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IN BRIEF

Assessing the Uncapped Transcriptome

Control of gene expression involves a variety of transcriptional and posttranscriptional processes that regulate the level of translatable mRNAs in a cell. The abundance of a given mRNA depends on the relative rates of its synthesis and degradation. In eukaryotes, mRNA degradation can occur by several processes: removal of the 3′ poly(A)$^+$ tail by deadenylases followed by exosome-mediated degradation, binding of a small interfering RNA (siRNA) or a microRNA (miRNA) to a complementary internal site on the mRNA, which initiates endonucleolytic cleavage, and removal of the 7-methylguanosine cap at the 5′ end, rendering the mRNA vulnerable to degradation by XRN, a 5′ to 3′ exonuclease. The latter two of these decay pathways produce mRNA fragments with a free 5′ phosphate.

The presence of this free phosphate has recently been exploited by several groups to allow global sampling of partially degraded mRNAs in *Arabidopsis*. Following ligation of an adaptor to this 5′ phosphate and subsequent purification, reverse transcription, and PCR amplification, a collection of partially degraded mRNAs can be produced. Addo-Quaye et al. (2008) used this approach to isolate and identify specific targets of siRNA and miRNA-induced cleavage. Concurrently, German et al. (2008) combined this approach with high-throughput sequencing to isolate miRNA targets in the wild type and *xrn4* mutants. Similarly, Gregory et al. (2008) examined the roles of ABH1, a subunit of the cap binding complex, and XRN4 in miRNA-mediated RNA silencing.

Jiao et al. (pages 2571–2585) present a modification of this powerful method and combine it with microarray analysis to produce a global profile of the *Arabidopsis* transcriptome during early flower development. They detected uncapped mRNAs for >90% of expressed genes, showing some to be enriched and some depleted in the uncapped form (see figure). They demonstrated that the abundance of uncapped mRNAs is regulated, as evidenced by distinct uncapping profiles among different gene functional classes. For example, the authors detected a substantial enrichment in uncapped mRNAs among kinase gene transcripts. Also overrepresented in the uncapped form were, among others, mRNAs representing proteins targeted to the nucleus and plasma membrane, mRNAs representing developmental processes and signal transduction, and mRNAs known to be targets of miRNA and siRNA-induced silencing. By contrast, mRNAs for proteins involved in energy pathways or electron transport were deficient in uncapped mRNAs.

The authors also showed that the presence of short open reading frames, introns, or pseudoknots in the 5′ untranslated region were associated with higher levels of uncapping. By contrast, no 3′ untranslated region features correlated with uncapping levels. Interestingly, the total length of an mRNA was correlated with higher levels of uncapping, as was the presence of multiple introns. Finally, sequence analyses identified structural features of transcripts and *cis*-elements that were associated with different levels of uncapping. Most of these elements were identified from relatively uncapped transcript classes, suggesting that these *cis*-elements may be recruited to promote mRNA degradation.

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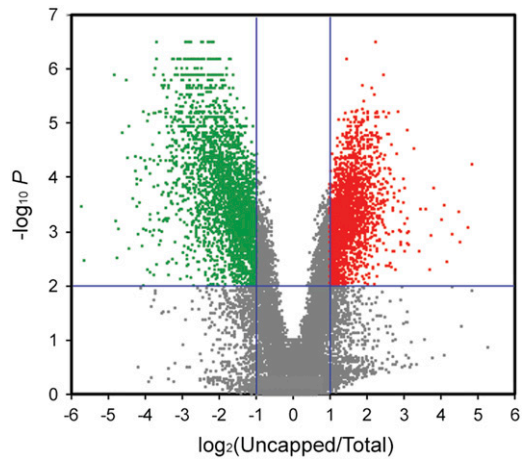

Volcano plot showing individual mRNAs that are relatively enriched (red) or depleted (green) in the uncapped form in developing *Arabidopsis* inflorescences.

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