The role of circular mRNA in green algae mitochondria

Mitochondria are often described as the ‘powerhouse’ of the cell as they play a critical role in the generation of metabolic energy. These rod-shaped organelles are the site of aerobic respiration, converting oxygen and nutrients into adenosine triphosphate (ATP). ATP is a nucleotide that contains a large amount of energy stored in its phosphate bonds. Therefore, when these bonds are hydrolysed the energy is released and is used to fuel the cell’s metabolic activities. Mitochondria are clearly essential for survival and are found in the majority of eukaryote cells. However, these vital eukaryote organelles actually originated from bacteria. Approximately 1.4 billion years ago, free-living bacteria were engulfed by primitive cells via a process known as endosymbiosis. Over time, changes occurred as the bacterium evolved into mitochondria and became integrated into the host cell. Mitochondria require fewer genes than a free-living bacterium and have lost many of the gene functions needed to survive independently. This means that mitochondria are completely reliant on the host cell. For example, the mitochondria genome of the land plant Arabidopsis thaliana encodes 32 proteins and in humans, only 13 proteins are encoded by mitochondrial genes. Furthermore, most of these proteins are involved in aerobic respiration and the production of ATP.

However, these organelles have maintained specific traits which are associated with the ancestral free-living bacteria. For example, they have their own set of circular chromosomes with genes that are expressed independently from those located in the nucleus. These mitochondrial genes are unique and expressed in ways that are completely unique in the biological world.

RNA EDITING

This inspired Dr Cahoon and his team to explore the mechanisms underlying gene expression in mitochondria and how they differ from bacterial and eukaryotic systems. The team began by investigating the possibility that RNA editing occurs in algae. RNA editing occurs after transcription, the first step of gene expression. Briefly, during transcription, a specific segment of DNA is copied into messenger RNA (mRNA) by the enzyme RNA polymerase. Ribosomes then translate the mRNA into a protein.

RNA editing is a process whereby enzymes can make changes to the mRNA nucleotide sequences i.e. by inserting, deleting or substituting specific nucleotides. This affects the amino acid and subsequent protein produced during translation. Interestingly, it is thought that RNA editing is an adaptive process, used to repair deleterious mutations to preserve proteins in organelles that are reliant on clonal reproduction – a process that is vulnerable to a high rate of mutation. This phenomenon is commonly seen in higher plants; however, it has remained unexplored in lower eukaryotes such as algae. This inspired Dr Cahoon and his colleagues to determine whether RNA editing occurs in two specific species of green algae – Chara vulgaris and Chlamydomonas reinhardtii. The team chose to perform the study on these freshwater algae because Chara is a ‘living fossil’ that may be similar to the evolutionary antecedent of all land plants and Chlamydomonas has been used as a model system for genetic analysis for nearly a century. The team performed an in-depth study of the transcriptome using molecular genetic tools such as ‘polymerase chain reaction’ (PCR) and Sanger Sequencing. PCR is used to amplify a single DNA segment into millions of copies and Sanger Sequencing is used to sequence these segments.

Overall, the results showed that RNA editing does not, in fact, occur in these two species of green algae.

POLYCYTIDYLATION IN MITOCHONDRIAL mRNA

However, the team did find evidence of long stretches of the nucleotide cytosine at the 3’ end of the RNAs. These were composed of up to 20 consecutive cytosines. This is a very unusual phenomenon whose discovery has only recently been reported. The team decided to confirm this observation by using circular ‘reverse transcription polymerase chain reaction’ (rtPCR) to analyse the transcriptome of Chlamydomonas reinhardtii. In PCR is a sensitive molecular genetic tool that is used to detect and quantify mRNA. The genome of the green algae is linear and compact, encoding only eight proteins – three transfer

Hypothetical model for Chlamydomonas mitochondrial mRNA processing proposed by Dr Cahoon and his team. Primary RNA transcripts (top) are transcribed from the genome. These are cleaved into individual mRNAs (middle) and poly-nucleotide additions are added to the 3’ (left) end of each mRNA to discourage enzymatic digestion (bottom).
Dr Cahoon and his team solved the 30-year old mystery of leaderless mRNA.

LEADERLESS mRNA
Next, Dr Cahoon and his colleagues focused their research efforts on determining the function of this unusual mRNA polycytidylation. A phenomenon found in Chlamydomonas mitochondria is leaderless mRNA. Typically, mRNAs are produced with extra nucleotides upstream of the start codon – this is the first codon of the mRNA. In eukaryotes, the start codon is typically AUG which is the code for the amino acid methionine. Those extra nucleotides, called a leader sequence, act as a signal for the ribosomes to find and translate the transcript into a protein. However, in 1988 Boer and Gray discovered that in C. reinhardtii mRNAs are cleaved upstream of their AUG start codons, creating leaderless mRNAs. This raises the question, what triggers translation in C. reinhardtii? This has remained a 30-year old mystery – until now.

RNA CIRCULARISATION
Using innovative experiments, Dr Cahoon and his team showed that the long cytosine stretches aids circularisation of the linear transcript, placing the 3’ untranslated region upstream of the 5’ start codon, creating a leader sequence. Polyacrylamide gel experiments were performed, and the results indicated that the circular mRNAs are ribosome-associated. This suggests that they are translated, supporting the evidence that circularisation aids translation initiation. Furthermore, the team sequenced the junction where the 3’ end joins the 5’ AUG. The results revealed that intra-molecular ligation occurs, enabling circularisation. Not only does circularisation negate the need for a different translation initiation mechanism, but it also has a protective role against exonucleases and can also increase translational productivity.

FUTURE RESEARCH
Overall, Dr Cahoon and his team solved the 30-year old mystery of leaderless mRNA. This ground-breaking research showed that polycytidylation of mitochondrial mRNA aids RNA circularisation – a novel translation initiation mechanism. The genetic architecture of green algae has been extensively exploited and characterised for nearly a century, so it is often difficult to discover new results in this field. However, Dr Cahoon’s discovery shows that there are always mysteries to be solved to continually enhance our knowledge. His team is continuing to explore RNA processing phenomena among other algae.