cytoplasmic domains of the import receptors Tom70 and Tom20 is determined by cytoplasmic chaperones, EMBO J. 16 (1997) 4267–4275.
[26] K. Terada, M. Kanazawa, M. Yano, B. Hanson, N. Hoogenraad, M. Mori, Participation of the import receptor Tom20 in protein import into mammalian mitochondria: analyses in vitro and in cultured cells, FEBS Lett. 403 (1997) 309–312.
[27] M. Yano, K. Terada, M. Mori, AIP is a mitochondrial import mediator that binds to both import receptor Tom20 and preproteins, J. Cell Biol. 163 (2003) 45–56.
[28] K. Terada, M. Kanazawa, B. Bukau, M. Mori, The human DnaJ homologue dj2 facilitates mitochondrial protein import and luciferase refolding, J. Cell Biol. 139 (1997) 1089–1095.
[29] K. Terada, M. Mori, Human DnaJ homologs dj2 and dj3, and bag-1 are positive cochaperones of hsc70, J. Biol. Chem. 275 (2000) 24728–24734.

[30] J.C. Young, N.J. Hoogenraad, F.U. Hartl, Molecular chaperones Hsp90 and Hsp70 deliver preproteins to the mitochondrial import receptor Tom70, Cell 112 (2003) 41–50.
[31] H. Suzuki, M. Maeda, K. Mihara, Characterization of rat TOM70 as a receptor of the preprotein translocase of the mitochondrial outer membrane, J. Cell Sci. 115 (2002) 1895–1905.
[32] T. Komiya, M. Sakaguchi, K. Mihara, Cytoplasmic chaperones determine the targeting pathway of precursor proteins to mitochondria, EMBO J. 15 (1996) 399–407.
[33] V. Haucke, T. Lithgow, S. Rospert, K. Hahne, G. Schatz, The yeast mitochondrial protein import receptor Mas20p binds precursor proteins through electrostatic interaction with the positively charged presequence, J. Biol. Chem. 270 (1995) 5565–5570.
[34] M. Kiebler, P. Keil, H. Schneider, I.J. van der Klei, N. Pfanner, W. Neupert, The mitochondrial receptor complex: a central role of MOM22 in mediating preprotein transfer from receptors to the general insertion pore, Cell 74 (1993) 483–492.
[35] N. Pfanner, A. Chacinska, The mitochondrial import machinery: preprotein-conducting channels with binding sites for presequences, Biochim. Biophys. Acta 1592 (2002) 15–24.
[36] P.J. Dekker, M.T. Ryan, J. Brix, H. Muller, A. Honlinger, N. Pfanner, Preprotein translocase of the outer mitochondrial membrane: molecular dissection and assembly of the general import pore complex, Mol. Cell. Biol. 18 (1998) 6515–6524.
[37] A.J. Johnston, J. Hoogenraad, D.A. Dougan, K.N. Truscott, M. Yano, M. Mori, N.J. Hoogenraad, M.T. Ryan, Insertion and assembly of human tom7 into the preprotein translocase complex of the outer mitochondrial membrane, J. Biol. Chem. 277 (2002) 42197–42204.
[38] H. Suzuki, Y. Okazawa, T. Komiya, K. Saeki, E. Mekada, S. Kitada, A. Ito, K. Mihara, Characterization of Rat TOM40, a central component of the preprotein translocase of the mitochondrial outer membrane, J. Biol. Chem. 275 (2000) 37930–37936.
[39] K. Saeki, H. Suzuki, M. Tsuneoka, M. Maeda, R. Iwamoto, H. Hasuwa, S. Shida, T. Takahashi, M. Sakaguchi, T. Endo, Y. Miura, E. Mekada, K. Mihara, Identification of mammalian TOM22 as a subunit of the preprotein translocase of the mitochondrial outer membrane (In Process Citation), J. Biol. Chem. 275 (2000) 31996–32002.
[40] Y. Nakamura, H. Suzuki, M. Sakaguchi, K. Mihara, Targeting and assembly of rat mitochondrial translocase of outer membrane 22 (TOM22) into the TOM complex, J. Biol. Chem. 279 (2004) 21223–21232.
[41] H. Suzuki, T. Kadowaki, M. Maeda, H. Sasaki, J. Nabekura, M. Sakaguchi, K. Mihara, Membrane-embedded C-terminal segment of rat mitochondrial TOM40 constitutes protein-conducting pore with enriched beta-structure, J. Biol. Chem. 279 (2004) 50619–50629.
[42] K. Hill, K. Model, M.T. Ryan, K. Dietmeier, F. Martin, R. Wagner, N. Pfanner, Tom40 forms the hydrophilic channel of the mitochondrial import pore for preproteins, Nature 395 (1998) 516–521.
[43] K.P. Kunkele, S. Heins, M. Dembowski, F.E. Nargang, R. Benz, M. Thieffry, J. Walz, R. Lill, S. Nussberger, W. Neupert, The preprotein translocation channel of the outer membrane of mitochondria, Cell 93 (1998) 1009–1019.
[44] D. Rapaport, How does the TOM complex mediate insertion of precursor proteins into the mitochondrial outer membrane? J. Cell Biol. 171 (2005) 419–423.
[45] N. Wiedemann, V. Kozjak, A. Chacinska, B. Schonfisch, S. Rospert, M.T. Ryan, N. Pfanner, C. Meisinger, Machinery for protein sorting and assembly in the mitochondrial outer membrane, Nature 424 (2003) 565–571.
[46] V. Kozjak, N. Wiedemann, D. Milenkovic, C. Lohaus, H.E. Meyer, B. Guiard, C. Meisinger, N. Pfanner, An essential role of Sam50 in the protein sorting and assembly machinery of the mitochondrial outer membrane, J. Biol. Chem. 278 (2003) 48520–48523.
[47] S.A. Paschen, T. Waizenegger, T. Stan, M. Preuss, M. Cyrklaff, K. Hell, D. Rapaport, W. Neupert, Evolutionary conservation of biogenesis of beta-barrel membrane proteins, Nature 426 (2003) 862–866.
[48] E. Schleiff, J. Soll, Membrane protein insertion: mixing eukaryotic and prokaryotic concepts, EMBO Rep. 6 (2005) 1023–1027.
[49] I. Gentle, K. Gabriel, P. Beech, R. Waller, T. Lithgow, The Omp85 family of proteins is essential for outer membrane biogenesis in mitochondria and bacteria, J. Cell Biol. 164 (2004) 19–24.
[50] A.D. Humphries, I.C. Streimann, D. Stojanovski, A.J. Johnston, M. Yano, N.J. Hoogenraad, M.T. Ryan, Dissection of the mitochondrial import and assembly pathway for human Tom40, J. Biol. Chem. 280 (2005) 11535–11543.
[51] D. Mokranjac, W. Neupert, Protein import into mitochondria, Biochem. Soc. Trans. 33 (2005) 1019–1023.
[52] K.N. Truscott, P. Kovermann, A. Geissler, A. Merlin, M. Meijer, A.J. Driessen, J. Rassow, N. Pfanner, R. Wagner, A presequence- and voltage-sensitive channel of the mitochondrial preprotein translocase formed by Tim23, Nat. Struct. Biol. 8 (2001) 1074–1082.
[53] N. Ishihara, T. Komiya, M. Sakaguchi, A. Ito, K. Mihara, Characterization of the initial steps of precursor import into rat liver mitoplasts, J. Biochem. (Tokyo) 124 (1998) 824–834.
[54] M. Donzeau, K. Kaldi, A. Adam, S. Paschen, G. Wanner, B. Guiard, M.F. Bauer, W. Neupert, M. Brunner, Tim23 links the inner and outer mitochondrial membranes, Cell 101 (2000) 401–412.
[55] A. Geissler, A. Chacinska, K.N. Truscott, N. Wiedemann, K. Brandner, A. Sickmann, H.E. Meyer, C. Meisinger, N. Pfanner, P. Rehling, The mitochondrial presequence translocase: an essential role of Tim50 in directing preproteins to the import channel, Cell 111 (2002) 507–518.
[56] H. Yamamoto, M. Esaki, T. Kanamori, Y. Tamura, S. Nishikawa, T. Endo, Tim50 is a subunit of the TIM23 complex that links protein translocation across the outer and inner mitochondrial membranes, Cell 111 (2002) 519–528.
[57] D. Court, F.E. Nargang, H. Steiner, R.S. Hodges, W. Neupert, R. Lill, Role of the intermembrane-space domain of the preprotein receptor Tom22 in protein import into mitochondria, Mol. Cell. Biol. 16 (1996) 4035–4042.
[58] M. Meinecke, R. Wagner, P. Kovermann, B. Guiard, D.U. Mick, D.P. Hutu, W. Voos, K.N. Truscott, A. Chacinska, N. Pfanner, P. Rehling, Tim50 maintains the permeability barrier of the mitochondrial inner membrane, Science 312 (2006) 1523–1526.
[59] A. Chacinska, M. Lind, A.E. Frazier, J. Dudek, C. Meisinger, A. Geissler, A. Sickmann, H.E. Meyer, K.N. Truscott, B. Guiard, N. Pfanner, P. Rehling, Mitochondrial presequence translocase: switching between TOM tethering and motor recruitment involves Tim21 and Tim17, Cell 120 (2005) 817–829.
[60] M. van der Laan, M. Rissler, P. Rehling, Mitochondrial preprotein translocases as dynamic molecular machines, FEMS Yeast Res. 6 (2006) 849–861.
[61] Q. Liu, P. D'Silva, W. Walter, J. Marszalek, E.A. Craig, Regulated cycling of mitochondrial Hsp70 at the protein import channel, Science 300 (2003) 139–141.
[62] H.C. Schneider, B. Westermann, W. Neupert, M. Brunner, The nucleotide exchange factor MGE exerts a key function in the ATP-dependent cycle of mt-Hsp70–Tim44 interaction driving mitochondrial protein import, EMBO J. 15 (1996) 5796–5803.
[63] P. D'Silva, Q. Liu, W. Walter, E.A. Craig, Regulated interactions of mtHsp70 with Tim44 at the translocon in the mitochondrial inner membrane, Nat. Struct. Mol. Biol. 11 (2004) 1084–1091.
[64] H.C. Schneider, J. Berthold, M.F. Bauer, K. Dietmeier, B. Guiard, M. Brunner, W. Neupert, Mitochondrial Hsp70/MIM44 complex facilitates protein import, Nature 371 (1994) 768–774.
[65] D. Mokranjac, M. Sichting, W. Neupert, K. Hell, Tim14, a novel key component of the import motor of the TIM23 protein translocase of mitochondria, EMBO J. 22 (2003) 4945–4956.
[66] K.N. Truscott, W. Voos, A.E. Frazier, M. Lind, Y. Li, A. Geissler, J. Dudek, H. Muller, A. Sickmann, H.E. Meyer, C. Meisinger, B. Guiard, P. Rehling, N. Pfanner, A J-protein is an essential subunit of the presequence translocase-associated protein import motor of mitochondria, J. Cell Biol. 163 (2003) 707–713.
[67] P.D. D'Silva, B. Schilke, W. Walter, A. Andrew, E.A. Craig, J protein cochaperone of the mitochondrial inner membrane required for protein import into the mitochondrial matrix, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 13839–13844.

[68] A.E. Frazier, J. Dudek, B. Guiard, W. Voos, Y. Li, M. Lind, C. Meisinger, A. Geissler, A. Sickmann, H.E. Meyer, V. Bilanchone, M.G. Cumsky, K. N. Truscott, N. Pfanner, P. Rehling, Pam16 has an essential role in the mitochondrial protein import motor, Nat. Struct. Mol. Biol. 11 (2004) 226–233.
[69] M. van der Laan, A. Chacinska, M. Lind, I. Perschil, A. Sickmann, H.E. Meyer, B. Guiard, C. Meisinger, N. Pfanner, P. Rehling, Pam17 is required for architecture and translocation activity of the mitochondrial protein import motor, Mol. Cell. Biol. 25 (2005) 7449–7458.
[70] D. Mokranjac, M. Sichting, D. Popov-Celeketic, A. Berg, K. Hell, W. Neupert, The import motor of the yeast mitochondrial TIM23 preprotein translocase contains two different J proteins, Tim14 and Mdj2, J. Biol. Chem. 280 (2005) 31608–31614.
[71] O. Gakh, P. Cavadini, G. Isaya, Mitochondrial processing peptidases, Biochim, Biophys. Acta 1592 (2002) 63–77.
[72] W.J. Ou, A. Ito, H. Okazaki, T. Omura, Purification and characterization of a processing protease from rat liver mitochondria, EMBO J. 8 (1989) 2605–2612.
[73] V.M. Saavedra-Alanis, P. Rysavy, L.E. Rosenberg, F. Kalousek, Rat liver mitochondrial processing peptidase. Both alpha- and beta-subunits are required for activity, J. Biol. Chem. 269 (1994) 9284–9288.
[74] A. Mukhopadhyay, P. Hammen, M. Waltner-Law, H. Weiner, Timing and structural consideration for the processing of mitochondrial matrix space proteins by the mitochondrial processing peptidase (MPP), Protein Sci. 11 (2002) 1026–1035.
[75] S. Kitada, E. Yamasaki, K. Kojima, A. Ito, Determination of the cleavage site of the presequence by mitochondrial processing peptidase on the substrate binding scaffold and the multiple subsites inside a molecular cavity, J. Biol. Chem. 278 (2003) 1879–1885.
[76] S. Kitada, K. Kojima, A. Ito, Glu(191) and Asp(195) in rat mitochondrial processing peptidase beta subunit are involved in effective cleavage of precursur protein through interaction with the proximal arginine, Biochem. Biophys. Res. Commun. 287 (2001) 594–599.
[77] F. Kalousek, G. Isaya, L.E. Rosenberg, Rat liver mitochondrial intermediate peptidase (MIP): purification and initial characterization, EMBO J. 11 (1992) 2803–2809.
[78] W. Voos, K. Rottgers, Molecular chaperones as essential mediators of mitochondrial biogenesis, Biochim. Biophys. Acta 1592 (2002) 51.
[79] M. Horst, W. Oppliger, S. Rospert, H.J. Schonfeld, G. Schatz, A. Azem, Sequential action of two hsp70 complexes during protein import into mitochondria, EMBO J. 16 (1997) 1842–1849.
[80] Y. Kubo, T. Tsunehiro, S. Nishikawa, M. Nakai, E. Ikeda, A. Toh-e, N. Morishima, T. Shibata, T. Endo, Two distinct mechanisms operate in the reactivation of heat-denatured proteins by the mitochondrial Hsp70/Mdj1p/Yge1p chaperone system, J. Mol. Biol. 286 (1999) 447–464.
[81] O. Deloche, K. Liberek, M. Zylicz, C. Georgopoulos, Purification and biochemical properties of *Saccharomyces cerevisiae* Mdj1p, the mitochondrial DnaJ homologue, J. Biol. Chem. 272 (1997) 28539–28544.
[82] N. Rowley, C. Prip-Buus, B. Westermann, C. Brown, E. Schwarz, B. Barrell, W. Neupert, Mdj1p, a novel chaperone of the DnaJ family, is involved in mitochondrial biogenesis and protein folding, Cell 77 (1994) 249–259.
[83] B. Westermann, B. Gaume, J.M. Herrmann, W. Neupert, E. Schwarz, Role of the mitochondrial DnaJ homolog Mdj1p as a chaperone for mitochondrially synthesized and imported proteins, Mol. Cell. Biol. 16 (1996) 7063–7071.
[84] D.J. Hartman, N.J. Hoogenraad, R. Condron, P.B. Hoj, Identification of a mammalian 10-kDa heat shock protein, a mitochondrial chaperonin 10 homologue essential for assisted folding of trimeric ornithine transcarbamoylase in vitro, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 3394–3398.
[85] P.V. Viitanen, G.H. Lorimer, R. Seetharam, R.S. Gupta, J. Oppenheim, J.O. Thomas, N.J. Cowan, Mammalian mitochondrial chaperonin 60 functions as a single toroidal ring, J. Biol. Chem. 267 (1992) 695–698.
[86] T.J. Corydon, J. Hansen, P. Bross, T.G. Jensen, Down-regulation of Hsp60 expression by RNAi impairs folding of medium-chain acyl-CoA dehydrogenase wild-type and disease-associated proteins, Mol. Genet. Metab. 85 (2005) 260–270.
[87] T.H. Lubben, A.A. Gatenby, G.K. Donaldson, G.H. Lorimer, P.V. Viitanen, Identification of a groES-like chaperonin in mitochondria that facilitates protein folding, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 7683–7687.
[88] Z. Lin, H.S. Rye, GroEL-mediated protein folding: making the impossible, possible, Crit. Rev. Biochem. Mol. Biol. 41 (2006) 211–239.
[89] G. Levy-Rimler, P. Viitanen, C. Weiss, R. Sharkia, A. Greenberg, A. Niv, A. Lustig, Y. Delarea, A. Azem, The effect of nucleotides and mitochondrial chaperonin 10 on the structure and chaperone activity of mitochondrial chaperonin 60, Eur. J. Biochem. 268 (2001) 3465–3472.
[90] Z. Xu, A.L. Horwich, P.B. Sigler, The crystal structure of the asymmetric GroEL-GroES-(ADP)7 chaperonin complex, Nature 388 (1997) 741–750.
[91] J. Martin, M. Mayhew, T. Langer, F.U. Hartl, The reaction cycle of GroEL and GroES in chaperonin-assisted protein folding, Nature 366 (1993) 228–233.
[92] S.E. Radford, GroEL: More than just a folding cage, Cell 125 (2006) 831–833.
[93] Y.C. Tang, H.C. Chang, A. Roeben, D. Wischnewski, N. Wischnewski, M.J. Kerner, F.U. Hartl, M. Hayer-Hartl, Structural features of the GroEL-GroES nano-cage required for rapid folding of encapsulated protein, Cell 125 (2006) 903–914.
[94] A.P. van Loon, A.W. Brandli, G. Schatz, The presequences of two imported mitochondrial proteins contain information for intracellular and intramitochondrial sorting, Cell 44 (1986) 801–812.
[95] B.S. Glick, A. Brandt, K. Cunningham, S. Muller, R.L. Hallberg, G. Schatz, Cytochromes c1 and b2 are sorted to the intermembrane space of yeast mitochondria by a stop-transfer mechanism, Cell 69 (1992) 809–822.
[96] W. Voos, B.D. Gambill, B. Guiard, N. Pfanner, E.A. Craig, Presequence and mature part of preproteins strongly influence the dependence of mitochondrial protein import on heat shock protein 70 in the matrix, J. Cell Biol. 123 (1993) 119–126.
[97] K. Tokatlidis, G. Schatz, Biogenesis of mitochondrial inner membrane proteins, J. Biol. Chem. 274 (1999) 35285–35288.
[98] C. Sirrenberg, M. Endres, H. Folsch, R.A. Stuart, W. Neupert, M. Brunner, Carrier protein import into mitochondria mediated by the intermembrane proteins Tim10/Mrs11 and Tim12/Mrs5, Nature 391 (1998) 912–915.
[99] T. Lutz, W. Neupert, J.M. Herrmann, Import of small Tim proteins into the mitochondrial intermembrane space, EMBO J. 22 (2003) 4400–4408.
[100] S. Hofmann, U. Rothbauer, N. Muhlenbein, W. Neupert, K.D. Gerbitz, M. Brunner, M.F. Bauer, The C66W mutation in the Deafness Dystonia Peptide 1 (DDP1) affects the formation of functional DDP1–TIM13 complexes in the mitochondrial intermembrane space, J. Biol. Chem. 277 (2002) 23287–23293.
[101] C.T. Webb, M.A. Gorman, M. Lazarou, M.T. Ryan, J.M. Gulbis, Crystal structure of the mitochondrial chaperone TIM9.10 reveals a six-bladed alpha-propeller, Mol. Cell 21 (2006) 123–133.
[102] A. Chacinska, S. Pfannschmidt, N. Wiedemann, V. Kozjak, L.K. Sanjuan Szklarz, A. Schulze-Specking, K.N. Truscott, B. Guiard, C. Meisinger, N. Pfanner, Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins, EMBO J. 23 (2004) 3735–3746.
[103] N. Mesecke, N. Terziyska, C. Kozany, F. Baumann, W. Neupert, K. Hell, J.M. Herrmann, A disulfide relay system in the intermembrane space of mitochondria that mediates protein import, Cell 121 (2005) 1059–1069.
[104] M. Rissler, N. Wiedemann, S. Pfannschmidt, K. Gabriel, B. Guiard, N. Pfanner, A. Chacinska, The essential mitochondrial protein Erv1 cooperates with Mia40 in biogenesis of intermembrane space proteins, J. Mol. Biol. 353 (2005) 485–492.
[105] S. Allen, V. Balabanidou, D.P. Sideris, T. Lisowsky, K. Tokatlidis, Erv1 mediates the Mia40-dependent protein import pathway and provides a functional link to the respiratory chain by shuttling electrons to cytochrome *c*, J. Mol. Biol. 353 (2005) 937–944.
[106] N. Wiedemann, N. Pfanner, A. Chacinska, Chaperoning through the mitochondrial intermembrane space, Mol. Cell 21 (2006) 145–148.

[107] R. Jensen, C. Dunn, Protein import into and across the mitochondrial inner membrane: role of the TIM23 and TIM22 translocons, Biochim. Biophys. Acta 1592 (2002) 25–34.
[108] B.R. Miller, M.G. Cumsky, An unusual mitochondrial import pathway for the precursor to yeast cytochrome *c* oxidase subunit Va, J. Cell Biol. 112 (1991) 833–841.
[109] E.E. Rojo, B. Guiard, W. Neupert, R.A. Stuart, Sorting of D-lactate dehydrogenase to the inner membrane of mitochondria. Analysis of topogenic signal and energetic requirements, J. Biol. Chem. 273 (1998) 8040–8047.
[110] J.M. Herrmann, H. Koll, R.A. Cook, W. Neupert, R.A. Stuart, Topogenesis of cytochrome oxidase subunit II. Mechanisms of protein export from the mitochondrial matrix, J. Biol. Chem. 270 (1995) 27079–27086.
[111] J.M. Herrmann, W. Neupert, R.A. Stuart, Insertion into the mitochondrial inner membrane of a polytopic protein, the nuclear-encoded Oxa1p, EMBO J. 16 (1997) 2217–2226.
[112] E.E. Rojo, R.A. Stuart, W. Neupert, Conservative sorting of F0-ATPase subunit 9: export from matrix requires delta pH across inner membrane and matrix ATP, EMBO J. 14 (1995) 3445–3451.
[113] R. Stuart, Insertion of proteins into the inner membrane of mitochondria: the role of the Oxa1 complex, Biochim. Biophys. Acta 1592 (2002) 79.
[114] N. Bonnefoy, M. Kermorgant, O. Groudinsky, M. Minet, P.P. Slonimski, G. Dujardin, Cloning of a human gene involved in cytochrome oxidase assembly by functional complementation of an oxa1- mutation in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 11978–11982.
[115] K. Hell, W. Neupert, R.A. Stuart, Oxa1p acts as a general membrane insertion machinery for proteins encoded by mitochondrial DNA, EMBO J. 20 (2001) 1281–1288.
[116] S. He, T.D. Fox, Membrane translocation of mitochondrially coded Cox2p: distinct requirements for export of N and C termini and dependence on the conserved protein Oxa1p, Mol. Biol. Cell 8 (1997) 1449–1460.
[117] M.F. Bauer, U. Rothbauer, N. Muhlenbein, R.J. Smith, K. Gerbitz, W. Neupert, M. Brunner, S. Hofmann, The mitochondrial TIM22 preprotein translocase is highly conserved throughout the eukaryotic kingdom, FEBS Lett. 464 (1999) 41–47.
[118] P. Rehling, N. Pfanner, C. Meisinger, Insertion of hydrophobic membrane proteins into the inner mitochondrial membrane–a guided tour, J. Mol. Biol. 326 (2003) 639–657.
[119] T. Endo, H. Yamamoto, M. Esaki, Functional cooperation and separation of translocators in protein import into mitochondria, the double-membrane bounded organelles, J. Cell Sci. 116 (2003) 3259–3267.
[120] C.M. Koehler, E. Jarosch, K. Tokatlidis, K. Schmid, R.J. Schweyen, G. Schatz, Import of mitochondrial carriers mediated by essential proteins of the intermembrane space, Science 279 (1998) 369–373.
[121] M.T. Ryan, H. Muller, N. Pfanner, Functional staging of ADP/ATP carrier translocation across the outer mitochondrial membrane, J. Biol. Chem. 274 (1999) 20619–20627.
[122] M.P. Murphy, D. Leuenberger, S.P. Curran, W. Oppliger, C.M. Koehler, The essential function of the small Tim proteins in the TIM22 import pathway does not depend on formation of the soluble 70-kilodalton complex, Mol. Cell. Biol. 21 (2001) 6132–6138.
[123] K.N. Truscott, N. Wiedemann, P. Rehling, H. Muller, C. Meisinger, N. Pfanner, B. Guiard, Mitochondrial import of the ADP/ATP carrier: the essential TIM complex of the intermembrane space is required for precursor release from the TOM complex, Mol. Cell. Biol. 22 (2002) 7780–7789.
[124] D. Leuenberger, N.A. Bally, G. Schatz, C.M. Koehler, Different import pathways through the mitochondrial intermembrane space for inner membrane proteins, EMBO J. 18 (1999) 4816–4822.
[125] P. Rehling, K. Model, K. Brandner, P. Kovermann, A. Sickmann, H.E. Meyer, W. Kuhlbrandt, R. Wagner, K.N. Truscott, N. Pfanner, Protein insertion into the mitochondrial inner membrane by a twin-pore translocase, Science 299 (2003) 1747–1751.
[126] F. Takakubo, P. Cartwright, N. Hoogenraad, D.R. Thorburn, F. Collins, T. Lithgow, H.H. Dahl, An amino acid substitution in the pyruvate dehydrogenase E1 alpha gene, affecting mitochondrial import of the precursor protein, Am. J. Hum. Genet. 57 (1995) 772–780.
[127] B.H. Robinson, Lacticacidemia. Biochemical, clinical, and genetic considerations, Adv. Hum. Genet. 18 (1989) 151–152.
[128] W. Lissens, L. De Meirleir, S. Seneca, I. Liebaers, G.K. Brown, R.M. Brown, M. Ito, E. Naito, Y. Kuroda, D.S. Kerr, I.D. Wexler, M.S. Patel, B. H. Robinson, A. Seyda, Mutations in the X-linked pyruvate dehydrogenase (E1) alpha subunit gene (PDHA1) in patients with a pyruvate dehydrogenase complex deficiency, Hum. Mutat. 15 (2000) 209–219.
[129] H.H. Dahl, S.M. Hunt, W.M. Hutchison, G.K. Brown, The human pyruvate dehydrogenase complex. Isolation of cDNA clones for the E1 alpha subunit, sequence analysis, and characterization of the mRNA, J. Biol. Chem. 262 (1987) 7398–7403.
[130] C.J. Danpure, P.R. Jennings, Peroxisomal alanine:glyoxylate aminotransferase deficiency in primary hyperoxaluria type I, FEBS Lett. 201 (1986) 20–24.
[131] C.J. Danpure, P.J. Cooper, P.J. Wise, P.R. Jennings, An enzyme trafficking defect in two patients with primary hyperoxaluria type 1: peroxisomal alanine/glyoxylate aminotransferase rerouted to mitochondria, J. Cell Biol. 108 (1989) 1345–1352.
[132] P.E. Purdue, Y. Takada, C.J. Danpure, Identification of mutations associated with peroxisome-to-mitochondrion mistargeting of alanine/glyoxylate aminotransferase in primary hyperoxaluria type 1, J. Cell Biol. 111 (1990) 2341–2351.
[133] P.E. Purdue, J. Allsop, G. Isaya, L.E. Rosenberg, C.J. Danpure, Mistargeting of peroxisomal L-alanine:glyoxylate aminotransferase to mitochondria in primary hyperoxaluria patients depends upon activation of a cryptic mitochondrial targeting sequence by a point mutation, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 10900–10904.
[134] A. Motley, M.J. Lumb, P.B. Oatey, P.R. Jennings, P.A. De Zoysa, R.J. Wanders, H.F. Tabak, C.J. Danpure, Mammalian alanine/glyoxylate aminotransferase 1 is imported into peroxisomes via the PTS1 translocation pathway. Increased degeneracy and context specificity of the mammalian PTS1 motif and implications for the peroxisome-to-mitochondrion mistargeting of AGT in primary hyperoxaluria type 1, J. Cell Biol. 131 (1995) 95–109.
[135] M.J. Lumb, C.J. Danpure, Functional synergism between the most common polymorphism in human alanine:glyoxylate aminotransferase and four of the most common disease-causing mutations, J. Biol. Chem. 275 (2000) 36415–36422.
[136] J.M. Leiper, P.B. Oatey, C.J. Danpure, Inhibition of alanine:glyoxylate aminotransferase 1 dimerization is a prerequisite for its peroxisome-to-mitochondrion mistargeting in primary hyperoxaluria type 1, J. Cell Biol. 135 (1996) 939–951.
[137] S. Subramani, A. Koller, W.B. Snyder, Import of peroxisomal matrix and membrane proteins, Annu. Rev. Biochem. 69 (2000) 399–418.
[138] W.J. Chen, M.G. Douglas, The role of protein structure in the mitochondrial import pathway. Unfolding of mitochondrially bound precursors is required for membrane translocation, J. Biol. Chem. 262 (1987) 15605–15609.
[139] U. Wienhues, K. Becker, M. Schleyer, B. Guiard, M. Tropschug, A.L. Horwich, N. Pfanner, W. Neupert, Protein folding causes an arrest of preprotein translocation into mitochondria in vivo, J. Cell Biol. 115 (1991) 1601–1609.
[140] C.J. Danpure, M.J. Lumb, G.M. Birdsey, X. Zhang, Alanine:glyoxylate aminotransferase peroxisome-to-mitochondrion mistargeting in human hereditary kidney stone disease, Biochim. Biophys. Acta 1647 (2003) 70–75.
[141] J.R. Wispe, J.C. Clark, M.S. Burhans, K.E. Kropp, T.R. Korfhagen, J.A. Whitsett, Synthesis and processing of the precursor for human manganosuperoxide dismutase, Biochim. Biophys. Acta 994 (1989) 30–36.
[142] D.C. Wallace, Animal models for mitochondrial disease, Methods Mol. Biol. 197 (2002) 3–54.
[143] J.S. Rosenblum, N.B. Gilula, R.A. Lerner, On signal sequence polymorphisms and diseases of distribution, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4471–4473.
[144] S. Shimoda-Matsubayashi, H. Matsumine, T. Kobayashi, Y. Nakagawa-Hattori, Y. Shimizu, Y. Mizuno, Structural Dimorphism in the Mitochon-

drial Targeting Sequence in the Human Manganese Superoxide Dismutase Gene, Biochem. Biophys. Res. Commun. 226 (1996) 561–565.
[145] A. Sutton, H. Khoury, C. Prip-Buus, C. Cepanec, D. Pessayre, F. Degoul, The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria, Pharmacogenetics 13 (2003) 145–157.
[146] A. Sutton, A. Imbert, A. Igoudjil, V. Descatoire, S. Cazanave, D. Pessayre, F. Degoul, The manganese superoxide dismutase Ala16Val dimorphism modulates both mitochondrial import and mRNA stability, Pharmacogenet. Genomics 15 (2005) 311–319.
[147] F. Degoul, A. Sutton, A. Mansouri, C. Cepanec, C. Degott, B. Fromenty, M. Beaugrand, D. Valla, D. Pessayre, Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease, Gastroenterology 120 (2001) 1468–1474.
[148] P. Nahon, A. Sutton, D. Pessayre, P. Rufat, F. Degoul, N. Ganne-Carrie, M. Ziol, N. Charnaux, G. N'Kontchou, J.C. Trinchet, L. Gattegno, M. Beaugrand, Genetic dimorphism in superoxide dismutase and susceptibility to alcoholic cirrhosis, hepatocellular carcinoma, and death, Clin. Gastroenterol. Hepatol. 3 (2005) 292–298.
[149] E. Kukielka, E. Dicker, A.I. Cederbaum, Increased production of reactive oxygen species by rat liver mitochondria after chronic ethanol treatment, Arch. Biochem. Biophys. 309 (1994) 377–386.
[150] D.C. Wallace, Mitochondrial diseases in man and mouse, Science 283 (1999) 1482–1488.
[151] J.B. Hoek, A. Cahill, J.G. Pastorino, Alcohol and mitochondria: a dysfunctional relationship, Gastroenterology 122 (2002) 2049–2063.
[152] S. Hiroi, H. Harada, H. Nishi, M. Satoh, R. Nagai, A. Kimura, Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese, Biochem. Biophys. Res. Commun. 261 (1999) 332–339.
[153] T. Nomiyama, Y. Tanaka, L. Piao, K. Nagasaka, K. Sakai, T. Ogihara, K. Nakajima, H. Watada, R. Kawamori, The polymorphism of manganese superoxide dismutase is associated with diabetic nephropathy in Japanese type 2 diabetic patients, J. Hum. Genet. 48 (2003) 138–141.
[154] L. Valenti, D. Conte, A. Piperno, P. Dongiovanni, A.L. Fracanzani, M. Fraquelli, A. Vergani, C. Gianni, L. Carmagnola, S. Fargion, The mitochondrial superoxide dismutase A16V polymorphism in the cardiomyopathy associated with hereditary haemochromatosis, J. Med. Genet. 41 (2004) 946–950.
[155] L. Tranebjaerg, C. Schwartz, H. Eriksen, S. Andreasson, V. Ponjavic, A. Dahl, R.E. Stevenson, M. May, F. Arena, D. Barker, A new X linked recessive deafness syndrome with blindness, dystonia, fractures, and mental deficiency is linked to Xq22, J. Med. Genet. 32 (1995) 257–263.
[156] H. Jin, M. May, L. Tranebjaerg, E. Kendall, G. Fontan, J. Jackson, S.H. Subramony, F. Arena, H. Lubs, S. Smith, R. Stevenson, C. Schwartz, D. Vetrie, A novel X-linked gene, DDP, shows mutations in families with deafness (DFN-1), dystonia, mental deficiency and blindness, Nat. Genet. 14 (1996) 177–180.
[157] C.M. Koehler, D. Leuenberger, S. Merchant, A. Renold, T. Junne, G. Schatz, Human deafness dystonia syndrome is a mitochondrial disease, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2141–2146.
[158] J. Binder, S. Hofmann, S. Kreisel, J.C. Wohrle, H. Bazner, J.K. Krauss, M.G. Hennerici, M.F. Bauer, Clinical and molecular findings in a patient with a novel mutation in the deafness-dystonia peptide (DDP1) gene, Brain 126 (2003) 1814–1820.
[159] M. Ezquerra, J. Campdelacreu, E. Munoz, E. Tolosa, M.J. Marti, A novel intronic mutation in the DDP1 gene in a family with X-linked dystonia-deafness syndrome, Arch. Neurol. 62 (2005) 306–308.
[160] L. Tranebjaerg, B.C. Hamel, F.J. Gabreels, W.O. Renier, M. Van Ghelue, A de novo missense mutation in a critical domain of the X-linked DDP gene causes the typical deafness-dystonia-optic atrophy syndrome, Eur. J. Hum. Genet. 8 (2000) 464–467.
[161] R.H. Swerdlow, G.F. Wooten, A novel deafness/dystonia peptide gene mutation that causes dystonia in female carriers of Mohr–Tranebjaerg syndrome, Ann. Neurol. 50 (2001) 537–540.
[162] H. Ujike, Y. Tanabe, Y. Takehisa, T. Hayabara, S. Kuroda, A family with X-linked dystonia-deafness syndrome with a novel mutation of the DDP gene, Arch. Neurol. 58 (2001) 1004–1007.
[163] L. Tranebjaerg, M. Van Ghelue, O. Nilssen, Jensen syndrome is allelic to Mohr–Tranebjaerg syndrome and both are caused by stop mutations in the DDP gene. Am. J. Hum. Genet. 61 (1997) A349 (suppl).
[164] L.A. Aguirre, I. del Castillo, A. Macaya, C. Meda, M. Villamar, M.A. Moreno-Pelayo, F. Moreno, A novel mutation in the gene encoding TIMM8a, a component of the mitochondrial protein translocase complexes, in a Spanish familial case of deafness-dystonia (Mohr–Tranebjaerg) syndrome, Am. J. Med. Genet., A 140 (2006) 392–397.
[165] U. Rothbauer, S. Hofmann, N. Muhlenbein, S.A. Paschen, K.D. Gerbitz, W. Neupert, M. Brunner, M.F. Bauer, Role of the deafness dystonia peptide 1 (DDP1) in import of human Tim23 into the inner membrane of mitochondria, J. Biol. Chem. 276 (2001) 37327–37334.
[166] K. Roesch, S.P. Curran, L. Tranebjaerg, C.M. Koehler, Human deafness dystonia syndrome is caused by a defect in assembly of the DDP1/TIMM8a–TIMM13 complex, Hum. Mol. Genet. 11 (2002) 477–486.
[167] C.M. Koehler, The small Tim proteins and the twin Cx3C motif, Trends Biochem. Sci. 29 (2004) 1–4.
[168] S. Hofmann, U. Rothbauer, N. Muhlenbein, K. Baiker, K. Hell, M.F. Bauer, Functional and mutational characterization of human MIA40 acting during import into the mitochondrial intermembrane space, J. Mol. Biol. 353 (2005) 517–528.
[169] K. Roesch, P.J. Hynds, R. Varga, L. Tranebjaerg, C.M. Koehler, The calcium-binding aspartate/glutamate carriers, citrin and aralar1, are new substrates for the DDP1/TIMM8a–TIMM13 complex, Hum. Mol. Genet. 13 (2004) 2101–2111.
[170] S.C. Hoppins, F.E. Nargang, The Tim8–Tim13 Complex of *Neurospora crassa* Functions in the Assembly of Proteins into Both Mitochondrial Membranes, J. Biol. Chem. 279 (2004) 12396–12405.
[171] N. Wiedemann, K.N. Truscott, S. Pfannschmidt, B. Guiard, C. Meisinger, N. Pfanner, Biogenesis of the protein import channel Tom40 of the mitochondrial outer membrane: intermembrane space components are involved in an early stage of the assembly pathway, J. Biol. Chem. 279 (2004) 18188–18194.
[172] K.M. Davey, J.S. Parboosingh, D.R. McLeod, A. Chan, R. Casey, P. Ferreira, F.F. Snyder, P.J. Bridge, F.P. Bernier, Mutation of DNAJC19, a human homologue of yeast inner mitochondrial membrane co-chaperones, causes DCMA syndrome, a novel autosomal recessive Barth syndrome-like condition, J. Med. Genet. 43 (2006) 385–393.
[173] E. Agsteribbe, A. Huckriede, M. Veenhuis, M.H. Ruiters, K.E. Niezen-Koning, O.H. Skjeldal, K. Skullerud, R.S. Gupta, R. Hallberg, O.P. van Diggelen, A fatal, systemic mitochondrial disease with decreased mitochondrial enzyme activities, abnormal ultrastructure of the mitochondria and deficiency of heat shock protein 60, Biochem. Biophys. Res. Commun. 193 (1993) 146–154.
[174] P. Briones, M.A. Vilaseca, A. Ribes, A. Vernet, M. Lluch, V. Cusi, A. Huckriede, E. Agsteribbe, A new case of multiple mitochondrial enzyme deficiencies with decreased amount of heat shock protein 60, J. Inherit. Metab. Dis. 20 (1997) 569–577.
[175] A. Huckriede, A. Heikema, K. Sjollema, P. Briones, E. Agsteribbe, Morphology of the mitochondria in heat shock protein 60 deficient fibroblasts from mitochondrial myopathy patients. Effects of stress conditions, Virchows Arch. 427 (1995) 159–165.
[176] A. Huckriede, E. Agsteribbe, Decreased synthesis and inefficient mitochondrial import of hsp60 in a patient with a mitochondrial encephalomyopathy, Biochim. Biophys. Acta 1227 (1994) 200–206.
[177] B. Fontaine, C.S. Davoine, A. Durr, C. Paternotte, I. Feki, J. Weissenbach, J. Hazan, A. Brice, A new locus for autosomal dominant pure spastic paraplegia, on chromosome 2q24–q34, Am. J. Hum. Genet. 66 (2000) 702–707.
[178] J.J. Hansen, A. Durr, I. Cournu-Rebeix, C. Georgopoulos, D. Ang, M.N. Nielsen, C.S. Davoine, A. Brice, B. Fontaine, N. Gregersen, P. Bross, Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60, Am. J. Hum. Genet. 70 (2002) 1328–1332.
[179] D.C. Wallace, Mitochondrial DNA in aging and disease, Sci. Am. 277 (1997) 40–47.

[180] G.C. Kujoth, A. Hiona, T.D. Pugh, S. Someya, K. Panzer, S.E. Wohlgemuth, T. Hofer, A.Y. Seo, R. Sullivan, W.A. Jobling, J.D. Morrow, H. Van Remmen, J.M. Sedivy, T. Yamasoba, M. Tanokura, R. Weindruch, C. Leeuwenburgh, T.A. Prolla, Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging, Science 309 (2005) 481–484.
[181] A.W. Dobson, Y. Xu, M.R. Kelley, S.P. LeDoux, G.L. Wilson, Enhanced Mitochondrial DNA Repair and Cellular Survival after Oxidative Stress by Targeting the Human 8-Oxoguanine Glycosylase Repair Enzyme to Mitochondria, J. Biol. Chem. 275 (2000) 37518–37523.
[182] B. Szczesny, T.K. Hazra, J. Papaconstantinou, S. Mitra, I. Boldogh, Age-dependent deficiency in import of mitochondrial DNA glycosylases required for repair of oxidatively damaged bases, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10670–10675.
[183] G. Wright, K. Terada, M. Yano, I. Sergeev, M. Mori, Oxidative stress inhibits the mitochondrial import of preproteins and leads to their degradation, Exp. Cell Res. 263 (2001) 107–117.
[184] M.K. Shigenaga, T.M. Hagen, B.N. Ames, Oxidative damage and mitochondrial decay in aging, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 10771–10778.
[185] S. Savitha, C. Panneerselvam, Mitochondrial membrane damage during aging process in rat heart: potential efficacy of L-carnitine and DL alpha lipoic acid, Mech. Ageing Dev. 127 (2006) 349–355.
[186] Y.T. Wong, R. Ruan, F.E. Tay, Relationship between levels of oxidative DNA damage, lipid peroxidation and mitochondrial membrane potential in young and old F344 rats, Free Radical Res. 40 (2006) 393–402.
[187] J.E. Kokoszka, P. Coskun, L.A. Esposito, D.C. Wallace, Increased mitochondrial oxidative stress in the Sod2 (+/−) mouse results in the age-related decline of mitochondrial function culminating in increased apoptosis, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 2278–2283.
[188] T.M. Hagen, D.L. Yowe, J.C. Bartholomew, C.M. Wehr, K.L. Do, J.Y. Park, B.N. Ames, Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 3064–3069.
[189] T. Kanamori, S. Nishikawa, M. Nakai, I. Shin, P.G. Schultz, T. Endo, Uncoupling of transfer of the presequence and unfolding of the mature domain in precursor translocation across the mitochondrial outer membrane, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 3634–3639.
[190] D. Rapaport, W. Neupert, R. Lill, Mitochondrial protein import. Tom40 plays a major role in targeting and translocation of preproteins by forming a specific binding site for the presequence, J. Biol. Chem. 272 (1997) 18725–18731.
[191] E.E. Craig, A. Chesley, D.A. Hood, Thyroid hormone modifies mitochondrial phenotype by increasing protein import without altering degradation, Am. J. Physiol. 275 (1998) C1508–C1515.
[192] M. Colavecchia, L.N. Christie, Y.S. Kanwar, D.A. Hood, Functional consequences of thyroid hormone-induced changes in the mitochondrial protein import pathway, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E29–E35.
[193] J.J. Schneider, D.A. Hood, Effect of thyroid hormone on mtHsp70 expression, mitochondrial import and processing in cardiac muscle, J. Endocrinol. 165 (2000) 9–17.
[194] O.I. Ornatsky, M.K. Connor, D.A. Hood, Expression of stress proteins and mitochondrial chaperonins in chronically stimulated skeletal muscle, Biochem. J. 311 (Pt 1) (1995) 119–123.
[195] M. Takahashi, A. Chesley, D. Freyssenet, D.A. Hood, Contractile activity-induced adaptations in the mitochondrial protein import system, Am. J. Physiol. 274 (1998) C1380–C1387.
[196] J.P. Mattson, C.R. Ross, J.L. Kilgore, T.I. Musch, Induction of mitochondrial stress proteins following treadmill running, Med. Sci. Sports Exerc. 32 (2000) 365–369.
[197] F.W. Booth, D.B. Thomason, Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models, Physiol. Rev. 71 (1991) 541–585.
[198] D. Pette, G. Vrbova, Adaptation of mammalian skeletal muscle fibers to chronic electrical stimulation, Rev. Physiol. Biochem. Pharmacol. 120 (1992) 115–202.
[199] M. Takahashi, D.A. Hood, Protein import into subsarcolemmal and intermyofibrillar skeletal muscle mitochondria. Differential import regulation in distinct subcellular regions, J. Biol. Chem. 271 (1996) 27285–27291.
[200] D.A. Krieger, C.A. Tate, J. McMillin-Wood, F.W. Booth, Populations of rat skeletal muscle mitochondria after exercise and immobilization, J. Appl. Physiol. 48 (1980) 23–28.
[201] L. Lan, S. Isenmann, B.W. Wattenberg, Targeting and insertion of C-terminally anchored proteins to the mitochondrial outer membrane is specific and saturable but does not strictly require ATP or molecular chaperones, Biochem. J. 349 (2000) 611–621.
[202] M. Nguyen, D.G. Millar, V.W. Yong, S.J. Korsmeyer, G.C. Shore, Targeting of Bcl-2 to the mitochondrial outer membrane by a COOH-terminal signal anchor sequence, J. Biol. Chem. 268 (1993) 25265–25268.
[203] Z.P. Zhuang, B. Marks, R.B. McCauley, The insertion of monoamine oxidase A into the outer membrane of rat liver mitochondria, J. Biol. Chem. 267 (1992) 591–596.
[204] F. Moro, A. Muga, Thermal adaptation of the yeast mitochondrial Hsp70 system is regulated by the reversible unfolding of its nucleotide exchange factor, J. Mol. Biol. 358 (2006) 1367–1377.
[205] A. Mukhopadhyay, L. Ni, H. Weiner, A co-translational model to explain the in vivo import of proteins into HeLa cell mitochondria, Biochem. J. 382 (2004) 385–392.
[206] U. Funfschilling, S. Rospert, Nascent polypeptide-associated complex stimulates protein import into yeast mitochondria, Mol. Biol. Cell 10 (1999) 3289–3299.
[207] R. George, P. Walsh, T. Beddoe, T. Lithgow, The nascent polypeptide-associated complex (NAC) promotes interaction of ribosomes with the mitochondrial surface in vivo, FEBS Lett. 516 (2002) 213–216.
[208] R. George, T. Beddoe, K. Landl, T. Lithgow, The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria in vivo, Proc. Natl. Acad. Sci .U. S. A. 95 (1998) 2296–2301.
[209] A.U. Ahmed, P.L. Beech, S.T. Lay, P.R. Gilson, P.R. Fisher, Import-associated translational inhibition: novel in vivo evidence for cotranslational protein import into *Dictyostelium discoideum* mitochondria, Eukaryot Cell 5 (2006) 1314–1327.
[210] S.O. Shan, P. Walter, Co-translational protein targeting by the signal recognition particle, FEBS Lett. 579 (2005) 921–926.
[211] J.A. MacKenzie, R.M. Payne, Ribosomes specifically bind to mammalian mitochondria via protease-sensitive proteins on the outer membrane, J. Biol. Chem. 279 (2004) 9803–9810.
[212] K.S. Crowley, R.M. Payne, Ribosome binding to mitochondria is regulated by GTP and the transit peptide, J. Biol. Chem. 273 (1998) 17278–17285.
[213] A.D. Bergemann, F. Cole, K. Hirschhorn, The etiology of Wolf–Hirschhorn syndrome, Trends Genet. 21 (2005) 188–195.
[214] S. Endele, M. Fuhry, S.J. Pak, B.U. Zabel, A. Winterpacht, LETM1, a novel gene encoding a putative EF-hand Ca(2+)-binding protein, flanks the Wolf–Hirschhorn syndrome (WHS) critical region and is deleted in most WHS patients, Genomics 60 (1999) 218–225.
[215] K. Nowikovsky, E.M. Froschauer, G. Zsurka, J. Samaj, S. Reipert, M. Kolisek, G. Wiesenberger, R.J. Schweyen, The LETM1/YOL027 gene family encodes a factor of the mitochondrial K+ homeostasis with a potential role in the Wolf–Hirschhorn syndrome, J. Biol. Chem. 279 (2004) 30307–30315.
[216] S. Schlickum, A. Moghekar, J.C. Simpson, C. Steglich, R.J. O'Brien, A. Winterpacht, S.U. Endele, LETM1, a gene deleted in Wolf–Hirschhorn syndrome, encodes an evolutionarily conserved mitochondrial protein, Genomics 83 (2004) 254–261.
[217] K.S. Dimmer, S. Fritz, F. Fuchs, M. Messerschmitt, N. Weinbach, W. Neupert, B. Westermann, Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*, Mol. Biol. Cell 13 (2002) 847–853.
[218] A.E. Frazier, R.D. Taylor, D.U. Mick, B. Warscheid, N. Stoepel, H.E. Meyer, M.T. Ryan, B. Guiard, P. Rehling, Mdm38 interacts with ribosomes and is a component of the mitochondrial protein export machinery, J. Cell Biol. 172 (2006) 553–564.
[219] B. Dworniczak, M.E. Mirault, Structure and expression of a human gene coding for a 71 kd heat shock 'cognate' protein, Nucleic Acids Res. 15 (1987) 5181–5197.

[220] K.M. Abdul, K. Terada, M. Yano, M.T. Ryan, I. Streimann, N.J. Hoogenraad, M. Mori, Functional analysis of human metaxin in mitochondrial protein import in cultured cells and its relationship with the Tom complex, Biochem. Biophys. Res. Commun. 276 (2000) 1028–1034.
[221] M. Yano, N. Hoogenraad, K. Terada, M. Mori, Identification and functional analysis of human Tom22 for protein import into mitochondria, Mol. Cell. Biol. 20 (2000) 7205–7213.
[222] A.M. Edmonson, D.K. Mayfield, V. Vervoort, B.R. DuPont, G. Argyropoulos, Characterization of a human import component of the mitochondrial outer membrane, TOMM70A, Cell Commun. Adhes. 9 (2002) 15–27.
[223] I.S. Goping, D.G. Millar, G.C. Shore, Identification of the human mitochondrial protein import receptor, huMas20p. Complementation of delta mas20 in yeast, FEBS Lett. 373 (1995) 45–50.
[224] N. Muhlenbein, S. Hofmann, U. Rothbauer, M.F. Bauer, Organization and function of the small Tim complexes acting along the import pathway of metabolite carriers into mammalian mitochondria, J. Biol. Chem. 279 (2004) 13540–13546.
[225] M.F. Bauer, K. Gempel, A.S. Reichert, G.A. Rappold, P. Lichtner, K.D. Gerbitz, W. Neupert, M. Brunner, S. Hofmann, Genetic and structural characterization of the human mitochondrial inner membrane translocase, J. Mol. Biol. 289 (1999) 69–82.
[226] U. Bomer, J. Rassow, N. Zufall, N. Pfanner, M. Meijer, A.C. Maarse, The preprotein translocase of the inner mitochondrial membrane: evolutionary conservation of targeting and assembly of Tim17, J. Mol. Biol. 262 (1996) 389–395.
[227] Y. Guo, N. Cheong, Z. Zhang, R. De Rose, Y. Deng, S.A. Farber, T. Fernandes-Alnemri, E.S. Alnemri, Tim50, a component of the mitochondrial translocator, regulates mitochondrial integrity and cell death, J. Biol. Chem. 279 (2004) 24813–24825.
[228] T. Bhattacharyya, A.N. Karnezis, S.P. Murphy, T. Hoang, B.C. Freeman, B. Phillips, R.I. Morimoto, Cloning and subcellular localization of human mitochondrial hsp70, J. Biol. Chem. 270 (1995) 1705–1710.
[229] A.A. Choglay, J.P. Chapple, G.L. Blatch, M.E. Cheetham, Identification and characterization of a human mitochondrial homologue of the bacterial co-chaperone GrpE, Gene 267 (2001) 125–134.
[230] R.L. Strausberg, E.A. Feingold, L.H. Grouse, J.G. Derge, R.D. Klausner, F.S. Collins, L. Wagner, C.M. Shenmen, G.D. Schuler, S.F. Altschul, B. Zeeberg, K.H. Buetow, C.F. Schaefer, N.K. Bhat, R.F. Hopkins, H. Jordan, T. Moore, S.I. Max, J. Wang, F. Hsieh, L. Diatchenko, K. Marusina, A.A. Farmer, G.M. Rubin, L. Hong, M. Stapleton, M.B. Soares, M.F. Bonaldo, T.L. Casavant, T.E. Scheetz, M.J. Brownstein, T. B. Usdin, S. Toshiyuki, P. Carninci, C. Prange, S.S. Raha, N.A. Loquellano, G.J. Peters, R.D. Abramson, S.J. Mullahy, S.A. Bosak, P.J. McEwan, K.J. McKernan, J.A. Malek, P.H. Gunaratne, S. Richards, K.C. Worley, S. Hale, A.M. Garcia, L.J. Gay, S.W. Hulyk, D.K. Villalon, D.M. Muzny, E.J. Sodergren, X. Lu, R.A. Gibbs, J. Fahey, E. Helton, M. Ketteman, A. Madan, S. Rodrigues, A. Sanchez, M. Whiting, A. Madan, A.C. Young, Y. Shevchenko, G.G. Bouffard, R.W. Blakesley, J.W. Touchman, E.D. Green, M.C. Dickson, A.C. Rodriguez, J. Grimwood, J. Schmutz, R.M. Myers, Y.S. Butterfield, M.I. Krzywinski, U. Skalska, D. E. Smailus, A. Schnerch, J.E. Schein, S.J. Jones, M.A. Marra, Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16899–16903.
[231] E. Petek, C. Windpassinger, J.B. Vincent, J. Cheung, A.P. Boright, S.W. Scherer, P.M. Kroisel, K. Wagner, Disruption of a novel gene (IMMP2L) by a breakpoint in 7q31 associated with Tourette syndrome, Am. J. Hum. Genet. 68 (2001) 848–858.
[232] M. Mao, G. Fu, J.S. Wu, Q.H. Zhang, J. Zhou, L.X. Kan, Q.H. Huang, K. L. He, B.W. Gu, Z.G. Han, Y. Shen, J. Gu, Y.P. Yu, S.H. Xu, Y.X. Wang, S.J. Chen, Z. Chen, Identification of genes expressed in human CD34(+) hematopoietic stem/progenitor cells by expressed sequence tags and efficient full-length cDNA cloning, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 8175–8180.
[233] T. Nagase, N. Seki, A. Tanaka, K. Ishikawa, N. Nomura, Prediction of the coding sequences of unidentified human genes. IV. The coding sequences of 40 new genes (KIAA0121–KIAA0160) deduced by analysis of cDNA clones from human cell line KG-1, DNA Res. 2 (1995) 167–74, 199–210.
[234] A. Chew, G. Sirugo, J.P. Alsobrook II, G. Isaya, Functional and genomic analysis of the human mitochondrial intermediate peptidase, a putative protein partner of frataxin, Genomics 65 (2000) 104–112.
[235] S. Jindal, A.K. Dudani, B. Singh, C.B. Harley, R.S. Gupta, Primary structure of a human mitochondrial protein homologous to the bacterial and plant chaperonins and to the 65-kilodalton mycobacterial antigen, Mol. Cell. Biol. 9 (1989) 2279–2283.
[236] G. Legname, G. Fossati, G. Gromo, N. Monzini, F. Marcucci, D. Modena, Expression in *Escherichia coli*, purification and functional activity of recombinant human chaperonin 10, FEBS Lett. 361 (1995) 211–214.
[237] J. Syken, T. De-Medina, K. Munger, TID1, a human homolog of the *Drosophila* tumor suppressor l(2)tid, encodes two mitochondrial modulators of apoptosis with opposing functions, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 8499–8504.
[238] B. Lu, N. Garrido, J.N. Spelbrink, C.K. Suzuki, Tid1 isoforms are mitochondrial DnaJ-like chaperones with unique carboxyl termini that determine cytosolic fate, J. Biol. Chem. 281 (2006) 13150–13158.