Non-coding RNAs in Organelles

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Introduction

Mitochondria and chloroplasts are sub-cellular organelles that exhibit both prokaryotic and eukaryotic features of gene expression. Although they carry their own genetic material and transcription machineries, they are highly dependent on the proteins translated on cytoplasmic ribosomes and post-translationally imported into the organelle [1]. Over the course of evolution, a large chunk of genetic material from the organelles has got translocated to the nucleus, also known as "endosymbiotic gene transfer". This has resulted in their reduced genetic complexity in terms of genomic size and the number of genes harboured by their circular ds genome [2]. Though they have a relatively simple genome organization, their transcription machinery is surprisingly very complex and the gene expression is highly regulated both at the transcriptional and post-transcriptional level [3]. Various post-transcriptional modifications include RNA processing, generation of 5' and 3' termini, RNA editing, cis-trans splicing and RNA turnover [4].

To add another level of complexity in regulation of gene expression in the organelles, many small non-coding RNAs (sncRNA) have been detected in mitochondria and chloroplasts with some of them being imported from the cytosol and some being transcribed within these organelles [5]. ncRNAs are now considered as one of the biggest regulators of gene expression in the cytoplasm, controlling a wide variety of biological functions. Traditionally, non-coding functional RNAs comprise of ribosomal RNA (rRNA) and transfer RNA (tRNA) but this category now has new members in the form of microRNAs (miRNAs), small interfering RNAs (siRNAs), long non-coding RNAs (lncRNAs) and antisense RNAs (asRNA) [6]. Recent boom in the field of bioinformatics, next generation sequencing, specially strand-specific RNA sequencing has revealed an unexpectedly large number of ncRNAs in Arabidopsis and barley chloroplasts [18,19]. Many of these ncRNAs are transcribed antisense to the protein-coding genes. Such antisense transcripts bind near the 3' end of the mRNA and stabilize the target transcripts by protecting the 3' ends from 3' 5' exoribonucleases [19].

ncRNA's in Plastids

Relaxed plastid transcription and translation results in many extended transcripts getting transcribed from one promoter and many a times both strands can get transcribed. After their downstream processing, a pool of metastable RNA species is formed including a distinct class of plastid encoded ncRNA which may have yet unexplored role in plastid gene regulation [8]. Over the past two years, strand-specific RNA sequencing has revealed an unexpectedly large number of ncRNAs in Arabidopsis and barley chloroplasts [18,19]. Many of these ncRNAs are transcribed antisense to the protein-coding genes. Such antisense transcripts bind near the 3' end of the mRNA and stabilize the target transcripts by protecting the 3' ends from 3' 5' exoribonucleases [19].

Experimental RNomics, wherein specialized cDNA libraries are made exclusively for detecting potential novel ncRNA species, has identified a number of novel ncRNA candidates in chloroplasts [5]. As in case of mitochondria, ncRNAs in chloroplasts are of two types—one category belongs to the nucleus-derived ncRNAs and another is of plastid encoded novel candidates which map to the intergenic regions in chloroplast genome. The striking features of almost all the ncRNAs detected in this study have been the stable stem-loop structure that they form and that they all are less than 50 bp in size. In fact, many of these candidates have a size range of 18-25 bp, same as the miRNAs present in the nucleocytosolic compartment of plant cells [5,8]. However, some recent findings suggest that these ncRNAs could just be the binding sites of Pentatricopeptide Repeat proteins (PPR) which bind near the 5' end of chloroplast transcripts to protect them against exonucleases. The sequences of these sncRNA are conserved across Arabidopsis species and bear similarities with the binding sequences of PPR proteins. The conservation at the sequence level reflect the conservation amongst PPR proteins across in land plants suggesting that they could just be the footprints of RNA binding proteins, thus intensifying the debate on how important these sncRNAs really are in regulating gene expression in chloroplasts [20].

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Conclusion and Future prospects

Exploring the possibility of ncRNAs as the regulators of gene expression in the organelles is an exciting venture. Unlike in mitochondria, where the presence and active role of miRNAs in regulating gene expression have been experimentally proven, the future of ncRNAs in chloroplasts still look a little uncertain. Although there is a huge amount of data being generated every passing day, the biggest challenge is to functionally validate these findings. This task would include expression check by northern blotting, determining transcript-abundance by real time RT-PCR, exploring sequence conservation across different species, and most importantly, creating stable mutated lines to prove the exclusivity of their proposed function [8]. One major difficulty one faces while studying organellar ncRNAs and their functional relevance is their low abundance, contaminating cytosolic/nuclear ncRNAs and the fact that they may be expressed only at particular developmental stages which may lead us to miss their existence in the organelles.

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