Distinct characteristics and functions of plant circular RNAs

Emese Xochitl Szabo, Julian Fratte, Margaux Kaster and Sascha Laubinger
emese.xochitl.szabo@uni-oldenburg.de

Institut für Biologie und Umweltwissenschaften (IBU), Carl von Ossietzky Universität, Oldenburg, Germany
Chemical Genomics Centre (CGC), Max Planck Institute of Molecular Physiology, Dortmund, Germany
Max-Planck Institute for Developmental Biology, Tübingen, Germany

Introduction

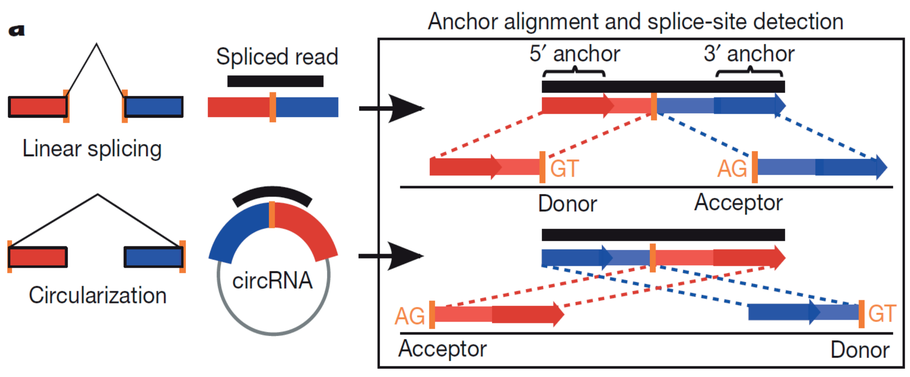


Figure 1: Non-canonical backsplicing [1].

Circular RNAs (circRNAs) are the products of back-splicing, which has long been considered as a splicing error. Canonical splicing occurs during pre-mRNA maturation and results in exon joining between adjacent 5' and 3' splice sites. Back-splicing occurs when downstream donor 3' splice sites are joined with upstream accepeptor 5' splice site, resulting in a circular RNA molecule (Figure 1). Recently, circRNA joined the group of regulatory non-coding RNAs (ncRNAs) and several function and roles have been discovered (Figure 2). For instance, circRNAs act as a decoy (sponge) for miRNAs to regulate the expression of the target mRNAs. circRNAs can function as backbones in ribonuclear particles (RNPs) to regulate transcription. Also, circRNAs associate with ribosome and can encode proteins.

Here we present an approach combining bioinformatic tools and molecular techniques to identify, characterize and reveal various functions of circRNAs in plants.

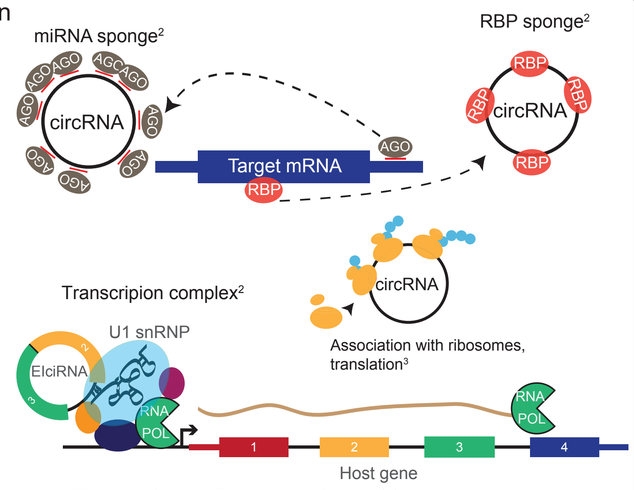
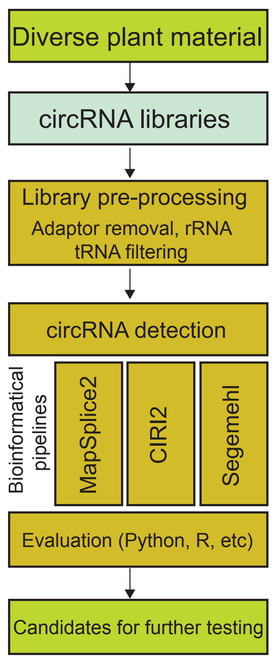


Figure 2: circRNA functions [2,3].

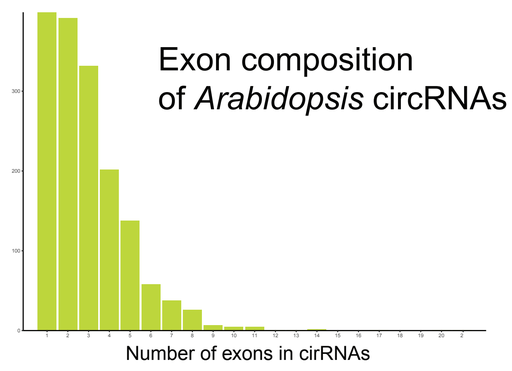
Overview of project workflow

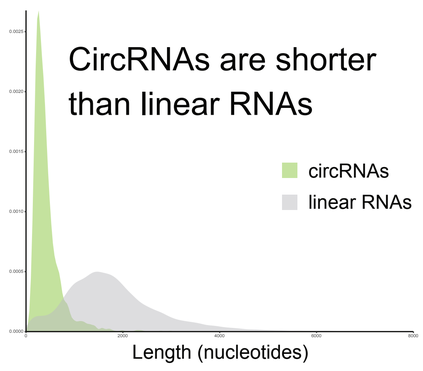
Key points:

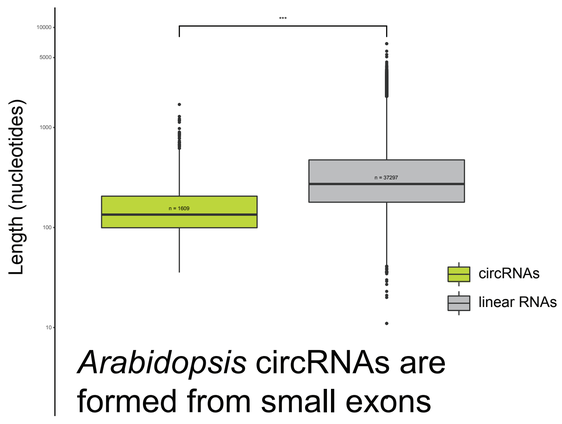
- *Arabidopsis thaliana* seedlings, grown under diverse conditions and treated with different hormones
- RNA isolation and ribosomal RNA depletion
- circRNA enrichment using RNAse R (which degrades linear RNAs)
- Prefiltering ribosomal and tRNA reads
- Detecting and evaluating candidates with independent angorithmic solutions[4,5,6]
- Selection of strong circRNA candidates
- Validation of selected candidates
- Characterization of selected circRNAs



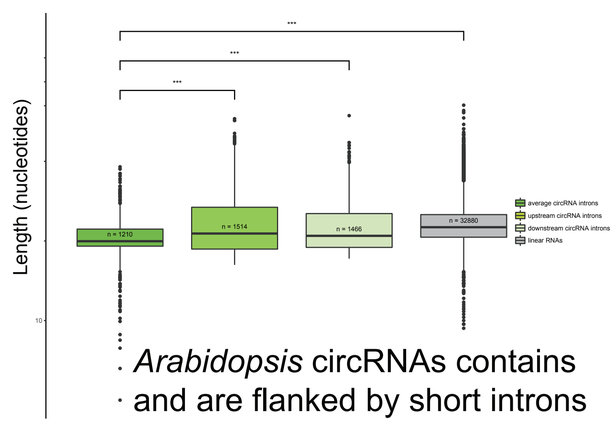
Characteristics of Arabidopsis circRNAs







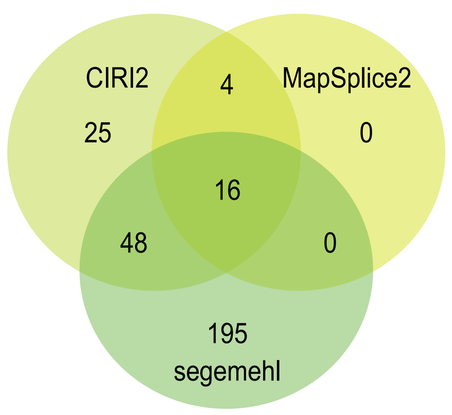
Arabidopsis circRNAs are formed from small exons



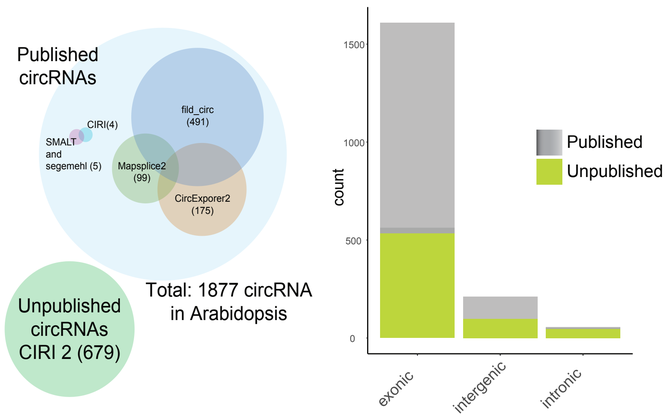
Arabidopsis circRNAs contains and are flanked by short introns

Evaluation of bioinformatic tools

We selected three circRNA detection tools and compared the results of our circRNA with recently published circRNAs from Arabidopsis.

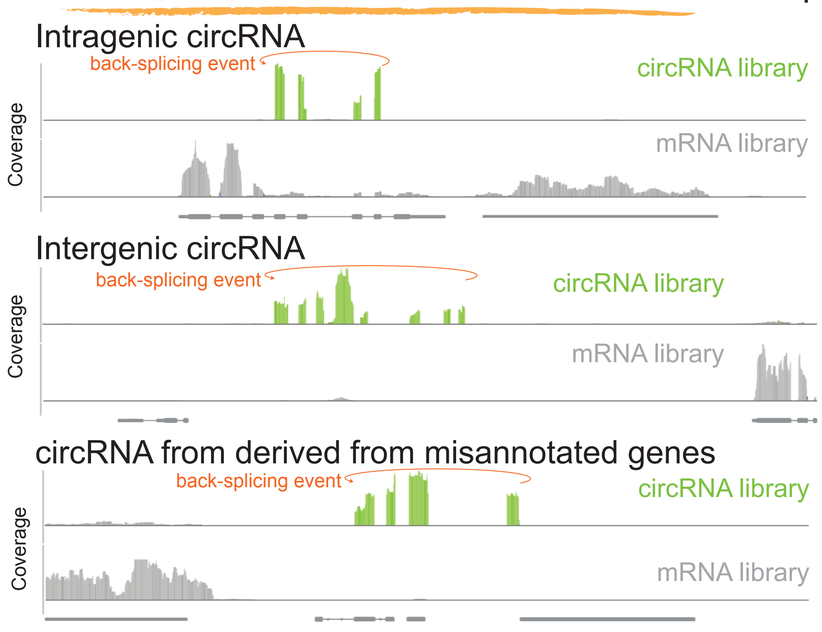


Performance test on a small data set

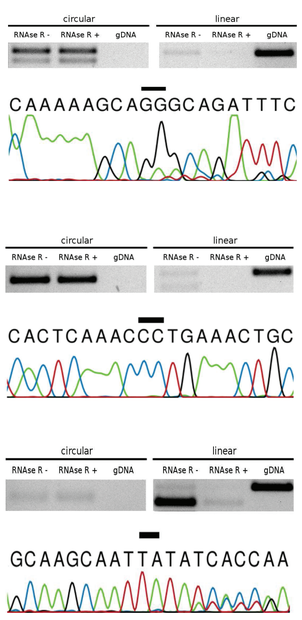


Comparison with published circRNAs. In total, we detected 679 new circRNAs, often from intronic regions

Validation of circRNAs



PCR validation of circRNAs



Outlook: Characterization of circRNAs

CircRNA expression analysis

- Expression of circRNAs and linear RNAs under different developmental and stress conditons

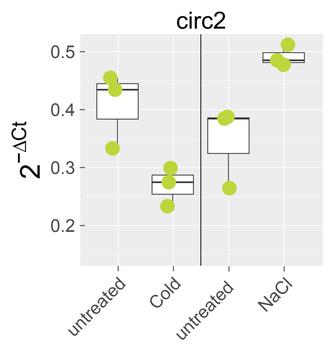
CircRNA mutants and knockdowns

- T-DNA insertion lines targeting circRNA loca - tions
- amiRNA constructions against the backspliced region
- overexpression lines using constitutive promoters

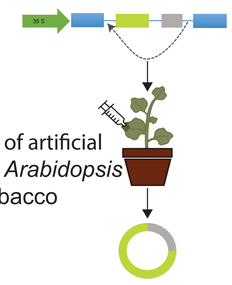
CircRNAs as a tool

- circRNAs are very stable[2]
- Overexpression of artificial circRNAs containing desired sequences
- Spray application[7] of circRNAs

Stress-specific expression



Overexpressionof artificial circRNAs



Spray application of circRNAs

Isolation of in vivo or in vitro generated circRNAs followed by spray application[7] onto plants

Ideas and Suggestions

References

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(2) Barrett and Salzman. 2016. Circular RNAs: analysis, expression and potential functions. Nature, 495(7441), 333-338.
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(5) Hoffmannet al. 2014. A multi-split mapping algorithm for circular RNA, splicing, trans-splicing, and fusion detection. Genome Biol 15.2 (2014): R34.
(6) Gao et al. 2015. CIRI: an efficient and unbiased algorithm for de novo circular RNA identification. Genome Biol 16.4.
(7) Tenllado and Llave. 2004. RNA interference as a new biotechnological tool for the control of virus diseases in plants."Virus Research 102.1.85-96.