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ARABIDOPSIS DCP5, A DECAPPING COMPLEX PROTEIN INTERACTS WITH UBIQUITIN-5 IN THE PROCESSING BODIES

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Abstract

Exclusively in eukaryotes, turnover of messenger RNA (mRNA) involves the expulsion of methylated-7-guanosine-di-phosphate (m7GDP) present at the 5' end, referred to as decapping of mRNA. The decapping event modulates plant developmental processes. Primarily the decapping process is executed by several decapping complex protein factors, of which DCP1, DCP2 and DCP5 are indispensable. In plants, DCP5 participates in proper functioning of processing bodies (P bodies), whereby the aberrant mRNAs are accumulated for decay. The molecular mechanisms underlying the DCP5 mediated processing of mRNAs are appealing. The results of the present investigation showed that DCP5 physically interacts with Ubiquitin 5. The ubiquitin is a 76-amino acid polypeptide that acts as a covalent modifier of innumerable proteins, resulting in cellular homeostasis. Ubiquitin protein initiates the process of ubiquitination resulting in paving path for the proteins to their final destination, protein decay. Future studies may reveal novel role of ubiquitin proteins in the process of mRNA decay.

Key words: mRNA decapping, Decapping protein complex, Ubiquitin, Ubiquitination

Introduction

In eukaryotes, mRNA turnover primarily involves the elimination of m^7 GDP from the 52 end. The 5' monophosphate is a substrate for the 5' exonuclease XRN1 (Poole and Audrey, 1997) resulting in the quick decay of the mRNA. Primarily, a protein complex mediates the decapping reaction. The decapping protein complex is well characterized in yeast and human but not in plants. In *Arabidopsis thaliana*, DCP1, DCP2, DCP5 are the central decapping protein factors. The m^7GDP cap is specifically removed by the DCP2 which posses the pyrophosphatase activity (Xu *et al.,* 2006; Iwasaki *et al.,* 2007). DCP1, DCP2 and DCP5 localize in *Arabidopsis* processing bodies (P bodies), depicting their role in mRNA decay (Xu *et al.,* 2006; Xu *et al.,* 2009). mRNA decapping event occurring inside P bodies is tightly regulated, chiefly a irreversible process. In eukaryotes, messenger ribo-nucleoproteins (mRNPs) assemble together into the P bodies (Sheth and Parker, 2003). Most importantly, P bodies function includes mRNA storage, mRNA decapping and translational repression (Yu *et al.,* 2019). Regulation of mRNA translatability is associated with the P-body assembly. But, the translational arrest may be reversed, enabling the reengagement of some mRNAs into the translational machinery to resume translation (Brengues *et al.,* 2005). The mechanisms regulating the mRNA decapping process are still unknown.

Ubiquitination, a unique post-translational modification event in eukaryotes is vital for maintaining the protein homeostasis (Hershko and Ciechanover, 1998; Khoury *et al.,* 2011; Walsh, 2006). Mainly, ubiquitination involves covalent add-on of a small ubiquitin protein into the target protein through a series of reactions, essentially facilitated by three classes of enzymes, E1, E2 and E3. E1 is the ubiquitin-activating enzyme (UBA), E2 is the ubiquitin-conjugating enzyme (UBC) and E3 is the ubiquitin ligase (Callis, 2014). Ubiquitin, a highly conserved protein contains 76-amino acid residues (Callis and Vierstra, 1989). In eukaryotes, multiple genes encode ubiquitin resulting into two dissimilar types of translational fusions referred to as homomeric fusions and heteromeric fusions. Homomeric fusions comprise of multimers of ubiquitin coding regions (polyubiquitin) whereas, heteromeric fusions, the 76-amino acid residue ubiquitin is followed by a different protein (ubiquitin-extension proteins), mostly small ribosomal proteins or else, a ubiquitin-like protein, RUB (Burke *et al.,* 1988; Callis and Vierstra, 1989; Callis *et al.,* 1990; Callis *et al.,* 1995). Till date, ubiquitin gene

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count in *Arabidopsis thaliana* is twelve (Table 1: Callis and Vierstra, 1989; Callis *et al.*, 1990; Sun and Callis, 1993; Callis *et al.*, 1995). The present investigation shall try to show the physical interaction between *Arabidopsis thaliana* UBQ5 and DCP5. It is intriguing to reveal the basis of this interaction as it would facilitate in one hand to understand the role of DCP5 beyond its canonical activity and in other hand, the role of ubiquitin in maintaining the protein homeostasis.

Materials and Methods

Plant materials and growth conditions

Nicotiana benthamiana plants for transient expression of target proteins, particularly for confocal experiments were grown in soil. Temperature was maintained at 22° C with 70–80% relative humidity under long day (12/12 h light/dark) conditions.

Plasmids

DCP5 and UBQ5 full-length cDNAs were obtained by RT-PCR using Col-0 RNA. The PCR products were cloned into pDONR207 and sequenced. Wild type (WT) coding sequences of DCP5 and UBQ5 were cloned into expression vectors (GFP, RFP and BiFC vectors).

Agro-infiltration and confocal laser scanning microscopy (CLSM)

The binary clones were transformed into *Agrobacterium tumefaciens* strain (GV3010), and agro-infiltrated into *Nicotiana benthamiana* leaves. Two days post-agro-infiltration, the infected leaves were analyzed under CLSM.

Statistical analysis

For calculating the number of P bodies observed in a definite cell, total area of the cell in terms of μm^2 and total number of P bodies occurring in the cell were recorded. Then, average number of P bodies per unit area of the cell was calculated. Experiments were done in triplicates to validate the results.

Results and Discussion

UBQ5 and DCP5 localizes to cytoplasm and P bodies respectively:

One of the central decapping protein factors, DCP5 is comprised of several vital domains including DFDF positioned from 453-489 amino acid residues and FFD/TFG positioned from 512-554 amino acid residues (Fig. 2a, 2b, 2c). Likewise, UBQ5 contains ubiquitin coding domain fused with the 40S ribosomal protein S27a-3 (Fig. 1a, 1b, 1c). UBQ5 when over-expressed under *35S* Cauli Mosaic Virus (CMV) promoter localized to the cytoplasm (Fig. 3). Under similar conditions, over-expressed DCP5 localized to P bodies. Canonically, DCP5 is involved in mRNA decapping, primarily which occurs at P bodies. Moreover, UBQ5 localization points out towards the role of ubiquitin, mainly in the protein homeostasis.

UBQ5 and DCP5 co-localize and physically interact in the P bodies:

UBQ5 re-localized to the P bodies when co-expressed with the DCP5 (Fig. 4a). To validate whether there is any physical interaction among the DCP5 and UBQ5, we co-infiltrated DCP5-VYNE and UBQ5-CYCE

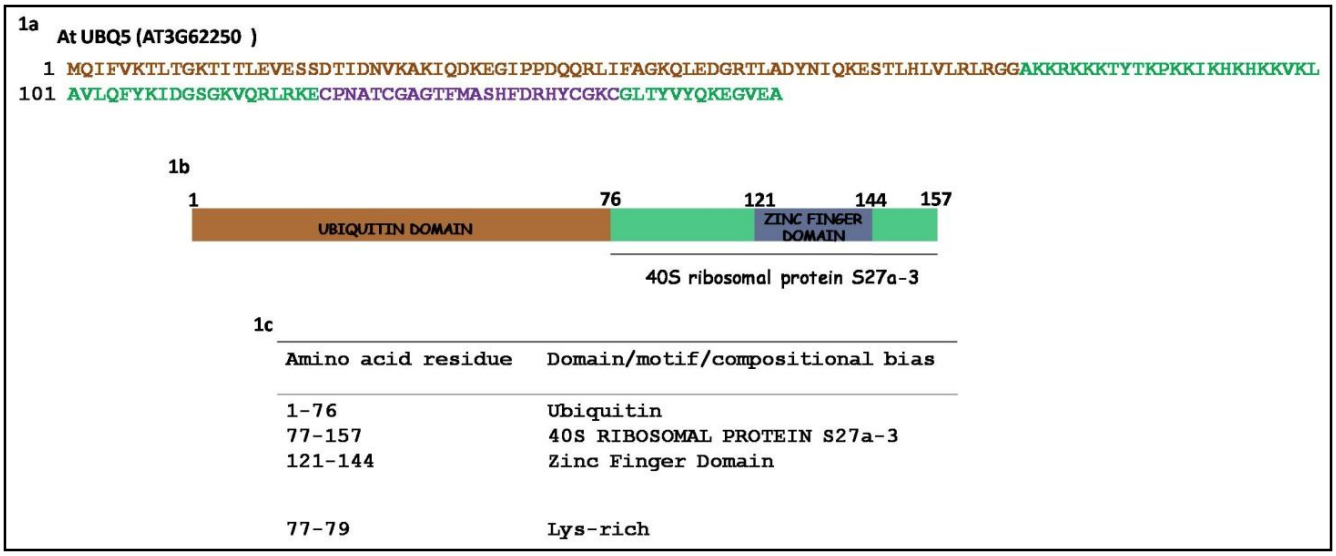

Fig. 1: Amino acid sequence and protein domain organization of UBQ5:
1a. Sequence of UBQ5 protein is shown using single-letter codes for amino acids.
1b. Domain organization of *Arabidopsis* UBQ5: Domain information was generated with UniProt.
1c. Amino acid residues of UBQ5 representing domain/motif/compositional bias.

Table 1: List of Ubiquitin proteins in *Arabidopsis thaliana*.

AGI Number	Gene	Gene product
AT3G52590	UBQ1	Fusion of ubiquitin and 52 amino acid residue RP L40 (RP-Ribosomal product)
AT2G36170	UBQ2	Fusion of ubiquitin and 52 amino acid residue RP L40 (RP-Ribosomal product)
AT5G03240	UBQ3	Polyubiquitin
AT5G20620	UBQ4	Polyubiquitin
AT3G62250	UBQ5	Fusion of ubiquitin and RP S27a-3
AT2G47110	UBQ6	Fusion of ubiquitin and RP S27a-2
AT2G35635	UBQ7	Ubiquitin-RUB1 fusion
AT4G05320	UBQ10	Polyubiquitin
AT4G05050	UBQ11	Polyubiquitin
AT4G02890	UBQ14	Polyubiquitin
AT1G31340	UBQ15	Ubiquitin-RUB2 fusion
AT1G23410	UBQ17	Fusion of ubiquitin and RP S27a-1

(BiFC fusion proteins). BiFC studies pointed out the DCP5-UBQ5 interaction as the fluorescence signal was seen in the P bodies (Fig. 4b), suggesting that the DCP5 is involved in re-localization of the UBQ5 into the P bodies by physical interaction. This is quite interesting, since; previously it was known that DCP5 is well involved in targeting mRNA (Xu *et al*., 2009) whereas, UBQ5 performs its canonical role by targeting proteins (Callis, 2014). Interaction among two distantly related protein factors particularly in terms of their targets possibly may provide new directions to understand the underlying molecular mechanisms, mainly the role of mRNA decapping factors in the protein degradation pathway. Also, the number of P bodies formed by the DCP5 when co-expressed with UBQ5 was reduced up to 35% as compared to the number of P bodies formed when DCP5

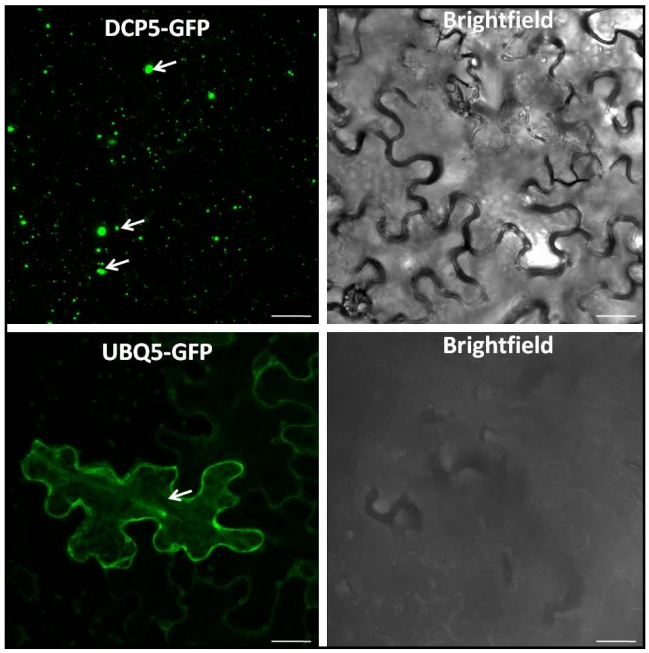

Fig. 3: Independently, DCP5 and UBQ5 localizes to P bodies and cytoplasm respectively. DCP5-GFP localizes to P bodies, whereas, UBQ5-GFP localizes to the cytoplasm when transiently expressed in the *Nicotiana benthamiana* as indicated by the arrow marks. (Scale bar: 10µm).

2a

At DCP5 (AT1G26110)

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  1 MAADNTGSKSSSAADSYVGSLISLTSKSEIRYEGILYNINTDESSIGLQNVRSFGTEGRKKDGPQVPPSDKVYEYILFRGTDIKDLQVKASPPVQPPAST
101 INNDPAIIQSHYPSPMPTSGSLPSTASGSLPDISSHNGQPGQHGMGFQNAMPLYQPGGNLGSWGASPQPPMYWQGFYTPPPNGLPQLHQQSLIRPPHGLP
201 MPNSLQQPLQYPNFNTPPPPTGSSSLQGSSLPEAPSSLFPFSTSSQMLAPSSLPFPGLPPVTLSSSLQSTLQSAPSPSLASEMAPPLLSNKAPITAPPTL
301 PQDTNLLSFSLSTTRATEASTGLPLSNKPSVVTGPISPPQTTPLTSAPVAGVSSSISQDKPKPLLVTPGQLLQSGSSAVSLSPPSTNADKDVEVVQVSSS
401 AGLEQSVPVTSEAQPPILPLPSSARPTQKPNGHSFPNHNGYRGRGRGRGRGAGRSHQVMKFTEDFDFTAMNEKFNKDEVWGHLGKSTTLDGDEDDDSPTV
501 DEAELPKIEAKPVYNKDDFFDSLSSNTIDRESQNSRPRFSEQRKLDTETFGEFSRFRGGRGGRGGYGRNNGYSRGGYGGRGYGGYGGRGGGGGGYGYGGR
601 GQGRGVSNRTT
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2b

2c

Amino acid residue	Domain/motif/compositional bias
453-489	DFDF (Domain)
512-527	FFD box (motif)
534-554	TFG box (motif)
92-301	Pro-rich (compositional bias)
558-605	Gly-rich (compositional bias)

Fig. 2: Amino acid sequence and protein domain organization of DCP5:
2a. Sequence of DCP5 protein is shown using single-letter codes for amino acids.
2b. Domain organization of *Arabidopsis* DCP5: Domain information was generated with UniProt.
2c. Amino acid residues of DCP5 representing domain/motif/compositional bias.

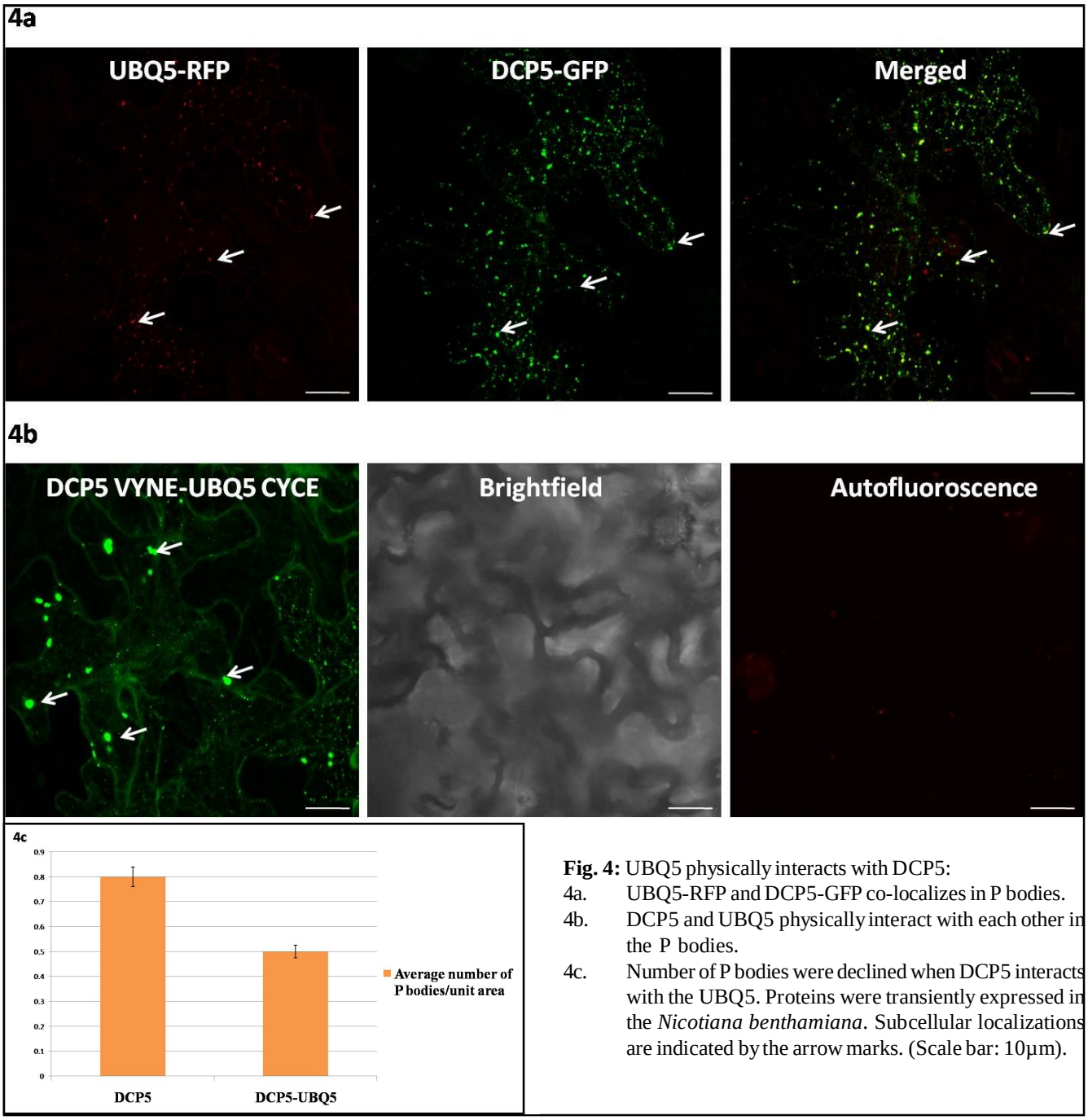

Fig. 4: UBQ5 physically interacts with DCP5:
4a. UBQ5-RFP and DCP5-GFP co-localizes in P bodies.
4b. DCP5 and UBQ5 physically interact with each other in the P bodies.
4c. Number of P bodies were declined when DCP5 interacts with the UBQ5. Proteins were transiently expressed in the *Nicotiana benthamiana*. Subcellular localizations are indicated by the arrow marks. (Scale bar: 10µm).

was singly expressed (Fig. 4c). This shows that UBQ5 may regulate the function of DCP5, resulting in the unavailability of DCP5 for its canonical function in the P bodies.

Conclusion

Decapping of mRNA is quite vital, primarily for maintaining the homeostasis of functional mRNA. Even though several studies have revealed the underlying principles behind this significant molecular event, still the role of decapping complex proteins are at primitive level. In plants, DCP5 being a key player in maintaining the P body function by actively participating in the mRNA decapping process, its non-canonical function may also be quite intriguing. Ubiquitin, primarily being responsible for targeting specific proteins into the protein degradation pathway interacts with DCP5 and moreover effects the sub-cellular localization of the DCP5, thus suggesting its role in the turnover of mRNA. In future studies, it would be quite interesting to reveal the role of plant ubiquitins beyond their canonical function.

Author contribution statement

Kunja Bihari Satapathy and Gagan Kumar Panigrahi

conceived the idea. Gagan Kumar Panigrahi performed the experiments. Kunja Bihari Satapathy and Gagan Kumar Panigrahi analyzed the idea. Kunja Bihari Satapathy and Gagan Kumar Panigrahi wrote the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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