The Dictyostelium model for mitochondrial biology and disease

XAVIER G. PEARCE, SARAH J. ANNESLEY and PAUL R. FISHER*

Discipline of Microbiology, La Trobe University, Victoria, Australia

ABSTRACT The unicellular slime mould Dictyostelium discoideum is a valuable eukaryotic model organism in the study of mitochondrial biology and disease. As a member of the Amoebozoa, a sister clade to the animals and fungi, Dictyostelium mitochondrial biology shares commonalities with these organisms, but also exhibits some features of plants. As such it has made significant contributions to the study of eukaryotic mitochondrial biology. This review provides an overview of the advances in mitochondrial biology made by the study of Dictyostelium and examines several examples where Dictyostelium has and will contribute to the understanding of mitochondrial disease. The study of Dictyostelium’s mitochondrial biology has contributed to the understanding of mitochondrial genetics, transcription, protein import, respiration, morphology and trafficking, and the role of mitochondria in cellular differentiation. Dictyostelium is also proving to be a versatile model organism in the study both of classical mitochondrial disease e.g. Leigh syndrome, and in mitochondria-associated neurodegenerative diseases like Parkinson’s disease. The study of mitochondrial diseases presents a unique challenge due to the cryptic nature of their genotype-phenotype relationship. The use of Dictyostelium can contribute to resolving this problem by providing a genetically tractable, haploid eukaryotic organism with a suite of readily characterised phenotype readouts of cellular signalling pathways. Dictyostelium has provided insight into the signalling pathways involved in multiple neurodegenerative diseases and will continue to provide a significant contribution to the understanding of mitochondrial biology and disease.

KEY WORDS: Dictyostelium, mitochondrial biology, mitochondrial disease, AMPK, neurodegeneration

Introduction

Mitochondria are double-membrane-bounded organelles present in all nucleated eukaryotic cells, with few exceptions. Beyond their primary role of ATP synthesis via oxidative phosphorylation, mitochondria are also involved in many cellular processes such as calcium signalling, cellular differentiation and proliferation, and programmed cell death (Bertero and Maack, 2018, Umemoto et al., 2018, Vakifahmetoglu-Norberg et al., 2017). The mitochondrion is thought to have originated from an endosymbiotic event between a primitive eukaryote and a \( \alpha \)-proteobacterium (Leger et al., 2015). The strongest evidence of this is that mitochondria possess their own genome, and like bacteria, this genome is circular in structure, while the proteins it encodes are more closely related to bacterial than to eukaryotic counterparts. Indeed, eukaryotic mitochondria have retained a division system akin to that of their prokaryotic ancestors (Leger et al., 2015). Over time, many of the requisite genes for the proper functioning of mitochondria have migrated to the nuclear genome, a process that has continued more or less independently in the different phylogenetic lineages. The result is that the gene composition and size of the mitochondrial genome is different in each eukaryotic lineage, with the human mitochondrial genome being one of the most severely reduced in size (13 protein-encoding genes, 16 kb in size) (Wallace, 2005), while the least reduced is that of the protist \textit{Reclinomonas americana} whose 69 kilobases encode 65 proteins (Burger et al., 2013).

In humans, mutations affecting either the mitochondrial genome or nuclear genes encoding mitochondrial proteins can cause complex diseases whose diverse clinical presentations break the nexus between genotype and phenotype (Stenton and Prokisch, 2018). Thus, the same genetic defect can cause very diverse clini-
Mitochondrial outcomes in patients and similar disease phenotypes can be produced by multiple genetic causes (Sofou et al., 2018). Because of this complexity, the study of mitochondrial biology and disease has relied upon contributions from several model organisms, including the yeast Saccharomyces cerevisiae, and the cellular slime mould Dictyostelium discoideum.

D. discoideum belongs to the Amoebozoa, a sister clade to the Opisthokonta, the group containing fungi and animals. Dictyostelium mitochondria share many similarities with all eukaryotes, but have some features found in either animals or plants. D. discoideum has been used extensively as an eukaryotic model organism in the study of cellular processes such as chemotaxis, thermotaxis, motility, programmed cell death, cell differentiation, signal transduction, and human disease modelling (Maniak, 2011, Steinert and Heuner, 2005). It is also a well-established model organism for the investigation of mitochondrial genetics and in mitochondrial disease modelling (Barth et al., 2007; Francione et al., 2011). Due to its biology including several cellular processes more akin to higher eukaryotes e.g. phagocytosis, chemotaxis, and differentiation, Dictyostelium is an ideal model for the study of conserved genes not found in yeast (Torija et al., 2006). A fully sequenced nuclear and mitochondrial genome, tractability to genetic modification, and a life cycle that provides numerous phenotypic readouts of cellular processes make D. discoideum an attractive model for mitochondrial biology and disease. This review aims to provide an overview of D. discoideum mitochondrial biology and its utility as a model in understanding human mitochondrial disease mechanisms.

Mitochondrial biology

Mitochondrial genetics

The mitochondrial genome of D. discoideum is fully sequenced and is 55,564 bp in length with an A + T content of 72.6% (Ogawa et al., 2000). The genome is circular and each cell contains approximately 200 copies of the mitochondrial genome. This A + T richness is a feature shared with other protzoa, fungi, nematodes, and invertebrates (Nakao et al., 2002, Stewart and Beckenbach, 2005). Despite this, unlike other organisms with similar A + T richness, D. discoideum appears to use the universal genetic code in its translation of mitochondrial mRNAs (Angata et al., 1995). The mitochondrial genome contains 61 genes, the majority of which, like all mitochondrial genomes, encode gene products related to respiration and translation (Ogawa et al., 2000). Interestingly, all genes are encoded on the same DNA strand, in the same orientation, and the genome is transcribed from a single start site (Le et al., 2009). This primary transcript is processed into eight major polycistrionic transcripts which are additionally further processed into di- and tri-cistrionic transcripts (Barth et al., 1999, Barth et al., 2001). D. discoideum mtDNA encodes 33 proteins, 2 ribosomal RNAs, and 18 transfer RNA genes with many of these genes ortho-/homologues to Homo sapiens mtDNA genes (Table 1). Notably, in humans the genes encoding ribosomal proteins have all transferred to the nuclear genome. However, the majority of common respiratory chain constituents remain encoded in the mtDNA. As expected from this “loss” of genes, the human mitochondrial genome is substantially smaller at 16,569 bp (Anderson et al., 1981). As many of the requisite proteins for mitochondria are now encoded on the nuclear genome, many required proteins are imported into the mitochondria, including those required for transcription.

Mitochondrial transcription

Mitochondrial DNA in fungi, plants, and animals is transcribed by a nuclear-encoded mitochondrial RNA polymerase (mtRNAP) that has a high sequence similarity to bacteriophage RNA polymerases (Filée et al., 2002, Gustafsson et al., 2016, Ringel et al., 2011). Unlike bacteriophage mtRNAPs, eukaryotic mtRNAPs require additional proteins for efficient transcription, known as mitochondrial transcription factors (Falkenberg et al., 2002). Consisting of two families, mitochondrial transcription factors A & B (mtTFA; mtTFB), together with mtRNAP, form the machinery required for mitochondrial transcription. D. discoideum similarly has a nuclear-encoded phage-like mitochondrial RNA polymerase, RpmA. The rpmA gene consists of a continuous open reading frame of 2,850 nucleotides in length and codes for a protein of 950 amino acid residues, within which all catalytically essential residues are conserved (Le et al., 2009). While being shown to initiate transcription, RpmA, did so inefficiently, suggesting that, like other eukaryotes D. discoideum requires mitochondrial transcription factors for mtDNA transcription. Manna et al., (2013) identified and characterised a D. discoideum mitochondrial transcription factor B. Identified as tfb1m, this gene is 1,458 bp in size, with a corresponding amino acid sequence 485 residues in length, and approximately 56 kDa in molecular weight. Tfb1m was shown to localise to the mitochondria and function both as a rRNA adenosine dimethyltransferase, but also to activate mitochondrial transcription in concert with RpmA. The dual role of methyl transferase activity and mitochondrial transcription initiation is concordant with the human homologues of Tfb1m (Cotney and Shadel, 2006). While RpmA expressed without Tfb1m

| Mitochondrial genome of D. discoideum | Homo sapiens |
|--------------------------------------|-------------|
| NADH-dehydrogenase: MT-ND1, 2, 3, 4, 4L, 5, 6, 7, 8, 11 | NADH–dehydrogenase: MT-ND1, 2, 3, 4, 4L, 5, 6 |
| Apocytochrome b: cob | Cytochrome b: MT-CYB |
| Cytochrome oxidase: cox1/2, cox3 | Cytochrome c oxidase: MT-CO1, 2, 3 |
| F1− ATPase: ap6, ap8, ap9 | F− ATP synthase: MT-ATP6, 8 |
| psb2, psd4, psd7, psd8, rps8, rps11, rps12, rps13, rps14, rps19 | *nDNA encoded |
| Large subunit: rps2, rpl5, rpl6, rpl11, rpl14, rpl16 | |
| Large subunit: rnl | |
| Small subunit: rps4 | |
| Small subunit: rps12 | |
| Small subunit: rps19 | |
| Small subunit: rps2 | |
| Small subunit: rps4 | |
| Small subunit: rps7 | |
| Small subunit: rps8 | |
| Small subunit: rps11 | |
| Small subunit: rps12 | |
| Small subunit: rps13 | |
| Small subunit: rps14 | |
| Small subunit: rps19 | |
| Small subunit: rpl5 | |
| Small subunit: rpl6 | |
| Small subunit: rpl11 | |
| Small subunit: rpl14 | |
| Small subunit: rpl16 | |
| Small subunit: rpl2 | |
| Small subunit: rpl5 | |
| Small subunit: rpl6 | |
| Small subunit: rpl11 | |
| Small subunit: rpl14 | |
| Small subunit: rpl16 | |
| Small subunit: rpl2 | |
| Small subunit: rpl5 | |
| Small subunit: rpl6 | |
| Small subunit: rpl11 | |
| Small subunit: rpl14 | |
| Small subunit: rpl16 | |
| Small subunit: rps4 | |
| Small subunit: rps7 | |
| Small subunit: rps8 | |
| Small subunit: rps11 | |
| Small subunit: rps12 | |
| Small subunit: rps13 | |
| Small subunit: rps14 | |
| Small subunit: rps19 | |
produced undetectable amounts of transcripts, coexpression with Tfb1m improved transcription efficiency dramatically, to the point where it was suggested that Tfb1m may be the sole transcription factor necessary for mitochondrial transcription in *D. discoideum* (Manna et al., 2013). As illustrated above, mitochondrial function is dependent on nuclear-encoded protein import and this is facilitated by the TOM/TIM complexes in mitochondria.

**Protein import**

As most mitochondrial proteins are encoded in the nuclear genome rather than the mitochondrial genome, mitochondria rely upon protein import and subsequent assembly for correct functioning and biogenesis. After early controversy, the demonstration that mitochondrial protein import could occur posttranslationally *in vitro* resulted in a long-held belief that this was the predominant mechanism, with protein synthesis occurring in the cytosol and subsequent being imported (Ahmed and Fisher, 2009, Fox, 2012). However, *Dictyostelium* provided an early counter demonstration that some proteins are imported into the mitochondria posttranslationally and others cotranslationally as a result of selective trafficking of the mRNA to the mitochondrial surface (Ahmed et al., 2006). It is now clear that both mechanisms are used in parallel: approximately half of the proteins that make up the mitochondrial proteome are translated at the surface of mitochondria, with the remainder being synthesized in the cytosol and posttranslationally imported (Fox, 2012). Most mitochondrial inner membrane proteins were found to be cotranslationally targeted in *Saccharomyces* (Williams et al., 2014). This localised translation of proteins is presumed to facilitate their correct import and assembly, however why not all mitochondrial proteins are cotranslationally imported is not fully understood (Fox, 2012). Localised synthesis of proteins coupled with their simultaneous transport is not mitochondria-specific (Aviram and Schuldiner, 2017). Similar cotranslational import of proteins also occurs in the endoplasmic reticulum, and chloroplasts (Dudek et al., 2015, Króliczewski et al., 2016). Cotranslational import of proteins now appears to be a commonplace occurrence not limited to any particular organelle, and a system used in concert with posttranslational import (Allen et al., 2019, Wiedemann and Pfanner, 2017).

Whether it occurs cotranslationally or posttranslationally, the import of proteins into mitochondria is a particularly challenging problem that eukaryotes needed to “solve”, due to the complex architecture of the mitochondria, requiring the bridging of two membrane delineated spaces i.e. the intermembrane space and matrix (Wojtkowska et al., 2015). The reference model of mitochondrial import machinery is that of *Saccharomyces cerevisiae* (Demishtein-Zohary and Azem, 2017). The complete protein import system consists of three outer membrane (TOM, TOB/SAM, MIM), four inner membrane (TIM22, TIM23, TIM23+PAM, OXA) and three mitochondrial intermembrane space assembly (MIA) complexes. Comparative sequence analysis of genome and transcriptome data by Wojtkowska et al., (2017) indicates that in *D. discoideum*, protein import complexes of the canonical *Saccharomyces* appear to be conserved with the exception of MIA (albeit with some subunit variation/loss) (Fig. 1).

**Fig. 1. The mitochondrial protein import complexes of the Amoebozoa supergroup.** *D. discoideum* (and other Amoebozoa) share a highly similar organisation of their protein import complexes to the canonical *Saccharomyces* protein import complexes and other members of the Amoebozoa. Diagram reused under the terms of the Creative Commons Attribution 4.0 International License. Diagram modified from Wojtkowska et al., (2017).
cation, and protein sorting and assembly, respectively (Wenz et al., 2015). TIM22 & 23 (translocase of the inner membrane) mediate precursor protein import through the IMM, dependant on the location of the protein’s import targeting signals (within sequence or N-terminus, respectively). PAM (presequence translocase-associated motor) functions to drive translocation of proteins into the matrix. OXA (oxidase assembly factor) mediates insertion of proteins from the matrix into the inner membrane (Wenz et al., 2015).

Most protein import from the cytosol through the outer mitochondrial membrane (OMM) is facilitated by the TOM complex (Rapaport, 2002). In D. discoideum, the TOM complex consists of Tom 7, 20, 22, 40, 70 (Macasev et al., 2004, Wojtkowska et al., 2017). Tom22 and Tom40 are conserved in all eukaryotes with the addition of Tom7 after the divergence of the Excavates supergroup (Mani et al., 2016).

Harbauer et al., (2014) suggest that the mitochondrial import machinery should be considered a regulatory hub as impairment of protein import activity acts as a sensor for mitochondrial health and regulates biogenesis and mitochondrial cycling. Dysfunction in protein import can have deleterious effects on mitochondrial membrane potential and cause the accumulation of misfolded proteins in the intermembrane space inducing the mitochondrial stress response (Haynes et al., 2013). This triggers retrograde signalling to the nucleus and upregulation of molecular chaperones, proteases, import complex subunits and proteins in an attempt to alleviate mitochondrial stress and restore mitochondrial homeostasis (Haynes et al., 2013).

Due to its intrinsic role in cellular and mitochondrial health, and association with mitochondrial disease, a complete understanding of mitochondrial import may prove critical in the management of neurological disorders and mitochondrial diseases. Dysfunction of mitochondrial protein import mechanisms is associated with several neurological disorders. Accumulation of amyloid precursor protein in mitochondrial import channels of Alzheimer’s patient brains results in the inhibition of the transport of cytochrome c oxidase subunits into mitochondria, decreased cytochrome c oxidase activity and increase ROS production (Devi et al., 2006). Oligomeric α-synuclein (the putative toxic form of the pathological agent of Parkinson’s disease) binds to the TOM20 subunit of the translocase of the outer membrane (TOM), impairing protein import and causing dysfunction of respiratory Complex I (Di Maio et al., 2016).

Mitochondrial morphology and trafficking

Previously (still currently in undergraduate textbooks) depicted to be discrete bean-shaped organelles free in the cytoplasm of cells, mitochondria are now known to be more complex and dynamic in their morphology, arrangement and subcellular distribution (Tilokani et al., 2018, Wai and Langer, 2016). Mitochondria undergo continuous cycles of fusion and fission and are redistributed within the cell to areas requiring energy production (Eisner et al., 2018). Mitochondrial movement, fusion and fission are dependent on the cell cytoskeleton, both actin and microtubular, and mediated by actin and microtubule motor proteins (Woods et al., 2016).

D. discoideum mitochondria have been reported to be present in various proportions of spherical (66 %), rod (24 %), and tubular (10%) forms in fixed cells (Gilson et al., 2003). Live cell imaging via laser scanning confocal imaging, also showed they are in the main, spherical (Schimmel et al., 2012). Gilson et al., (2003) reported that D. discoideum has two homologues of the α-proteobacterial bacterial fission protein, FtsZ, FszA and FszB. Homologues are also found in other eukaryotic lineages, but not in metazoan, fungi or higher plants (which do however retain plastid homologues of the cyanobacterial FtsZ). When FszA and/or FszB are knocked out, the proportions of the mitochondrial shape classes change greatly, the tubular shape being promoted, consistent with a conserved role in mitochondrial fission. Submitochondrial localisation patterns of FszA suggest that it plays a direct role in mitochondrial division with the distribution of FszA along tubular mitochondria at sites of recent or likely future divisions. The nature of FszB’s role in mitochondrial division is unclear but may be indirect.

While mutation of FszA and/or FszB impaired mitochondrial fission it did not cause the complete cessation of mitochondrial division (Gilson et al., 2003). Other eukaryotes use dynamin-like proteins during mitochondrial fission and two D. discoideum dynamin orthologues, DymA and DymB were suggested as candidate proteins for involvement in fission. Electron micrographs of Dictostelium dymA knockouts show the accumulation of mitochondria into dense clusters and most mitochondria taking an interconnected tubular, branching form (Wienke et al., 1999). These defects were attributed to defects in membrane trafficking processes, rather than defects in mitochondrial division directly. DymB, while being targeted to the mitochondria and playing other diverse roles in the cell, such as stabilisation of actin filaments in cytokinesis, has no obvious role in mitochondrial division (Masud Rana et al., 2013, Rai et al., 2011). Indeed, using a live cell fission and fusion assay and laser scanning confocal microscopy, Schimmel et al., (2012) demonstrated that fusion/fission rates were not affected by loss of DymA, DymB or MidA (Schimmel et al., 2012), a mitochondrial Complex I assembly factor whose loss causes a specific Complex I respiratory defect (Carilla-Latorre et al., 2010). Thus dymA D. discoideum cells have aggregated spherical mitochondria, with tubular structures, but not altered fusion or fission rates, while the mitochondria in dynB- cells also approximated wildtype fission/fusion rates. It appears that uniquely in D. discoideum (thus far), DymA and DymB are not essential for mitochondrial replication.

However, mitochondrial fission and fusion are affected by loss of CiuA, a protein first discovered in Dictostelium and found to be essential for normal localization of mitochondria in the cell (Zhu et al., 1997). Knocking out D. discoideum cluA caused clustering of the mitochondria in the perinuclear region at the centre of the cell along with some moderate growth and viability defects (Zhu et al., 1997). CiuA homologues present as open reading frames in all sequenced eukaryotic organisms (Fields et al., 2002). The mammalian homologue, CLUH, binds a specific subset of mRNAs for nuclear encoded mitochondrial proteins and its loss not only causes clustering of the mitochondria, but reduced mitochondrial biogenesis (Gao et al., 2014). Knocking out the Drosophila homologue causes morphologically abnormal mitochondria, reduced ATP levels and clustering in germ line cells that suggested unequal inheritance (Cox and Spradling, 2009, Sen et al., 2013). When the Saccharomyces cerevisiae homologue (CLU1) was expressed in cluA D. discoideum, the aberrant mitochondrial localisation and growth defects were remedied, indicating that CLU1 and cluA encode functional homologues (Fields et al., 1998). Despite a similar result of interconnected mitochondria as in the case of dymA- mutants, this seemed not to be due to cytoskeletal disruption, but to the late blocking of mitochondrial outer membrane fission (Fields et al., 2002). Previously hypothesised to function as a mitochondria-
cytoskeleton linker protein, CluA has since been demonstrated not to be required for mitochondrial motility but does play an as yet unidentified role in mitochondrial fusion and fission (Woods et al., 2016). While it is known that FsZA, FsZB, and CluA are all essential for mitochondrial fusion and fission, it is not known whether these can account for all of the mitochondrial division activity, since there are no triple mutants available.

Pharmacological disruption of microtubules demonstrated that they are the primary cytoskeletal element involved in D. discoideum mitochondrial movement and distribution (Woods et al., 2016). Actin filaments appear to play a secondary role, affecting the number of mitochondria moving at one time, but not their movement speed or cellular distribution (Woods et al., 2016). The Ras-related GTPase Miro was shown in humans to play a role in the calcium-dependent regulation of mitochondrial transport along microtubules. Miro was found to play a similar role in mammals and other metazoans such as Drosophila. However in Saccharomyces, Arabidopsis, and D. discoideum, Miro is involved in mitochondrial morphology, inheritance and homeostasis rather than trafficking (Yamaoka and Hara-Nishimura, 2014). The D. discoideum orthologue of the human Miro gene is gemA. When knocked-out, gemA- mutants exhibited significant growth defects, particularly in liquid media, attributed to reduced mitochondrial mass and ATP content. Mitochondrial O$_2$ consumption was increased in gemA- mutants, however glucose consumption and mitochondrial membrane potential remained equivalent to wildtype. Mitochondrial mass and morphology were unaffected, and mitochondrial trafficking in vivo showed no impairment (Vlahou et al., 2011). Given the commonalities in function in plants, yeasts and amoebae, Miro appears to have an ancestral function in the homeostasis of mitochondria that has been later modified in metazoans for mitochondrial transport (Yamaoka and Hara-Nishimura, 2014). An understanding of the regulation of mitochondrial dynamics is crucial, as they are essential for key cellular processes in D. discoideum such as differentiation and multicellular development.

**Mitochondrial involvement in development and differentiation**

Mitochondria have functions in regulation of the growth/differentiation transition, cell-type determination, taxis and pattern formation. D. discoideum cells with depleted mtDNA had a delayed entry into differentiation and a cessation of development at the slug stage (Chida et al., 2004). The proportion of prestalk cells was significantly increased with concomitant reduction in prespore cells, in addition, prespore/prestalk cell patterning was disrupted (Chida et al., 2004). Similar findings of shortened fruiting body stalks indicating an increase in the proportion of prestalk cells were made in mutant strains in which expression of the essential mitochondrial protein chaperonin 60 was knocked down (Bokko et al., 2007, Kotsifas et al., 2002).

Further evidence of mitochondria’s role in differentiation is that the expression of mitochondrial ribosomal protein S4 (mt-RPS4) in response to starvation is essential for the initiation of differentiation (Inazu et al., 1999, Maeda and Chida, 2013). Disruption of mrps4 within mitochondria impaired differentiation. Knockouts of mrps4 do not aggregate after 16 h, but do grow normally, suggesting that mt-RPS4 is not needed for growth but is essential for differentiation. Perhaps serendipitously, mt-RPS4 has several nuclear localisation signals in D. discoideum and was shown to localise to the nucleus in mrps4 ectopic overexpression models. Surprisingly, this overexpression of mt-RPS4 in the nucleus enhanced early differentiation, while stable nuclear expression of mrps4 antisense RNA produced the opposite outcome. The mechanisms for these extramitochondrial actions of the mitochondrially encoded mt-RPS4 are unknown. Although not required for mitochondrial fusion/fission, the Complex I assembly factor MidA is essential for correct cellular and mitochondrial function and development (Torija et al., 2006). MidA knock-out in D. discoideum leads to diverse phenotype outcomes: cell growth, size, macropinocytosis and phagocytosis were all reduced. In addition, multicellular development was arrested at the end of the slug stage. While oxygen consumption remained similar to wildtype, a significant reduction of mitochondrial ATP generation in midA- mutants was noted but without any defect in mitochondrial membrane potential or in mitochondrial number, indicating MidA involvement in ATP synthesis. Later work determined that MidA interacts with, and is required for mitochondrial Complex I function (Carilla-Latorre et al., 2010). Further discussion of MidA’s role in mitochondrial respiration is discussed in the mitochondrial disease modelling section of this document.

In mitochondrially diseased D. discoideum, multicellular morphogenesis is disrupted, in particular fruiting body stalk and basal disk formation are abnormal, generally having shortened, thickened stalks (Kotsifas et al., 2002). Stalk-cell differentiation in D. discoideum is coordinated by a group of chlorinated hexaphones known as differentiation-inducing factors (DIF) (Kubohara et al., 2017, Thompson and Kay, 2000). These DIFs also are involved in chemotaxis in D. discoideum, a role that has created interest in these compounds for their potential as anti-metastatic cancer drugs. There are three DIF factors: DIF-1, DIF-2, and DIF-3. DIF-1 is the primary inducer of stalk cell differentiation, with DIF-2 playing a lesser role, and DIF-3 (the first metabolite resulting from the degradation of DIF-1) having effectively no activity on stalk differentiation induction (Thompson and Kay, 2000).

While their phenotypic outcomes have been well examined, the signalling pathways the DIFs act upon remain to be understood. It has been proposed that DIF-1 may function via increases in cytosolic calcium or proton concentrations (Kubohara et al., 2017). As mitochondria are integral to intracellular calcium homeostasis, they appear to be good candidate targets for DIF activity (Paupe and Prudent, 2018). A fluorescently labelled DIF-1 was shown to localise to mitochondria under early starvation or after a 21 hour incubation with cAMP (Kubohara et al., 2017). This may indicate that DIF-1 interacts with the mitochondria in its induction of stalk cell differentiation. DIFs have been shown to act as a mitochondrial uncoupler in mammalian cells (Kubohara et al., 2015). When D. discoideum mitochondria were uncoupled pharmacologically using CCCP (2-[2-(3-Chlorophenyl)hydrazinylidene]propanedinitrile) or DNP (2,4-dinitrophenol), a significant but small induction of stalk cell formation was observed. This suggests that uncoupling of the mitochondria by DIF-1 only partially explains its induction of stalk cell differentiation (Kubohara et al., 2017).

DIF-1 was also shown to bind to and inhibit malate dehydrogenase (MDH) in HeLa cells (Matsuda et al., 2010). Importantly, this inhibition was isolated to the mitochondrial malate dehydrogenase (mMDH) not the cytoplasmic isoform. MDH is an essential enzyme of the tricarboxylic acid cycle and malate-aspartate shuttle pathway. Inhibition of mMDH by DIF-1 lowered cellular ATP levels and inhibited proliferation (Matsuda et al., 2010). Interestingly, DIF-3 was able to bind to mMDH, but did not inhibit mMDH enzymatic
activity nor reduce cellular ATP levels, despite inhibiting HeLa cell proliferation. This parallels the observation that the DIF analogues play different roles in *D. discoideum*, i.e. DIF-3 is not a strong inducer of *D. discoideum* stalk cell differentiation whereas DIF-1 is. While it is currently unknown whether DIF-1 interacts with *D. discoideum* mitochondrial malate dehydrogenase, it appears to be a valid target for the understanding of DIF-1 induction of the stalk cell differentiation pathway and its interaction with mitochondria. The fact that DIF-3 was able to inhibit HeLa cell proliferation without affecting ATP steady-state levels may make it a more attractive candidate as an anti-cancer compound than DIF-1 (Matsuda et al., 2010). Fluorescently labelled DIF-3 localised to the mitochondria and had deleterious effects on mitochondrial morphology and activity in HeLa cells (Kubohara et al., 2013). DIF derivative compounds also inhibited the migration of metastatic murine osteosarcoma cells *in vitro* (Kubohara et al., 2015). DIF-3 promotes mitochondrial fission, induces autophagy, and induces caspase-independent cell death in human leukaemia (K562 CML) lines. K562 cells treated with DIF-3 showed mitochondrial morphology transitioning from the typical network of interconnected mitochondria, to individual organelles with disrupted cristae after 3 - 6 hours, and complete absence of mitochondria after 24 hours. This had significant deleterious effects on ATP production, induced superoxide production and depleted the mitochondrial membrane potential. DIF-3 induces autophagy in K562 cells by rapid inhibition of mTOR pathway signalling as a result of dephosphorylation of mTOR at Ser2481 (Dubois et al., 2016). DIF-3 triggered caspase-independent cell death through the induction of Ca^{2+} influx into the cytoplasm, and the subsequent recruitment of DRP1 to the mitochondria. K652 tumours implanted in mice were inhibited by DIF-3 treatment in food. Beneficially, the inhibition of tumour growth was not apoptotic in nature, so could bypass the apoptotic drug resistance frequently encountered in tumour cells (Dubois et al., 2016). It appears that the DIFs have significant interactions with mitochondria both in *D. discoideum* and human cancer cells and further pursuit of this relationship may provide some insight into mitochondrial roles in cellular morphogenesis.

**Mitochondrial respiratory function**

**Oxidative phosphorylation**

The primary function of the mitochondria is the production of adenosine triphosphate (ATP) by oxidative phosphorylation (OXPHOS) via the electron transport chain (ETC). The OXPHOS pathway in *D. discoideum* is fundamentally the same as its mammalian counterpart, aside from the presence of an additional alternative oxidase (discussed in the following section) (Fig. 2). As the primary means of ATP production, OXPHOS function is central to cellular health and dysregulation of respiration is a common indicator of many mitochondrial diseases and disorders.

The first and largest respiratory complex in the OXPHOS pathway is NADH:ubiquinone oxidoreductase or Complex I (CI), consisting of 45 subunits in mammals with a combined weight of 970 kDa. Fourteen of these are considered "core" subunits as they comprise the minimal functional CI conserved in bacteria, the remainder, so-called "accessory" subunits, in mammals have unknown roles (Fiedorczuk et al., 2016). *D. discoideum* possesses proteins homologous to the core subunits, and a subset of the mammalian accessory proteins. Three subunit genes that are now encoded on the nuclear genome in mammals are encoded on the mitochondrial genome in *D. discoideum*. CI couples the oxidation of NADH to the reduction of ubiquinone, and the pumping of protons from the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space. This forms an H⁺ gradient, the proton motive force. CV uses the proton motive force to drive the phosphorylation of adenosine diphosphate (ADP), regenerating ATP. TCA, tricarboxylic acid cycle; AOX, alternative oxidase; Q, ubiquinone; Cyt C, cytochrome c; UCP, uncoupling protein; IMS, intermembrane space.
a side effect of this, it is also the main site of production of reactive oxygen species which form by leakage of electrons directly to molecular O$_2$ at the point where electrons are normally transferred via ubiquinone to Complex III. A common cause and indicator of mitochondrial disease, respiratory complex I dysfunction has been successfully modelled in D. discoideum, as discussed in a later section of this article.

Alternative oxidase and uncoupling protein

In addition to the classic pathway, the D. discoideum OXPHOS pathway has both a cyanide-resistant ubiquinol alternative oxidase (AOX), similarly to many plant and fungi mitochondria, and uncoupling proteins (UCPs) as do most eukaryotes (Jarmuszkiewicz et al., 2002, McDonald, 2008). D. discoideum AOX (AoxA) is a monomeric oxidase able to accept electrons generated via the electron transport chain, reduce oxygen to water, and generates heat from the dissipation of free-energy (McDonald, 2008). This decreases the yield of oxidative phosphorylation overall but provides a cyanide-resistant alternative pathway for mitochondrial respiration to continue if the cytochrome c oxidase pathway is inhibited. UCP also reduces oxidative phosphorylation yield by dissipation of the H$^+$ gradient generated by the ETC.

AOX and UCPs are used for thermogenesis in plants and mammals, respectively. However, in unicellular eukaryotes, like D. discoideum, any thermal differential with the environment would be quickly dissipated. Therefore, their purpose in D. discoideum is likely that of an energy-dissipation system for modulation of the reduction of ETC components. AOX and UCP both decrease the formation of reactive oxygen species (ROS), so may protect mitochondria from DNA damage, and the initiation of programmed cell death by preventing the over-reduction of the ETC chain (McDonald, 2008, Popov et al., 1997). AoxA was also suggested to play a role in multicellular differentiation of D. discoideum, since pharmacological inhibition of AoxA in starved amoebae triggered differentiation into stalk-like sterile cells (Kimura et al., 2010). However, AoxA null strains developed normally under unstressed conditions. This apparent contradiction suggests that the phenotypes exhibited during pharmacological inhibition may not be due solely to the inhibition of AoxA (Kimura et al., 2010). It is possible that the AOX inhibitor also caused oxidative or some other stress in the treated cells, in addition to inhibiting AoxA. When stressed with hydrogen peroxide, the development of AoxA null strains was significantly impaired, in that the mutants were unable to aggregate (Kimura et al., 2010).

Since AoxA is needed in oxidatively stressed cells for the transition from growth to aggregation, it must be expressed at that time. Jarmuszkiewicz et al., (2002) used antibodies against plant AOX to assay expression of Dictyostelium AoxA. They found that the protein is expressed continuously in the lifecycle of D. discoideum, but its expression levels changed significantly during development. AoxA expression was significantly higher during exponential growth than during stationary phase (20-fold) (Jarmuszkiewicz et al., 2002). However, inducing aggregation of exponentially growing cells by starvation caused the maintenance of AoxA levels at 50% of exponential growth. Maintenance of AoxA levels during starvation-induced development may be necessary for the correct developmental stalk/spore ratio in the fruiting body stage (Jarmuszkiewicz et al., 2002).

A similar approach to studying D. discoideum UCP expression revealed it to be constant throughout growth and development, suggesting a constant need for its activity whatever the life-stage (Jarmuszkiewicz et al., 2004). While its expression level remained constant, D. discoideum UCP was activated by free fatty acids, and consequently dissipated the mitochondrial membrane potential. Doing so should reduce the generation of ROS and so may provide a “safety valve” for the cell, with UCP acting indirectly as an antioxidant (Jarmuszkiewicz et al., 2004).

More recent bioinformatic analysis indicates that Dictyostelium has three uncoupling protein isoforms: UcpA, UcpB, and UcpC (Satre et al., 2007). Due to their similar size (33 kDa, 31 kDa, and 35 kDa, respectively) it is unclear whether an individual isoform or the sum of all their expression was detected using the plant-derived antibodies. Therefore, it is possible that individual isoforms may differ in their expression during growth and development, and further investigation of this is warranted. Overall, AOX and UCP appear to provide a means of relieving ROS generation during high metabolic activity e.g. during exponential growth, and additionally AOX has a role in multicellular development in D. discoideum.

Mitochondrial disease modelling in Dictyostelium

Mitochondrial disease characteristics

Diseases involving mitochondrial dysfunction can be broadly divided into two groups: diseases that arise from a direct primary mitochondrial cause, typically mutations directly affecting the activity or expression of mitochondrial proteins, and those that originate elsewhere but have effects on the mitochondria. Primary mitochondrial diseases in humans cause a range of diverse outcomes including myopathy, diabetes, kidney disease, epilepsy, autism, blindness, deafness, heart disease, and cancer (Kalyanaraman et al., 2018). Diseases of nonmitochondrial origin that involve mitochondrial dysfunction include many neurodegenerative and neurological disorders such as retinitis pigmentosa, Parkinson’s disease, Alzheimer’s disease, and amyotrophic lateral sclerosis (Onyango et al., 2017, Park et al., 2006, Reddy and Beal, 2005).

A primary mitochondrial disease may be caused by inherited or acquired mutations in mitochondrial or nuclear-encoded mitochondrial genes and manifest phenotypically by impacting the ATP production of mitochondria (Alston et al., 2017). As mitochondrial genomes exist in multiple copies in each mitochondrion, and each mitochondrion is one of hundreds in each cell, mutations in the mtDNA may occur in only a proportion of the mitochondrial population, a state known as heteroplasmy (Stefano and Kream, 2016). The proportions of mutant mitochondrial genomes can vary between individuals, between tissues in an individual, and change with age. Phenotypic outcomes of mitochondrial diseases are determined by how large a proportion of the mitochondrial population is affected (mutant load) (Stewart and Chinnery, 2015). The appearance of a phenotype requires a mutant load threshold to be reached. For example, mutations in ATPase 6 (m.8993T>G) will cause neuropathy, ataxia and retinitis pigmentosa (NARP) when heteroplasmy is between 60 and 90% and develop Leigh syndrome when >90% (Claeyss et al., 2016). If the same position is mutated differently, i.e. m8993T>C then Leigh syndrome develops in homoplasmy and a >90% heteroplasmcy will cause NARP (Weerasinghe et al., 2018). Furthermore, Leigh syndrome can also be caused by more than 75 other mutations in entirely separate genes e.g. loss of function of a subunit of Respiratory Complex II, sdhA (Lake et al., 2016). Defects
in the same respiratory complex can also give rise to multiple disorders. Dysfunction of Respiratory Complex I can cause a number of diseases including MELAS syndrome, Parkinsonism, Leber’s disease, and Leigh syndrome (Rodenburg, 2016). In addition to mutant load, cells at particular risk of mitochondrial dysfunction are those that have high energy requirements such as the cerebrum, muscles, and neurons (Zhao et al., 2019). Therefore, in some tissues where energy requirements are lower, the cells are not diseased, whereas in others with high ATP demand, the mutant load may be high enough to cause disease e.g. retinitis pigmentosa (Petit et al., 2018). The complexity and difficulty in studying mitochondrial diseases emphasises the need for a simpler model organism such as Dictyostelium discoideum to clarify these complex phenotypes and provide the basis for investigating the signalling pathways involved and the downstream cellular consequences.

**Dictyostelium models of mitochondrial disease**

_D. discoideum_ provides a well-established model for the study of mitochondrial disease. Presented here is a discussion of some mitochondrial disease modelling from all areas of mitochondrial cellular involvement. _D. discoideum_ mitochondrial disease models have been developed that fall into two categories: those with direct primary involvement of mitochondrial genes and proteins, and those where there is mitochondrial involvement, but the disease aetiology is not mitochondrial.

The first _D. discoideum_ model of mitochondrial disease was created via the mutation of the mitochondrial encoded large ribosomal RNA subunit gene, _rnl_ (Wilczynska et al., 1997). This created a heteroplasmonic mitochondrial mutant strain with aberrant phototaxis. Further investigation of heteroplasmonic mitochondrial mutations in _D. discoideum_ revealed that a common phenotypic signature was shared across strains (Francione, 2008, Wilczynska et al., 1997). This signature phenotype includes impaired growth, both on bacterial lawns and in liquid media, deranged phototaxis and thermotaxis, defective multicellular morphogenesis with shortened and thickened fruiting body stalks, and susceptibility to *Legionella* proliferation within the amoeba (Francione et al., 2009). Interestingly, the antisense inhibition of the _D. discoideum_ chaperonin 60 gene, a protein required for the correct folding of proteins within mitochondria, caused the same aberrant phenotypes (Bokko et al., 2007). Chaperonin 60 knockdown causes an overall knockdown in mitochondria, caused the same aberrant phenotypes (Bokko et al., 2016). Several known genetic mutations in familial Parkinson’s (PD) affect human mitochondria-associated proteins such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) (Viscomi et al., 2016). Several known genetic mutations in familial Parkinson’s (PD) affect human mitochondria-associated proteins such as PRKN, PINK1, DJ-1, HTRA2, and LRRK2 with corresponding mitochondrial dysfunction (Kalinderi et al., 2016, Narendra, 2016). Attractively, _D. discoideum_ does not endogenously express some of these proteins so they can be studied in a naïve cell to determine their putative interacting partners and possible cytotoxic mechanisms. Alternatively, where native homologues of human disease related proteins exist in _D. discoideum_, they can be readily manipulated and analysed.

How might these phenotypes arise? Kotsifas et al., (2002) suggested that signal transduction is more readily disrupted by energy depletion than cellular activities. Signal transduction is energetically expensive and the most obvious _a priori_ prediction of mitochondrial genome dysfunction in mitochondrial disease is that many of the manifested phenotypes would be due to a shortfall in the production of ATP. The depletion of ATP would cause disease through disruption of cellular activities, and those activities/tissues with high energy requirements would be most affected. However, it has been shown in _D. discoideum_, that mitochondrial disease phenotypes in these cases are attributable to chronic AMP-activated protein kinase (AMPK) signalling rather the direct result of ATP insufficiency (Bokko et al., 2007, Francione et al., 2009). AMPK is an energy-sensing protein kinase responsible for maintaining ATP homeostasis by inhibiting anabolic processes, such as growth and proliferation, and promoting ATP-generating pathways, such as mitochondrial biogenesis and fatty acid oxidation. The net result is that ATP homeostasis in maintained by chronic activation of AMPK with the downstream phenotypic consequences of this activation being a permanent feature. Antisense inhibition of AMPK in the _D. discoideum_ mitochondrial disease models rescued the defective phenotypes where the respiratory activity of the mitochondria was broadly affected (Bokko et al., 2007).

Bolstering the case for AMPK’s role, similar phenotypic consequences were observed when specific respiratory complexes were impaired. As previously discussed, MidA plays a role in _D. discoideum_ development. MidA is the _D. discoideum_ orthologue of human NDUFAF7 protein, an arginine methyltransferase that is required for the assembly and function of mitochondrial complex I (CI) (Carilla-Latorre et al., 2010, Hameed et al., 2018). _D. discoideum_ midA knockout disrupted phototaxis and thermotaxis, growth and multicellular development. The defects in phototaxis and thermotaxis were found to be entirely and the growth defects partially mediated by chronic activation of AMPK (Carilla-Latorre et al., 2010). Analysis of respiration in midA null and midA catalytic site point mutants by Seahorse respirometry confirmed the critical and specific role of MidA in proper functioning of CI, with a significant reduction of protonophore-uncoupled, whole cell O₂ consumption attributable to CI, unaccompanied by defects in other respiratory complexes measured (CII & CV). The proportion of basal O₂ consumed for ATP production was unchanged in the midA mutants, as expected if AMPK is chronically activated to maintain ATP homeostasis (Hameed et al., 2018).

The second category of _D. discoideum_ mitochondrial dysfunction models are those that have mitochondrial involvement not directly related to respiration. While not considered classical mitochondrial diseases, there is evidence that mitochondrial dysfunction is involved in the pathogenesis of neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) (Viscomi et al., 2016). Several known genetic mutations in familial Parkinson’s (PD) affect human mitochondria-associated proteins such as _D. discoideum_ midA knockout disrupted phototaxis and thermotaxis, growth and multicellular development. The defects in phototaxis and thermotaxis were found to be entirely and the growth defects partially mediated by chronic activation of AMPK (Carilla-Latorre et al., 2010). Analysis of respiration in midA null and midA catalytic site point mutants by Seahorse respirometry confirmed the critical and specific role of MidA in proper functioning of CI, with a significant reduction of protonophore-uncoupled, whole cell O₂ consumption attributable to CI, unaccompanied by defects in other respiratory complexes measured (CII & CV). The proportion of basal O₂ consumed for ATP production was unchanged in the midA mutants, as expected if AMPK is chronically activated to maintain ATP homeostasis (Hameed et al., 2018).
affecting ATP levels (Chernivec et al., 2018). Disrupted mitochondrial dynamics and ROS damage were implied to possibly contribute to the loss of neurons in PD due to their high energy requirement and reliance on mitochondrial movement (Chernivec et al., 2018).

HTRA2 is a nuclear encoded serine protease which, when mutated, can cause late-onset Parkinson’s disease (Fu et al., 2017). HTRA2 is localised to the mitochondrial intermembrane space in normal conditions and is believed to perform a positive, protective role against cellular stress and also function as a transducer of mitochondrial stress signals (Strauss et al., 2005). Exposure to cellular stresses causes the release of HTRA2 to the cytosol, where it binds to inhibitors of apoptosis, deactivating their inhibition of caspases, initiating programmed cell death (Martins et al., 2002). HTRA2 deletion causes mitochondrial dysfunction and PD-like features (Martins et al., 2004). PD patient-associated mutations in HTRA2 caused the deactivation of the protease activity of HTRA2 when replicated in neuroblastoma SH-SY5Y-derived cell lines (Strauss et al., 2005). In addition, some of these mutant cell lines were more susceptible to staurosporine-induced cellular stress (Strauss et al., 2005). Furthermore, HTRA2 interacts with PINK1, a protein kinase associated with early onset PD, and forming part of the same stress-sensing pathway. HTRA2 phosphorylation is PINK1-dependent and occurs adjacent to a site of a known PD-associated mutation (Plun-Favre et al., 2007). However, it is not entirely understood how HTRA2 mutations cause PD, and whether this is due to a reduction or increase in its serine protease activity or related to its role in cell death.

The single D. discoideum homologue of human HTRA2, also called HTRA2, appears to play a conserved cytoprotective role (Chen et al., 2018). Antisense inhibition and overexpression of a catalytically inactive form of D. discoideum HTRA2 both resulted in defects in morphogenesis and growth but did not affect phototaxis. This suggests that HTRA2 inhibition does not affect mitochondrial ATP production and therefore does not chronically activate AMPK (Chen et al., 2018). The protective role of HTRA2 appears contingent on its expression level. Overexpressed wildtype HTRA2 was cytotoxic in D. discoideum, even without translocation to the cytosol, evidently due to its serine protease activity, since a protease-dead mutant form could be overexpressed. Overexpressing active HTRA2 may cause the depletion of proteins essential to mitochondrial function and thereby have triggered cell death pathways (Chen et al., 2018). An alternative hypothesis is that overexpression may have caused an accumulation of active, cytotoxic protease in the cytosol by breaching the maximum capacity for mitochondrial import of the protein. However, the protease-dead GFP-tagged HtrA did not accumulate in the cytosol when overexpressed but was correctly targeted to the mitochondria.

**Conclusion**

It is clear that the study of mitochondrial biology and disease in D. discoideum has yielded valuable insight into the inner workings of eukaryotic mitochondria and begun to tease out the complexities of human mitochondrial disease. Some of the proteins with conserved roles in mitochondrial biology, notably MidA and CluA, were first discovered and studied in Dictyostelium. In other cases, Dictyostelium has help revealed ancient protein functions, such as the role of Miro in mitochondrial homeostasis or FszA in mitochondrial fission, that have been conserved in some eukaryotic lineages but modified or lost in metazoa.

Both classical mitochondrial disease and mitochondria-associated neurodegenerative disease models have been successfully created in D. discoideum. Mitochondrial disease modelling in D. discoideum first revealed that the deficiency in ATP production may not directly cause disease phenotypes by inhibition of cellular activities, rather, that they may be caused by the chronic activation of AMPK in an attempt to restore energy homeostasis. As no effective therapies currently exist for mitochondrial disorders, this knowledge may be critical in the treatment or alleviation of mitochondrial disease symptoms. By bridging the gap between yeast models and higher eukaryotes, D. discoideum is well placed to continue contributing further in our understanding of mitochondrial biology, and by extension, eukaryotic cell biology.

**Acknowledgements**

XGP received an Australian Postgraduate Research Award. This work was supported by the Australian Research Council Discovery Project grant to PRF and SJA (DP140104276). We are grateful to Claire Storey for her invaluable assistance drafting the manuscript.

**References**

Ahmed, A.U., Gilson, P.R., Lay, S.T., Beech, P.L. and Fisher, P.R. (2006). Import-associated translational inhibition—novel in vivo evidence for cotranslational protein import into Dictyostelium mitochondria. Eukaryotic Cell 5: 1314-1327.

Ahmed, A.U. and Fisher, P.R. (2009). Chapter 2 Import Of Nuclear-Encoded Mitochondrial Proteins: A Cotranslational Perspective. In International Review of Cell and Molecular Biology. vol. 273. Academic Press, pp.49-68.

Alam, M. and Schmidt, W.J. (2002). Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. Behav. Brain Res. 136: 317-324.

Allen, W.J., Collinson, I. and Römisch, K. (2019). Post-Translational Protein Transport by the Sec Complex. Trends Biochem. Sci.

Alston, C.L., Rocha, M.C., Lax, N.Z., Turnbull, D.M. and Taylor, R.W. (2017). The genetics and pathology of mitochondrial disease. J. Pathol. 241: 236-250.

Anderson, S., Bankier, A.T., Barrett, B.G., De Bruijn, M.H., Coulsen, A.R., Douin, J., Eperon, I.C., Nierlich, D.P., Roe, B.A. and Sanger, F. (1981). Sequence and organization of the human mitochondrial genome. Nature290: 457.

Angata, K., Kuroe, K., Yanagisawa, K. and Tanaka, Y. (1995). Codon usage, genetic code and phylogeny of Dictyostelium discoideum mitochondrial DNA as deduced from a 7.3-kb region. Curr. Genet. 27: 249-256.

Aviram, N. and Schuldiner, M. (2017). Targeting and translocation of proteins to the endoplasmic reticulum at a glance. J. Cell Sci 130: 4079-4085.

Barch, C., Greferath, U., Kotsifas, M. and Fisher, P.R. (1999). Polycistronic transcription and editing of the mitochondrial small subunit (SSU) ribosomal RNA in Dictyostelium discoideum. Curr Genet 36: 55-61.

Barch, C., Greferath, U., Kotsifas, M., Tanaka, Y., Alexander, S., Alexander, H. and Fisher, P. (2001). Transcript mapping and processing of mitochondrial RNA in Dictyostelium discoideum. Curr Genet. 39: 355-364.

Bertero, E. and Maack, C. (2018). Calcium signaling and reactive oxygen species in mitochondria. Circulation Res. 122: 1460-1478.

Betarbet, R., Sherer, T.B., Mackenzie, G., Garcia-Osuna, M., Panov, A.V. and Greenamyre, J.T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson’s disease. Nat Neurosci 3: 1301-6.

Bokko, P.B., Francione, L., Bandala-Sanchez, E., Ahmed, A.U., Annesley, S.J., Huang, X., Khurana, T., Kimmel, A.R. and Fisher, P.R. (2007). Diverse cytopathologies in mitochondrial disease are caused by AMP-activated protein kinase signaling. Mol Biol Cell 18: 1874-86.

Burger, G., Gray, M.W., Forget, L. and Lang, B.F. (2013). Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists. Genome Biol. Evol. 5: 418-438.

Capizzi, C., Passamonti, M. and Breton, S. (2016). The human mitochondrial genome may code for more than 13 proteins. Mitochondrial DNA Part A27: 3098-3101.
CARILLA-LATORRE, S., GALLARDO, M.E., ANNESLEY, S.J., CALVO-GARRIDO, J., GRANA, C., ACCARI, S.L., SMITH, P.K., VALENCIA, A., GARESSE, R., FISHER, P.R. et al. (2010). MidA is a putative methyltransferase that is required for mitochondrial complex I function. J Cell Sci 123: 1674-83.

CHEN, S., SANISLAV, O., ANNESLEY, S.J. and FISHER, P.R. (2018). Mitochondrial HTAR2 Plays a Positive, Protective Role in Dictyostelium discoideum but is Cytotoxic When Overexpressed. Genes (Basel) 9.

CHERNIVEC, E., COOPER, J. and NAYLOR, K. (2018). Exploring the Effect of Rotenone-A Known Inducer of Parkinson’s Disease-On Mitochondrial Dynamics in Dictyostelium discoideum. Cells 7.

CHIDA, J., YAMAGUCHI, H., AMAGAI, A. and MAEDA, Y. (2004). The necessity of mitochondrial genome DNA for normal development of Dictyostelium cells. J Cell Sci 117: 3141-52.

CLAEYS, K.G., ABICHT, A., HÄUSLER, M., KLEINLE, S., WIESMANN, M., SCHULZ, J.B., HORVATH, R. and WEISS, J. (2016). Novel genetic and neuropathological insights in neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP). Muscle Nerve 54: 328-333.

COTNEY, J. and SHADEL, G. (2006). Evidence for an Early Gene Duplication Event in Yeast. Science 314: 608-613.

CUBAS, E., BECKER, C., ALTMUELLER, J., FROMMOLT, P. and SARDELLI, M. (2014). CLUH regulates mitochondrial biogenesis by binding mRNAs of nuclear-encoded mitochondrial proteins. J Cell Biol 207: 213-223.

GILSON, P.R., YU, X.C., HERDEL, D., BARTH, C., SAVAGE, A., KIEFEL, B.R., LAY, S., FISHER, P.R., MARGOLIN, W. and BEECH, P.L. (2003). Two Dictyostelium orthologs of the prokaryotic cell division protein FtsZ localize to mitochondria and are required for the maintenance of normal mitochondrial morphology. Eukaryot Cell 2: 1315-1326.

GUSTAFSSON, C.M., FALKENBERG, M. and LARSSON, N.-G. (2018). Maintenance and expression of mammalian mitochondrial DNA. Ann. Rev. Biochem. 85: 133-160.

HAMEED, U.F.S., SANISLAV, O., LAY, S.T., ANNESLEY, S.J., JOBICHEN, C., FISHER, P.R., SWAMINATHAN, K. and ARÖLD, S.T. (2018). Proteobacterial Origin of Protein Agrinine Methylation and Regulation of Complex I Assembly by MidA. Cell Rep. 24: 1966-2004.

HARBAUER, A.B., ZAHEDI, R.P., SICKMANN, A., PFANNER, N. and MEISINGER, C. (2014). The protein import machinery of mitochondria—a regulatory hub in metabolism, stress, and disease. Cell Metab. 19: 357-372.

HAYNES, C.M., FIORESE, C.J. and LIN, Y.-F. (2013). Evaluating and responding to mitochondrial dysfunction: the mitochondrial unfolded-protein response and beyond. Trends Cell Biol. 23: 311-318.

HENDERSON, B., FARES, M.A. and LUND, P.A. (2013). Chaperonin 60: a paradoxi
cally, evolutionarily conserved protein family with multiple moonlighting functions. Biological Rev. 88: 955-987.

INOZU, Y., CHAE, S. and MAEDA, Y. (1999). Transient expression of a mitochondrial gene cluster including rps4 is essential for the phase-shift of Dictyostelium cells from growth to differentiation. Dev. Genet. 25: 339-352.

JARMSZUKIEWICZ, W., BEHRENST, M., NAVET, R. and SLUSE, F.E. (2002). Uncoupling protein and alternative oxidative of Dictyostelium discoideum: occurrence, properties and protein expression during vegetative life and starvation-induced early development. FEBS Lett 532: 459-464.

JARMSZUKIEWICZ, W., CZARNA, M., SWIDA, A. and BEHRENST, M. (2004). Uncoupling Proteins in Amoeboid Eukaryotes, Acanthamoeba castellanii, and Dictyostelium discoideum. Toxicol Mech Methods 14: 3-6.

JASIM, R.A.F. (2018). The Role of Calcium Signalling in Neurodegenerative and Mitochondrial Disease using Dictyostelium discoideum as a Simple Model. In Department of Physiology, Anatomy, and Microbiology, vol. Doctor of Philosophy (ed. Victoria, Australia: La Trobe University.): Thesis (Ph.D.) -- La Trobe University, 2008.

KUBOHARA, Y., KIKUCHI, H., NGUYEN, V.H., KUWAYAMA, H. and OSHIMA, Y. (2017). Familial Parkinson’s Disease-Associated L166P Mutant DJ-1 is Cleaved by Mitochondrial Serine Protease Omi/HtrA2. Neurosci. Bull. 33: 685-694.

FU, K., WANG, Y., GUO, D., WANG, G. and REN, H. (2017). Familial Parkinson’s Disease-Associated L166P Mutant DJ-1 is Cleaved by Mitochondrial Serine Protease Omi/HtrA2. Neurosci. Bull. 33: 685-694.

FU, K., WANG, Y., GUO, D., WANG, G. and REN, H. (2017). Familial Parkinson’s Disease-Associated L166P Mutant DJ-1 is Cleaved by Mitochondrial Serine Protease Omi/HtrA2. Neurosci. Bull. 33: 685-694.

GARG, S.K., VILLANUEVA, C. and MITCHELL, P.A. (2010). Mitochondrial protein synthesis, import, and assembly. Genetics 192: 1203-1234.

FRANCIONE, L.M. (2008). Mitochondrial disease in dictyostelium discoideum, (ed. LA TROBE, U.): Thesis (Ph.D.) – La Trobe University, 2008.

KUBOHARA, Y., KIKUCHI, H., MATSUO, Y., OSHIMA, Y. and HOMMA, Y. (2013). Mitochondria are the target organelle of differentiation-inducing factor-3, an anti-tumor agent isolated from Dictyostelium discoideum [corrected]. PLoS One 8: e72118.

KUBOHARA, Y., KIKUCHI, H., NGUYEN, V.H., KUWAYAMA, H. and OSHIMA, Y. (2017). Evidence that differentiation-inducing factor-1 controls chemotaxis and cell differentiation, at least in part, via mitochondria in D. discoideum. Biof Open 6: 741-751.
KUBOHIRA, Y., KOMACHI, M., HOMMA, Y., KIKUCHI, H. and OSHIMA, Y. (2015). Derivatives of Dicytostelium differentiation-inducing factors inhibit lysophosphatidic acid-stimulated migration of murine osteosarcoma LM8 cells. Biochim Biophys Acta 843: 800-805.

LAKE, N.J., COMPTON, A.G., RAHMAN, S. and THORBURN, D.R. (2016). Leigh syndrome: one disorder, more than 75 monogenic causes. Ann. Neurol. 79: 190-203.

LARSEN, S., HANSS, Z. and KRÜGER, R. (2018). The genetic architecture of mitochondrial dysfunction in Parkinson’s disease. Cell Tissue Res. 373: 21-37.

LE, P., FISHER, P.R. and BARTH, C. (2009). Transcription of the Dicytostelium discoideum mitochondrial genome occurs from a single initiation site. RNA 15: 2321-2330.

LEGER, M.M., PETRUI, M., ZARSKY, V., EME, L., VLCEK, C., HARDING, T., LANG, B.F., ELIAS, M., DOLEZAL, P. and ROGER, A.J. (2015). An ancestral bacterial division system is widespread in eukaryotic mitochondria. Proc Natl Acad Sci USA 112: 10239-10246.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWBIGIN, E., SILVA-FILHO, M.C., MULHERN, T.D. and LEMBE, W. (2016). Ca<sup>2+</sup>-mitochondria axis drives cell division in hematopoietic stem cells. Nature 537: 62-66.

MACASEV, D., WHELAN, J., NEWB
Neurodegenerative Disease. In Mitochondrial Dysfunction in Neurodegenerative Disorders. Springer, pp.81-106.

VLAHOU, G., ELIAS, M., VON KLEIST-RETZOW, J.C., WIESNER, R.J. and RIVERO, F. (2011). The Ras related GTPase Miro is not required for mitochondrial transport in Dictyostelium discoideum. *Eur J Cell Biol* 90: 342-355.

WAI, T. and LANGER, T. (2016). Mitochondrial dynamics and metabolic regulation. *Trends Endocrinol. Metab.* 27: 105-117.

WALLACE, D.C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* 39: 359-407.

WEERASINGHE, C.A.L., BUI, B.H.T., VU, T.T., NGUYEN, H.L.T., PHUNG, B.K., NGUYEN, V.M., PHAM, V.A., CAO, V.H. and PHAN, T.N. (2018). Leigh syndrome T8993C mitochondrial DNA mutation: Heteroplasmy and the first clinical presentation in a Vietnamese family. *Molec. Med. Rep.* 17: 6919-6925.

WENZ, L.-S., OPALIŃSKI, Ł., WIEDEMANN, N. and BECKER, T. (2015). Cooperation of protein machineries in mitochondrial protein sorting. *BBA - Molec. Cell Res.* 1853: 1119-1129.

WIEDEMANN, N. and PFANNER, N. (2017). Mitochondrial machineries for protein import and assembly. *Annu. Rev. Biochem.* 86: 195-222.

WIEDEMANN, N. and PFANNER, N. (2017). Mitochondrial machineries for protein import and assembly. *Annu. Rev. Biochem.* 86: 685-714.

WENZ, L.-S., OPALIŃSKI, Ł., WIEDEMANN, N. and BECKER, T. (2015). Cooperation of protein machineries in mitochondrial protein sorting. *BBA - Molec. Cell Res.* 1853: 1119-1129.

WIEDEMANN, N. and PFANNER, N. (2017). Mitochondrial machineries for protein import and assembly. *Annu. Rev. Biochem.* 86: 685-714.

OHLSSON, M., OHLSSON, M., OHLSSON, M., OHLSSON, M. and OHLSSON, M. (1997). Disruption of a Dynamin Homologue Affects Endocytosis, Organelle Morphology, and Cytokinesis in Dictyostelium discoideum. *Molec. Biol. Cell* 10: 225-243.

WILCZYŃSKA, Z., BARTH, C. and FISHER, P.R. (1997). Mitochondrial mutations impair signal transduction in Dictyostelium discoideum slugs. *Biochem Biophys Res Commun* 234: 39-43.

WILLIAMS, C.C., JAN, C.H. and WEISSMAN, J.S. (2014). Targeting and plasticity of mitochondrial proteins revealed by proximity-specific ribosome profiling. *Science* 346: 748-751.

WOJTKOWSKA, M., BUCZEK, D., STOBIEŃ, O., KARACHITOS, A., ANTONIEWICZ, M., SŁOCINSKA, M., MAKALOWSKI, W. and KMITA, H. (2015). The TOM Complex of Amoebzoans: the Cases of the Amoebo Acanthamoeba castellanii and the Slime Mold Dictyostelium discoideum. *Protist* 166: 349-362.

WOJTKOWSKA, M., BUCZEK, D., SUZUKI, Y., SHABARDINA, V., MAKALOWSKI, W. and KMITA, H. (2017). The emerging picture of the mitochondrial protein import complexes of Amoebozoa supergroup. *BMC Genomics* 18: 997.

WOODS, L.C., BERBUSSE, G.W. and NAYLOR, K. (2016). Microtubules Are Essential for Mitochondrial Dynamics: Fission, Fusion, and Motility in Dictyostelium discoideum. *Front Cell Dev Biol* 4: 19.

YAMAOKA, S. and HARA-NISHIMURA, I. (2014). The mitochondrial Ras-related GTPase Miro: views from inside and outside the metazoan kingdom. *Front Plant Sci* 5: 350.

ZHANG, X.Y., LU, M.H., YUAN, D.J., XU, D.E., YAO, P.P., JI, W.L., CHEN, H., LIU, W.L., YAN, C.X., XIA, Y.Y. et al., (2019). Mitochondrial Dysfunction in Neural Injury. *Front Neurosci* 13: 30.

ZHOU, Q., HULEN, D., LIU, T. and CLARKE, M. (1997). The cluA- mutant of Dictyostelium identifies a novel class of proteins required for dispersion of mitochondria. *Proc Natl Acad Sci USA* 94: 7308-7313.
Further Related Reading, published previously in the *Int. J. Dev. Biol.*

**Mitochondria and lipid metabolism in mammalian oocytes and early embryos**  
Josephine Bradley and Karl Swann  
*Int. J. Dev. Biol.* (2019) 63: 93-103  
https://doi.org/10.1387/ijdb.180355ks

**Dying to survive - apoptosis, necroptosis, autophagy as supreme experiments of nature**  
Mauro Piacentini and Guido Kroemer  
*Int. J. Dev. Biol.* (2015) 59: 5-9  
https://doi.org/10.1387/ijdb.150167mp

**Transformation of the mitochondrial genome**  
Véronique Larosa and Claire Remacle  
*Int. J. Dev. Biol.* (2013) 57: 659-665  
https://doi.org/10.1387/ijdb.130230cr

**Modulation of mitochondrial biogenesis and bioenergetic metabolism upon in vitro and in vivo differentiation of human ES and iPS cells**  
Alessandro Prigione and James Adjaye  
*Int. J. Dev. Biol.* (2010) 54: 1729-1741  
https://doi.org/10.1387/ijdb.103198ap

**Sperm mitochondria diaphorase activity – a gene mapping study of recombinant inbred strains of mice**  
Aniela Golas, Paulina Malek, Małgorzata Piasecka and Jozefa Styrna  
*Int. J. Dev. Biol.* (2010) 54: 667-673  
https://doi.org/10.1387/ijdb.082778ag

**Organelles in developing neurons: essential regulators of neuronal morphogenesis and function**  
Sayaka Sekine, Masayuki Miura and Takahiro Chihara  
*Int. J. Dev. Biol.* (2009) 53: 19-27  
https://doi.org/10.1387/ijdb.082618ss