The plant nucleolus functions in mRNA export and NMD

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Introduction

- The nucleolus is a multi-functional nuclear compartment involved in ribosomal RNA gene transcription/processing and ribosomal subunit assembly as well as many other aspects of RNA metabolism and cellular functions such as cell division, aging or sensing stress.
- Proteomic and cell biological analysis shows that plant EJC proteins are associated with the nucleolus (Pendle et al 2005, Mol. Biol. Cell 16, 260-269) 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 and the localization of Arabidopsis UPF proteins.

Results - Nucleolar cDNAs are enriched in mis-spliced transcripts

Figure 1: 500 full-length cDNA clones were obtained and fully sequenced from three cDNA libraries constructed from total RNA of Arabidopsis cell cultures and from isolated nuclei and nucleoli. Transcripts were classified as fully spliced mRNAs, transcripts from single exon (non-spliced) genes and "aberrantly" spliced transcripts. Aberrant mRNAs were enriched in the nucleolar library compared to the whole cell and nuclear libraries. The "aberrantly" spliced transcripts fell into different classes: intron unspliced, cryptic exon or intron removed, and cryptic 5' or 3' splice site selection. The majority of "aberrantly" transcripts were potential NMD substrates.

Figure 2: Nuclei were fractionated into nucleolar and nucleoplasmic fractions. Approximately 4 times more total RNA was isolated from the nucleolar fraction compared to the nucleoplasmic fraction. RT-PCR (RT with oligo dT; PCR with gene-specific primers; 24 cycles) on the same amount of RNA showed more fully spliced product in the nucleoplasmic fraction and more higher molecular weight products ("aberrant" transcripts) in the nucleolar fraction. Accounting for the different amounts of RNA isolated from nucleoli and nucleoplasm, fully spliced mRNAs are represented at approximately similar levels in the nucleolus and nucleoplasm but aberrant mRNAs are enriched in the nucleolus.

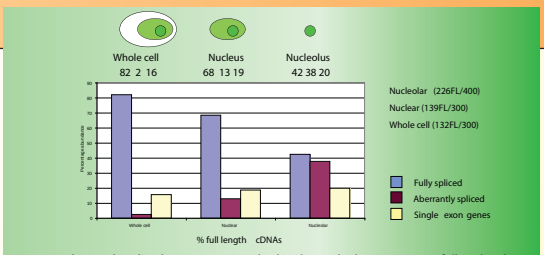


Figure 1. Aberrantly spliced mRNAs are enriched in the nucleolus. Percentage fully spliced, mis-spliced and single exon full-length cDNA transcripts found in the whole cell, nuclear and nucleolar libraries.

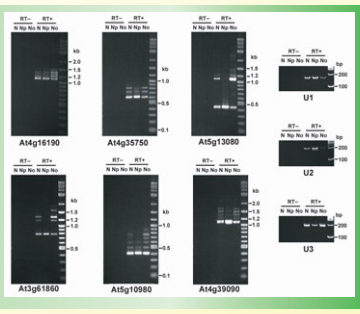


Figure 2. RT-PCR of transcripts of different genes from total RNA from nuclei (N), nucleoplasm (Np) and nucleoli (No). The smaller bands represent fully spliced products. HMW products are observed mainly in the nucleolar fraction. RT-PCR with U1 and U2snRNAs and U3snoRNAs show enrichment of the fractions.

Results - Localisation of UPF-GFP fusion proteins

Figure 3: GPF fusion proteins of full-length cDNAs of UPF1, UPF2 and UPF3 were expressed in Arabidopsis cell cultures following Agrobacterium-mediated transfection. UPF3 was predominantly localised to the nucleolus; UPF2 localised to the nucleolus and cytoplasm; UPF1 localised to the cytoplasm . As a control, a fibrillarin-mRFP fusion protein labels the nucleolus.

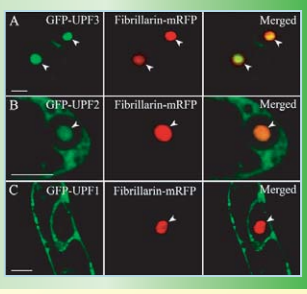
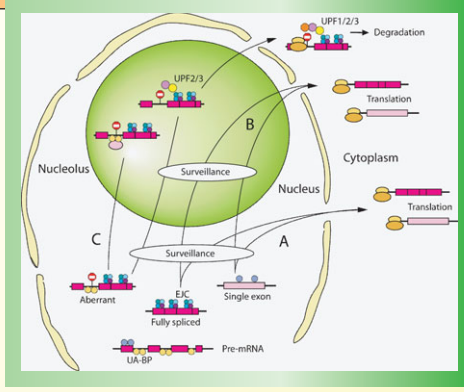


Figure 3. Localisation of UPF-GFP fusion proteins

Conclusions - Models of nucleolar function in export and surveillance/NMD

The association of EJC components with the nucleolus and the presence of mRNAs in the nucleolus suggest that the nucleolus is involved in mRNA biogenesis. Fully spliced transcripts and single exon gene transcripts are expected to be exported from the nucleoplasm (A) but the levels of fully spliced mRNAs in the nucleolus suggest free diffusion between the nucleolus and nucleoplasm which may reflect a nucleolar export pathway (B) (suggested in yeast and in the export of some human viruses). Partially, alternatively or mis-spliced transcripts potentially contain EJCs and UA-binding proteins associated with remaining intronic sequences and the majority contain premature termination codons (stop sign). The accumulation of aberrant mRNAs/potential NMD substrates in the nucleolus suggests that mRNA surveillance of all transcripts occurs in the nucleoplasm or even in the nucleolus itself. Alternatively, the accumulation of aberrant transcripts may reflect the targetting or retention (at least temporarily) through an interaction with UA-binding proteins (C). In the nucleolus, assembly of UPF3 and UPF2 on PTC+ transcripts could occur prior to export and formation of the degradation complex with UPF1 in the cytoplasm.



Acknowledgements - Research supported by the Scottish Executive Environment and Rural Affairs Department (SEERAD) and the Biotechnology and Biological Sciences Research Council (BBSRC). UPF2 was obtained from Dr. Daniel Silhavy (Agricultural Biotechnology Centre, Gödöllö, Hungary.