Nuclear RNA Export and Its Importance in Abiotic Stress Responses of Plants

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Abstract Transduction of developmental and environmental cues into the nucleus to induce transcription and the export of RNAs to the cytoplasm through the nuclear pore complex (NPC) play pivotal roles in regulation of gene expression. The process of bulk export of mRNAs from nucleus to cytoplasm is highly conserved across eukaryotes. Assembly of export-competent mRNA ribonucleoprotein (mRNP) is coupled with both transcription and mRNA processing. The export-competent mRNP consists of mRNAs and a dozen nucleocytoplasmic shuttling nuclear proteins, including RNA export factors (Mex67-Mtr2 heterodimer, Npl3), poly(A)-binding proteins, DEAD-box protein 5 (Dbp5), and nucleoporins (NUPs) in yeast. Mobile NUPs help docking of mRNP to the NPC nuclear basket. A partially

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unfolded mRNP complex appears to be pulled through the NPC by using energy from Dbp5-catalyzed ATP hydrolysis. Dbp5 probably catalyzes the release of mRNA from mRNP in the cytoplasm. In contrast to bulk export of mRNAs by a Mex67-Mtr2/Npl3-dependent pathway, a specific subset of mRNA export under stress and export of microRNAs are mediated through the karyopherin (importin β) family of proteins in a Ran-GTPase-dependent pathway. Our knowledge of mRNA export mechanisms in flowering plants is in its infancy. Some proteins of the NUP107–160 complex, NUPs and DEAD-box proteins (DBPs), have been studied in flowering plants. *Arabidopsis* NUP160/SAR1 plays a critical role in mRNA export, regulation of flowering, and hormone and abiotic stress responses, whereas NUP96/SAR3/MOS3 is required for mRNA export to modulate hormonal and biotic stress responses. DEAD-box proteins have been implicated in mRNA export and abiotic stress response of yeast and higher plants. *Arabidopsis* DBP CRYOPHYTE/LOS4 plays an important role in mRNA export, abiotic stress response, germination, and plant development. Further studies on various components of nuclear mRNA export in plants during nonstress and stress conditions will be necessary to understand the link between mRNA export and stress-responsive gene expression.

Introduction

Abiotic stresses such as drought, salinity, and temperature extremes pose severe limitations to plant growth and development, thus limiting agricultural productivity. Drought is a major production constraint for agriculture in 45% of the world's geographical areas, where 38% of the human population lives (Bot et al. 2000). This problem will further increase as per capita fresh water availability is expected to decrease drastically in the near future. About 20% of irrigated agricultural land is adversely affected by salinity (Flowers and Yeo 1995). Approximately 93% of the continental area of Earth experiences, at least some of the time, temperatures below +15°C, which is cold stress for many crops. Further, high-temperature stress is a major problem, affecting about 40% of the irrigated areas of wheat alone (Fischer and Byerlee 1991). Global climate changes may increase drought and temperature stresses in many parts of the world by the end of this century. Hence, concerted efforts are being made worldwide to understand the mechanisms of abiotic stress tolerance in plants and to employ these mechanisms for genetic improvement of crop plants. Significant progress has been made in understanding the molecular genetic basis of abiotic stress tolerance in plants.

The perception and transduction of abiotic stresses to switch on genes involved in adaptive responses are critical to the survival and reproduction of plants exposed to adverse environments. Plants have evolved multiple stress response pathways, some of which are specific, but others may be common for various abiotic stresses (Chinnusamy et al. 2004). Transcriptional regulation of gene expression is relatively well understood (Zhu 2002; Chinnusamy et al. 2005, 2006; Yamaguchi-Shinozaki and Shinozaki 2006), but only limited progress has been made in unraveling

posttranscriptional regulation of gene expression under various abiotic stresses (Sunkar and Zhu 2004; Borsani et al. 2005; Sunkar et al. 2006) (see the chapter by G. S. Ali and A. S. N. Reddy, this volume).

Eukaryotes have a well-defined nucleus separated from the cytoplasm by a nuclear envelope, the hallmark of eukaryotic organisms. Therefore, nucleocytoplasmic trafficking—entry of developmental/environmental signals for gene expression and export of expressed gene products from the nucleus through nuclear pores—plays a fundamental role in gene expression in eukaryotic organisms. The processes of nucleocytoplasmic trafficking are fairly well understood in animals and yeast, but only a beginning has been made in higher plants (Cullen 2003; Cole and Scarcelli 2006). In eukaryotes, gene expression is regulated posttranscriptionally by pre-mRNA processing, mRNA stability, RNA export from the nucleus, and translation. Thus nuclear export of mRNA and microRNAs (miRNAs)/short interfering RNAs (siRNAs) is an integral part of gene regulation in response to abiotic stresses. This chapter discusses the processes of nuclear export of mRNAs and miRNAs/siRNAs in nonstress and stress environments, mainly based on studies from yeast and higher plants.

Nuclear mRNA Export

In eukaryotes, the nucleus is surrounded by a double-layered membrane called the nuclear envelope. Therefore, transduction of environmental/development signals into the nucleus to regulate gene transcription and export of RNA molecules from the nucleus depends highly on the macromolecular traffic through the nuclear envelope. The composition of the outer membrane of the nuclear envelope is similar to that of the endoplasmic reticulum (ER), whereas the inner membrane of the nuclear envelope contains a distinct protein composition. The space between the two layers/lumen is called the perinuclear space. The nucleocytoplasmic transport of macromolecules occurs through nuclear pores present on the nuclear envelope, except during cell division, when dissolution of the nuclear envelope occurs. The aqueous channel of the nuclear pore is about ~9 nm in diameter at rest but during active transport expands up to ~25 nm. Larger molecules of >40 kDa are selectively transported through nuclear pores with the help of transport receptors or nuclear export factors. Although the nuclear pore permits the diffusion of molecules <40 kDa in size, even small proteins and RNAs are actively transported. Transport through the nuclear pore is energy dependent (Cullen 2003; Cole and Scarcelli 2006).

Nuclear Pore Complex

The nuclear pore is composed of several proteins forming a nuclear pore complex (NPC). Proteins that constitute the NPC are collectively called nucleoporins

(NUPs). In yeast, the molecular mass of the NPC is ~50 MDa, and the core is composed of about 30 NUPs, multiple copies that form an octagonal symmetry. Most NUPs are stationary, and some shuttle between the nucleus and cytoplasm (Rout et al. 2000). The NPC is octagonally symmetric around its cylindrical axis. Peripheral filaments emanating from the core into the nucleoplasm conjoin distally to form a basketlike structure that extends up to 100 nm into the nucleoplasm, whereas peripheral filaments emanating from the core into the cytoplasm spread outward, and eight fibrils extend ~50 nm into the cytoplasm. This architecture and many of the NUPs are conserved in all eukaryotes (Rout et al. 2000).

NUPs bind to a cargo (proteins and transported RNAs) or nuclear transporter that in turn binds to a cargo. Transport of the cargo through nuclear pores requires binding of the cargo to soluble NUPs and nuclear transport receptors or nuclear export factors (NEFs) (Weis 2002; Pemberton and Paschal 2005). Nuclear transport receptors/NEFs transiently interact with NUPs in the NPC and transport the cargo across the nuclear envelope. About one-third of NUPs provide binding sites for transport receptors and thus play crucial roles in the transport of cargo-receptor complexes. The receptor/export factor binding sites on these NUPs have multiple phenylalanineglycine (FG) repeat motifs (FxFG, GLFG, or FG) flanked by polar residues (Suntharalingam et al. 2003). Different FG motifs may facilitate interaction of NUPs with distinct subsets of transport receptors, as evident from yeast mutants that affect the translocation of particular transport receptors (Blevins et al. 2003). Interaction between FG repeats of NUPs with export factors helps in docking of cargo and its transport through NPCs. Binding of the export factors to FG motifs (FXFG, GLFG, and FG) of NUPs is necessary for mRNA export (Strasser et al. 2000). Understanding the interaction between the transport complex and specific NUPs will shed further light on the regulation of nucleocytoplasmic transport.

Nuclear RNA Export Factors

Nuclear transport receptors can be classified into the karyopherin/importin-β family of proteins and non-karyopherin NEFs. Export of proteins, tRNA, U-rich snRNAs, 5S RNAs, and miRNAs/siRNAs is mediated through importin-β family export receptors. The non-karyopherin NEFs mediate export of mRNAs (Cullen 2003; Cole and Scarcelli 2006). Karyopherin binding to a nuclear cargo requires the GTP-bound form of the Ran GTPase, whereas cytoplasmic hydrolysis of Ran-GTP to Ran-GDP induces cargo release. However, NEFs function independently of Ran-GTPase. Ran-dependent karyopherins play a limited role in mRNA export but a vital role in miRNA export (Kimura et al. 2004; Cole and Scarcelli 2006). Some of the NEFs involved in mRNA export in yeast and their homologs in other eukaryotes are listed in Table 1. Under nonstress conditions, yeast MEX67p (a homolog of vertebrate Tap/NXF1), an export factor unrelated to the importin-β family, mediates bulk export of mRNAs (Segref et al. 1997). MEX67p binds to

Table 1 Nuclear export factors involved in mRNA export in yeast

Yeast gene for nuclear export factors	Metazoan homolog	Remarks	References
MEX67p (mRNA export factor of 67 kDa)	Human Tap/Nuclear Export Factor 1 (NXF1)	Yeast mutant deficient in Mex67p accumulates mRNA in the nucleus. MEX67p interacts with FG of NUPs in NPC.	Segref et al. 1997
Mtr2p (mRNA transport)	Human NXT1/p15	Heterodimer formation between Mtr2p and Mex67p is required for the export of mRNA in yeast.	Strasser et al. 2000
Yra1p (yeast RNA annealing protein 1)	Mice Aly/ human REF (RNA export factor)	Yra1p is a member of the REF (RNA and export factor binding proteins) family. It binds to mRNA and to Mex67p. Yra2p can substitute for Yra1p function. Mutation in Yralp results in mRNA accumulation in the nucleus.	Strasser and Hurt 2000; Zenklusen et al. 2001
Npl3 (nuclear protein localization 3)		mRNA export factor that functions in proper packaging of the mRNP; loss-of-function temperature-sensitive *NPL3* alleles accumulate poly(A)$^+$ RNA in the nucleus at the nonpermissive temperature.	Lee et al. 1996

both mRNAs and NUPs. The yeast MEX67p-Mtr2p heterodimer interacts with the mRNA ribonucleoprotein (mRNP) complex for export through NPCs (Vinciguerra and Stutz 2004; Cole and Scarcelli 2006).

Export of mRNAs requires processing, packaging by RNA-binding proteins, recognition by export factors, and translocation through the NPC into the cytoplasm. mRNA export does not depend on a specific motif in the cargo, but sequestration of mRNA into the mRNP complex is necessary for export (Cullen 2003; Dimaano and Ullman 2004). However, in some specific cases, such as karyopherin-dependent stress mRNA export, an adenylate uridylate-rich element (ARE) in the 3′ UTR of mRNAs is necessary for recognition by karyopherins. Formation of the mRNP complex is coupled with transcription and processing of pre-mRNA.

Transcription-Coupled mRNP Formation

Pre-mRNA transcribed by RNA polymerase (Pol) II is processed to add a 5′ monomethyl cap soon after transcription initiation, whereas splicing of introns and 3′ cleavage and polyadenylation occur immediately after transcription in the nucleus. mRNA processing occurs cotranscriptionally, and RNA Pol II coordinates these activities. The carboxy-terminal domain (CTD) of Pol II plays a crucial role in these processes in a manner dependent on the state of CTD phosphorylation (Hirose and Manley 2000). mRNPs are formed in the nucleus by packing of mRNAs into heterogenous nuclear RNPs (hnRNPs). A subset of these hnRNP proteins is retained in the nucleus, whereas others accompany the mRNA into the cytoplasm, where they dissociate to release mRNA. Then these RNPs move back into the nucleus for further rounds of export. In *Saccharomyces cerevisiae*, Npl3 (also termed Mtr13/Mts1/Nab1/Nop3), a shuttling NEF, contains two RNA-recognition motifs. Npl3p is a major mRNA-binding protein. The role of Npl3p in mRNA export was revealed by nuclear accumulation of mRNA in *npl3* mutants (Lee et al. 1996). Furthermore, Npl3p, along with other nuclear proteins, packages pre-mRNA into an export-competent RNP (Shen et al. 1998). In the cytoplasm, Npl3 releases mRNA and is transported back into the nucleus by the importin Mtr10 (Senger et al. 1998). In *S. cerevisiae*, Npl3p is recruited into the transcription complex with RNA Pol II. Mutations in both Npl3 and TATA-binding protein block mRNA export. Chromatin immunoprecipitation assays in yeast showed that Npl3 is recruited to mRNA during transcription at an early stage, whereas another mRNA export factor, Yra1p (=Aly/REF in metazoans), is recruited cotranscriptionally at a later step (Lei et al. 2001). This finding is consistent with the role of Yra1p, because it appears to tag the completely processed mRNP for nuclear export (Strässer and Hurt 2000). Np13 purified from yeast showed 17 methylated arginines and 10 Arg-Gly-Gly tripeptides exclusively dimethylated. Arginine methylation of Npl3 appears to facilitate export directly by weakening contacts with nuclear proteins (McBride et al. 2005).

Yra1p, Sub2p, THO (suppressor of the transcriptional defect of Hpr1 by overexpression) complex, Tex1, and Hpr1p (hyperrecombination) form a protein complex named TREX (transcription-export) complex in yeast (Strasser et al. 2002). THO complex consists of four proteins, namely, Tho2, Hpr1, Mft1 (mitochondrial fusion targeting 1), and Thp2 (THO2-HPR1 phenotype). The TREX complex is specifically recruited to genes during transcription and plays a conserved role in coupling transcription to bulk mRNA export (Strasser et al. 2002). Hpr1p genetically and physically interacts with Yra1p and Sub2p (an RNA helicase). Hpr1p is necessary for efficient targeting of Yra1p and Sub2p to genes undergoing active transcription (Zenklusen et al. 2002). Cotranscriptional recruitment to the mRNA export receptor Mex67p contributes to nuclear pore anchoring of activated genes (Dieppois et al. 2006). Yra1 interacts directly with the mRNA export factor Mex67, which localizes primarily to nuclear pores. This finding suggests that Yra1 can function to bridge the mRNP formation with the actual translocation machinery (Strässer and Hurt 2000). Thus cotranscriptional recruitment of RNA export factors into mRNP is a conserved mechanism of bulk mRNA export.

An RNA helicase DEAD-box protein, Dbp5, has been implicated in transcription-coupled formation of export competent mRNPs. Yeast Dbp5 associates with mRNA early during transcription and accompanies it to the cytoplasm (Zhao et al. 2002). Yeast Dbp5 interacts with TFIIH, which may help in loading Dbp5 onto nascent mRNP (Estruch and Cole 2003). Further evidence for the involvement of TFIIH in mRNA export came from the finding that *Schizosaccharomyces pombe ptr8+* mutation and *S. cerevisiae ssl2* mutation cause nuclear accumulation of poly(A)$^+$ RNA. *PTR8* and *SSL2* genes encode a component of TFIIH homologous to human XPB, a protein involved in nucleotide excision repair and transcription. Expression of human XPB in these yeast mutants rescues them from mRNA export defects. Moreover, Ptr8p functionally interacts with Tho2p, a component of the TREX complex involved in mRNA export (Mizuki et al. 2007). Thus Dbp helps in formation of mRNPs for export.

mRNA Processing Coupled with mRNP Formation

In addition to transcription-dependent mRNP formation, mRNA processing such as 5′ capping, splicing, and polyadenylation also helps in formation of export-competent mRNPs. Splicing appears to promote mRNA export but is not necessary for mRNA export. The exon junction complex (EJC) is a multisubunit complex deposited by the spliceosome 20 to 24 nucleotides 5′ to the site of intron removal (Le Hir et al. 2000). The EJC helps binding of factors involved in mRNA export and nonsense-mediated mRNA decay. EJC consists of SRM160, RNPS1, Y14, Magoh, and Aly (=Yra1p) proteins. As discussed above, Aly (=Yra1p), a member of the REF binding proteins, plays a crucial role in mRNA export (Table 1). EJC formed during splicing provides a binding site for the Tap-Nxt (=MEX67p-Mtr2p in yeast) heterodimer, and thus splicing enhances mRNA export (Le Hir et al. 2001). The recruitment of Yra1p to EJC appears to be mediated by Sub2p, a member of the DEAD-box family of RNA helicases, which play an important role in spliceosome assembly. Sub2p mutation blocks poly(A)$^+$ mRNA export in yeast. Sub2p and Yralp directly interact both in vitro and in vivo, and Sub2p helps in recruitment of Yralp to the mRNP (Strässer and Hurt, 2001). Since Yra1p can interact with Mex67p (Strasser et al. 2000), Yra1p in turn recruits the Mex67p-Mtr2p heterodimer to form an export-competent mRNP.

mRNA export depends upon proper 3′ end processing such as poly(A) tail formation (see the chapter by A.G. Hunt, this volume). Yeast mutants defective in 3′ mRNA processing are also impaired in mRNA export. Furthermore, deletion of the *cis*-acting sequences required to couple 3′ processing and termination results in production of transcripts that fail to exit the nucleus. This evidence suggests that cleavage, termination, and export are coupled (Hammell et al. 2002). Yra1p is preferentially recruited to mRNPs of intron-containing genes in a splicing-dependent pathway, whereas Yra1p recruitment into mRNPs of all genes depends on 3′-end formation, regardless of intron status (Lei and Silver 2002). Furthermore, yeast poly(A)-binding protein Pab1 has been shown to shuttle between the nucleus

and the cytoplasm and plays a role in mRNA export. Inhibition of nuclear import of Pab1 results in a kinetic delay in the export of mRNA. In addition to bulk mRNA export by the Mex67p-Mtr2p pathway, Pap1 also plays a role in the karyopherin chromosome region maintenance 1 (CRM1)-dependent pathway of nuclear export. A *pab1* deletion strain is rescued by a mutation in the 5′–3′ exoribonuclease *RRP6,* a component of the nuclear exosome. Thus nuclear Pab1 may be required for efficient mRNA export and quality control of mRNA in the nucleus (Brune et al. 2005).

These results explain why splicing enhances the nuclear export of mRNA but is not necessary for mRNA export. This observation may be due to the fact that cotranscriptional splicing- and 3′-end processing–coupled mRNP formation–recruits the same nuclear factor, Yra1p. Thus Yra1p is recruited into mRNP by any one of the processes of mRNA synthesis and helps in recruitment of the Mex67p-Mtr2p heterodimer to form export-competent mRNP.

Export of mRNP Through NPCs

As discussed above, assembly of export-competent mRNP occurs cotranscriptionally and is coupled to mRNA processing. Export-competent mRNP consists of mRNA and a dozen nuclear proteins, including REFs (Mex67-Mtr2 heterodimer, Npl3), poly(A)-binding proteins (Pab1 and Pap2), Dead box protein Dbp5, and mobile NUPs. Mutations in yeast Mex67, Gle1, and Dbp5 result in nuclear accumulation of poly(A)$^+$ mRNAs (Segref et al. 1997; Tseng et al. 1998). Studies of Balbiani ring mRNP granule export revealed that at least partial unfolding of mRNPs is necessary for their transport through NPCs, and the 5′ end of the mRNP enters the channel first (Daneholt 2001). mRNPs are possibly pulled through NPCs by using energy from ATP hydrolysis. Dbp5 probably acts as the ATPase and unwinds mRNP during transport through the NPC. Mutations that impair ATPase activity of Dbp5 also impair the function of Dbp5 in vivo (Schmitt et al. 1999). Gle1 stimulates ATPase activity of Dbp5 in an IP6-dependent manner; this activation may facilitate the remodeling of mRNP protein composition during directional transport and provide energy to power transport cycles (Alcazar-Roman et al. 2006; Weirich et al. 2006). In yeast, a mutation in Gle1 affects the overall structural integrity of NPCs and impairs mRNA export (Murphy et al. 1996). A Gle1 homolog in humans, hGle1, is also a nuclear-cytoplasmic shuttling protein involved in mRNA export (Watkins et al. 1998). Proteomic analysis of vertebrate NPCs and the nucleocytoplasmic shuttling nature of Gle1 suggested that the association of hGle1 with NPCs is transient. The N-terminal region of hGle1 interacts with the nucleoporin hNup155. NPC localization of hGle1p requires hNup155p and hNup42p/hCG1/NPL1 (Rayala et al. 2004; Kendirgi et al. 2005). Another NUP-interacting export factor, Gle2p/Rae1p, was shown to play a crucial role in mRNA export (Murphy et al. 1996) through its interaction with yeast Nup116p, which shares similarity with human hNUP98 (Pritchard et al. 1999).

Yeast Nup145p is similar to yeast Nup116p and hNup98. Depletion of Nup145p in vivo leads to rapid accumulation of mRNA in the nucleus. Genetic analysis in yeast revealed that Nup145p, Nup116p, and Nup100p play redundant functions in nucleocytoplasmic transport (Fabre et al. 1994). Nup160 and Nup133 are localized on the basket side of the NPC. Nucleoporins Nup160, Nup133, Nup107, and Nup96 exist as a complex and are collectively termed the Nup160 complex. Among these, Nup160 and Nup133 interact with Nup98 and play a role in mRNA export (Vasu et al. 2001). Nup153p is a highly mobile protein recruited to the mRNP export complex during transcription (Griffis et al. 2004).

The mechanism of translocation of mRNP through NPC is much less known. Interactions between nuclear export factors and FG repeats on NUPs drive the translocation of receptor-cargo complexes through nuclear pores. Tap, the metazoan homolog of yeast nuclear export factor MEX67, contains UBA-like and NTF2-like folds that can associate directly with FG repeats. Mutations in the Tap-UBA domain abolished the interaction of Tap with nucleoporins Nup98, p62, and RanBP2, whereas mutations in the NTF2-like domain of Tap impaired Nxt1 (=yeast Mtr2) binding. Although both of these mutations impaired mRNA export, Tap interaction with the NPC in vivo or its nucleocytoplasmic shuttling was not affected. Thus Tap requires both the UBA- and NTF2-like domains to mediate the export of RNA cargo, but it shuttles through the NPC independently of these domains when free of RNA cargo (Levesque et al. 2006).

Cytoplasmic filaments of NPC consist of Nup159, Gle1, Nup82, and Nup42/Rip1. Mutations in these genes affect mRNA export (Cole and Scarcelli 2006). Dbp5 travels along with the mRNP complex through NPCs. Once mRNP reaches the cytoplasmic side of the NPC, Dbp5 interacts with the cytoplasmic filament Nup159 of the NPC. The N-terminal region of Nup159 is necessary for Dbp5 binding, and deletion of this domain significantly reduces the Dbp5 at the nuclear periphery. In addition to Dbp5, Nup159 also interacts with nucleoporins Nup82 and NSP1 (nucleoskeletal-like protein 1). Nsp1p contains multiple repeats of the amino acids FXFG. Nup159 mutant yeast is impaired in mRNA export (Del Priore et al. 1997). Mutations in highly conserved residues of the N-terminal region, which interact with Dbp5, render Nup159-Dbp5 interaction temperature sensitive and also result in temperature-sensitive mRNA export. These findings suggest that the Nup159 N-terminal domain functions in mRNA export as a binding platform, tethering shuttling Dbp5 molecules at the nuclear periphery (cytoplasmic side), and the Dbp5 may release mRNA from mRNP at the cytoplasmic face of the NPC (Weirich et al. 2004).

Nuclear Export of miRNAs and siRNAs in Plants

Small RNAs, which do not code for proteins but negatively regulate gene expression, are classified into two types—miRNAs and siRNAs—on the basis of their biogenesis. miRNAs are synthesized from single-stranded primary miRNA (pri-miRNA)

transcripts, which are transcribed from miRNA genes (*MIR* genes) by RNA Pol II. In plants, the pri-miRNA transcript forms one or more stem-loop secondary structures, which is(are) cleaved by a ribonuclease III-like enzyme called Dicer-like 1 (DCL1) protein to produce an miRNA-miRNA* duplex in the nucleus. DCL1 protein interacts with a nuclear dsRNA-binding protein, HYPONASTIC LEAVES 1 (HYL1) (Kurihara et al. 2006), to precisely cleave pri-miRNA into the miRNA-miRNA* duplex (stem region of the hairpin) of ~21-nt length. A nuclear methyltransferase protein, HUA ENHANCER 1 (HEN1), methylates the three terminal nucleotides on their 2′-OH group in the miRNA-miRNA* duplex, which is then exported from the nucleus into the cytoplasm by HASTY (HST), a member of the importin β family of nucleocytoplasmic transporters, via the Ran-GDP-Ran-GTP transport system (Yi et al. 2003; Lund et al. 2004; Park et al. 2005). The miRNA-miRNA* duplex is then unwound into a single-stranded mature miRNA by an unknown helicase. The miRNA (~21-nt length) then enters the RNA-induced silencing complex (Bartel 2004; Kidner and Martienssen 2005; Jones-Rhoades et al. 2006; Mallory and Vaucheret 2006).

Endogenous siRNAs are synthesized from long double-stranded RNAs (dsRNAs) of endogenous origin such as (a) miRNA-directed cleavage products of noncoding single-stranded RNAs (ssRNAs)/transgene mRNAs, which are then converted into dsRNAs by RNA-dependent RNA polymerases (RDRs), generating *trans*-acting siRNAs (ta-siRNAs); (b) dsRNAs formed from the mRNAs encoded by natural *cis*-antisense gene pairs (nat-siRNAs; Borsani et al. 2005); and (c) dsRNAs generated from heterochromatin and DNA repeats (Mallory and Vaucheret 2006). RDRs and DCL-like proteins process the dsRNAs formed from different sources. Biogenesis of different classes of siRNAs is carried out by specific RDR-DCL protein combinations in the nucleus (Jones-Rhoades et al. 2006; Mallory and Vaucheret 2006).

Small RNAs are incorporated into an RNA-induced silencing complex (RISC) or a RNAi-induced transcriptional silencing (RITS) complex, which contain Argonaute (AGO) family proteins. miRNA and siRNAs regulate gene expression by (a) cleavage of target mRNAs that are complementary to the miRNA or siRNA, (b) miRNA- or siRNA-mediated translational repression, and (c) transcriptional silencing mediated mainly by heterochromatic siRNAs and, in some cases, by miRNA (Bartel 2004; Bao et al. 2004; Chan et al. 2005). Repression of gene expression by mRNA cleavage and translational repression necessitates the export of miRNAs or siRNAs from the nucleus to the cytoplasm. *Arabidopsis* HST is an ortholog of mammalian exportin 5 (Exp5) and yeast Msn5p. Msn5p exports phosphorylated proteins and imports replication protein A, involved in DNA replication and repair, whereas EXP5 exports pre-microRNAs (pre-miRNAs), tRNAs, and short hairpin RNAs into the cytoplasm (Yi et al. 2005). However, loss-of-function mutations in *HST* reduced the export of most but not all miRNAs but did not affect the export of tRNAs and endogenous siRNAs or transgene silencing in *Arabidopsis*. These results suggest that HST plays a crucial role in miRNA export, but other nuclear export pathways are also involved in export of small RNAs in *Arabidopsis* (Park et al. 2005).

Stress-Specific mRNA Export Pathways

In yeast, in situ hybridization showed that abiotic stresses (heat shock and ethanol) blocked the nuclear export of most poly(A)$^+$ RNA, whereas the heat shock protein 70 (HSP70) mRNAs were exported through functional NPCs under stress. These results suggest a separate nuclear traffic for normal mRNAs and HSP mRNAs under heat stress (Saavedra et al. 1996). In yeast, bulk export of mRNA is independent of Ran but dependent on nuclear export factors MEX67, MTR2, and NPL3. In yeast, an FG repeat containing 42-kDa nucleoporin, NUP42/Rip1p, is associated with NPCs and is necessary for mRNA export under heat stress. NUP42 is dispensable for growth and mRNA export under normal conditions, because *HSP70* mRNAs transcribed from the *GAL* promoter::SSA4 under non-heat-stress conditions was efficiently exported to the cytoplasm in the *nup42* mutant. Yeast Nup42p interacts with the REFs Gle1p (GLFG LEthal), Gle2p, and Nup100p (Saavedra et al. 1997); Gle1p and Gle2p are required for specific mRNA export under stress.

Heat shock (42°C) to yeast cells results in rapid dissociation of mRNA export factor Gle2p from the nuclear envelope into the cytoplasm, which is accompanied by inhibition of bulk export of mRNAs. Acclimation treatment (37°C for 1 h) helped to stabilize association of Gle2p to NPC at 42°C and thus permitted export of bulk poly(A)$^+$ mRNA. Yeast deletion mutants *gle2*Δ and *nup42*Δ could not induce the acclimation response and thus failed to adapt the export of bulk poly(A)$^+$ mRNA to heat shock. These results suggest that Gle2p and Nup42p are necessary for mRNA export, especially under heat-shock conditions (Izawa et al. 2004), whereas nucleoporins Nup159p/Rat7p, Nup120p/Rat2p, and Nup145p/Rat10p are required for mRNA export in both normal and heat stress pathways (Saavedra et al. 1997).

Gle1 is a conserved mRNA export factor from yeast to humans. hGle1 interacts with hNup155, which is necessary to target hGle1 to NPCs. In addition to hNup155, hCG1/NPL1 (=yNup42) and hGle1B are required for Gle1 targeting to NPCs. siRNA-directed suppression of hCG1 resulted in decreased levels of hCG1, which in turn resulted in hGle1 accumulation in cytoplasmic foci and also inhibited export of the Hsp70 mRNA in HeLa cells. Thus the Gle1-yNup42 (=hGle1-hCG1) mRNA export mechanism is highly conserved and plays a pivotal role in specific mRNA export under heat stress (Kendirgi et al. 2005).

Gle1 and Nup42p involved in mRNA export are in the Nup82p subcomplex, on the cytoplasmic face of the NPC in yeast. The Nup82p subcomplex contains Nup82p, Rat7p/Nup159p, Nsp1p, Gle1p/Rss1p, and Rip1p/Nup42p. Nup159p and Gle1p contain binding sites for Dbp5p. In the yeast Nup42p deletion mutant *nup42*Δ, both Gle1p and Dbp5p dissociate from NPCs after heat shock at 42°C. Efficient export of HSP mRNA after heat shock depends upon a novel 6-amino acid motif of Dbp5p. This motif is not required for mRNA export under normal growth conditions or ethanol shock (Rollenhagen et al. 2004).

In addition to Nup42p-Gle1p-Dbp5p, yeast RNA Pol II subunit Rpb4p also appears to mediate mRNA export under abiotic stresses in yeast. Yeast Rpb4p is required for transcription, mRNA export, and cell survival under heat stress conditions. Class II

mutants of RBP4 (*Rbp4-2*), which affect only the export role but are normal in transcription function, could not acquire thermotolerance even under acclimation treatment. Rpb4p appears to perform different roles in export of bulk mRNA at 37°C and heat shock mRNAs at 42°C (Farago et al. 2003). Rpb4p and Rpb7p form a dissociable heterodimeric complex, which can bind to mRNA and shuttle between the nucleus and cytoplasm as a heterodimer. Shuttling of Rpb4p and Rpb7p depends on ongoing transcription under normal conditions, but during the severe stresses of heat shock, ethanol, and starvation, the two proteins shuttle via a transcription-independent pathway (Selitrennik et al. 2006).

The karyopherin CRM1 is involved in export of proteins, HIV mRNA, U small nuclear RNAs, and all rRNAs in humans. Although bulk mRNA export is independent of karyopherins, a specific subset of mRNA appears to be exported through CRM1 under stress. Human RNA binding protein (HuR) binds to an adenylate uridylate-rich element (ARE) in the 3′ UTRs. The interaction of HuR, pp32 and APRIL, the leucine-rich nuclear export signal (NES)-containing ligands, with CRM1 enhances the export of mRNAs of certain early response genes (ERGs) (Gallouzi and Steitz, 2001). In addition, the nuclear export of human *interferon-α1* (*IFN-α1*) mRNA, an ERG mRNA induced upon viral infection, has been shown to be exported through CRM1, and ARE in the 3′ UTR of *IFN-α1* mRNA is not essential for this (Kimura et al. 2004). Thus the CRM1 pathway appears to mediate both ARE-dependent and -independent export of specific mRNAs under stress.

Role of mRNA Export in Hormonal and Biotic Stress Responses of Plants

Relatively little information exists on the NUPs and NEFs required for mRNA export in plants. Bioinformatic analyses have identified several conserved NUPs from rice and *Arabidopsis* that are homologous to yeast and vertebrate NUPs (Neumann et al. 2006). Since the nuclear mRNA export pathway is highly conserved across species (fungi, insects, vertebrate) and many proteins similar to NUPs and NEFs have been found in higher plants, the mRNA export process in higher plants appears to be similar to that of other eukaryotes. The following section briefly discusses the NUPs characterized in plants and their role in mRNA export (Table 2).

The animal NUP107–160 complex is functionally equivalent to the yeast NUP84 complex, which is involved in mRNA export (Bai et al., 2004). Most subunits of the NUP107–160 complex (NUP160, NUP133, NUP107, NUP96, NUP85, NUP43, and two proteins similar to Sec13) have identifiable homologs in the *Arabidopsis* proteome, which suggests that the NUP107–160 complex is also conserved in *Arabidopsis* (Parry et al. 2006). *Arabidopsis snc1 (*suppressor of npr1-1, constitutive 1), a gain-of-function mutant in a Toll Interleukin1 receptor-nucleotide binding-Leu-rich repeat-type resistance gene (*R*-gene), shows constitutive expression of disease resistance genes. A suppressor screen for *snc1* led to the isolation of *mos3* (modifier of snc1,3). mos3 is susceptible to bacterial diseases,

Table 2 Higher plant homologs of yeast/vertebrate NUPs involved in mRNA export

Plant Protein	Function	Remarks	References
Arabidopsis suppressor of auxin resistance 1 (SAR1)/ AtNUP160	Nucleoporin, part of Nup107–160 subcomplex	Similar to human Nup160; sar1 mutants accumulate poly(A) RNA within the nucleus	Parry et al. 2006
Arabidopsis NUP160	Nucleoporin, part of Nup107–160 subcomplex	*atnup160–1* is defective in poly(A) mRNA export, reduces induction of *CBF3-LUC* under cold stress, and is hypersensitive to chilling and freezing stress	Dong et al. 2006
Suppressor of auxin resistance 3 (SAR3) of *Arabidopsis*	Nucleoporin, part of Nup107–160 subcomplex	Similar to human Nup96; sar3 mutants accumulate poly(A) RNA within the nucleus	Parry et al. 2006
Arabidopsis modifier of *SNC*1,3 (MOS3/SAR3)	Nucleoporin, part of Nup107–160 subcomplex	Required for disease resistance; similar to human Nup96, which is required for reversal of inhibition of mRNA nuclear export by a viral protein	Zhang and Li 2005
Lotus NUP133	Nucleoporin, part of Nup107–160 subcomplex	NUP133 participates in host-plant recognition of symbiotic microbes; Nup133 in yeast and vertebrate plays crucial role in mRNA export	Kanamori et al. 2006

which suggests that MOS3 is required for basal resistance to pathogens. *MOS3* encodes a putative NUP, which is localized on the nuclear envelope. *MOS3* shows high sequence similarity to human Nup96, which is involved in mRNA export. Thus nucleocytoplasmic trafficking appears to play a crucial role in biotic stress resistance (Zhang and Li 2005).

Direct evidence for the role of Nup96 in mRNA export came from studies of *sar* (suppressor of auxin resistance) mutants. A screen to identify suppressors of the *axr1* (auxin-resistant 1) mutant resulted in identification of *sar1* and *sar3* mutants in *Arabidopsis*. *sar1* and *sar3* mutations affect the nuclear localization of the transcriptional repressor AXR3/INDOLE ACETIC ACID17 and thus suppress the phenotype conferred by *axr1*. *SAR1* and *SAR3* encode proteins with similarities to vertebrate NUP160 and NUP96, respectively. SAR1 and SAR3 localize to the nuclear membrane. *sar1* and *sar3* mutant plants accumulate poly(A) RNA within

the nucleus. These results suggest that SAR1 and SAR3 nucleoporins are required for mRNA export and modulate plant responses to the hormone auxin (Parry et al. 2006). Another member of the NUP107–160 complex, NUP133, characterized from *Lotus*, also showed a participation in host-plant recognition of symbiotic microbes (Kanamori et al. 2006).

Role of mRNA Export in Abiotic Stress Response of Plants

Dead box proteins (DBPs) with RNA helicase activity are involved in RNA metabolism such as transcription, RNA processing, RNA decay, and nucleocytoplasmic transport. As discussed previously, in yeast and vertebrates, Dbp5 plays a crucial role in export competent mRNP formation in nucleus, transport of mRNP through the NPC, and release of mRNA from mRNP in the cytosol. The role of DBP in mRNA export and abiotic stress response of flowering plants came from the analysis of the mutant *los4* (low expression of osmotically responsive genes 4) of *Arabidopsis*.

Screening for mutants with altered expression of the *RD29A-LUC* reporter gene under abiotic stresses led to the isolation of the mutant *los4-1*. *los4-1* shows reduced *RD29A-LUC* expression in response to cold but not ABA or high salt. In *los4-1*, *CBF3* expression is reduced and *CBF1* and *CBF2* expression is delayed, and thus the mutant exhibits hypersensitivity to chilling temperatures. Map-based cloning of *LOS4* showed that it encodes a DEAD-box RNA helicase (Gong et al 2002). Later, the *cryophyte/los4–2* mutant (allelic to *los4–1*) was isolated; the mutant showed superinduction of *CBF2* under cold stress and enhanced cold tolerance. The CRYOPHYTE/LOS4-GFP protein is enriched in the nuclear rim. Consistent with the cold-sensitive phenotype of *los4–1*, *los4–1* showed inhibited mRNA export under both normal and cold stress conditions. In contrast, the cold-tolerant but heat-sensitive *cryophyte/los4-2* showed normal mRNA export under cold stress but was defective in mRNA export from the nucleus at warm temperatures (Gong et al. 2005). The results from *los4* mutants demonstrated that LOS4 DEAD-box RNA helicase plays an important role in mRNA export, which is necessary for abiotic stress response. LOS4 helicase is also involved in many physiological processes such as germination (ABA hypersensitivity of *los4–2*) and plant development (*los4* mutant flowers earlier), in addition to its role in low-temperature responses (Gong et al. 2005).

In addition to their presence in *Arabidopsis*, abiotic stress-responsive DEAD-box-related helicases have been reported from pea. Pea DNA helicases 45 (*PDH45*) and *PDH47* are upregulated by various abiotic stresses. Tissue-specific differences in expression of *PDH47* were observed, because ABA upregulated *PDH47* expression in roots but not in shoots. PDH47 was localized to both the cytosol and nucleus. Similar to the Dbp5 of yeast, PDH47 also showed ATPase activity (Vashisht et al. 2005). Transgenic tobacco plants overexpressing PDH45 showed enhanced tolerance to salt stress (Sanan-Mishra et al. 2005). Thus DEAD-box RNA helicases play a pivotal role in mRNA export and abiotic stress tolerance. Further

studies will be necessary to understand the link between DBP-mediated mRNA export and abiotic stress-responsive gene expression.

The role of NUPs in abiotic stress response was demonstrated recently. Use of a *CBF3* promoter-driven LUC reporter gene screen isolated *atnup160-1* mutant, which was hypersensitive to chilling stress and also defective in acquired freezing tolerance. Map-based cloning of At*NUP160* revealed it to encode a putative NUP, homologous to the mammal Nup160. Microarray analysis revealed that *atnup160* mutation impaired the expression of *CBFs* and a number of other genes involved in plant cold tolerance. *atnup160* flowers early and shows retarded seedling growth, especially at low temperatures. The AtNUP160-GFP fusion protein is localized at the nuclear rim. *atnup160–1* mutant plants are also impaired in mRNA export. AtNUP160 is ubiquitously expressed in all tissues and not regulated by cold stress. Thus AtNUP160 is critical for mRNA export, cold-responsive gene expression, cold tolerance, as well as plant development at normal temperatures (Dong et al. 2006). AtNUP160/SAR1 plays a pivotal role in nucleocytoplasmic transport of RNAs and regulates plant development (flowering), abiotic stress, and hormonal responses in plants (Dong et al. 2006; Parry et al. 2006).

Nucleocytoplasmic shuttling of NUPs and transport receptors is important for mRNA export. Once mRNA is delivered into the cytosol, the proteins (NUPs and export factors) are imported back into the nucleus. Importin β proteins interact with importin α and a cytoplasmic cargo containing the nuclear localization signal; then this complex is imported into the nucleus through the NPC. For example, nuclear mRNA export factor Npl3p is imported back by importin Mtr10 (Senger et al. 1998). Moreover, the importin β-domain protein CRM1-dependent pathway plays a crucial role in export of specific mRNAs under stress in humans. We used a *P RD29A:LUC* genetic screen to identify a SAD2/Importin β-domain protein involved in nucleocytoplasmic trafficking. *sad2-1* knockout mutation enhanced the expression of *RD29A:LUC* reporter gene and ABA- and stress-responsive genes under cold, salt, polyethylene glycol (PEG), and ABA treatments. However, the expression of SAD2 is not regulated by stress or ABA. *sad2–1* also exhibited ABA hypersensitivity in seed germination and seedling growth. SAD2 is predominantly localized in the nucleus. These results suggest that SAD2 may play a crucial role in nuclear import of a negative regulator of cold, salt, PEG, and ABA responses (Verslues et al. 2006).

Arabidopsis homologs for some of the yeast proteins involved in nuclear export of mRNA have been identified by sequence similarity. Our studies of *AtNUP160*, *LOS4*, and *SAD2* showed that these genes involved in mRNA export are critical for abiotic stress responses, although their transcript levels are not regulated by abiotic stresses. We examined the expression pattern of some of the *Arabidopsis* genes potentially involved in nuclear export of mRNA by using the Genevestigator response viewer (Zimmermann et al. 2004). Interestingly, some of the genes showed more than twofold induction under stress (Table 3). Putative *Arabidopsis* nucleoporin At2g45000, a homolog of yeast NSP1, appears to be upregulated by drought, salt, cold, and heat stresses. Some of the genes showed more than twofold induction under hypoxia. Further molecular genetic analysis of these genes will help unravel their role in abiotic stress responses of plants.

Table 3 *Arabidopsis* homologs of yeast NUPs/nuclear proteins involved in mRNA export

		Fold change in expression of target genes under stress (https://www.genevestigator.ethz.ch)						
Yeast	*Arabidopsis*	Drought	Salt	Cold	Heat	ABA	Oxidative	Maximum increase*
NSP1	At2g45000[1]	2.50	2.00	1.50	1.75	1.00	0.75	2.50, drought
Nup42/Rip1	At1g75340[1]	0.93	0.92	0.93	1.12	0.72	0.87	2.02, hypoxia
Nup82	At5g05680[1]	0.99	0.68	0.91	0.94	0.58	1.06	2.63, *A. tumefaciens*
Nup84	At3g14120[1]	1.03	0.77	0.72	1.30	0.66	1.04	1.68, *A. tumefaciens*
Nup100/	At1g59660/At1g	1.25	1.23	1.29	1.09	0.69	1.67	13.22, hypoxia
Nup116	10390[1]	1.15	0.89	1.14	1.31	0.98	1.32	
Nup120	At1g33410[1]/AtNUP160/SAR1	0.93	0.80	0.72	1.16	0.99	0.92	1.45, low nitrate
Nup133	At2g05120[1,a]	0.88	0.93	0.82	1.26	0.98	0.89	2.10, anoxia
Nup145	At1g80680 [1]/MOS3/SAR3	1.00	0.73	0.85	1.45	0.83	1.07	1.45, heat
Gle1	At1g13120[1]	0.93	0.84	0.96	1.16	0.80	0.86	1.68, BL
Gle2	At1g80670[1]	1.07	0.84	0.91	1.37	0.79	1.70	7.85, hypoxia
Yra1	At5g59950	1.16	1.15	1.61	1.64	1.00	1.36	1.97, hypoxia
Dbp5	At3g53110/LOS4	0.95	1.01	0.91	1.15	0.88	0.99	1.59, anoxia
	At1g14850/NUP155	0.97	0.84	0.91	0.93	1.14	0.98	1.50, cold 24 h/7 days
Mago	At1g02140[b]	0.93	0.74	0.93	1.06	0.76	0.97	1.43, heat; 1.39 salicylic acid
Y14	At1g51510[b]	0.94	0.69	1.32	0.95	0.82	0.87	1.53, cold 24 h/7 days

[1]*Arabidopsis* homologs of yeast proteins identified by Neumann et al. 2006

*Maximum fold increase under stress/hormone treatment

[a]*Arabidopsis* homologs of Lotus *NUP133*

[b]Park and Muench 2007

Conclusion and Perspectives

Our knowledge of mRNA export mechanisms in flowering plants is in its infancy. Being poikilothermic and sessile, plants need a high order of gene regulation to respond to environmental cues, including abiotic stresses. Transduction of developmental and environmental cues into the nucleus to induce transcription and export of mRNA and regulatory small RNAs (miRNAs/siRNAs) to the cytoplasm through the NPC plays a pivotal role in regulation of gene expression. The process of bulk export of mRNA from the nucleus to the cytoplasm takes place through a distinct pathway independent of Ran-regulated karyopherins. The assembly of export-competent mRNP is coupled to transcription and 3′ mRNA processing. Export-competent mRNP consists of mRNA and a dozen nuclear proteins, including RNA-export factors (Mex67-Mtr2), Npl3, poly(A)-binding proteins, mobile NUPs, and the DEAD-box protein Dbp5. Dbp5 appears to power the transport through NPCs and catalyzes the release of mRNA from mRNP in the cytoplasm. It is known that in yeast and humans under stress conditions, a specific subset of mRNA is exported through specific pathways other than the bulk mRNA export pathway. However, in plants such pathways are yet to be discovered.

Bioinformatic analysis of *Arabidopsis* and rice genome sequences led to the in silico identification of nucleoporin and NEF homologs of yeast/vertebrates. Although the mRNA export pathway appears to be conserved in vertebrates, insects, and yeast, whether it is completely conserved in flowering plants is still unknown. Current evidence suggests that some variations in RNA export pathways exist in plants. For example, the mammalian and yeast nuclear export receptor EXP5 exports pre-microRNAs (pre-miRNAs) as well as tRNAs into the cytoplasm. However, the EXP5 ortholog of *Arabidopsis*, HST, is involved in nuclear export of miRNA but not tRNAs. In plants, NUP107–160 complex proteins have been shown to participate in mRNA export and regulate germination, flowering, hormone response, host-plant symbiotic interaction, and biotic and abiotic stress tolerance. Furthermore, the DBP RNA helicases have been implicated in mRNA export and abiotic stress tolerance of plants. Further studies on various components of the nuclear mRNA export machinery in plants and their role in abiotic stress response will be necessary to better understand the link between mRNA export and abiotic stress-responsive gene expression.

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