Components of the *Arabidopsis* nuclear pore complex play multiple diverse roles in control of plant growth

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Abstract

The nuclear pore complex (NPC) is a multisubunit protein conglomerate that facilitates movement of RNA and protein between the nucleus and cytoplasm. Relatively little is known regarding the influence of the *Arabidopsis* NPC on growth and development. Seedling development, flowering time, nuclear morphology, mRNA accumulation, and gene expression changes in *Arabidopsis* nucleoporin mutants were investigated. Nuclear export of mRNA is differentially affected in plants with defects in nucleoporins that lie in different NPC subcomplexes. This study reveals differences in the manner by which nucleoporins alter molecular and plant growth phenotypes, suggesting that nuclear pore subcomplexes play distinct roles in nuclear transport and reveal a possible feedback relationship between the expression of genes involved in nuclear transport.

Key words: Flowering time, nuclear pore, nuclear pore complex (NPC), nuclear transport, nucleoporin, nucleus.

Introduction

The nuclear pore complex (NPC) is a massive macromolecular conglomerate that sits within invaginations of the nuclear envelope and controls nucleo-cytoplasmic transport of RNA and protein (Raices and D’Angelo, 2012). Proteomic and microscopic analysis have deciphered that the entire NPC is comprised of distinct subcomplexes that are octagonally arranged around a central channel (Cronshaw et al., 2002; Alber et al., 2007a, b; Fiserova et al., 2009; Degrasse and Devos, 2010; Tamura et al., 2010). The size of a single NPC varies between ~60 MDa in yeast and ~120 MDa in metazoans but contain similar sets of core proteins. NPC subcomplexes are comprised of individual nucleoporin (NUP) proteins. These NUPs play roles in maintaining the structural integrity of the NPC (Walther et al., 2003), directly influencing gene expression (Capelson et al., 2010; Kalverda et al., 2010; Van de Vosse et al., 2013), controlling differentiation (D’Angelo et al., 2012), maintaining regions of chromatin exclusion (Krull et al., 2010), or modulating nuclear transport (Walde and Kehlenbach, 2010).

Over the past 15 years much of the work aimed at elucidating the nature and function of the NPC has taken place in *Saccharomyces cerevisiae* and in metazoan cell culture. However, a growing portfolio of research in *Drosophila* (Capelson et al., 2010; Kalverda et al., 2010), *Caenorhabditis elegans* (Galy et al., 2003; Rodenas et al., 2012), fission yeast (Bai et al., 2004), and trypanosomes (DeGrasse et al., 2009) now indicates that general NPC and specific NUP function may vary significantly between eukaryotes.

The plant NPC remained somewhat of a mystery until recent studies using proteomics (Tamura et al., 2010), electron microscopy (Fiserova et al., 2009), and bioinformatics (Neumann et al., 2006, 2010) determined that the structure of the plant NPC is probably similar to that observed in other eukaryotes. The proteomic study of Tamura and co-workers (Tamura et al., 2010) demonstrated that the composition of...
the plant NPC is more closely aligned to the metazoan NPC than the yeast NPC. In that study, the NUPs that were identified indicate that the broad composition of the NPC sub-complexes is maintained (Tamura et al., 2010). In addition, a plant-specific nucleoporin, NUP136, was identified, and suggests that in future, other plant-specific NUPs will be discovered (Tamura et al., 2010).

In addition to this information about the composition of the NPC, it has been found that certain NUPs from Arabidopsis, tobacco, and Lotus are involved in diverse signalling pathways. These studies show that the NPC is a control point for the interaction of the plants with both pathogenic (Zhang and Li, 2005; Cheng et al., 2009; Wiermer et al., 2012) and symbiotic microorganisms (Kanamori et al., 2006; Saito et al., 2007; Groth et al., 2010). In addition, plants with defects in NUP function have altered responses to hormone signalling (Parry et al., 2006; Robles et al., 2012) and abiotic stresses (Dong et al., 2006a, b; Lazaro et al., 2012; MacGregor et al., 2013). A pattern is emerging that places the NPC as a participant in many signalling cellular pathways even though the molecules whose nucleo-cytoplasmic transport is controlled by different NUPs are largely unknown (Parry, 2013). This study presents phenotypic and molecular analysis of a range of Arabidopsis NUP mutants. As evidence emerges from other experimental systems that individual NUPs play specific cellular roles, it appears that NUPs influence plant growth by different molecular mechanisms. In addition, it is shown that in certain nup mutants, expression of genes involved in nuclear transport is up-regulated, suggesting a mechanism of feedback control.

**Materials and methods**

**Growth conditions**

Seedlings were grown at 22 °C for 16 hr light [termed long days (LDs)] or 12 hr light [termed short days (SDs)] on 1% agar plates with 1% sucrose, half-strength Murashige and Skoog (MS) salts pH 6, and germinated after 3 d at 4 °C. Identities of SALK T-DNA insertion lines (Alonso et al., 2003) are outlined in Supplementary Table S1 available at JXB online. The primers used for identification of homozygous insertion lines are shown in Supplementary Table S2.

**RNA extraction and real-time PCR**

After growth for 7 d in LD conditions, RNA was extracted from 100 mg of seedling tissue using the Spectrum RNA kit (Sigma-Aldrich). A 1–2 μg aliquot of RNA was used to produce cDNA with a Superscript III (Life Technologies) or cDNA synthesis kit (Bioline). Non-quantitative PCR for mutant analysis was performed using Red-Hot Taq (Bioline). Real-time PCR was performed with SYBR-Green, Platinum Taq (Life Technologies), using primers for gene expression shown in Supplementary Table 2 at JXB online on an MJ Research Opticon 2 machine with Opticon Monitor 3 software. Quantification of expression was determined from ≥3 experiments and the values derived using the comparative C_T method (2–ΔΔC_T) (Schmittgen and Livak, 2008) with ACTIN7 (At5g09810) as the internal control. Control genes At4g33060 and At3g10040 were selected at random from a data set produced from study of the Arabidopsis hypoxia response (Licausi et al., 2011). From the microarray data described in Fig. 6, the fold change in log2 expression compared with Col-0 in At4g33060 is 0.054 (nup62-2) or 0.048 (nup6-2) and in At3g10040 is 0.144 (nup62-2) or 0.104 (nup62-2).

**mRNA localization**

Seven-day-old seedlings were prepared similarly to previous methods (Parry et al., 2006) with modifications. Briefly, seedlings were incubated with slow shaking at room temperature in glass universals with Buffer 1 [120 mM NaCl, 7 mM Na_2HPO_4, 3 mM Na_2PO_4, 2.7 mM KCl, 80 mM EGTA, 0.1% Tween, 10% dimethylsulfoxide (DMSO)] + formaldehyde (5%, PFA) for >30 min followed by 5 min washes in 100% methanol (2×), 100% ethanol (2×), and 1:1 Buffer 1: methanol before post-fixing in Buffer 1 + PFA for >30 min. Samples were washed for 5 min in Buffer 1 (2×) and Perfect Hyb Plus Buffer (Sigma-Aldrich), the latter in a 12-well plastic tissue culture dish. Seedlings were pre-hydrized at 50 °C in fresh Perfect Hyb Plus Buffer for >1 h after addition of 0.6 pmol μl⁻¹ 25-mer oligo(DT) primers tagged with fluorescein and incubated overnight. Samples were then washed with 2× SSC, 0.1% SDS and 0.2× SSC, 0.1% SDS for 1 h and >20 min, respectively. For visualization, samples were mounted either in propidium podide (PI; 1 μg ml⁻¹) with Vectashield (Vector lab) or in Vectashield+4’,6-diamidino-2-phenylindole (DAPI; 1.5 μg ml⁻¹) and viewed using the fluorescein isothiocyanate (FITC) filter on a Zeiss Axioskop 2 plus the position of the nuclei was determined using either a tetramethylrhodamine isothiocyanate (TRITC) filter to view PI-stained nuclei or a UV filter to view DAPI-stained nuclei. For quantification, ImageJ was used to compare pixel intensity between the nucleus and cytoplasm within individual cells and expressed as a ratio where a higher value represented higher nuclear expression, as detailed in Supplementary Fig. S5 at JXB online. The EVOS XL Imaging system was used (Life Technologies) to visualize ×10 images of roots.

**Measuring nuclear morphology**

Seedlings were prepared as in the mRNA localization experiments except that roots were mounted in PI (1 μg ml⁻¹) with Vectashield (Vector lab) or in Vectashield+DAPI (1.5 μg ml⁻¹) and visualized using the TRITC (PI) or UV (DAPI) filter on the Axioskop 2 plus. ImageJ was used to measure the nuclear circularity and nuclear perimeter.

**Microarray expression**

RNA was extracted from three independent sets of 7-day-old wild-type, nup62-2, and nup160-1 seedlings using the Spectrum Plant RNA kit (Sigma-Aldrich). A 1 μg aliquot of RNA from each sample (triplicate for each genotype) was sent to the NASCarrays facility (http://affy.arabidopsis.info) where it was prepared, processed, and hybridized to the Affymetrix AraGene-1.0-st-v1 chip using their established protocols. The resulting data was converted using R/Bioconductor2.12 (http://www.bioconductor.org) to CSV files following normalization using the GCRMA protocol. The triplicated expression values were averaged and this value from each mutant was compared with wild type. The gene ID of each sample whose expression has altered 2-fold was determined by comparison with an appropriate probe set [AraGene-1.0-st-v1.na32.tair10.probeset (version 1)] using a Perl script kindly supplied by Ben Wareham (University of Liverpool). The entire data set can be downloaded via the IPlant collaborative: http://data.iplantcollaborative.org/quickshare/aad660k79af44e93a/Exp654.zip (last accessed 4 August 2014).

**Results**

Nucleoporin mutants exhibit a range of phenotypes

A comprehensive analysis of the composition of the plant NPC revealed that most of the known metazoan and yeast NPC components could be identified in Arabidopsis (Tamura et al., 2010). However, the contribution of many of these components to plant growth and development is unknown, while those plants with mutations in NUP genes have been
investigated as part of isolated studies. Therefore, a direct comparative study of insertion mutants within many of the NUP genes was performed. This involved investigating general growth responses such as flowering time and root elongation, changes in nuclear morphology and nuclear mRNA transport, as well as global alterations in gene expression. The aim was to ascertain how alterations in the function of the nucleus might correspond to changes in plant growth. Viable homozygous mutants were selected from across NPC subcomplexes that each contribute to the different proposed functions of the NPC, as suggested from studies in other eukaryotes.

Figure 1 shows the proposed arrangement of the plant NUPs into their putative subcomplexes, based on analogy to yeast and metazoan NUPs. In earlier studies, certain null nup mutants were identified as exhibiting embryo lethality in Arabidopsis, including MOS7/NUP88 (Cheng et al., 2009), NUP214/LNO1, GLE1 (Braud et al., 2012), NUP1/NUP136 (Lu et al., 2010), nup62-3 and nup205-1 (Meinke et al., 2008). Therefore, it was not unexpected to be unable to isolate homozygous mutants in new alleles of NUP214 (nup214-3, Salk_220_H11), NUP205 (nup205-2, Sail_874_A02), and an allele of NLP1/CG (nlp1-1 Sail_006526) (Supplementary Table S1 at JXB online).

The proposed NUP62 subcomplex comprises the NUP62, NUP58, and NUP54 proteins and is thought to reside in the central pore of the NPC (Fig. 1) (Solmaz et al., 2011). In this study, another lethal NUP62 allele (nup62-4 Salk_071521) was identified and the nup62-2 (Sail_127_F01) and nup62-1 (Salk_037337) alleles were investigated. These plants are viable although significantly smaller in stature compared with wild-type plants (Fig. 2; Supplementary Fig. 3 at JXB online). nup62-2 plants contain a T-DNA insertion at position 2061 bp of the cDNA, causing a premature stop codon that presumably creates a truncated protein lacking 53 C-terminal amino acids. Nup62-1 plants have a T-DNA insertion at the border of the fifth exon and fifth intron (equivalent to the position of amino acid 612), introducing a stop codon after an additional 47 amino acids.

The nup54-2 (Salk_015252) and nup58-2 (Salk_099638) mutant alleles share a similar growth phenotype to nup62-2 reminiscent of previously identified nup mutants, characterized by a reduction in root elongation, a decrease in stature, and early flowering, when grown in both in LD (Fig. 2; Supplementary Fig. S3 at JXB online) and SD conditions (Supplementary Fig. S2). The nup54-2 and nup58-2 alleles do not produce a full-length transcript yet have 5’ and 3’ mRNA expression, indicating that these alleles, like nup62-2, probably form truncated proteins. It remains to be determined how these truncated proteins specifically impact nuclear transport in Arabidopsis. A structural analysis of the rat NUP62 subcomplex indicates that NUP54 individually binds both NUP62 and NUP58 to form the intact NUP62 complex (Solmaz et al., 2011). These authors show that NUP62 and NUP54 interact by their N-terminal domains, so it is feasible that the truncated NUP62 and NUP54 proteins are still able to make this interaction in Arabidopsis. The nup54, nup58, and nup62 mutant alleles were designated in a recent study that characterized vegetative phenotypes of these alleles (Ferrandez-Ayela et al., 2013). Interestingly double mutant combinations of nup58nup62 and nup58nup54 alleles are viable and do not have a significantly more severe phenotype than single mutant plants.

The NUP107–160 subcomplex is predicted to play a structural role in the NPC. It is the largest NPC subcomplex comprising eight members including NUP85, NUP96, NUP160, and SEH1 (Fig. 1) and has been characterized as playing a role in the auxin and defence responses (Zhang and Li, 2005; Parry et al., 2006; Wiermer et al., 2012). New alleles of NUP85 (nup85-2, Salk_133369 and nup85-3, Salk_131200) were identified, both of which show a wild-type growth phenotype (Fig. 2; Supplementary Fig. S4 at JXB online). The T-DNA insertion in nup85-2 is located in the 5’ part of the gene so probably causes a null mutation, but the insertion in nup85-3 causes introduction of a premature stop codon after amino acid 477. Interestingly, nup83 and seh1 mutants do not share the strong nup160-4 (Salk_126801) or nup96 mutant phenotypes (Fig. 2; Parry et al., 2006).

Therefore, the phenotypes observed in the viable nup mutant plants appear to fall into two broad classes: those whose growth is similar to that of wild-type plants and those that display a consistent altered growth phenotype (Supplementary Table S1 at JXB online).
The NPC influences nuclear morphology

The size and shape of plant nuclei are affected by alterations in NPC and nuclear envelope composition (Tamura et al., 2010; Tamura and Hara-Nishimura, 2011; Zhou et al., 2012). Nuclei from mature root cells across the range of nup mutants were visualized (Fig. 3) and it was found that certain mutant nuclei have a smaller nuclear perimeter. Whereas in wild-type root cells, nuclei in this cell type have an elongated morphology, the nup160-1 [which has the same growth phenotype as nup160-4 (Parry et al., 2006)] mutant displays significantly more circular nuclei in common with sun1KOsm2KD mutant nuclei (Fig. 3B–E) (Zhou et al., 2012). This suggests that the function of the NPC has a more profound role in controlling nuclear morphology rather than purely size. Interestingly, although other nup mutant plants such as nup62, nup58-2, and nup54-2 have reduced size and are early flowering (Fig. 2), the nuclear morphology of these plants is not significantly different from that of wild-type plants. This suggests that alterations in nuclear morphology do not completely correlate with the broader nup mutant growth phenotypes.

Nucleoporins play differing roles in the control of mRNA nuclear export

Work performed in other eukaryotic systems has demonstrated that cells with altered NPC composition exhibit defects in bulk mRNA nuclear export. This has been observed
in Arabidopsis as mRNA is preferentially held within the nucleus of certain nup mutants (Dong et al., 2006b; Parry et al., 2006; Jacob et al., 2007; Xu et al., 2007b; Lu et al., 2010; Wiermer et al., 2012; MacGregor et al., 2013). This analysis was extended by quantifying mRNA nuclear accumulation in a new set of nup mutants. In this experiment, roots are incubated with a labelled oligo(dT) probe and the fluorescent signal inside and outside of nuclei was measured in order to quantify the amount of mRNA that has remained within the nucleus (Supplementary Fig. S5 at JXB online). Consistent with previous observations, wild-type plants show little nuclear mRNA accumulation as the fluorescent intensity is equivalent inside and outside the nucleus (Fig. 4A, B, O; Supplementary Fig. S5). In nup160, seh1, and nup85 nuclei, stronger nuclear fluorescence was quantified that represents nuclear mRNA accumulation (Fig. 4I–O, Supplementary Figs S4, S5). This agrees with the hypothesis that a role of the Arabidopsis NUP107–160 complex is to influence mRNA transport. Interestingly although seh1-1 and nup85 mutant alleles display a mild defect in mRNA export, their general growth in similar to wild-type plants.

Nuclei from nup62, nup54-2, and nup58-2 roots each display levels of mRNA nuclear accumulation equivalent to wild-type nuclei (Fig. 4C–H, O, Supplementary Figs S3, S5 at JXB online) despite having significantly altered growth phenotypes. Importantly this is the first time that, when tested, plants with a nup mutation do not exhibit a defect in mRNA accumulation. This indicates that the NUP62 subcomplex plays a role distinct from that of other tested NUPs in the control of mRNA nuclear export.
Plants lacking other tested NUPs do show defects in nuclear mRNA export, whether these NUPs are situated within the NUP107–160 complex or lie in peripheral NPC locations (Parry et al., 2006; Jacob et al., 2007; Xu et al., 2007a; Lu et al., 2010; Wiermer et al., 2012; MacGregor et al., 2013). Therefore, this may suggest that there is a distinct route of mRNA transport through the plant NPC that does not involve the NUP62 subcomplex.

**Nucleoporin double mutants display a range of growth and molecular phenotypes**

Previous work has shown that NUP160 plays an important role in plant NPC function (Fig. 2) (Dong et al., 2006b; Parry et al., 2006; Robles et al., 2012). Therefore, to better understand the genetic relationship between NUP160 and other NUPs, certain nup mutants were crossed with alleles of nup160. These included nup98a-1, nup85-2, and nup62-2 plants. In the resulting double mutants a range of phenotypes were observed that highlight the differing genetic relationships of NUP160 with other NUPs.

Single nup160-1 mutants have reduced root elongation and smaller stature, while nup85-2 plants resemble wild-type plants. The nup160nap85 double mutant grows similarly to the nup160 mutant with no increase in phenotypic severity (Fig. 5). This indicates that the loss of NUP160 removes any requirement for NUP85 function and, as discussed below, it is difficult to explain this phenotype with current presumed knowledge of how these proteins are arranged in the NUP107–160 complex (see the Discussion).

NUP98 is a peripheral FG-repeat NUP that in metazoans has multiple functions, not least as a determinant of transport through the NPC (Hulsmann et al., 2012), control of gene expression (Kalverda et al., 2010), and as a potent oncoprotein (Xu and Powers, 2009). While there is a single NUP98 gene in metazoans, in plants a duplication event (Arabidopsis Genome Consortium, 2000) created both NUP98a and NUP98b genes, where the amino acid sequences are 61% identical. Plants with a T-DNA insertion in each of these genes were identified (Supplementary Fig. S6 at JXB online) and it was shown that these plants have wild-type phenotypes (Fig. 5; Supplementary Fig. S6). The T-DNA insertion within

![Image](https://academic.oup.com/jxb/article-abstract/65/20/6057/2485450)

**Fig. 5.** Nup double mutants show different phenotypes. Root elongation was measured in light-grown (A) 7-day-old seedlings. Rosette leaf number (B) at the time of flowering in plants grown in LD conditions. Error bars represent the SE. Student t-test, compared with Col-0(*) or nup160-1(+), *P*<0.01. Representative images of plants grown for 23 d under LD conditions with the following genotypes; Col-0 (C), nup85-2 (D), nup98a-1 (E), nup160-1 (F), nup160nap85 (G), nup160nap98a (H). Comparison of 34-day-old plants (I) from L–R: nup98a-1, nup160-1, nup98anup160. Scale bars represent 13mm.
**nup98a-1** lies in a 5’ location and appears to prevent transcription of the gene, whereas **nup98b-2** the insertion lies within the last exon, suggesting that a truncated protein will be produced.

When compared with **nup160-1**, the **nup160nup98a** double mutant has significantly smaller roots and reduced stature (Fig. 5). These more severe phenotypes indicate that the NUP107–160 complex and NUP98 play distinct roles in plant growth. This also suggests that NUP98a performs different functions from NUP98b, as the assumed presence of the latter is unable to make up for the lack of the former. The relationship between these two plant NUP98 proteins are under evaluation and, from current knowledge of the NPC and of NUP98 function in other organisms, it is expected that **nup98anup98b** double mutant plants will not be viable.

Following a cross between **nup160-4** and **nup62-2**, it was not possible to identify viable **nup160-4nup62-2** double mutant plants. This was unsurprising since the **nup62-2** and **nup160-4** single mutants both demonstrate pleiotropic mutant phenotypes and it suggests that the plant is unable to overcome reduced function of both NUP107–160 and NUP62 subcomplexes. The underlying reason for this will become clearer once a better understanding is obtained about certain NUP proteins.

### Gene expression change in nucleoporin-deficient plants

In order to understand the phenotypic changes that are observed in **nup160-4** and **nup62-2** plants, an analysis of global gene expression was performed using the NASCarray service at the Nottingham Arabidopsis Stock Centre. In the context of the plant NPC, the only previous global expression analysis has investigated either **tpr1** or **hosl** mutant plants. First, post-flowering **tpr1** mutants were compared with pre-flowering wild-type plants and, more recently, early-flowering 14-day-old **hosl** was compared with wild-type plants (Jacob et al., 2007; MacGregor et al., 2013). Interestingly, ~8% of genes showed up-regulated expression in these experiments. In the case of **hosl**, these included a high proportion of genes under circadian regulation, which reflects the multiple roles that HOS1 appears to play in plant development (MacGregor et al., 2013; Jung et al., 2014).

In order to avoid the inevitable expression changes that occur during floral transition, gene expression was compared between 7-day-old wild-type, **nup62-2**, and **nup160-4** seedlings (Fig. 6A). Transcript levels from triplicate samples were assessed using the ATH1 Genome Array and, following comparison of average expression levels of wild-type and mutant genes, relatively few genes showed a 2-fold change in gene expression. When **nup160-4** seedlings were compared with the wild type, 159 genes showed 2-fold up-regulation and 174 genes showed similar levels of down-regulation. In **nup62-2**, 76 genes were 2-fold up-regulated and 58 were 2-fold down-regulated. Comparison of these data sets showed that 18 annotated genes were 2-fold down-regulated in both mutants. When the gene ontology (GO) annotations of these genes were analysed, no apparent similarity in their proposed function was revealed (Supplementary Fig. S7 at JXB online).

Most notable were the 20 annotated genes that were 2-fold up-regulated in both **nup62-2** and **nup160-4** (Fig. 6B–D). This group includes five genes involved in nuclear transport, namely the nucleoporins NUP98b and RAEl, and the nuclear transport proteins RAN1, RAN2, and XPO1 (Haizel et al., 1997; Blanvillain et al., 2008; Lee et al., 2009; Tamura et al., 2010). Subsequently the expression of RAN1 was assessed in other 7-day-old **nup** mutant seedlings and found to be significantly up-regulated in **nup54-2**, **nup58-2**, seh1-1, and **nup85-2** plants (Supplementary Fig. S8 at JXB online).

These findings provide an interesting clue to a potential feedback mechanism that may exist to regulate the activity of the plant NPC, namely that in the absence of a particular NUP, expression of other genes involved in nuclear transport is altered. Importantly, if this suggests that a certain level of ‘buffering’ exists in the regulation of nuclear transport components, it may begin to explain why plants appear better able than other eukaryotes to overcome a decline in function of certain NUP proteins.

### Discussion

In this study, the function of a number of *Arabidopsis* NUPs were investigated using phenotypic analysis, cell biological techniques, and measurement of global gene expression.

Although the architecture of the entire NPC is similar across all eukaryotes, the function of individual NUPs can vary greatly in an organism-specific manner. Therefore, nuclear transport will influence plant-specific signalling pathways that respond to phytohormones, biotic and abiotic stresses. It appears that the general phenotypic consequence of removing *Arabidopsis* NUPs takes one of three different forms. First, the plants are unable to survive, as in the case of removing NLP1/CG, NUP205, NUP62, GP210 (Meinke et al., 2008), NUP88 (Wiermer et al., 2010), NUP214, or AtGle1 (Braud et al., 2012). The second group, comprising mutants that mostly produce truncated proteins, have an intermediate phenotype characterized by retarded growth and early flowering, and occurs in plants lacking wild-type function of NUP54, NUP58, NUP62, NUP160, NUP96, NUP136/NUP1, HOS1, and TPR1/NUA (Zhang and Li, 2005; Dong et al., 2006a, b; Parry et al., 2006; Jacob et al., 2007; Xu et al., 2007b; Lu et al., 2010; Tamura et al., 2010; Lazaro et al., 2012; Robles et al., 2012; Wiermer et al., 2012; Ferrandez-Ayela et al., 2013; Jung et al., 2013; MacGregor et al., 2013). Finally, reducing the function of certain nucleoporins causes no obvious phenotype, namely NUP98a/b, NUP133, NUP107, NUP85, Seh1, and Sec13 (Wiermer et al., 2012). However certain double mutants can have more severe phenotypes than constituent single mutants, as in the case of **nup160nup62-2**, **nup160nup98a**, or **nup160nup96** (Parry et al., 2006). Overall it appears that NUPs that are not part of NPC subcomplexes are essential for growth, while those within a subcomplex may be functionally ‘buffered’ by the...
other complex members (Fig. 1). However, gaining a fuller understanding of how these mutants cause their phenotypes requires a greater knowledge of the structural interactions between NUPs. Obtaining this information would enable better predictions as to the effect of any truncated proteins, and is certainly an important task to accomplish as understanding of the plant NPC increases.

Arguably the non-lethal nup mutants are most interesting as they provide a platform to understand how the NPC influences different signalling pathways. The data presented here indicate that even though nup mutants may share similar phenotypes, the molecular basis for this change is not the same, most notably in the control of nuclear mRNA transport. The mRNA accumulation observed in mutants within the NUP107–160 complex is interesting as it is shown that seh1-1 and nup85 seedlings have defective nuclear mRNA accumulation yet do not exhibit major growth defects.

The NUP107–160 complex is a significant structural component of the NPC (Walther et al., 2003; Alber et al., 2007a) and, if one can reasonably assume that this structure exists in the plants, then it is surprising that the plant is able to survive with relatively minor phenotypic consequences when the function of members of this complex are reduced, removed, or altered. However, the phenomenon of varied phenotypes that result from changes in different NUP107–160 complex members is somewhat recapitulated across other eukaryotes. In C. elegans, reduction of NUP expression by RNA interference (RNAi) shows a wide variation in the amount of embryo lethality, namely NUP107, Seh1 (100% survival), NUP133 (99%), NUP85 (63%), NUP160, and NUP96 (~0%) (Galy et al., 2003).
The function of the NUP107–160 subcomplex is relatively well studied in other organisms. The orthologous yeast NUP84 complex has a Y-shaped structure with the arms of the ‘Y’ formed by NUP120 (orthologous to AtNUP160) and NUP85 (Lutzmann et al., 2002). A more comprehensive dissection of the yeast complex also found that certain members are more important for fitness of the organism, namely both the NUPs lying within the ‘arms’ of the Y-complex and also scNUP145C (orthologous to vertebrate/plant NUP96) (Fernandez-Martinez et al., 2012). Given these seemingly important structural positions, it is difficult to explain why the nup160nup85 double mutant does not have a more severe growth phenotype than a single nup160 mutant. This contrasts with the severe growth defects observed in nup160nup96 double mutants (Parry et al., 2006). Perhaps NUP160 and NUP96 have other functions away from the NPC that are reflected in their more severe phenotypes. Studies across different eukaryotes show that the function of the NUP107–160 complex is determined by a number of key components, although how this corresponds to the structural alignment of the entire NPC remains largely unknown.

Given the limits of the mRNA accumulation assay, it was surprising to discover that members of the NUP62 subcomplex do not appear to participate in mRNA export. This subcomplex sits within the central channel of the NPC yet its role in nuclear transport remains somewhat controversial. One set of largely structural experiments shows that the complex acts as a variable ring allowing transit of molecules through the pore (Solmaz et al., 2011), while a set of transport assays in Xenopus oocytes reveals that the complex makes a minimal contribution to passive and active transport (Hulsman et al., 2012). Currently, this depth of information for the role of the complex in Arabidopsis is not available, but it is clear from the lethality of certain nup62 alleles and the growth phenotypes of mutants in other members that it plays a major functional role. One theory that explains movement of the molecules across the NPC posits that there are distinct paths through the pore (Schoch et al., 2012), and the apparent lack of mRNA accumulation phenotype in the NUP62 complex mutants may suggest that the complex participates in one type of transport pathway but not in all of them. Future investigations into how the complex influences both nuclear import and export will hopefully clarify these roles.

The alteration in nuclear morphology in certain nup mutants (Fig. 3) mirrors what has been observed in Arabidopsis nup136-1 plants where nuclear shape is more significantly affected than nuclear size (Tamura et al., 2010). Similar phenotypes are also observed in plants that lack function of inner nuclear membrane-localized SUN proteins (Fig 3), outer nuclear membrane (ONM)-localized WIP proteins, or a novel plant myosin that links the ONM to the actin cytoskeleton (Zhou et al., 2012; Tamura et al., 2013). The recent identification of the KAKU1 myosin protein, which links the ONM and cytoskeleton, begins to explain how these changes in nuclear morphology might occur (Tamura et al., 2013). In wild-type plants, elongated nuclei are observed in leaf epidermal cells as well as in mature root cells, and it is likely that this elongation occurs as a mechanical consequence of an increase in cell volume. As seen in kakul1 mutants, removing the linkage between the nuclear membrane and cytoskeleton prevents this change in nuclear shape/size and results in more circular nuclei (Tamura and Hara-Nishimura, 2011; Tamura et al., 2013). Therefore, Fig. 3 suggests that the structural NUP107–160 complex of the NPC directly or indirectly interacts with cytoplasmic or nucleoplasmic proteins that help maintain nuclear morphology. During their proteome analysis of the plant NPC, Tamura and colleagues (Tamura et al., 2010) failed to identify structural proteins of the cytoplasm or nucleoplasm. However, they did not perform immunoprecipitations using NUP160 that was shown in Fig. 3 to have altered morphology, so the possibility remains that these members of the NPC interact with proteins in the direct nuclear periphery.

In experiments to analyse the gene expression changes that result from altered NPC function, surprisingly few genes showed 2-fold changes in either nup160 or nup62 mutants (Fig. 6; Supplementary Fig. S7 at JXB online). However it is intriguing to discover that genes involved in nuclear transport were up-regulated in both mutants. Performing this type of experiment in other multicellular organisms is difficult due to the general phenotypic severity of nup mutants. As a result, there is no published record of this possible feedback between NUP function and expression of genes involved in nuclear transport. Ongoing research at both a global level to investigate nuclear mRNA transport and more precisely looking at individual transcripts will further decipher this relationship, but as it stands these results might indicate that reducing NPC function in nup mutants causes a concomitant up-regulation of some genes that may increase the rate of nuclear transport, in order to maintain the status quo and ultimately the viability of the cell. However, in light of these findings, it is difficult to explain why, if there is a compensatory mechanism, certain mutants show strong mutant phenotypes. This probably highlights the complexity of the system where the NPC lies at the nexus of mRNA export and protein import. Any perturbation in the system will undoubtedly lead to numerous cellular changes that amount to pleiotropic phenotypes. However, this again raises the questions as to why some NUPs are more essential than others, and can only be answered with a more targeted study of individual subcomplexes. Although the overall significance of this result remains to be seen, it could have major ramifications regarding our understanding of how the components of the NPC are regulated.

This study highlights the NPC as a central player in the biology of the plant nucleus. Over the coming decade, it is hoped that many of these findings will be extended to gain an understanding as to how the NPC specifically influences plant growth and how this compares with mechanisms of control in other eukaryotes.

Supplementary data
Supplementary data are available at JXB online.
Figure S1. T-DNA insertions and NUP expression.
Figure S2. Growth of nup mutants.
Figure S3. Phenotypes of nup62 mutant plants.
Figure S4. Phenotypes of nup85 mutants.
Figure S5. mRNA accumulation in nup mutants.
Figure S6. Phenotypes of nup98 mutants.
Figure S7. Gene expression change in nup mutants.
Figure S8. RAN1 expression in selected nup mutants.
Table S1. Details of Arabidopsis nucleoporin mutants.
Table S2. Primer sequences used in mutant characterization or expression analysis.

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References
Alber F, Dokudovskaya S, Veenhoff LM, et al. 2007a. Determining the architectures of macromolecular assemblies. Nature 450, 683–694.
Alber F, Dokudovskaya S, Veenhoff LM, et al. 2007b. The molecular architecture of the nuclear pore complex. Nature 450, 695–701.
Alonso JM, Stepanova AN, Leisse TJ, et al. 2003. Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science 301, 633–637.
Arabidopsis Genome Consortium. 2000. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 408, 736–815.
Bai SW, Rouquette J, Umeda M, Faigle W, Loew D, Sazer S, Doye V. 2004. The fission yeast Nup107–120 complex functionally interacts with the small GTPase Ran/Spr1 and is required for mRNA export, nuclear pore distribution, and proper cell division. Molecular and Cellular Biology 24, 6379–6392.
Bianvillain R, Boavida LC, McCormick S, Ow DW. 2008. Exportin1 genes are essential for development and function of the gametophytes in Arabidopsis thaliana. Genetics 180, 1493–1500.
Braud C, Zheng W, Xiao W. 2012. LONO1 encoding a nucleoporin is required for embryogenesis and seed viability in arabidopsis. Plant Physiology 160, 823–836.
Capelson M, Liang Y, Schulte R, Mair W, Wagner U, Hetzer MW. 2010. Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. Cell 140, 372–383.
Cheng YT, Germain H, Wiermer M, et al. 2009. Nuclear pore complex component MOS7/Nup88 is required for innate immunity and nuclear accumulation of defense regulators in Arabidopsis. The Plant Cell 21, 2503–2516.
Cronshaw JM, Krutchinsky AN, Zhang W, Chait BT, Matunis MJ. 2002. Proteomic analysis of the mammalian nuclear pore complex. Journal of Cell Biology 158, 915–927.
D’Angelo MA, Gomez-Cavazos JS, Mei A, Lackner DH, Hetzer MW. 2012. A change in nuclear pore complex composition regulates cell differentiation. Developmental Cell 22, 446–458.
Degrasse JA, Devos D. 2010. A functional proteomic study of the Trypanosoma brucei nuclear pore complex: an informatic strategy. Methods in Molecular Biology 673, 231–238.
DeGrasse JA, DuBois KN, Devos D, Siegel TN, Sali A, Field MC, Rout MP, Chait BT. 2009. Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor. Molecular and Cellular Proteomics 8, 2119–2130.
Dong CH, Agarwal M, Zhang Y, Xie Q, Zhu JK. 2006a. The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1. Proceedings of the National Academy of Sciences, USA 103, 8281–8286.
Dong CH, Hu X, Tang W, Zheng X, Kim YS, Lee BH, Zhu JK. 2006b. A putative Arabidopsis nucleoporin, ATNUP160, is critical for RNA export and required for plant tolerance to cold stress. Molecular and Cellular Biology 26, 9533–9543.
Fernandez-Martinez J, Phillips J, et al. 2012. Structure-function mapping of a heptameric module in the nuclear pore complex. Journal of Cell Biology 196, 419–434.
Fernandez-Ayela A, Alonso-Peral MM, Sanchez-Garcia AB, Micol P, Perez-Perez JM, Micol JL, Ponce MR. 2013. Arabidopsis TRANSCURVATA1 encodes NUP58, a component of the nucleopore central channel. PLoS One 8, e67661.
Fiseroja J, Kiseleva E, Goldberg MW. 2009. Nuclear envelope and nuclear pore complex structure and organization in tobacco BY-2 cells. The Plant Journal 59, 243–255.
Galy V, Mattaj IW, Askjaer P. 2003. Caenorhabditis elegans nucleoporins Nup93 and Nup205 determine the limit of nuclear pore complex size exclusion in vivo. Molecular Biology of the Cell 14, 5104–5115.
Groth M, Takeda N, Perry J, et al. 2010. NENA, a Lotus japonicus homolog of Sec13, is required for rhizodermal infection by arbuscular mycorrhiza fungi and rhizobia but dispensable for cortical endosymbiotic development. The Plant Cell 22, 2509–2526.
Haizel T, Merkle T, Pay A, Fejes E, Nagy F. 1997. Characterization of proteins that interact with the GTP-bound form of the regulatory GTPase Ran in Arabidopsis. The Plant Journal 11, 93–103.
Hulsman BB, Labokha AA, Gorlich D. 2012. The permeability of reconstructed nuclear pores provides direct evidence for the selective phase model. Cell 150, 739–751.
Jacob Y, Mongkolsirivatana C, Veley KM, Kim SY, Michaels SD. 2007. The nuclear pore protein ATPR is required for RNA homeostasis, flowering time, and auxin signaling. Plant Physiology 144, 1383–1390.
Jung HJ, Lee HJ, Park MJ, Park CM. 2014. Beyond ubiquitination: proteolytic and nonproteolytic roles of HOS1. Trends in Plant Science 19, 538–545.
Jung HJ, Park JH, Lee S, To TK, Kim JM, Seki M, Park CM. 2013. The cold signaling attenuator HIGH EXPRESSION OF OSOMATICALLY RESPONSIVE GENE1 activates FLOWERING LOCUS C transcription via chromatin remodeling under short-term cold stress in Arabidopsis. The Plant Cell 25, 4378–4390.
Kalverda B, Pickersgill H, Shloma VV, Fornerod M. 2010. Nucleoporins directly stimulate expression of developmental and cell-cycle genes inside the nucleoplasm. Cell 140, 360–371.
Kanamori N, Madsen LH, Raditoua S, et al. 2006. A nucleoporin is required for induction of Ca2+ spiking in legume nodule development and essential for rhizobial and fungal symbiosis. Proceedings of the National Academy of Sciences, USA 103, 359–364.
Kroll S, Dorries J, Boysen B, Reidenbach S, Magnus L, Norder H, Thybø J, Gordens V. 2010. Protein Tpr is required for establishing nuclear pore-associated zones of heterochromatin exclusion. EMBO Journal 29, 1659–1673.
Lazarov A, Valverde F, Pineiro M, Jarillo JA. 2012. The Arabidopsis E3 ubiquitin ligase HOS1 negatively regulates CONSTANS abundance in the photoperiodic control of flowering. The Plant Cell 24, 982–999.
Lee JY, Lee HS, Wi SJ, Park KY, Schmit AC, Pai HS. 2009. Dual functions of Nicotiana benthamiana Rae1 in interphase and mitosis. The Plant Journal 59, 278–291.
Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LA, Perata P, van Dongen JT. 2011. Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. Nature 479, 419–422.
Lu Q, Tang X, Tian G, et al. 2010. Arabidopsis homolog of the yeast TREX-2 mRNA export complex: components and anchoring nucleoporin. The Plant Journal 61, 259–270.
Lutze Mann M, Kunze R, Buerer A, Aub E, Hurt E. 2002. Modular self-assembly of a Y-shaped multiprotein complex from seven nucleoporins. EMBO Journal 21, 387–397.
MacGregor DR, Gould P, Foreman J, et al. 2013. HIGH EXPRESSION OF OSOMATICALLY RESPONSIVE GENE1 is required for circadian periodicity through the promotion of nuclear-cytoplasmic mRNA export in Arabidopsis. The Plant Cell 25, 4391–4404.
Meinke D, Muralla R, Sweeney C, Dickerman A. 2008. Identifying essential genes in Arabidopsis thaliana. Trends in Plant Science 13, 483–491.
Neumann N, Jeffares DC, Poole AM. 2006. Outsourcing the nucleus: nuclear pore complex genes are no longer encoded in nucleomorph genomes. Evolutionary Bioinformatics Online 2, 23–34.
Neumann N, Lundin D, Poole AM. 2010. Comparative genomic evidence for a complete nuclear pore complex in the last eukaryotic common ancestor. PLoS One 5, e13241.

Parry G. 2013. Assessing the function of the plant nuclear pore complex and the search for specificity. Journal of Experimental Botany 64, 833–845.

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

Raices M, D’Angelo MA. 2012. Nuclear pore complex composition: a new regulator of tissue-specific and developmental functions. Nature Reviews Molecular Cell Biology 13, 687–699.

Robles LM, Deslauriers SD, Alvarez AA, Larsen PB. 2012. A loss-of-function mutation in the nucleoporin ATNUP160 indicates that normal auxin signalling is required for a proper ethylene response in Arabidopsis. Journal of Experimental Botany 63, 2231–2241.

Rodenas E, González-Aguilera C, Ayuso C, Askjaer P. 2012. Dissection of the NUP107 nuclear pore subcomplex reveals a novel interaction with spindle assembly checkpoint protein MAD1 in Caenorhabditis elegans. Molecular Biology of the Cell 23, 930–944.

Saito K, Yoshikawa M, Yano K, et al. 2007. NUCLEOPORIN85 is required for calcium spiking, fungal and bacterial symbioses, and seed production in Lotus japonicus. The Plant Cell 19, 610–624.

Schmittgen TD, Livak KJ. 2008. Analyzing real-time PCR data by the comparative C(T) method. Nature Protocols 3, 1101–1108.

Schoch RL, Kapinos LE, Lim RY. 2012. Nuclear transport receptor binding avidity triggers a self-healing collapse transition in FG-nucleoporin molecular brushes. Proceedings of the National Academy of Sciences, USA 109, 16911–16916.

Solmaz SR, Chauhan R, Blobel G, Melcak I. 2011. Molecular architecture of the transport channel of the nuclear pore complex. Cell 147, 590–602.

Tamura K, Fukao Y, Iwamoto M, Haraguchi T, Hara-Nishimura I. 2010. Identification and characterization of nuclear pore complex components in Arabidopsis thaliana. The 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.

Tamura K, Iwabuchi K, Fukao Y, Kondo M, Okamoto K, Ueda H, Nishimura M, Hara-Nishimura I. 2013. Myosin X-I links the nuclear membrane to the cytoskeleton to control nuclear movement and shape in Arabidopsis. Current Biology 23, 1776–1781.

Van de Vosse DW, Wan Y, Lapetina DL, Chen WM, Chiang JH, Aitchison JD, Wozniak RW. 2013. A role for the nucleoporin nup170p in chromatin structure and gene silencing. Cell 152, 969–983.

Walde S, Kehlenbach RH. 2010. The part and the whole: functions of nucleoporins in nucleocytoplasmic transport. Trends in Cell Biology 20, 461–469.

Walther TC, Alves A, Pickersgill H, et al. 2003. The conserved Nup107–160 complex is critical for nuclear pore complex assembly. Cell 113, 195–206.

Wiermer M, Cheng YT, Imkampe J, Li M, Wang D, Lipka V, Li X. 2012. Putative members of the Arabidopsis Nup107–160 nuclear pore subcomplex contribute to pathogen defense. The Plant Journal 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.

Xu S, Powers MA. 2009. Nuclear pore proteins and cancer. Seminars in Cell and Developmental Biology 20, 620–630.

Xu XM, Rose A, Meier I. 2007a. NUA activities at the plant nuclear pore. Plant Signaling and Behavior 2, 553–555.

Xu XM, Rose A, Muthuswamy S, Jeong SY, Venkatakrishnan S, Zhao Q, Meier I. 2007b. 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. The Plant Cell 19, 1537–1548.

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. The Plant Cell 17, 1306–1316.

Zhao Q, Meier I. 2011. Identification and characterization of the Arabidopsis FG-repeat nucleoporin Nup62. Plant Signaling and Behavior 6, 330–334.

Zhou X, Graumann K, Evans DE, Meier I. 2012. Novel plant SUN-KASH bridges are involved in RanGAP anchoring and nuclear shape determination. Journal of Cell Biology 196, 203–211.