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Abstracts

S12 Mitochondrial Genome Expression

Lectures

12L.1 Knowing when to stop — human mitochondrial translation termination

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Sequencing of the human mitochondrial DNA (mtDNA) over 20 years ago revealed a number of unusual features including a modified codon usage. Deviations from the standard genetic code include the recoding of UGA to tryptophan and strikingly the apparent recoding of 2 arginine triplets to termination signals. This use of both AGA and AGG occurs rarely in other mammals and the precise mechanism that has driven the change from encoding arginine to dictating a translational stop has posed a challenging conundrum. Most other mitochondrial systems have reduced the number of stop signals from three to two retaining only UAA and UAG, so why did human organelles choose a more complicated route and alter the use of AGA and AGG? Furthermore this recoding would require a concomitant change in the domains of mitochondrial release factor(s) that would ensure sequence specific recognition of these non-cognate stop codons. Closer inspection of the human mt-genome sequence shows that the 2 open reading frames that employ AGA/AGG codons, MTCOI and MTND6 respectively are both immediately downstream of a U residue. Thus, by a single nucleotide shift each could use the conventional UAA or UAG stop codon and follow the same route as many other mitochondrial genomes. Rearrangement of mRNA within ribosomes to alter the linear readout of the message has been well characterised, with 1 frameshifting used as a viral strategy to decode overlapping reading frames (ORFs). Similarities between viral- and human mt-DNA exist that include overlapping ORFs and multiple repeat sequences and interestingly it would require a 1 frameshift to reposition the mtDNA AGA/AGG codons to UAG. Commonly conserved features of classical programmed ribosomal 1 frameshifting (PRF) include a stable downstream secondary structure, a hungry codon, each of which can contribute to stalling the ribosome and heptanucleotide 'slippery' sequence. To identify if some form of 1 frameshift takes place, we had to define the precise codon present in the mitoribosomal A-site at translation termination. We targeted the endoribonuclease RelE, to mitochondria, and confirmed that our mtRelE retained specificities for sequence and mitoribosomal A-site cleavage between residues 2 and 3 of the A-site codon. By the use of our engineered mtRelE we show that PRF does indeed occur negating the apparent reassignment of AGA/AGG as stop codons.

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12L.2 Yeast models of ATPase-based diseases

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Human neurological disorders (NARP, Leigh, BCSL) have been associated with primary deficiencies in the mitochondrial ATP synthase caused by specific mutations in the mitochondrially-encoded subunit a (referred to as Atp6p in yeast). We have introduced five of these pathogenic mutations (T8993G, T8993C, T9176G, T9176C, and T8851C) in the yeast mitochondrial ATP6 gene in order to better understand how they affect the ATP synthase. They all resulted in a decrease in the rate of mitochondrial ATP synthesis, from 30% to 95%. Two mutations (T8993G and T8851C) had little effect on the assembly/stability of the ATP synthase while the three others (T9176G, T8993C, and T9176C) compromised more or less severely the incorporation or stability of Atp6p within the complex. Notwithstanding the caveats in the extrapolation of data from yeast to humans, we argue that the five ATP6 pathogenic mutations created to model the human syndromes of NARP/MILS/BCSL, have similar impacts on the ATP synthase of yeast and human origins. Due to the lack of relevant animal models, development of effective treatment for human mitochondrial diseases, in particular those resulting from defects in the ATP synthase, are very limited. We thus exploited our yeast disease models for the search of rescuing mechanisms through the isolation of genetic and pharmacological suppressors. Several drugs that proved to be active both in yeast and human cells deficient in ATP synthase have been found [1].

Reference

[1] Couplan E, Ayar R, Kucharczyk R, Ezkurdia N, Salin B, Gagneur J, St. Onge RP, Steinmetz L, di Rago J-P, Blondel M, *in preparation*.

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