

Melbourne Dictyostelium conference

**Institute for Advanced Study
La Trobe University**

21st of July 2005 (9.30am-5pm)

Sponsored by the La Trobe University Institute for
Advanced Study as part of the "Showcasing the
Future" programme.

Schedule of Oral Presentations

Welcome (9.30am-9.40am)

Phototaxis

Signalling Pathways (9.40am-10.20am)

Sarah Annesley
Esther Bandala

Mitochondrial Disease (10.20am-11.00am)

Lisa Said
Sui Lay

MORNING TEA (11.00am-11.20am)

Enzymes involved in Metabolism and Signalling (11.20am-12.40pm)

Paul Bokko
Paige Smith
Sandra Accari
Claire Allan

LUNCH (12.40pm-1.40pm)

Mitochondrial Biogenesis

Division and Protein Import (1.40pm-3.00pm)

Afsar Ahmed
Keith Niven
Marie Murphy
Ben Kiefel

AFTERNOON TEA (3.00pm-3.20pm)

Transcription of Mitochondrial Genome (3.20pm-4.40pm)

Phuong Le
Luke Kennedy
Elin Berger
Maggie Mokbel

The role of filamin in phototaxis and identification of potential filamin binding partners

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Filamin is an actin binding protein that is ubiquitous in eukaryotes. Actin is one of the most highly conserved proteins in evolution and the actin cytoskeleton is required for most movements of the cell at the cellular and intracellular level. The actin cytoskeleton must be able to rapidly rearrange its structure in response to various stimuli. This fast response is concerted by numerous actin binding proteins. The actin binding proteins can be grouped according to their function and filamin belongs to the F-actin crosslinking proteins. These proteins are important for stabilizing the 3D cortical actin network. Unlike other actin binding proteins filamin has been shown to be involved in slug phototaxis.

The filamin protein is comprised of two major domains, the actin binding domain situated at the N terminus is involved in binding actin filaments and is conserved amongst many actin binding proteins. Following the actin binding domain is the rod domain, this domain is comprised of six repeat segments that each form an immunoglobulin like fold which may facilitate interactions with other proteins. It is thought that the role of filamin in slug phototaxis must lie in this rod domain. This study seeks to determine which segment(s) of filamin are required for phototaxis, and also to identify binding partners of filamin. In order to achieve these aims the filamin mutant was transformed with various constructs containing specific deletions of the gene and observing the transformants phototactic behaviour. If the defective phototactic phenotype was not restored by a particular construct then it could be assumed that the missing section is important for slug phototaxis. To date it appears that the actin binding domain is essential for filamins role in slug phototaxis and the other segments are currently being tested. In order to identify binding partners of filamin many techniques have been employed. The most success has come from immunoprecipitation experiments which have shown that rasD, a known signaling protein, is a potential binding partner of filamin. Other potential binding partners are being investigated.

THE ROLE OF GTP-BINDING PROTEINS CONTROLLING *Dictyostelium discoideum* PHOTOTAXIS.

Esther Bandala, Paul R. Fisher.

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Proteins control and mediate many of the biological activities of cells in association with partner molecules, or as components of large molecular assemblies. GTP-binding proteins have an important role in order to coordinate many different signals that the cell receives. GTP-binding proteins are divided in three main groups: the large mammalian G $\alpha\beta\gamma$ heterotrimers, the small Ras and Ras-like GTPases and the intermediate-sized GTPases such as elongation factors. It has been shown that some members of these GTP-binding proteins family are involved in the phototaxis process in *Dictyostelium*. Cells lacking RasD protein are affected in phototaxis and thermotaxis but the signalling partners of RasD are not known. Heterotrimeric G proteins have important regulatory functions during *Dictyostelium* development. AIMS: To determine possible binding partners of the RasD protein and to investigate the functions of the G β subunit in the phototaxis process in *Dictyostelium*. RESULTS: Using the immunoprecipitation technique we found that Filamin, GRP125, ERK2 and PKB are possible binding partners of RasD, when RasD is overexpressed and constitutively active. In order to investigate the functions of the G β subunit, a temperature-sensitive G β mutant and an overexpression G β (wt) mutant were used. We found that in cells overexpressing the G β (wt) at the permissive temperature 22°C, phototaxis was bidirectional in comparison to the wild type. Using the temperature-sensitive G β mutant we found that the phototaxis was not affected in temperature-shift experiments at 22-27 °C, but the development in the temperature-sensitive G β mutant was impaired at higher temperatures. PRELIMINARY CONCLUSIONS: We have some evidence of a possible signalling complex that participates in *Dictyostelium* phototaxis. Ras D is part of this complex, but G β probably is not involved.

Mitochondrial gene disruption in *Dictyostelium discoideum*.

L. M. Said, P.R. Fisher.

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Recent evidence indicates that signal transduction may be more sensitive than other cellular activities to sublethal mitochondrial defects. This could be either because signal transduction which is energetically expensive is more sensitive to cytosolic ATP depletion than other cellular activities or because some other mitochondrial function is affected eg. mitochondria may have a direct role in signal transduction.

To understand how mitochondrial dysfunction affects phototactic signal transduction, various mitochondrial genes in *D. discoideum* have been targeted for disruption and the effects on phototaxis examined. The genes selected for disruption involved those that encode subunits of oxidative phosphorylation complexes including *atp1* (atp synthase subunit 1), *cob* (cytochrome b of cytochrome reductase), *atp6* (atp synthase subunit 6), *cox3* (cytochrome c oxidase) and *nd2* (NADH-Q reductase subunit 1). Open reading frames (ORFs) termed ORF796 and ORF1740 were also targeted for disruption. The products of these genes have unknown functions (not thought to be linked to oxidative phosphorylation (OXPHOS)).

It was found that disruption experiments targeted at all the genes led to a significantly high level of phototaxis mutants regardless of whether the encoded gene product is known to be critical for OXPHOS. It is possible that the disruption of the ORFs had an indirect effect on OXPHOS, via a downstream affect on other mitochondrial genes. Alternatively the ORFs may be essential for some unknown mitochondrial function and indirectly effect OXPHOS as a result.

The mutants were further characterised and found to also have slower growth rates and abnormal morphologies. Preliminary data also suggests that the mutants have defects in phagocytosis and are more susceptible to *Legionella* infection.

Characterisation of succinate dehydrogenase (SdhA) in *Dictyostelium discoideum*.

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The cloning, sequencing and functional characterization of the flavoprotein subunit gene (SdhA) of succinate dehydrogenase in *Dictyostelium discoideum* is described. Sequence comparison of the *Dictyostelium sdhA* gene with homologs from other organisms reveal that the gene sequence has been highly conserved throughout evolution. The *sdhA* gene contains no introns and encodes a protein of about 64 kDa that is targeted to the mitochondria. This localization was supported by the expression and observation of SdhA-GFP fusion protein in *Dictyostelium* cells. Transcription of the gene is down regulated during early *Dictyostelium* development in response to starvation, while the level of the flavoprotein subunit remained constant throughout the life cycle. In an attempt to understand how mitochondrial dysfunction affects phototactic signal transduction, targeted disruption, gene knockout, antisense inhibition and interference RNA (RNAi) experiments were performed using cloned gene sequences. Consistent with the essential role of succinate dehydrogenase in cellular energetics, mutants in which the *sdhA* gene had been disrupted could not be isolated. Transformants containing an antisense RNA-expressing plasmid showed varying degrees of decreased *sdhA* mRNA levels and protein, but no significant effects on phototaxis accuracy or growth was observed. In contrast, orientation in phototaxis as well as growth and morphogenesis was impaired in *sdhA* transformants containing low copy numbers of the RNAi-expressing plasmid in the genome. The RNAi construct was lethal in cells at higher copy numbers. Northern and Western blot analysis and measurement of the energy status of transformants were also determined.

THE ROLE OF AMP-ACTIVATED PROTEIN KINASE (AMPK) IN PHOTOTAXIS AND MITOCHONDRIAL DISEASES IN *Dictyostelium discoideum*.

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The AMP-activated protein kinase (AMPK) is a metabolic-stress-sensing protein kinase that regulates metabolism in response to energy demand and supply by directly phosphorylating rate-limiting enzymes in metabolic pathways as well as controlling gene expression (Kemp et al 2003). Thus a decrease in the energy charge would activate the enzyme. Activated AMPK switches off anabolic pathways (fatty acid, triglyceride, cholesterol and protein synthesis and gene transcription) and switches on catabolic pathways such as glycolysis and fatty acid oxidation. We describe the cloning, sequencing and functional characterisation of AMPK α subunit of the *D. discoideum*. Sequence comparison of the *D. discoideum* AMPK α subunit with homologues from other eukaryotes reveals that the gene sequence is highly conserved. The *D. discoideum* AMPK α subunit encodes a protein about 39kDa, contains 3 introns and APE & DFG motifs. Preliminary pharmacological study showed that treatment of AX2 cells with graded concentrations of AICAR (a specific AMPK activator) caused dose-dependent phototaxis defects. In an attempt to understand the role of AMPK in mitochondrial dysfunction and photosensory signal transduction, ectopic overexpression, antisense inhibition and AMPK/hspA double antisense cotransformation using the cloned sequences as well as pharmacological study on AX2 cells were performed. Northern blot and RT-PCR show that the AMPK α subunit gene is expressed throughout growth and development (life cycle). Plasmid construct copy numbers in all transformants were determined. Mutants overexpressing AMPK α (catalytic) subunit gene showed phototaxis defects without obvious effects on development. Phototaxis defects became severe in transformants carrying higher copy numbers of the ectopic overexpression construct. The mRNA levels were high but only slight increases in the protein levels were observed. In contrast, antisense inhibition led to strong reductions of mRNA levels. These reductions resulted in only moderately decreased levels of protein. Transformants containing an antisense RNA-expressing plasmid showed impaired aggregation on bacterial lawns but exhibited improved phototaxis on charcoal agar plates. The accuracy of phototaxis improved while development impairment became severe in transformants carrying higher plasmid construct copy numbers. Slugs of AMPK α subunit antisense transformants also migrate shorter distances, make fewer and smaller fruiting bodies. At very high copy numbers the mutant cells do not aggregate even on charcoal agar. The result suggests that AMPK functional attenuation (antisense-RNA) impairs the progress of aggregation and thus slowed or prevented development. This indicates that AMPK α subunit plays a role at least in initiation of development in *D. discoideum*. Although AMPK is widely studied in relation to diabetes, exercise physiology and energy homeostasis, its possible role in mitochondrial diseases appears not to have been considered previously. To investigate the possible role of AMPK in mitochondrial disease, we cotransformed AX2 cells with antisense plasmid constructs of hspA and AMPK α subunit. Antisense inhibition of chaperonin 60 expression (hspA) was shown previously to impair phototaxis and (at high copy numbers) development. Phototaxis and development were wild type in

Cotransformants. The result suggests that there is genetic interaction between AMPK and mitochondrial function (*hspA*) in phototaxis and development.

The interaction demonstrates that ATP depletion caused by mitochondrial dysfunction activates AMPK whose downstream targets include proteins belonging to other signal transduction pathways thereby initiating other signalling cascades. The resulting perturbation of these pathways would result in impaired responses to corresponding signals (for instance in *D. discoideum* leading to deranged phototaxis). This may explain why mitochondrial diseases present mostly as central nervous system and muscle disorders as some of the pathological consequences of ATP depletion in mitochondrial diseases could be due to chronic activation of AMPK.

Tripeptidyl-peptidase I and its role in *Dictyostelium discoideum*

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Tripeptidyl-peptidase I (TPP-I) is one of over 200 lysosomal enzymes identified in mammals. Its precise role is at present unknown, but it is thought to be involved in initial cleavage of the mitochondrial ATP synthase subunit c protein. This is believed to allow further breakdown by other lysosomal enzymes. Mutations in the gene encoding TPP-I in humans lead to the fatal neurodegenerative disease known as late infantile neuronal ceroid lipofuscinosis type 2 or Batten disease.

The TPP-I gene sequence has been identified in the cellular slime mould *Dictyostelium discoideum*. The aim of this project is to characterise the *tppA* gene to determine its function in *D. discoideum* and thereby to contribute to Batten disease research. By creating mutant constructs, strains may be created with both decreased and increased TPP-I expression levels and various phenotypes may then be tested and compared to wild type. The gene may also be manipulated to discover more about the function of lysosomal enzymes in general and how they are targeted to the lysosomes in *D. discoideum*.

Preliminary results suggest that the *tppA* gene is single copy and expressed in all stages of the lifecycle. Antisense inhibition and overexpression constructs have been created and transformed into *D. discoideum*. The outcome of these transformation events suggests that increasing *tppA* gene expression may be lethal, whilst only a small number of antisense inhibited transformants have been obtained from 10 transformation experiments. This suggests that the enzyme may play a significant role in cellular functioning in *D. discoideum*.

Some phenotypic properties have also been tested using the *tppA* antisense inhibited transformants that were obtained. These strains show a significantly slower growth rate than wild-type when grown both axenically and on *E.coli* B2 lawns. This suggests that the *tppA* gene may play a role in pinocytosis and phagocytosis which will be studied later in the project.

The role of Phospholipase A₂ in *Dictyostelium discoideum*

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Phospholipases are ubiquitous in nature. They belong to the family of lipolytic enzymes involved in the cleavage of phospholipids at various sites present in the cell. There are four major classes of phospholipases A, B, C and D. Phospholipase A₂ is one member of the A group which plays an important role in cellular pathways.

Phospholipase A₂ has been shown to be a superfamily, consisting of 3 major classes (secretory, cytosolic and calcium-independent) and 1 minor class (Platelet Activating Factor). Each of these classes has its own role to play in the cell. However they all cleave at the same point on phospholipids, the *sn*-2 position. This cleavage results in the release of a free fatty acid and a lysophospholipid. Both these products can involve themselves in different pathways.

In *Dictyostelium discoideum* phospholipase A₂ has been proposed to be involved in the influx of calcium from the extracellular environment. My work involves the identification and characterisation of a putative phospholipase A₂ from the cytosolic class. Through the creation of various mutants, it is possible to study the role this enzyme may have in *Dictyostelium discoideum* and whether this is involved in the influx of calcium from the extracellular environment.

Work carried out so far on antisense mutants have indicated that there is a defect in growth depending on method of obtaining food. In liquid, growth rates are comparable to wild-type Ax2 indicating that pinocytosis is unaffected, whereas when grown on solid media with *E. coli* B/2 as a food source several strains grow significantly faster than wild-type Ax2. Again using antisense mutants measurements of calcium influx when stimulated with cAMP was tested. It was seen that there is a definite decrease with respect to the control strain of calcium influx, which could be due to either slower development or to impaired Ca²⁺ signalling.

G-PROTEIN COUPLED RECEPTORS AND CALCIUM SIGNALLING IN *DICTYOSTELIUM*

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G-protein coupled receptors (GPCRs) are present in all eukaryotes and are involved in many biological processes such as olfaction, light reception, hormone regulation and chemotaxis. Chemotaxis is an important biological response that is fundamental for inflammation, neurogenesis, angiogenesis and other morphogenetic processes. Many of the mechanisms by which GPCRs mediate chemotaxis are not well understood at the molecular level. A valuable tool for studying GPCR functionality has been the production of chimeric receptors that allow different domains to be isolated and investigated.

The *Dictyostelium discoideum* G-protein coupled receptor CAR-1 mediates chemotaxis to cAMP during aggregation. Occupancy of CAR-1 activates intracellular signalling cascades via G-proteins and also independent of G-proteins. As well as other processes, these intracellular signals induce of an influx of extracellular calcium into the cytosol. This calcium is thought to influence oriented cell movement by contributing to modulation of the cytoskeleton dynamics.

AIMS: To investigate the molecular mechanisms of CAR-1 signal transduction that induce a calcium influx. **EXPERIMENTAL METHODS:** Chimeric receptors were constructed from CAR-1 and the GPCR odr-10 that binds the odorant diacetyl. They are being utilised to investigate the domains of CAR-1 involved in signalling a calcium influx. **PRELIMINARY CONCLUSIONS:** The chimeric receptors tested so far have failed to mediate a response to the ligand diacetyl. However, in the course of these experiments, a developmentally regulated response to diacetyl was observed that seems likely to be mediated by one of the 60 GPCRs encoded in the genome. **FUTURE WORK:** To continue use of chimeric receptors in investigation of CAR-1 functionality and signalling mechanisms. To identify the cell surface receptor responsible for calcium influx in response to diacetyl. To investigate putative *Dictyostelium* membrane calcium pumps and channels, and their roles in calcium signalling.

Cotranslational protein import into *Dictyostelium* mitochondria an - *in vivo* study

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To study mitochondrial protein import in *Dictyostelium discoideum*, we utilized two different reporter proteins - green fluorescent protein (GFP) and aequorin (a Ca²⁺ - sensitive luminescent protein). Both GFP and aequorin were fused either to variable N-terminal regions of chaperonin 60 (the first 23, 40 and 150 amino acids), or to the mitochondrial targeting sequence of DNA topoisomerase II and expressed in AX2 cells under actin 15 promoter. The activities of both the mitochondrially targeted GFP and aequorin fusion proteins was unexpectedly much lower than the non-targeted (cytoplasmic) forms. We found that targeting GFP to the mitochondria results in reduced levels of fusion protein even though transcription of the fusion gene and the stability of the protein are unaffected. The results indicate a novel phenomenon for GFP fusion proteins *import-mediated translational inhibition* where by protein import into the mitochondria limits the rate of translation. The simplest explanation for this is that the GFP fusion protein import occurs cotranslationally i.e. protein synthesis and import into mitochondria are coupled events. Consistent with cotranslational import, Northern analysis showed that GFP mRNA is associated with isolated mitochondria. The leader peptide of GFP fusions is susceptible to degradation in the cytoplasm but not in the mitochondria, whereas GFP itself is quite stable in both compartments. We suggest that the cotranslational import of GFP fusion proteins results in their avoidance of cytosolic degradation of the leader peptide. By contrast, the results with aequorin transformants have shown that aequorin expressions are quite comparable at both transcriptional and translational levels regardless of whether or not it has been successfully targeted to the mitochondria. The reduced activity of aequorin in mitochondrially targeted fusion proteins presumably results from interference by the additional N-terminal amino acids either in proper folding or with some aspect of their catalytic activity.

FszB shows a polar localisation in *Dictyostelium* mitochondria

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FtsZ is a key bacterial division protein forming a ring at the site of cell division. Though FtsZ is absent from the bacterially derived mitochondrial organelles of animals, fungi and higher-plants, the mitochondria of many protists, including *Dictyostelium*, have retained FtsZ. We previously showed that *Dictyostelium discoideum* has two nuclear-encoded FtsZs, FszA and FszB, targeted to the mitochondria. Null mutations of *fszA* and/or *fszB* resulted in decreased mitochondrial division, and anti-FszA and FszA-GFP localisations showed belts and/or puncta in mitochondria that represented recent or future sites of organelle division. FszB-GFP localised to a sub-mitochondrial body that was usually located at one end of the organelle and not at the site of division. This was unexpected because multiple FtsZs present in plant chloroplasts all localise to the division plane and appear to have a direct role in division. To ascertain if GFP had caused the FszB-GFP fusion to mis-target to the mitochondrial poles, we raised antibodies to a synthetic FszB peptide (amino acids A289 to T310). Immunofluorescence revealed anti-FszB to have a polar localisation within mitochondria – similar to that observed in FszB-GFP cells, confirming for the first time the differential localisations of two independent FtsZ proteins within the one organelle.

Localisation of FtsZ in the secondary plastid of *Phaeodactylum tricorutum*

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Plastids are fundamental organelles of all photosynthetic and some non-photosynthetic eukaryotes of endosymbiotic origin. Two plastid-derived, nuclear-encoded FtsZ proteins have been previously described for the diatom *Thalassiosira pseudonana* (Kiefel *et al.*, 2004), and following sequence analysis I have located a third. Sequence comparison to the genome of the genetically manipulable diatom *Phaeodactylum tricorutum* has uncovered three homologous plastid-encoded FtsZ sequences. FtsZ is an elementary bacterial division protein that also plays an important role in the division of the inner membrane of plastids. As diatoms are of secondary endosymbiotic origin, they are encased by four membranes. Studying the localisation of the three FtsZs may lead to elucidating the mechanisms by which these additional two membranes divide. Here I have completed phylogenetic studies to determine the origin of the FtsZ sequences and attempted to determine the subcellular localisation of the three FtsZs by implementing FtsZ-GFP fusion proteins. This study is the first to be conducted on eukaryotes of secondary endosymbiotic origin to elucidate the mechanisms involved in secondary plastid division.

References

Kiefel, B., Gilson, P., and Beech, P. (2004). Diverse eukaryotes have retained mitochondrial homologues of the bacterial division protein FtsZ. *Protist* 155, 105-115.

Bioinformatic search for FtsZ-associated proteins

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Mitochondria cannot be produced *de novo*, and therefore pre-existing organelles must be divided prior to cell division to ensure each daughter cell receives their mitochondrial allotment. Mitochondria are descended from free-living α -proteobacterial ancestors, and like chloroplasts which are also descended from bacteria (in this case cyanobacteria) many still use the bacterial division protein FtsZ for fission. Unlike the case for chloroplasts which still use other components of the bacterial division system (namely the Min proteins), no other known bacterial division proteins have been identified as having a role in mitochondrial division (our unpublished data and that of others). Recently, the genomes of the red alga *Cyanidioschyzon merolae* (Matsuzaki *et al.*, 2004) and the diatom *Thalassiosira pseudonana* (www.jgi.doe.gov/) and the have become publicly available, both of which encode mitochondrial FtsZ (mtFtsZ) proteins (Kiefel *et al.*, 2004; Miyagishima *et al.*, 2004; Takahara *et al.*, 2000). When added to the genome of *Dictyostelium discoideum*, we thus have at our disposal the complete genomes of three species that contain mitochondrial FtsZs. Here we are attempting to perform, *in silico*, the first comparative mitochondrial proteomics of mtFtsZ-containing organisms. We will use this information to identify common features of the mitochondrial division apparatus in those organisms that use MtFtsZ, and produce candidate mitochondrial division proteins.

Transcription of the *Dictyostelium discoideum* mitochondrial genome occurs from a single initiation site.

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In Northern hybridisation and primer extension analyses, Barth *et al.* (2001) compiled a detailed transcript and processing map of the *Dictyostelium* mitochondrial genome. The results obtained suggested the mitochondrial DNA to be transcribed into eight major transcripts. In order to determine whether these transcripts could possibly derive from processing of even larger transcripts, Reverse Transcriptase Polymerase Chain Reactions (RT-PCR) were performed in an attempt to amplify the overlapping regions between the eight major transcripts. Except for one region, all overlapping regions could be reverse transcribed and amplified, indicating that even larger transcripts existed and that the eight major transcripts observed in Northern hybridisation studies were the products of transcript processing. In Southern hybridisation analyses of DNA fragments representing the regions between the eight major transcripts with *in vitro* capped mitochondrial RNA, only one DNA fragment was detected, demonstrating that the 5' end of only one of the eight major transcripts represents a genuine transcription start site. From this we conclude that transcription of the *Dictyostelium* mitochondrial genome is initiated at a single site. The single transcription initiation site is found in a non-coding region located upstream of the large ribosomal subunit RNA gene *rml*. In mobility shift assays, the DNA sequences located upstream of the transcription initiation site were bound by DNA binding proteins, suggesting the presence of promoter and possibly other regulatory sequences in this region.

Mitochondrial RNA processing in *Dictyostelium discoideum*

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The mitochondrial genome of *Dictyostelium discoideum* is approximately 55 KB in length and is transcribed into eight large polycistronic transcripts. The formation of polycistronic precursor RNA molecules is possibly a mechanism for the coordinate regulation and expression of mitochondrial genes.

In order to obtain functional gene transcripts, the polycistronic precursors need to be processed into smaller mono-, di-, and tri-cistronic molecules. Presumably, these modifications require the actions of various nuclear- encoded proteins that could be putatively identified by sequence homology to known processing enzymes. Furthermore, the presence of a mitochondrial targeting signal could be identified using various software. A nuclear-encoded gene with sequence homology to an *A.thaliana* RNA helicase was previously identified and has been shown, via GFP expression studies, to localise to the mitochondria *in vivo*. The present study aims to further characterise this protein, termed DdHelA, through gene disruption and RNAi experiments.

Transcript mapping and primer extension studies have shown that in most cases both ends of the large precursor RNA molecules are flanked by regions encoding tRNAs. It is proposed that a portion of the tRNA molecules are recognised by processing enzyme/s and subsequent excision of the tRNA results in the functional gene transcripts. In some cases where flanking tRNAs are not present it is proposed that the processed region of the larger transcript contains structures similar to those recognised in the tRNA.

The present study is an investigation into the nature of these processing events and the proteins involved.

Identification and characterisation of a mitochondrial DNA polymerase in *Dictyostelium discoideum*

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Mitochondrial DNA polymerase gamma (mtDNAP γ) is the sole enzyme found so far in a variety of organisms to be devoted to mitochondrial DNA (mtDNA) replication. Database searches of the *Dictyostelium discoideum* genome database have suggested that a mtDNAP γ -like enzyme is not involved in *D. discoideum* mtDNA replication. The results obtained from the searches indicated that there are no homologous mtDNAP γ sequences present in the *D. discoideum* genome. This also seems to be the case in plants, where a mtDNAP γ -like enzyme has not been discovered yet. To date, the enzyme that mediates the replication of the *D. discoideum* mtDNA has not been identified.

In order to identify the gene of a *D. discoideum* mitochondrial DNA polymerase (mtDNAP), searches of the *D. discoideum* genome database were performed based on sequence homology to known *Escherichia coli* DNA polymerase (DNAP) sequences. One potential *D. discoideum* mtDNAP gene sequence was edited to obtain an open reading frame and translated into a protein sequence that was used for BLAST searches and sequence alignments. The alignments demonstrated that the potential *D. discoideum* mtDNAP has very low similarity to known mtDNAP γ sequences from different organisms, but it shows a high similarity to DNAP A sequences. Using the software Predator, MitoProt and HelicalWheel, it was demonstrated potential *D. discoideum* mtDNAP encodes a protein that is highly likely to be translocated into the mitochondria. Various molecular techniques were used to isolate, characterise and clone the gene for the potential *D. discoideum* mtDNAP. These included Southern and Northern hybridisation analysis to demonstrate the presence of the gene in the *D. discoideum* genome and to assess its transcription. Reverse Transcription polymerase Chain Reaction (RT-PCR) has been performed to obtain a cDNA copy of the gene, demonstrating the presence of an intron in the 5' portion of the gene. To confirm the subcellular localisation of the potential *D. discoideum* mtDNAP, a 5' portion of the gene has been cloned into the *D. discoideum* expression vector pA15GFP to create a fusion protein that allows targeting of the green fluorescent protein (GFP) to the mitochondria. With the identification and characterisation of *D. discoideum* mtDNAPs, more can be learnt about the molecular processes involved in *D. discoideum* mtDNA replication and repair.