Front. Biol. 2011, 6(3): 246–250
DOI 10.1007/s11515-011-1060-7

REVIEW

The mRNA export pathway in plants

Oliver X. DONG[1,2], Kaeli JOHNSON[2], Xin LI[2], Yuelin ZHANG (✉)[1]

[1] *National Institute of Biological Sciences, Beijing 102206, China;*
[2] *Michael Smith Laboratories, Room 301, 2185 East Mall, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada*

Abstract **A double lipid bilayer separating the nucleus from the cytoplasm, termed the nuclear envelope, is a defining feature of eukaryotes. Nucleocytoplasmic transport of macromolecules through the nuclear pores enables fine-tuned regulation of biologic processes. All mature mRNAs are delivered to the cytoplasm from the nucleus via an mRNA export pathway. Much work has been done in yeast and animals to study the machinery of mRNA export. However, until recently, research on plant mRNA export has been quite limited. Genetic, bioinformatic, and biochemical investigations have expanded our understanding of the mRNA export process in plants. Here, we review recent progress that has been made elucidating the components of the mRNA export pathway in plants. MOS3 (MODIFIER OF SNC1, 3) /AtNup96 and AtNup160 are both components of the highly conserved Nup107-160 nucleoporin complex and were shown to play key roles in mRNA export. MOS11 (MODIFIER OF SNC1, 11), which is homologous to the RNA helicase enhancer CIP29 in human, was recently found to be involved in the same pathway as MOS3. A DEAD Box RNA helicase, LOS4 (low expression of osmotically responsive genes 4) was also found to play a role in the mRNA export process, putatively by carrying mRNA molecules through the nuclear envelope. Recently, a protein complex homologous to the yeast TREX-2 complex was also found to play important roles in mRNA export in plants. It appears that most players in the mRNA export pathway are highly conserved among plants, yeast and animals.**

Introduction

The mRNA export pathway plays an important role in eukaryotic gene expression (Chinnusamy et al., 2008). Unlike prokaryotic cells, where no clear boundary separates the nuclear region from the cytoplasm, eukaryotic cells contain nuclei enclosed by the nuclear envelope comprised of a double-layered membrane structure. Nuclear pores embedded in the nuclear envelope allow the passage of macromolecules such as proteins and RNAs across the membrane.

In eukaryotic cells, mRNA undergoes series of modifications in the nucleus before being exported into the cytoplasm for translation (Keene, 2010). Modifications of the mRNA, such as 5′ capping, 3′ polyadenylation and the removal of introns, take place both co-transcriptionally and post-transcriptionally. Incorrectly processed mRNAs are degraded. Heterogeneous nuclear ribonucleoproteins (hnRNPs) are recruited to the mRNA at this stage to form mRNA-protein complexes termed ribonucleoproteins (mRNPs) (Iglesias and Stutz, 2008; Chaudhury et al., 2010). Some hnRNPs facilitate the export of the mRNA. The passage of the export-competent mRNPs through the nuclear pore also requires the recruitment of certain nuclear export factors (NEFs). NEFs bridge the mRNPs and proteins in the nuclear pore complex (NPC) called nucleoporins (NUPs) (Vinciguerra and Stutz, 2004; Carmody and Wente, 2009; Stewart, 2010). NUPs which are responsible for such interaction usually contain multiple FG (phenylalanine-glycine) repeats in their amino acid sequence and the difference in the patterns of these repeats determines the specificity of the interaction with different NEFs (Chinnusamy et al., 2008; Strambio-De-Castillia et al., 2010). After mRNPs are docked onto the NPC, they are pulled through the nuclear pore. The energy for this process is provided by RNA helicase-mediated hydrolysis of NTPs (nucleoside triphosphate) (Tanner and Linder, 2001). RNA helicases are also known as RNA chaperones due to their role in the correction of the misfolded RNA molecules.

Therefore, successful export of mRNA requires the processing of the pre-mRNA, the formation of mRNP, the targeting of mRNP to the nuclear pore complex and the unidirectional release of the mRNP into the cytoplasm. The coordination of the function of components involved in the mRNA export pathway, such as hnRNP proteins, RNA

Received November 2, 2010; accepted December 2, 2010
Correspondence: Yuelin ZHANG
E-mail: zhangyuelin@nibs.ac.cn

helicases, NEFs and NUPs, ensures the efficiency and accuracy of mRNA export. Here we summarize and discuss some components involved in the mRNA export pathway in plants (Fig. 1).

The AtNup107-160 complex in mRNA export

In the *Arabidopsis thaliana* mutant *snc1* (*suppressor of npr1-1*, *constitutive*, *1*), a gain-of-function mutation leads to constitutive activation of the expression of *PR* (*Pathogenesis-Related*) genes and enhanced resistance against virulent pathogens including the bacteria *Pseudomonas syringae* pv. *maculicola* ES4326 (*P.s.m.* ES4326) and the oomycete *Hyaloperonospora arabidopsidis* Noco2 (Zhang et al., 2003). The *snc1* single mutant plants accumulate higher levels of salicylic acid (SA) and are smaller than wild type plants due to fitness costs associated with autoimmunity. The mutation in *snc1* results in an E (glutamate) to K (lysine) change in the linker region between the NB (nucleotide binding) domain and the LRR (leucine-rich repeat) region of a Resistance (R) protein. To identify additional components required for SNC1 function, genetic screens were performed in either a *snc1* or a *snc1 npr1* background to search for mutants able to suppress the *snc1* phenotype. One of the mutants characterized in detail was *mos3* (*modifier of snc1*, *3*) (Zhang and Li, 2005). Compared with the *snc1* single mutant, the *mos3 snc1* double mutant is larger in size, has suppressed pathogen resistance and reduced SA levels.

MOS3 was found to encode a nucleoporin homologous to Nup96 in human and C-Nup145p in yeast. In human cells, the expression of *Nup96* is upregulated by interferon gamma. The increase in the expression of *Nup96* plays an important role as it reverses the inhibition of mRNA nuclear export by a viral toxin (Enninga et al., 2002). In yeast cells, the C-Nup145p seems to be a limiting factor for proper mRNA export under certain conditions. In mutant yeast cells grown at 37°C,

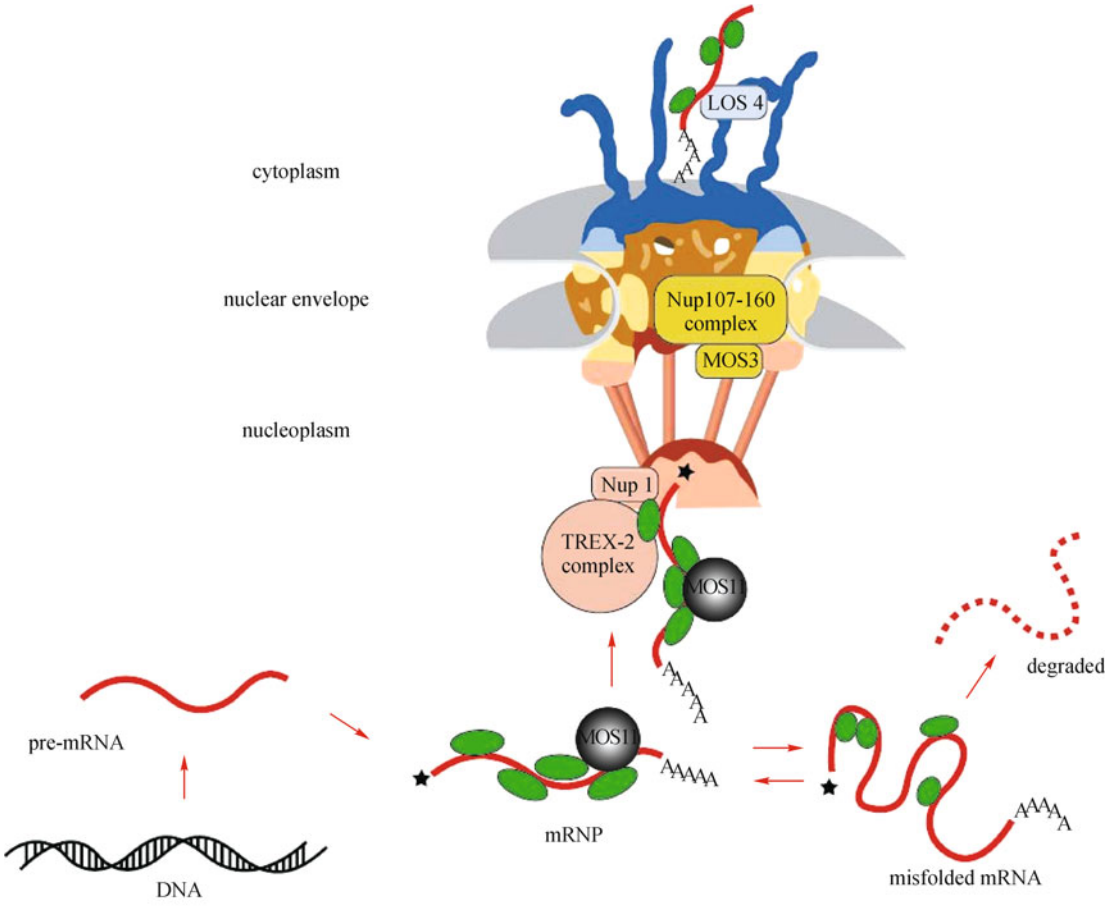

Figure 1 Components involved in the mRNA export pathway in plants. MOS11 enhances the activity of RNA chaperones to keep the pre-mRNA molecules in proper conformation and to correct the misfolded mRNA molecules. The TREX-2 complex, attached to the nuclear pore complex through Nup1, facilitates the export of mRNA. MOS3, a component of the Nup107-160 complex, is located in the core region of the nuclear pore complex. The Nup107-160 complex is required for mRNA export. LOS4, a DEAD-Box helicase, helps pulling the mRNA through the nuclear pore using the energy from ATP hydrolysis.

poly-adenylated (poly-A) RNA accumulated in the nucleus and the cells were unable to grow (Dockendorff et al., 1997). However, the viability of these cells was not affected at 30°C (Dockendorff et al., 1997). In *mos3/sar3* (*suppressor of auxin resistance*, *3*) mutant plant cells, poly-A RNA accumulates in the nucleus (Parry et al., 2006). With the structural homology between MOS3, Nup96 and C-Nup145p as well as the functional similarity between Nup96 and C-Nup145p, it is not surprising that MOS3 plays a role in mRNA export. Suppression of *snc1* phenotypes by *mos3* is probably caused by impaired export of mRNA of defense regulators.

MOS3 homologs in yeast and human belong to the Nup107-160 complex, which also contains Nup160 (Zuccolo et al., 2007). Nup160 is conserved among various eukaryotic organisms including plants, yeast, mice and humans. Nup160 associates with Nup133, Nup107 and Nup96 to form the Nup107-160 complex in human cells. It is likely that these proteins function together in the same mRNA export pathway. AtNup160 is also referred to as SAR1 (suppressor of auxin resistance, 1) and *sar1* cells have been shown to accumulate poly-A RNA in the nucleus (Dong et al., 2006; Parry et al., 2006).

In a genetic screen to look for components involved in the control of plant tolerance to cold, a recessive mutation, *atnup160-1*, was identified. Transcriptional levels of cold-induced genes *CBF1*, *CBF2* and *CBF3* were substantially decreased in *atnup160-1*, whereas the mutation had little effect on the global transcription levels compared with the wild type plants. The *atnup160-1* mutant plants were more sensitive to chilling and freezing stresses and displayed early flowering phenotypes. Significant poly-A RNA accumulation was observed in the nuclei of the *atnup160-1* mutant cells but not in wild type plant cells, indicating that *atnup160-1* is defective in mRNA export. AtNup160 is required for mRNA export under both warm and cold temperatures. The reduced expression of cold-induced genes in *atnup160-1* suggests that mRNA export is critical for the proper expression of these genes (Dong et al., 2006).

MOS11 functions in the same mRNA export pathway as MOS3

In the screen for mutants that suppress the *snc1* phenotype, *mos11* (*modifier of snc1, 11*) was found to partially suppress the dwarfism and enhance the disease resistance of *snc1* (Germain et al., 2010). MOS11 shows close homology with human protein CIP29 and yeast Tho1. In human cells, CIP29 was identified as a primary DDX39 interacting protein, which enhances the RNA-unwinding activity of DDX39 (Sugiura et al., 2007; Gatfield et al., 2001). DDX39 belongs to the DEAD/DExH box RNA helicase family. Members of this RNA helicase family are involved in transcription as well as RNA editing, splicing, export, translation, and degradation (Tanner and Linder, 2010). The major function of these proteins is to keep the RNA in its unwound state. Therefore, MOS11 may act as a co-chaperone in the nucleus to co-catalyze the unwinding of mRNA before its export into the cytoplasm. Similar to *mos3* and *atnup160-1*, *mos11* mutant plant cells accumulates poly-A RNA in the nucleus, suggesting that MOS11 also plays a crucial role in mRNA export (Germain et al., 2010). Unlike MOS3, which is a member of the nuclear pore complex, MOS11 was found inside the nucleus (Germain et al., 2010). To analyze the relationship between MOS3 and MOS11, susceptibility of the *mos3* and *mos11* single mutants to *P.s.m.* ES4326 was compared with that of the *mos3 mos11* double mutant. Since *mos3* and *mos3 mos11* mutant plants showed similar levels of enhanced susceptibility to the pathogen, MOS3 and MOS11 probably function in the same mRNA export pathway. As MOS11 is located inside the nucleus while MOS3 is a nucleoporin, MOS11 may be associated with mRNA molecules before they are delivered into the cytoplasm through the nuclear envelope by Nup107-160 complex.

The function of the DDX39 homolog in *Arabidopsis* has yet to be determined. DDX39 has been shown to interact with another human protein ALY, which is believed to be recruited to the mRNP during spliceosome assembly and plays a role specifically in the export of mRNA, but not other types of RNA (Zhou et al., 2000). Future analysis of proteins associated with MOS11 will enhance our understanding of the mRNA export process in plants.

LOS4 is an RNA helicase involved in mRNA export

In the study of cold stress-induced response in *Arabidopsis*, a recessive mutant *los4-1 (low expression of osmotically responsive genes 4, 1)* showed impaired accumulation of *CBF* gene transcripts and enhanced cold susceptibility (Gong et al., 2005). Another recessive mutation allelic to *los4-1*, *los4-2,* displayed enhanced induction of *CBF2* and its downstream target genes under chilling or freezing conditions (Gong et al., 2005). However, the response of *los4-2* plants to abscisic acid was greatly reduced under high temperature conditions compared with wild type plants (Gong et al., 2005). *los4-2* plants were lethal when incubated at high temperatures (26°C–28°C), while wild type plants were not adversely affected (Gong et al., 2005). *LOS4* encodes another putative DEAD box helicase (Gong et al., 2005). The LOS4 protein was shown to be highly enriched at the nucleus rim, indicating that the LOS4 protein is located at the nuclear envelope (Gong et al., 2005). Accumulation of poly-A RNA within the nucleus was observed in *los4-2* plant cells under normal temperature but not under cold conditions (4°C) (Gong et al., 2005). This suggests that the LOS4 protein may play an important role in RNA export. One explanation for the opposite changes in the expression level of cold-tolerance genes in *los4-1* and *los4-2* is that LOS4 acts as a direct

temperature sensor and the two mutations affected its function in different ways.

It is unclear how LOS4 functions in plants cells to facilitate the export of RNA molecules. In yeast and vertebrate cells, DEAD Box Protein Dbp5 is directly involved in the mRNA export process, which is believed to use the energy from ATP to remodel the mRNPs to help them pass through the nuclear pore (Tanner and Linder, 2010). It is possible that there is a functional similarity between Dbp5 and LOS4.

Arabidopsis has mRNA export complex homologous to the yeast TREX-2 complex

In yeast, the TREX-2 complex is also known as the Thp1-Sac3-Cdc31-Sus1 complex based on its protein composition. It is anchored to the nuclear face of the nuclear pore complex through the interaction between Sac3 and Nup1, a protein located at the basket-like part of NPC. It is believed that TREX-2 is involved in coupling SAGA (Spt-Ada-Gcn5 acetyltransferase)-dependent transcription and mRNA export, as Sus1 was found to associate with the SAGA transcriptional co-activator complex (Lu et al., 2010). Interestingly, homologs of a number of components and binding partners of the yeast TREX-2 complex were identified in *Arabidopsis* based on bioinformatic prediction and yeast two-hybrid analysis (Lu et al., 2010; Yelina et al., 2010; Jauvion et al., 2010). Orthologs of yeast Thp1, Sac3 and Cdc31 were all found to physically associate with the *Arabidopsis* TREX-2 complex (Lu et al., 2010). Nuclear accumulation of poly-A mRNA has been observed in *atthp1* (Lu et al., 2010), suggesting that plants possess a protein complex similar to the yeast TREX-2 complex. This complex appears to be important for plant mRNA export as well as other RNA processing pathways (Yelina et al., 2010; Jauvion et al., 2010).

Perspectives

The mRNA export pathway is not only essential for normal plant growth and development, but also contributes greatly in mediating plant responses to both biotic and abiotic stresses. Our understanding of this process in plants is far from complete. Since genes involved in mRNA export appear to be largely conserved between yeast, animals, and plants, work conducted in non-plant systems can provide a foundation for investigations using plant models.

Our understanding of mRNA export in plants is still in its infancy. An increasing number of papers investigating the mRNA export pathway in plants are published each year. One advantage of using *Arabidopsis* to study mRNA export is that it allows us to look at the functions of the mRNA export-related genes at the whole organism level, while studies using yeast and human cells focus solely on gene function at a cellular level. With the help of modern genetic, bioinformatic and biochemical approaches, a rapid advancement in our understanding of the mRNA export process in plants is expected.

Acknowledgements

The work from YZ laboratory was supported by a grant from the Chinese Ministry of Science and Technology.

References

Carmody S R, Wente S R (2009). mRNA nuclear export at a glance. J Cell Sci, 122(Pt 12): 1933–1937

Chaudhury A, Chander P, Howe P H (2010). Heterogeneous nuclear ribonucleoproteins (hnRNPs) in cellular processes: Focus on hnRNP E1's multifunctional regulatory roles. RNA, 16(8): 1449–1462

Chinnusamy V, Gong Z, Zhu J K (2008). Nuclear RNA export and its importance in abiotic stress responses of plants. Curr Top Microbiol Immunol, 326: 235–255

Dockendorff T C, Heath C V, Goldstein A L, Snay C A, Cole C N (1997). C-terminal truncations of the yeast nucleoporin Nup145p produce a rapid temperature-conditional mRNA export defect and alterations to nuclear structure. Mol Cell Biol, 17(2): 906–920

Dong C H, Hu X, Tang W, Zheng X, Kim Y S, Lee B H, Zhu J K (2006). A putative *Arabidopsis* nucleoporin, AtNUP160, is critical for RNA export and required for plant tolerance to cold stress. Mol Cell Biol, 26(24): 9533–9543

Enninga J, Levy D E, Blobel G, Fontoura B M (2002). Role of nucleoporin induction in releasing an mRNA nuclear export block. Science, 295(5559): 1523–1525

Gatfield D, Le Hir H, Schmitt C, Braun I C, Köcher T, Wilm M, Izaurralde E (2001). The DExH/D box protein HEL/UAP56 is essential for mRNA nuclear export in *Drosophila.* Curr Biol, 11(21): 1716–1721

Germain H, Qu N, Cheng Y T, Lee E K, Huang Y, Dong O X, Gannon P, Huang S, Ding P, Li Y, Sack F, Zhang Y, Li X (2010). MOS11: a new component in the mRNA export pathway. PLoS Genet, 6(12): e1001250

Gong Z, Dong C H, Lee H, Zhu J, Xiong L, Gong D, Stevenson B, Zhu J K (2005). A DEAD box RNA helicase is essential for mRNA export and important for development and stress responses in *Arabidopsis.* Plant Cell, 17(1): 256–267

Iglesias N, Stutz F (2008). Regulation of mRNP dynamics along the export pathway. FEBS Lett, 582(14): 1987–1996

Jauvion V, Elmayan T, Vaucheret H (2010). The conserved RNA trafficking proteins HPR1 and TEX1 are involved in the production of endogenous and exogenous small interfering RNA in *Arabidopsis.* Plant Cell, 22(8): 2697–2709

Keene J D (2010). Minireview: Global regulation and dynamics of ribonucleic acid. Endocrinology, 151: 1391

Lu Q, Tang X, Tian G, Wang F, Liu K, Nguyen V, Kohalmi S E, Keller W A, Tsang E W, Harada J J, Rothstein S J, Cui Y (2010). *Arabidopsis* homolog of the yeast TREX-2 mRNA export complex: components and anchoring nucleoporin. Plant J, 61(2): 259–270

Parry G, Ward S, Cernac A, Dharmasiri S, Estelle M (2006). The *Arabidopsis* SUPPRESSOR OF AUXIN RESISTANCE proteins are nucleoporins with an important role in hormone signaling and development. Plant Cell, 18(7): 1590–1603

Stewart M (2010). Nuclear export of mRNA. Trends Biochem Sci, 35 (11): 609– 617

Strambio-De-Castillia C, Niepel M, Rout M P (2010). The nuclear pore complex: bridging nuclear transport and gene regulation. Nat Rev Mol Cell Biol, 11(7): 490–501

Sugiura T, Sakurai K, Nagano Y (2007). Intracellular characterization of DDX39, a novel growth-associated RNA helicase. Exp Cell Res, 313 (4): 782–790

Tanner P, Linder (2010). 2001. DExD/H Box RNA Helicases: From generic motors to specific dissociation functions. Mol Cell, 8(2): 251–262

Vinciguerra P, Stutz F (2004). mRNA export: an assembly line from genes to nuclear pores. Curr Opin Cell Biol, 16(3): 285–292

Yelina N E, Smith L M, Jones A M E, Patel K, Kelly K A, Baulcombe D C (2010). Putative *Arabidopsis* THO/TREX mRNA export complex is involved in transgene and endogenous siRNA biosynthesis. Proc Natl Acad Sci USA, 107(31): 31

Zhang Y, Goritschnig S, Dong X, Li X (2003). A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in *suppressor of npr1-1, constitutive 1*. Plant Cell, 15(11): 2636–2646

Zhang Y, Li X (2005). A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by *suppressor of npr1-1, constitutive 1*. Plant Cell, 17(4): 1306–1316

Zhou, Luo M, Straesser R, Katahira J, Hurt E, Reed R (2000). The protein Aly links pre-messenger-RNA splicing to nuclear export inmetazoans. Nature, 407(6802): 256

Zuccolo M, Alves A, Galy V, Bolhy S, Formstecher E, Racine V, Sibarita J B, Fukagawa T, Shiekhattar R, Yen T, Doye V (2007). The human Nup107-160 nuclear pore subcomplex contributes to proper kinetochore functions. EMBO J, 26(7): 1853–1864