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The *Arabidopsis* ortholog of the 77 kDa subunit of the cleavage stimulatory factor (AtCstF-77) involved in mRNA polyadenylation is an RNA-binding protein

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ABSTRACT

The 77 kDa subunit of the polyadenylation cleavage stimulation factor (CstF77) is important in messenger RNA 3′ end processing. Previously, we demonstrated that AtCstF77 interacts with AtCPSF30, the *Arabidopsis* ortholog of the 30 kDa subunit of the Cleavage and Polyadenylation Specificity Factor. In further dissecting this interaction, it was found that the C-terminus of AtCstF77 interacts with AtCPSF30. Remarkably, we also found that the C-terminal domain of AtCstF77 possesses RNA-binding ability. These studies therefore reveal AtCstF77 to be an RNA-binding protein, adding yet another RNA-binding activity to the plant polyadenylation complex. This raises interesting questions as to the means by which RNAs are recognized during mRNA 3′ end formation in plants.

Structured summary:
MINT-7712550: *AtCstF77* (uniprotkb:Q8LKG5) *binds* (MI:0407) to *AtCPSF30* (uniprotkb:A9LNK9) by *pull down* (MI:0096)

1. Introduction

The processing of pre-mRNAs in eukaryotic cells is a complicated, yet critical process involving numerous players [1–3]. Briefly, the events involved in RNA processing include addition of the 5′ methyl guanosine cap, intron/exon splicing, and addition of a 3′ polyadenosine [poly(A)] tail. These processes are highly coordinated, co-transcriptional nuclear events that show no true separation spatially or temporally from other events in gene expression [3–5]. The formation of the 3′ end of the pre-mRNA has been well studied in several eukaryotes spanning multiple kingdoms [1,2]. This process involves several multisubunit complexes that can be separated, purified, and assayed for activities important for the complete process.

One of the complexes involved in polyadenylation in mammals is the so-called cleavage stimulation factor, or CstF [6]. In mammals, this complex is comprised of 50 kDa, 64 kDa, and 77 kDa subunits [6]. CstF64 possesses an RNA recognition motif (RRM)-type RNA-binding domain near the N-terminus [7–13], while the C-terminal region interacts with other members of the polyadenylation complex [14–16]. The sequence preference of the CstF64 RRM has been determined to be G/U-rich sequences downstream of the poly(A) site (AAUAAA) in mammals and A-rich sequences in yeast [9–12]. CstF50 is a WD repeat protein that interacts with CstF77 and also with the BRCA-associated ubiquitin ligase subunit BARD [16,17].

CstF77 possesses a series of so-called HAT (for the *half-a-TPR*) domains [18]; this feature of CstF77 is conserved in all eukaryotes. The HAT domain is flanked at the C-terminus of the protein by a C-terminal domain that exhibits very lower evolutionary conservation. Functionally, CstF77 acts as a scaffold and a bridge to other polyadenylation factors, as it has multiple interactions with the other CstF subunits and with the cleavage and polyadenylation specificity factor (CPSF) subunits CPSF160 and symplekin [16,19–21]. CstF77 exists as a dimer [20,22] that is held together by subunit–subunit interactions involving HAT motifs in the central third of the protein. This dimer may be modeled as a concave shape, with the C-terminus residing within the cavity formed by the rest of the dimer (see Fig. 1A).

As with its mammalian counterpart, the *Arabidopsis* CstF77 ortholog (AtCstF77) interacts with CstF64 and CPSF160 [23,24]. Additionally, AtCstF77 also binds the *Arabidopsis* ortholog of the 30 kD subunit of CPSF, AtCPSF30 [24]. In this study, we describe an analysis of the interaction between AtCstF77 and AtCPSF30. We also report an unexpected finding arising from these studies, that AtCstF77 can bind RNA. Together, these results corroborate

Abbreviations: CstF, cleavage stimulatory factor; CPSF, cleavage and polyadenylation specificity factor; RRM, RNA recognition motif; MBP, maltose binding protein

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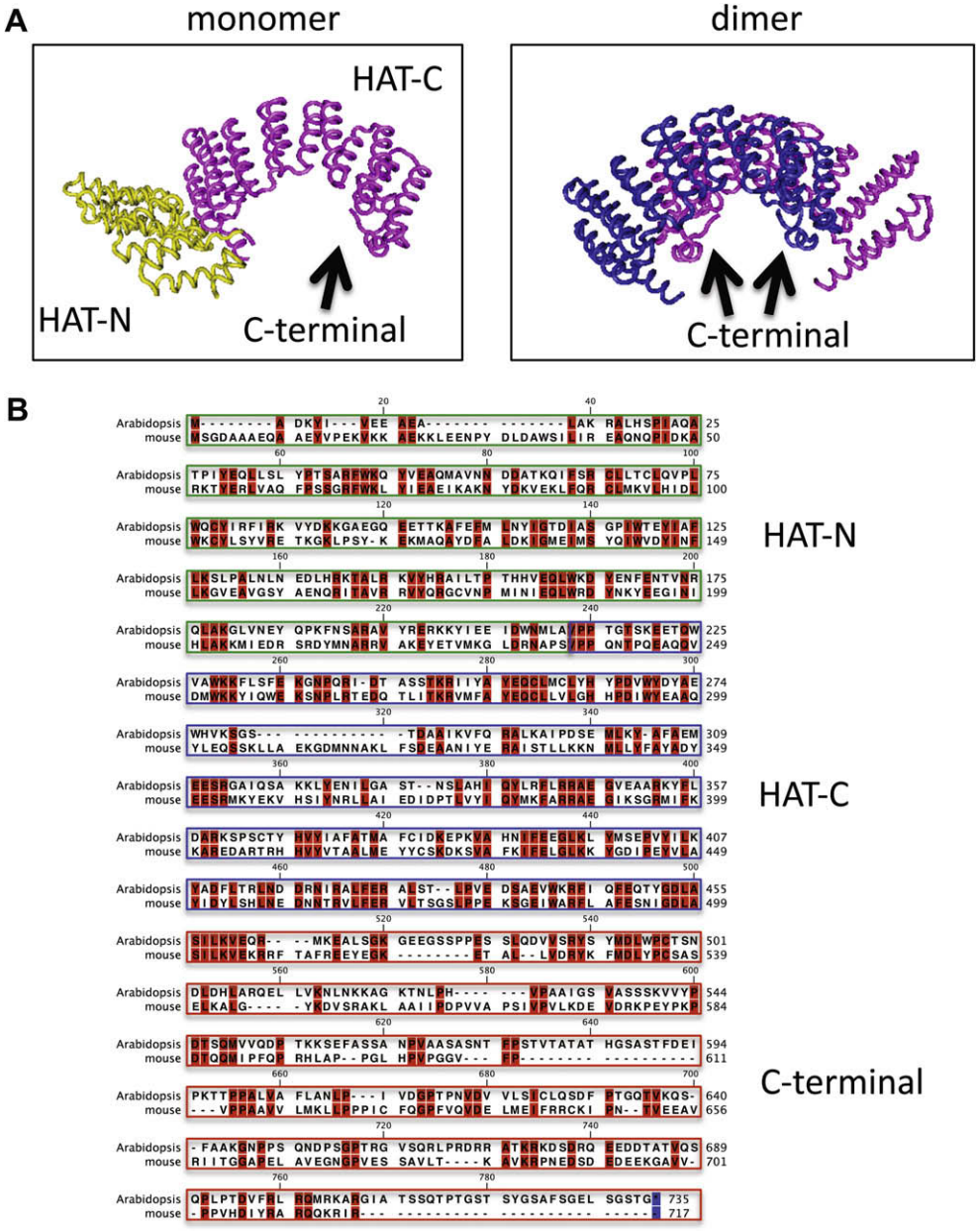

Fig. 1. Structures of the AtCstF77 derivatives used in this study. (A) Illustrations of the 3-dimensional structures of the monomeric (left) and dimeric (right) HAT domains of the mouse CstF77. These illustrations were made using Cn3D 4.1 and are based on the PDB accessions 2OOE and 2OND, respectively. The portion corresponding to the HAT-N domain is colored in yellow, and the HAT-C domain in purple or blue. The C-terminal domain of CstF77 was not in the crystal structure; the position of this domain, based on the location of the C-termini of the chains shown, is noted with black arrows. (B) Amino acid sequence alignment of the *Arabidopsis* and mouse CstF77 proteins. The HAT-N, HAT-C, and C-terminal domains are set off with boxes with differently-colored outlines.

those of previous studies purporting an interaction between AtCstF77 and AtCPSF30. Additionally, they add a new RNA-binding capability to the plant polyadenylation complex, and with it an added complexity to this aspect of polyadenylation in plants.

2. Results

Previously, it was reported that the C-terminal half (approximately) of AtCstF77 interacted with AtCPSF30 in a yeast two-hybrid assay [24]. To confirm this interaction, in vitro pull-down assays were performed. Using the crystal structure of murine CstF77 [22] as a guide, it was determined (Fig. 1A) that AtCstF77 could be subdivided into three domains, with a C-terminal region that, while not apparent in the structures, could be placed within a conceptual cavity formed by the dimeric HAT part of the protein (see the right panel of Fig. 1A). The C-terminal domain is not well-conserved evolutionarily (Fig. 1B). For AtCstF77, the CTD consisted of residues 500–734, (Fig. 1B).

Thus, to test the interaction between the C-terminus of AtCstF77 and AtCPSF30, the maltose binding protein (MBP) tag was fused with amino acids 500–734 of AtCstF77; the MBP tag was used for pull-down assays and for purification of the proteins. Subsequently, the co-purification of biotinylated AtCPSF30 with MBP-AtCstF77 CTD was assayed. The results of these experiments showed that AtCPSF30 co-purified with the MBP-AtCstF77 CTD ("MBP-77 CTD" in Fig. 2B). In contrast, AtCPSF30 did not bind to MBP (Fig. 2B). This binding of the AtCstF77 CTD was specific for AtCPSF30 (and not for the biotin tag), as the major biotinylated