Genome-wide view on RNA-protein interactions by iCLIP

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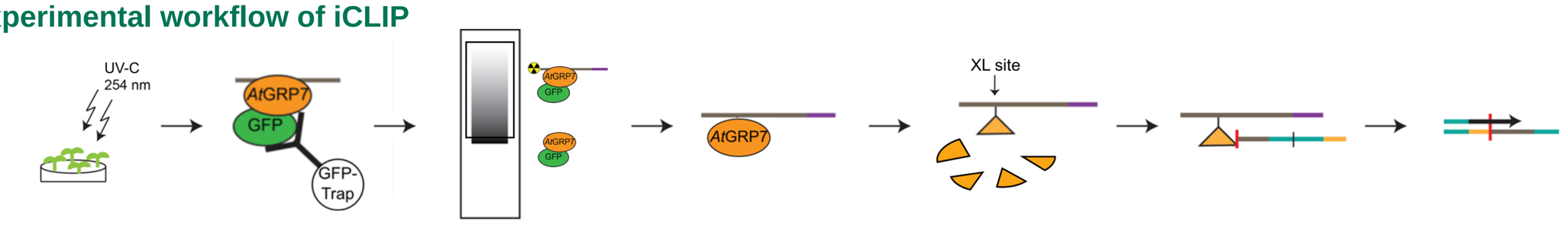
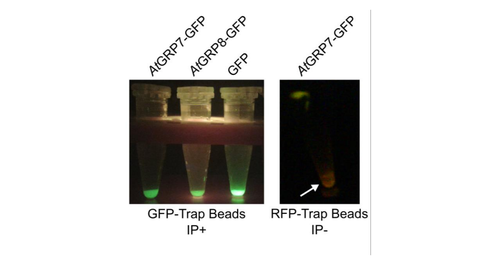
Abstract

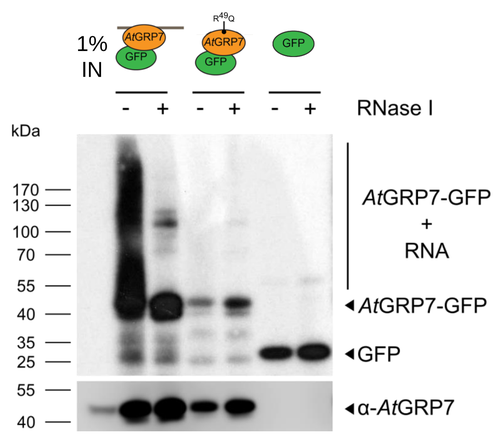
mRNAs associate with a multitude of RNA-binding proteins throughout their life cycle and these interactions play essential roles in the posttranscriptional regulation of the mRNAs. To characterize an RNA-binding protein, it is crucial to globally identify its mRNA targets. This knowledge is still limited in plants, mainly due to the lack of established *in vivo* techniques to analyze RNA-protein interactions, Therefore, we adapted the iCLIP (individual nucleotide resolution crosslinking and immunoprecipitation) procedure (König et *al*., 2010) for its use in plants, thereby identifying 858 mRNA targets of the RNA-binding protein *At*GRP7 (*Arabidopsis thaliana* glycine-rich RNA-binding protein 7) in *Arabidopsis thaliana*.

1. Experimental workflow of iCLIP

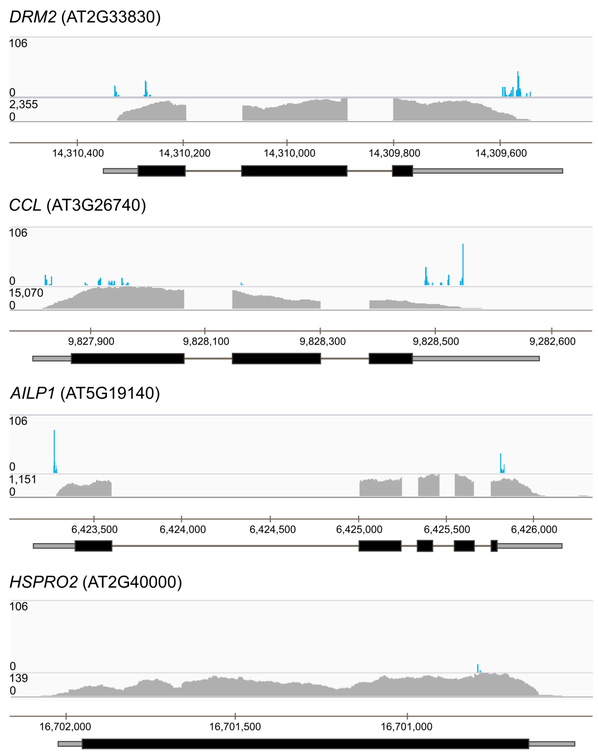


2. RNA-protein complexes are successfully precipitated

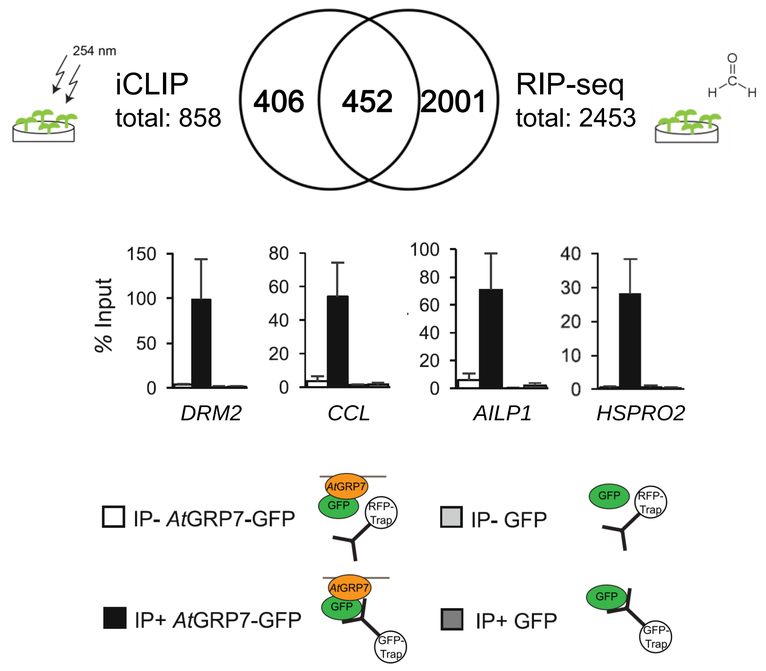




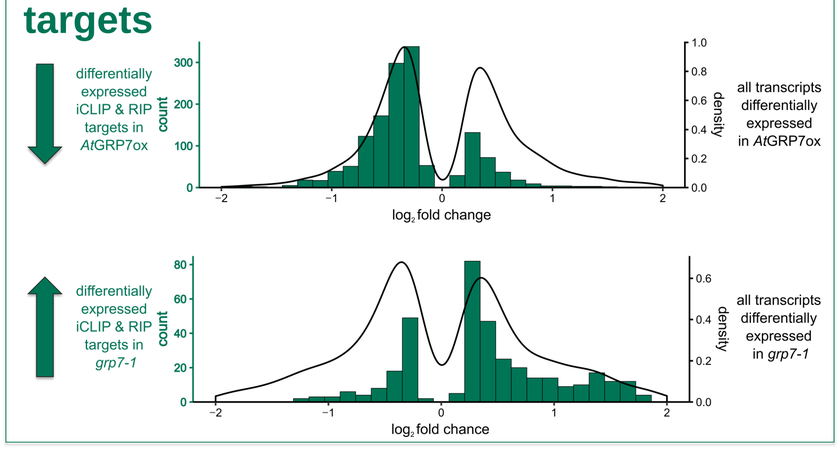
3. Identification of crosslink sites



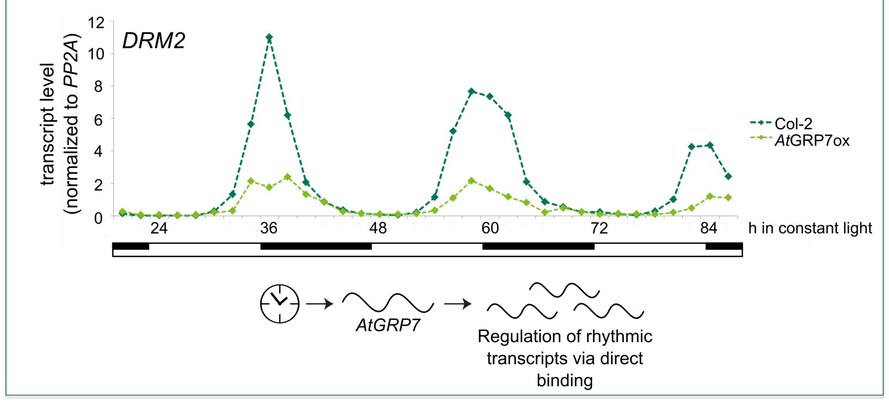
4. iCLIP results are independently validated by RIP-seq



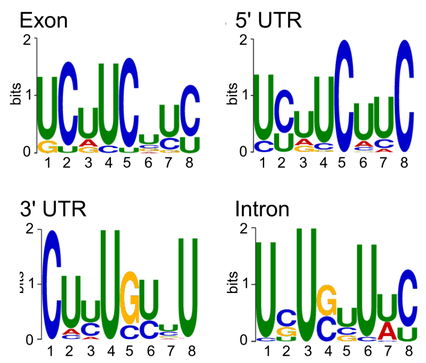
5. *At*GRP7 negatively regulates its direct targets



6. *At*GRP7 influences the circadian expression of its targets



7. High resolution allows binding motif determination



Conclusion

By establishing iCLIP in *Arabidopsis thaliana* for the RNA-binding protein *At*GRP7 in its *in vivo* context, we were able to identify crosslink sites in 858 mRNAs, of which 53% were also independently validated by RIP-seq. Furthermore, we observed a predominantly negative effect of *At*GRP7 on the targets. A subset of targets is also influenced in their circadian expression pattern. These results represent a first step in the global understanding of the posttranscriptional network controlled by the RNA-binding protein *At*GRP7. As the presented iCLIP protocol was optimized for precipitation of a GFP-tag, it grants the possibility to easily apply it to other fusion proteins to explore the target repertoire of more RNA-binding proteins. For instance, we applied iCLIP on the *At*GRP7 paralog *At*GRP8 and identified 476 targets common to both proteins.

Meyer K, Köster T, Nolte C, Weinholdt C, Lewinski M, Grosse I, Staiger D, 2017. Adaptation of iCLIP to plants determines the binding landscape of the clock-regulated RNA-binding protein *At*GRP7. *Genome Biology* 18(1), 204.
Köster T, Meyer K, 2018. Plant Ribonomics: Proteins in Search of RNA Partners. *Trends in Plant Science*, 10.1016/j.tplants.2018.01.004.
König J, Zarnack K, Rot G, Curk T, Kayikci M, Zupan B, Turner DJ, Luscombe N, Ule J, 2010. iCLIP reveals the function of hnRNP particles in splicing at individual nucleotide resolution. *Nature Structural & Molecular Biology* 17, 909-915.

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