Pak. J. Agri. Sci., Vol. 51(4), 919-924; 2014
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

CLEAVAGE AND POLYADENYLATION SPECIFICITY FACTOR (CPSF) SUBUNIT INTERACTION IN TOMATO, *Solanum lycopersicum*

Saba Riaz[1,*], Munir Ahmed Sheikh[1], Muhammad Shahid[1] and Abdul Wahid[2]

[1]Department of Biochemistry, University of Agriculture Faisalabad, Pakistan;
[2]Department of Botany, University of Agriculture Faisalabad, Pakistan.
[*]Corresponding author's e-mail: sabi aem@yahoo.com

Cleavage and polyadenyation is an important step in eukaryotic mRNA biogenesis that also controls gene expression. The process undergoes with interaction of poly(A) factors with the poly(A) sites and with each other. Among different poly(A) factors, Cleavage and Polyadenylation Specificity Factor (CPSF) has a central role in the process. Tomato, *Solanum lycopersicum*, is an important member of the Solanaceae family and grown worldwide with high production. We investigated interaction of different CPSF subunits with each other and with some other proteins using TAP (tandem affinity purification) method in tomato. Four CPSF subunit genes were transformed in *Agrobacterium* followed by transformation in tomato callus. The interaction was studied by proteomics approach. Proteomics results have shown the interactions among CPSF subunits with each other and with other neighboring proteins that may have important implications in gene regulation eventually leading to the control of many biological processes such as flowering time. This further demonstrates that the 3'-end formation of mRNA involves extensive protein-protein interaction network which is coupled with other processes of mRNA biogenesis such as transcription, capping, splicing and translation.
Keywords: CPSF, tomato, poly(A) factors, proteomics

INTRODUCTION

Cleavage and polyadenylation, sometimes referred to as polyadenylation, of pre-mRNA is a crucial step for producing mature mRNA (Tian and Graber, 2012). It is composed of a tract of adenosines (polyA) to the cleaved RNA (Zhao *et al*., 2009). The polyadenylation is not only an essential step for the production of correctly processed mRNA but also has other crucial functions (Koh and Wong, 2007). The polyadenylation process requires two essential components; the cis-elements on mRNA and trans-elements in the form of protein factors that recognize and interact with the cis-elements to complete the process (Hunt *et al.*, 2008). A macromolecular machinery comprising of more than twenty proteins bring about 3'-end processing of mRNA (Chan *et al.*, 2011).

The trans factors include multiple poly(A) factors organized into complexes. Among these factors CPSF multisubunit complexes have the central role in the polyadenylation process. These subunits are classified on the basis of molecular masses which are CPSF30, CPSF 73, CPSF 100 and CPSF 160 (Cohen *et al.*, 1999). In *Arabidopsis* the CPSF 73 has been further divided into CPSF73-I and CPSF73-II (Xu *et al.*, 2006). The CPSF subunits in plants are not only involved in cleavage and polyadenylation process but also found to have different roles in plant metabolism (Xu *et al.*, 2004).

The polyadenylation process in plants has mostly been reported from the model plants Arabidopsis and rice that mainly includes bioinformatics analysis rather than experimental work (Li *et al.*, 2008). Tomato, *Solanum lycopersicum* L., is a model plant of Solanaceae family (Aoki *et al.*, 2010) that comprises more than 3000 species including potato, eggplant, peppers etc (Mueller *et al.*, 2005a). However, many important molecular processes, including 3'-end processing of mRNA, have still not been studied in tomato. Therefore, a project was designed to study CPSF gene expression pattern and CPSF subunits interaction in this important plant, and are reported in this paper.

MATERIALS AND METHODS

***Germination of tomato seeds*:** Tomato (*Solanum lycopersicum* L.) seeds, var. Roma, were procured from Ayub Agriculture Research Institute, Faisalabad, Pakistan and soaked in 5% sodium hypochlorite for 15 min under sterilized conditions followed by washing with distilled autoclaved water for 6 times at 5 min intervals and then at 20 min intervals twice. The seeds were placed in autoclaved Petri plates having Whattman filter paper soaked in distilled autoclaved water under sterilized conditions and germinated under sterile conditions on wet filter paper at 28°C in growth chamber (Artificial Climat Chamber [RTOP series]).

CPSF subunit interaction study by proteomic approach:

***Construction of TAP-tagged vectors with CPSF genes*:** The TAP (Tandem Affinity Purification) tag (TAPi) vectors (Rohila *et al.*, 2004) were very kindly gifted by Quinn Li, Department of Botany, Miami University, Oxford, Ohio,

USA. It was Gateway-compatible binary vector. The CPSF gene sequences (cDNA) were first cloned into pENTR vector (Invitrogen) following manufacturer's protocol. To a 1.5 mL microcentrifuge tube 1 µL of cDNA sequence (50 fmol) was added to 2 µL pDONR 221 vector (150 ng/µL), 4 µL 5X BP clonase reaction buffer and made up to 16 µL with TE buffer, pH 8.0. Added 4 µL of the BP clonase enzyme, mixed well and incubated at 25°C for 60 min. Added 2 µL of 2 µg/µL proteinase K and incubated at 37°C for 10 min. The ligated mixture was transformed in competent *E. coli* cells and selected on kanamycin.

The ENTRY clones containing the CPSF genes were subjected to LR recombination in nTAPi and cTAPi vectors. The plasmids were isolated with the help of GeneJet Plasmid miniprep kit (Fermentas) following manufacturer's protocol.

***Transformation of CPSF-TAP tagged vectors in Agrobacterium tumefaciens*:** The CPSF-TAP-tagged vectors were transformed in *Agrobacterium tumefaciens* LBA4404 by electroporation in AARI, Faisalabad.

***Transformation of vectors in tomato*:** To prepare tomato suspension culture approximately 5-10 mm diameter segments of the tomato leaves were excised and transferred to the callus induction medium containing Murashige and Skoog basal salts, Nitsch's vitamins, myo-inositol (100 mg/L) and 3% sucrose supplemented with plant growth regulators 2,4-dichlorophenoxyacetic acid (2 mg/L) and 6-benzylaminopurine (0.1 mg/L) and solidified agar. The suspension culture was prepared following Lu *et al.* (2008).

The transformation was done using *Agrobacterium* transformation by employing the method reported by Zhao *et al*. (2009).

***TAP purification and MS analysis*:** TAP purification was done after Rohila *et al.* (2004). Briefly, about 100 grams of cells (fresh weight) were ground in liquid nitrogen with acid washed quartz to a fine powder. Extraction was carried out in extraction buffer (20 mMTris/HCl, pH 8.0, 150 mM NaCl, 0.1% NP-40, 2.5 mM EDTA, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride (PMSF), 2 mM Benzamide, 20 mM NaF, and 0.1% [V/V] of protease inhibitors cocktail [Sigma]). The mixture was passed through Ig G beads and calmodulin affinity beads. Finally, the proteins were eluted with 1 to 1.5 mL of elution buffer (10 mM Tris/HCl, 150 mM NaCl, 1 mM Mg-acetate, 1 mM imidazole, 2 mM ethylene glycol tetraacetic acid (EGTA), 0.1 % NP40 (V/V), 10 mM β-mercaptoethanol, 1 mM PMSF and 1/1000 protease inhibitor cocktail, pH 8.0) into the desired number of fractions, which were monitored by absorbance at 280 nm. Eluted proteins were precipitated by trichloroacetic acid (TCA) and sodium deoxycholate (DOC) before loading onto a SDS-PAGE gel. The proteins were analyzed by mass spectrometry after trypsin digestion from the proteomics facility at Danforth Plant Science Center, USA.

RESULTS

Proteomics approach was used to study the CPSF subunit interaction. Tandem Affinity Purification (TAP) system of (Rohila *et al.*, 2004) was applied to study the interaction. A TAP tag is attached with the gene of interest and transformed in plant cells. It helps purification of protein complexes containing proteins of interest attached to the TAP-tag. The TAP tag comprises of Protein A domains having affinity with IgG-agarose, A tobacco etch virus (TEV) protease site so that the fusion protein may be cleaved from the IgG-agarose and A calmodulin-binding protein domain.

The protein complexes bound to the bait protein fused to TAP-tag are isolated from the plant cells transformed with the TAP-tagged vectors. The tandem protein A domains attached to the TAP-tagged protein complexes bind to IgG-agarose, and the unbound proteins are washed out. The TAP-tagged protein complexes are separated from IgG-agarose by cleaving with TEV protease at the TEV-protease cleavage site. The remaining TAP-tagged protein complexes are applied to the calmodulin column where calmodulin-binding domain of the TAP-tag binds in the presence of Ca^{+2}. This gives further purification of the desired protein complexes as the unbound proteins are washed out again. The TAP-tagged proteins complexes are relieved from calmodulin binding with the help of EGTA that chelates out Ca^{+2} ions, hence the interaction between calmodulin and TAP-tagged protein complexes is disrupted. The protein complexes thus isolated are subjected to mass spectrometric analysis to identify the proteins in the protein complexes.

The CPSF subunit genes were introduced in TAP vectors nTAPi and cTAPi (Fig. 1). The CPSF100 was fused to TAP tag at N-terminus whereas all other subunit genes were ligated in cTAPi. The CPSF subunit genes were under the control of cauliflower mosaic virus (CaMV) 35S promoter and CaMV terminator. The CPSF subunits in the TAP vectors were transformed in *E. coli* followed by miniplasmid preparation. The plasmids were digested with *Hind*III for confirmation of the ligation of CPSF subunits in the plasmids. All the plasmids showed positive results as expected fragment sizes (Table 1) were obtained after the restriction digestion except for CPSF73II that could not be transformed due to some unknown reason.

Table 1. CPSF gene sizes and restricted fragment sizes after miniplasmid preparation

Gene name	Size (bp)	Plasmid	Fragment size after *Hind*III restriction (bp)
CPSF30	753	CTAPi-CPSF30	2514
CPSF73	2338	CTAPi-CPSF73	4099
CPSF100	2417	NTAPi-CPSF100	4178
CPSF160	4329	CTAPi-CPSF160	6090

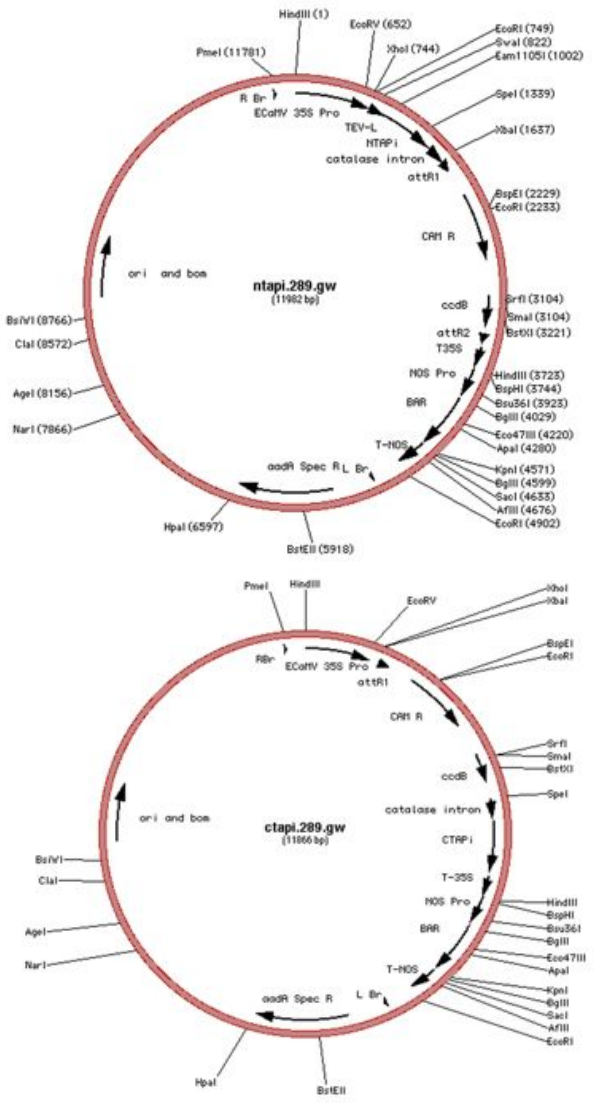

Figure 1. nTAPi and cTAPi vectors

After confirmation the plasmids were transformed in *Agrobacterium tumefaciens* LBA4404 and selected with spectinomycin antibiotic. The positive colonies were picked and the CPSF subunit genes were confirmed by colony PCR. The successful transformants were transformed in tomato cells by electroporation and the TAP-tagged protein complexes were isolated by passing the cell lysate over IgG-agarose column, cleaved at TEV cleavage site and then passing over calmodulin-Ca^{2+} column. The protein complexes were sequenced by mass spectrometric analysis from USA. The poly(A) factors and other proteins identified were tagged with each of the TAP-tagged vector are given in Table 2.

Table 2. Proteins identified after transformation of TAP- tagged CPSF genes in tomato

TAP-tagged plasmid	Proteins identified
CTAPi-CPSF30	CPSF30, CPSF73II, CPSF160, FIPS5
CTAPi-CPSF73I	CPSF73I, CLPS3, CPSF73II, FY, CPSF100, CPSF160, PCFS4
NTAPi-CPSF100	CPSF100, CPSF160, FY, CPSF73I
CTAPi-CPSF160	CPSF160, CPSF100, CPSF30, CPSF73I, PAP, SYM5, FY

DISCUSSION

The interaction with CPSF subunits was studied by proteomics analysis of the proteins purified from tomato cell suspension after transformation with TAP-tagged-CPSF gene vectors. Each of the TAP-tagged fraction fractionates the proteins that have interaction with the CPSF subunit and with other proteins. As seen in Figure 2 the CPSF30 had interaction with FIPS5 which in turn interacts with poly(A) polymerase (PAP) and stimulates it (Forbes *et al.*, 2006). The CPSF30 also had interaction with CPSF73II that has been shown to have functions other than housekeeping (Xu *et al.*, 2004). The CPSF73II interacts with CPSF73I. The CPSF73I also has interaction with CPSF100, CPSF160, FY protein, CLPS3 (cleavage family complex protein) and PCFS4 (protein 1 of cleavage factor). CPSF100 also has interaction with CPSF160 and FY, and CPSF160 with FY, PAP and SYM5 (symplekin). Symplekin is also involved in post-transcriptional gene silencing by RNA (Herr *et al.*, 2006) and has interaction with CstF64.

Another poly(A) factor CLPS3 (CLP1-Similar Protein 3), an ortholog of human CLP1, was also found to be essential for embryo development and female gamete transmission in Arabidopsis (Xiang *et al.*, 2008). (Zhao *et al.*, 2009) investigated CPSF *in vivo* in *A. thaliana* by proteomic studies using tandem affinity purification for isolation of the protein complexes followed by mass spectrometry and Western immunoblots. They found all the above mentioned CPSF subunits plus *At*FY and *At*FIPS5. They further demonstrated that AtCPSF100 served as a core to which all other factors were associated except *At*FIPS5. Similarly, *At*CPSF73I has been shown to be an endonuclease having a role in cleavage of the mRNA during 3'end formation (Mandel *et al.*, 2006). CPSF30 has also been shown as an RNA-binding endonuclease associated with other factors (Rao *et al.*, 2009). Moreover, it was found that the *At*CPSF30 itself was localized to the cytoplasm when expressed by itself; however, it was found to be localized in nucleus when co-expressed with other factors such as *At*CPSF160 and *At*CPSF73 probably due to interaction. (Rao *et al.*, 2009) further demonstrated that the CPSF30 was speculated to have multiple novel protein-protein interactions among CPSF factors. (Delaney *et al.*, 2006)

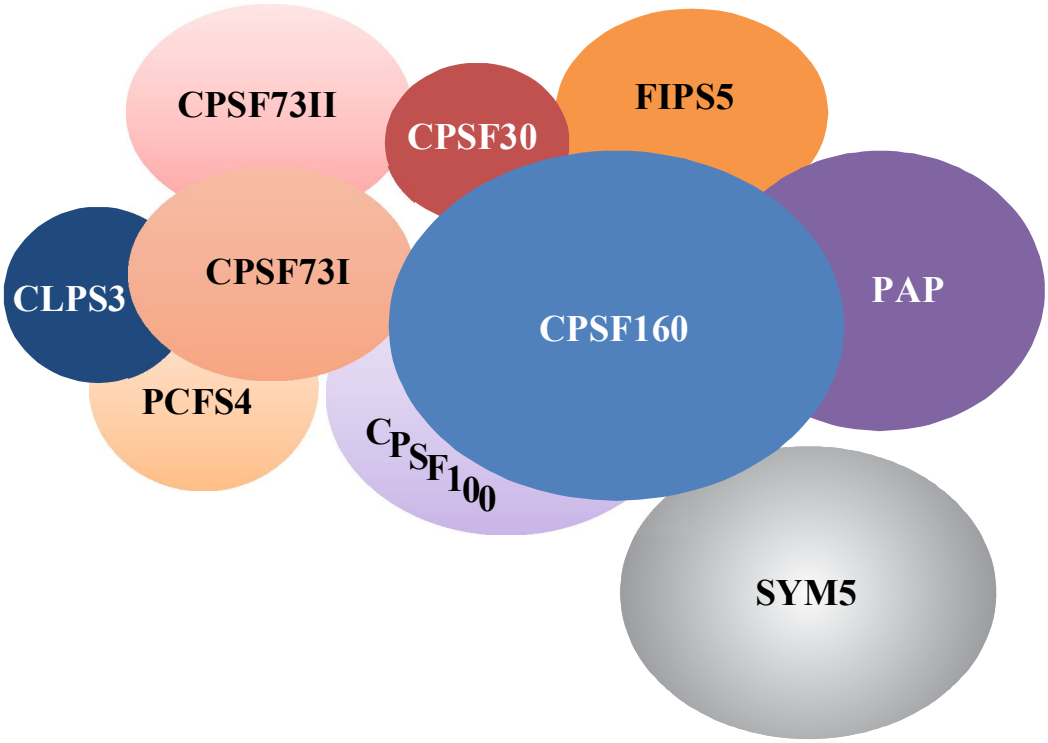

Figure 2. Interaction among CPSF subunits and other protein factors in tomato.
The interactions were determined by the proteomics analysis. The protein factors are drawn to nearest possible scale. Abbreviations of proteins other than CPSF: PAP (Poly(A) polymerase), FIPS5 (factor interacting with poly(A) polymerase, PCFS4 (Pcf1-similar protein 4), SYM5 (Symplekin5), CLPS3 (CLP1-similar protein 3), FY (a homologue of yeast Pfs2p factor).

studied CPSF30 interactions using calmodulin binding assay and found that the CPSF30 was not only capable of self-interactions but also potentially involved in possible interplay between splicing and polyadenylation and their regulation. In another study (Addepalli and Hunt, 2007) the CPSF30 was found to have zinc finger motifs, and it was indicated that interactions of the protein were probably via Fip1. The CCCH zinc finger motif of AtCPSF30 has also been shown to have a disulphide linkage (Addepalli *et al.*, 2010). Fip1 (Factor Interacting with poly(A) polymerase) is another protein that is known to provide a bridge between poly(A) polymerase, poly(A) apparatus and RNA in eukaryotes (Forbes *et al.*, 2006; Meinke *et al.*, 2008); rather it was reported that the Fip1 from *Arabidopsis* had plant-specific interactions between the protein and other factors.

The CPSF100 gene is ca 2400 nucleotide long and encodes a polypeptide of 100 kDa (Elliott *et al.*, 2003). The factor was found not only in *Arabidopsis* but the nuclear extracts from pea and wheat germ also recognized the antibodies raised against the Arabidopsis CPSF100. Moreover, it was found that 60 amino acid domain of AtCPSF100 had an interaction with 220 amino acids of poly(A) polymerase. Dominski *et al.* (2005) demonstrated that the CPSF73 and CPSF100 belonged to a superfamily of zinc-dependent β-lactamase fold proteins.

Several RNA binding proteins other than poly(A) factors have been reported to influence different post-transcriptional processes including splicing, RNA decay, polyadenylationetc (Staiger and Koster, 2011). Among these Fip1 is a subunit of the poly(A) apparatus and acts as a bridge between poly(A) polymerase, other poly(A) subunits and substrate RNA (Forbes *et al.*, 2006; Meinke *et al.*, 2008). The protein was found to have 1196 amino acids, and 137 amino acids had interaction with poly(A) polymerase.

Another RNA binding protein FCA has also been shown to interact with 3'-end processing machinery of mRNA (Simpson *et al.*, 2010). It was found to be an alternative polyadenylation regulator. The FCA expression was speculated to have its role in regulating flowering time in Arabidopsis. Whole-genome tiling array studies regarding FCA and FPA proteins revealed that the proteins had an important role in 3'end processing and transcription termination in Arabidopsis (Sonmez *et al.*, 2011). The FCA promotes flowering and autoregulates its own expression via alternative polyadenylation (Quesada *et al.*, 2005) in Arabidopsis. Physical interaction of FCA with a 3'end

processing factor (FY) was reported to be mandatory for the FY function in flowering control.

***Conclusion*:** Different plant proteins have been found to interact with 3'end processing of mRNA in tomato that may have important implications in gene regulation eventually leading to the control of many biological processes such as flowering time. This further demonstrates that the 3'-end formation of mRNA in the plant involves extensive protein-protein interaction network which is coupled with other processes of mRNA biogenesis such as transcription, capping, splicing and translation. The results would have practical implications in improving tomato cultivars by applying outcome of this research.

***Acknowledgment*:** The research was conducted under Higher Education Commission, Islamabad, Pakistan funded Project No. 20-890. The help by Dr Ihsan Ullah from Agricultural Biotechnology Research Institute, AARI, Faisalabad is also deeply appreciated.

REFERENCES

Addepalli, B. and A.G. Hunt. 2007. A novel endonuclease activity associated with the Arabidopsis ortholog of the 30-kDa subunit of cleavage and polyadenylation specificity factor. Nucleic Acids Res. 35: 4453-4453.

Addepalli, B., P.A. Limbach and A.G. Hunt. 2010. A disulfide linkage in a CCCH zinc finger motif of an Arabidopsis CPSF30 ortholog. FEBS Lett. 584: 4408-4408.

Aoki, K., K. Yano, A. Suzuki, S. Kawamura, N. Sakurai, K. Suda, A. Kurabayashi, T. Suzuki, T. Tsugane, M. Watanabe, K. Ooga, M. Torii, T. Narita, I.T. Shin, Y. Kohara, N. Yamamoto, H. Takahashi, Y. Watanabe, M. Egusa, M. Kodama, Y. Ichinose, M. Kikuchi, S. Fukushima, A. Okabe, T. Arie, Y. Sato, K. Yazawa, S. Satoh, T. Omura, H. Ezura and D. Shibata. 2010. Large-scale analysis of full-length cDNAs from the tomato (*Solanum lycopersicum*) cultivar Micro-Tom, a reference system for the Solanaceae genomics. BMC Genomics 11: 210-226.Bai, Y. and P. Lindhout. 2007. Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? Ann. Bot. 100: 1085-1094.

Chan, S., E.A. Choi and Y. Shi. 2011. Pre-mRNA 3'-end processing complex assembly and function. Wiley Interdiscip. Rev. RNA 2: 321-335.

Cohen, L.A., Z. Zhao, B. Pittman and F. Khachik. 1999. Effect of dietary lycopene on N-methylnitrosourea-induced mammary tumorigenesis. Nutr. Cancer 34: 153-159.

Delaney, K.J., R. Xu, J. Zhang, Q.Q. Li, K.Y. Yun, D.L. Falcone and A.G. Hunt. 2006. Calmodulin interacts with and regulates the RNA-binding activity of an Arabidopsis polyadenylation factor subunit. Plant Physiol. 140: 1507-1521.

Dominski, Z., X.C. Yang, M. Purdy, E.J. Wagner and W.F. Marzluff. 2005. A CPSF-73 homologue is required for cell cycle progression but not cell growth and interacts with a protein having features of CPSF-100. Mol. Cell. Biol. 25: 1489-1500.

Elliott, B.J., T. Dattaroy, L.R. Meeks-Midkiff, K.P. Forbes and A.G. Hunt. 2003. An interaction between an Arabidopsis poly(A) polymerase and a homologue of the 100 kDa subunit of CPSF. Plant Mol. Biol. 51: 373-384.

Forbes, K.P., B. Addepalli and A.G. Hunt. 2006. An Arabidopsis Fip1 homolog interacts with RNA and provides conceptual links with a number of other polyadenylation factor subunits. J. Biol. Chem. 281: 176-186.

Herr, A.J., A. Molnar, A. Jones and D.C. Baulcombe. 2006. Defective RNA processing enhances RNA silencing and influences flowering of Arabidopsis. Proc. Natl. Acad. Sci. USA 103: 14994-15001.

Hunt, A.G., R. Xu, B. Addepalli, S. Rao, K.P. Forbes, L.R. Meeks, D. Xing, M. Mo, H. Zhao, A. Bandyopadhyay, L. Dampanaboina, A. Marion, C. Von Lanken and Q.Q. Li. 2008. Arabidopsis mRNA polyadenylation machinery: comprehensive analysis of protein-protein interactions and gene expression profiling. BMC Genomics 9: 220-235.

Jimenez-Gomez, J.M. and J.N. Maloof. 2009. Sequence diversity in three tomato species: SNPs, markers, and molecular evolution. BMC Plant Biol. 9: 85-96.

Koh, C.H. and L. Wong. 2007. Recognition of polyadenylation sites from Arabidopsis genomic sequences. Genome Inform. 19: 73-82.

Li, Y., Z. Zhang, Y. Jia, Y. Shen, H.He, R. Fang, X. Chen and X. Hao. 2008. 3-Acetonyl-3-hydroxyoxindole: a new inducer of systemic acquired resistance in plants. Plant Biotechnol. J 6: 301-308.

Lu, C.H., N.J. Engelmann, M.A. Lila and J.W. Erdman, Jr. 2008. Optimization of lycopene extraction from tomato cell suspension culture by response surface methodology. J. Agric. Food Chem. 56: 7710-7714.

Mandel, C.R., S. Kaneko, H. Zhang, D. Gebauer, V. Vethantham, J.L. Manley and L. Tong. 2006. Polyadenylation factor CPSF-73 is the pre-mRNA 3'-end-processing endonuclease. Nature 444: 953-956.

Meinke, G., C. Ezeokonkwo, P. Balbo, W. Stafford, C. Moore and A. Bohm. 2008. Structure of yeast poly(A) polymerase in complex with a peptide from Fip1, an intrinsically disordered protein. Biochemistry 47: 6859-6869.

Mueller, L.A., T.H. Solow, N. Taylor, B. Skwarecki, R. Buels, J. Binns, C. Lin, M.H. Wright, R. Ahrens, Y.

Wang, E.V. Herbst, E.R. Keyder, N. Menda, D. Zamir and S.D. Tanksley. 2005a. The SOL Genomics Network: a comparative resource for Solanaceae biology and beyond. Plant Physiol. 138: 1310-1317.

Quesada, V., C. Dean and G.G. Simpson. 2005. Regulated RNA processing in the control of Arabidopsis flowering. Int. J. Dev. Biol. 49: 773-780.

Rao, S., R.D. Dinkins and A.G. Hunt. 2009. Distinctive interactions of the Arabidopsis homolog of the 30 kD subunit of the cleavage and polyadenylation specificity factor (AtCPSF30) with other polyadenylation factor subunits. BMC Cell Biol. 10: 51-63.

Rohila, J.S., M. Chen, R. Cerny and M.E. Fromm. 2004. Improved tandem affinity purification tag and methods for isolation of protein heterocomplexes from plants. Plant J. 38: 172-181.

Simpson, G.G., R.E. Laurie, P.P. Dijkwel, V. Quesada, P.A. Stockwell, C. Dean and R.C. Macknight. 2010. Noncanonical translation initiation of the Arabidopsis flowering time and alternative polyadenylation regulator FCA. Plant Cell 22: 3764-3777.

Sonmez, C., I. Baurle, A. Magusin, R. Dreos, S. Laubinger, D. Weigel and C. Dean. 2011. RNA 3' processing functions of Arabidopsis FCA and FPA limit intergenic transcription. Proc. Natl. Acad. Sci, USA 108: 8508-8513.

Staiger, D. and T. Koster. 2011. Spotlight on post-transcriptional control in the circadian system. Cell. Mol. Life Sci. 68: 71-83.

Tian, B. and J.H. Graber. 2012. Signals for pre-mRNA cleavage and polyadenylation. Wiley Interdiscip. Rev. RNA 3: 385-396.

Xiang, T., N. Zong, Y. Zou, Y. Wu, J. Zhang, W. Xing, Y. Li, X. Tang, L. Zhu, J. Chai and J.M. Zhou. 2008. Pseudomonas syringae effector AvrPto blocks innate immunity by targeting receptor kinases. Curr. Biol. 18: 74-80.

Xu, R., H. Zhao, R. D. Dinkins, X. Cheng, G. Carberry and Q.Q. Li. 2006. The 73 kD subunit of the cleavage and polyadenylation specificity factor (CPSF) complex affects reproductive development in Arabidopsis. Plant Mol. Biol. 61: 799-815.

Xu, R., X. Ye and Q. Quinn Li. 2004. AtCPSF73-II gene encoding an Arabidopsis homolog of CPSF 73 kDa subunit is critical for early embryo development. Gene 324: 35-45.

Zhao, H., D. Xing and Q.Q. Li. 2009. Unique features of plant cleavage and polyadenylation specificity factor revealed by proteomic studies. Plant Physiol. 151: 1546-1556.