The plant nuclear envelope and regulation of gene expression

Geraint Parry*

University of Liverpool, Institute of Integrative Biology, Crown Street, University of Liverpool, Liverpool L69 7ZB, UK

* To whom correspondence should be addressed. E-mail: geraint.parry@liverpool.ac.uk

Received 3 November 2014; Revised 30 December 2014; Accepted 8 January 2015

Abstract

The nuclear envelope (NE) separates the key mechanisms of transcription and translation, and as such is a critical control point in all eukaryotic cells. In plants, the proteins of the NE influence a number of processes including the control of nucleo-cytoplasmic transport of RNA and protein, chromatin localization to the nuclear periphery, and direct chromatin binding by members of the nuclear pore complex (NPC). In this review I attempt to bring these roles under the umbrella of their effect on gene expression, even though the complex nature of this cellular environment means there is considerable overlap of effects. Although the volume of research in plant cells has greatly improved over recent years, it is clear that our understanding of how the components of the NE either directly or indirectly influence gene expression is still in its infancy.

Key words: Gene expression, nuclear envelope, nuclear pore, nuclear transport, nucleoporin, nucleus.

Introduction

Eukaryotic cells are defined by the presence of membrane-bound organelles, and none of these is more important for cell function than the nucleus. It is hoped that every high school biology student understands that the central dogma of molecular biology describes that transcription occurs within the nucleus, mRNA is transported to the cytoplasm where polypeptides are produced, and the resulting proteins remain in the cytoplasm or move to an appropriate organelle, which includes the nucleus. However, often overlooked are the processes that control how both RNA and proteins move between the nucleus and cytoplasm, which is clearly a critical point of regulation (Fig. 1).

The double-membrane nuclear envelope (NE) is impermeable to macromolecules so in simplest terms the nuclear pore complex (NPC) is the conduit through which all nucleo-cytoplasmic transport occurs. This movement is facilitated by the activity of karyopherin proteins that rely on a gradient of the Ran-GTP protein across the NE. The NPC is a massive protein grouping comprised of upwards of 200 individual nucleoporins (NUPs) arranged in distinct subcomplexes that play unique and overlapping cellular roles (Alber et al., 2007a, b). In most cases these are single-copy proteins whose function is usually essential for correct growth. Bapste et al. (2005) described the NPC as containing ‘highly adapting proteins in an ancient frozen structure’ whereby the general architecture is conserved throughout all eukaryotes but the individual subunits have undergone significant sequence change, even though in many cases equivalent NUPs maintain a common function. Arguably it is when the function of these proteins diverges that study of the role of the NPC becomes more interesting. In some cases, genetic studies of equivalent NUPs in different organisms have revealed distinct functions. This is perhaps demonstrated most clearly when expression of the NUP160 gene is altered. Knocking down expression of the npp-6/nup160 gene by RNA interference (RNAi) in Caenorhabditis elegans causes almost 100% embryo lethality (Galy et al., 2003), whereas although Arabidopsis nup160 mutants do exhibit a growth phenotype these plants are able to survive (Cernac et al., 1997; Dong et al., 2006; Parry et al., 2006). This highlights that the interaction of the nuclear...
transport system with its cellular environment will differ in an organism-specific manner and that valuable information can be gained by investigating a wide breadth of eukaryotes to understand how this system is regulated.

The NPC contains a number of distinct subcomplexes that are made up of a varying number of individual NUP proteins (Alber et al., 2007a, b). These subcomplexes play discrete roles during nucleocytoplasmic transport. At the periphery of the NPC lies the nuclear basket that plays a number of roles connected to the movement of cargoes out of the nucleus, including heterochromatin exclusion and anchoring of active genes (see below). Cytoplasmic filaments play an analogous role on the other face of the NPC where they facilitate the hydrolysis of RanGTP and appear to exclude ribosomes from the space immediately surrounding the nuclear pore (Fiserova et al., 2014). The central subcomplexes are concerned either with the structural stability of the NPC (the NUP107–160 complex) or with transport through the pore (the NUP62 subcomplex). However, even during nuclear transport, certain NUPs have promiscuous roles where they may interact with multiple NPC subcomplexes as well as being necessary for interaction with karyopherin cargos. In the past 2 years, matched reviews that outline the structure and function of the plant NPC have been published and contain a level of detail that is outside the focus of this review (Parry, 2013; Tamura and Hara-Nishimura, 2013).

Although nucleo-cytoplasmic transport occurs via the NPC, the complex does not reside as a lone protein island within the NE. Over recent years the identity and function of a range of proteins that lie in the inner (INM) or outer nuclear membrane (ONM) have been defined. These include the INM Sad1p, UNC-84 (SUN) proteins and the membrane-spanning Klarsicht, ANC-1, Syne Homology (KASH) domain proteins (Evans et al., 2014). SUN proteins are well conserved throughout eukaryotes, while outside of the definitive KASH domain these proteins show little similarity between different organisms. Using innovative database searches and yeast two-hybrid (Y2H) screening, a number of novel plant KASH domain proteins have recently been identified and shown to reside within the NE (Graumann et al., 2014; Zhou et al., 2014). In addition, a series of plant-specific WPP domain proteins play an analogous role to metazoan KASH proteins including the critical function of anchoring the RanGAP GTPase to the ONM (Xu et al., 2007a; Zhao et al., 2008; Zhou et al., 2012). In general, the function of INM- and ONM-localized proteins is to provide a link between the NE and the cytoplasm and nucleoplasm. The protein complex that is formed by the linkages between KASH and SUN proteins is termed the ‘Linker of cytoskeleton and nucleoskeleton’ (LINC) complex. In vertebrate cells these linkages are well established, but it is encouraging that the plant-specific proteins that provide these linkages have recently also been identified (Evans et al., 2014). Using fluorescence resonance energy transfer (FRET), Arabidopsis SUN1 and SUN2 were shown to interact with the CROWDED NUCLEUS1 (CRWN1) protein that lies at the periphery of the nucleoplasm, providing shape and size to the nucleus (Graumann, 2014). On the other hand, SUN proteins also interact with the WPP-INTERACTING PROTEIN (WIP1) protein that lies on the ONM (Zhou et al., 2012). WIP1 is closely related to WIT proteins, and they share ONM localization (Zhao et al., 2008). The WIT1 protein has been shown to link the NE to actin filaments via, at the very least, the newly defined, plant-specific KAKU1 myosin-like protein (Tamura et al., 2013). These WIP and WIT proteins comprise the plant-specific WPP family of proteins and are responsible for anchoring the RanGAP GTPase to the ONM (Zhou et al., 2012). This localization of RanGAP only occurs in plants and plays an analogous role to the NUP358 protein localized in the cytoplasmic filaments of the metazoan NPC (Hutten et al., 2008). In the past year, a number of review articles have discussed the role of plant NE proteins and can be found in a special edition of Frontiers in Plant Biology (http://journal.frontiersin.org/ResearchTopic/1848#articles, accessed 29 January 2015). Interestingly there has been no evidence from plants describing a direct link between members of the NPC and proteins that lie in the NE. However certain nucleoporin (nup) mutants share defects in nuclear...
morphology that are observed in sun and kaku mutant cells (Tamura et al., 2010; Zhou et al., 2012; Parry, 2014).

Over the past 5 years, the research community made up of the ‘International Plant Nuclear Consortium’ has made great strides in understanding the contribution of NPC and NE proteins to many cellular processes including gene expression (Graumann et al., 2013). By providing a physical barrier while remaining a critical link between transcription and translation, proteins residing in the NE can have profound effects on gene expression. In this review, I will attempt to describe how the plant NE influences gene expression and will separate these effects under three broad and often overlapping headings that mirror movement through the central dogma (Fig. 1): (i) Direct control of gene expression: How is gene expression altered in nuclear transport mutants and do NE-associated proteins directly interact with chromatin? (ii) Influencing chromatin positioning: How do nuclear envelope proteins alter positioning of intranucleus domains? (iii) Control of nuclear transport: How do alterations in mRNA export and protein import affect gene expression?

**Direct control of gene expression**

Arguably the simplest way to assess how an individual protein influences gene expression is to identify an organism that has a mutation in your gene of interest. One of the great benefits of working with plants, and in particular Arabidopsis thaliana, is the ability to identify plants with stable T-DNA insertions, that will often alter the function of a particular gene.

Clearly this attribute of Arabidopsis genetics has been utilized many times and, recently, many NUP mutants have been identified (Parry, 2013, 2014). The growth phenotype of Arabidopsis mutants with a defect within a member of the NPC takes one of three forms: in the first instance, the plant is unable to survive the loss of this protein and exhibits embryo lethality. Secondly, the general growth of the plant is unaffected and it retains a wild-type phenotype [although in some cases it exhibits a specific phenotype to an inductive signal (Wiermer et al., 2012)]. Lastly the nup mutant exhibits a phenotype that is conserved in a number of examples that includes shorter roots, premature phase change resulting in early flowering, decreased stature, and a loss of fertility (Parry, 2013, 2014). This is clearly a general phenotype so a major aim in this research area is to elucidate the changes in gene expression that occur in this situation.

**Gene expression changes in nup mutants**

In order to attempt to answer this question, an analysis of global gene expression has been conducted in Arabidopsis TRANSLOCATED PROMOTER REGION (attp1), NUCLEOPORIN160 (nup160), nup62 (by microarray), and HIGH EXPRESSION OF OSOTOMICALLY RESPONSIVE GENES1 (hos1) [by RNA sequencing (RNA-seq)] mutant plants (Jacob et al., 2007; MacGregor et al., 2013; Parry, 2014). Each of these nup mutants shares the early flowering phenotype described above, even though, by analogy with vertebrate or yeast NUPs, they are predicted to lie in different NPC subcomplexes, namely the nuclear basket (TPR), the central channel (NUP62), or the structural NUP107–160 subcomplex (NUP160, HOS1) (Tamura and Hara-Nishimura, 2013).

These experiments were each conducted using whole seedlings but at slightly different times during plant development. This makes direct comparisons difficult even though many patterns are clear. In order to understand this situation more fully, it would be necessary to compare levels of gene expression in different tissues, but currently that information is not available.

Overall the amount of gene expression change in each of these mutants is relatively small, with ~8% of genes undergoing 2-fold changes in ttp1 and hos1 mutants (Jacob et al., 2007; MacGregor et al., 2013) whereas <1% of genes had this level of change in nup160 or nup62 (Parry, 2014). In part, the differences between these outcomes can be explained by the timing of the experiments: in ttp1 plants, expression was compared between post-flowering mutants and pre-flowering wild-type plants, which would account for a significant amount of gene expression change (Jacob et al., 2007). Hos1 samples were taken at 14 days post-germination (dpg), at which time gene expression changes leading to floral transition will be advanced in the mutant plants when compared with the wild type (MacGregor et al., 2013). Gene expression change in nup160 and nup62 was assessed at 7 dpg, at which point the mutant seedlings were smaller than the wild type, but should not have begun to undergo the gene expression changes that lead to floral transition (Parry, 2014).

Another factor that explains the greater gene expression change in hos1 mutants is the pleiotropic function of this protein (Jung et al., 2014). The vertebrate homologue of HOS1 is called EMBRYONIC LARGE MOLECULE DERIVED FROM YOLK SAC (ELYS) and was identified as associating with the NUP107–160 subcomplex during mitosis (Rasala et al., 2006). Subsequently HOS1 was identified in immunoprecipitations with the Arabidopsis nucleoporin, RNA-EXPORT FACTOR1 (RAE1), indicating that it also associates with the NPC in plants (Tamura et al., 2010). However, over the past decade, a range of experiments from different labs have demonstrated that HOS1 directly binds to DNA (Jung et al., 2013), acts as an E3 ubiquitin ligase, a role that influences the response to cold treatment and the control of flowering time (Lazarov et al., 2012; Lee et al., 2012), as well as influencing bulk mRNA export (MacGregor et al., 2013) (see later). Hos1 mutant plants also have a defect in the timing of the circadian clock, and the gene expression changes in these mutants reflect this phenotype, as expression of many of the core clock-regulated genes is significantly altered (MacGregor et al., 2013). Interestingly the authors also show that nup160 mutants exhibit a circadian phenotype, yet the expression of the same core-regulated genes are not significantly altered in 7 dpg nup160 plants, which might reflect the differences in plant growth at the time these experiments were conducted (MacGregor et al., 2013; Parry, 2014).

One intriguing result obtained from nup62 and nup160 mutants was that amongst the list of significantly up-regulated
genes were a number whose protein products are involved in nuclear transport, including RAN1, EXPORTIN1 (XPO1), and NUP98b (Parry, 2014). Interestingly, some of these genes are also significantly up-regulated in *hos1* plants, including NUP98b and a previously uncharacterized RNA-binding protein that may be involved in nuclear mRNA export (MacGregor et al., 2013). The functional significance of these expression changes is not yet clear, but might suggest that in certain *nup* mutants, the cell is ‘sensing’ the defect in nuclear transport and attempting to compensate for this defect by increasing the expression of other genes involved in the process. This is probably a simplistic explanation, and a full understanding of this process requires further study of the relevant protein products. Even if this compensatory mechanism is occurring in these mutant plants, it appears to have limited efficacy given the mutant phenotypes in *nup160*, *nup62*, and *hos1* plants. Interestingly the *RAN1* gene is also up-regulated in 7-day-old *nup85* and *sehl* seedlings, both of which have a wild-type growth phenotype (Parry, 2014). It is tantalizing to speculate that gene expression change in these mutants is sufficient to retain ‘wild-type growth’. However, this will require a more global assessment of expression change in order to draw definitive conclusions. Regardless, this type of potential compensatory gene expression change has not yet been documented in any other multicellular eukaryote. As many *C. elegans nuplpp* mutants are viable, it would be intriguing to discover whether these mutants have similar alterations in gene expression to compensate for a partial loss of nuclear pore activity (Galj et al., 2003).

A limitation of these gene expression studies is that they do not provide information as to whether the observed changes are direct or indirect. Green fluorescent protein (GFP)-tagged NUP160, NUP62, and TPR1/NUA all localize to the nuclear rim (Dong et al., 2006; Xu et al., 2007b; Tamura et al., 2010), but it is possible that more dynamic pools of these proteins also interact with chromatin within the interphase nucleoplasm, as is the case with HOS1 (Lee et al., 2001; Lazaro et al., 2012; Jung et al., 2013). This sort of detailed information is not yet available to the plant cell biologist, so it remains to be seen if plant NUPs can directly interact with chromatin or whether their effects are mediated due to secondary changes resulting from a general defect in nuclear transport.

**Direct interactions with chromatin**

When attempting to define how an individual protein affects gene expression, a researcher can assess the chromatin to which that protein is binding or investigate the cascade of expression change that occurs when the activity of that protein is altered. Historically the primary function of the NPC was thought to be in the control of nucleo-cytoplasmic transport. However, more recently, evidence has emerged that individual NUPs play roles away from the NPC. Experiments in *Drosophila* using both ChIP-chip and DamID-chip demonstrated that certain NUPs bind directly to chromatin (Capelson et al., 2010; Kalverda et al., 2010). In general, these NUPs associate with active histone marks such as H3K4me2 and H4K16Ac, indicating that NUPs associate more readily with actively transcribed loci (Kalverda et al., 2010). Somewhat surprisingly, NUP50, NUP62, and NUP98 all associate with active genes, even though these NUPs are predicted to lie at different positions within the NPC (Tamura and Hara-Nishimura, 2013). NUP50 is localized to the nuclear basket, NUP98 is a mobile protein found on both sides of the nuclear envelope, whereas NUP62 is an FG-repeat protein thought to be embedded within the central pore of the NPC.

However, another set of experiments performed in yeast show an opposing role for the core nucleoporin NUP170. This NUP interacts with heterochromatin and is required for silencing of subtelomeric genes (Van de Vosse et al., 2013). This information sheds new light on ideas about what the ‘function’ of a particular NPC subcomplex might be when its constituent NUPs are not associated as part of the entire complex.

As a supplement to the NUP–chromatin interaction experiments described above (Kalverda et al., 2010), the authors also created RNAi and overexpression constructs of NUP98 and showed that in these cell lines, genes with altered expression match those that directly interacted with NUP98. These experiments used cultured *Drosophila* cell lines, highlighting one limitation in the study of gene expression in metazoan experimental systems: the difficulty in creating organisms having stable mutations in NUPs.

As briefly mentioned above, the *Arabidopsis* HOS1 protein has been shown to interact with chromatin. More specifically, HOS1 interacts with FLOWERING LOCUS C (FLC) chromatin, a linkage that occurs following short-term cold stress (Jung et al., 2013). HOS1 also interacts with the FVE1 protein, which is another regulator of FLC expression. It is unclear whether the interaction of HOS1 with FLC chromatin is a direct interaction or whether it requires the prior interaction between HOS1 and FVE1. Currently this is the only line of evidence linking a plant NUP to chromatin, but, given what is known about the general similarity of the NPC throughout eukaryotes, it is likely that other interactions occur in plants.

Over the past few years, great strides have been made in the identification of proteins that reside within the NE, most notably the INM SUN proteins and the ONM WPP or KASH domain proteins (Evans et al., 2014). Genetic studies in *Arabidopsis* have shown that there is functional redundancy in these gene families. There are five *Arabidopsis* SUN proteins that are divided into ‘classic’ or ‘mid’ SUN clades dependent on the position of the SUN domain in the polypeptide’s secondary structure (Murphy et al., 2010; Tatout et al., 2014). Plants with single or double mutants in the mid-SUN proteins have no developmental phenotype yet triple *sun3sun4sun5* plants cannot be isolated (Graumann et al., 2014). Similarly, null *sun1sun2* plants are not viable but *sun1* mutants with reduced SUN2 expression have a wild-type growth phenotype, indicating that a small but functionally important amount of the ‘classic’ SUN proteins is necessary for survival (Oda and Fukuda, 2011; Zhou et al., 2012).

As mentioned above, the WPP protein family anchor the RanGAP protein to the NE (Zhou et al., 2012). Given the importance of RanGAP (see later), it is surprising that in
a wifi (wip1wip2wip3wit1wit2) quintuple mutant the only reported developmental defect occurs in male fertility as a result of defective nuclear migration (Zhou and Meier, 2014). However, in an earlier study, Arabidopsis RNAi lines with reduced expression of the WPP gene family were shown to exhibit reduced root growth (Patel et al., 2004).

There are currently no reported experiments about the gene expression change that occurs in plants deficient in any INM- or ONM-localized proteins. It will be interesting to ascertain how any change in these plants compare with that observed in the nup mutants. Discussed below are the roles that these NE-localized proteins play in defining chromosome domains, indicating that the effect these proteins have on gene expression is likely to be a secondary consequence due to changes in chromatin structure.

Influencing nuclear chromatin positioning

The interphase nucleoplasm is far from a static environment. The nucleus contains a variety of non-membranous aggregates that include Cajal bodies and the nucleolus. In addition to these ‘nuclear bodies’, each chromosome occupies a distinct ‘territory’ (CT) within the nucleus. Within these CTS, the arrangement of chromatin broadly reflects its transcriptional state. Our understanding of these CTS has largely relied upon fluorescent in situ hybridization (FISH). Recent advances in imaging technology have allowed a greater appreciation of these historically mysterious intranuclear domains (Guo and Fang, 2014). Similarly, researchers now appreciate that the nuclear periphery is also a dynamic, changing region, and that proteins associated with the NE play a critical role in determining the localization of chromatin to these regions and its resulting transcriptional state. Tightly packed heterochromatin has long been known to localize preferentially to the nuclear periphery, but the conventional wisdom that this is a transcriptionally quiet region has been challenged by evidence showing that chromatin close to the NPC is more active. This so-called ‘gene-gating’ hypothesis (Blobel, 1985) proposes that highly expressed genes move toward the NPC in order to facilitate rapid translocation of mRNA through the pore. Although the idea of ‘gene-gating’ still holds true, more recent experimentation has refined this theory, with evidence suggesting that not all eukaryotes follow the example first proposed from studies in yeast cells.

‘Gene-gating’ at the nuclear periphery

In yeast, a variety of experiments support the idea of ‘gene-gating’ and show that induced genes move to the vicinity of the NPC. The GAL genes are induced with a requirement for galactose metabolism, and the upstream activation sequence (UAS) of these genes interacts with the NPC (Schmid et al., 2006). More recently this mechanism was shown to require SUMOylation of chromatin-associated factors. In cells lacking the function of the SUMO protease UBL-SPECIFIC PROTEASE (Ulp1), these induced genes were not tethered to the NPC (Texari et al., 2013). Furthermore, a large protein complex responsible for mRNA export, termed the TREX2 complex (TRANSCRIPTION ELONGATION AND RNA EXPORT), is also necessary for induction of GAL genes and importantly interacts with the NPC via the NUP1 protein (Cabal et al., 2006).

However, evidence from Drosophila cells suggests that ‘gene-gating’ may not be a universal phenomenon. In studies highlighted above showing that full-length NUP98 directly associated with actively transcribed chromatin, the authors also created constructs whereby NUP98 was either localized free in the nucleoplasm or tethered to a protein that resides within the NPC (Kalverda et al., 2010). Interestingly these experiments showed that NPC-tethered NUP98 associated with genes that did not contain active histone marks, suggesting that movement of genes to the NPC causes transcriptional repression (Kalverda et al., 2010). However, drawing strong conclusions from either of these data sets probably does not capture the entire situation where the movement of active genes to the vicinity of the NPC is dependent on the level of transcription, timing of induction, or the regulatory sequences associated with each gene.

In Arabidopsis, the orthologues of the TREX2 complex were characterized following initial identification of athlp1 mutant plants in an unrelated screen for ectopic β-glucuronidase (GUS) expression driven by the promoter of the 7S storage gene (Lu et al., 2010). Subsequent Y2H and reverse genetic approaches identified other members of the TREX2 complex and showed by bimolecular fluorescence complementation (BiFC) that the Athlp1 protein interacts with the nuclearoporin NUP1 in vivo (Lu et al., 2010). In a different study, NUP1 was also identified as NUP136 and, whereas null mutant alleles of nup1 are lethal, knockdown or truncated mutant alleles of this NUP1/NUP136 are able to survive, showing the ‘classic-nup’ phenotype of early flowering and reduced stature (Lu et al., 2010; Tamura et al., 2010). While Athlp1::YFP (yellow fluorescent protein) is found throughout the nucleoplasm, NUP1::YFP localizes to the nuclear periphery; therefore, in analogy with other eukaryotes, NUP1 is predicted to tether the TREX2 complex to the NPC. Arabidopsis plants deficient in members of the TREX2 complex exhibit mild growth phenotypes; thus, reducing the function of the complex appears not to be essential for plant growth. As stated above, the athlp1 mutant was identified due to a change in gene expression of a promoter-GUS transgene, but this was a tissue-level alteration and therefore does not provide any information regarding how the TREX2 complex might influence gene expression within the nucleus (Lu et al., 2010). Given its localization within the nucleus, a defective TREX2 complex might lead to defects in mRNA export, which is discussed in more detail later in this review. However, the TREX2 complex is also linked to the nuclear protein degradation apparatus via its interaction with the UBIQUITIN C-TERMINAL HYDROLASE enzymes UCH1 and UCH2, as well as indirectly with the 26S proteasome (Lu et al., 2010; Tian et al., 2012). This considerably widens the potential influence of the TREX2 complex and as such heightens the need for more specific evidence to link the complex to the transcriptional machinery in the vicinity of the NPC.
Plant cells use SUMOylation to affect protein function in a manner analogous to other eukaryotes (Park et al., 2011). The EARLY in SHORT DAYS4 (ESD4) and ESD4-LIKE SUMO PROTEASE (ELS1) are the closest orthologues of the yeast SUMO proteases ULP1 and ULP2 (Hermkes et al., 2011). As the name suggests, the Arabidopsis esd4 mutant was discovered due to its early flowering phenotype, which is accompanied by severely reduced stature in mature plants, a phenotype that is surprisingly not accentuated in esd4els1 double mutants (Reeves et al., 2002; Murtaz et al., 2003; Hermkes et al., 2011). Like ULP1 in yeast, ESD4–GFP localizes to the NE, so it was surprising that the only nucleoplasmically localized ELS1 but not ESD4 was able to complement a yeast ulp1 mutant (Hermkes et al., 2011). This leads to the conclusion that ESD4 and ELS1 have slightly different roles in the SUMO pathway from those of their yeast orthologues. Therefore, the extent to which either of these proteins influences transcription in the vicinity of the NPC is yet to be determined.

As predicted by its mutant phenotype, ESD4 affects the expression of the flowering time regulators FLC and FLOWERING TIME (FT), yet the precise mechanism for this is unknown (Reeves et al., 2002). Similar to observations in other eukaryotes, SUMOylation in plants does appear to be linked to the NPC. The NUCLEAR PORE ANCHOR protein (NUA/TPR1) is the major structural component of the nuclear basket and, as in esd4 mutants, nua1 mutant seedlings show an increase in SUMOylated proteins (Xu et al., 2007b; Muthuswamy and Meier, 2011). Given the localization of these proteins, it might be assumed that NUA would affect the ESD4 binding at the NE. However somewhat surprisingly there was no difference in the expression pattern of GFP–ESD4 in nua1 mutants (Xu et al., 2007b).

The nucleoporin NUP160 resides within the structural NUP107–160 complex so in this context it is somewhat surprising that nup160 mutants also accumulate SUMOylated proteins (Muthuswamy and Meier, 2011). In these mutants, NUA remains localized to the nuclear rim so the increased SUMOylation does not rely on an interaction between these two NUPs. The authors show that environmental and chemical stress leads to an increase in both SUMO conjugates and mRNA accumulation, suggesting that there is a (currently unknown) relationship between these phenomena (Muthuswamy and Meier, 2011). Therefore, this indicates that changes in SUMOylation might affect gene expression via perturbations in mRNA export and not by any change in the preferential localization of highly expressed transcripts. More research is needed to clarify the roles of SUMO as at the present time it is unknown whether changing the amount of SUMOylation is required for moving transcriptionally active genes close to the NPC.

Heterochromatin at the nuclear periphery

Nuclear shape in metazoan cells is determined by the lamina, a filamentous layer of lamin protein that is attached to the NE by the interaction with SUN proteins (Burke and Stewart, 2013). The lamina is proposed to act as a ‘gene-silencing hub’ since lamina-associated domains (LADs) are mostly gene-poor regions (Shevelyov and Nurminsky, 2012). In silico searches reveal that orthologues to lamin proteins cannot be found in plant genomes. However, electron microscopy in tobacco, onion, and carrot cells has demonstrated that a layer of filamentous proteins lies ‘beneath’ the INM and is thought to play an analogous role to the metazoan lamina (Fiserova et al., 2009; Ciska et al., 2013; Kimura et al., 2014). The first characterized member of this ‘plant lamina’ was the NUCLEAR MATRIX CONSTITUENT PROTEIN 1 (NMCP) from carrot cells, which was later shown to have sequence similarity throughout plant genomes, including four Arabidopsis CRWN/LINC proteins (Dittmer et al., 2007). In an excellent recent genetic study, T-DNA mutants were isolated from each of crwn1–crwn4 and, whereas single mutant plants have a wild-type phenotype, plants with higher order mutations exhibit reduced stature and early flowering, with nuclei that are smaller and more circular, especially in triple mutants (Wang et al., 2013). Although there is functional redundancy between these CRWN proteins, the authors show that CRWN1 and CRWN4 have independent effects on nuclear morphology, and their inability to identify a crwn quadruple mutant shows that at least one functional CRWN protein is essential for survival (Wang et al., 2013).

Chromocentres (CCs) are nuclear regions that contain densely packed silenced heterochromatin. Wild-type Arabidopsis nuclei have up to 10 CCs, maximized by the number of diploid chromosomes. In theory, any alteration in CC number and location will affect gene expression in heterochromatic regions, and in crwn mutants there are alterations in both the number and intranuclear organization of CCs (Wang et al., 2013). Dispersal of CCs can occur when the correct repressive histone marks are not added to heterochromatin. One example of this occurs in Arabidopsis atrx5/6 mutants that lack the repressive H3K27me1 mark, causing CC dispersal and activation of heterochromatin gene expression (Jacob et al., 2009). Although crwn mutants also show changes in CC location, it is not known whether the expression of transposable elements or other repetitive DNA contained within CCs is altered (Wang et al., 2013). In atrx5/6 mutants, reactivation of gene expression within heterochromatin regions occurs due to a ‘chemical’ change to the DNA, so it will be interesting to discover whether the more ‘structural’ alteration to CCs that occurs in crwn mutants has the same effect on gene expression.

Although the CRWN/NMCP proteins do not reside within the NE, their close association was confirmed when their nuclear morphology, and their inability to identify a crwn quadruple mutant shows that at least one functional CRWN protein is essential for survival (Wang et al., 2013).
CCs and the CRWN proteins (Wang et al., 2013). Breaking any link in this chain of interactions can alter nuclear morphology, resulting in formation of more circular nuclei (Griffis et al., 2014). However, at the current time, the effect of ‘breaking the chain’ on the reactivation of heterochromatin, the localization of euchromatin, and on changes in global gene expression is unknown in the plant nucleus.

Active transcription at the nuclear periphery in plants

In the past year, evidence has emerged indicating that certain plant genes undergoing active transcription do indeed move to the nuclear periphery (Feng et al., 2014). Using a novel protocol termed padlock-FISH, these authors labelled individual CAB loci and showed that following a light signal, the locus moved from a nucleoplasmic location toward the nuclear periphery (Feng et al., 2014). Dark-grown Arabidopsis seedlings were transferred to red light and within a 3h time period the amount of FISH label that resided within 0.2 μm of the nuclear periphery increased from 20% to 50%. This nuclear movement was stalled in plants that have a defect in photomorphogenesis such as cop1 and det (Feng et al., 2014). This technique is a robust experimental tool so it will be very interesting to discover whether this nucleoplasmic movement of loci occurs under a range of inductive conditions in plants with defects in NE-associated proteins. Furthermore, it is still unclear whether movement of the loci to the nuclear periphery is necessary for its activation or whether this occurs after binding to the transcriptional apparatus. Using the genetic tools that are available in Arabidopsis, teasing apart these different aspects of gene regulation should, in theory, be relatively straightforward (Fig. 2).

Control of nuclear transport

Arguably the most obvious alteration to the cellular dynamics that occurs when the function of NE-associated proteins is altered is during the movement of molecules between the nucleus and cytoplasm. All RNAs and proteins that move between the compartments do so via the NPC. Cargoes smaller than 40 kDa are able to move freely through the NPC, but larger cargoes are actively transported via interaction with karyopherin transport proteins and NUPs that reside within the NPC. One would predict that perturbing nuclear transport would have a profound effect on gene expression. However, the difficulty in investigating these phenomena lies in resolving both: (i) direct from indirect effects; and (ii) how individual proteins specifically affect expression of particular genes.

For the purpose of this review, I will attempt to separate the effects on RNA export from those on protein import, although in vivo these two mechanisms are inextricably linked.

Control of RNA export

In all eukaryotes, perturbing the function of the NPC usually results in the accumulation of polyadenylated mRNA. This general effect is demonstrated by whole-mount in situ localizations using a fluorescently labelled oligo(dT) probe (Gong et al., 2005). Undoubtedly this is a crude method for showing that bulk mRNA is held within the nucleus as it provides no information about the movement of specific transcripts. In metazoan cell culture, researchers now use high-resolution imaging techniques to map the movement of single mRNP cargoes through the NPC, but the difficulties of working with plant cells have meant that these techniques have not yet been adopted in plants (Ma et al., 2013).

Table 1 describes the mutants in which an accumulation of nuclear mRNA has been investigated, mostly in NUP mutants, but also in plants with defects in RNA maturation and transport. Interestingly this shows that not all NUP mutants exhibit nuclear mRNA accumulation. Seedlings lacking normal function of NUP62, NUP54, or NUP58 each exhibit nuclear mRNA accumulation equivalent to the wild type (Parry, 2014). Even though these tested alleles are probably not null mutants, the plants each share growth defects characterized by shorter roots and early flowering (Zhao and Meier, 2011; Ferrandez-Ayela et al., 2013; Parry, 2014). Each of these NUPs contains hydrophobic FG repeats and together they are thought to comprise the central NUP62 subcomplex. In other experimental systems, NUPs with FG repeats interact with transport cargoes to facilitate movement through the

![Fig. 2. Repositioning of loci within the nucleus. (A) The Chlorophyll a/b (CAB) gene is activated by light, and a higher proportion of the loci move from the nucleoplasm to be situated closer to the nuclear envelope (NE) based on (B) It is unknown whether the loci are activated and then move to the nuclear periphery (B1) or whether the activation first requires repositioning to the NE (B2). (C) It is also unknown whether interaction (represented by dashed lines) with the nuclear pore complex (NPC) or other proteins at the inner nuclear membrane, such as SUN proteins, is necessary for activation and/or repositioning.](image-url)
Table 1. Arabidopsis mutants in which the accumulation of mRNA has been investigated

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Proposed function</th>
<th>Mutant allele</th>
<th>Nuclear mRNA accumulation</th>
<th>Mutant phenotype*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS4</td>
<td>RNA helicase</td>
<td>los4-1, los4-2</td>
<td>Yes</td>
<td>Yes</td>
<td>Gong et al. (2005); MacGregor et al. (2013)</td>
</tr>
<tr>
<td>MOS11</td>
<td>RNA binding</td>
<td>mos11-1, mos11-2</td>
<td>Yes</td>
<td>No</td>
<td>Germain et al. (2010)</td>
</tr>
<tr>
<td>THP1</td>
<td>TREX2 complex</td>
<td>Athtp-3</td>
<td>Yes</td>
<td>No</td>
<td>Lu et al. (2010)</td>
</tr>
<tr>
<td>NUP96/MOS3</td>
<td>NUP, NUP107–160 subcomplex</td>
<td>mos3-1, sar3-1</td>
<td>Yes</td>
<td>Yes</td>
<td>Parry et al. (2006); Germain et al. (2010); Wiermer et al. (2012)</td>
</tr>
<tr>
<td>NUP160/SAR1</td>
<td>NUP, NUP107–160 subcomplex</td>
<td>nup160-1, nup160-3, nup160-4</td>
<td>Yes</td>
<td>Yes</td>
<td>Dong et al. (2006); Parry et al. (2006); Muthuswamy and Meier (2011); Wiermer et al. (2012); MacGregor et al. (2013); Parry (2014)</td>
</tr>
<tr>
<td>NUP85</td>
<td>NUP, NUP107–160 subcomplex</td>
<td>nup85-2, nup85-3</td>
<td>Yes</td>
<td>No</td>
<td>Parry (2014)</td>
</tr>
<tr>
<td>NbNUP75</td>
<td>NUP, NUP107–160 subcomplex</td>
<td>VIGS suppression</td>
<td>Yes</td>
<td>–</td>
<td>Ohtsu et al. (2014)</td>
</tr>
<tr>
<td>Seh1</td>
<td>NUP, NUP107–160 subcomplex</td>
<td>seh1-1</td>
<td>Yes</td>
<td>No</td>
<td>Wiermer et al. (2012); Parry (2014)</td>
</tr>
<tr>
<td>HOS1</td>
<td>NUP, NUP107–160 subcomplex</td>
<td>hos1-1, hos1-3</td>
<td>Yes</td>
<td>Yes</td>
<td>MacGregor et al. (2013)</td>
</tr>
<tr>
<td>NUP98a</td>
<td>NUP</td>
<td>nup98a-1</td>
<td>No</td>
<td>No</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>NUP98b</td>
<td>NUP</td>
<td>nup98b-2</td>
<td>No</td>
<td>No</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>NUP58</td>
<td>NUP, NUP62 subcomplex</td>
<td>nup58-2</td>
<td>No</td>
<td>Yes</td>
<td>Parry (2014)</td>
</tr>
<tr>
<td>NUP54</td>
<td>NUP, NUP62 subcomplex</td>
<td>nup54-2</td>
<td>No</td>
<td>Yes</td>
<td>Parry (2014)</td>
</tr>
<tr>
<td>NUA/TPR1</td>
<td>NUP, nuclear basket</td>
<td>nua-1/attpr1, nua-2, nua-3</td>
<td>Yes</td>
<td>Yes</td>
<td>Jacob et al. (2007); Xu et al. (2007b)</td>
</tr>
<tr>
<td>NUP1/NUP136</td>
<td>NUP, nucleoplasmic location</td>
<td>nup1-1/nup136-1, nup136-2</td>
<td>Yes</td>
<td>Yes</td>
<td>Lu et al. (2010); Tamura et al. (2010)</td>
</tr>
<tr>
<td>NbRAE1</td>
<td>NUP, nucleoplasmic</td>
<td>Tobacco RNAi</td>
<td>Yes</td>
<td>–</td>
<td>Lee et al. (2009)</td>
</tr>
</tbody>
</table>

* The mutant phenotype is defined as being different from the wild-type phenotype, including early flowering and reduced stature.

 pore, and the NUP62 subcomplex is predicted to play an important role in this process (Labokha et al., 2013). For this reason, given the proposed central role for this subcomplex in nuclear transport, it is surprising that these plant mutants exhibit a wild-type level of nuclear mRNA accumulation (Parry, 2014). It is possible that truncated proteins produced in the nup62-1, nup62-2, nup54-2, or nup58-2 alleles retain the integrity of the subcomplex, maintaining its function. In the case of NUP62, this might be possible as the nup62-3 allele is embryo lethal and is predicted to disrupt the N-terminal portion of the protein (Meinke et al., 2008; Parry, 2014). Research focused on the FG-repeat regions from Xenopus laevis NUP58, NUP54, and NUP62 reveals that each region is able to interact individually with nuclear transport receptors (NTRs) but that a composite hydrogel of amalgamated FG-repeat regions from each NUP interacted with NTRs with much greater strength. This indicates that individual NUPs are able to bind transport receptors but that the entire complex does so with greater efficacy (Labokha et al., 2013).

Transferring this information into the Arabidopsis situation suggests that even if the entire NUP62 subcomplex cannot form, then the individually localized NUPs might retain transport properties and provide enough nuclear transport for the plant to remain viable. However, when comparing between eukaryotes, it is dangerous to draw firm conclusions as the similarity of analogous NUPs is not high. Therefore, it is important to perform appropriate experiments in your organism of choice to tease apart important functional differences between organisms. Interestingly, although Arabidopsis double mutant combinations of these alleles are slightly smaller in stature than the equivalent single mutants, these plants do survive (Ferrandez-Ayela et al., 2013). This further suggests that individual NUPs are performing a transport function outside of an interaction with the rest of the subcomplex.

When evaluating what might cause a change in gene expression, the significance of altering mRNA accumulation is difficult to assess. For example, when plants are subjected to environmental or chemical stress, nuclear mRNA will also accumulate (Muthuswamy and Meier, 2011). Therefore, the similar response in nup mutants might be a consequence of the plants being under internal stress due to a defect in nuclear
transport. However, what argues against this hypothesis is that NUP62 subcomplex mutants (nup62, nup58, and nup54) exhibit an early flowering, ‘stressed’ growth phenotype yet do not accumulate nuclear mRNA (Ferrandez-Ayela et al., 2013; Parry, 2014). In contrast, nup85 and seh1 plants display wild-type growth yet do exhibit mRNA accumulation (Wiermer et al., 2012; Parry, 2014). This indicates that preventing correct bulk mRNA export does not play a wholly definitive role in controlling Arabidopsis growth. This is a significant departure from the a priori hypothesis that mRNA accumulation is a major driver of phenotype. It would be dangerous to dismiss the functional influence of mRNA accumulation, but probably what is most important is the retention/transport of specific mRNAs in each mutant and not simply bulk accumulation. However, understanding how mRNA export influences this process is not a simple task. Preventing a specific mRNA from exiting the nucleus will have major ramifications regarding the amplitude and timing of how that gene is expressed, yet currently we have no information about the rate of transport of different mRNAs. Therefore, to define the significance of mRNA accumulation in Arabidopsis, it is important to discover whether there are specific mRNAs that are retained or transported in different nup mutants. One ongoing experimental strategy to answer this question is to use polysome profiling to separate differentially exported mRNAs (Liu et al., 2012).

MicroRNAs (miRNAs) play an important role in regulation of gene expression by signalling for the cleavage of complementary mRNAs. Nuclear transport of miRNAs occurs via a different set of transport factors from that of mRNAs (Kohler and Hurt, 2007). Unsurprisingly, a number of plant proteins that participate in nuclear transport also alter levels of miRNA accumulation. However, this mechanism does not seem to be a consequence of altered miRNA transport but rather is probably due to changes in the transport of unknown regulatory proteins. Arabidopsis plants that have a defect in HASTY/EXPORTIN5, the Importinβ SAD2/EMA1, or the nuclear basket protein TPR1/NUA each exhibit an overall reduction in the amount of miRNA accumulation (Park et al., 2005; Jacob et al., 2007; Wang et al., 2011). However, none of these mutant plants alters the relative levels of nuclear versus cytoplasmic miRNA, so the authors suspect that the early flowering phenotypes of hasty, sad2/ema1, and attpr1/nu are, in part, a consequence of altered localization of unknown proteins that are responsible for regulation of miRNA function (Park et al., 2005; Jacob et al., 2007; Wang et al., 2011). This will result in an alteration in gene expression by miRNA-mediated cleavage of specific mRNAs, some of which are involved in regulation of flowering time and/or hormone signalling.

Control of protein import

In this trip along the central dogma of molecular biology, the final role that the NE might play in the control of gene expression lies in the influence it has on protein synthesis and transport. Translation occurs on ribosomes either located free in the cytoplasm or most often attached to the rough endoplasmic reticulum (ER). The ER membrane is continuous with the NE (Mattaj, 2004), yet we have no information regarding how ribosomes might localize to the plant ONM and therefore influence translation of mRNAs as they exit the NPC. As mentioned above, the WIP and WIT WPP domain localize to the ONM and are needed for correct anchoring of the nucleus to the cytoskeleton (Tamura et al., 2013). In addition, they are responsible for localizing the AtRanGAP GTPase to the ONM, where it functions to uncouple export cargoes from karyopherins by hydrolysis of Ran-GTP to Ran-GDP (Xu et al., 2007a; Zhao et al., 2008). In Arabidopsis there are two closely related functionally redundant AtRanGAP proteins (Rodrigo-Peiris et al., 2011). In atrangap double mutants, male gametogenesis is unaffected yet female gametogenesis aborts following meiosis. Interestingly this ONM localization of AtRanGAP is seemingly not essential for its function, as wip123 triple mutants lack AtRanGAP nuclear localization yet grow normally (Xu et al., 2007a). This suggests that AtRanGAP has residual activity when not tethered to the ONM but has optimum function when anchored at the location where it can directly interact with export cargoes as they exit the NPC.

The target of AtRanGAP is the RAN1 protein, of which there are three family members in Arabidopsis. An excellent recent study characterized a ranl mutant that had significantly reduced levels of the RAN1 protein. Interestingly, in homozygous ranl mutants, <10% of seeds aborted, whereas mutant flowers fertilized with wild-type pollen showed >90% seed abortion (Liu et al., 2014). The authors do not offer an explanation for the molecular mechanisms at work, but it clearly demonstrates a complex relationship of how RAN1 influences gene expression, particularly during endosperm development.

Once polypeptides are correctly folded, many of these proteins will need to be imported back into the nucleus to fulfil their assigned function. This involves passage through the NPC, and a key aim in the study of the NPC is the attempt to define whether any signalling pathways are preferentially affected by interaction with particular NUPs (Parry, 2013). Within the current literature, signalling pathways that respond to auxin and bacterial pathogens, have, in some way, been associated with different NUPs.

NUPs and the auxin response

The plant hormone auxin is responsible for controlling a wide range of developmental processes (Moon et al., 2004). One of its key cellular signalling pathways involves the regulated protein degradation of negative transcriptional regulators termed Aux1IAA proteins (Mockaitis and Estelle, 2008). One set of auxin receptors is a family of F-box proteins termed AFBs (Auxin F-Box) that are contained within an SCF E3 ubiquitin ligase (Dharmasiri et al., 2005). The activity of the SCF complex is controlled by the activity of a ubiquitin-like protein called RUB1, whose addition is reliant on the AXR1 enzyme (Moon et al., 2004). Axr1 mutant plants display pleiotropic auxin-related growth defects; therefore, during the course of a suppressor screen, it was surprising
to discover that two NUP mutants, nup160/sar1 and nup96/sar3, were able partially to suppress this phenotype (Cernac et al., 1997; Parry et al., 2006). The basis for this suppression is not entirely clear, but a best guess is that nup mutants delay the nuclear import of AuxIAA negative regulators, thus alleviating the defect in axr1 that initially dampens auxin gene expression (Parry et al., 2006). Although a subtle redistribution of only a single AuxIAA protein, IAA17, was shown, if this response is repeated across 20+ family members, then it surely would have a more profound effect (Parry et al., 2006). Subsequent experiments have shown that this auxin response is not specific to NUP160/SAR1 or NUP96/SAR3, as attrp1 and nup58/cule mutant plants are able to suppress the axr1 mutant phenotype (Jacob et al., 2007; Ferrandez-Ayela et al., 2013). As expected in seedlings that suppress an auxin-resistant phenotype, nup58 mutants are hypersensitive to auxin (Ferrandez-Ayela et al., 2013). However, somewhat surprisingly, neither nup160 nor nup96 single mutants exhibit auxin hypersensitivity (Parry et al., 2006; Robles et al., 2012), which is perplexing considering the strong suppression of axr1 and indicates that there is a complex relationship between the auxin response and the NPC.

**Nuclear import of pathogen effectors**

A number of proteins situated in the NE have been shown to be involved in the response to different pathogens (for review see Parry, 2013). However, defining the identity of the molecules that are transported across the NPC following infection is less simple. The MODIFIER OF SNC1 3 (MOS3/NUP96), MOS7/NUP88, NUP160, and Seh1 proteins are NUPs, and the equivalent mutants are all hypersensitive to bacterial pathogens (Zhang and Li, 2005; Cheng et al., 2009; Wiermer et al., 2012). In addition, transient down-regulation of *Nicotiana benthamiana* NbNUP75/NUP85 also makes plants more susceptible to the fungus *Phytophthora infestans*.

In some of these mutants, aspects of protein import are altered after exposure to pathogens. *Mos7-1* mutants show a reduction in the nuclear localization of the SUPPRESSOR OF NPR1-1, CONSTITUTIVE (SNC1), NONEXPRESSER OF PR (NPR1), and ENHANCED DISEASE SUSCEPTIBILITY (EDS1) proteins, all of which are important for the defence response (Cheng et al., 2009). Importantly, the authors show that the nuclear accumulation of other proteins unrelated to the defence response are unaffected in these mutants. Therefore, it is reasonable to suggest that mislocalization of defence-related proteins will affect gene expression in response to the certain pathogens (Cheng et al., 2009). The function of the MOS7/NUP88 protein is notably different from that of MOS3/NUP96, NUP160, or Seh1 as they reside within different NPC subcomplexes. In nup160 mutants, the overall cellular level of EDS1 is reduced but there is no change in the relationship between cytoplasmic and nuclear pools of this protein. Currently there is no information about specific protein mislocalization in the nup96, nup160, or seh1 mutants. As there is no evidence that MOS7/NUP88 plays a role in nuclear mRNA export, this might suggest that in *Arabidopsis* MOS7/NUP88 impacts the defence response by affecting nuclear protein import while members of the NUP107–160 complex may affect this process by altering mRNA export.

**Nuclear transport and the interaction with symbiotic microorganisms**

In *Lotus japonicus*, plants with a mutation in any of NUP85, Seh1, or NUP133 show defects in the root interaction with symbiotic microorganisms, even though the general plant growth is largely unaffected (Kanamori et al., 2006; Saito et al., 2007; Groth et al., 2010). These three proteins are members of the NUP107–160 subcomplex and their protein levels are partially dependent on each other (Binder and Parniske, 2013). Interestingly, *ljnup85/ljnup133* double mutants exhibit a temperature-sensitive seedling growth phenotype that is much more severe than that of either single mutant (Binder and Parniske, 2013). Although these studies offer no clue as to the precise changes in nuclear transport that are occurring, the roots of mutant plants exhibit dampened calcium oscillations (Kanamori et al., 2006; Saito et al., 2007; Groth et al., 2010). Changing calcium levels is an important early response in the symbiotic pathway, and a recent study from the related legume, *Medicago truncatula*, showed that calcium signalling occurs via changes in the NE and that the cation channel DOES NOT MAKE INFECTIONS1 (DMI1) localizes to the INM (Capoen et al., 2011). Therefore, in *Lotus* NUP mutants, DMI1 localization might be altered, resulting in changes in the ability of the INM to transmit a calcium signal (Charpentier and Oldroyd, 2013).

Overall we currently have very little understanding regarding the nuclear protein import that is altered in NUP mutants and whether, either in the steady state or after an inductive signal, the movement of proteins is affected in a NUP-specific manner. Although a number of investigations have attempted to catalogue the components of the plant nuclear proteome, these studies have had limited success (Erhardt et al., 2010; Narula et al., 2013; Petrovska et al., 2014). Overall the number of identified proteins has been small and extremely variable, depending on the extraction protocol and type of mass spectrometric analysis. However, as technologies improve, it is hoped that it will be possible to better define the nuclear proteome in the context of a defective NE and under different growth conditions. With this type of large-scale data it will be possible to begin to understand how the NE/NPC specifically impacts the flow of proteins into the nucleus and their relevance for different signalling pathways.

**Concluding remarks**

In this review I have attempted to describe how NE-associated proteins might affect gene expression, either by directly binding chromatin, altering chromatin patterning within the nucleoplasm, or by altering nuclear transport of RNA and/or protein. Although in the past decade a great deal of progress has been made in describing the proteins that reside at the NE and within the NPC, what has yet to be broadly achieved is determining how each of these proteins interacts
with different signalling molecules and therefore to define how they affect cellular responses. It is heartening that in the 2 years since reviews were published in this journal that described the structure and function of the NPC (Parry, 2013; Tamura and Hara-Nishimura, 2013), there have been a significant number of extra studies describing the function of many NE-localized proteins. The members of the International Plant Nuclear Consortium will continue to study this important area of research (Graumann et al., 2013) and are beginning to link with other researchers in order to understand how the NE interacts with other nuclear locations and signalling pathways.

Acknowledgements

The author wishes to thank Yi Fang for helpful discussions over the past year, and Jiayun Zhu for generating unpublished data as part of her undergraduate research project at the University of Liverpool.

References


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.


