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► To cite this version:

Eduardo Muñoz Díaz, Anne Debures, Alicia Camuel, Magali Rompais, Christine Carapito, et al.. Facts or fiction? Nucleolar sequestration of proteins in *Arabidopsis thaliana* under heat stress. OddPols 2023 - International Conference on Transcription mechanism and regulation by archeal RNA polymerases I, III, IV and V, May 2023, Jaén, Spain. . hal-04842133

HAL Id: hal-04842133

<https://hal.science/hal-04842133v1>

Submitted on 17 Dec 2024

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Facts or Fiction? Nucleolar Sequestration of Proteins in *Arabidopsis thaliana* under Heat Stress

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The nucleolus is the most prominent nuclear body found in eukaryotes, whose main function is the synthesis of ribosomes. However, a new nucleolar function was characterized in human cells. Upon abiotic stresses, i.e., acidosis or heat stress, certain proteins move from their usual subcellular localization to be retained in the nucleolus. This sequestration leads to the rearrangement of the nucleolar architecture, forming the detention center. Further analysis revealed a peptide sequence responsible of this translocation: the nucleolar detention signal (NoDS). Moreover, an association of the NoDS with lncRNAs transcribed from rDNA intergenic spacer (IGS) was observed. This novel nucleolar retention function is considered as a new post-translational mechanism happening under adverse cellular conditions (Audas et al., 2012). Our main goal is to determine whether this nucleolar protein sequestration mechanism is conserved in plants, specifically in *Arabidopsis thaliana*, under heat stress.

An initial bioinformatic analysis using a human NoDS aimed to find NoDS-containing proteins in the proteome of *Arabidopsis*. Among 123 candidates, the selected protein was Las1-like family protein (AtLAS1), based on its function and the sequence of its potential NoDS. More precisely, AtLAS1 participates in the processing of the ITS2 in the 45S rRNA. To test whether the potential NoDS from AtLAS1 was functional in *Arabidopsis*, it was fused to the green fluorescent protein (GFP) to create AtNoDS-GFP and GFP-AtNoDS. The subcellular localization was analyzed in transformed *Arabidopsis* plants exposed at 22°C and 37°C. Whereas fluorescence from AtNoDS-GFP and GFP-AtNoDS was distributed in the cytoplasm and nucleus at 22°C, the fluorescence concentrated in the “nucleolar space” at 37°C, also being present as cytosolic aggregates. This change in distribution is transient, since it is no longer observed after 4 hours at 37°C. On top of that, cytoplasmic and nuclear fluorescence is restored once plants are no longer exposed to the stressor.

On the other hand, the accumulation of IGS transcripts was characterized at 22°C and 37°C by RT-PCR and RT-qPCR. IGS transcripts were not detected at 22°C. In contrast, they strongly accumulated under heat stress. Additionally, RNA FISH experiments showed the formation of IGS transcripts foci in nucleus of *Arabidopsis* seedlings at 37°C, more precisely in the “nucleolar space”.

Taken altogether these results into account, it is suggested that the nucleolar sequestration of proteins may be conserved in *Arabidopsis*, since both NoDS fusion proteins and IGS transcripts are located in the “nucleolar space” upon heat stress. However, the presence of the detention center is not observed, as *Arabidopsis* nucleoli become morphologically and functionally disrupted at 37°C (Darriere et al., 2022).