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Osmotic stress-responsive promoter upstream transcripts (PROMPTs) act as carriers of MYB transcription factors to induce the expression of target genes in *Populus simonii*

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Summary

Complex RNA transcription and processing produces a diverse range catalog of long noncoding RNAs (lncRNAs), important biological regulators that have been implicated in osmotic stress responses in plants. Promoter upstream transcript (PROMPT) lncRNAs share some regulatory elements with the promoters of their neighbouring protein-coding genes. However, their function remains unknown. Here, using strand-specific RNA sequencing, we identified 209 differentially regulated osmotic-responsive PROMPTs in poplar (*Populus simonii*). PROMPTs are transcribed bidirectionally and are more stable than other lncRNAs. Co-expression analysis of PROMPTs and protein-coding genes divided the regulatory network into five independent subnetworks including 27 network modules. Significantly enriched PROMPTs in the network were selected to validate their regulatory roles. We used delaminated layered double hydroxide lactate nanosheets (LDH-lactate-NS) to transport synthetic nucleic acids into live tissues to mimic overexpression and interference of a specific PROMPT. The altered expression of *PROMPT_1281* induced the expression of its *cis* and *trans* targets, and this interaction was governed by its secondary structure rather than just its primary sequence. Based on this example, we proposed a model that a concentration gradient of *PROMPT_1281* is established, which increases the probability of its interaction with targets near its transcription site that shares common motifs. Our results firstly demonstrated that *PROMPT_1281* act as carriers of MYB transcription factors to induce the expression of target genes under osmotic stress. In sum, our study identified and validated a set of poplar PROMPTs that likely have regulatory functions in osmotic responses.

Introduction

Large-scale RNA sequencing analysis has indicated that more than 90% of eukaryotic genomes are actively transcribed to yield a highly complex network of protein-coding transcripts and non-coding RNAs (Djebali *et al.*, 2012; Hangauer *et al.*, 2013). Protein-coding genes make up only 1%–2% of all transcripts, indicating the widespread occurrence of noncoding RNAs in eukaryotic genomes (Hangauer *et al.*, 2013; Kim and Sung, 2012). Functional noncoding RNAs are divided into housekeeping and regulatory RNAs (Chen and Carmichael, 2010; Shuai *et al.*, 2014). Based on their extraordinary differences in transcript lengths and biogenesis, classification of regulatory noncoding RNAs remains difficult. Long noncoding RNAs (lncRNAs) are usually classified as RNAs greater than 200 nucleotides (nt) that lack significant protein-coding capacity (Ulitsky and Bartel, 2013). Depending on their orientation and/or proximity to protein-coding genes, ncRNAs are annotated as promoter upstream transcripts (PROMPTs), enhancer RNAs (eRNAs), long intervening/intergenic ncRNAs (lincRNAs) and natural antisense transcripts (NATs). Additionally, many lncRNAs are annotated as small nucleolar RNA-ended lncRNAs (sno-lncRNAs), 5′snoRNA-ended and 3′-polyadenylated lncRNAs (SPAs), circular RNAs (circRNAs) and circular intronic RNAs (ciRNAs) depending on their RNA processing pathways (Wu *et al.*, 2017).

LncRNAs are key regulators of gene expression at both the transcriptional and the post-transcriptional levels in diverse cellular contexts and biological processes (Chen, 2016; Quinn and Chang, 2016). LncRNAs can regulate gene expression in *cis*- or *trans*-acting. *Cis*-acting lncRNAs function near the site of their synthesis and act directly on one or several contiguous genes on the same strand or chromosome. Thus, we speculated that the orientation and/or proximity of lncRNAs to protein-coding genes might be the main factor for determining whether they act in *cis*. The eRNAs have enhancer-like functions and can control promoter and enhancer interactions (Li *et al.*, 2013; Melo *et al.*, 2013). COOLAIR, a NAT transcribed from the *FLOWERING LOCUS C* (*FLC*) gene, mediates the formation of a stable RNA–DNA triplex and an R-loop (Sun *et al.*, 2013a,b; Wahba and Koshland, 2013). The R-loop recruits a transcription repressor, which results in repression of *FLC*. By contrast, *trans*-acting lncRNAs diffuse from the site of their synthesis and can act directly on many genes at

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