

# Novel functions for the nucleolus from proteomic and RNomic analysis of *Arabidopsis* nucleoli

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## Introduction

- The nucleolus is the site of ribosomal RNA gene transcription and processing and ribosomal subunit assembly.
- The nucleolus is a multi-functional nuclear compartment involved in 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 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.

## Results - Nucleolar cDNAs are enriched in mis-spliced transcripts

Nearly 1000 cDNA clones were sequenced from three cDNA libraries 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 (Figure 1).

Initial RT-PCR experiments show that there are similar amounts of mRNA in the nucleus and nucleolus with fully spliced mRNAs being more abundant in the nucleus (Figure 2).

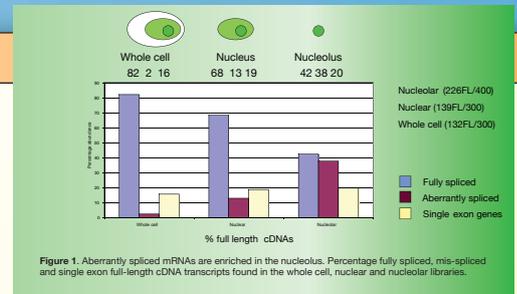


Figure 1. Aberrantly spliced mRNAs are enriched in the nucleolus. Percentage fully spliced, mis-spliced and single exon full-length cDNA transcripts found in the whole cell, nuclear and nucleolar libraries.

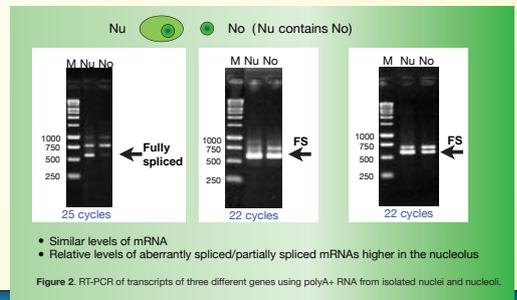


Figure 2. RT-PCR of transcripts of three different genes using polyA+ RNA from isolated nuclei and nucleoli.

## Results - The nature of mis-spliced transcripts

The “aberrantly” spliced transcripts were grouped into different classes: intron unspliced, cryptic exon or intron removed, and cryptic 5' or 3' splice site selection (examples in Figure 3).

Around 80% of the introns affected were unspliced.

Of the over 100 “aberrantly” transcripts, around two-thirds were classical NMD substrates.

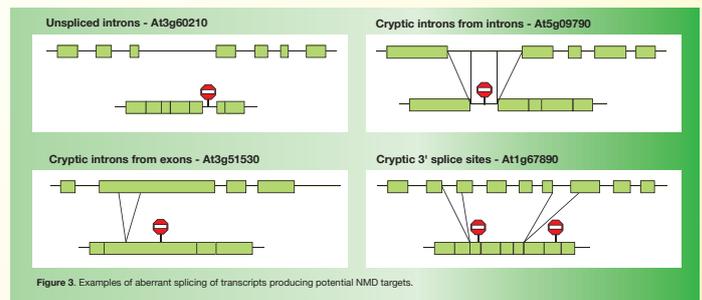


Figure 3. Examples of aberrant splicing of transcripts producing potential NMD targets.

## Conclusions

The presence of mRNAs in the nucleolus and the enrichment of aberrantly spliced mRNAs suggests that the plant nucleolus is involved in aspects of mRNA transport/export or in mRNA surveillance (Figure 4).

This is consistent with the localization of EJC components to the nucleolus (Pendle et al., Mol. Biol. Cell 16, 260-269 2005) and, in particular UPF3 (unpublished data).

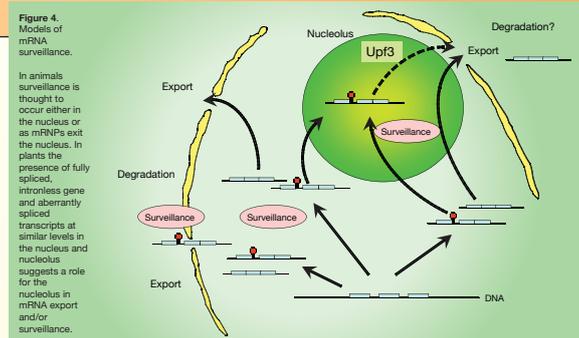


Figure 4. Models of mRNA surveillance.

In animals surveillance is thought to occur either in the nucleus or as mRNPs exit the nucleus. In plants the presence of fully spliced, intronless gene and aberrantly spliced transcripts at similar levels in the nucleus and nucleolus suggests a role for the nucleolus in mRNA export and/or surveillance.