PLANT SIGNALING & BEHAVIOR
2020, VOL. 15, NO. 11, e1812878 (4 pages)
https://doi.org/10.1080/15592324.2020.1812878

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SHORT COMMUNICATION

The function of the WRI1-TCP4 regulatory module in lipid biosynthesis

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ABSTRACT

The plant-specific TCP transcription factors play pivotal roles in various processes of plant growth and development. However, little is known regarding the functions of TCPs in plant oil biosynthesis. Our recent work showed that TCP4 mediates oil production via interaction with WRINKLED1 (WRI1), an essential transcription factor governing plant fatty acid biosynthesis. *Arabidopsis* WRI1 (AtWRI1) physically interacts with multiple TCPs, including TCP4, TCP10, and TCP24. Transient co-expression of *AtWRI1* with *TCP4*, but not *TCP10* or *TCP24*, represses oil accumulation in *Nicotiana benthamiana* leaves. Increased TCP4 in transgenic plants overexpressing a miR319-resistant *TCP4* (*rTCP4*) decreased the expression of AtWRI1 target genes. The *tcp4* knockout mutant, the *jaw-D* mutant with significant reduction of *TCP4* expression, and a *tcp2 tcp4 tcp10* triple mutant, display increased seed oil contents compared to the wild-type *Arabidopsis*. The APETALA2 (AP2) transcription factor WRI1 is characterized by regulating fatty acid biosynthesis through cross–family interactions with multiple transcriptional, post-transcriptional, and post-translational regulators. The interacting regulator modules control the range of AtWRI1 transcriptional activity, allowing spatiotemporal modulation of lipid production. Interaction of TCP4 with AtWRI1, which results in a reduction of AtWRI1 activity, represents a newly discovered mechanism that enables the fine-tuning of plant oil biosynthesis.

ARTICLE HISTORY
Received 29 July 2020
Revised 16 August 2020
Accepted 17 August 2020

KEYWORDS
Arabidopsis; WRI1; TCP4; protein–protein interaction; transcriptional activity; plant oil biosynthesis

Introduction

The plant-specific TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR (TCP) transcription factors play pivotal roles in various physiological processes, such as leaf development, cell cycle, plant hormone signaling, defense responses, and the circadian clock.[1–3] The 24 *Arabidopsis* TCPs are divided into class I and class II subfamilies.[2–5] The Class II *TCP2-4, TCP10*, and *TCP24* are post-transcriptionally controlled by microRNA319 (miR319), and the *jaw-D* mutant overproducing miR319 significantly reduces the expression of the five Class II *TCPs*.[5] Yeast-two-hybrid (Y2H) assays show that several miRNA319-regulated TCPs are ASYMMETRIC LEAVES 2 (AS2)-interacting proteins, which play roles in repressing class-I *KNOX* genes.[6] TCP3 interacts with R2R3-MYB transcription factors that regulate flavonoid biosynthesis.[7] A subset of TCPs are SUPPRESSOR OF rps4-RLD1 (SRFR1)-interacting transcriptional regulators, and the SRFR1-TCP interacting modules equilibrate plant development and immunity.[8] Despite numerous known roles of TCPs in plant physiological and biochemical processes, participation of TCPs in plant oil accumulation was not reported.

WRINKLED1 (WRI1; Figure 1(a)), a member of APETALA2 (AP2) transcription factor family,[9,10] plays an essential role in the transcriptional regulation of plant oil biosynthetic pathways.[11–13] *Arabidopsis WRI1 (AtWRI1)* loss-of-function mutant (*wri1-1*) displays an approximately 80% reduction in seed oil content compared to wild-type (WT).[14] Transcriptomic analysis of developing seeds revealed that a majority of the down-regulated genes in *wri1-1* encode fatty acid biosynthetic and glycolytic enzymes.[15] Numerous genes encoding enzymes in the late glycolysis and fatty acid biosynthesis are validated as AtWRI1 targets in subsequent studies.[16–18] The AW-box, $[CnTnG](n)_7[CG]$, in the gene promoters is characterized as the binding sequence of AtWRI1.[17] *WRI1* orthologs have been discovered in diverse monocot and dicot species, and have shown to be functional in mediating plant oil accumulation.[19–27] Ectopic expression of *AtWRI1* or *WRI1* orthologs leads to elevated oil accumulation in seeds and vegetative tissues of the transgenic plants.[9,13,20,23,24,28,29] Transient overexpression of *WRI1s* in tobacco leaves also stimulates oil accumulation.[27,30–33] Recent progress advanced our understanding of the structure/function of WRI1, particularly in the functional domains/motifs, protein structural features, and the interacting regulators (summarized in Figure 1). AtWRI1 interacts with CULLIN3-based E3 ligase adaptor BTB/POZMATH (BPM) proteins which mediate 26S proteasomal degradation of AtWRI1.[34] *In silico* analysis discovered that AtWRI1 protein contains three intrinsically disordered regions (IDRs).[30] Particularly, the IDR3 comprises a PEST motif that mediates AtWRI1 stability.[30] When the IDR3-PEST is removed or the putative phosphorylation residues in IDR3-PEST are mutated, the variants are more stable and capable of inducing a higher oil accumulation in plant cells compared to the native AtWRI1, suggesting a possible regulation of AtWRI1 at the IDR3-PEST motif by phosphorylation.[30]

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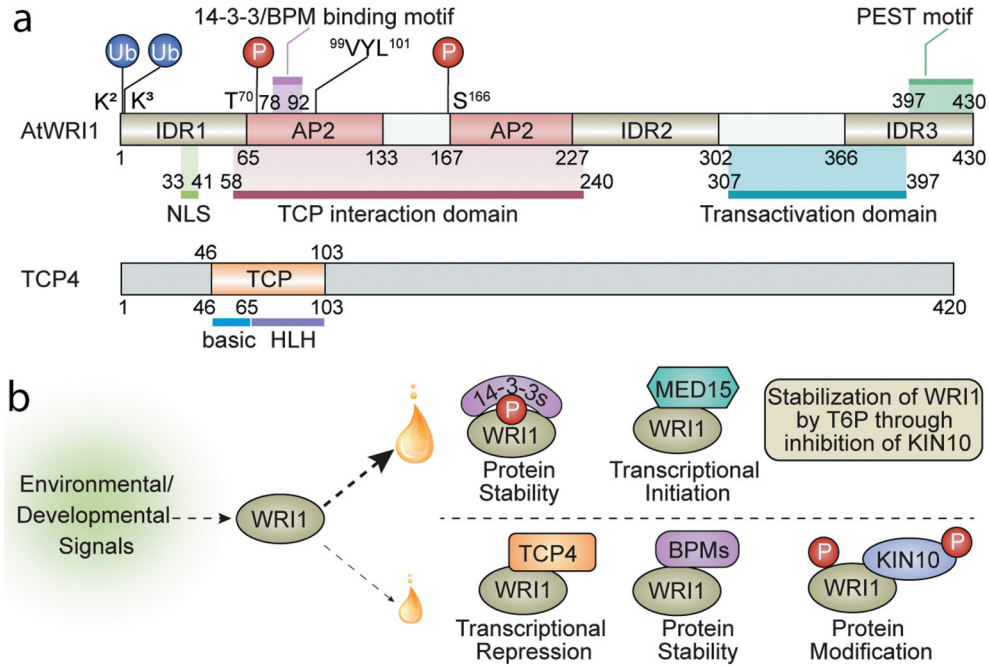

Figure 1. Structural features of WRI1 and TCP4 and molecular mechanisms of WRI1-regulated oil biosynthesis. (a) Schematic diagram of AtWRI1, including two AP2 domains, three intrinsically disordered regions (IDRs), a nuclear localization signal (NLS), a functional motif of "VYL", the 14-3-3 and E3 ligase adaptor (BPM) binding motifs, TCP4-interacting domain, the transactivation domain (TAD), the ubiquitination sites, and the KIN10 phosphorylation sites. The schematic diagram of TCP4 shows the basic region and the helix-loop-helix (HLH) region of the TCP domain. (b) The fine-tuning of lipid biosynthesis through modulating WRI1 activity leads to an increase (large oil drop) or a decrease (small oil drop) of oil accumulation. In response to environmental or developmental signals, various WRI1 modules are formed, which either positively or negatively regulate the transcriptional activity of WRI1. Interaction of WRI1 with 14-3-3s or MED15 leads to enhanced protein stability, transcriptional activity, or assembly of the transcriptional machinery, resulting in higher oil accumulation. T6P stabilizes WRI1 by inhibition of KIN10, which positively mediates oil biosynthesis. Modules formed between WRI1 and TCP4, BPMs, or KIN10 reduce transcriptional activity or protein stability, resulting in lower oil accumulation. 14-3-3s, 14-3-3 proteins; MED15, Mediator subunit 15; T6P, trehalose 6-phosphate; BPMs, CULLIN3-based E3 ligase adaptor BTB/POZMATH (BPM) proteins.

AtWRI1 also interacts with 14-3-3 proteins in yeast and plant cells.[35] Co-expression of *14-3-3* with *AtWRI1* enhances AtWRI1-mediated oil production, likely by increasing the stability and transcriptional activity of AtWRI1.[35,36] AtWRI1 is a substrate of the SNF1-related protein kinase KIN10, which catalyzes AtWRI1 phosphorylation that mediates AtWRI1 degradation.[33] Phosphorylation deficient mutations at T70 and S166 positions abolish KIN10-triggered phosphorylation and enhance the stability of AtWRI1.[33] Further study found that trehalose 6-phosphate (T6P) plays a role in stabilizing AtWRI1 protein and enhancing fatty acid production through repression of KIN10.[37] Recruitment of the mediator subunits by transcriptional regulators to initiate transcription is a conserved mechanism in eukaryotic cells.[38] *Arabidopsis* mediator subunit MED15 physically interacts with AtWRI1, and the transgenic *Arabidopsis* plants overexpressing *MED15* shows elevated expression of AtWRI1 targets.[39]

The involvement of TCP4 in WRI1-mediated plant oil accumulation

Our recent study uncovered a previously unknown relationship between WRI1 and TCP4 that affects the plant oil biosynthesis.[40] We screened an *Arabidopsis* transcription factor library and identified several TCP transcription factors (including TCP4, TCP10, and TCP24) as new AtWRI1-interacting partners. We validated the interaction between AtWRI1 and TCPs in yeast and plant cells. In a plant transient expression system, TCP4 represses AtWRI1-mediated oil production, as well as the transcriptional activity of AtWRI1.[40] Overexpressing miR319-resistant *TCP4* (*rTCP4*) reduced expression of AtWRI1 targets in transgenic plants. TCP4 represses the transcriptional activity of AtWRI1, and *TCP4* mutants, including *tcp4* T-DNA knockout, the *jaw-D* mutant with reduced *TCP4* expression, and a *tcp2 tcp4 tcp10* triple mutant, display increased seed oil content compared to WT.[40]

The WRI1-TCP4 module: combinatorial transcriptional control of plant oil biosynthesis

Intra- and inter-family transcription factor interactions greatly expand the complexity of combinatorial transcriptional regulation. In plant cells, protein complex formation within a transcription factor family has been well documented; however, cross-family transcription factor interactions are increasingly recognized.[41] A transcription factor family comprises a group of sequence-specific DNA-binding factors with distinct or overlapping functions. Different families vary in their transactivation activity (e.g. as activators or repressors), DNA-binding specificity, and response to various biotic and abiotic stimuli. Cross-family transcription factor interactions provide multi-layer regulatory mechanisms that are required for fine-tuning gene expression in response to various developmental and environmental signals. Increasing evidence demonstrates that TCP transcription factors serve as transcriptional hubs via interaction with diverse transcriptional regulators in numerous biological processes.[41] However, only recently we revealed the

involvement of TCP in plant lipid biosynthesis through interaction with the AP2 transcription factor WRI1.[40]

The involvement of TCP4, acting as a transcriptional repressor, in lipid biosynthesis is intriguing. Fatty acid biosynthesis is tightly controlled, especially during the seed development to maintain the proportional balance of lipids, proteins, and carbohydrates. As such, plants have evolved mechanisms that allow dialing the amplitudes of transactivation by critical fatty acid regulator, such as WRI1. While several activators of WRI1 activity have been characterized, negative regulation of AtWRI1 activity, for example, through phosphorylation and proteasomal degradation, have also been reported.[13,25,30,33,34] However, little is known regarding transcriptional repressors that negatively regulate the AtWRI1 activity. Our recent discovery highlights the possible role of TCP4 as a transcriptional repressor of AtWRI1 to modulate seed oil biosynthesis.[40] Nevertheless, our understanding of the WRI1-TCP4 regulatory module is by no means complete. Using *in silico* phosphorylation analysis[40] and mass spectrometry,[42] multiple phosphorylation residues in TCP4 have been identified. Given the importance of phosphorylation in mediating protein functions, such as protein–protein interaction, subcellular localization, and transcriptional activity, we speculate that phosphorylation might play a role in regulating the AtWRI1-TCP4 module. Questions remain as to how the upstream developmental or environmental signals trigger the phosphorylation and what kinases are involved in these processes.

Acknowledgments

The WRI1 work in the Ma Lab was supported by a Nanyang Technological University Startup grant and a Ministry of Education (MOE) of Singapore Tier 1 to W.M. (2018-T1-002-019).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

The WRI1 work in the Ma Lab was supported by a Nanyang Technological University Startup grant and a Ministry of Education (MOE) of Singapore Tier 1 to W.M. (2018-T1-002-019).

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