Definition plant microRNA primary transcripts and their splicing patterns using RNAseq

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Motivation

The prediction of conserved mature microRNAs and their precursor hairpins has been addressed through several computational tools, while the detection of novel and lineage specific microRNAs is typically approached through deep sequencing of small RNA species. However, a meaningful understanding of both the regulation of miRNA transcription and the potential roles of alternative splicing in post-transcriptional regulation of microRNA biogenesis require accurate, high throughput methods to describe primary microRNA transcript structure.

Methods

Given that at least most primary miRNAs in plants are believed to be transcribed by RNA polymerase II, we reasoned that, despite the expected short physiological half life of such species, ultra high-throughput sequencing of cDNA should provide evidence of primary miRNA transcripts and splicing of these molecules. We tested this hypothesis using Illumina RNAseq data from the Grapevine Vitis vinifera. Reads were mapped to the genome sequence and “islands” of transcription including known miRNA precursors were analysed in detail. All possible canonical splice junctions within such islands were generated computationally and used as targets for mapping of RNAseq reads that did not map to the genome sequence (reads potentially covering splice junctions).

Results

We show that for many microRNA precursors, convincing estimates of primary transcript coordinates can be obtained from RNAseq data. Furthermore, estimates of splicing events obtained from our approach can often be validated experimentally. Our data suggest that splicing and alternative splcing of primary miRNAs may be widespread, at least in the grapevine, and that alternative splicing may represent a mechanism of post-transcriptional regulation of miRNA biogenesis.

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