LONO1 Encoding a Nucleoporin Is Required for Embryogenesis and Seed Viability in Arabidopsis\textsuperscript{1[C][W][OA]}

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Early embryogenesis in Arabidopsis (Arabidopsis thaliana) is distinguished by a predictable pattern of cell divisions and is a good system for investigating mechanisms of developmental pattern formation. Here, we identified a gene called LONO1 (LNO1) in Arabidopsis in which mutations can abolish the first asymmetrical cell division of the zygote, alter planes and number of cell divisions in early embryogenesis, and eventually arrest embryo development. LNO1 is highly expressed in anthers of flower buds, stigma papilla of open flowers, and embryo and endosperm during early embryogenesis, which is correlated with its functions in reproductive development. The homozygous lno1-1 seed is not viable. LNO1, a homolog of the nucleoporin NUP214 in human (Homo sapiens) and Nup159 in yeast (Saccharomyces cerevisiae), encodes a nucleoporin protein containing phenylalanine-glycine repeats in Arabidopsis. We demonstrate that LNO1 can functionally complement the defect in the yeast temperature-sensitive nucleoporin mutant nup159. We show that LNO1 specifically interacts with the Arabidopsis DEAD-box helicase/ATPase LOS4 in the yeast two-hybrid assay. Furthermore, mutations in AtGLE1, an Arabidopsis homolog of the yeast Gle1 involved in the same poly(A) mRNA export pathway as Nup159, also result in seed abortion. Our results suggest that LNO1 is a component of the nuclear pore complex required for mature mRNA export from the nucleus to the cytoplasm, which makes LNO1 essential for embryogenesis and seed viability in Arabidopsis.

In Arabidopsis (Arabidopsis thaliana), early embryogenesis follows a predictable pattern of cell divisions. For example, the zygote first divides asymmetrically to form a small apical cell and a large basal cell after fertilization, establishing two cell lineages with fundamentally different growth patterns and developmental fates (Scheres and Benfey, 1999). The apical cell then undergoes two rounds of longitudinal cell divisions and one round of transverse division to form eight cells, which divide periclinally to produce a 16-cell pro-embryo. The spherical proembryo continues to develop and passes through a series of stages that can be defined morphologically as globular, transition, heart, torpedo, and walking stick stages (Goldberg et al., 1994). The basal cell elongates and divides transversely to form a structure of seven to nine cells called the suspensor, which follows an extraembryonic cell fate.

However, mutations in some genes can make the basal lineage cells adopt an embryonic cell fate by undergoing longitudinal cell divisions (Vernon and Meinke, 1994; Zhang and Somerville, 1997; Lotan et al., 1998; Lukowitz et al., 2004).

The normal pattern formation is important in embryogenesis, and many genes have been shown to regulate the process. The PIN-FORMED (PIN) genes encode transporter-like membrane proteins that function as auxin efflux carriers (Friml, 2003). PIN proteins control polar auxin transport and establish auxin gradients during embryogenesis, which are required for early embryo pattern formation in Arabidopsis (Friml et al., 2003; Weijers et al., 2005). Genes encoding transcription factors, such as AUXIN RESPONSE FACTORS, MONOPTEROS, BODENLOS, TARGET OF MONOPTEROS5 (TM05), TM07, HANABA TARANU, PLETHORA1 (PLT1), and PLT2, are also involved in pattern formation and/or embryonic root initiation in early embryogenesis (Berleth and Jurgens, 1993; Hamann et al., 1999; Aida et al., 2004; Nayw et al., 2010; Schlereth et al., 2010).

Determination of the apical and basal cell fates requires the signaling pathways regulated by YODA (a MEK kinase; Lukowitz et al., 2004), SHORT SUPPRESSOR (an IL-1 receptor-associated kinase/Pelle-like kinase; Bayer et al., 2009), WUSCHEL-LIKE HOMEOBOX (Haecker et al., 2004; Breuninger et al., 2008), and GROUNDED or RKD4, a putative transcription factor of the RWP-RK motif family (Jeong et al., 2011; Waki et al., 2011). RECEPTOR-LIKE PROTEIN KINASE1 and TOADSTOOL2 are also critical for embryonic pattern formation (Nodine et al., 2007). These genes function in...
diverse pathways in plants, but how the signal events regulated by these genes converge during early embryogenesis is not well understood. Recently, genomic approaches have been employed to understand how many genes are expressed in seeds and gametophyte as well as which genes are essential for making a seed (Meinke et al., 2008; Le et al., 2010; Autran et al., 2011; Drews et al., 2011; Hsieh et al., 2011; Nodine and Bartel, 2012), but it seems that there is no definitive answer to what the minimal gene set is for making a seed in plants.

In eukaryotic cells, the nuclear envelope forms a physical barrier to separate the cytosol from the nucleus. The nuclear pore complexes (NPCs) are channels embedded between the inner and outer nuclear membranes and consist of multiple copies of approximately 30 different proteins (Hetzer and Wente, 2009; Strambio-De-Castilla et al., 2010). NPCs are the only channels that all nucleocytoplasmic traffic must go through, such as mRNA export from the nucleus to the cytoplasm and protein import from the cytoplasm to the nucleus. As a macromolecular complex, NPC is not easy to study, but recent progress has shed light on its structure, assembly, and functions. Nucleoporins, the building blocks of NPCs, can be generally divided into three classes: a small group of membrane-anchored proteins, a large group of barrier proteins containing Phe-Gly (FG) repeats, and a large group of scaffold proteins that form a stable architectural framework of the NPC (Brohawn et al., 2009; Onischenko and Weis, 2011). NPC biogenesis occurs in both mitosis and interphase (Hetzer and Wente, 2009). NPCs disassemble into subcomplexes during mitosis. Subcomplexes and endoplasmic reticulum membranes are then recruited to chromatin at the end of mitosis (Dultz et al., 2008; Lupu et al., 2008). For example, it has been shown that recruitment of the Nup107/160 subcomplex initiates the NPC assembly during nuclear envelope reforma-

tion in *Xenopus* spp. and is followed by the recruitment of endoplasmic reticulum membranes containing POM121 and Ndc1 and the subsequent integration of Nup155 and Nup53 (Rasala et al., 2008). During interphase, other newly synthesized nucleoporins are targeted and incorporated into the nuclear envelope to form NPCs (Rasala et al., 2008). While the main function of NPCs is to mediate trafficking between the nucleus and the cytoplasm, functions of individual nucleoporins in growth and development have only begun to be understood. For example, the nucleoporin Nup98 stimulates the expression of cell cycle genes in *Drosophila* spp. (Kalverda et al., 2010).

**CAN/NUP214** was originally discovered due to its involvement in myeloid leukemia in humans (*Homo sapiens*; von Lindern et al., 1992a, 1992b). NUP214 is an FG repeat-containing nucleoporin that is likely to serve as a docking site in the receptor-mediated import of substrates across the NPC (Bastos et al., 1997). It has been shown that *nup214−/−* mouse embryonic stem cells are not viable and that embryos die early in utero (van Deursen et al., 1996). Interestingly, these defective *nup214−/−* embryos do not display any obvious abnormal morphology in their nuclear envelopes or NPCs (van Deursen et al., 1996). The Nup159/RAT7 gene (for RNA trafficking) was identified in a screen for genes required for nucleocytoplasmic transport of mRNA in yeast (*Saccharomyces cerevisiae*; Gorsch et al., 1995). Nup159p/Rat7p contains FG repeats, is the yeast homolog of human NUP214, and is located on the cytoplasmic side at the nuclear rim (Kraemer et al., 1995). mRNA export in the yeast temperature-sensitive rat7-1 mutant was quickly stopped when the temperature was shifted from 23°C to 37°C, while the protein import from the cytoplasm to the nucleus did not seem to be affected (Gorsch et al., 1995). Recent studies in yeast have revealed the mechanism of the sequential interaction between DEAD-box protein5 (Dbp5) and Gle1 and Nup159 in regulating mRNA export (Hodge et al., 2011; Noble et al., 2011). The association of Dbp5 with NPCs is dynamic and correlated with translocation rates of mRNA protein (mRNP) through NPCs (Hodge et al., 2011; Noble et al., 2011). Inositol hexakisphosphate (IP6)-bound Gle1 promotes ATP binding to Dbp5 and activates its ATPase activity, and hydrolysis of Dbp5-ATP to Dbp5-ADP mediates the remodeling of mRNP complexes. Nup159 then functions to promote ADP release from Dbp5-ADP, and the resulting nucleotide-free Dbp5 is then ready for ATP loading upon stimulation from Gle1-IP6, starting a new round of nucleotide cycling, mRNP remodeling, and export (Hodge et al., 2011; Noble et al., 2011).

Although the structure of NPCs is conserved in eukaryotes, not much is known about the structure of NPCs and functions of nucleoporins in plants (Meier and Brkljacic, 2009). Ten nucleoporins have been isolated and functionally characterized in plants: NUP160/SARI, NUP133, NUP96/SAR3/MOS5, NUP88/MOS7, NUP75/NUP85, NUP1/NUP136, RAEL, TPR/NUA, NUP107, and NUP62 (Zhang and Li, 2005; Dong et al., 2006; Kanamori et al., 2006; Parry et al., 2006; Jacob et al., 2007; Saito et al., 2007; Xu et al., 2007; Lee et al., 2009; Tamura et al., 2010; Tamura and Hara-Nishimura, 2011; Wiemer et al., 2010, 2012; Zhao and Meier, 2011). These nucleoporins have been shown to regulate cell division, hormone signaling, response to stress, rhizobial and fungal symbiosis, flowering, and other aspects of plant development. Recently, 22 nucleoporins have been identified through the mass spectrometry-based interactive proteomics in Arabidopsis, including Arabidopsis NUP214 (Tamura et al., 2010), but the biological functions of most nucleoporins in plant growth and development remain unknown.

Here, we report the isolation and characterization of the *lno1* (*lon1*) mutant during early embryogenesis and seed development. *LNO1* encodes nucleoporin-containing FG repeats in Arabidopsis, a homologous protein to the nucleoporin NUP214 in humans, and Nup159 in yeast. We show that *LNO1* can functionally complement the yeast nucleoporin temperature-sensitive mutant *nup159*. We also show that the nucleoporin LNO1 interacts with Arabidopsis DEAD-box helicase/ATPase, CRYOPHYTE/
LOS4, in the yeast two-hybrid assay. Mutations in LNO1 can abolish the first zygotic asymmetrical cell division, alter normal pattern formation in early embryogenesis, and lead to aborted seed. Remarkably, AtGLE1, an Arabidopsis homolog of the yeast Gle1 functioning in the same pathway as Nup159, is also required for seed viability. Our studies suggest that LNO1 is a functionally conserved nucleoporin protein that is required for embryogenesis and seed development in Arabidopsis.

RESULTS
Identification of the LNO1 Gene and T-DNA Insertional Mutants
To understand embryogenesis and seed development, we took a genetic approach (see “Materials and Methods”) and identified genetic mutations that interrupt seed development. By screening a set of T-DNA insertional mutant lines, we identified a few mutants that had abnormal embryogenesis or seed abortion. We named these genes LONO, after a god who was associated with fertility, agriculture, rainfall, and music in Hawaiian mythology. We found that LNO1 (At1G55540) was required for seed viability in Arabidopsis. When lno1-1 became homozygous, the seed was not viable (Fig. 1), and we were not able to obtain viable lno1-1/lno1-1 plants. In self-pollinated heterozygous LNO1/lno1-1 plants, we counted seed abortion and found 357 aborted seeds among a total of 1,439 seeds (1,082:357, $\chi^2 = 0.009$ for the 3:1 ratio, $P > 0.9$; Fig. 1; Table I), suggesting that the lno1-1 mutation results in seed abortion and follows simple Mendelian inheritance. We backcrossed the lno1-1 mutant to wild-type plants five times, and the result showed that the seed abortion phenotype was correlated with the lno1-1 mutation in each progeny and each generation. To further confirm that the seed abortion phenotype was caused by the T-DNA insertional mutation in LNO1, we transformed the LNO1 transgene under the control of the 35S promoter into LNO1/lno1-1 heterozygous plants, and the result showed that the transgene was able to complement the mutant phenotype and restored the seed viability in the homozygous mutant lno1-1/lno1-1 background.

We did not detect any morphological phenotype or defect in ovule or pollen development of the lno1-1 mutant allele. We further examined whether the lno1-1 mutant allele has any defects in transmission through female or male by reciprocally crossing the heterozygous LNO1/lno1-1 with wild-type plants. In the F1 progeny, there were no empty positions in siliques, suggesting that there were no degenerated ovules or unfertilized ovules, and almost all the seeds were viable whether the LNO1/lno1-1 plants were used as female or male in the cross (Table II). After the F1 progeny were planted, we genotyped each plant in the F1 and found that the lno1-1 allele was transmitted to the next generation as efficiently as the wild-type allele either by female or male (Table II).

Figure 1. Effect of the lno1-1 mutation on seed viability in Arabidopsis. Siliques of wild-type (LNO1/LNO1) and heterozygous (LNO1/ lno1-1) plants were dissected and photographed at 6 DAP (A), at 12 DAP (B), and at 18 DAP (C). Brown and green seeds in the silique on the right in C were aborted and viable seeds, respectively.
LNO1 Encodes a Nucleoporin Protein in Arabidopsis

In Arabidopsis, LNO1 (At1G55540) has 18 exons and 17 introns (Fig. 2A) and encodes a protein with 1,819 amino acids that can be divided into an N-terminal β-propeller domain, a coiled-coil domain in the middle, and C-terminal FG repeats (Fig. 2B). LNO1 encodes a nucleoporin protein with the FG repeats and is a homolog of NUP214/CAN in humans and Nup159 in yeast (Fig. 2C). In the N-terminal β-propeller domain, LNO1 shares 25% and 14% amino acid sequence identity with NUP214 and Nup159, respectively, and the C-terminal FG repeats of LNO1 show 33% and 30% sequence identity to those of NUP214 and Nup159, respectively (Fig. 2C). We have identified two lno1 mutant alleles: lno1-1 and lno1-2. lno1-1 and lno1-2 had a T-DNA insertion in the second exon (+609 bp relative to the transcription start) and in the promoter (−187 bp relative to the transcription start), respectively. To examine whether the lno1 mutant is a knockout allele, we used semiquantitative reverse transcription (RT)-PCR to examine LNO1 expression in seeds. We found that lno1-1 was a null allele (Fig. 2D) and homozygous lethal (Fig. 1, B and C), whereas lno1-2 was a knockdown allele (Fig. 2D) that had no obvious defect in growth and development (Table I). This result indicates that a low expression of the nucleoporin LNO1 is sufficient to sustain normal embryogenesis and seed development, although LNO1 is essential.

LNO1 Is Important for Embryogenesis

The seed with homozygous null allele lno1-1 was aborted before seed maturation (Fig. 1, B and C), but it appeared the same as the wild-type seed before 6 d after pollination (DAP) by regular light microscopy (Fig. 1A). To examine whether lno1-1 had any defects in early embryogenesis, we cleared the seed and observed that 47% (n = 121) of the putative homozygous lno1-1/lno1-1 embryos developed abnormally. As described below, these homozygous lno1-1 mutant embryos displayed a variety of developmental abnormalities that were consistently observed.

Abnormalities in the lno1-1 mutant embryos were first detected during the first zygotic division. At 1 DAP, the wild-type zygote usually elongates and divides asymmetrically to give rise to a small apical cell and a large basal cell (Fig. 3A). However, we observed that lno1-1 mutant zygotes divided almost symmetrically (Fig. 3G). Approximately 31% (n = 86) of the lno1-1/lno1-1 embryos had basal cells that failed to elongate (Fig. 3, G and H) as the wild-type embryos did (Fig. 3, A and B). This result shows that the lno1 mutation affects the earliest stage of embryogenesis in Arabidopsis. In wild-type embryos, the basal cell elongates and divides transversely to form a suspensor with seven to nine cells (Fig. 3, C and D), and longitudinal divisions do not occur in suspensors (Fig. 3, C and D). By contrast, longitudinal cell divisions in the basal cell lineage were detected in approximately 36% (n = 117) of the lno1-1/lno1-1 embryos at 3 to 4 DAP (Fig. 3, I and J). In wild-type embryos, there is a clear boundary between the spherical proembryo and the linear file of suspensor cells (Fig. 3, C and D) at 3 to 4 DAP. The hypophysis, the uppermost cell of the basal lineage, becomes prominent at 4 DAP (Fig. 3D). This clear demarcation between embryo and suspensor was often not detected in the lno1-1 mutant embryos because of many longitudinal cell divisions in the suspensor (Fig. 3, I and J). These results indicate that mutations in LNO1 can make the basal cell assume an embryonic cell fate that divides longitudinally.

Abnormalities in number and planes of cell division persisted throughout embryogenesis in the lno1-1 mutant. In wild-type Arabidopsis, the embryo passes through a series of stages that are defined morphologically as globular, transition, and heart stages at 4 to 6 DAP (Fig. 3, D–F), but in the abnormal lno1-1 mutant, the typical embryo morphology was not observed (Fig. 3, J–L). At the transition and early heart stages of wild-type embryos, two symmetrical cotyledons are initiated.

Table I. The lno1-1 mutation affects embryogenesis and seed viability in Arabidopsis

| Self-Pollinated | Abnormal F1 Embryo | F1 Seed Abortion |
|-----------------|--------------------|-----------------|
|                 | Abnormal | Total | Percentage | Aborted | Total | Percentage |
| Wild type       | 0        | 386   | 0          | 2       | 467   | 0.4        |
| LNO1/lno1-1     | 57       | 483   | 11.8       | 357     | 1439  | 24.8       |
| lno1-2/lno1-2   | 0        | 437   | 0          | 6       | 875   | 0.7        |

Table II. The lno1-1 mutant has neither parent-of-origin effect nor defects in transmission

| Parental Genotype (Female × Male) | Viable Seed | Aborted Seed | Total Seed | LNO1/LNO1 | LNO1/lno1-1 |
|----------------------------------|-------------|--------------|------------|-----------|-------------|
| Col-0 × Col-0                    | 137         | 2            | 139        |           |             |
| LNO1/lno1-1 × LNO1/LNO1 F1 progeny | 248         | 3            | 251        |           |             |
| LNO1/LNO1 × LNO1/lno1-1 F1 progeny | 331         | 1            | 332        |           |             |

χ² = 0.70, P > 0.40

χ² = 1.49, P > 0.22
from lateral domains of the embryo and an embryonic shoot apical meristem is differentiated from the medial domain between the two cotyledons (Fig. 3F; Berleth and Chatfield, 2002; Prigge et al., 2005). Approximately 19% (n = 58) of lno1-1/lno1-1 embryos failed to differentiate two cotyledons (Fig. 3, K and L). Mutant embryos having one cotyledon also lacked a medial domain where the embryonic shoot meristem could be generated (Fig. 3, K and L). In summary, these results indicate that the knockout lno1 mutation alters the number and planes of cell divisions required for generating the normal embryo proper and suspensor, apical-basal axis, cotyledons, and meristem.

To examine whether the lno1-1 mutant allele has any parent-of-origin effects on embryogenesis, we crossed the heterozygous LNO1/lno1-1 female plants with wild-type (LNO1/LNO1) male plants and examined 271, 254, 423, and 316 embryos at 2, 3, 5, and 10 DAP, respectively, but we did not find any abnormal mutant embryos. In the reciprocal cross (LNO1/LNO1 × LNO1/lno1-1), we

Figure 2. LNO1 encodes a nucleoporin protein containing FG repeats in Arabidopsis. A, Gene structure of LNO1 and locations of T-DNA insertions in two lno1 mutant alleles. Boxes represent exons, and lines represent promoters or introns. B, The three domains of the LNO1 protein in Arabidopsis. Numbers represent positions of amino acids in LNO1. C, Protein sequence alignments at the N terminus and FG repeats of Arabidopsis LNO1, human NUP214, and yeast Nup159. The alignment was performed using ClustalW2 and followed by using BOXSHADE 3.21. Identical residues are highlighted in dark gray, and similar residues are highlighted in light gray. D, Expression of LNO1 in the lno1-1 and lno1-2 mutant plants. Two sets of primers were used to examine LNO1 expression in lno1-2 and wild-type plants. ACT, ACTIN; WT, wild type.
examined 246, 168, 374, and 281 embryos at 2, 3, 5, and 10 DAP, respectively, but we did not observe any abnormal mutant embryos either. This result showed that both the maternal and paternal mutant alleles are required for producing the abnormal embryo phenotype and that mutations in LNO1 do not have any detectable parent-of-origin effects on embryogenesis.

**LNO1 Is Highly Expressed in Reproductive Tissues**

To obtain a preliminary overview of the microarray expression data, we searched a database (https://www.genevestigator.com/gv/) and found that LNO1 has very low expression in all the tissues examined except sperm cells. To examine the expression of LNO1 in Arabidopsis, we fused the LNO1 promoter with the reporter gene GUS (LNO1 promoter:GUS) and examined the temporal and spatial expression patterns of the transgene in Arabidopsis. We did not detect expression of the transgene LNO1 promoter:GUS in any vegetative tissues, such as leaves, shoots, stems, or roots, although we employed several GUS staining protocols as described (Debrouwe and De Block, 1992; Sessions et al., 1999; Luo et al., 2000; Yu et al., 2005). However, we were able to observe expression of the transgene LNO1 promoter:GUS in reproductive tissues, such as flowers and early developing seeds (Fig. 4). We examined more than 70 flowers from seven independent transgenic lines and observed a consistent expression pattern (Fig. 4, A and B). When we dissected out the flowers, we found that LNO1 promoter:GUS was expressed in anthers but not in other floral organs before flower stage 11 (Fig. 4, C–F). The LNO1 promoter:GUS transgene was not detected in early flower buds at stages 1 to 5 (Fig. 4, A and B), then started to express in flower buds at stages 6 and 7 (Fig. 4, A and B), reached the highest expression in flower buds at stages 8 and 9 (Fig. 4C), had reduced expression in flowers at stages 10 and 11 (Fig. 4, D and E), and was almost undetected in its expression in flowers at stage 12 (Fig. 4F). Interestingly, when flowers reached stage 13, LNO1 promoter:GUS was specifically expressed in stigma papilla (Fig. 4G) and reached its highest expression in the stage 14 flower (Fig. 4H). We further checked the expression of LNO1 promoter:GUS in the developing seed and observed that the transgene was expressed in both embryo and endosperm.
as early as 2 DAP (Fig. 4I), continually expressed at 3 DAP (Fig. 4J), and reached its highest expression at 4 DAP in embryo and endosperm (Fig. 4K). Then, its expression decreased, but it could still be detected at the outer layer cells of the embryo proper at the transition stage (Fig. 4L).

In short, the temporal and spatial expression patterns of the LNO1 promoter:GUS transgene are correlated with its functions in reproductive development in Arabidopsis.

Although we tried several GUS staining protocols (Debrouwe and De Block, 1992; Sessions et al., 1999; Luo et al., 2000; Yu et al., 2005) and also modified some parameters, we were not able to detect any expression.

Figure 4. Temporal and spatial expression of LNO1 in Arabidopsis. A to L, Expression of the transgene LNO1 promoter:GUS in Arabidopsis reproductive tissues. A and B, Vertical views of the apex of floral buds (stages 1–12) of the transgenic plant. C and D, Side views of floral buds at stages 9 and 10, respectively, with sepals and petals dissected away. E to H, Side views of floral buds at stages 11, 12, 13, and 14, respectively, with sepal removed. I to L, Seed photographs of the LNO1 promoter:GUS transgenic plant after GUS staining at 2, 3, 4, and 6 DAP, respectively. EM, Embryo; EN, endosperm; EP, embryo proper; S, suspensor. Arrowheads indicate locations of GUS expression.

Figure 5. LNO1 functions as a nucleoporin protein in Arabidopsis. LNO1 can functionally complement the defect in the yeast mutant nup159. The full-length LNO1, the N-terminal domain (NTD), and LNO1 without the N-terminal domain (LNO1ΔNTD) were cloned into the vector pAS1 and transformed into the nup159Δ1-456 and nup159Δ1-141 mutants. Yeast cultures were serially diluted (10⁻³, 2⁻³, 10⁻⁴, 2⁻⁴, 1,000⁻³, and 2,000⁻³) and spotted on YPD plates.
of the LNO1 promoter:GUS transgene in vegetative tissues. To examine whether LNO1 was expressed in vegetative tissues, we employed more sensitive quantitative RT-PCR to examine LNO1 temporal and spatial expression. We found that LNO1 was expressed in all the tissues we examined (Fig. 4M). We could detect the expression of LNO1 in young seedlings (4-, 7-, and 14-d-old seedlings), mature plants (21-d-old plants), and rosette and cauline leaves. Expression of LNO1 was relatively low in very early stage flowers (stage 1–4 flowers) but was increased in later stages (stage 7–11 flowers). LNO1 was also expressed in stamens during flower stages 10 and 11, carpels at flower stage 12, and embryo and endosperm at 7 DAP. This quantitative RT-PCR result is consistent with the expression pattern of the LNO1 promoter:GUS transgene (high expression in flowers at flower stages 8 and 9) and also shows that LNO1 expression was detected in the tissues where expression of the transgene LNO1 promoter:GUS was not detected.

LNO1 Can Complement the Temperature-Sensitive Yeast Mutant nup159

Nup159, a homolog of Arabidopsis LNO1, has been well studied in yeast. The crystal structure shows that the N terminus of Nup159 forms an asymmetric seven-bladed β-propeller that is conserved in eukaryotes (Weirich et al., 2004). The yeast mutant nup159Δ1–1456L, which has a deletion of the entire N-terminal domain and part of the central domain, has growth defects and is not able to grow at 37°C (Del Priore et al., 1997). The mutant nup159VI–LE has also been shown to be temperature sensitive (Weirich et al., 2004). To test whether LNO1 can functionally complement the growth defects in the yeast mutant, we cloned the Arabidopsis LNO1 full-length complementary DNA (cDNA) into the yeast expression vector pAS1 (Durfee et al., 1993; Wang et al., 2005) and transformed the construct into the yeast mutant nup159Δ1–1456L and nup159VI–LE. Both mutants (nup159Δ1–1456L and nup159VI–LE) with the LNO1 construct (pAS1-LNO1) grew well at the nonpermissive temperature (37°C; Fig. 5). We further did the complementation experiment by using the N-terminal domain of LNO1 (pAS1-LNO1-NTD) alone or LNO1 sequence with deletion of NTD (pAS1-LNO1ΔNTD). The result showed that neither of the constructs can complement the growth defect of nup159 at the nonpermissive temperature (37°C; Fig. 5). These results demonstrate that the full-length LNO1 can functionally complement the growth defects of the yeast mutant nup159 with the N-terminal deletion or mutations at residues Val-323 and Ile-326 but not the N-terminal or C-terminal domain of LNO1 by itself. These experiments suggest that LNO1 is a functionally conserved nucleoporin in eukaryotes.

LNO1 Interacts with the DEAD-Box Helicase/ATPase LOS4

The yeast Nup159, localized to the cytoplasmic side of the NPC, plays a critical role in exporting mRNA from the nucleus to the cytoplasm. The N terminus of Nup159 forms a β-propeller, which mediates protein-protein interaction, and recruits Dbp5, which functions in mRNP export (Hodge et al., 1999; Schmitt et al., 1999). The N-terminal domain of the human nucleoporin NUP214 has also been shown to interact with the DEAD-box RNA helicase Ddx19 in the crystal structure (Napetschnig et al., 2009). We searched the database and found an Arabidopsis protein called LOS4 sharing high homology with the yeast Dbp5 and human Ddx19 (Gong et al., 2005). To examine whether LNO1 can interact with LOS4, we cloned the N-terminal domain, full-length, and C-terminal domain of LNO1 with the Gal4 DNA activation domain in pACT2 (pACT2-NTD, -LNO1, and -LNO1ΔNTD, respectively), fused LOS4 with the Gal4 DNA-binding domain in pAS1 (pAS1-LOS4), and examined the fusion protein interaction in a yeast two-hybrid assay. The result showed that

Figure 6. LNO1 interacts with Arabidopsis DEAD-box helicase/ATPase and LOS4 in yeast two-hybrid assays. The N-terminal domain of LNO1 (NTD), full-length LNO1, and LNO1 with deletion of NTD (LNO1ΔNTD) were fused with the Gal4 DNA activation domain (AD), and LOS4 was fused with the Gal4 DNA binding domain (BD). pACT2 (AD) and pAS1 (BD) empty vectors were included as negative controls. Yeast transformants were spotted on synthetic dropout plates without Leu (L) and Trp (W) or without Leu, Trp, and His (H) and with 20 mM 3-aminotriazole (3-AT). The 5-bromo-4-chloro-indolyl-β-D-galactopyranoside filter-lift assay was as described (Rea et al., 2012). Yeast cultures were serially diluted (10X, 100X, and 1,000X). [See online article for color version of this figure.]
the N-terminal domain and full-length LNO1 interacted with RNA helicase LOS4 in yeast, but the C-terminal domain of LNO1 alone (LNO1\(^{\text{NTD}}\)) did not (Fig. 6). When we swapped the Gal4 DNA-binding domain with the Gal4 DNA activation domain in the yeast two-hybrid constructs, we obtained the same result as that shown in Figure 6 (Supplemental Fig. S1): the N-terminal domain and full-length LNO1 specifically interact with RNA helicase LOS4. This result implies that LNO1 is part of the mRNA export machinery in nucleocytoplasmic transport across the nuclear membrane in eukaryotes.

**AtGLE1 Is Also Critical for Seed Viability**

Nup159 functions together with other nucleoporins including Gle1 in exporting mRNAs from the nucleus to the cytoplasm (Alcázar-Román et al., 2006; Noble et al., 2011). It has been reported that there is a homolog of the yeast Gle1 in Arabidopsis, **AtGLE1** (At1G13120; Tamura et al., 2010). **AtGLE1** is a relatively small protein with 611 amino acids and has a putative coiled-coil domain in the middle and a GLE1 domain in the C terminus (Fig. 7A). The GLE1 domain of AtGLE1 shares 17% and 26% amino acid sequence identity with its homolog Gle1 in yeast and HsGle1 in humans, respectively (Fig. 7B). To examine if **AtGLE1** is required for seed viability, we searched the Arabidopsis database (www.arabidopsis.org) and found a T-DNA insertional line, CS16088 or emb1745 (Tzafrir et al., 2004), and to be consistent with the nomenclature in Arabidopsis, we called the mutant **Atgle1-1**. Through genotyping, we found that there is a T-DNA insertion in the 5' untranslated region (16 bp upstream of the translation start codon ATG) in the **Atgle1-1** mutant (Fig. 7C). We used RT-PCR to examine **AtGLE1** expression in seeds and showed that the expression of **AtGLE1** was almost not detectable in the **Atgle1** mutant seed (Fig. 7D). In self-pollinated heterozygous **AtGLE1**/**Atgle1-1** plants, we found aborted seeds (Fig. 8). There were 127 aborted seeds among a total of 647 seeds (19.6% seed abortion). The **Atgle1-1** mutant was backcrossed and self-pollinated, and we found that the seed abortion phenotype was cosegregating with the mutant allele in each generation and plant. This result indicates that **AtGLE1**, the Arabidopsis homolog of yeast Gle1 involved in the same poly(A) mRNA export pathway as **Nup159**, is also pivotal for seed viability in plants.

**Figure 7.** **AtGLE1** encodes an Arabidopsis putative homolog of yeast Gle1 and human HsGle1. A, The two domains of the **AtGLE1** protein in Arabidopsis. Numbers represent positions of amino acids in **AtGLE1**. B, Protein sequence alignment of the Gle1 domain in the C terminus of Arabidopsis **AtGLE1**, human HsGle1, and yeast ScGle1. The alignment was performed using ClustalW2 and followed by BOXSHADE 3.21. Identical residues are highlighted in dark gray, and similar residues are highlighted in light gray. C, Gene structure of **AtGLE1** and location of T-DNA insertion in the **Atgle1-1** mutant (16 bp upstream of the ATG start codon). D, Expression of **AtGLE1** in the **Atgle1-1** mutant and wild-type plants. **ACT**, **ACTIN**; WT, wild type. [See online article for color version of this figure.]
DISCUSSION

The NPCs are important structures on the nuclear envelope that mediate trafficking between the nucleus and the cytoplasm. Nucleoporins participate in forming the nuclear pore structure and in nucleocytoplasmic transport. Genetic and biochemical evidence has shown that mutations in nucleoporins affect the structure of NPCs and the nuclear envelope (Doye and Hurt, 1997). NPCs can also affect growth and development. In *Caenorhabditis elegans*, 17 out of 20 nucleoporins are required for embryonic development (Galy et al., 2003). The *C. elegans* homologs of vertebrate Nup93 and Nup205 were found to be essential for normal NPC distribution in the nuclear envelope in vivo and for cell viability, and depletion of Nup93 or Nup205 caused defects in NPC exclusion, abnormal chromatin condensation, and early embryonic arrest (Galy et al., 2003). In mice, NUP214 is required for embryonic stem cell and embryo viability, and the *nup214*−/− null embryos die in utero between 4.0 and 4.5 d post coitum (van Deursen et al., 1996). However, its yeast homolog Nup159 is not essential: null mutant *nup159* can grow at permissive temperature (Weirich et al., 2004). Here, we show that LNO1, a homolog of NUP214, is essential for embryogenesis and seed viability in plants. The *lno1-1* mutation can affect cell divisions and pattern formation in early embryogenesis (Fig. 3). Approximately 47% of the homozygous *lno1-1/lno1-1* embryos showed abnormal cell division and pattern formation in early embryogenesis, but more than half of the *lno1-1/lno1-1* embryos could continue to develop without obvious morphologic defects to certain levels. There are no other genes showing high homology with LNO1 in Arabidopsis, so it is not clear why more than 50% of the null *lno1-1/lno1-1* embryos could continue to develop until the heart or torpedo stage during early embryogenesis and then abort at a late stage. One possibility is that LNO1 plays a role in normal endosperm development in Arabidopsis. Since LNO1 was expressed in early endosperm tissues (Fig. 4), mutations in LNO1 might affect normal endosperm development in Arabidopsis, thus leading to late-stage seed abortion of those normal-looking *lno1-1/lno1-1* embryos at an earlier stage. Alternatively, LNO1 is required for mRNA export, and mutations in LNO1 might abolish the export of mRNA of many essential genes in embryogenesis from the nucleus to the cytosol, thus causing seed abortion in all the *lno1-1/lno1-1* embryos at the late stage.

The NPC consists of approximately 30 nucleoporins in eukaryotic cells. The nucleoporin CAN/NUP214 was originally found to be a putative oncogene product associated with myeloid leukemogenesis and is localized to the cytoplasmic side of the NPC (Kraemer et al., 1994). However, in cells overexpressing NUP214, NUP214 can bind to both the cytoplasmic and the nucleoplasmic sides of the NPC (Boer et al., 1997). NUP214 plays a role in nuclear protein import, mRNA export, and cell cycle progression and interacts with DDX19 (Napetschnig et al., 2009; von Moeller et al., 2009). In yeast, the nucleoporins Nup159 and Gle1 are both localized to the cytoplasmic side of the NPC and function in the same pathway in exporting mRNA. The N-terminal domain of Nup159 forms a β-propeller that functions in mRNA export by tethering the shuttling helicase Dpb5 at the nuclear periphery and locally concentrating this mRNA-remodeling factor at the cytoplasmic face of the NPC (Weirich et al., 2004). Nup159 and Nup82 form a cytoplasmically oriented subcomplex
of the NPC that is essential for RNA export but not for classical nuclear localization sequence-mediated nuclear protein import (Hurwitz et al., 1998). LNO1 (AtNUP214) is an Arabidopsis homolog of human NUP214 and yeast Nup159. AtNUP214 was localized to the NPC in the root tip cells (Tamura et al., 2010). We showed that LNO1 complemented the yeast temperature-sensitive mutant nup159 (Fig. 5). LOS4, a homolog of ATPase DDX19 in human and Dbp5 in yeast, was shown to function in mRNA export in Arabidopsis (Gong et al., 2005), and we showed that LOS4 interacts with LNO1 in yeast (Fig. 6). Furthermore, the Glei homolog, ATG1E1, is also required for seed viability in Arabidopsis (Figs. 7 and 8). These results suggest that LNO1 (AtNUP214), LOS4, ATG1E1, and perhaps other plant nucleoporins might form a functionally conserved pathway as those in human and yeast, which plays an essential role in nuclear and cytoplasmic trafficking in plants.

Patterning formation in early embryogenesis is regulated by auxin gradients and other signaling pathways. Interestingly, SUPPRESSOR OF AUXIN RESISTANCE1 (SAR1) and SAR3, encoding nucleoporins NUP160 and NUP96, respectively, are involved in hormone auxin signaling and development (Parry et al., 2006). The defects in the mutant lno1 are similar to those embryogenesis mutants in the auxin signaling pathway. One possibility is that LNO1 as a nucleoporin might interact with other signaling pathways and regulate embryogenesis (e.g. the auxin signaling) or, at least in part, LNO1 can affect the transport of mRNAs of components of those signaling pathways involved in early embryogenesis, thus affecting early pattern formation. Since the homozygous lno1/lno1 seed is not viable, we are not able to examine the role of LNO1 at the seedling stage or in mature plants. Thus, it is possible that LNO1 can be an essential gene throughout the whole life cycle of plants. We expect that further biochemical characterization of LNO1 and genetic study will reveal its function in signal transduction or transporting other signaling components in early embryogenesis and perhaps in other developmental processes in plants.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

We took a genetic approach to identify mutants that affect seed development or embryogenesis in Arabidopsis (Arabidopsis thaliana). We performed a cDNA microarray using the F1 seed of the DNA methyltransferase met1 mutant (Xiao et al., 2003, 2006) crossed with wild-type ecotype Columbia (Col-0) and a control wild-type cross and found over 1,300 genes that had altered expression in the met1 mutant cross compared with the wild type (W. Xiao, C. Braid, and W. Zhang, unpublished data). We then chose 116 putative genes that affect embryogenesis, seed development, epigenetics, transcription, translation, or chromatin remodeling based on annotations in The Arabidopsis Information Resource, and there were putative T-DNA insertion lines available for these genes at the Arabidopsis Biological Resource Center. lno1-1 is one of the mutants that we obtained and characterized in this screen. We have ordered all available putative T-DNA insertion lines (CS16007, CS423371, CS804562, CS810290, CS810299, CS813163, CS813171, CS842534, CS842651, SALK_037979, SALK_108182, and SALK_108199) in the LNO1 locus (At1G55540). However, we were only able to genotype and find T-DNA insertion in the At1G55540 locus in two lines: CS810299 (lno1-1) and CS842651 (lno1-2).

LNO1 Required for Embryogenesis and Seed Viability

CS16007 was identified as emb101-1 (Tazfiri et al., 2004), but it was later withdrawn from the database (www.seedgenes.org). The T-DNA insertion in lno1-1 (CS810299) was detected in the second exon by amplifying a PCR fragment with the following primers: forward primer, 5’-CGGAAAA-GAGGAGAAGGACG-3’, and LB3, 5’-TTGATCCTGAACTTAAACC-ATCTC-3’. The T-DNA insertion in lno1-2 (CS842651) was detected in the promoter by amplifying a PCR fragment with two primers: 5’-CAGTCCTT-CATCGGAATACCC-3’ and LB3 (see above). Once T-DNA insertions were found in a plant, PCR amplification was used to determine homozygosity by amplifying an endogenous gene fragment across the T-DNA insertion. An endogenous gene fragment was amplified in lno1-1 with the following primers: forward, 5’-TAGATCCTGAAACCTCTCC-3’, and reverse, 5’-CAGTCCTT-CATCGGAATACCC-3’, and in lno1-2 with the following primers: forward, 5’-GAAGATCCCTTTGTTATGCTGGA-3’, and reverse, 5’-TGCTT-AGACCTTCAACCTCTTGCAAGT-3’. By using the same approach, we found a T-DNA insertion in the 5’ untranslated region and 14 bp upstream of Atgol-1 (CS16088, emb1745) by amplifying a PCR fragment with two primers: 5’-GTCTGTTGTTGTCGATT-3’ and LB1, 5’-GCCCCTTTACAG-AATGAAATAATTGAC-3’. Arabidopsis plants were grown in growth chambers or a greenhouse under 16 h of light and 8 h of dark at 23°C.

Complementation Assay for the lno1-1 Mutant

The full-length coding region of LNO1 was amplified by PCR using forward primer 5’-CCGCTCTTAGAATGAGCAGTACGAGAAGA-3’ (Xnl site underlined) and reverse primer 5’-CCGCGCTCTATCTTCTTTCATCTCAGT-GAAGAG-3’ (SacI site underlined) and cloned into pBlII121 vector (Clontech). The resulting construct was transformed into Agrobacterium tumefaciens strain GV3101 and infiltrated into the LNO1/lno1-1 heterozygous plants by floral dipping. Positive transformants were selected based on their resistance to kanamycin on Murashige and Skoog medium, and the transgenic plants in the lno1-1 mutant background were chosen for analysis of seed viability.

Whole-Mount Seed Clearing and Microscopy

Whole-mount immature seeds were dissected from siliques 1 to 6 DAP, cleared in Hoyer’s fluid (70% (v/v) chloral hydrate, 4% (v/v) glycerol, and 5% (v/v) gum arabic), and observed with a Zeiss Axioskop 50 microscope using differential interference contrast optics in the Department of Pathology of the Medical School at Saint Louis University.

Gene Expression Analysis

For the examining the expression of LNO1 in the lno1-1 mutant allele, viable (LNO1/LNO1) and aborted (lno1-1/lno1-1) seeds at 10 DAP from the heterozygous LNO1/lno1-1 plants were isolated, and total RNA was extracted. For examining the expression of LNO1 in the lno1-1 mutant allele, seeds of wild-type (LNO1/LNO1) and homozygous mutant (lno1-2/lno1-2) seeds at 10 DAP were isolated, and total RNA was extracted. Gene expression was examined by using semiquantitative RT-PCR using 28 to 30 PCR amplification cycles or quantitative RT-PCR. First-strand cdNA was synthesized using the Proto-Script First Strand cDNA Synthesis Kit (New England Biolabs). PCR product was amplified using SABiosciences’ SYBR Green qPCR Mastermix, and ACTIN was used as an internal control. Quantitative RT-PCR was performed using MJ Research PTC-200 Thermal Cycler and MJ Opticon Monitor Analysis Software (Bio-Rad). The primer pairs for amplifying cDNA of LNO1 were as follows: forward 9, 5’-TGCTGTTGTTGTCGATT-3’ and reverse 9, 5’-CAGTCCTT-CATCGGAATACCC-3’. The second set of primer pairs for amplifying cDNA of LNO1 were as follows: forward 4, 5’-GGATGCTTCTG-CAGGAAA-3’, and reverse 4, 5’-GAAGATCCCTTTGTTATGCTGGA-3’. The primer pairs for amplifying cDNA of ATGLE1 were as follows: forward 3, 5’-CCTATGAGTACGAAACAATTTG-3’, and reverse 3, 5’-AAGGACGTT-CAAAACATC-3’.

The LNO1 Promoter:GUS Construct and GUS Histochemical Staining

The 5’ promoter region of LNO1 was PCR amplified with forward primer 5’-CGGCGCTCTAGGAATGAGGAC-3’, containing a SalI restriction site, and reverse primer 5’-CCGCGCTCTAGGAATGAGGAC-3’, containing a BamH restriction site, and cloned into the binary
vector pH101. The construct was confirmed by sequencing, transformed into A. tumefaciens strain GV3101, and infiltrated into Col-0. Stable transgenic T2 plants were used for GUS staining analysis. GUS staining was performed as described with minor modifications (Stangeland and Salehian, 2002). In short, plant tissues were harvested and immersed into the GUS staining buffer (10 mM sodium phosphate buffer, pH 7.2, 0.5% Triton X-100, 1 mg mL\(^{-1}\) 3-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, and 2 μM potassium ferricyanide) on ice. Samples in the staining buffer were vacuum infiltrated on ice for 20 min and then incubated at 37°C with rotation for 24 h. Tissues were destained using 70% ethanol. Samples were imbibed in 50% glycerol on slides for imaging.

Yeast Two-Hybrid Assay
(Yeast (Saccharomyces cerevisiae) two-hybrid experiments were performed using the system described previously (Rea et al., 2012). To make Gal4 DNA activation domain (AD) constructs, the full-length LNO1 was PCR amplified with primers LON1-ADF (5'-CCCCGATATCGGATGAGCAATGTTAGTGAA-3'; EcoRV site underlined) and LNO1-ADR (5'-CCCCGATATCGTACTATATAAACGCAGACAGTCTT-3'; SalI site underlined) and inserted into pACT2 vector digested with BamHI and XhoI; the LNO1 N-terminal domain (1–761 amino acids; NTD) coding region was PCR amplified with primers LNO1-NTD-ADF (5'-CCCCGATATCGGATGAGCAATGTTAGTGAA-3'; BamHI site underlined) and LNO1-NTD-ADR (5'-CCCCGATATCGTACTATATAAACGCAGACAGTCTT-3'; SalI site underlined) and inserted into pACT2 digested with BamHI and XhoI; the LNO1 C-terminal domain (996–1485 amino acids; CTD) coding region was PCR amplified with primers LNO1-CTD-ADF (5'-CCCCGATATCGTACTATATAAACGCAGACAGTCTT-3'; EcoRV site underlined) and LNO1-CTD-ADR (5'-CCCCGATATCGTACTATATAAACGCAGACAGTCTT-3'; SalI site underlined) and inserted into pACT2 digested with SalI and XhoI. For the Gal4 binding domain (BD) constructs, the Arabidopsis LOS4 coding region was PCR amplified with primers LOS4-BDF (5'-CCCCGATATCGTACTATATAAACGCAGACAGTCTT-3'; EcoRV site underlined) and LOS4-BDR (5'-CCCCGATATCGTACTATATAAACGCAGACAGTCTT-3'; XhoI site underlined) and inserted into pGAD vector digested with SalI and XhoI. Positive transformants were selected on complete minimal (CM) dropout medium minus Trp and Leu. Positive interactions and positive transformants were tested as described above.

Yeast Complementation Test
The plasmid DNA of pAS1-LNO1, pAS1-NTD, or pAS1-LNO1\(^{-}\)CTD was transformed into the yeast strain Nup159 mutant strains KWY126 (nup159\(^{1455}\)Δ\(^{542}\)) and KWY1269 (nup159\(^{1455}\)Δ\(^{542}\)). Positive transformants were selected on CM medium minus Trp and Leu. Five microliters of liquid culture in yeast extract peptone dextrose (YPD) medium for each positive transformant was spotted on YPD plates after serial dilutions. Complementation tests were performed at 37°C (nonpermissive temperature). Control plates were incubated at 23°C for growth.

Supplemental Data
The following materials are available in the online version of this article.

Supplemental Figure S1. LNO1 interacts with LOS4 in yeast.

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LITERATURE CITED
Aida M, Beis D, Heidstra R, Willemsen V, Bilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B (2004) The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. Cell 119: 109–120
Alcázar-Román AR, Tran EJ, Guo SL, Wente SR (2006) Inositol hexaki-

Plant Physiol. Vol. 160, 2012
important for development and stress responses in Arabidopsis. Plant Cell 17: 256–267

Gorsch LC, Dockendorff TC, Cole CN (1995) A conditional allele of the novel repeat-containing yeast nucleoporin RAT7/NUP159 causes both rapid cessation of mRNA export and reversible clustering of nuclear pore complexes. J Cell Biol 129: 939–955

Haeker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of WOX genes mark cell fate decisions during early embryonic pattern formation in Arabidopsis thaliana. Development 131: 657–668

Hamann T, Mayer U, Jürgens G (1999) The auxin-insensitive bodenlos mutation affects primary root formation and apical-basal patterning in the Arabidopsis embryo. Development 126: 1387–1395

Hetzer MW, Wente SR (2009) Border control at the nucleus: biogenesis and organization of the nuclear membrane and pore complexes. Dev Cell 17: 606–616

Hodge CA, Colot HV, Stafford P, Cole CN (1999) Ratlp/Rbp5p is a shuffling transport factor that interacts with Rat7p/Nup159p and Gclp1p and suppresses the mRNA export defect of xpo1p1 cells. EMBO J 18: 5778–5788

Hodge CA, Tran EJ, Noble KN, Alcazar-Roman AR, Ben-Yishay R, Colot HV, Stafford P, Cole CN (2012) Histone H1 affects gene imprinting and DNA methylation in Arabidopsis. Proc Natl Acad Sci USA 109: 1755–1760

Hurwitz ME, Strambio-de-Castillia C, Blobel G (1998) Two yeast nuclear pore complex proteins involved in mRNA export form a cytoplasmically oriented subcomplex. Proc Natl Acad Sci USA 95: 11241–11245

Jacob Y, Mongolsikirwatana C, Veley KM, Kim SY, Michaels SD (2000) Exon–side the nucleoplasm. Cell 100: 1383–1390

Jeong S, Bayer M, Lukowitz W (2011) Taking the very first steps from polarity to axial domains in the early Arabidopsis embryo. J Exp Bot 62: 1687–1697

Kalverda B, Pickersgill H, Shloma VV, Fornerod M (2010) Nuclearoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. Cell 146: 360–371

Kanemori N, Madsen LH, Radutoiu S, Frantescu M, Quistgaard EM, Hodge CA, Tran EJ, Noble KN, Alcazar-Roman AR, Ben-Yishay R, Hodge CA, Colot HV, Stafford P, Cole CN (2011) Taking the very important roles of the yeast nucleoporin Nup159 and the DEAD-box helicase Ddx19. Proc Natl Acad Sci USA 108: 3089–3094

Kawano H, Benavidez AF, Miyazaki M, Dorfman H, Tchekhovskoy Z, et al. (2011) The GATA factor HANABA TARANU is required to position the proembryo boundary in the early Arabidopsis embryo. Dev Cell 19: 103–113

Kilian KW, Tran EJ, Alcazar-Roman AR, Hodge CA, Cole CN, Wente SR (2011) The Dbp5 cycle at the nuclear pore complex during mRNA export. II. Nucleotide cycling and mRNA remodeling by Dbp5 are controlled by Nup159 and Gcl1. Genes Dev 25: 1065–1077

Kilian KW, Benavidez AF, Miyazaki M, Dorfman H, Tchekhovskoy Z, et al. (2011) The GATA factor HANABA TARANU is required to position the proembryo boundary in the early Arabidopsis embryo. Dev Cell 19: 103–113

Kilian KW, Tran EJ, Alcazar-Roman AR, Hodge CA, Cole CN, Wente SR (2011) The Dbp5 cycle at the nuclear pore complex during mRNA export. II. Nucleotide cycling and mRNA remodeling by Dbp5 are controlled by Nup159 and Gcl1. Genes Dev 25: 1065–1077

Kilman M, Brkljajic J (2009) Adding pieces to the puzzling plant nuclear envelope. Curr Opin Plant Biol 12: 752–759

Meinke D, Muralla R, Sweeney C, Dickerman A (2008) Identifying essential genes in Arabidopsis thaliana. Trends Plant Sci 13: 483–491

Napetschnig J, Kassube SA, Debler EW, Wong RW, Blobel G, Hoelz A (2009) Structural and functional analysis of the interaction between the nucleoporin Nup214 and the DEAD-box helicase Ddx19. Proc Natl Acad Sci USA 106: 3089–3094

Nawy T, Bayer M, Mravec J, Friml J, Birnbaum KD, Lukowitz W (2010) The GATA factor HANABA TARANU is required to position the protoplasmic bridge in the early Arabidopsis embryo. Dev Cell 19: 103–113

Noble KN, Tran EJ, Alcazar-Roman AR, Hodge CA, Cole CN, Wente SR (2011) The Dbp5 cycle at the nuclear pore complex during mRNA export. II. Nucleotide cycling and mRNA remodeling by Dbp5 are controlled by Nup159 and Gcl1. Genes Dev 25: 1065–1077

Nodine MD, Bartel DP (2012) Maternal and paternal genomes contribute equally to the transcriptome of early plant embryos. Nature 482: 94–97

Nodine MD, Yadegari R, Tax FE (2007) RPK1 and TOAD2 are two receptor-like kinases redundantly required for Arabidopsis embryonic pattern formation. Dev Cell 12: 943–956

Onischenko E, Weis K (2011) Nuclear pore complex: a coat specifically targeted to the nuclear envelope. Curr Opin Cell Biol 23: 283–301

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

Piggee MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE (2005) Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in Arabidopsis development. Plant Cell 17: 61–76

Rasala BA, Ramos C, Harel A, Forbes DJ (2008) Capture of AT-rich chromatin by ELYS recruits POM121 and NDC1 to initiate nuclear pore assembly. Mol Biol Cell 19: 3982–3996

Rea M, Zheng W, Chen M, Braud C, Bhangdu D, Rognan TN, Xiao W (2012) Histone H1 affects gene imprinting and DNA methylation in Arabidopsis. Plant J 71: 776–786

Saito K, Yoshikawa M, Yano K, Miwa H, Uchida H, Asamizu E, Sato E, Tabata S, Imaizumi-Anraku H, Umehara Y, et al. (2007) NUCLEOPORIN5 is required for calcium spiking, fungal and bacterial symbiosis, and seed production in Lotus japonicus. Plant Cell 19: 610–624

Scheres B, Benfey PN (1999) Asymmetric cell division in plants. Annu Rev Plant Physiol Plant Mol Biol 50: 505–537

Schierath A, Müller B, Liu W, Krentz M, Flippe J, Rademacher EH, Schmid M, Jürgens G, Weiers D (2010) MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. Nature 464: 913–916

Schmitz C, von Kobbe C, Bachi A, Panté N, Rodrigues JP, Boscheron C, Rigaut G, Wilim M, Séraphin B, Carmona-Fonseca M, et al. (1999) Dbp5, a DEAD-box protein required for mRNA export, is recruited to the cytoplasmonic fibres of nuclei in lepidopteran cells via a conserved interaction with CAN/Nup159p. EMBO J 18: 4332–4347

Sessions A, Weigel D, Yanofsky MF (1999) The Arabidopsis thaliana MERISTEM LAYER 1 promoter specifies epidermal expression in meristems and young primordia. Plant J 20: 259–263

Stangeland B, Salehian Z (2002) An improved clearing method for GUS assay in Arabidopsis endosperm and seed. Plant Mol Biol Rep 20: 107–114

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

Tamura K, Fukao Y, Iwamoto M, Haraguchi T, Hara-Nishimura I (2010) Identification and characterization of nuclear pore complex components in Arabidopsis thaliana. Plant Cell 22: 4084–4097

Tamura K, Hara-Nishimura I (2011) Involvement of the nuclear pore complex in morphology of the plant nucleus. Nucleus 2: 168–172

Tazfir I, Pena-Muralla R, Dickerman A, Berg M, Rogers R, Hutchens S, Sweeney TC, McElver J, Aux G, Patton D, et al. (2004) Identification of genes required for embryo development in Arabidopsis. Plant Physiol 135: 1206–1220

Veijov K, Pena-Muralla R, Dickerman A, Berg M, Rogers R, Hutchens S, Sweeney TC, McElver J, Aux G, Patton D, et al. (2004) Identification of genes required for embryo development in Arabidopsis. Plant Physiol 135: 1206–1220

Vernon DM, Meinke DW (1994) Embryogenic transformation of the suspensor in twin, a polyembryonic mutant of Arabidopsis. Dev Biol 165: 566–573

von Lindern M, Fornerod M, van Baal S, Jaegel M, de Wit B, Buijs A, Grosveld G (1992a) The translocation (6;9), associated with a specific...
subtype of acute myeloid leukemia, results in the fusion of two genes, dek and can, and the expression of a chimeric, leukemia-specific dek-can mRNA. Mol Cell Biol 12: 1687–1697

von Lindern M, van Baal S, Wiegant J, Raap A, Hagemeijer A, Grosveld G (1992b) Can, a putative oncogene associated with myeloid leukemia, may be activated by fusion of its 3’ half to different genes: characterization of the set gene. Mol Cell Biol 12: 3346–3355

von Moeller H, Basquin C, Conti E (2009) The mRNA export protein DBP5 binds RNA and the cytoplasmic nucleoporin NUP214 in a mutually exclusive manner. Nat Struct Mol Biol 16: 247–254

Waki T, Hiki T, Watanabe R, Hashimoto T, Nakajima K (2011) The Arabidopsis RWP-RK protein RKD4 triggers gene expression and pattern formation in early embryogenesis. Curr Biol 21: 1277–1281

Wang Y, Manotti LA Jr, Lee MJ, Dohlman HG (2005) Differential regulation of G protein alpha subunit trafficking by mono- and polyubiquitination. J Biol Chem 280: 284–291

Weijers D, Sauer M, Meurette O, Friml J, Ljung K, Sandberg G, Hooykaas P, Offringa R (2005) Maintenance of embryonic auxin distribution for apical-basal patterning by PIN-FORMED-dependent auxin transport in Arabidopsis. Plant Cell 17: 2517–2526

Weirich CS, Erzberger JP, Berger JM, Weis K (2004) The N-terminal domain of Nup159 forms a beta-propeller that functions in mRNA export by tethering the helicase Dpb5 to the nuclear pore. Mol Cell 16: 749–760

Wiermer M, Cheng YT, Imakempe J, Li M, Wang D, Lipka V, Li X (2012) Putative members of the Arabidopsis Nup107-160 nuclear pore subcomplex contribute to pathogen defense. Plant J 70: 796–808

Wiermer M, Germain H, Cheng YT, Garcia AV, Parker JE, Li X (2010) Nucleoporin MOS7/Nup88 contributes to plant immunity and nuclear accumulation of defense regulators. Nucleus 1: 332–336

Xiao W, Custard KD, Brown RC, Lemmon BE, Harada JJ, Goldberg RB, Fischer RI (2006) DNA methylation is critical for Arabidopsis embryogenesis and seed viability. Plant Cell 18: 805–814

Xiao W, Gehring M, Choi Y, Margossian L, Pu H, Harada JJ, Goldberg RB, Pennell RI, Fischer RI (2003) Imprinting of the MEA Polycomb gene is controlled by antagonism between MET1 methyltransferase and DME glycosylase. Dev Cell 5: 891–901

Xu XM, Rose A, Muthuswamy S, Jeong SY, Venkatakrishnan S, Zhao Q, Meier I (2007) NUCLEAR PORE ANCHOR, the Arabidopsis homolog of Tpr/Mlp1/Mlp2/megator, is involved in mRNA export and SUMO homeostasis and affects diverse aspects of plant development. Plant Cell 18: 1537–1548

Yu HJ, Hogan P, Sundaresan V (2005) Analysis of the female gametophyte transcriptome of Arabidopsis by comparative expression profiling. Plant Physiol 139: 1853–1869

Zhang JZ, Somerville CR (1997) Suspensor-derived polyembryony caused by altered expression of valyl-tRNA synthetase in the twn2 mutant of Arabidopsis. Proc Natl Acad Sci USA 94: 7349–7355

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: 1306–1316

Zhao Q, Meier I (2011) Identification and characterization of the Arabidopsis FG-repeat nucleoporin Nup62. Plant Signal Behav 6: 330–334