The plant nucleolus: functions in mRNA export or NMD?

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Introduction

- The nucleolus is a multi-functional nuclear compartment involved in ribosomal RNA transcription/processing and ribosomal subunit assembly as well as many other aspects of RNA metabolism and cellular functions such as cell division, aging and stress sensing.
- Proteomic and GFP fusion protein localisation shows that plant EJC proteins are associated with the nucleolus suggesting that mRNAs will also be present in the nucleolus, perhaps as part of the mRNA export or nonsense-mediated decay (NMD) pathways.
- To address this we have isolated cDNAs from whole cells and isolated nuclei and nucleoli; examined the distribution of transcript types in the nucleolus and nucleoplasm; examined the behaviour of transcripts in mutants of the NMD proteins UPF1 and UPF3; and determined the localisation of Arabidopsis UPF proteins.

Nucleolar cDNAs are enriched in aberrantly spliced transcripts

Figure 1 - 500 full-length cDNA clones were fully sequenced from cDNA libraries constructed from poly A+ RNA of Arabodopsis cell cultures and from isolated nuclei and nucleoli. Transcripts were classified as fully spliced mRNAs, transcripts from single exon (non-spliced) genes and ìaberrantlyî spliced transcripts. Aberrant mRNAs were enriched in the nucleolar library compared to the whole cell and nuclear libraries. The ìaberrantlyî spliced transcripts fell into different classes: intron unspliced, exon skip, cryptic exon or intron removed, and cryptic 5í and 3í splice site selection. The majority contained premature termination codons (PTC).

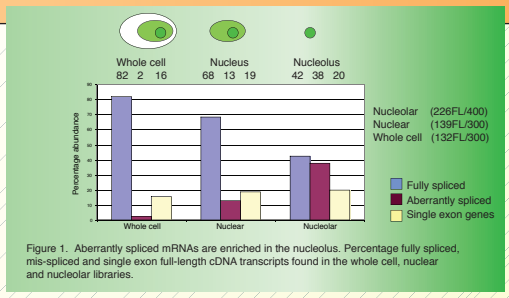

Figure 1. Aberrantly spliced mRNAs are enriched in the nucleolus. Percentage fully spliced, mis-spliced and single exon full-length cDNA transcripts found in the whole cell, nuclear and nucleolar libraries.

Figure 2 - Nuclei were fractionated into nucleolar and nucleoplasmic fractions. Semi-quantitative RT-PCR (RT with oligo dT; PCR with gene-specific primers; in linear amplification range - 24 cycles) on the same amount of RNA showed more fully spliced product in the nucleoplasmic fraction and more higher molecular weight products (îaberrantî transcripts) in the nucleolar fraction.

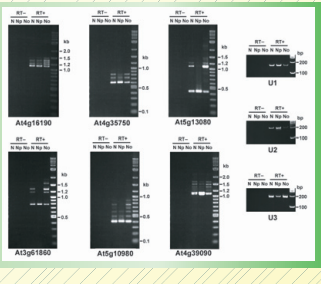

Figure 2. RT-PCR of transcripts of different genes from total RNA from nuclei (N), nucleoplasm (Np) and nucleoli (No). The smaller bands represent fully spliced products. HMW bands are observed mainly in the nucleolar fraction. RT-PCR of U1 and U2snRNAs and U3snoRNAs show enrichment of fractions.

Many aberrant transcripts are nonsense-mediated decay substrates

Figure 3 - Using the current rules for nonsense-mediated decay in animals and plants, around 90% of the aberrantly spliced transcripts were potential NMD substrates. To examine this, semi-quantitative RT-PCR was carried out on total RNA from seedlings of wild type plants and from mutants in NMD proteins: *upf1-5* and *upf3-1*. Transcripts which are turned over by NMD should increase in intensity in the mutants which are defective in NMD (see arrowed bands). Some transcripts did not alter in intensity in the mutants despite containing PTCs suggesting that they are not substrates for NMD.

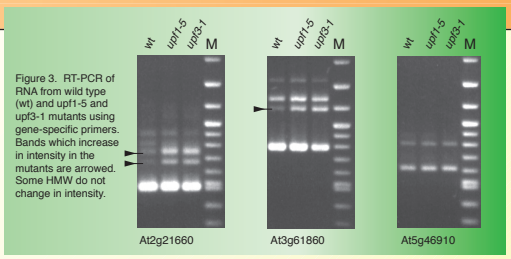

Figure 3. RT-PCR of RNA from wild type (wt) and upf1-5 and upf3-1 mutants using gene-specific primers. Bands which increase in intensity in the mutants are arrowed. Some HMW do not change in intensity.

UPF3 and UPF2 localise to the nucleolus

Figure 4 - GFP fusion proteins of full-length cDNAs of UPF1, UPF2 and UPF3 were expressed in Arabidopsis cell cutures following Agrobacterium-mediated transfection. UPF3 was predominantly localised to the nucleolus; UPF2 localised to the nucleolus and cytoplasm; UPF1 localised to the cytoplasm. Fibrillarin-mRFP (red) fusion protein identified the nucleolus.

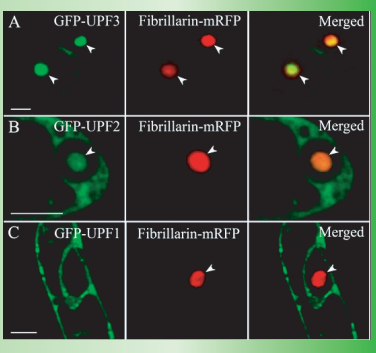

Figure 4. Co-localistion of UPF-GFP and fibrillarin-mRFP fusion proteins. Nucleoli are indicated by arrowheads.

Summary:

- plant EJC components are associated with the nucleolus;
- the nucleolus contains mRNAs;
- the nucleolus is enriched with partially, alternatively or mis-spliced transcripts;
- the majority of aberrant transcripts contain PTCs;
- some aberrant transcripts are NMD substrates, and
- UPF2 and UPF3 localise to the nucleolus.

Taken together, the nucleolus may have a role in nonsense-mediated decay of mRNAs. One model is that mRNA surveillance occurs in the nucleoplasm and aberrant transcripts are targetted to the nucleolus where assembly of UPF2 and UPF3 occurs prior to export and formation of the degradation complex with UPF1 in the cytoplasm.

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