PLANT SIGNALING & BEHAVIOR
2018, VOL. 13, NO. 8, e1482176 (3 pages)
https://doi.org/10.1080/15592324.2018.1482176

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ARTICLE ADDENDUM

WRINKLED1 as a novel 14-3-3 client: function of 14-3-3 proteins in plant lipid metabolism

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ABSTRACT

The conserved plant 14–3-3 proteins (14–3-3s) function by binding to phosphorylated client proteins to regulate their function. Previous studies indicate that 14–3-3s are involved in the regulation of plant primary metabolism; however, not much is known regarding the functions of 14–3-3s in plant oil biosynthesis. Our recent work shows that 14–3-3 plays a role in mediating plant oil biosynthesis through interacting with the transcription factor, WRINKLED1 (WRI1). WRI1 is critical for the transcriptional control of plant oil biosynthesis. *Arabidopsis* WRI1 physically interacts with 14–3-3s. Transient co-expression of *AtWRI1* with *14–3-3s* enhances plant oil biosynthesis in leaves of *Nicotiana benthamiana*. Transgenic plants overexpressing of a *14–3-3* show enhanced seed oil content. Co-expression of a *14–3-3* with *AtWRI1* results in increased transcriptional activity and protein stability of AtWRI1. Our transcriptional regulation model supports a concept that interaction of a 14–3-3 with a transcription factor enhances the transcriptional activity through protein stabilization.

ARTICLE HISTORY
Received 10 May 2018
Accepted 23 May 2018

KEYWORDS
Arabidopsis; 14-3-3; plant oil biosynthesis; protein-protein interaction; transcriptional activity; protein stability; phosphorylation

The 14–3-3 proteins (14–3-3s) are a family of phosphopeptide-binding proteins, which are conserved in all eukaryotes, and involved in the regulation of numerous biological and physiological processes.[1–3] Interactions of 14–3-3s with the phosphorylated client proteins lead to many alterations in protein characteristics such as stability, activity, subcellular localizations, and interactive property of the client proteins.[2–4] Thirteen functional 14–3-3s have been predicted in *Arabidopsis*.[2,3,5] Diverse clients of plant 14–3-3s have been identified, among which are transcription factors, ion channels, enzymes, proton pumps, and signaling proteins.[6–13] Despite numerous studies showing important roles of 14–3-3s in plant primary metabolism,[4,14] little is known about their involvement in plant oil biosynthesis.

The WRINKLED1 (WRI1) transcription factor is considered to be a "master regulator" in the transcriptional regulation of plant triacylglycerol (TAG) biosynthesis.[15] WRI1 is an APETALA2 (AP2) transcription factor [16,17] and its *Arabidopsis* loss-of-function mutant (*wri1-1*) shows an 80% reduction in seed oil content.[18] Transcriptome analysis indicated that the majority of the genes that show reduced expression in *wri1-1* encode enzymes for fatty acid biosynthesis and glycolysis.[19] Many genes encoding enzymes in the glycolytic and fatty acid biosynthetic pathways are recently characterized as AtWRI1 targets.[20–22] *WRI1* orthologs have been identified in other plant species (both monocots and dicots), and shown to be functional in terms of regulating plant oil biosynthesis.[23–29] Overexpression of *AtWRI1* and *WRI1* orthologs result in increased oil content in seeds and vegetative tissues of the transgenic plants.[23,26–28,30,31] Transient expression of *WRI1s* in tobacco leaves elevates oil production as well.[29,32–34] Recent work began to further elucidate AtWRI1 function by dissecting its functional domains/motifs, protein structural features, and interacting partners. AtWRI1 was recently discovered to interact with CULLIN3-based E3 ligase adaptor BTB/POZMATH (BPM) proteins to mediate its stability.[35] Computational analysis revealed that AtWRI1 protein displays a hallmark of intrinsic disorder and three intrinsically disordered regions (IDRs) are identified.[33] In particular, the IDR3 contains a PEST motif, which is known to mediate protein stability.[33]

Recently we have demonstrated that a novel function of 14–3-3s in plant oil biosynthesis.[36] 14–3-3s physically interact with AtWRI1 in yeast and plant cells, suggesting that AtWRI1 is a novel client for 14–3-3s. Stable transgenic plants overexpressing a *14–3-3* show increased seed oil content. In a *Nicotiana benthamiana* transient expression system, a 14–3-3 is found to be able to increase AtWRI1-mediated TAG generation, as well as transcriptional activity and protein stability of AtWRI1.[36] In mammalian cells, 14–3-3s are capable of interacting with transcription factors, to enhance the transcriptional activity and protein stability. The interaction of 14–3-3σ with transcription factor p53 results in increased transcriptional activity and enhanced stability of p53.[37] Co-expression of the ETV1 transcription factor with 14–3-3τ in 293T cells also leads to enhanced stability and transcriptional activity.[38] In plant cells, the activity of barley transcription factor HvABI5 is increased through interacting with a 14–3-3.[13] Thus, our work further supports the concept that 14–3-3s mediate the activity and stability of their client substrates through protein-protein interaction.[2–4]

In mammalian cells, kinases trigger the phosphorylation of transcription factors to create 14–3-3 binding sites which

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Addendum to: Ma W, Kong Q, Mantyla JJ, Yang Y, Ohlrogge JB, Benning C. 14-3-3 protein mediates plant seed oil biosynthesis through interaction with AtWRI1. Plant J. 2016; 88: 228–235. doi: 10.1111/tpj.13244.

is important for mediating the activity of transcription factors in signal transduction pathways.[37–39] Currently, we do not know whether the interaction between a 14–3-3 and AtWRI1 is dependent on environmental signals or unidentified kinase(s) in charge of phosphorylating AtWRI1. Therefore, an insightful future work will be focused on the identification of kinases that interact with and phosphorylate AtWRI1. It is highly possible that diverse kinases are involved in the regulation of AtWRI1 activity which displays tempo-spatial variations, depending on different embryo developmental stages and upstream signaling events. Recent work by Zhai et al. has shown that KIN10 (an important SNF1-related protein kinase in sugar signaling pathways) interacts with AtWRI1 to trigger the phosphorylation and degradation of AtWRI1 protein.[40] The 14–3-3 binding region[36] and KIN10 phosphorylation site[40] are adjacent, suggesting a possible overlap between these two described occurrences. In addition, our prior work finds that phosphorylation of the IDR3–PEST motif mediates the protein stability of AtWRI1.[33] Taken together, these work[33,36,40] suggests that the phosphorylation might play a dual role in terms of mediating the activity of AtWRI1.

Due to the functional redundancy, it is not surprising to see that some other plant 14–3-3s not tested in our work can also interact with AtWRI1 and enhance the transcriptional activity of AtWRI1. So what are the 14–3-3s' specific roles in modulating AtWRI1 activity and how can they coordinate during the embryo development? Therefore, the precise mechanistic roles of these 14–3-3s need to be further elucidated in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

The work on WRI1 in the Ma Lab is currently supported by a Nanyang Technological University Startup grant (to W.M.).

Acknowledgments

We thank Dr. Ling Yuan (University of Kentucky) for critical reading the manuscript. The work on WRI1 in the Ma Lab is currently supported by a Nanyang Technological University Startup grant (to W.M.).

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