In the Transcripts: Long-Read Transcriptomics Enables a Novel Type of Transposable Element Annotation in Plants

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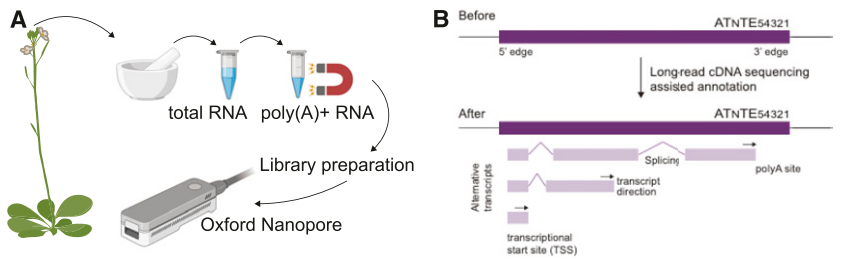
IN BRIEF

In the Transcripts: Long-Read Transcriptomics Enables a Novel Type of Transposable Element Annotation in Plants[OPEN]

Transposable elements (TEs) are mobile genetic elements and major constituents of eukaryotic chromosomes. TEs promote genetic and epigenetic variation within genomes and are a major source of evolutionary novelty and adaptation (Lisch, 2013). In plants, TEs represent from 20% of the genomic content in Arabidopsis (*Arabidopsis thaliana*) to 85% in maize (*Zea mays*). Structural characterization of TEs and identification of transcriptionally active copies are critical to understand how TEs alter gene expression, chromatin topology, and cellular and organismal growth, development, and response to the environment. Compared with genes, the repetitiveness and structural complexity of TEs have thus far restricted their annotation to minimal features and hampered their analysis. Notably, annotation of transcriptionally active elements and identification of transcript features, such as the transcription start site (TSS) and splicing patterns, are still lacking.

In this issue of *The Plant Cell*, **Panda and Slotkin (2020)** used Oxford Nanopore Technology long-read sequencing of cDNAs to establish a transcript-based annotation of TEs in Arabidopsis (see figure, A). To expose TE transcripts for sequencing and annotation, the authors used a combination of Arabidopsis mutants compromised in multiple layers of TE silencing. After generating over 5 million reads, the authors used the Oxford Nanopore Pinfish pipeline to annotate TE transcripts and identified a total of 2188 TE transcript models originating from 1292 individual TEs. The TE transcript models include newly identified TSSs, polyadenylation sites, transcription orientation, and splicing patterns (see figure, B). When overlaying these new TE transcript features onto the community-standard Arabidopsis TAIR10 TE annotation, the authors found that only 4% of previously annotated TEs can produce transcripts in the conditions tested, a large majority being *Gypsy*-like LTR retrotransposons and *Mutator* DNA transposons.



Long-Read cDNA Sequencing Allows a Genome-Wide Transcriptional Annotation of Transposons.

(A) Experimental procedure to capture TE transcripts by long-read cDNA sequencing. After isolation of total RNA, polyadenylated RNAs were purified and cDNA libraries prepared from the poly(A)$^+$ RNA. The resulting barcoded cDNAs were processed on a portable MinION sequencer.
(B) Long-read cDNA sequencing enables transcriptional annotation of TEs and reveals TE transcript features such as TSS, poly(A) site, transcript orientation, and splicing pattern.
(*Adapted from Panda and Slotkin [2020], Figures 1 and 2.*)

A major issue in performing genome-wide TE analyses is the ambiguous mapping of reads to individual TE copies belonging to the same family. This is mostly due to the short read size of second-generation sequencing technologies that cannot discriminate between identical or near-identical TEs and results in multimapping reads that limit the resolution of the analysis. Using the multicopy *Evadé* retrotransposon as an example, Panda and Slotkin (2020) demonstrated that short-read RNA sequencing data fail at accurately identifying transcriptionally active *Evadé* copies due to identical multimapping reads. The same analysis using the Oxford Nanopore Technology-generated long reads accurately pinpointed the *Evadé* copies producing transcripts. In addition, using the transcript-based TE annotation as a guide for distributing the multimapping short reads dramatically enhanced the mapping specificity, and reads were faithfully assigned to the corresponding transcribed *Evadé* copy. The authors further expanded their analysis to maize to validate the utility of such an approach in a larger and more complex plant genome. They took advantage of publicly available PacBio Iso-Seq data obtained from a diverse set of maize tissues, including endosperm and pollen that display developmental relaxation of TE silencing (Martínez and Slotkin, 2012; Wang et al., 2016). They detected over 1000 TE transcripts originating from 745 unique TEs, mostly shared between tissues but including a subset of pollen-specific TE transcripts. Similar to Arabidopsis *Evadé*, they identified element-specific transcripts from the high-copy (over 12,000) maize *Opie* transposons.

Because of their mutagenic potential, TEs are usually maintained in a repressed state by DNA methylation. In plants, TEs are decorated with peaks of CHH (in which H is any base other than G) methylation at their 5′ edges that insulates the TE from the surrounding genomic environment (Zemach et al., 2013). Panda and Slotkin (2020) applied their transcript-based annotation strategy to look at the prevalence of 5′ edge CHH peaks in transcriptionally potent transposons. They observed that TEs identified through transcript-based annotation exhibit higher CHH methylation at their 5′ edge than nontranscribed TEs, suggesting efficient

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targeting and silencing of these mutagenic elements by the genome. In addition to DNA methylation, TEs are silenced through the production of small interfering RNAs (siRNAs). The molecular mechanism by which plant TE transcripts are processed to generate primary siRNAs is still obscure. In other systems, TE transcripts display reduced splicing accuracy compared with genes, and this perturbed splicing is thought to fuel the production of primary siRNAs. The authors thus investigated the splicing features of transcribed TEs identified in their analysis. They found that transcribed TEs have generally larger exons, fewer introns, and reduced splicing accuracy compared with protein-coding genes. However, in Arabidopsis, splicing accuracy does not correlate with the propensity of a TE transcript to generate primary siRNAs.

This long-read transcript-based transposon annotation represents a valuable resource for the plant community and enables genome-wide analyses and family-specific studies on TEs that have so far been hampered by the use of short-read sequencing approaches. Refinements of long-read single-molecule sequencing technologies offer an unprecedented opportunity to study transposon activities and their molecular control at scale in notoriously complex plant genomes (Shahid and Slotkin, 2020).

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