SHORT COMMUNICATION

Plant Signaling & Behavior 8:5, e23975; May 2013; © 2013 Landes Bioscience

Role of *Arabidopsis* Pumilio RNA binding protein 5 in virus infection

Sung Un Huh and Kyung-Hee Paek*

School of Life Sciences and Biotechnology; Korea University; Seoul, Republic of Korea

Keywords: RNA-binding proteins (RBPs), Pumilio/FBF (Puf) protein, Pumilio homology domain (PHD), *hunchback* (*hb*), Cucumber mosaic virus (CMV)

Regulation of gene expression is mediated by diverse RNA binding proteins which play important roles in development and defense processes. Pumilio/FBF (Puf) protein in mammals functions as a posttranscriptional/translational repressor by binding to the 3' UTR regions of its target mRNAs. Previous study reported that APUM5 provides protection against CMV infection by directly binding to CMV RNAs in *Arabidopsis*. CMV RNAs contain putative Pumilio-binding motifs and APUM5 bound to the 3' UTR and some of its internal motifs both in vitro and in vivo. APUM5 works as a negative regulator of the 3' UTR of CMV and it might regulate CMV replication. Our findings suggest that APUM5 acts as a defensive repressor in plants during CMV infection. However, functions of APUM5 and other APUM members are still not clear and more studies are needed to find out the interacting partners and target mRNAs in host plant.

Pumilio/FBF (Puf) proteins have an evolutionary conserved Pumilio homology domain (PHD) and are found in organisms as diverse as protozoa, fungi, mammals and plants.[1-3] Puf is proposed to function as a posttranscriptional/translational repressor by interacting with sequence-specific motifs in the 3' UTR of its target mRNAs[4] or by associating with rRNA processing, possibly in a sequence non-specific manner in the nucleolus.[5,6] The targets of Puf proteins are involved in differentiation, development, cell cycle, stem cell renewal and synaptic functions.[7-9] In plant, Puf-like proteins are encoded by more copies of genes than mammalian Puf proteins. For example, *Arabidopsis* and rice genomes contain 25 and 19 putative *Puf* genes whereas human, Drosophila, *C. elegans* and yeast genomes contain two, one, nine and six *Puf* genes, respectively. In a bioinformatics analysis, the expression of some plant *Puf* genes increased in response to biotic and abiotic stresses and exhibited tissue-dependent differential expression.[10] This demonstrates that plant Puf proteins could play diverse roles in plant development, differentiation and biotic and abiotic stresses.

Recently, targets of the *Arabidopsis* Puf protein were selected by yeast three-hybrid (Y3H) screening using APUM2 as bait, and five target mRNA sequences were found.[11] Unexpectedly, these candidate genes were not associated with well-known target genes, such as those involved in development, stem cell renewal and differentiation. One of the candidate target mRNAs was *RD19* (At4g39090), *RESPONSIVE TO DEHYDRATION 19,* which responds to desiccation as an abiotic stress.[11] RD19 interacts with the *Ralstonia solanacearum* type III effector PopP2.[12] In terms of biotic stress regulation, the direct binding of mammalian Puf proteins to target genes has not yet been reported. However, the expression of 13 genes that encode proteins involved in antibacterial and antifungal activity was upregulated in Drosophila Pum mutant, as determined by genome-wide analysis,[13] although homologs of these genes do not exist in *Arabidopsis*.

Previous studies indicated that some Pufs localize predominantly to the cytoplasm, but diverse Pufs are also known to localize to multiple subcellular organelles.[14-16] CMV RNAs replicate in the vacuolar membranes and tonoplasts by means of replicase protein complexes.[17,18] In our study, some co-localization events were detected between APUM5-GFP and FM4-64 in the plasma membrane (**Fig. 1A and B**) and tonoplast-like structures (**Fig. 1A and C**). However, some of the regions labeled with APUM5-GFP did not coincide with FM4-64 labeling (**Fig. 1A**). In addition, some of the APUM5-GFP signal was detected in vesicle-like structures and in parts of the nucleus (**Fig. 1D-F**). Thus, APUM5 might have other functions to regulate endogenous target mRNAs because APUM5 localizes to multiple subcellular organelles.

APUM5 bound to the putative Pumilio-binding motifs in the CMV RNAs and *hb*NRE2.[19] Drosophila Pum binds to *hb*NRE2 mRNA and represses its ability to regulate embryo development via deadenlyase-dependent and -independent pathways.[20,21] This indicates that APUM5 might be associated with deadenylase-dependent and -independent pathways to repress CMV RNAs, even though CMV has a tRNA-like structure (TLS) instead of a poly(A) tail at its 3' end.[22] Interestingly, only CMV group-I but not CMV group-II strains contained conserved Pumilio-binding motifs at the 3' UTR. Furthermore, some internal, putative

*Correspondence to: Kyung-Hee Paek; Email: khpaek95@korea.ac.kr

Submitted: 01/14/13; Accepted: 02/12/13

Citation: Huh SU, Paek K-H. Role of *Arabidopsis* Pumilio RNA binding protein 5 in virus infection. Plant Signal Behav 2013; 6: e23075; http://dx.doi.org/10.4161/psb.23975

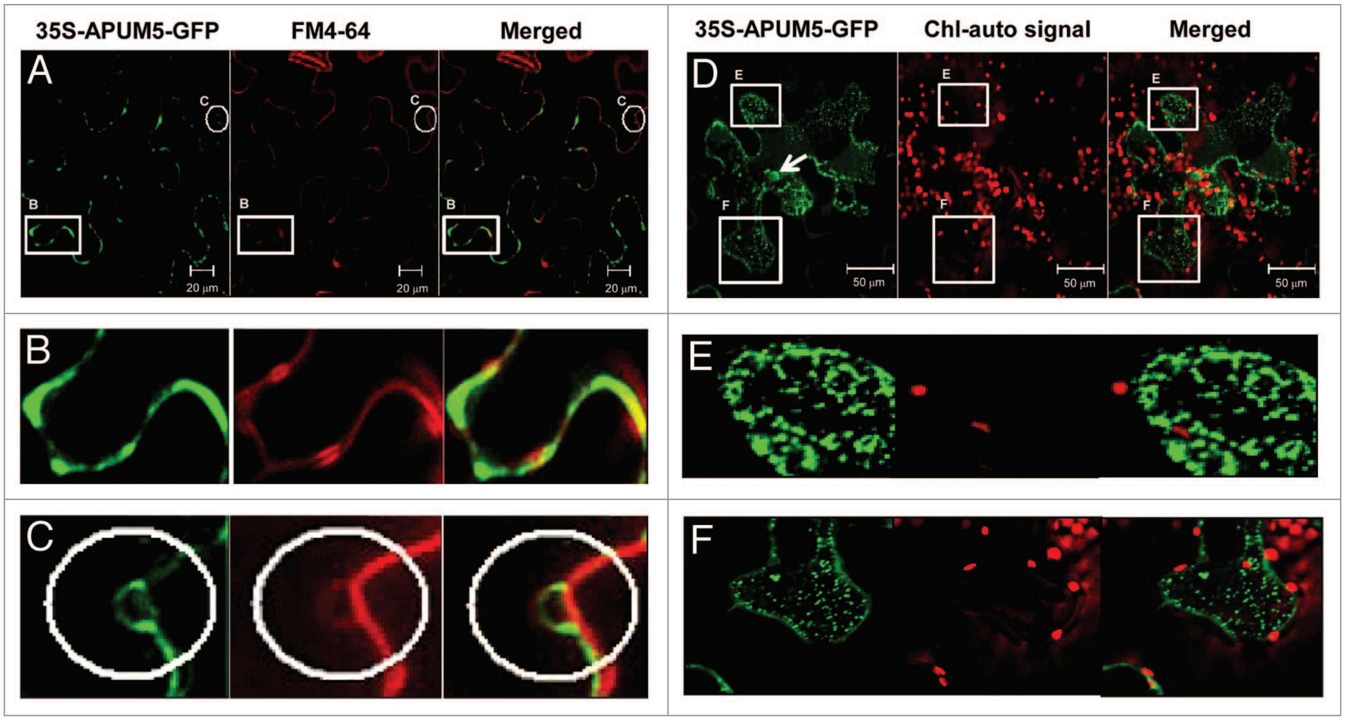

Figure 1. Subcellular Localization of APUM5-GFP in *N. benthamiana*. (**A**) C-terminal GFP-tagged *APUM5* was transiently expressed in *N. benthamiana*. The FM4-64 stain was used as a plasma membrane marker. Merged image shows colocalized regions. Scale bars are indicated at the pictures. (**B**) APUM5-GFP signals were merged with FM4-64 in the plasma membrane. (**C**) APUM5-GFP signals were merged with FM4-64 in vacuolar-like membrane. (**B and C**) Images are enlarged images of those indicated as boxes and circles in the (**A**) image. (**D**) APUM5-GFP was localized to vesicle-like structures and nucleus. The arrow indicates the nucleus. Red signals are chloroplast-auto signals. Scale bars are indicated at the pictures. (**E and F**) Enlarged images of those indicated by boxes in (**D**).

Pumilio-binding motifs of CMV RNAs could also serve as targets for the repression of CMV infection (**Fig. 2**).

mRNA repression by yeast Puf3, 4 and 5, human Pum, Drosophila Pum and *C. elegans* FBF seems to follow a general regulatory mechanism.[4,23] For example, yeast Puf5 directly interacts with Pop2p and forms the Ccr4-Pop2p-NOT mRNA deadenylase complex that then attacks the 3' UTR of target mRNA.[4,24] We investigated the *Arabidopsis* homologs of mammalian Pop2p deadenylase. Pop2p is also known as carbon catabolite repressor 4-CCR4-associated factor1 (CCR4-CAF1) and catalyzes mRNA deadenylation.[25] The CAF1 family in *Arabidopsis* has 11 members, some of which respond to environment and biotic stresses.[26] AtCAF1a and AtCAF1b have active involvement in biotic and abiotic stresses.[26] Thus, *Arabidopsis* APUM5 might interact with similar partners that are associated with deadenylation in planta through a more general model, as suggested by the Puf interaction with the Ccr4-Pop2p-NOT complex. Unlike Xenopus Pum2, APUM5 did not bind to the 5' m7G cap structure for blocking the assembly of the initiation complex (**Fig. 2**).[19,27] Thus, APUM5 might inhibit CMV infection together with unknown repression complexes.

Puf protein is very useful to regulate target RNA because of sequence-specific binding capacity. PHD of Puf could be engineered to specifically recognize different Pumilio binding site in animal system.[28-30] However, plant Puf protein function is still not fully known compared with animal Pufs. Thus, more accurate analysis of the function of APUM5 and other APUM family needs to be done in plant. And further finding of its endogenous mRNA targets and de novo interacting partners could also be helpful in dissecting the function of plant Puf proteins.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by the Wujangchoon Project (PJ007850) from the Rural Development Administration, Republic of Korea.

References

1. Wickens M, Bernstein DS, Kimble J, Parker R. A PUF family portrait: 3'UTR regulation as a way of life. Trends Genet 2002; 18:150-7; PMID:11858839; http://dx.doi.org/10.1016/S0168-9525(01)02616-6.
2. Wharton RP, Aggarwal AK. mRNA regulation by Puf domain proteins. Sci STKE 2006; 2006:pe37; PMID:17003467.
3. Quenault T, Lithgow T, Traven A. PUF proteins: repression, activation and mRNA localization. Trends Cell Biol 2011; 21:104-12; PMID:21115348; http://dx.doi.org/10.1016/j.tcb.2010.09.013.
4. Goldstrohm AC, Hook BA, Seay DJ, Wickens M. PUF proteins bind Pop2p to regulate messenger RNAs. Nat Struct Mol Biol 2006; 13:533-9; PMID:16715093; http://dx.doi.org/10.1038/nsmb1100.
5. Droll D, Archer S, Fenn K, Delhi P, Matthews K, Clayton C. The trypanosome Pumilio-domain protein PUF7 associates with a nuclear cyclophilin and is involved in ribosomal RNA maturation. FEBS Lett 2010; 584:1156-62; PMID:20153321; http://dx.doi.org/10.1016/j.febslet.2010.02.018.

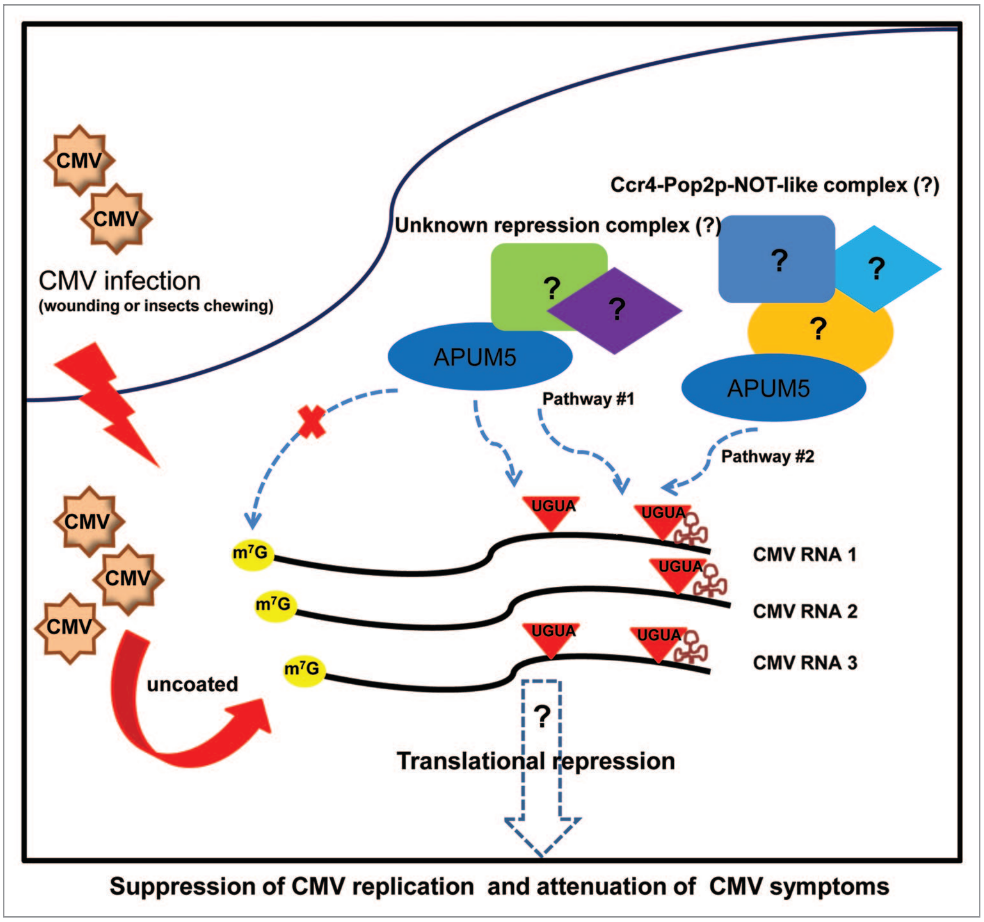

Figure 2. Proposed working model for APUM5 suppression of CMV infection. First, CMV entry into the host cells occurs by mechanical wounding or insects chewing. Then CMV RNAs replicate and synthesize viral proteins. CMV RNAs are stabilized by a tRNA-like structure (TLS) which is recognized by tRNA-specific enzymes. APUM5 recognizes the CMV 3' UTR and some internal Pumilio binding motifs in the CMV genome either with unknown repression complexes or in association with Ccr4-Pop2p-NOT-like complex. Finally, APUM5 suppresses CMV infection via the direct binding of viral RNAs and attenuates CMV symptoms.

6. Abbasi N, Kim HB, Park NI, Kim HS, Kim YK, Park YI, et al. APUM23, a nucleolar Puf domain protein, is involved in pre-ribosomal RNA processing and normal growth patterning in *Arabidopsis.* Plant J 2010; 64:960-76; PMID:21143677; http://dx.doi.org/10.1111/j.1365-313X.2010.04393.x.
7. Szakmary A, Cox DN, Wang Z, Lin H. Regulatory relationship among *piwi, pumilio*, and *bag-of-marbles* in *Drosophila* germline stem cell self-renewal and differentiation. Curr Biol 2005; 15:171-8; PMID:15668175; http://dx.doi.org/10.1016/j.cub.2005.01.005.
8. Vessey JP, Schoderboeck L, Gingl E, Luzi E, Riefler J, Di Leva F, et al. Mammalian Pumilio 2 regulates dendrite morphogenesis and synaptic function. Proc Natl Acad Sci USA 2010; 107:3222-7; PMID:20133610; http://dx.doi.org/10.1073/pnas.0907128107.
9. Archer SK, Luu VD, de Queiroz RA, Brems S, Clayton C. *Trypanosoma brucei* PUF9 regulates mRNAs for proteins involved in replicative processes over the cell cycle. PLoS Pathog 2009; 5:e1000565; PMID:19714224; http://dx.doi.org/10.1371/journal.ppat.1000565.
10. Tam PP, Barrette-Ng IH, Simon DM, Tam MW, Ang AL, Muench DG. The Puf family of RNA-binding proteins in plants: phylogeny, structural modeling, activity and subcellular localization. BMC Plant Biol 2010; 10:44; PMID:20214804; http://dx.doi.org/10.1186/1471-2229-10-44.
11. Francischini CW, Quaggio RB. Molecular characterization of *Arabidopsis thaliana* PUF proteins--binding specificity and target candidates. FEBS J 2009; 276:5456-70; PMID:19682068; http://dx.doi.org/10.1111/j.1742-4658.2009.07230.x.
12. Bernoux M, Timmers T, Jauneau A, Brière C, de Wit PJ, Marco Y, et al. RD19, an *Arabidopsis* cysteine protease required for RRS1-R-mediated resistance, is relocalized to the nucleus by the *Ralstonia solanacearum* PopP2 effector. Plant Cell 2008; 20:2252-64; PMID:18708476; http://dx.doi.org/10.1105/tpc.108.058685.
13. Gerber AP, Luschnig S, Krasnow MA, Brown PO, Herschlag D. Genome-wide identification of mRNAs associated with the translational regulator PUMILIO in *Drosophila melanogaster.* Proc Natl Acad Sci USA 2006; 103:4487-92; PMID:16537387; http://dx.doi.org/10.1073/pnas.0509260103.
14. Gu W, Deng Y, Zenklusen D, Singer RH. A new yeast PUF family protein, Puf6p, represses *ASH1* mRNA translation and is required for its localization. Genes Dev 2004; 18:1452-65; PMID:15198983; http://dx.doi.org/10.1101/gad.1189004.
15. García-Rodríguez LJ, Gay AC, Pon LA. Puf3p, a Pumilio family RNA binding protein, localizes to mitochondria and regulates mitochondrial biogenesis and motility in budding yeast. J Cell Biol 2007; 176:197-207; PMID:17210948; http://dx.doi.org/10.1083/jcb.200606054.
16. Dallagiovanna B, Correa A, Probst CM, Holetz F, Smircich P, de Aguiar AM, et al. Functional genomic characterization of mRNAs associated with TcPUF6, a pumilio-like protein from *Trypanosoma cruzi.* J Biol Chem 2008; 283:8266-73; PMID:18056709; http://dx.doi.org/10.1074/jbc.M703097200.
17. Huh SU, Kim MJ, Ham BK, Paek KH. A zinc finger protein Tsip1 controls *Cucumber mosaic virus* infection by interacting with the replication complex on vacuolar membranes of the tobacco plant. New Phytol 2011; 191:746-62; PMID:21477206; http://dx.doi.org/10.1111/j.1469-8137.2011.03717.x.
18. Cillo F, Roberts IM, Palukaitis P. *In situ* localization and tissue distribution of the replication-associated proteins of *Cucumber mosaic virus* in tobacco and cucumber. J Virol 2002; 76:10654-64; PMID:12368307; http://dx.doi.org/10.1128/JVI.76.21.10654-10664.2002.
19. Huh SU, Kim MJ, Paek KH. *Arabidopsis* Pumilio protein APUM5 suppresses *Cucumber mosaic virus* infection via direct binding of viral RNAs. Proc Natl Acad Sci USA 2013; 110:779-84; PMID:23269841; http://dx.doi.org/10.1073/pnas.1214287110.
20. Chagnovich D, Lehmann R. Poly(A)-independent regulation of maternal *hunchback* translation in the *Drosophila* embryo. Proc Natl Acad Sci USA 2001; 98:11359-64; PMID:11562474; http://dx.doi.org/10.1073/pnas.201284398.

21. Wreden C, Verrotti AC, Schisa JA, Lieberfarb ME, Strickland S. Nanos and pumilio establish embryonic polarity in *Drosophila* by promoting posterior deadenylation of *hunchback* mRNA. Development 1997; 124:3015-23; PMID:9247343.
22. Rao AL. Genome packaging by spherical plant RNA viruses. Annu Rev Phytopathol 2006; 44:61-87; PMID:16480335; http://dx.doi.org/10.1146/annurev.phyto.44.070505.143334.
23. Campbell ZT, Menichelli E, Friend K, Wu J, Kimble J, Williamson JR, et al. Identification of a conserved interface between PUF and CPEB proteins. J Biol Chem 2012; 287:18854-62; PMID:22496444; http://dx.doi.org/10.1074/jbc.M112.352815.
24. Van Etten J, Schagat TL, Hrit J, Weidmann CA, Brumbaugh J, Coon JJ, et al. Human Pumilio proteins recruit multiple deadenylases to efficiently repress messenger RNAs. J Biol Chem 2012; 287:36370-83; PMID:22955276; http://dx.doi.org/10.1074/jbc.M112.373522.
25. Tucker M, Valencia-Sanchez MA, Staples RR, Chen J, Denis CL, Parker R. The transcription factor associated Ccr4 and Caf1 proteins are components of the major cytoplasmic mRNA deadenylase in *Saccharomyces cerevisiae.* Cell 2001; 104:377-86; PMID:11239395; http://dx.doi.org/10.1016/S0092-8674(01)00225-2.
26. Walley JW, Kelley DR, Nestorova G, Hirschberg DL, Dehesh K. *Arabidopsis* deadenylases AtCAF1a and AtCAF1b play overlapping and distinct roles in mediating environmental stress responses. Plant Physiol 2010; 152:866-75; PMID:19955262; http://dx.doi.org/10.1104/pp.109.149005.
27. Cao Q, Padmanabhan K, Richter JD. Pumilio 2 controls translation by competing with eIF4E for 7-methyl guanosine cap recognition. RNA 2010; 16:221-7; PMID:19933321; http://dx.doi.org/10.1261/rna.1884610.
28. Choudhury R, Tsai YS, Dominguez D, Wang Y, Wang Z. Engineering RNA endonucleases with customized sequence specificities. Nat Commun 2012; 3:1147; PMID:23093184; http://dx.doi.org/10.1038/ncomms2154.
29. Filipovska A, Rackham O. Designer RNA-binding proteins: New tools for manipulating the transcriptome. RNA Biol 2011; 8:978-83; PMID:21941129; http://dx.doi.org/10.4161/rna.8.6.17907.
30. Cooke A, Prigge A, Opperman L, Wickens M. Targeted translational regulation using the PUF protein family scaffold. Proc Natl Acad Sci USA 2011; 108:15870-5; PMID:21911377; http://dx.doi.org/10.1073/pnas.1105151108.