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An *Arabidopsis* 14-3-3 protein can act as a transcriptional activator in yeast

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Abstract The 14-3-3 proteins are a group of highly conserved and widely distributed eukaryotic proteins with diverse functions. One 14-3-3 protein, AFT1 from *Arabidopsis thaliana*, was found to be able to activate transcription in yeast. When fused to the DNA-binding domain of a bacterial protein LexA, AFT1 can activate transcription of reporter genes that contain LexA operator sequences in their promoters. Although the in vivo function of AFT1 is not completely known, its similarity to previously identified proteins found in transcription complexes of *Arabidopsis* and maize suggests that AFT1 and some other 14-3-3 proteins may activate gene expression in other systems as well.

Key words: 14-3-3 protein; Acidic activator; Transcription activation; *Arabidopsis*

1. Introduction

The yeast two-hybrid system [1] is a useful method for detecting protein-protein interactions in vivo. One modified version of the two-hybrid system, the interaction trap technique [2,3], uses the DNA-binding domain of the bacterial protein LexA as the DNA-binding protein instead of using the DNA-binding domain of the yeast Gal4 protein. In conducting an interaction trap experiment, a library that expresses cDNA-encoded proteins fused to a transcription activator domain (B42) is introduced into a host yeast strain. The host strain contains a plasmid which directs constitutive production of a transcriptionally inert LexA fusion protein which is called the 'bait' (LexA fused to the protein of interest) and two reporter genes. The transcription of these two reporter genes can be stimulated if the cDNA-encoded protein complexes with the bait. One reporter gene *LEU2* allows the strain to grow in the absence of leucine and the other reporter gene *LacZ* encodes β-galactosidase.

One 14-3-3 protein, AFT1 [4], encoded by an *Arabidopsis* cDNA can activate transcription with LexA protein alone, or with other different LexA fusion proteins, but not without LexA protein. The 14-3-3 proteins are implicated in playing important roles in signal transduction pathways (e.g. regulating protein kinase activities), stimulation of exocytosis, facilitation of protein transport into mitochondria, and transcriptional regulation of some stress-responsive genes [5,6]. Even though 14-3-3 proteins have been found in every eukaryotic system examined, their mode of action has not been understood in most cases. The fact that AFT1 can activate transcription in yeast suggests a possibility that 14-3-3 proteins may act as transcriptional activators under certain conditions, and if so, this would further broaden the known functions of 14-3-3 proteins.

2. Materials and methods

2.1. AFT1 and LexA interaction

The yeast strain EGY48 (*MAT*a *trp1 ura3 his3 LEU2*::*plexAop6-LEU2*), containing a plasmid pJK103 that directs expression of a *Gal1-lacZ* gene from two high-affinity ColE1 LexA operators, was used in the interaction trap experiments [2,3]. The prey plasmid that directs biosynthesis of the AFT1-B42 fusion protein in pJG4-5, and the bait plasmid that directs the biosynthesis of bait in pEG202 (e.g. LexA/AKR1–261, residues 1–261 of AKR fused to the DNA-binding protein LexA) were introduced into EGY48. The activation of transcription by AFT1 and its derivatives was measured by the growth of yeast on leucine-minus plates. The plasmids used to direct production of different LexA fusion proteins were as follows: pEG202 (LexA alone), pHM1-1 (LexA/Bicoid), pHM12 (LexA/Cdc2), pHM7-3 (LexA/Ftz homeo-domain), pAKR1–261 (LexA/AKR1–261), pAKR249–434 (LexA/AKR249–434), pAKR114–434 (LexA/AKR114–434). The plasmid pHMϕ, which does not produce LexA protein, was also used. The plasmids pHM1-1, pHM7-3, pHM12, pHMϕ, and pSH18–34 were from Roger Brents' laboratory (Massachusetts General Hospital, Boston, MA), and were described previously [2,3]. The LexA/AKR fusion plasmids, pAKR1–261, pAKR249–434, and pAKR114–434, were made in our laboratory by fusing various AKR fragments (residues 1–261, 249–434, and 114–434) to LexA. AKR is an *Arabidopsis* protein that plays a role in chloroplast development [7]. The protocols for conducting yeast two-hybrid experiments and other DNA manipulation techniques are described in Ausubel et al. [8].

2.2. Activation by B42/AFT1 and LexA/AFT1 fusion proteins

A series of AFT1 derivatives fused to B42 in the plasmid pJG4-5 were introduced into the strain EGY48 containing the plasmid pEG202 (which directs the constitutive production of LexA protein) and the plasmid pSH18–34 which contains the *LexAop-LacZ* reporter gene. The activation of transcription by AFT1-B42 and AFT1 derivatives was measured by the growth of yeast on leucine-minus plates and the activity of β-galactosidase. The assay for β-galactosidase was conducted as described in Zervos et al. [3]. A series of AFT1 derivatives fused to LexA in the plasmid pEG202 were introduced into the strain EGY48 containing the plasmid pSH18–34. The oligonucleotides used to amplify the desired AFT1 fragments which were later subcloned into pJG4-5 and pEG202 are shown below.

JW-5: CTGACTGAATTCATGGCGGCGACATTAGG
JW-6: GACTGAGTCGACCCTTCATCTAGATCCTC
JW-7: GACTGACTCGAGCCTTCATCTAGATCCTCA
JW-8: CTGACTGAATTCGAGTCTAAGGTCTTTTAC
JW-9: GACTGACTCGAGACTCGCTCCAGCAGATGG
JW-10: GACTGACTCGAGTGAAGAATTGAGAATCTC
JW-11: GACTGAGTCGACACTCGCTCCAGCAGATGG
JW-12: GACTGAGTCGACTGAAGAATTGAGAATCTC
JW-13: CTGACTGAATTCGTTACAGGCGCTACTCCAG

The oligonucleotide combinations used were: JW-5 and JW-6 (LexA/1–248); JW-5 and JW-12 (LexA/1–194); JW-5 and JW-11 (LexA/1–121); JW-13 and JW-6 (LexA/34–248); JW-8 and JW-6

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(LexA/122–248); JW-5 and JW-7 (B42/1–248); JW-5 and JW-9 (B42/1–121); JW-13 and JW-7 (B42/34–248); JW-8 and JW-7 (B42/122–248); JW-13 and JW-10 (B42/34–194).

3. Results

Transcriptional activation by the AFT1/B42 fusion protein in the yeast interaction trap system is shown in Fig. 1. When the yeast cells contained LexA or its fusion derivatives the expression of the reporter genes, *LEU2* and *LacZ*, was activated by the AFT1/B42 fusion protein. The LexA-dependent expression indicates that AFT1 may be able to interact with LexA so as to bring the activation domain B42 into the proximity of the reporter gene promoter. If this is the case, then it is B42 that is responsible for the observed transcriptional activation. We tested this hypothesis by making AFT1 deletion mutants and analyzing their impact on reporter gene expression. Deletion of the C-terminal half of AFT1 (B42/1–121) abolished its ability to activate, whereas deletion of either 33 or 121 residues from the N-terminus (B42/34–248 and B42/122–248) increased activation (Fig. 2A). It appears that it is the C-terminal half of AFT1 that is responsible for interacting with LexA. The reason for the increased activation is not known, but might be due to the tertiary structures of these two fusion proteins (B42/34–248 and B42/122–248) that interact with the transcriptional machinery more efficiently.

Since AFT1 is very acidic (p*I*=4.6), a feature shared by many transcription activators, and at least one of the other *Arabidopsis* 14-3-3 proteins was found in the transcriptional complex of the *Adh* gene promoter [9], we explored the possibility that AFT1 may activate gene expression. We fused AFT1 directly to the DNA-binding domain of LexA and found that the fusion protein can activate transcription (Fig. 2B). We then tested which part of AFT1 is important for the activation when AFT1 is fused to LexA. Deletion of 54 amino acids from the C-terminus (LexA/1–194) caused AFT1 to completely lose its ability to activate; whereas with deletion of 33 amino acids from the N-terminus (LexA/34–248), the activation dropped by about 75% (Fig. 2B). When both the N-terminal half (LexA/122–248) and the C-terminal half (LexA/1–121) were deleted, the activation dropped to basal levels (Fig. 2B). Thus, even though the C-terminal half is critical for activation and is more acidic than the N-terminal half, the N-terminal half also plays a role in activation.

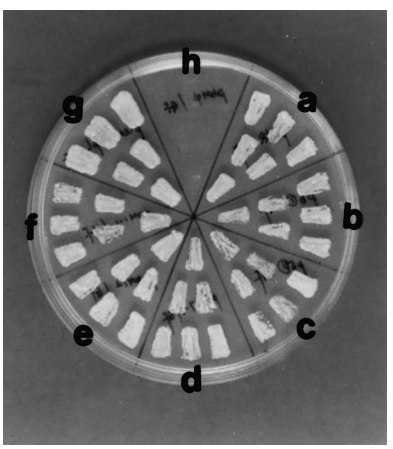

Fig. 1. The LexA-dependent activation of *LEU2* expression by AFT1. The activation was monitored by the growth of yeast on a leucine-minus plate. The AFT1 clone in vector pJG4-5, which directs the production of the AFT1/B42 fusion protein, was introduced into the yeast strain EGY48 where different plasmids had already been introduced. These different plasmids, which either direct production of different LexA fusion proteins or no LexA protein, were pEG202 (LexA alone, a); pHM1-1 (LexA/Bicoid, b); pHM12 (LexA/Cdc2, c); pHM7-3 (LexA/Ftz homeo-domain, d); pAKR1–261 (LexA/AKR1–261, e); pAKR249–434 (LexA/AKR249–434, f); pAKR114–434 (LexA/AK-R114-434, g); and pHMϕ (no LexA, h).

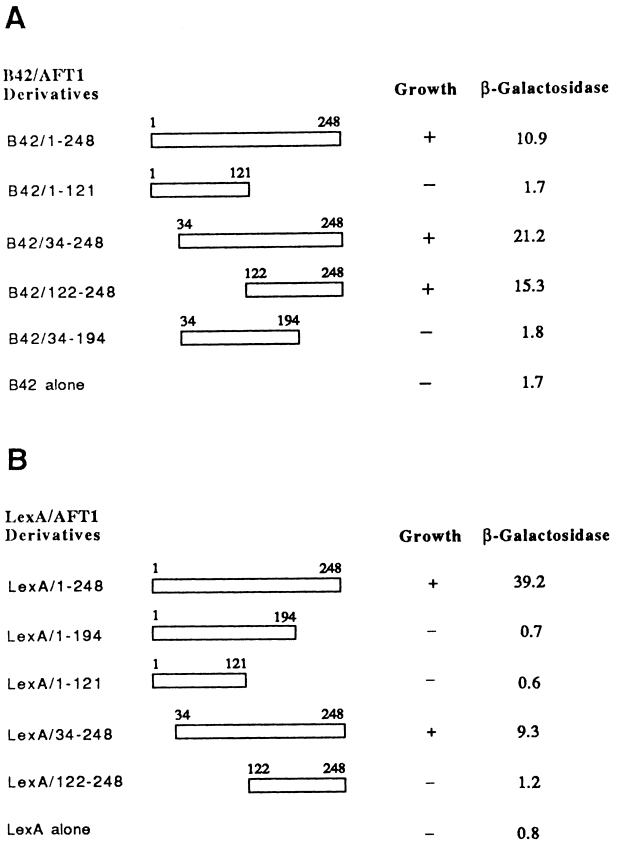

Fig. 2. Transcriptional activation by AFT1. The effects of various fusion proteins were monitored by the growth of yeast in the absence of leucine and quantified by measuring the activity of β-galactosidase. A: AFT1 and its derivatives fused to the activation domain B42 were introduced into the yeast strain EGY48 containing the plasmid pEG202 (which directs constitutive production of LexA protein) and plasmid pSH18–34 which contains the reporter gene *LexAop-LacZ*. B: AFT1 and its derivatives fused to the LexA protein in the plasmid pEG202 were introduced into the yeast strain EGY48 containing only the plasmid pSH18–34.

4. Conclusions

When a partial AFT1 fragment (residues 34–194) was used as bait in an effort to identify AFT1's interacting proteins, none of the identified candidates showed any relationship to proteins known to be involved in the transcription process. Two of the AFT1-interacting proteins are enzymes, ascorbate peroxidase [10] and *O*-methyltransferase [11]. This suggests that AFT1 may play an important role in regulating some enzymes' activities. However, this does not exclude the possibility that AFT1 may have other functions, since the AFT1 fragment that was used as the bait may not contain domains necessary for interaction with proteins such as transcriptional

factors or the transcription machinery. In fact, the in vivo function(s) of AFT1 may be quite diverse.

The reason that AFT1 activates transcription in yeast may be due to the fact that AFT1 is very acidic (15 negatively charged residues) and may act similarly to the acidic activators found in many transcriptional complexes, such as Gal4 [12], FOS [13], and Vp1 [14]. The acidic activators appear to function universally in all eukaryotic systems [15], but the molecular mechanism of activation is not understood. It is presumably the binding of the acidic activators to components of the transcription machinery that triggers transcription activation [16]. Binding could occur when the activator is in the proximity of a promoter, for example, LexA/AFT1 fusion proteins bind to the LexA operator near the TATA box. Our data indicate that the C-terminal half of AFT1 might interact with LexA, thereby bringing the B42/AFT1 fusion protein into the proximity of the TATA box of the reporter gene, which explains why B42/AFT1 can also activate transcription. There are two isoforms of 14-3-3 proteins in yeast [17], but they appear unable to interact with LexA or unable to activate transcription even if they interact with LexA in the interaction trap system we used. It is clear that 14-3-3 proteins from higher plants and yeast have acquired their own unique functions despite the fact that they still share some similar functions [18].

Based on our data, we propose that some 14-3-3 proteins may be, in addition to all their other functions, potential transcription activators. These acidic proteins are structurally similar to AFT1 and are small enough to be able to enter the nucleus freely (AFT1 has a MW of 28 kDa, and the exclusion limits of the nuclear pore are between 40 and 60 kDa [19]). Additional evidence in support of this proposal comes from the findings that GF14 proteins, the 14-3-3 proteins found in *Arabidopsis* and maize [8,20], are components of the transcriptional complex at the promoters of the *Adh* genes and were shown to be localized to nuclei of these plants [21]. There is no evidence that 14-3-3 proteins can bind to DNA. However, this is not unusual because several transcription factors, including Vp1 [14] and VP16 [22], contain activation domains but are not able to bind to DNA yet they often contain protein-protein interaction domains. In fact, GF14 proteins can physically interact with Vp1 [23], suggesting that they may play either structural or activational roles in the transcriptional complex of some plant genes. Although the large range of functions exhibited by the 14-3-3 proteins is difficult to reconcile since they are small proteins (around 250 residues) and are highly similar to each other (usually $>70\%$ identity), our hypothesis that some 14-3-3 proteins, including AFT1, can function as transcriptional activators deserves further testing.

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