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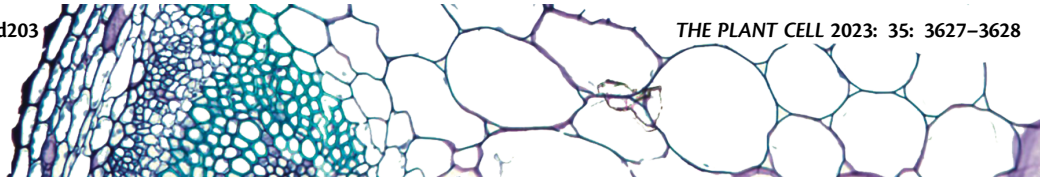

In Brief

From the archives: Nuclear import of pathogenic noncoding RNAs, ubiquitination and the control of heading date in rice, and the use of ribozymes to modulate gene expression

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October 2022: Nuclear import of pathogenic noncoding RNAs

RNAs are commonly considered to either remain inside the nucleus or be transported out of it after synthesis. However, functional RNAs often move from the cytoplasm to the nucleus. For example, viroids are single-stranded circular noncoding RNAs that can cause diseases by traveling into the nucleus. Although RNA export from the nucleus has been well studied, the RNA nuclear import machinery and the underlying molecular mechanism are not well understood. Ma et al. (2022) elucidated how potato spindle tuber viroid (PSTVd) RNA is recognized for nuclear import and which cellular proteins mediate this process. They identified a conserved RNA structure known as the C-loop in PSTVd, which serves as a signal for nuclear import. Disrupting the C-loop impaired viroid nuclear accumulation and pathogenicity. The authors demonstrated that the Arabidopsis viroid RNA-binding protein 1 (VIRP1) recognizes and binds to the C-loop. Importin is a well-known mediator of protein import into the nucleus. Interestingly, through RNA immunoprecipitation, the authors identified IMPORTIN ALPHA 4 (IMPa-4) in complex with PSTVd. Reducing the amount of IMPa-4 inhibited the nuclear localization of PSTVd and its infectivity. The nuclear accumulation of VIRP1 also depended on IMPa-4. Based on these key findings, the authors proposed a model in which IMPa-4 imports the VIRP1-PSTVd complex into the nucleus through the nuclear pore complex (see figure). Therefore, this study unraveled the molecular mechanism of RNA traffic from the cytoplasm to the nucleus and will inspire further investigations on motif-dependent RNA subcellular localization. The findings may also pave the way for future manipulation of RNA subcellular localizations for various applications.

October 2018: Ubiquitination and the control of heading date in rice

Flowering time, also known as heading date, is a crucial agronomic trait for rice adaptation in diverse regions and seasons (Molla 2022). Rice is a facultative short-day (SD) plant, meaning its flowering is induced by SD conditions and inhibited or delayed under long-day (LD) conditions. Ubiquitin-mediated protein degradation plays a significant role in regulating the rhythmicity of photoperiodic flowering. Heading Date Associated Factor 1 (HAF1), an E3 ubiquitin ligase, modulates the accumulation of Heading Date 1 (HD1) during flowering under SD conditions (Yang et al. 2015). On the other hand, EARLY FLOWERING3 (ELF3), a circadian clock gene, promotes flowering in rice under LD conditions. However, the mechanisms by which HAF1 controls flowering under LD conditions and the molecular mechanism of ELF3 accumulation are not yet understood.

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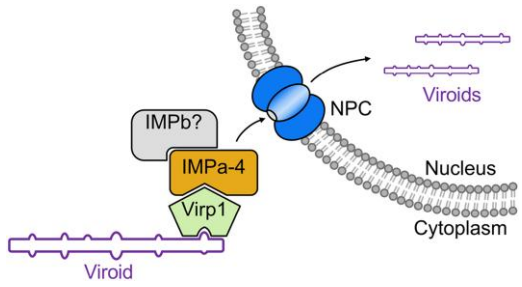

Figure. A proposed model of RNA nuclear import mediated by VIRP1/IMPa-4. IMPa-4 transports the RNP complex VIRP1-PSTVd into the nucleus. Figure adapted from Ma et al. (2022), Figure 5.

Zhu et al. (2018) revealed that HAF1 physically interacts with and controls the accumulation of ELF3 under LD conditions. The authors demonstrated that ELF3 directly interacts with HAF1 both in vivo and in vitro. HAF1 was found to mediate the ubiquitination and degradation of ELF3 through the 26S proteasome pathway. Genetic analysis conducted by the authors demonstrated that HAF1 is crucial for maintaining the diurnal accumulation of ELF3 during LD conditions. Since HAF1 does not affect the transcription of ELF3, it suggests that ELF3 function is primarily regulated at the protein level. Interestingly, the authors identified an amino acid variation (L558S) in the interacting domain of ELF3 with HAF1. This polymorphism strongly influences heading date differences in japonica germplasm. The OsELF3(L) allele was found to be prevalent in japonica germplasm from higher altitudes (≤35.7°N), whereas the OsELF3(S) allele was found in germplasm from latitudes below 35.7°N. These findings indicate that natural variation in OsELF3 may have contributed to regional adaptation and been selected during domestication. In summary, Zhu et al. (2018) provided valuable insights into how HAF1 modulates the circadian accumulation of OsELF3 through ubiquitin-mediated degradation to ensure accurate heading date under LD conditions.

October 1998: The use of ribozymes to modulate gene expression

Ribozymes are catalytic RNA molecules that can be designed to cleave specific target RNAs. Hammerhead is one of the most studied and smallest ribozymes. Due to its high target specificity and intolerance for 1–2 base mismatches, hammerhead ribozymes can be designed and employed to downregulate gene expression in eukaryotes. Merlo et al. (1998) demonstrated the use of hammerhead ribozymes in maize to downregulate a gene in the plant fatty acid biosynthetic pathway. The first step in C18-fatty acid desaturation is catalyzed by Δ9 desaturase, which converts stearic acid to oleic acid. Downregulating Δ9 desaturase would increase stearic acid content. Oil with high stearic content is desirable in the industry to produce margarine without additional hydrogenation. The authors optimized ribozyme constructs and targeted Δ9 desaturase in transgenic maize. Leaves of ribozyme transgenic plants exhibited increased accumulation of stearic acid, up to 4-fold compared with wild-type control leaves. The authors observed that the transcriptional context of the ribozyme had a strong impact on its cleavage efficiency. When the ribozyme gene was fused to the open reading frame of a selectable marker gene, it exhibited the highest activity. This fusion may enhance the protection of the ribozyme from nuclease attack, as translatable mRNAs are more stable in cells. Interestingly, the authors also showed the inheritance of the ribozyme-induced high stearate phenotype in subsequent generations of transgenic plants. Hence, this study demonstrated that ribozymes can be used to regulate the expression of endogenous genes in plants at the post-transcriptional level.

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