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Two *Arabidopsis* orthologs of the transcriptional coactivator ADA2 have distinct biological functions

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ABSTRACT

Histone acetylation is an example of covalent modification of chromatin structure that has the potential to regulate gene expression. Gcn5 is a prototypical histone acetyltransferase that associates with the transcriptional coactivator Ada2. In *Arabidopsis*, two genes encode proteins that resemble yeast ADA2 and share approximately 45% amino acid sequence identity. We previously reported that plants harboring a T-DNA insertion in the *ADA2b* gene display a dwarf phenotype with developmental defects in several organs. Here we describe T-DNA insertion alleles in the *ADA2a* gene, which result in no dramatic growth or developmental phenotype. Both *ADA2a* and *ADA2b* are expressed in a variety of plant tissues; moreover, expression of *ADA2a* from a constitutive promoter fails to complement the *ada2b-1* mutant phenotype, consistent with the hypothesis that the two proteins have distinct biochemical roles. To further probe the cellular roles of ADA2a and ADA2b, we studied the response of the transcriptional coactivator mutants to abiotic stress. Although *ada2b* seedlings display hypersensitivity to salt and abscisic acid and altered responses to low temperature stress, the responses of *ada2a* seedlings to abiotic stress generally parallel those of wildtype plants. Intriguingly, *ada2a;ada2b* double mutant plants display an intermediate, *gcn5*-like phenotype, suggesting that ADA2a and ADA2b each work independently with GCN5 to affect genome function in *Arabidopsis*.

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1. Introduction

The genomes of eukaryotic organisms are packaged into chromatin, the basic unit of which is a nucleosome containing approximately 150 base pairs of double-stranded DNA wrapped around an octamer of histone proteins [1]. While chromatin clearly serves a packaging role for eukaryotic DNA, the mechanisms underlying the dynamic and regulatory nature of chromatin have come to light in the last decade or so [2–4]. Chromatin can be modified in ways that affect DNA function through the action of two main types of multiprotein complexes. The first involves the adjustment of specific histone-DNA contacts by ATP-dependent nucleosome remodeling enzymes [5]. The second involves the post-translational covalent modification of histones through the addition or removal of phosphoryl, methyl, or acetyl groups, among other covalent modifications [6].

Acetylation of the N-terminal tails of histones was one of the first modifications to be characterized and is generally correlated with increased accessibility and transcription of the associated DNA [7]. The identification of the transcriptional regulator Gcn5 as a histone acetyltransferase opened the door to characterization of enzymes and their regulatory partners that form large multiprotein complexes which function to alter chromatin states [8]. Of particular interest here, Gcn5 physically associates with Ada2 in several larger transcriptional coactivator complexes that are particularly well characterized in the yeast *Saccharomyces cerevisiae* [9–12]. It has also been shown that Gcn5 requires Ada2 for full function *in vivo* [13]. Notably, yeast strains harboring mutations in either *Gcn5* or *Ada2* have essentially identically phenotypes, which is consistent with these two factors working together to modify chromatin [14].

More recently, our research group and others have set out to characterize these factors in multicellular eukaryotes, including their roles in response to developmental or environmental signals. Epigenetic effects on regulation of plant gene expression are well-documented [15,16], and thus exploration of these chromatin

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modifiers in plant systems seemed warranted. We and others have characterized *gcn5* mutants in *Arabidopsis* [17–20]. Disruption of *GCN5* (also known as *HAG1*) gene function in *Arabidopsis* results in pleiotropic effects on plant development including dwarfism, loss of apical dominance, and floral defects affecting fertility [18].

These pleiotropic effects in plants mirror diverse developmental effects in metazoans. *Drosophila* also appears to require *Gcn5* for normal developmental processes, as flies harboring a null allele display defects in metamorphosis and oogenesis [21]. Two orthologs of the yeast *GCN5* gene (*GCN5* and *PCAF*) have been identified in the laboratory mouse *Mus musculus*. Knockouts of *GCN5* result in lethality approximately one-third of the way through embryonic development, whereas null mutants of *PCAF* survive, suggesting that the two paralogs have diverse functions [22,23]. Taken together, these reverse genetic approaches confirm the importance of GCN5 action for proper growth and development of higher eukaryotes.

Comparable studies have explored the *in vivo* roles of ADA2. We identified two orthologs of *ADA2* in *Arabidopsis* [24], designated *ADA2a* and *ADA2b*. A T-DNA insertion in the *ADA2b* gene (*ada2b-1*) resulted in mutant plants that are dwarf and infertile, sharing some similarities to the *gcn5* mutant phenotype [18]. In this report, we characterize three T-DNA disruption alleles of the *ADA2a* gene. In comparison to *ada2b* mutants, we find no dramatic mutant phenotype under standard growing conditions. This evidence for distinct roles of *ADA2* orthologs is echoed in *Drosophila*, where disruption of different *ADA2* paralogs results in distinct phenotypes [25–27]. Two *ADA2* paralogs have also been identified in the human genome, and several GCN5–ADA2 transcriptional coactivator complexes have been characterized [28]. In both *Drosophila* and humans, the two ADA2 paralogs associate with GCN5 in distinct protein complexes, suggesting different functional roles [25,26,28–30].

We were particularly interested in examining the *ada2a*, *ada2b*, and *gcn5* mutants in response to abiotic stress. Initially we focused on transcriptional regulation involved in promoting a plant's ability to withstand low-temperature stress. CBF1 is a member of a family of transcriptional activators that function as master regulators of the cold acclimation pathway in *Arabidopsis* by stimulating the transcription of cold-responsive (*COR*) genes [31]. Previous work has shown that CBF1 activation of a reporter gene in yeast required Ada2 and Gcn5 [24]. We have also shown that *COR* gene expression during cold acclimation is delayed and reduced in *ada2b-1* and *gcn5-1* mutants [18]. Taken together, these results suggest that ADA2b and GCN5 may work with CBF1 to promote the cold acclimation response.

In this report, we probe the response of *ada2a* and *ada2b* mutants to several abiotic stresses including low temperatures, high salt concentrations, and treatment with abscisic acid (ABA). In all cases, we find that *ada2b-1* displays altered responses whereas the *ada2a* mutants generally show a response comparable to that of wildtype plants. By ruling out differences in spatial or temporal expression, we conclude that the two ADA2 proteins differ inherently in their biochemical activities. Analysis of *ada2a-2;ada2b-1* double mutants suggests a role for ADA2a in aiding GCN5 function in *Arabidopsis* as predicted by *in vitro* studies [24,32].

2. Materials and methods

2.1. Identification and molecular characterization of mutants

The *ada2b-1* and *gcn5-1* mutants have been previously described [18]. The *ada2a-1* and *ada2a-2* mutants were identified in a PCR-based reverse genetics screen of T-DNA mutagenized populations (Wassilewskija-2 ecotype, hereinafter Ws) available from the University of Wisconsin. The *ada2a-3* mutant plants (Columbia-0 ecotype, Col) were obtained from collections at the Salk Institute. The genotypes of mutant plants were confirmed by PCR using gene-specific primers as well as primers recognizing the T-DNA border sequences (Table 1).

Table 1
Nucleotide sequences of gene-specific PCR primers used to identify and characterize *ada2a* and *ada2b* mutants

Primer	Sequence	Location	Strand
JL202	5′-cattttataataacgctgcggacatctac-3′	T-DNA left border (UW)	NA
XR-2	5′-tgggaaaacctggcgttacccaacttaat-3′	T-DNA right border (UW)	NA
LBa1	5′-tggttcacgtagtgggccatcg-3′	T-DNA left border (Salk)	NA
LBb1	5′-gcgtggaccgcttgctgcaact-3′	T-DNA left border (Salk)	NA
ST463	5′-ccgtagggtttaaagatggcgtgaacata-3′	5′ end of *ADA2a*	Forward
ST464	5′-tgagtcacctattcccttatgaaccagca-3′	3′ end of *ADA2a*	Reverse
ST841	5′-gttgatggtctccttggagctgactta-3′	Exon 12 of *ADA2a*	Forward
ST842	5′-cttgttacttctgattggaatgccg-3′	Exon 4 of *ADA2a*	Forward
ST843	5′-ttgactccagctaaggcagacaaag-3′	Exon 8 of *ADA2a*	Reverse
ST947	5′-ctagcttctcgtcctgctgagg-3′	Exon 1 of *ADA2a*	Forward
ST948	5′-aaggccaggtttcctctcattc-3′	Exon 3 of *ADA2a*	Reverse
ST705	5′-aagcggaggaaggaatttgt-3′	Exon 11 of *ADA2a*	Forward
ST706	5′-agtcagctccaaggagacca-3′	Exon 12 of *ADA2a*	Reverse
ST461	5′-agggttcttcttctctgtggtttcgcata-3′	5′ end of *ADA2b*	Forward
ST468	5′-catcgtcatctcacttcttcttcctttccttg-3′	3′ end of *ADA2b*	Reverse
ST628	5′-actcctcacaaatgtgatcacccataccg-3′	Exon 3 of *ADA2b*	Forward
ST629	5′-ggtggaaacaggtttcttccctccaaaac-3′	Exon 9 of *ADA2b*	Reverse
ST646	5′-agtgggggcacactcgtttcaaattattc-3′	Exon 11 of *GCN5*	Forward
ST647	5′-tccgcaacaaacatatccaatgtcacgta-3′	Exon 13 of *GCN5*	Reverse
ST881	5′-ctgatgaaatgctctctcgtggttt-3′	*EF4α*	Forward
ST882	5′-tgggaggtcaaagtttatgaccaga-3′	*EF4α*	Reverse
KB51	5′-ggaaaggatctgtacggtaac-3′	*ACT2*	Forward
KB52	5′-tgtgaacgattcctggac-3′	*ACT2*	Reverse
KP23	5′-ggtcgtactactggtattgtgct-3′	*ACT3*	Forward
KP24	5′-tgacaatttcacgctcagct-3′	*ACT3*	Reverse
ST981	5′-atggcttcttctttccacagc-3′	*COR15a*	Forward
ST982	5′-gaagctttctttgtggcctc-3′	*COR15a*	Reverse
ST1045	5′-ctggcaaagctgaggagaag-3′	*COR6.6*	Forward
ST1046	5′-actgccgcatccgatatact-3′	*COR6.6*	Reverse
KP21	5′-gaaaggaggaggaggaatgg-3′	*COR78*	Forward
KP22	5′-aaccagccagatgattttgg-3′	*COR78*	Reverse
KP11	5′-ggagtggttggtctggctaa-3′	*GolS*	Forward
KP12	5′-ttggttatccggtgggtaaa-3′	*GolS*	Reverse
ST1099	5′-cgggtgacggagaattagg-3′	18S rRNA	Forward
ST1100	5′-ttgtcactacctccccgtgt-3′	18S rRNA	Reverse

NA, not applicable.

Total RNA from various *Arabidopsis* tissues was prepared using the RNeasy Plant Mini Kit (Qiagen) or the Concert™ Plant RNA Reagent (Invitrogen). Levels of specific mRNAs were assayed using reverse transcription followed by PCR. Gene-specific primers for *ADA2a*, *ADA2b*, *GCN5*, *EF4α*, *ACT2*, *ACT3*, *COR6.6*, *COR15a*, *COR78*, *GolS*, and 18S rRNA are listed in Table 1. For gel-based reverse transcriptase (RT)-PCR assays, RT reactions were carried out using 0.5 µg of DNase-treated RNA and the Access RT-PCR System (Promega). PCR products were subject to gel electrophoresis and visualized by ethidium bromide staining. For Real-time PCR, 1 µg of total RNA was used in cDNA synthesis in a final reaction volume of 20 µl. After the reaction, the samples were diluted 10-fold in nuclease-free water. Real-time PCR was carried out using 3 µl of a given diluted cDNA sample and 0.25 µM of each primer in a 30 µl reaction volume containing SYBR Green, 3 mM MgCl2, 12.4 mM dNTPs and 0.15 µl Ampli Taq Gold DNA polymerase enzyme (all reagents were obtained from ABI). For each sample, the Ct values thus obtained for the *COR* genes were normalized to the corresponding value obtained for *ACT3*.

For northern blot analyses, 5–10 µg of RNA was subjected to electrophoresis and transferred to a Hybond N+ membrane (Amersham Pharmacia) using standard blotting procedures. [^{32}P]-labeled probes were generated using a random priming method (Random Primers DNA Labeling System, Gibco BRL). Blots were hybridized in Perfect Hyb Plus (Sigma) and washed to a stringency of 0.2×SSC, 0.1% SDS, and 0.01% sodium pyrophosphate at 65 °C before exposing to X-ray film or phosphoimager analyses.

2.2. Plant growth and phenotypic analysis

Plants were routinely grown at 20–25 °C with a constant overhead light source (cool-white fluorescent lights, 100–150 µmol m^{-2} s^{-1}).

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