Identification and analysis of LNO1-Like and AtGLE1-Like Nucleoporins in plants

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Abbreviations: LNO1, LONO1; AtGLE1, Arabidopsis GLE1; Nup, Nucleoporin; NPC, Nuclear pore complex; FG repeats, phenylalanine-glycine repeats; LOS4, LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 4; mRNP, mRNA-protein complex; IP6, Inositol hexakisphosphate

Nucleoporins (Nups) are building blocks of the nuclear pore complex (NPC) that mediate cargo trafficking between the nucleus and the cytoplasm. Although the physical structure of the NPC is well studied in yeast and vertebrates, little is known about the structure of NPCs or the function of most Nups in plants. Recently we demonstrated two Nups in *Arabidopsis*: LONO1 (LNO1), homolog of human NUP214 and yeast Nup159, and AtGLE1, homolog of yeast Gle1, are required for early embryogenesis and seed development. To identify LNO1 and AtGLE1 homologs in other plant species, we searched the protein databases and identified 30 LNO1-like and 35 AtGLE1-like proteins from lower plant species to higher plants. Furthermore, phylogenetic analyses indicate that the evolutionary trees of these proteins follow expected plant phylogenies. High sequence homology and conserved domain structure of these nucleoporins suggest important functions of these proteins in nucleocytoplasmic transport, growth and development in plants.

A defining characteristic of eukaryotic cells is the presence of the double-layered nuclear envelope that surrounds the nucleus, thus isolating the genetic material in the nucleus from the rest of the cell and separating the nucleoplasm from the cytoplasm. Nuclear pores are channels that cross the nuclear envelope and mediate the transportation of macromolecular components between the cytoplasm and the nucleus.¹ Nuclear pores are formed by an intricate structure called the nuclear pore complex (NPC) that consists of multiple copies of approximately 30 different proteins called nucleoporins (Nups).² NPCs play an essential role in cargo trafficking as well as genetic information flow between the nucleus and the cytoplasm. Nups form a channel comprising the scaffold proteins, the nuclear ring, and the cytoplasmic and nuclear filaments. About one third of the Nups contain phenylalanine-glycine (FG) repeats and serve as a sieve-like meshwork for the selective traffic through NPCs.² The physical structure of NPCs is relatively well characterized, but the formation of NPCs and the function of many individual Nups are not well understood.

The structure of NPCs is conserved in eukaryotes and has been well studied in yeast and vertebrates.^{3–5} However, not much is known about the structure of NPCs and functions of nucleoporins in plants.^{6,7} Only 13 Nups have been isolated and functionally characterized in plants so far (NUP160/SAR1,

NUP133, NUP96/SAR3/MOS3, NUP88/MOS7, NUP75/NUP85, NUP1/NUP136, RAE1, TPR/NUA, NUP107, NUP62, LNO1/NUP214, AtGLE1, and NUP58).^{8–21} These plant NUPs have been shown to regulate cell division, hormone signaling, response to stress, rhizobial and fungal symbiosis, plant immune responses to pathogens, flowering, plant development, and reproduction. By performing an *in vivo* pull-down followed by mass-spectrometry-based interactive proteomics, Tamura et al. were able to identify 22 proteins belonging to the nuclear pore complex in *Arabidopsis*.¹⁵ However, biological functions of most nucleoporins in plant signaling and development remain unknown.

Nucleoporins are required for embryogenesis, e.g., Nup98 in zebrafish plays a role in nucleocytoplasmic trafficking and is required for normal embryogenesis.²² In *Caenorhabditis elegans*, it has been shown that 17 Nups are required for embryonic development acting either alone or in combinations.²³ Recently we identified two Nups in *Arabidopsis*: LONO1 (LNO1) and AtGLE1 and showed they are involved in seed development.²⁴ *LNO1*, a homolog of *NUP214* in human and *Nup159* in yeast, encodes a nucleoporin containing FG-repeats in *Arabidopsis*. *LNO1* is highly expressed in the anthers of flower buds, stigma papilla of open flowers, and embryo and endosperm during early embryogenesis. *NUP214* was originally identified as result of its

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association with certain types of leukemia.²⁵ The *nup214* mutant mice were aborted *in utero*.²⁶ *Nup159* was uncovered in yeast during a screen to identify genes that are responsible for mRNA export from the nucleus to the cytoplasm.²⁷ We have shown that LNO1 in *Arabidopsis*, like NUP214 in mice, is required for embryogenesis, and that the homozygous *lno1/lno1* seed is aborted.²⁴ AtGLE1, an *Arabidopsis* homolog of the yeast Gle1 involved in the same poly(A) mRNA export pathway as Nup159, was recently discovered.¹⁵ Gle1 was first identified in yeast that contains a nuclear export signal. It is localized predominantly at NPCs, and mediates RNA export from the nucleus to the cytoplasm.²⁸ Gle1 in yeast and its homologs in mammals are known to be associated with the NPC and to shuttle between the nucleus and the cytoplasm.²⁸ Gle1 is a highly conserved protein and is important for transporting mRNA out of the nucleus.²⁹ Inositol hexakisphosphate (IP6) has been shown to interact with Gle1, and this interaction is required for mRNA export to occur.³⁰ As in the *Arabidopsis lno1* mutant, mutations in *AtGLE1* also result in embryo abortion.²⁴

We have shown that LNO1 specifically interacts with LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 4 (LOS4) in a yeast two-hybrid assay. *LOS4* encodes a DEAD box RNA helicase/ATPase in *Arabidopsis*.³¹ It was originally discovered in a screen for mutants that had a reduced expression of cold response genes in *Arabidopsis*.³² *LOS4* is homologous to Dbp5 and DDX19 in yeast and humans, respectively. As an RNA helicase, *LOS4* is responsible for unwinding double-stranded RNA using ATP as an energy source. DEAD box family RNA helicases have a conserved D (Asp)-E (Glu)-A (Ala)-D (Asp) amino acid sequence and are found in diverse species and have a role in cell growth and division as well as embryogenesis.³³ Like LNO1 and AtGLE1, homologs of *LOS4* have been implicated in mRNA export in yeast and mammals.³⁴ For example, Dbp5 accumulated around the nucleus and was found to be essential for mRNA export in yeast because of its role in remodeling mRNA-protein complex (mRNP) during export through the NPC. The temperature-sensitive yeast mutant *dbp5* accumulated high levels of poly-A mRNA in the nucleus when submitted to non-permissive temperatures.^{34,35} Our results show that LNO1 and AtGLE1 are required for embryogenesis in *Arabidopsis* and LNO1 can complement the yeast conditionally lethal *nup159* mutant and physically interacts with *LOS4*, suggesting that LNO1 and AtGLE1 are highly evolutionarily conserved components of the NPC and are vital for exporting mRNA from the nucleus to the cytoplasm in plants. To understand whether LNO1 and AtGLE1 are conserved in various plant species during evolution, we searched the Phytozome (Phytozome V9.1) and NCBI databases, and identified and analyzed homologous sequences of these Nup proteins in a variety of green plants.

LNO1-Like Proteins are conserved in different plant species

In order to identify LNO1 homologs in other plant species, the full-length amino acid sequence of LNO1 (Atlg55540) was used to search the Phytozome and NCBI databases using

BLASTp and hits from 30 different plant species were obtained. Amino acid sequences of these putative homologs were then put through the PSIPRED DomPred (protein domain recognition) software to ensure they were authentic as LNO1 homologs and had the conserved 7-fold β -propeller domain of *Arabidopsis* LNO1 (CATH database domain code 1xipA00, cathdb.info). The coiled-coil domain and FG repeats were also found in these putative LNO1 homologous proteins (Fig. 1). The 30 different plant species that have putative LNO1 homologs are: *Gossypium raimondii* (Gorai.004G097900.1), *Theobroma cacao* (Cocoa tree; EOY32268), *Vitis vinifera* (Grape vine; GSVIVT01025508001), *Citrus clementina* (mandarin orange; Ciclev10014017 min), *Citrus sinensis* (sweet orange; orange1.lg000229 min), *Malus domestica* (Apple; MDP0000233952), *Prunus persica* (Peach; EMJ06137), *Fragaria vesca* (strawberry; XP_004296151.1 or mrna01579.1), *Eucalyptus grandis* (Flooded gum or rose gum; Eucgr.J01744.1), *Linum usitatissimum* (common flax or linseed; Lus10010334), *Linum usitatissimum* (Lus10021859), *Populus trichocarpa* (poplar; Potri.003G223400.1), *Ricinus communis* (Castor oil plant; XP_002523390), *Aquilegia coerulea* (Colorado Blue Columbine; Aquca_009_00103.1), *Solanum lycopersicum* (Tomato; XP_004231682), *Cucumis sativus* (Cucumber; XP_004147125.1), *Glycine max* (Soybean; XP_003526034 and XP_003552784), *Phaselous vulgaris* (string bean or snap bean; Phvul.007G078700.1), *Cicer arietinum* (Chickpea; XP_004495016), *Arabidopsis lyrata* (XP_002894510), *Capsella rubella* (Shepherd's Purse; EOA39137), *Brassica rapa* (canola or field mustard; Bra030852), *Thellungiella halophila* (a genus in the mustard family closely related to *Arabidopsis*; Thhalv10011181 min), *Panicum virgatum* (switchgrass; Pavirv00002473 min, Pavirv00061104 min), *Setaria italica* (Foxtail millet; Si028651 min), *Zea mays* (Corn; DAA64173), *Oryza sativa* (Rice; *Oryza sativa* Indica EEC82708 and *Oryza sativa* Japonica EEE67839), *Aegilops tauschii* (Goatgrass; EMT14894), *Triticum urartu* (the wild wheat progenitor with the A genome; EMS44986), *Physcomitrella patens* (moss; Pp1s5_110V6.1), and *Coccomyxa subellipsoidea* (microalgae; 63339). Relative domain positions were shown in Figure 1 based on the location of the β -propeller and coiled-coil domains as well as FG repeats. Strikingly, the relative location of these domains is quite consistent except in cucumber. In almost all of the plant species and in human, the β -propeller domain is located in the N-terminus, the coiled-coil domain is located in the middle followed by a high density of FG repeats in the C-terminus. Nup159 in *Saccharomyces cerevisiae* had its coiled-coil domain and FG repeats region inverted, which is different from those in human and most plants.

Notably, the evolutionary tree of LNO1 homologous proteins (Fig. 2) generally follows known plant phylogenies. *Arabidopsis thaliana* was closely related to *Arabidopsis lyrata* and then to *Capsella rubella* (a genus in the Brassicaceae or mustard family), *Brassica rapa*, and *Thellungiella halophila* (a genus of plants in the Brassicaceae or mustard family). Soybean LNO1 homolog was closely related to LNO1 homologs in *Phaselous vulgaris* (string bean) and *Cicer arietinum*, a genus in the Fabaceae (legume) family. The LNO1 homologs from *Cucumis sativus* (Cucumber; XP_004147125.1) and *Solanum lycopersicum*

Figure 1A. Identification of LNO1-like proteins in different plant species and domain locations of the β -propeller (black), coiled-coil (gray), and FG repeats (white). Numbers represent positions of amino acids in the protein. The full-length amino acid sequence of LNO1 (At1g55540) was used to search the NCBI and Phytozome databases using BLASTp and hits from 30 different plant species were obtained. Amino acid sequences of these putative homologs were then put through the PSIPRED DomPred (Protein Domain Prediction) software to ensure they were authentic as LNO1 homologs and had the conserved 7-fold β -propeller domain of *Arabidopsis* LNO1 (CATH database domain code 1xipA00, cathdb.info). The homologous sequences of β -propeller, coiled-coil, and FG Repeats were also aligned together using ClustalW2 and followed by using BOXSHADE 3.21 (data now shown).

(Tomato; XP_004231682) are relatively more related to those from dicots *Arabidopsis*, soybean and chickpeas than other trees or monocots. In the monocot, *Triticum urartu* and *Aegilops tauschii*, which are the diploid wild progenitors of the A and D genomes of polyploid wheat, respectively, have LNO1-like homologs in their genomes that are very closely grouped together. LNO1 homologous sequences from the grass family: *Setaria italica* (Foxtail millet), *Zea mays*, *Oryza sativa*, *Triticum urartu* and *Aegilops tauschii* were grouped together and were

separated from LNO1 homologs from dicots and trees with a high confidence. All LNO1 homologous proteins are more closely group together than its homolog NUP214 in human and Nup159 in yeast.

AtGLE1-Like Proteins are conserved in green plants

To identify Gle1 homologs in plant species, the full-length amino acid sequence of AtGLE1 (At1g13120) was used to

Figure 1B. Identification of LNO1-like proteins in different plant species and domain locations of the β -propeller (black), coiled-coil (gray), and FG repeats (white). Numbers represent positions of amino acids in the protein. The full-length amino acid sequence of LNO1 (At1g55540) was used to search the NCBI and Phytozome databases using BLASTp and hits from 30 different plant species were obtained. Amino acid sequences of these putative homologs were then put through the PSIPRED DomPred (Protein Domain Prediction) software to ensure they were authentic as LNO1 homologs and had the conserved 7-fold β -propeller domain of *Arabidopsis* LNO1 (CATH database domain code 1xipA00, cathdb.info). The homologous sequences of β -propeller, coiled-coil, and FG Repeats were also aligned together using ClustalW2 and followed by using BOXSHADE 3.21 (data now shown).

search the Phytozome and NCBI databases using BLASTp and hits from 35 different plant species were obtained. SMART genomic protein analysis software was used to identify the conserved PFAM Gle1 family domain of *Arabidopsis* AtGLE1 in other plant species. The coiled-coil domain was also found and located upstream of the Gle1 family domain in most of these putative AtGLE1 homologous proteins (Fig. 3). The 35 different plant species that have putative AtGLE1 homologs are: *Ricinus communis* (Castor oil plant;

XP_002515056.1), *Manihot esculenta* (cassava4.1_003555 min.g), *Linum usitatissimum* (Lus10001317), *Populus trichocarpa* (Black cottonwood or poplar tree; XP_002311751.1 or Potri.008G183500.1), *Eucalyptus grandis* (Eucgr. B03244.1), *Malus domestica* (MDP0000298548), *Prunus persica* (ppa003840 min), *Citrus sinensis* (orange1.lg006315 min), *Citrus clementina* (Ciclev10030923 min), *Medicago truncatula* (Medtr3g086460.1), *Glycine max* (Soybean; XP_003537556.1), *Phaseolus vulgaris* (Phvul.001G256000.1),

Figure 2. Evolution tree of human NUP214, yeast Nup159, *Arabidopsis thaliana* LNO1, and other LNO1-like proteins in plants. The amino acid sequences of LNO1 and its homologs were aligned by ClustalW, and then subjected to phylogenetic tree construction using MegAlign software (MegAlign, DNASTar Lasergene 8). Dashed lines represent nodes that collapsed in the strict consensus tree.

Gossypium raimondii (Gorai.012G173500.1), *Theobroma cacao* (Thecc1EG010631t1), *Carica papaya* (Papaya; evm.model.supercontig_13.88), *Thellungiella halophila* (Thhalv10007079 min), *Brassica rapa* (Bra026928), *Capsella rubella* (Carubv10008625 min), *Arabidopsis lyrata* (XP_002892728.1), *Cucumis sativus* (Cucsa.286770.1), *Vitis vinifera* (Grape vine; CAN81849.1), *Aquilegia coerulea* (Aqua_001_00165.1), *Solanum lycopersicum* (Soly05_g013790.2.1), *Mimulus guttatus* (mgv1a003066 min), *Oryza sativa* (Rice, LOC_Os02_g38250.1; *Oryza sativa Indica*, EEC73520.1; *Oryza sativa Japonica*, BAD16837.1), *Brachypodium distachyon* (purple false brome related to the major cereal species; XP_003567589.1), *Hordeum vulgare* (barley; BAJ99175.1), *Panicum virgatum* (switchgrass; Pavirv00021326 min), *Setaria italica* (Si016696 min), *Sorghum bicolor* (sorghum; XP_002463669.1), *Zea mays* (GRMZM2G417217_T02), *Selaginella moellendorffii* (lycopod; XP_002960959.1), *Physcomitrella patens* (moss; XP_001781965.1 and Pp1s316_12V6.1), *Micromonas pusilla* (algae; 50155), and

Chlamydomonas reinhardtii (algae; XP_001691275.1 or Cre06.g300900.t1.3). Based on the data acquired from the SMART protein analyses, the domain locations in AtGLE1-like proteins were constructed (Fig. 3). It is striking that all the putative AtGLE1-like proteins have very similar domain topography, with an approximately 80 amino acids of coiled-coil domain followed by a 200–250 amino acids of the Gle1 family domain.

To understand evolution of these putative AtGLE1-like proteins, we entered the protein sequence alignment data into MEGA and created a maximum likelihood evolutionary tree (Fig. 4). The phylogenetic tree followed known phylogenies for the most part. The monocot monophyletic group (*Oryza sativa*, *Brachypodium distachyon*, *Hordeum vulgare*, *Panicum virgatum*, *Setaria italica*, *Sorghum bicolor*, and *Zea mays*) was separated from the dicot monophyletic group with relatively high confidence. The *Brassicaceae* or mustard family is clustered together (*Arabidopsis thaliana*, *Arabidopsis lyrata*, *Capsella rubella*, *Brassica rapa*, and *Thellungiella halophila*). The *Fabaceae* (legume)

Figure 3A. Identification of AtGLE1-like proteins in different plant species and locations of the coiled-coil (gray) and Gle1 family domains (black). Numbers represent positions of amino acids in the protein. The full-length amino acid sequence of AtGLE1 (At1g13120) was employed to search the NCBI and Phytozome databases using BLASTp and hits from 35 different plant species were obtained. SMART genomic protein analysis software was used to identify the conserved PFAM Gle1 family domain of *Arabidopsis* AtGLE1 in other plant species. The homologous domain sequences of Gle1 family and coiled-coil were also aligned together using ClustalW2 and followed by using BOXSHADE 3.21 (data now shown).

family is also closely clustered together (*Medicago truncatula*, *Glycine max*, and *Phaseolus vulgaris*). The algae, *Chlamydomonas reinhardtii*, branched off the tree early as expected. Unusually, *Physcomitrella patens* and *Selaginella moellendorffii*, moss and lycopod respectively, form a monophyletic group.

Discussion

Based on the sequence alignment (data not shown), domain locations, and evolution trees presented here, it is evident that LNO1 and AtGLE1 homologous proteins are highly conserved

Figure 3B. Identification of AtGLE1-like proteins in different plant species and locations of the coiled-coil (gray) and Gle1 family domains (black). Numbers represent positions of amino acids in the protein. The full-length amino acid sequence of AtGLE1 (At1g13120) was employed to search the NCBI and Phytozome databases using BLASTp and hits from 35 different plant species were obtained. SMART genomic protein analysis software was used to identify the conserved PFAM Gle1 family domain of *Arabidopsis* AtGLE1 in other plant species. The homologous domain sequences of Gle1 family and coiled-coil were also aligned together using ClustalW2 and followed by using BOXSHADE 3.21 (data now shown).

in plant species. This is likely due to the basic function of the NPC that exports mRNA from the nucleus into the cytoplasm. These proteins are vital and mutations in *AtGLE1* and *LNO1* result in non-viable seeds in *Arabidopsis*.²⁴ Both *los4-1* and *los4-2* mutants show abnormal mRNA localization, but are viable,³¹ and this could be due to that both of *los4-1* and *los4-2* are missense mutations instead of null alleles. Therefore, it is tempting to speculate that a null allele of the *los4* mutant might have a similar seed abortion phenotype as *lno1* and *Atgle1*.

The N-terminal domain of LNO1/Nup214/Nup159 forms a unique asymmetrical 7 bladed β -propeller that is conserved

and found in all Nup159 homologs.³⁶ In addition to mediating protein-protein interaction and localizing LOS4/DDX19/Dbp5 to the nuclear pore, Nup159 is responsible for the release of ADP to recycle Dbp5.³⁵ Considering the immense biological importance of this pathway, it is reasonable that the β -propeller would have a degree of conservation. In addition to the N-terminal β -propeller, LNO1 and their homologs in other plant species have two other domains: the coiled-coil domain and the FG repeats. The FG repeats are found in approximately one-third of nucleoporins,³⁷ and its function is not completely understood. One prominent theory hypothesizes that FG nucleoporins extend

Figure 4. Phylogenetic tree of human GLE1, yeast Gle1, *Arabidopsis thaliana* AtGLE1, and other GLE1-like proteins in plants. The amino acid sequences of AtGLE1 and its homologs were aligned by MAFFT (Version 7).⁴⁵ A maximum likelihood tree was created by using MEGA software (Version 5.2.2).⁴⁶

into the nuclear pore and form a physical barrier to non-specific molecules.³⁸ On the other hand, the “Forest” model hypothesizes that the coiled-coil domain of these FG repeat proteins may switch from a globular to extended conformation and change the structure of the nuclear pore to accommodate different cargos.³⁸ These FG repeats can form a gel at local concentrations that may be dissolved by the binding of importins and exportins carrying their cargo through the NPC, which is the basis of the “hydrogel” or “selective phase” model.³⁹ In their experiments on reconstituted *Xenopus* nuclei, Hullsman et. al. found that Nup98 alone was both necessary and sufficient for the formation of a proper barrier to the passive transport of large molecules. Despite the result that Nup98 is the only FG repeat nucleoporin necessary for creating an effective barrier, these features are conserved in NUP214 and its homologs. This indicates that the FG repeats and coiled-coil domain function in the “hydrogel” is not completely understood. One possibility is the FG repeats region helps localize newly

synthesized proteins to the NPC, where they perform their functions in nucleocytoplasmic transport.

Another interesting feature of the conservation of LNO1 homologs is domain locations. In yeast Nup159, the FG-repeat region is medial while the coiled-coil domain is in the C-terminus, unlike human NUP214 or plant LNO1. In some plant species, no LNO1 homologous sequences can be found and this might be due to a lack of properly annotated LNO1 homologs in the database. For example, the original LNO1 sequence incorrectly annotated into two distinct genes in *Arabidopsis* until recently.¹⁵ Expression of *LNO1* is quite low in all tissues except for sperm cells (www.genevestigator.com).²⁴ If this trend of generally low expression of the *LNO1*-like genes holds true in other plant species and perhaps in other eukaryotes, acquiring sufficient EST data including LNO1 homologs would be difficult. *Zea mays* is another example and until very recently only a fragment of the β -propeller domain was available in the database. Another

problem is a lack of sequence similarity in the protein. Since the majority of these proteins have the FG repeats, which are defined by its primary structure and have little secondary structure, there is little selective pressure to prevent amino acid substitutions. Furthermore, while the β -propeller domain itself has a very conserved structure, its sequence is less conserved.³⁶ This could result in rapid divergence as long as the tertiary structure is maintained, making identification of LNO-like proteins using programs such as BLAST ineffective in some distant relatives of *Arabidopsis*.

Gle1 is an essential mRNA export factor in yeast.⁴⁰ The Gle1 protein contains predicted coiled-coil domain and IP₆ binding domain for regulating DED-box proteins (Dbp5 in yeast, DDX19 in human, or LOS4 in plants).⁴¹ By interacting with the small molecule IP₆, Gle1 stimulates ATPase activity of Dbp5.^{42,43} Then Gle1/IP₆-activated Dbp5 is converted from a Dbp5-ATP to Dbp-ADP state that causes mRNP remodeling, thus facilitating export directionality.⁴⁴ A recent study shows that Gle1 also regulates the initiation and termination of translation. Furthermore, Gle1 can form an oligomer through its coiled-coil domain during mRNA export. The mutation in human *GLE1* is causally linked to human lethal congenital contracture syndrome-1.⁴¹ In our study, we found the *Atgle1* mutation gives approximately 19% seed abortion in the heterozygous plants (*AtGLE11/Atgle1*),²⁴ suggesting its mutation is not homozygous lethal. However, when we tried to isolate the homozygous mutant (*Atgle1/Atgle1*), we were not successful after genotyping more than 200 plants from the heterozygous plant (*AtGLE11/Atgle1*) progeny. One possible explanation is that the non-aborted homozygous mutant seeds do not germinate or do not survive during early seedling growth. This suggests that AtGLE1 is required for mRNA export germination and early seedling development. Broad existence of AtGLE1-like proteins (Figs. 3 and 4) suggests the important functions of these proteins in plants. It would be interesting to know functions of AtGLE1-like proteins

in various plant species, and what would be the phenotype if there is a mutation in these genes.

Conclusion

The nucleoporins LNO1 and AtGLE1 are important for early embryogenesis and seed development in *Arabidopsis*. By searching protein database and sequence analysis, we have identified 30 LNO1-like and 35 AtGLE1-like proteins in various plant species including agriculturally important cereal crops (rice, wheat, maize, and barley), legumes (soybean, *Brassica rapa*, and *Medicago truncatula*), fruit and vegetable crops (cucumber, tomato, citrus, grape, and cacao). The phylogenetic analysis shows that the evolutionary trees of these proteins follow expected plant phylogenies. We conclude that nucleoporins LNO1-like and AtGLE1-like proteins are conserved from lower plant species (*Physcomitrella patens* and *Selaginella moellendorffii*) to higher C3 (*Brassica* and legume family) and C4 (sorghum, maize, and switchgrass) plants as well as perennial trees (poplar, flooded gum, citrus, peach, and apple trees). The conservation of sequence and domain structure of these nucleoporins reflects the essential function of these proteins in regulating cargo transport between the nucleus and cytoplasm in plant cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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