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Characterization of *Arabidopsis* decapping proteins AtDCP1 and AtDCP2, which are essential for post-embryonic development

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Abstract Although decapping is an important process in eukaryotic mRNA turnover, little is known about this process in plants. Here, we identified *Arabidopsis thaliana* decapping proteins AtDCP1 and AtDCP2 and showed that (I) AtDCP2 is an active decapping enzyme, (II) AtDCP1 interacts with itself, (III) AtDCP1 and AtDCP2 are localized to cytoplasmic foci (putative *Arabidopsis* processing body), and (IV) AtDCP1 and AtDCP2 are essential for post-embryonic development. Our findings provide new insights into the role of decapping-dependent mRNA turnover.

Keywords: mRNA turnover; Decapping; DCP1; DCP2; Processing body; *Arabidopsis thaliana*

1. Introduction

mRNA turnover is important for the regulation of gene expression. Especially, decapping of mRNA is a critical step in the 5′ to 3′ mRNA decay pathway (reviewed in [1]). In yeast, Dcp1 is copurified with decapping activity [2,3] then Dcp2 is isolated as a suppressor of the *dcp1* mutation [4]. Recent studies have shown that Dcp2 is a catalytic subunit of the decapping complex [5–7]. Dcp1 directly interacts with Dcp2 in yeast [4,8], whereas in humans, Dcp1 indirectly interacts with Dcp2 through binding with Hedls [9]. Decapping proteins colocalize to cytoplasmic foci known as processing bodies (P-bodies) (also called Dcp-bodies or GW-bodies [7,10–13]), in which mRNA degradation ([1] and references therein) and mRNA storage have been observed [14].

Decapping proteins are important components not only in the 5′ to 3′ decay pathway but also in nonsense-mediated decay (NMD), AU-rich element (ARE)-mediated decay (reviewed in [15]) and RNA interference pathways [16,17]. That is, they function in several mRNA decay pathways in both yeast and animals. However, the roles of plant decapping proteins remain unknown. Here, we therefore searched the whole genome of *Arabidopsis thaliana* and identified yeast Dcp1 and Dcp2 homologs designated AtDCP1 [7] and AtDCP2, respectively. Biochemical analyses showed that AtDCP2 is an *Arabidopsis* decapping enzyme, microscopic analyses showed that AtDCP1 and AtDCP2 are colocalized to cytoplasmic foci, and *dcp1* and *dcp2* mutant analysis indicated that both AtDCP1 and AtDCP2 have essential roles in *Arabidopsis* post-embryonic development.

2. Materials and methods

2.1. Plasmids

Plasmids with the prefix 'pBIC' were used for agroinfiltration [18]. pBICDCP1-GFP, pBICDCP1-RFP, and pBICDCP1-HA have the full-length open reading frame (ORF) for AtDCP1 fused with GFP, RFP, and HA, respectively, to their C-termini. pBICDCP2-GFP, pBICDCP2-RFP, and pBICDCP2-HA have the full-length ORF for AtDCP2 fused with GFP, RFP, and HA, respectively, to their C-termini. These plasmids were therefore used for agroinfiltration along with pBICP35 as a negative control. pBICDCP1-GFP was used to transform Col-0. pIGDCP2-GFP, which encodes full-length AtDCP2 fused with GFP to its C-terminus, was used to complement *dcp2-1*. pGEX4T2 (GE-Healthcare) was used for expression of GST. pGEXDCP1 and pGEXDCP2, which contain the full-length ORFs of AtDCP1 and AtDCP2, respectively, were used for the expression of GST-fusion recombinant proteins in *Escherichia coli*. pUCTMVΩ, which contains the *Tobacco mosaic virus* 5′ leader sequence, was used for in vitro transcription. See Supplementary information for details on construction of the plasmids used in this study.

2.2. In vitro decapping assay

pUCTMVΩ was digested with *Eco*RI and then uncapped RNA was transcribed by MEGAshortscript (Ambion). RNA (10 pmol) was then capped with 15 pmol [α-^{32}P]GTP (3000 Ci/mmol) and guanylyltransferase (Ambion). Cap-labeled RNA was passed four times over Microspin G-25 columns (GE-Healthcare) then 0.5 pmol cap-labeled RNA was used for the decapping reaction.

The decapping reaction was essentially carried out as described previously [5] with some modifications. See Supplementary information for details on the expression and purification of the recombinant proteins and the decapping reaction.

2.3. Agroinfiltration and immunoprecipitation

Agroinfiltration using *Agrobacterium tumefaciens* GV3101 was performed as described previously [18]. The concentration of *Agrobacterium* was normalized to 0.8 OD at 600 nm. See Supplementary information for details on immunoprecipitation.

2.4. Microscopy

Microscopic analyses were performed with OLYMPUS IX70 using an EGFP/DsRed filter (CHROMA). Micrographs were processed electronically using Adobe Photoshop software.

2.5. Plant materials and analyses

Arabidopsis T-DNA insertion lines, *dcp1-2* (FLAG_563G05) and *dcp2-1* (Salk_000519), were isolated by PCR-based genotyping using

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Abbreviations: DCP1, decapping protein 1; DCP2, decapping protein 2; P-body, processing body

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the following sets of primers: *dcp1-1* (LP 5′-ATCTGGTGGAGAATCTCCTGG-3′, RP 5′-GAGAGGAGGATTGTAGAGGCG-3′, and INRA-LB3 5′-TCCAGGGCGTGTGCCAGGTGC-3′) and *dcp2-1* (LP 5′-TTTGCTATTCTTTGACCTCCG-3′, RP 5′-ATTGGGCTCGAATTGATTTTC-3′, and LBb1 5′-GCGTGGACCGCTTGCTGCAACT-3′), respectively. Seeds were grown on agar-solidified medium and observed at 8 days post-germination (dpg). For RT-PCR, total RNA was extracted with Isogen (Nippongene) from seedlings at 8 dpg. cDNA was synthesized with SuperscriptIII (Invitrogen) and an oligo(dT) primer. PCR was then carried out with the following set of primers: AtDCP1 (5′-ATGTCTCAAAACGGGAAGA-3′ and 5′-TCATTGTTGAAGTGCATTTT-3′), AtDCP2 (5′-ATGTCGGGCCTCCATCG-3′ and 5′-TCAAGCTGAATTACCAGATT-3′), and EF1a (5′-GCTGTCCTTATCATTGACTCCACC-3′ and 5′-TCATACCAGTCTCAACACGTCC-3′). *A. tumefaciens* GV3101 carrying either pIGDCP2-GFP and pBICDCP1-GFP were used to complement *dcp2-1* and transform Col-0, respectively.

3. Results

3.1. Identification of AtDCP1 and AtDCP2

To identify *Arabidopsis* decapping proteins, we searched the *Arabidopsis* genomic database revealing AtDCP1 (At1g08370) and AtDCP2 (At5g13570). (AtDCP1 was reported previously [7].) Schematic diagrams of these homologs are presented in Fig. 1. AtDCP1 and AtDCP2 contain domains highly conserved among yeast and human decapping proteins. AtDCP1 has consensus amino acid residues in an N-terminal EVH1 domain (Fig. 1A), a putative binding site for decapping regulatory proteins [8]. On the other hand, AtDCP2 has two well-conserved Box A and Nudix hydrolase domains (Fig. 1B). The box A domain of hDcp2 has a role in recognition of the correct phosphate within the cap pyrophosphate linkage [19], while the Nudix domain contains a Nudix motif, which is a catalytic center of Dcp2 [5–7,19–22]. In the Nudix motif, AtDCP2 contains conserved glutamate residues (Fig. 1C), essential for the decapping activity of yeast Dcp2p [9]. Because of these highly conserved domains, we considered AtDCP1 and AtDCP2 to be homologs of the decapping proteins.

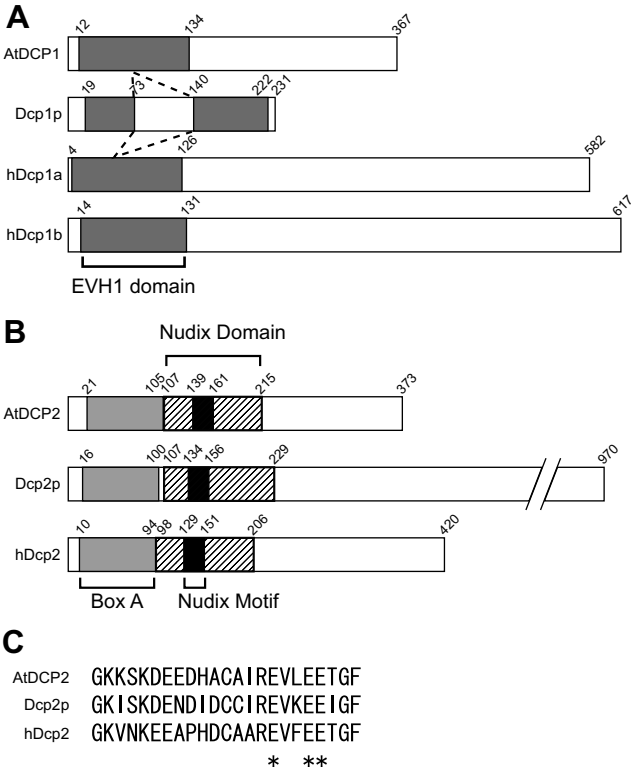

Fig. 1. *Arabidopsis* decapping proteins. (A) Schematics of yeast Dcp1p, human hDcp1a and hDcp1b, and *A. thaliana* AtDCP1. The gray box represents the EVH1 domain conserved among the Dcp1s. (B) Schematics of yeast Dcp2p, human hDcp2, and *A. thaliana* AtDCP2. The gray box, shaded box, and black box represent the Box A domain, Nudix domain, and Nudix motif, respectively. (C) Alignment of the Nudix Motif in AtDCP2, Dcp2p, and hDcp2. Asterisks indicate glutamate residues essential for the decapping activity of yeast Dcp2p.

3.2. AtDCP2 is an active decapping enzyme

To confirm the decapping activity of AtDCP1 and AtDCP2, we carried out an in vitro decapping assay. As shown in Fig. 2, decapping activity was detected with GST-AtDCP2, but not with GST alone nor with GST-AtDCP1 (Fig. 2, lanes 1–3). The addition of GST-AtDCP1 did not enhance the decapping activity of GST-AtDCP2 under our experimental conditions (Fig. 2, lanes 4–6). These results indicate that AtDCP2 is an active decapping enzyme.

3.3. AtDCP1 and AtDCP2 colocalize to cytoplasmic foci

Because Dcp1 and Dcp2 colocalize to P-bodies in yeast and humans, we examined the subcellular localization of AtDCP1 and AtDCP2. As shown in Fig. 3A, in agroinfiltrated *Nicotiana benthamiana* leaves, AtDCP1-RFP clearly localized to cytoplasmic granule-like foci (Fig. 3A, left panel) and AtDCP2-GFP was observed throughout the cytoplasm and in punctate spots (Fig. 3A, center panel). The AtDCP1-RFP and AtDCP2-GFP dots overlapped in merged images

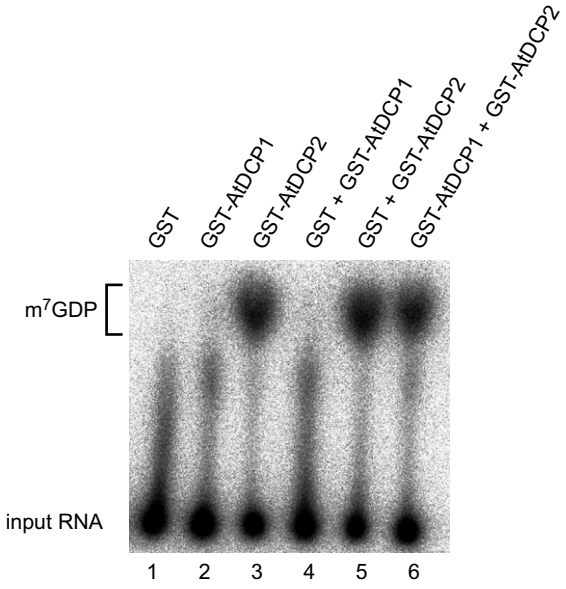

Fig. 2. In vitro decapping assay with AtDCP1 and AtDCP2. Cap-labeled RNA was incubated with either GST (lane 1), GST-AtDCP1 (lane 2), GST-AtDCP2 (lane 3), GST plus GST-AtDCP1 (lane 4), GST plus GST-AtDCP2 (lane 5), or GST-AtDCP1 plus GST-AtDCP2 (lane 6). The position of the input RNA, which remained at the origin and the m^7GDP are indicated on the left. The positions of m^7GDP on the TLC plates were confirmed using unlabeled m^7GDP standards.

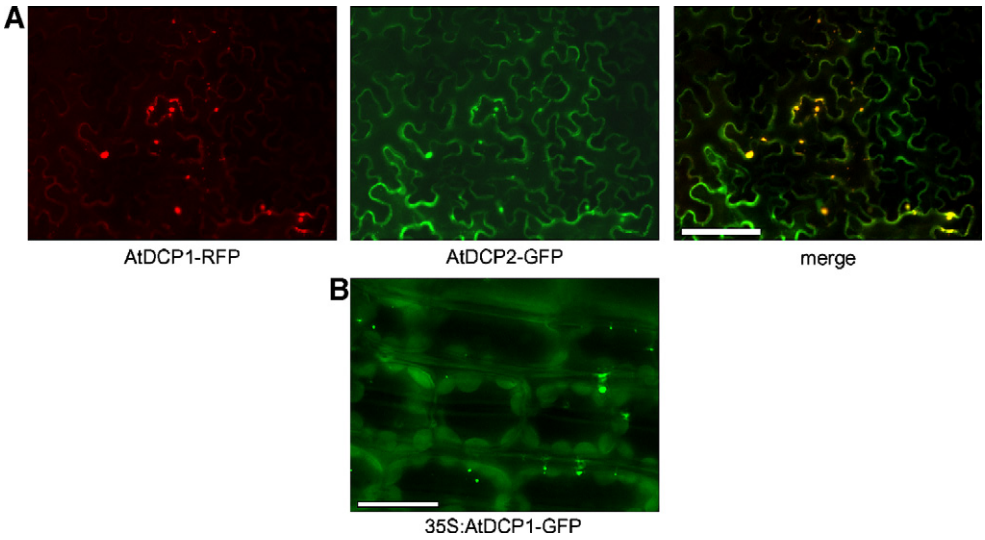

Fig. 3. Localization of the *Arabidopsis* decapping proteins. (A) Transiently expressed AtDCP1 and AtDCP2 colocalized to cytoplasmic foci. Epidermal cells of agroinfiltrated *Nicotiana benthamiana* leaves were observed under a fluorescent microscopy at 3 days post infiltration (dpi). (B) AtDCP1-GFP localized in cytoplasmic foci in epidermal cells of an inflorescence stem of a transgenic *Arabidopsis*. Scale bars: (A) 100 μm; (B) 20 μm.

(Fig. 3A, right panel), showing that both AtDCP1 and AtDCP2 colocalize in the cytoplasmic foci. Similar colocalization patterns were observed using AtDCP1-GFP and AtDCP2-RFP (data not shown). In addition, similar dot structures were observed in the transgenic *Arabidopsis* plants (Fig. 3B). Collectively, these data suggest that AtDCP1 and AtDCP2 colocalize in *Arabidopsis* P-bodies.

3.4. Coimmunoprecipitation analysis of decapping proteins

In yeast, Dcp1p interacts with Dcp2p [4,8]. To test whether AtDCP1 interacts with AtDCP2, we carried out immunoprecipitation analysis. No interaction was detected between AtDCP1 and AtDCP2 under our experimental conditions (Fig. 4A). This result suggests that AtDCP1 does not directly interact with AtDCP2 or that the interaction between AtDCP1 and AtDCP2 is very weak.

During the course of the immunoprecipitation experiments, we observed extra bands with a higher molecular weight than that of AtDCP1-GFP in the inoculation with AtDCP1-GFP plus an empty vector (data not shown). We therefore hypothesized that AtDCP1 interacts with itself to form a homooligomer. Immunoprecipitation experiments confirmed that AtDCP1 interacts with itself (Fig. 4B).

3.5. dcp1 and dcp2 mutants exhibit seedling lethality and defect in vascular development

To examine the roles of AtDCP1 and AtDCP2 in plants, we isolated two T-DNA insertion mutants of *Arabidopsis*, *dcp1-2* and *dcp2-1*. We determined T-DNA insertion sites (Fig. 5A) and confirmed no detectable accumulation of AtDCP1 and AtDCP2 transcripts in *dcp1-2* and *dcp2-1*, respectively (Fig. 5B). Morphological observation of *dcp1-2* and *dcp2-1* further showed that both mutants exhibit similar phenotypes; both mutants were seedling lethal, showed arrested post-embryonic development including cotyledon expansion (Fig. 5C, top panels), development of vascular networks (Fig. 5C, middle panels), root elongation (Fig. 5C, bottom panels), and shoot development (Fig. 5C, top panels). In *dcp2-1*, these developmental defects were recovered by the expression AtDCP2-GFP (Fig. 5D). (Because these lines formed no flower buds, we could not have the seeds of these lines.) These results indicate that both AtDCP1 and AtDCP2

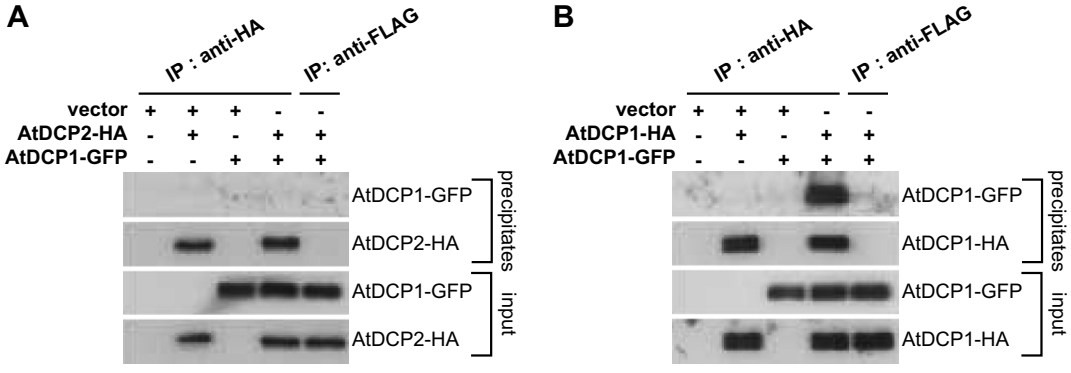

Fig. 4. AtDCP1 interacts with itself. (A) GFP-tagged AtDCP1 and HA-tagged AtDCP2 transiently expressed in *Nicotiana benthamiana* leaves and then subjected to anti-HA or anti-FLAG immunoprecipitation. (B) GFP-tagged AtDCP1 and HA-tagged AtDCP1 transiently expressed in *Nicotiana benthamiana* leaves and then subjected to anti-HA or anti-FLAG immunoprecipitation.

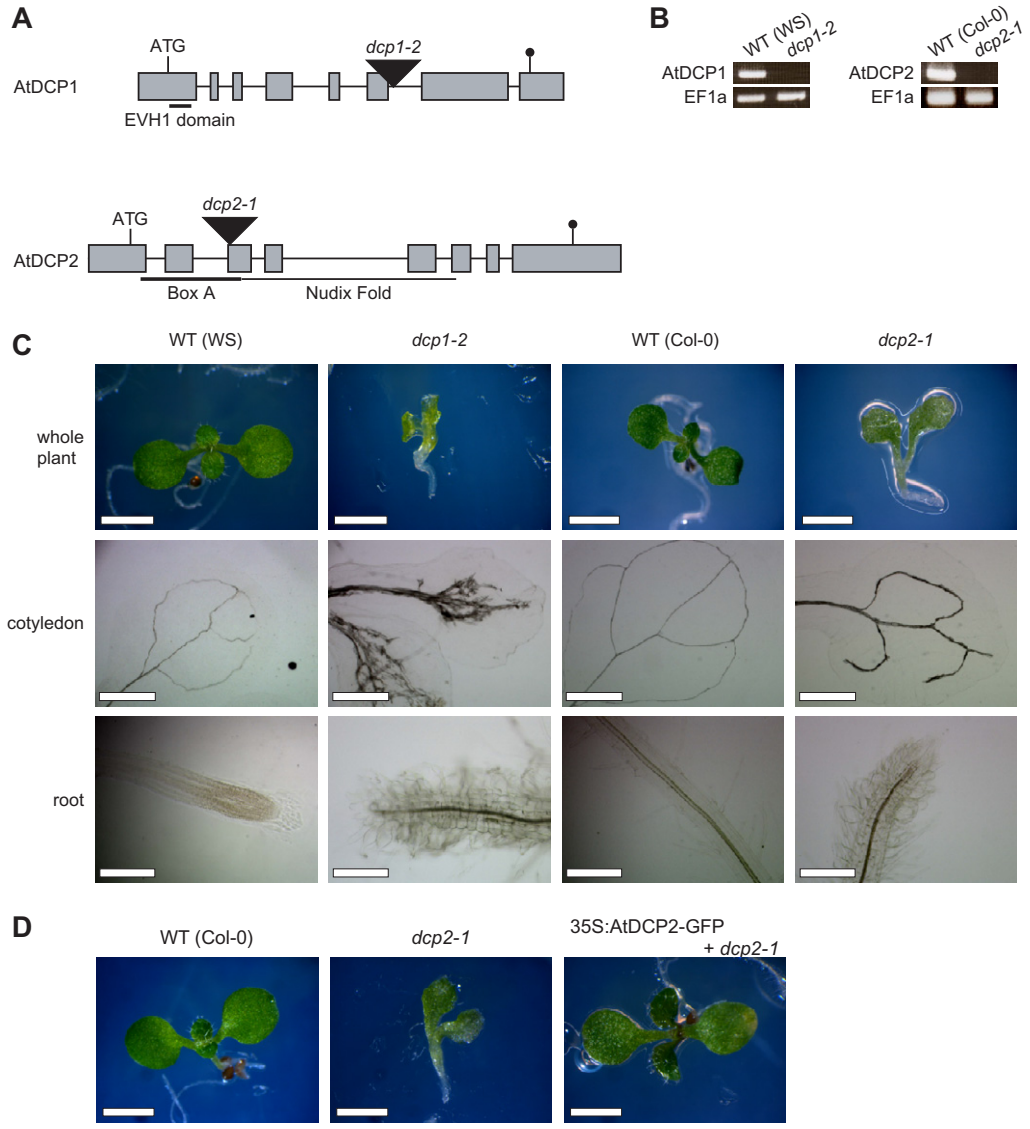

Fig. 5. *Arabidopsis* decapping proteins are essential for post-embryonic development. (A) Exon-intron structures of AtDCP1 and AtDCP2. Triangles show the positions of the T-DNA insertion in *dcp1-2* and *dcp2-1*. (B) RT-PCR analysis of *dcp1-2* and *dcp2-1*. The translation elongation factor EF1a was used as a control. (C) Phenotypes of decapping protein mutants. Top, middle, and bottom panels represent whole plants, cotyledons, and roots, respectively. (D) The expression of AtDCP2-GFP-complemented dcp2-1 phenotypes. Scale bar: (C top panels, D) 2 mm; (C middle panels) 500 μm; (C bottom panels) 200 μm.

are essential for post-embryonic development and strongly suggest that AtDCP1 and AtDCP2 function in the same pathway in plant development.

4. Discussion

In this study, we identified and characterized two plant decapping proteins. AtDCP1 and AtDCP2 are thought to work together in plant development, because of their similar phenotypes with *dcp1-2* and *dcp2-1* (Fig. 5) and their colocalization (Fig. 3). Considering the decapping activity of AtDCP2 (Fig. 2), we propose that AtDCP1/AtDCP2-mediated mRNA turnover has an essential role in post-embryonic development. Typical phenotypic defects in *dcp1-2* and *dcp2-1* are an open venation pattern lacking distal vein meeting in cotyledons and reduced meristematic activity in the shoots and roots (Fig. 5). Because similar defects have been observed in some auxin mutants [23,24], genes downregulated in response to auxin in post-embryonic development may be plausible candidates degraded in AtDCP1/AtDCP2-mediated pathways. Interestingly, seedlings of both *dcp1-2* and *dcp2-1* had the ability to produce a callus (data not shown), suggesting that AtDCP1 and AtDCP2 are not indispensable for cell division or viability under a dedifferentiated status.

Although in yeast Dcp1p enhances decapping activity of Dcp2p [20], we did not detect AtDCP1-mediated enhancement of decapping activity (Fig. 2). Our decapping data are consistent with those of previous studies in humans and *Caenorhabditis elegans* [5,7,22]. Yeast Dcp1p is thought to directly interact with Dcp2p [4,8], whereas human hDcp1a indirectly interacts with hDCP2 through the decapping component Hedls, which enhances decapping activity of hDcp2 [9]. Interestingly, *Arabidopsis* contains a Hedls homolog called VARI-

COSE (VCS). Because *vcs* mutants show abnormal vein patterning [25] similar to *dcp1-2* and *dcp2-1* (Fig. 5), VCS is thought to be a decapping component that works together with decapping proteins in *Arabidopsis*. AtDCP1 may therefore bind with AtDCP2 through VCS. In this context, the lack of an interaction between AtDCP1 and AtDCP2 in our assay may be explained by the shortage of VCS as observed in human cells [9]. Collectively, we hypothesize that AtDCP1 does not directly interact with AtDCP2, and therefore, does not enhance the decapping activity of AtDCP2.

Although in yeast Dcp1p is required for decapping activity in vivo, roles of Dcp1 in higher eukaryotes remain unknown. The similar phenotypes of *dcp1-2* and *dcp2-1* suggest that AtDCP1 is probably involved in decapping in vivo. Western analysis of AtDCP1 showed an extra band (Fig. 4) similar to the result of human hDcp1 [5,9]. This extra band may correspond to a phosphorylated AtDCP1, as with phosphorylated Dcp1p in yeast [3]. Notably, we showed that AtDCP1 interacts with itself (Fig. 4). Further experiments need to be conducted to clarify not only the molecular mechanisms of AtDCP1 but also the modification of AtDCP1 and roles of AtDCP1-AtDCP1 interactions in decapping activity in vivo.

Here, we identified plant P-bodies using AtDCP1-GFP (Fig. 3B). Currently, it is unknown whether mRNA degradation proteins including 5′ to 3′ exonuclease, deadenylase, decapping regulators, Upf1 (an NMD factor), and Argonaute proteins localize to P-bodies and whether plant P-bodies store untranslated mRNAs. We believe therefore that AtDCP1-GFP is a marker for further analysis of P-bodies in plants.

Notes added in proof

During our submission, Xu et al. (Plant Cell 18, 3386-3398 (2006)) reported a similar characterization of DCP1, DCP2 and VARICOSE proteins. To avoid unnecessary confusion in the future, we adopted their mutant allele numbering for *dcp1-2*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2007.04.051.

References

[1] Coller, J. and Parker, R. (2004) Eukaryotic mRNA decapping. Annu. Rev. 73, 861–890.
[2] Beelman, C.A., Stevens, A., Caponigro, G., LaGrandeur, T.E., Hatfield, L., Fortner, D.M. and Parker, R. (1996) An essential component of the decapping enzyme required for normal rates of mRNA turnover. Nature 382, 642–646.
[3] LaGrandeur, T.E. and Parker, R. (1998) Isolation and characterization of Dcp1p, the yeast mRNA decapping enzyme. EMBO J. 17, 1487–1496.
[4] Dunckley, T. and Parker, R. (1999) The DCP2 protein is required for mRNA decapping in *Saccharomyces cerevisiae* and contains a functional MutT motif. EMBO J. 18, 5411–5422.
[5] Lykke-Anderson, J. (2002) Identification of a human decapping complex associated with hUpf proteins in nonsense-mediated decay. Mol. Cell. Biol. 22, 8114–8121.
[6] Wang, Z., Jiao, X., Carr-Schmid, A. and Kiledjian, M. (2002) The hDcp2 ptotein is a mammalian mRNA decapping enzyme. Proc. Natl. Acad. Sci. USA. 99, 12663–12668.
[7] van Dijk, E., Cougot, N., Meyer, S., Babajko, S., Whale, E. and Séraphin, B. (2002) Human Dcp2: a catalytically active mRNA decapping enzyme located in specific cytoplasmic structures. EMBO J. 21, 6915–6924.
[8] She, M., Decker, C.J., Sundramurthy, K., Liu, Y., Chen, N., Parker, R. and Song, H. (2004) Crystal structure of Dcp1p and its functional implications in mRNA decapping. Nat. Struct. Mol. Biol. 11, 249–256.
[9] Fenger-Grøn, M., Fillman, C., Norrild, B. and Lykke-Andersen, J. (2005) Multiple processing body factors and the ARE binding protein TTP activate mRNA decapping. Mol. Cell 20, 905–915.
[10] Sheth, U. and Parker, R. (2003) Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. Science 300, 805–808.
[11] Ingelfinger, D., Arndt-Jovin, D.J., Luhrmann, R. and Achsel, T. (2002) The human LSm1-7 proteins colocalize with the mRNA-degrading enzymes Dcp1/2 and Xrnl in distinct cytoplasmic foci. RNA 8, 1489–1501.
[12] Eystathioy, T., Jakymiw, A., Chan, E.K., Séraphin, B., Cougot, N. and Fritzler, M.J. (2003) The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. RNA 9, 1171–1173.
[13] Cougot, N., Babajko, S. and Séraphin, B. (2004) Cytoplasmic foci are sites of mRNA decay in human cells. J. Cell Biol. 165, 31–40.
[14] Brengues, M., Teixeira, D. and Parker, R. (2005) Movement of eukaryotic mRNAs between polysomes and cytoplasmic processing bodies. Science 310, 486–489.
[15] Fillman, C. and Lykke-Andersen, J. (2005) RNA decapping inside and outside of processing bodies. Curr. Opin. Cell Biol. 17, 326–331.
[16] Sen, G.L. and Blau, H.M. (2005) Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. Nat. Cell Biol. 7, 633–636.
[17] Liu, J., Valencia-Sanchez, M.A., Hannon, G.J. and Parker, R. (2005) MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat. Cell Biol. 7, 719–723.
[18] Takeda, A., Tsukuda, M., Mizumoto, H., Okamoto, K., Kaido, M., Mise, K. and Okuno, T. (2005) A plant RNA virus suppresses RNA silencing through viral RNA replication. EMBO J. 24, 3147–3157.
[19] Piccirillo, C., Khanna, R. and Kiledjian, M. (2003) Functional characterization of the mammalian mRNA decapping enzyme hDcp2. RNA 9, 1138–1147.
[20] Steiger, M., Carr-Schmid, A., Schwartz, D.C., Kiledjian, M. and Parker, R. (2003) Analysis of recombinant yeast decapping enzyme. RNA 9, 231–238.
[21] She, M., Decker, C.J., Chen, N., Tumati, S., Parker, R. and Song, H. (2006) Crystal structure and functional analysis of Dcp2p from *Schizosaccharomyces pombe*. Nat. Struct. Mol. Biol. 13, 63–70.
[22] Cohen, L.S., Mikhli, C., Jiao, X., Kiledjian, M., Kunkel, G. and Davis, R.E. (2005) Dcp2 decaps $m^{2,2,7}$GpppN-capped RNAs, and its activity is sequence and context dependent. Mol. Cell. Biol. 25, 8779–8791.
[23] Steynen, Q.J. and Schultz, E.A. (2003) The FORKED genes are essential for distal vein meeting in Arabidopsis. Development 130, 4695–4708.
[24] Hardtke, C.S. and Berleth, T. (1998) The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J. 17, 1405–1411.
[25] Deyholos, M.K., Cavaness, G.F., Hall, B., King, E., Punwani, J., Van Norman, J. and Sieburth, L.E. (2003) VARICOSE. A WD-domain protein, is required for leaf blade development. Development 130, 6577–6588.