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Mitochondrial Protein Import Dysfunction: Mitochondrial disease, Neurodegenerative Disease and Cancer.

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Keywords: Mitochondria, Protein Import, Neurodegeneration, Cancer, Huntington's disease, Alzheimer's disease, Parkinson's disease, TOM, TIM

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/1873-3468.14022

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Abstract

The majority of proteins localised to mitochondria are encoded by the nuclear genome, with approximately 1500 proteins imported into mammalian mitochondria. Dysfunction in this fundamental cellular process is linked to a variety of pathologies including neuropathies, cardiovascular disorders, myopathies, neurodegenerative diseases and cancer, demonstrating the importance of mitochondrial protein import machinery for cellular function. Correct import of proteins into mitochondria requires the co-ordinated activity of multimeric protein translocation and sorting machineries located in both the outer and inner mitochondrial membranes, directing the imported proteins to the destined mitochondrial compartment. This dynamic process maintains cellular homeostasis, and its dysregulation significantly affects cellular signalling pathways and metabolism. This review summarises current knowledge of the mammalian mitochondrial import machinery and the pathological consequences of mutation of its components. In addition, we will discuss the role of mitochondrial import in cancer, and our current understanding of the role of mitochondrial import in neurodegenerative diseases including Alzheimer's disease, Huntington's disease, Parkinson's disease.

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Introduction

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Mitochondria are often described as the powerhouse of the cell due to their central role in energy production [1]. However, mitochondrial function extends far beyond energy generation and includes roles in metabolism, calcium homeostasis, iron-sulfur cluster biogenesis and apoptosis, to name a few [1,2]. Mitochondrial size, shape, distribution and protein complement varies between different cell types and in response to specific metabolic demands of each cell type [3-5]. The plasticity of the mitochondrial proteome enables the cell to rapidly respond to environmental stress and to local demands in energy metabolism. Mitochondria contain four compartments, the mitochondrial outer membrane and the mitochondrial inner membrane, which segregate two aqueous compartments, the intermembrane space and mitochondrial matrix ***(*Fig.1*)***. The mitochondrial inner membrane is highly folded forming specialised compartments called cristae which are connected to the inner boundary membrane by cristae junctions [6]. The formation of cristae significantly increase the surface area of the membrane, with cristae density dynamically responding to bioenergetic demands of the cell [6,7]. The mammalian mitochondrial proteome consists of approximately 1500 proteins, of which 13 proteins are encoded by the mitochondrial genome. The remaining 99% of proteins localised to mitochondria are nuclear encoded and are translated in the cytosol prior to import into mitochondria [8]. Import of these proteins to the correct sub-mitochondrial localisation is coordinated by sophisticated multimeric protein machineries, referred to as translocases [9].

In recent years it has become apparent that perturbations to the pathways and machines that drive mitochondrial protein import has severe consequences on human health. This includes, mitochondrial diseases that are broadly defined as energy generation disorders, in addition to metabolic disorders, cardiovascular disorders, neurodegenerative disorders and cancer [10-12]. This review will summarise the current understanding of mitochondrial protein import in mammalian cells, and how dysfunction of these pathways/machines results in mitochondrial disease. In addition, we will discuss the role of defective mitochondrial import in neurodegenerative diseases such as Alzheimer's disease, Huntington's disease and Parkinson's disease. We will also discuss the role of mitochondrial import in cancer, providing a snapshot of the wide-reaching implications of defects in mitochondrial import machineries.

Overview of Mitochondrial import

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Much of what we know about the mitochondrial import machineries comes from studies using fungal model organisms, with many of the core proteins highly conserved from yeast to mammals [13,14]. The last decade has seen a significant advancement in the understanding of mammalian translocase machines and their associated import pathways [15]. This review will focus on mammalian translocase machineries and their connection to disease. For further information on yeast mitochondrial import machineries we recommend the following comprehensive reviews [13,16,17].

Protein translocation across the mitochondrial outer membrane: The TOM Complex

The Translocase of the Outer Membrane (TOM) complex is the main entry gate for proteins into mitochondria, with over 90% of precursor protein translocation at mitochondria occurring through this complex [18,19]. The human TOM complex is a highly conserved heterooligomeric protein complex which is comprised of 7 subunits: Tom40, Tom70, Tom22, Tom20, and the small TOM proteins Tom5, Tom6 and Tom7 (**Fig.1**) [18,20-22]. Utilising cryo-electron microscopy, the structure of the TOM complex in Bakers' yeast (*Saccharomyces cerevisiae*) and *Neurospora crassa* have revealed the dynamic nature of this complex with dimeric, trimeric and tetrameric conformations detected [20-22]. Tom40 is a β-barrel protein that forms the central pore of the TOM complex which associates with Tom22, Tom5, Tom6 and Tom7 by their transmembrane helices to form a stable complex [18]. Tom70 and Tom20 are the preprotein receptors of the TOM complex and interact with internal targeting signals and N-terminal targeting signals of preproteins respectively [23-25]. Tom70 contains a tetratricopeptide repeat (TPR) domain which interacts with cytosolic chaperones and Tom20 in a proposed mechanism of competitive binding to facilitate substrate recognition and import [26]. Tom22 has been described to tether two Tom40 subunits and is curved to increase association with the Tom40 barrel [20,27,28]. The helical domain of Tom22 extends into the intermembrane space and may assist TOM complex association with the inner membrane translocase machinery [20]. Although their role in mammalian cells is poorly defined, the small Tom proteins Tom5, Tom6 and Tom7 are proposed to provide structural stability to the TOM complex, with loss of Tom7 affecting the stability of the TOM complex [29,30].

The Sorting and assembly machinery complex: The SAM Complex

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The mitochondrial outer membrane contains a number of β-barrel proteins including Tom40 and VDAC (Voltage Dependent Anion Channel) that are sorted and assembled by specialized machinery termed the SAM (Sorting and Assembly Machinery) complex [31,32]. Following import across the outer membrane, β-barrel proteins are transferred to the SAM complex for insertion into the membrane (**Fig. 1**). The core β-barrel subunit of the SAM complex Sam50 is conserved from bacteria through to mammalian systems, showcasing the bacterial origin of mitochondria [32]. In mammalian cells, metaxin-1 and metaxin-2 have weak homology to the yeast SAM components Sam37 and Sam35 respectively, and have been shown to function in mitochondrial protein import [31,33-36]. Recent data has described a role for the SAM complex as a structural component of the Mitochondrial Intermembrane space Bridging (MIB) complex along with the mitochondrial contact site (MICOS) complex, which serve to maintain the cristae morphology of the inner membrane [36-41]. Sam50 along with the inner membrane proteins CHCHD3 and mitofilin have been shown to function in the MIB complex [37]. The MIB complex also likely contains the paralog metaxin-3 and DnaJC11 [40]. Complexome analysis of the MICOS complex and the MIB complexes in human cells suggest that Sam50/metaxin-2/metaxin-3 and DnaJC11/metaxin-1 form subassemblies of the MIB complex, progressively adding to the MICOS complex [40]. In human cells knockdown of Sam50 results in reduced levels of metaxin-1 and metaxin-2 suggesting that they act in the same pathway, however the role of these proteins in import has yet to be clearly defined [31]. Depletion of Sam50 also causes aberrant cristae morphology and loss of cristae junctions, demonstrating a role for the SAM complex in both β-barrel biogenesis and cristae formation [41].

Import of proteins via the pre-sequence pathway: The Translocase of the Inner Membrane 23 (TIM23) complex.

Following translocation across TOM, precursor proteins containing N-terminal targeting sequences are directed to the Translocase of the Inner Membrane 23 (TIM23) complex where they are laterally inserted into the inner membrane, or directed to the mitochondrial matrix in a membrane potential-dependent manner (**Fig. 1**) [42]. N-terminal presequences are poorly conserved at the sequence level, however they have conserved structural properties including a length of 20 – 60 amino acids, an α-helical structure with a net positive charge and the potential to form and amphipathic

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helix [43,44]. The core of the TIM23 complex (TIM23^{CORE}) consists of Tim17, Tim23, and Tim50. Tim23 and Tim17 form the core channel of the complex, while Tim50 acts as a preprotein receptor [45,46]. There are two isoforms of Tim17 (Tim17a and Tim17b) that have different tissue expression profiles, with Tim17a expressed highly in the heart and brain, while Tim17b is highly expressed in skeletal muscle [47]. Tim17a and Tim17b isoforms are found in distinct TIM23 complexes, however it is unclear if these proteins are functionally redundant [47,48]. Proteins destined for the mitochondrial matrix are translocated via the TIM23^{MOTOR} complex that consists of the core TIM23 subunits in association with the Presequence-Associated Motor (PAM). In humans, the PAM motor is comprised of the proteins Mortalin (mtHsp70 in yeast), Tim44, DNAJC15/19 (Pam18 in yeast), Magmas (Pam16 in yeast) and GrpE (Mge1 in yeast) [48]. Tim44 associates loosely with the matrix face of the TIM23^{CORE} and acts as a scaffold for Mortalin [47]. Mortalin is the central component of PAM and provides the mechanical force to drive preproteins into the matrix, while Magmas, DnaJC15/19 and GrpE regulate the activity of Mortalin [49-51]. DnaJC15/19 associate with distinct forms of the TIM23 complex based on the presence of Tim17a or Tim17b respectively [48]. Following translocation of preproteins into the mitochondrial matrix, the matrix targeting pre-sequence is removed proteolytically by the heterodimeric Mitochondrial Processing Peptidase (MPP) [52]. PMPCA is the alpha subunit of the heterodimeric MPP complex which contains a glycine-rich-loop that is the contact site for substrates [53,54]. PMPCB is the beta subunit of the MPP complex which is a metalloprotease that contains the catalytic site of the MPP complex [55,56]. Secondary cleavage of proteins is performed by Mitochondrial Intermediate Peptidase (MIPEP/MIP) at the N-terminal octapeptide that is yielded by MPP [52].

Presequence-containing proteins that are destined for insertion into the inner membrane interact with the TIM23^{SORT} complex, which contains the TIM23^{CORE}, membrane anchored Tim21 and ROMO1 (Mgr2 in yeast) [48,57]. ROMO1 modulates the interaction of Tim21 with the TIM23^{CORE} complex, and may have an additional role in maintaining protein quality control, with the absence of ROMO1 resulting in the loss of the mitochondrial protease Yme1L [57]. Tim21 also associates with the MITRAC (mitochondrial translation regulation assembly intermediate of cytochrome *c* oxidase) complex where it assists in the assembly of Complex IV [58]. During import of nuclear

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encoded subunits of Complex IV, Tim21 associates with preproteins and shuttles them to assembly intermediates in concert with the MITRAC complex [58].

Protein insertion into the inner mitochondrial membrane: The Translocase of the Inner Membrane 22 (TIM22) complex.

The import of multi-membrane spanning proteins with internal targeting signals into the inner membrane utilises the Translocase of the Inner Membrane 22 (TIM22) complex. The mammalian TIM22 complex is comprised of the central pore subunit Tim22, and the transmembrane anchored Acylglycerol kinase (AGK) and Tim29 (**Fig. 1**) [59-63]. Carrier preproteins are translocated across the outer membrane by the TOM complex, and are then passed to the TIM22 complex by the small TIM chaperones [63,64]. There are six small TIM proteins in mammalian cells that chaperone proteins through the inter-membrane space: Tim8a, Tim8b, Tim9, Tim10a, Tim10b (Tim12 in yeast) and Tim13 [65-68]. These small TIM proteins dynamically interact with the TIM22 complex to transfer the preprotein for sorting and insertion into the inner membrane by preventing aggregation within the aqueous inter-membrane space [67]. The small TIM proteins form hetero-hexameric complexes and are proposed to chaperone precursor proteins from the TOM complex to the TIM22 complex (**Fig. 1**) [69,70]. While Tim9, Tim10a and Tim10b function in the transport of carrier protein substrates, the true function of the Tim8-Tim13 still requires clarification. Human Tim8a was recently shown to be involved in Complex IV assembly in neuronal cells, potentially acting as an assembly factor for Complex IV biogenesis [71].

Oxidative folding of proteins in the inter-membrane space: The Mitochondrial Import and Assembly (MIA) pathway.

The MIA pathway couples import and oxidative folding of proteins that are cystine-rich (**Fig. 1**) [72]. The central component, Mia40 (also known as CHCHD4) is located in the intermembrane space and binds incoming precursor proteins. Mia40 is anchored to the inner membrane through an association with the membrane integrated protein AIF (Apoptosis-inducing factor) [73,74]. Here, Mia40 functions as a disulfide donor to induce folding of precursor proteins through oxidation of cystines and the formation of stable disulfide bonds [75,76]. Following oxidation of substrates, Mia40 is re-oxidised by the sulfhydryl oxidase ALR (augmenter of liver regeneration, also known as GFER)

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(Erv1 in yeast) which can utilise either molecular oxygen or cytochrome *c* as electron receptors, linking this process to cellular respiration [77-79].

Mitochondrial disease as a consequence of dysfunctional protein import

Mitochondrial disease encompasses a diverse group of disorders that involve defects in oxidative phosphorylation (OXPHOS) due to mutations in genes encoded by mitochondrial DNA (mtDNA) or nuclear DNA [12]. Mutations in almost 290 genes have been identified to cause mitochondrial disease which are classified into two groups; those that have a primary role in OXPHOS and those that have an indirect role in OXPHOS biogenesis or involve other cellular processes [12,19]. Mitochondrial disorders are largely treated symptomatically [80]. Gene therapy represents a promising treatment option for mitochondrial disease; however, a number of challenges are faced including multi-organ involvement, crossing the blood brain barrier, import of proteins into the matrix and the ethical justification of mitochondrial replacement therapy [81]. Here we discuss the consequence of mutation in genes involved in mitochondrial import (summarised in **Table 1**).

Mitochondrial disease and the TOM complex

A recent report by Dutta et al., 2020 described two patients with *de novo* mutations in the C-terminus of the TOM70 protein (patient 1 T607I and patient 2 I554F) (**Table 1**) [82]. Both patients presented with white matter abnormalities, hypotonia, hyper-reflexia, dystonia and cognitive deficits, however differences in clinical presentation were also noted [82]. Homozygous null drosophila models of patient mutations resulted in pupae lethality, indicating that the mutations are loss of function alleles [82]. A further patient identified with a compound heterozygous mutation (T265M and A582V) presented with severe anaemia, lactic acidosis and developmental delay (**Table 1**) [83]. The T265M mutation is not in a known Tom70 domain, where the A582V mutation is in a TPR domain, potentially affecting substrate binding of Tom70 [83,84]. Patient cells had reduced levels of the Tom70 dimer, and Tom70 was not detected in the TOM complex by Blue Native PAGE [83]. Mitochondrial respiratory complexes I, IV and V levels were reduced in patient cells, and the activity of Complex IV was reduced compared to control cells [83]. Few pathogenic mutations have been identified previously in proteins of the TOM complex. The T607I and I554F mutations identified by Dutta et al., 2020 are both partial loss of function alleles resulting in a

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severe neurological phenotype, highlighting the significant role of the TOM complex in neurological function [82]. These results identify mutations in *TOMM70* as a novel disease-causing gene, however further investigation is required to determine the role of Tom70 in neurological disease and the maintenance of Complex IV. More significant loss of function mutations in TOM subunits are potentially embryonic lethal, hence why there are very few identified in humans.

Mitochondrial diseases and the TIM23 complex

Magmas and Spondylometaphyseal Dysplasia, Megarbane-Dagher-Melki type

Homozygous missense mutations in Magmas (N76D and Q74P) results in Spondylometaphyseal dysplasia, Megarbane-Dagher-Melki type (SMDMDM) (**Table 1**) [85-88]. SMDMDM is a rare autosomal recessive disorder characterised by skeletal abnormalities, severe chondrodysplasia, dysmorphic facial appearance, growth retardation, developmental delay and early death [85-88]. The skeletal dysplasia seen in SMDMDM is uncommon in mitochondrial disorders, however Magmas expression detected in the distal femurs of mice suggests a role for this protein in skeletogenesis [87]. The missense mutations N76D and Q76P are located in the region of Magmas that interacts with DnaJC19 [87], potentially resulting in disruption of the PAM complex, however further studies are required to explore the consequence of these mutations on the $TIM23^{MOTOR}$ complex.

DnaJC19 and Dilated Cardiomyopathy with Ataxia

Mutations in DnaJC19 result in Dilated Cardiomyopathy with Ataxia (DCMA) and is also referred to as 3-methylglutaconic aciduria type V. DCMA is an autosomal recessive disorder that is primarily characterised by severe early onset dilated cardiomyopathy. Additional clinical features include increased 3-methylglutaric acid and 3-methylglutaconic acid, optic atrophy, cerebellar ataxia, and prenatal or postnatal growth failure [89-93]. The majority of pathogenic mutations in DnaJC19 result in premature stop codons within the protein, in one case producing a truncated protein product encoding the transmembrane domain alone (**Table 1**) [89-93]. The molecular basis for DCMA is currently unclear, however a reduction in respiratory chain activity was observed in some patients [89]. In addition, the clinical presentation of elevated 3-methylglutaconic acid is a common feature of respiratory chain disorders [94]. A recent study identified DnaJC19 as a binding partner of Prohibitin 2, a protein that

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forms part of large lipid-protein scaffolds located in the mitochondrial inner membrane and implicated DnaJC19 in cardiolipin metabolism [95,96]. It is possible that DnaJC19 fulfils multiple roles in mitochondria, with both a role in protein import and cardiolipin metabolism, however this remains to be examined further in knockout cell models and patient cells.

Tim50 and 3-Methylglutaconic aciduria type IX

3-Methylglutaconic aciduria (3-MGA-uria) syndromes comprise a group of diseases with elevated 3-MGA-uria in urinary secretions and mitochondrial membrane defects (**Table 1**). A variety of mutations (missense and nonsense) in Tim50 result in the autosomal recessive disorder 3-MGA-uria type IX (MGCA9), which is characterised by early onset seizures, 3-MGA-uria, intellectual disability and delayed development, and hypotonia or spasticity [97-99]. Reyes *et al*., (2018) identified a patient with heterozygous mutations in Tim50 (S112* and G190A), levels of Complex I, II and IV respiratory components were reduced, decreased respiration and increased reactive oxygen species (ROS) [97]. The presentation of the disease in these patients was rapid and severe leading to death at age 2 [97]. In contrast, patients with the heterozygous Tim50 mutation R217W and T252M present with 3-MGA-uria and variable deficiency of Complex V [98]. Similar results were found in patients with heterozygous mutations in Tim50 R114Q and G269S who present with 3-MGA-uria, a generalised reduction in the activity and assembly of the respiratory chain complexes, reduced respiration and aberrant cristae [99]. Patients with heterozygous Tim50 mutations (S112* and G190A; R114Q and G269S) also had reduced Tim50 protein levels, potentially contributing to the observed heterozygous patient phenotypes [97,99]. The variable effects on protein import, respiration and severity of disease progression may be a consequence of the specific mutation and how that mutation affects protein structure and function. Further examination of the effect of the pathogenic Tim50 mutations on mitochondrial import is required to provide insight into the effect of these mutations on function of the Tim23 complex.

PMPCA and Spinocerebellar ataxia, autosomal recessive 2

Pathogenic missense mutations in PMPCA result in the autosomal recessive disorder Spinocerebellar ataxia, autosomal recessive 2 (SCAR2) [100-102] (**Table 1**). SCAR2 is a neurological disorder that is characterised by impaired movement and ataxic gait

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which is non- or slowly progressive [101]. Additional features may include moderate to severe intellectual disability, loss of fine motor skills, dysarthria, hearing loss and blindness [100-102]. The variability in phenotype may be explained by the functional impact of the mutations, with those close to the glycine-rich-loop resulting in a severe form of SCAR2 [101,102]. Mutations in PMPCA have been shown to affect kinetics of processing of the matrix protein frataxin [100,102]. Frataxin acts in iron metabolism, with frataxin deficiency leading to defects in iron-sulfur cluster biogenesis and ATP production [103].

PMPCB and Multiple Mitochondrial Dysfunctions syndrome 6

Vögtle et al., (2018) reported on 5 children of 4 unrelated families with the early onset neurodegenerative disorder Multiple Mitochondrial Dysfunctions syndrome 6 (MMD6) (**Table 1**) [104]. MMD6 is characterised by episodic neurological regression, developmental delay, cerebellar atrophy and hypotonia; with variable features including seizures, optic atrophy, failure to thrive, ataxia and dystonia [104]. A variety of different mutations were identified in the beta subunit of MPP (PMPCB) that contains the catalytic domain for substrate cleavage [104]. Based on the crystal structure of yeast MPP, the identified mutations were not located in the active site of PMPCB, as such Vögtle et al., (2018) suggest that the mutations may affect the stability or dimer formation of MPP [104]. As seen with PMPCA, mutations in PMPCB result in altered processing of frataxin in patient cells, while other MPP substrates were unaffected [104].

MIPEP and Combined oxidative phosphorylation deficiency 31

Combined oxidative phosphorylation deficiency-31 (COXPD31) is a severe autosomal recessive disorder that is the result of pathogenic mutations (missense and nonsense) in MIPEP (**Table 1**) [105]. COXPD31 has been identified in 4 unrelated patients presenting with left ventricular non-compaction, developmental delay, seizures and severe hypotonia [105]. Utilising a yeast model to examine the functional consequence of two of these mutations, Eldomery et al., (2016) demonstrated a loss of protein expression (L83Q) or loss of peptidase activity (L339F and K376E) in the yeast homologue of MIPEP, Oct1 [105]. The severity and early onset of COXPD31 highlights the significance of mitochondrial processing in the maturation of matrix destined proteins.

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Mitochondrial diseases associated with the TIM22 complex.

TIMM22 and Combined oxidative phosphorylation deficiency 43

Pacheu-Grau et al., (2018) reported on a single patient with Combined oxidative phosphorylation deficiency 43 (COXPD43) (**Table 1**) [106]. COXPD43 is characterised by intrauterine growth retardation, feeding difficulties, delayed myelination and decreased Complex I, II and IV activity in muscle [106]. Two pathogenic heterozygous mutations in the TIM22 gene were identified resulting in nonsense and missense mutations (Y25* and V33L) in Tim22, with the Y25* mutations likely resulting in loss of protein expression [106]. Analysis of the TIM22 complex by blue-native page showed a significant loss of this complex in patient fibroblasts, and this could be restored following protein complementation [106]. Analysis of steady state levels of mitochondrial proteins revealed a reduction in the level of Tim22 and Tim29, as well as the carrier protein ANT3, but not the TIM22 component AGK [106]. Additional analysis in patient or CRISPR knockout cells is required to determine the molecular mechanism of the oxidative phosphorylation deficiency.

AGK and Sengers Syndrome

Sengers syndrome is an autosomal recessive disorder characterised by congenital cataracts, hypertrophic cardiomyopathy, skeletal myopathy and lactic acidosis (**Table 1**) [107-115]. Sengers syndrome is caused by a variety of mutations in the TIM22 complex component AGK, including missense, nonsense and frameshift mutations [107-115]. The severity of the phenotype varies significantly, even within families with the same mutation, although it should be noted that the less affected sibling in this case received intervention at an early age potentially affecting disease progression [113]. Sengers syndrome has two distinct forms, a severe infantile form and a mild form, with the severe infantile form frequently a result of homozygous nonsense mutations [111]. Biochemical analysis of Sengers patient and *AGK* knockout cell lines has revealed defects in TIM22 mediated carrier import and impaired lipid metabolism [60,61], suggesting multiple pathways that may be affected upon *AGK* mutation providing a possible mechanism for the variability of disease presentation.

TIMM8a and Mohr-Tranebjærg syndrome

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Mutations in the *TIMM8A* gene (also known as *DDP*), which encodes the protein Tim8a, result in the X-linked recessive disorder Mohr-Tranebjærg syndrome (MTS) (**Table 1**) [65,71,116-119]. MTS is characterised by progressive sensorineural deafness beginning in early childhood, with progressive dystonia, spasticity, optic atrophy and neurodegeneration [116-119]. The loss of function of Tim8a was initially thought to result in impeded import of the Tim23 protein, which utilises the TIM22 import pathway [66]. However, recent findings have highlighted that loss of Tim8a does not affect the import of Tim23, but has a significant effect on the protein level of Complex IV subunits and assembly factors in a neuronal-like SH-SY5Y cells [71]. Cells lacking Tim8a have greater levels of reactive oxygen species, which makes the cells more sensitive to apoptotic induction [71]. This work has led to the suggestion that the molecular mechanism underscoring MTS is Complex IV deficiency and downstream sensitivity to apoptosis in neuronal cells.

Mitochondrial diseases associated with the MIA pathway.

AIF missense mutations

The X-linked *AIFM1* gene encodes the oxidoreductase AIF which interacts with Mia40 at the inner membrane [74]. AIF also has a well described role in apoptosis, where upon mitochondrial permeabilization AIF is released into the cytosol and translocates to the nucleus to induce DNA fragmentation, DNA condensation and cell death [120]. Missense mutations in *AIFM1* result in a range of pathologies including Combined oxidative phosphorylation deficiency 6 (COXPD6) [121], Cowchock syndrome [122-124], Spondyloepimetaphyseal dysplasia with hypomyelinating leukodystrophy (SEMDHL) [125,126], progressive mitochondrial encephalomyopathy [127-129], deafness x-linked 5 (DFNX5) [130], axonal polyneuropathy [131], cerebellar ataxia [122] and prenatal ventriculomegaly without lactic acidosis (**Table 1**) [121]. The severity of these disorders varies significantly even within families [132]. Common clinical features include developmental disorders, motor dysfunction, peripheral sensory neuropathy, peripheral motor neuropathy, abnormal MRI, and hearing loss [122,124,125,128-130,133]. Defects in the respiratory chain are also a feature of a number of the identified AIF mutations, particularly in Complex I, III and IV [121,128-131,133], however this was not an identified phenotype of all mutations. A number of subunits and assembly factors of Complex I, III and IV are identified substrates of AIF, containing Cx_9C motifs that are oxidised by the MIA40 pathway to ensure correct

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folding [134]. Consequently, it is not surprising that mutations in AIF affect the activity of Complex I, III and IV. A puzzling aspect of this group of disorders, is the range in phenotypic presentation following mutation in different regions of AIF. There has yet to be a comprehensive analysis of the position of all the identified mutations, and how this may affect the function of AIF during protein oxidation. AIF is widely expressed in tissues, however it is essential for neuronal survival during development in mice, with specific defects in mitochondrial respiration and structure following loss of AIF [135]. Given the significant neurological component in many of these disorders, it would also be informative to look at the consequence of these mutations in neuronal cell models.

ALR and Mitochondrial progressive myopathy with congenital cataract and developmental delay

Missense and nonsense mutations in the sulfhydryl oxidase ALR result in mitochondrial progressive myopathy with congenital cataract and developmental delay (MPMCD) (**Table 1**) [136-139]. Additional variable features of MPMCD include hearing loss, respiratory chain deficiency and hypotonia [136-139]. The molecular mechanism of a number of these mutants has yet to be characterised, however characterisation of the R194H mutation revealed a COX deficiency, with reduced immunostaining of COX17, Tim13 and COX6B1 in patient fibroblasts [137]. The activity of ALR is linked to the oxidative folding of respiratory chain proteins via the MIA pathway, it is therefore not surprising that a respiratory deficiency would be present in patients [74]. Complex IV is the terminal oxidase of the electron transport chain, with ALR shuttling electrons from cytochrome *c* to Complex IV [78,79]. Given this, it would be interesting to determine if any of the reported mutations in ALR also effect the membrane potential or the presence of reactive oxygen species, further affecting the health of mitochondria.

Mitochondrial protein import dysfunction and neurodegenerative diseases

Neurons are especially dependent on mitochondria for ATP production, metabolite synthesis and calcium buffering at the synapses. Due to the size and length of axonal processes, they are dependent on the efficient anterograde and retrograde transport of mitochondria to the synapses, to ensure neurotransmission and generation of membrane potential along the axon [140]. It is therefore not surprising that dysfunctional mitochondria are found in a number of neurodegenerative disorders

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including Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Parkinson's disease, Huntington's disease, Optic atrophy, Spastic paraplegia and Charcot-Marie-Tooth [140,141]. A number of mitochondrial defects have been described in neurodegenerative diseases including defects in mitochondrial dynamics, transport, energy production, ROS production, protein quality control, metabolism and biogenesis [141,142]. Here we will discuss the role of mitochondrial import in Alzheimer's disease, Parkinson's disease and Huntington's disease.

Mitochondrial import and Alzheimer's disease

The aetiology of Alzheimer's disease is still unknown, and while it is likely to be a multifactorial disorder there are a number of characteristics that are known to correlate with late-onset sporadic Alzheimer's disease (LOAD) [143]. LOAD is characterised by the presence of Aβ plaques, neurofibrillary tangles, inflammation and oxidative damage in the brain. Amyloid precursor protein (APP) is cleaved by β and γ secretases to form the Aβ peptide, which is then exported from the cell [144]. APP has been shown to be targeted to mitochondria in neuronal cells by a positively charged N-terminal domain [145]. Chemical crosslinking demonstrated that the APP preprotein interacts with the Tom40, Tim44 and Tim23 and arrests in the import channels resulting in reduced respiration and reduced membrane potential (**Fig. 2A**) [145,146]. The accumulation of APP across the import channels inhibited mitochondrial import, and increased hydrogen peroxide production [146]. Similar results have also been found with Aβ peptide, which interacts with TOM complex independent of membrane potential [147]. While there is some conflicting reports regarding the import of Aβ into mitochondria, the association of Aβ (particularly Aβ42) with the TOM complex is sufficient to prevent mitochondrial import [148]. The mitochondrial Presequence Peptidase (PreP) degrades Aβ peptide, with increased expression of PreP found to attenuate Aβ accumulation in an Alzheimer's mouse model [149], demonstrating a downstream clearance mechanism for Aβ within mitochondria. Aβ mediated inhibition of precursor processing machinery in AD patient mitochondria has also been shown to result in accumulation of mitochondrial preproteins and intermediates, suggesting that precursor accumulation may contribute to AD pathology [150]. Alteration to mortalin expression has also been shown to ameliorate Aβ–induced mitochondrial fragmentation and cell death in neuroblastoma cells [151]. A variable polyT repeat in

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an intronic polymorphism in the Tom40 gene (rs10524523) has also been associated with an increased risk of Alzheimer's disease, in association with the apolipoprotein E genotype *APOE* ε3 and *APOE* ε4 [152-155]. The very long (VL) polyT repeat and the long (L) polyT repeat in the *TOMM40* gene are linked with *APOE* ε3 and *APOE* ε4 gene variants respectively, and an earlier age of onset in LOAD [152-154]. However, there is some disparity in the literature regarding the risk factor of the Tom40 polymorphism, potentially due to the parameters defined in the analyses [156,157]. In addition, apolipoprotein E acts in extracellular lipid transport, with a apoE4 fragment (1-274) which interacts with mitochondrial respiratory chain subunits and causes a reduction in the activity of complex III and IV [158]. This suggests a possible mechanism by which apoE4 may cause neurodegeneration through reduced mitochondrial function and mitochondrial neurofibrillary tangles [158].

Mitochondrial import and Parkinson's disease

Parkinson's disease is a progressive neurodegenerative disorder that affects movement. One of the causative agents of inherited autosomal dominant or recessive Parkinson's disease are mutations in the gene encoding the PTEN-induced putative kinase 1 (PINK1) and Parkin E3-ubiquitin ligase (Parkin) [159,160]. PINK1 is imported into mitochondria and localised to the inner membrane, however upon dissipation of the mitochondrial membrane potential, PINK1 localises to the outer membrane where it interacts with the TOM complex (**Fig. 2B**) [161,162]. Upon accumulation of PINK1 at the outer membrane, Parkin is subsequently recruited and mitophagy is induced to clear the damaged mitochondria [163,164]. Parkin and PINK1 have been shown to interact with proteins of the TOM complex (Tom7, Tom20, Tom22, Tom40, Tom70) [162,164-169]. Parkin ubiquitylates a number of mitochondrial outer membrane proteins including the TOM complex, targeting the damaged mitochondria for degradation, while the mitochondrial outer membrane deubiquitinase USP30 removes Parkin attached ubiquitin [169-171]. In addition to a role in mitophagy, recent research has suggested Parkin mediated ubiquitylation of TOM proteins may stimulate mitochondrial import, with import efficiency found to be reduced in PD patient cells [168]. The deubiquitinase USP30 has also been shown to have a role in TOM mediated import, with loss of USP30 causing accumulation of substrates [172]. Pathogenic mutations in Parkin have been shown to disrupt interactions with Tom70

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and Tom40 [165,173]. Tom7 is required for stabilisation of PINK1 in the outer membrane upon loss of membrane potential, while Tom70 has been shown to be required for PINK1 import into mitochondria [167,174,175].

Alpha-synuclein (α-Syn) is an abundant neuronal protein that can interact with phospholipids at the presynaptic terminal of neurons [176]. Aberrant deposition, accumulation and aggregation of α-Syn in Lewy bodies and Lewy neurites is a causative factor in Parkinson's disease [176]. Accumulation of α-Syn at mitochondria has been found to result in mitochondrial dysfunction characterised by reduced oxidative phosphorylation, oxidative stress and impaired mitochondrial import (**Fig. 2B**) [177-180]. In neural tissue from Parkinson's patients, an interaction of α-Syn and Tom20 was detected, with a concomitant loss of mitochondrial import and disrupted interaction of Tom20 with Tom22 [180]. Tom40 has also been found to be significantly reduced in the brain of Parkinson's patients and in transgenic mouse models, with re-expression of Tom40 decreasing α-Syn accumulation [177]. These results indicate that modulation of the TOM complex represents a potential therapeutic target for Parkinson's disease.

Mitochondrial import and Huntington's disease

Huntington's disease (HD) is a fatal autosomal dominant disorder that is caused by the abnormal expansion of CAG repeats in the huntingtin gene [181]. This expansion stimulates misfolding and aggregation of mutant Huntingtin protein (mHTT) which is cytotoxic [181]. Mitochondrial dysfunction has been described to contribute to the pathophysiology of the disease with evidence of oxidative stress, mitochondrial DNA depletion, calcium imbalance and disrupted mitochondrial cristae in HD and mHTT mouse models [181]. Both wild type and mutant HTT have been found in the mitochondrial inter-membrane space where the mHTT protein binds specifically to Tim23 (**Fig. 2C**) [182,183]. The binding of mHTT to Tim23 has been shown to reduce import of nuclear encoded mitochondrial proteins as suggested by reduced levels of matrix localised proteins in cells expressing mHTT and in HD patient brain tissue [182,183]. This mHTT induced import defect is sufficient to trigger neuronal cell death [182,183]. In addition, in a HD mouse model defective protein import was an early defect found in the forebrain but not in the liver indicating that dysfunction in

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mitochondrial import may be an early indicator of mHTT pathophysiology [183]. However, a recent report by Hamilton et al., (2020) contradicts these previous findings proposing that mHTT interacts with the outer membrane only [184]. These studies utilised different mouse models of HD with different CAG repeat lengths and different methods to examine the submitochondrial localisation of mHTT in patient samples, potentially affecting interpretation of the results [183,184]. The mechanism of mitochondrial dysfunction in Huntington's disease is still unclear, and more analysis needs to be done to determine the role of mHTT and mitochondrial import, including unravelling the kinetics of mitochondrial import in response to different mHTT CAG repeat lengths.

Mitochondrial protein import and cancer

The best described change in mitochondrial function during cancer cell growth is the reprogramming of cellular respiration from oxidative phosphorylation to aerobic glycolysis, known as the Warburg effect [10,185]. However within the tumour microenvironment cancer cells are both glycolytic and oxidative, with glycolytic cells utilising metabolites from the surrounding oxidative cells for fuel [186,187]. Many different aspects of mitochondrial biology directly influence tumorigenesis, including alterations to cell death pathways, mitochondrial dynamics, mitochondrial biogenesis and mTOR signalling, and oxidative stress [10]. A significant number of components of the mitochondrial import machineries are overexpressed in a variety of different cancers, including subunits of the TOM complex (Tom20, Tom40, Tom7, and Tom70) [186,188-192], the TIM23 complex (Tim17a, Mortalin, Magmas, Tim50 and Tim23) [186,193-207], the TIM22 complex (Tim22, AGK) [186,208-217], the small Tim chaperones (Tim8a, Tim8b, Tim9, Tim13) [186,218-220] and Mia40 [221,222]. In addition, two variants of Tim44 (C925A and G1274A) have been identified in oncocytic thyroid carcinomas [223]. Understanding the role of mitochondrial import and the protein profile of different cancers may provide useful information for both diagnostic and treatment markers. Those proteins for which a mechanism of action in oncogenesis has been explored are discussed below (**Fig. 3**).

Variation in mitochondrial function plays a central role in neoplastic transformation. Upon oncogenic transformation mitochondria are reprogrammed to utilise aerobic glycolysis for ATP production [224]. While glycolysis produces less ATP molecules

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than oxidative phosphorylation, cancer cells can compensate for this with a subsequent increase in mitochondrial biomass and increased speed of ATP production [10,224]. Determining if the upregulation of mitochondrial import proteins has an oncogenic function has yet to be explored for a number of the subunits identified as upregulated in various cancers.

Cancer and the TOM complex

Tom20 overexpression in colorectal cancer and Tom40 overexpression in ovarian cancer correlates with increased cell proliferation, migration and invasion [190,192]. To investigate the significance of overexpression of Tom20 and Tom40 in cancer, the knockdown of Tom20 or Tom40 in cancer cells was examined. Tom40 siRNA in colorectal cancer and Tom20 siRNA in epithelial ovarian cancer reduces levels of ATP, cellular proliferation, migration and invasion [190,192]. In addition, knockdown of Tom20 or Tom40 in mouse xenograft models suppressed tumor growth, indicating that modulation of these proteins can directly affect tumorgenesis [190,192]. The knockdown of Tom40 results in reduced levels of Tom20 and Tom22, and increased membrane potential [192]. In contrast to this, siRNA of Tom20 causes a reduction in membrane potential [190], indicating potentially different consequences to Tom40 knockdown despite both proteins located in the same complex. In yeast mutants lacking Tom20 and Tom70, the core import pore can still form and protein import was functional, albeit with reduced kinetics [225], however the consequence to mitochondrial import upon siRNA of Tom20 and Tom40 remains to be explored further in oncogenic cell models. Overexpression of Tom20 may also have a role in cancer transformation through interaction with the protein AIP (arylhyrocarbon receptor interacting protein) [226]. AIP interacts with the apoptotic inhibitor survivin which is imported into mitochondria upon apoptotic stimuli to inhibit caspase activation, with overexpression of survivin preventing apoptosis and promoting tumorigenesis [227]. Knockdown of Tom20 abolishes import of survivin into mitochondria and sensitises cells to apoptosis [228], indicating that Tom20 is a bona fide therapeutic target for oncogenesis.

Cancer and the TIM23 complex

Overexpression of Tim50 in non-small cell lung carcinoma patients facilitates tumor progression and cellular proliferation, which is proposed to stimulate cell growth

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through activation of extracellular signal related (ERK) signalling and Cyclin D1 activity [194]. Cyclin D1 binds to the mitochondrial outer membrane via an interaction with the voltage dependent anion channel (VDAC) and activates cell proliferation [229]. Overexpression of cyclin D1 has been linked to the progression of a number of different cancers through activation of cellular proliferation [230]. Zhang et al., (2019) demonstrated that treatment of cells with an ERK inhibitor U0126 reversed both Tim50 and cyclin D1 overexpression [194], however it is unclear how the overexpression of Tim50 regulate either cyclin D1 or phosphorylated ERK.

Mortalin expression in cancer cells correlates with malignancy in a p53-dependent manner [200,201]. p53 is a tumor suppressor that can induce apoptosis by initiating cell cycle arrest, interaction with apoptotic proteins and transcriptional activation of apoptosis [231]. p53 migrates to mitochondria where it can interact directly with mortalin and manganese superoxide dismutase (MnSOD) prior to translocation to the nucleus (**Fig. 3A**) [200,201,232]. Treatment of p53 positive cancer cells with the mortalin inhibitor MKT-077 has been shown to sensitise cells to apoptosis, identifying mortalin as a potential chemotherapeutic target [207]. Mortalin is also reported to modulate tumor cell survival through modulation of the MEK/ERK signalling pathway [205], although the mechanism by which mortalin regulates these pathways is not clear.

Cancer and the TIM22 complex

AGK was originally described as a mitochondrial lipid kinase that catalyses the phosphorylation of monoacylglycerol (MAG) and diacylglycerol (DAG) to lysophosphatidic acid (LPA) and phosphatidic acid (PA) [208]. However, AGK has also been identified as a subunit of the TIM22 complex in which it stabilises the complex and assists carrier protein import [60,61]. AGK has also been established as an oncogene, with elevated expression detected in a wide variety of cancers [208-217]. Overexpression of AGK enhances tumorigenicity, through increased proliferation, cell migration, metastasis and invasion; with elevated expression correlated with poor patient overall survival [208-217]. In prostate cancer cells, high AGK expression correlated with elevated levels of LPA and activation of epidermal growth factor (EGF) receptor and extracellular signal related kinase (ERK) [208]. AGK has also been shown to enhance proliferation of cancer cells through promotion of cell cycle

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transition from G1 to S phase of the cell cycle, and upregulation of cyclin D1 following repression of the transcription factor FOXO1 [208-210,213]. Increased AGK expression also promotes proliferation through activation of the PI3K/AKT/GSK3β signalling pathway resulting in nuclear accumulation of β-catenin which activates transcription factors associated with proliferation (**Fig. 3B**) [209,210,212,233]. Activation of the JAK2/STAT3 pathway has been reported to have a role in proliferation, survival and differentiation in tumours [234]. AGK has also been described to activate JAK2/STAT3 signalling, with elevated levels of AGK correlated with constitutive JAK2/STAT3 activity [216]. The mechanism of activation of JAK2/STAT3 by AGK is however unclear. AGK overexpression in hepatocellular carcinoma cells has also been shown to cause increased NF-κB signalling, resulting in inhibited apoptosis [215]. While the upregulation of oncogenic signalling pathways following elevated AGK has been well described, the effect on protein import remains an interesting avenue to explore.

Cancer and the MIA pathway

The oxidoreductase Mia40 has been shown to have a role in adapting to metabolic reprogramming during tumorigenesis through stabilisation of the hypoxia inducible factor subunit alpha (HIF1α) [221]. Upon stabilisation HIF1α translocates to the nucleus where it binds HIF1β and activates genes involved in cellular survival [235]. Stabilisation of HIF1α has been proposed to occur via a number of different mechanisms including stabilisation by reactive oxygen species (ROS) from complex III in response to hypoxia [236], and following elevated oxygen consumption by complex IV [221]. High expression of Mia40 has also been shown to regulate oxygen consumption and ATP production [221]. While overexpression of Mia40 is required for HIF1α stabilisation, this is independent of ROS production, suggesting that Mia40 regulates HIF1α in a mechanism independent of complex IV ROS production (**Fig. 3C**) [221]. The precise mechanism by which Mia40 stabilises HIF1α is currently unclear and may occur as a result of modification of respiratory chain biogenesis, however this remains to be explored further. Mia40 has also been shown to bind the tumor suppressor p53 at mitochondria (**Fig. 3C**) [222]. Mia40 is required for p53 translocation to mitochondria, with overexpression of Mia40 resulting in p53 exclusion

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from the nucleus, preventing transcriptional activation of tumor suppressor genes [222].

Conclusions and Perspectives

Mitochondrial translocases and the pathways they support are essential for mitochondrial function, with mutation in a large number of these components identified in mitochondrial disease, neurodegenerative disorders and cancer. The recent identification of pathological mutations in the mitochondrial outer membrane protein Tom70 has highlighted the need to expand the view on the role of the TOM complex in mitochondrial disease. The TOM complex has a significant role in the neurodegenerative disorders discussed in this review, indicating that mutations this complex are particularly sensitive to the specialised functions required in neuronal tissue. In particular, the early presentation of defective mitochondrial import in mutant Huntingtin mouse brains may represent a novel detection method for symptom onset in Huntington's disease, and potential therapeutic targets. A number of subunits of the TIM22 and TIM23 complexes are upregulated in a variety of different cancers, representing attractive targets for new therapies. Of note, many of these studies focussed on the ability of these components to alter cell growth, survival and tumorigenicity. However, there is significant scope for further investigation into how the upregulation of these proteins affects mitochondrial import and subsequently mitochondrial health.

Mitochondrial protein transport was once only viewed as a basic biological process. The pathologies detailed in this review highlight the importance of fundamental pathways to human health, and ultimately the importance of fundamental biology to driving medical research. Fundamental research in mitochondrial biology will shape future understanding of the role of mitochondrial protein import in mitochondrial disease, neurodegenerative disorders and cancer, and will hopefully feed into a pipeline that can facilitate therapeutic development and intervention.

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Figure legends

Figure 1. Mitochondrial protein import translocases and associated mitochondrial diseases. Mitochondria have a double-membrane architecture and rely on five translocase complexes for the import and sorting of proteins. The Translocase of the Outer Membrane complex (TOM, red) has 7 subunits which coordinate entry of nearly all mitochondrial proteins through the mitochondrial outer membrane. The Sorting and Assembly Machinery complex (SAM, green) has 3 subunits and facilitates insertion of β-barrel proteins into the mitochondrial membrane. The small TIM family of proteins form two heterotypic hexameric complexes that chaperone unfolded clients through the aqueous intermembrane space. A third small TIM hexamer is associated with the Translocase of the Inner Membrane 22 complex (TIM22, purple). The TIM22 complex has 6 subunits and imports both metabolite carriers and TIM subunits into the mitochondrial inner membrane. The Translocase of the Inner Membrane 23 complex (TIM23, orange) has 12 subunits which interact in a modular fashion. Arrangements of the TIM23 complex control import across the inner membrane to the matrix or release of membrane-anchored proteins into the mitochondrial inner membrane. The Mitochondrial Intermembrane space Assembly complex (MIA, blue) is comprised of 3 subunits and is responsible for the oxidative import of disulfide-containing proteins. Secondary mitochondrial diseases caused by mutation or loss of translocase subunits are indicated.

Figure 2. Mitochondrial protein import components implicated in proteopathic neurodegenerative disease. Disease-causing proteins perturb mitochondrial protein import in Alzheimer's, Parkinson's and Huntington's disease. **(A)** In Alzheimer's disease, amyloid precursor protein blocks the channel of TOM and TIM23 leading to Complex IV defects and increased reactive oxygen species (ROS). The cleavage product, amyloid-β42, aggregates precursors at the mitochondrial outer membrane and competes with mitochondrial precursors for the PrEP peptidase in the matrix; overall decreasing the import of mitochondrial proteins. Furthermore, decreased abundance or functionality of Mortalin exacerbates the impact of improper protein import in all three proteopathic neurodegenerative diseases. **(B)** In Parkinson's disease, α-synuclein binds Tom20 and decreases precursor import, which manifests as Complex I dysfunction and increased ROS levels. Loss of Tom40 in Parkinson's patients and models is associated with α-synuclein accumulation. Additionally, Tom7 and Tom70 are required for PINK1/Parkin-mediated mitophagy. **(C)** In Huntington's

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disease, mutant Huntingtin protein binds the TIM23 complex resulting in perturbed import and loss of membrane potential, ultimately causing mitochondrial dysfunction.

Figure 3. Mitochondrial translocase subunits contribute to oncogenesis and tumour survival. Many subunits of the mitochondrial protein import machinery contribute to oncogenesis or tumour survival. **(A)** Both Mortalin and Mia40 respectively sequester the transcription factor, p53, within mitochondria and preclude its tumour suppressor role. This diminishes genome stability, facilitating oncogenic mutations. **(B)** Acylglycerol kinase (AGK) overexpression promotes AKT activation and regulation of transcription factors, FOXO1 and β-catenin, which results in unrestrained cell division and loss of cell-to-cell adhesion. Additionally, AGK produces lysophosphatidic acid (LPA) which accumulates at the plasma membrane and induces EGFR signalling to promote cell growth. Therefore, AGK contributes to the epithelial-to-mesenchymal transition that precedes tumour metastasis. **(C)** Increased Mia40 expression in cancers leads to stabilisation of HIF-1 via increased Complex IV assembly and activity. This promotes malignant cell survival in the hypoxic tumour environment.

Table 1: Mitochondrial import translocase subunit variants linked to mitochondrial disease.

Gene symbol	Protein name	Disease(s)	Pathogenic variants	Molecular consequence	Ref.
TOMM70	Mitochondrial import receptor subunit TOM70 (Tom70)	Multi-OXPHOS defect with anaemia and lactic acidosis; White-matter neurodevelopmental disorder	p.T265M, p.I554F, p.A582V, p.T607I	Decreased OXPHOS CI, CIV and CV abundance; decreased CIV activity	[82,83]
PAM16	Mitochondria-associated granulocyte macrophage CSF signalling molecule (Magmas)	Spondylometaphyseal dysplasia, Megarbane-Dagher-Melike type (SMDMDM)	p.Q74P, p.N76D	Unknown	[85-88]
DNAJC19	DNAJ homolog, subfamily C, member 19 (DNAJC19)	Dilated cardiomyopathy with ataxia (DCMA)	p.T11I, p.F17fs, p.Y21*,	Unknown	[89-93]

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			p.Y98fs, p.A101fs		
TIMM50	Mitochondrial import inner membrane translocase subunit TIM50 (Tim50)	3-methylglutaconic aciduria type IX (MGCA9)	p.S9*, p.G87A, p.S112*, p.R114Q, p.G190A, p.R217W, p.T252M, p.A222T, p.R239W, p.G269S	Decreased OXPHOS CI, CII and CIV abundance; increased ROS; decreased CV abundance	[97-99]
PMPCA	Mitochondrial processing peptidase subunit alpha (MPPα)	Spinocerebellar ataxia autosomal recessive 2 (SCAR2)	p.R22W, p.S96L, p.R185Q, p.V256M, p.G356S, p.A377T, p.A377V, p.G515R	Unknown	[100-102]
PMPCB	Mitochondrial processing peptidase subunit beta (MPPβ)	Multiple mitochondrial dysfunctions syndrome 6 (MMD6)	p.R175C, p.R175H, p.V177G, p.A210P, p.I422T	Impaired matrix-targeted import, defective Fe-S cluster biogenesis, decreased OXPHOS activity	[104]
MIPEP	Mitochondrial intermediate peptidase (MIP)	Combined oxidative phosphorylation deficiency 31 (COXPD31)	p.L71Q, p.R175C, p.L306F, p.K343E, p.E602*	Impaired OXPHOS subunit import	[105]
TIMM22	Mitochondrial import inner membrane translocase subunit	Combined oxidative phosphorylation deficiency 43	p.Y25*, p.V33L	Loss of metabolite exchange;	[106]

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	22 (Tim22)	(COXPD43)		Decreased OXPHOS activity	
AGK	Acylglycerol kinase (AGK)	Sengers syndrome	p.M1I, p.W24*, p.Y102*, p.E111Q, p.R137*, p.Q173*, p.I175fs, p.Y224*, p.R281*, p.Q291*, p.I346fs	Loss of metabolite exchange, perturbed one-carbon metabolism	[107-115]
TIMM8A	Mitochondrial import inner membrane translocase subunit Tim8A (Tim8a)	Mohr-Tranebjærg syndrome/Deafness dystonia optic neuronopathy (DDON)	p.M1L, p.E24*, p.Q28X, p.Q38*, p.M39fs, p.C43fs, p.K50fs, p.C66W, p.R80*	Decreased OXPHOS CIV abundance, apoptotic sensitivity to mitochondrial ROS	[65,71, 116-119]
AIFM1	Apoptosis-inducing factor 1, mitochondrial (AIF)	Cowchock syndrome; Combined oxidative phosphorylation deficiency 6 (COXPD6); Deafness X-linked 5 (DFNX5); Spondyloepimetaphyseal dysplasia with hypomyelinating leukodystrophy (SEMDHL); Progressive mitochondrial encephalomyopathy;	p.G262S, p.G308E, p.G338E, p.R422Q, p.R422W, p.451Q, p.Q479R, p.E493V,	Impaired oxidative folding, decreased OXPHOS CI, CIII and CIV activity	[121-133]

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		Axonal polyneuropathy; Cerebellar ataxia; Prenatal ventriculomegaly			
GFER	FAD-linked sulfhydryl oxidase ALR (ALR)	Mitochondrial progressive myopathy with congenital cataract and developmental delay (MPMCD)	p.R67fs, p.A73fs, p.C74fs, p.Q125*, p.S189*, p.R194H, p.R196C	Impaired oxidative folding, decreased OXPHOS CIV activity	[136-139]

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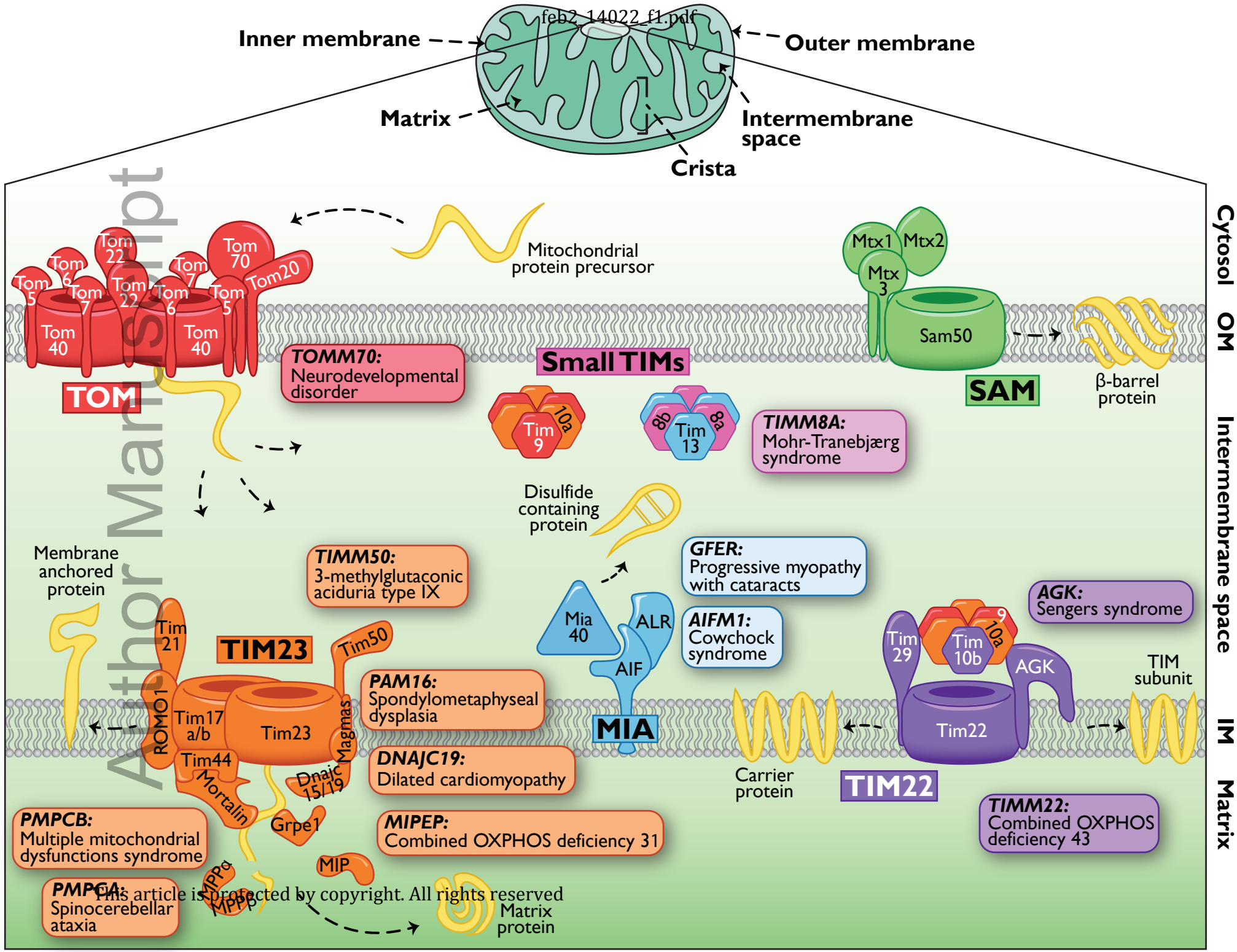

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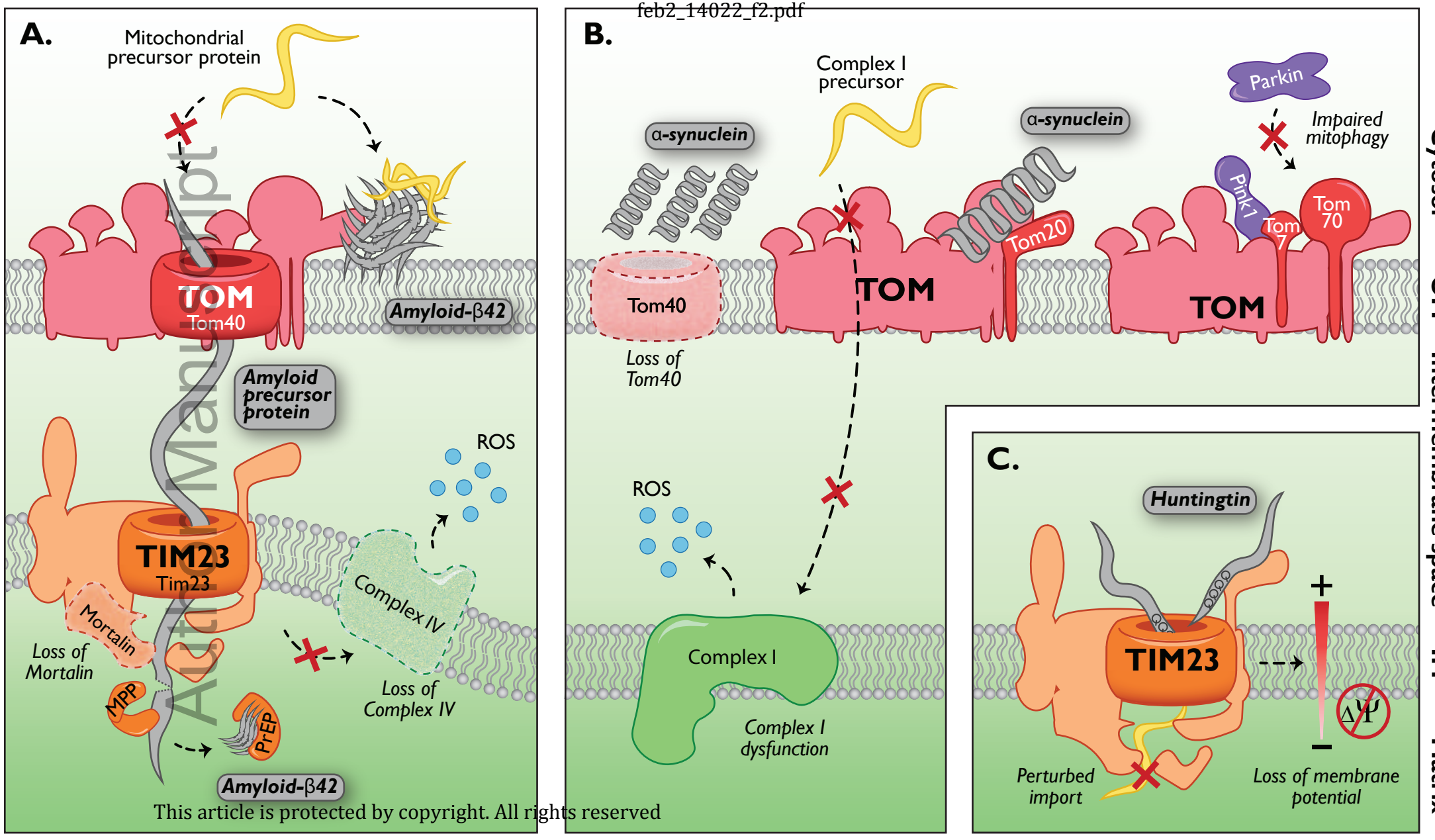

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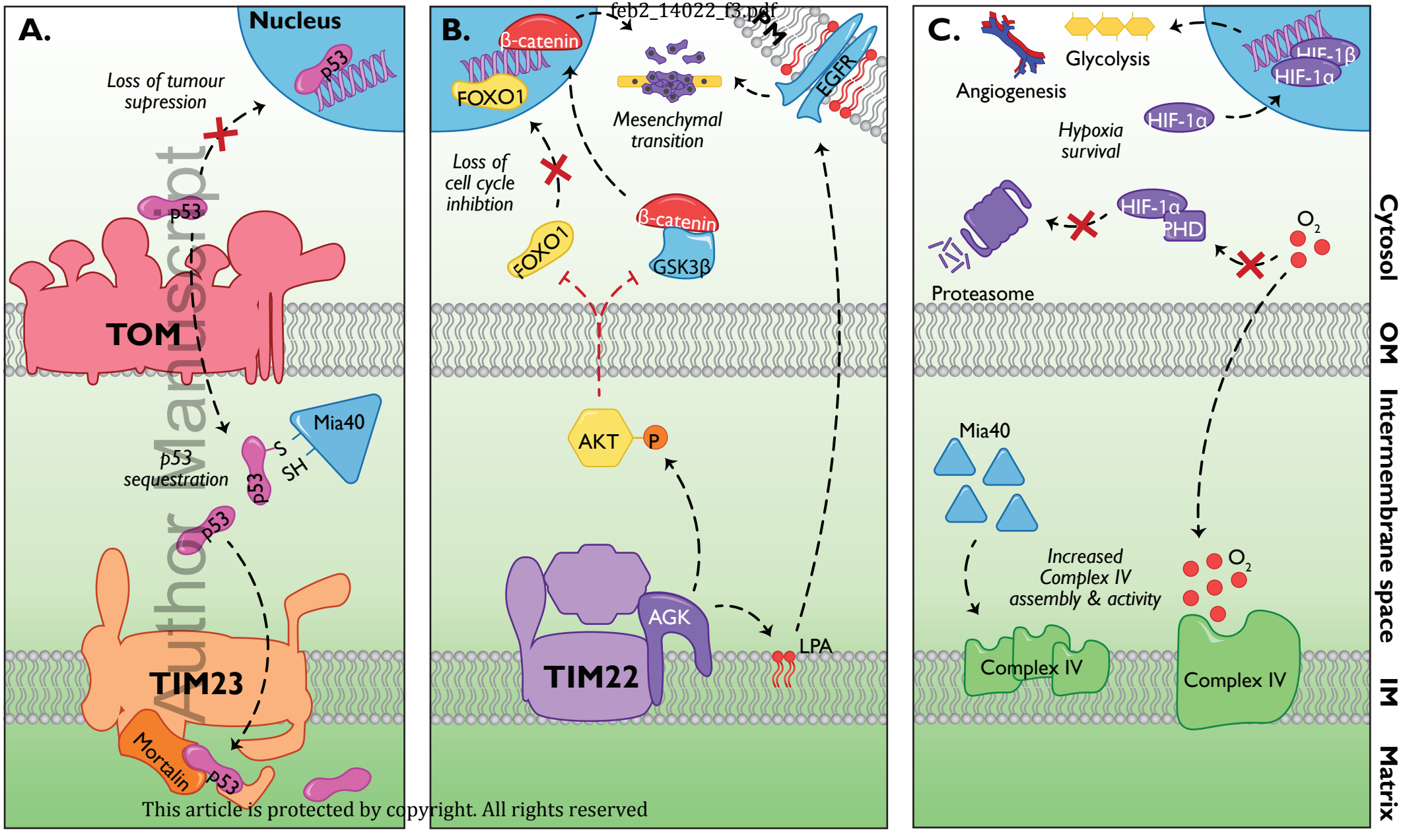

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