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Regulation of nuclear shape and size in plants

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Nuclear shape and size changes have long been used by cytopathologists to diagnose, stage, and prognose cancer. However, the underlying causalities and molecular mechanisms are largely unknown. The current eukaryotic tree of life groups eukaryotes into five supergroups, with all organisms between humans and yeast falling into the supergroup Opisthokonta. The emergence of model organisms with strong molecular genetic methodology in the other supergroups has recently facilitated a broader evolutionary approach to pressing biological questions. Here, we review what is known about the control of nuclear shape and size in the Archaeplastidae, the supergroup containing the higher plants. We discuss common themes as well as differences toward a more generalized model of how eukaryotic organisms regulate nuclear morphology.

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Introduction

In animals, changes in nuclear shape and size are associated with cell differentiation, development, and disease (recently reviewed by [1]). Cancer cell nuclear morphology is often altered, but the cause is not well understood [2,3]. In addition, changes in nuclear morphology are associated with several laminopathies and nuclear envelopathies [4,5]. Thus, understanding the physiological relevance and mechanism of nuclear morphology regulation is a topic of fundamental cell biology with potentially high translational impact.

In animal model systems, nuclear size and shape are influenced by DNA ploidy, nuclear structural components, cytoplasmic factors, nucleocytoplasmic transport, the cytoskeleton, and the extracellular matrix [3]. It is becoming increasingly evident that mitotic events also influence nuclear morphology [1]. However, our understanding of how nuclear shape and size are regulated is rather poor and our knowledge of the role that nuclear shape plays in nuclear function is limited [6].

The Archaeplastidae and Opisthokonta separated roughly one billion years ago as single-celled organisms [7]. While the fairly late recognition of the vastness of separate evolution has hampered fundamental plant cell biology research for a couple of decades, recent discoveries about how plants 'solve' shared cellular problems in different ways promise to enlighten research in all model organisms by introducing a broader evolutionary perspective. Here, we review what is known about nuclear size and shape regulation in plants, discuss the first emerging molecular players, and compare and contrast them to their opisthokont counterparts.

Nuclear size and shape in plants

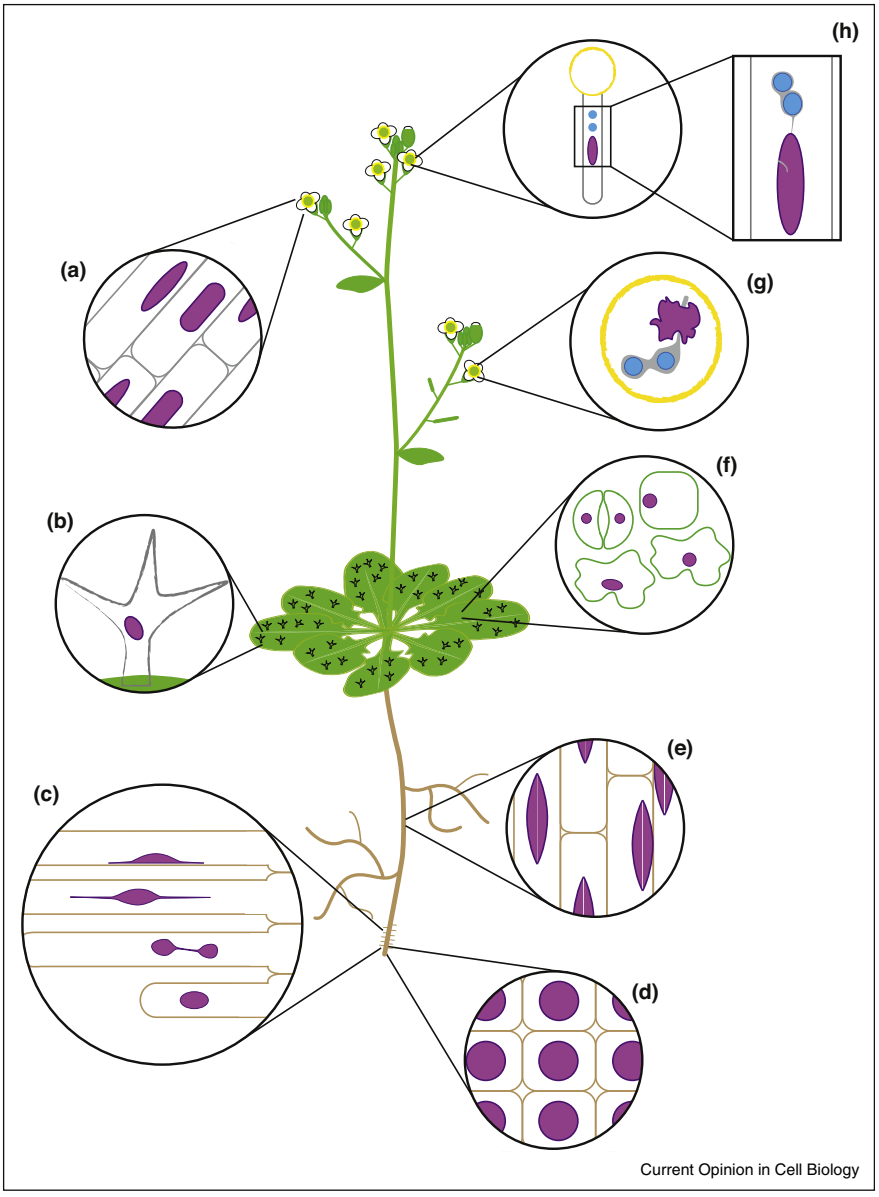
Embryonic and meristem nuclei in flowering plants are nearly spherical. During cell and tissue differentiation, however, nuclei adopt a variety of elongated and super-elongated shapes and increase their size, correlated to endoreplication [8]. In *Arabidopsis thaliana*, nuclei range from spherical in meristems and guard cells to hyper-elongated with extended membrane tails in root hairs [9,10••]. Some nuclei even divide into multiple subnuclear structures connected by thread-like projections (Figure 1) [11].

Elongated nuclei typically correlate with elongated cells. Nuclear size roughly correlates with cell size, with the smallest leaf nuclei in the diploid guard cells, bigger nuclei in epidermal cells, and the biggest nuclei in the large, highly polyploid unicellular trichomes [12].

The haploid pollen grain is the plant male gametophyte (Box 1). An Arabidopsis pollen grain contains three nuclei: the vegetative nucleus (VN) of the pollen cell and the two nuclei of the embedded germ cells (GCs). While the GC nuclei are small and spherical and contain highly condensed chromatin, the VN is larger and narrows during pollen tube growth, becoming oval to spindle-shaped, and has reduced chromatin compaction [13–16].

Nuclear size and volume decrease during seed maturation and increase again during imbibition and germination of Arabidopsis seeds. This correlates not with passive water uptake, but rather with heterochromatin distribution [17••]. There is, however, no clear correlation between nuclear size changes and seed dormancy or desiccation tolerance. Several mutants with defects in seed dormancy

Figure 1



Graphical depictions of nuclear shapes in different Arabidopsis cell types. **(a)** Nuclei in petal vascular tissue are rod-shaped and oval-shaped [9]. **(b)** Trichome nuclei are large and oval-shaped [9]. **(c)** Root hair nuclei adopt a range of shapes during development and after differentiation. Nuclei in elongating root hairs tend to be oval (bottom). Nuclei in mature root hairs can adopt a range of shapes. From top to second from bottom: flattened, with tails [9]; spindle-shaped [9]; and multiple connected subnuclear structures [9]. **(d)** Root meristem nuclei are generally spherical. **(e)** Root epidermal nuclei are spindle-shaped. **(f)** Nuclear shape in leaf cells varies between cell types. Nuclei in guard cells and mesophyll cells tend to be spherical. Pavement (epidermal) cell nuclei can be either ellipsoid or spherical. **(g)** In mature pollen, the vegetative nucleus (purple) is highly invaginated, including a channel that facilitates attachment to the cytoplasmic projections (gray) of the sperm cells (their spherical nuclei shown in blue) [14,15]. **(h)** In germinated Arabidopsis pollen, the sperm cells (spherical to oval nuclei shown in blue) are connected to each other and the vegetative nucleus (purple) by cytoplasmic projections (gray) [14,16,44]. The nucleus elongates during pollen germination, and is oblong in shape, with some invagination [14,16,45].

undergo wildtype (WT)-like nuclear size changes [17••]. Also, a *crwn1 crwn2* mutant (see below) that has imbibition and germination-insensitive small nuclei has no germination or dormancy defects. This suggests that specific properties of nuclei are an adaptation to desiccation, but independent of dormancy.

Several other studies have observed changes in seed nuclear size. *Phaseolus vulgaris* seed pith and plumule cell nuclei shrink when they approach the dormant phase [18]. *Phaseolus lunatis* and *Zea mays* have smaller nuclei in dormant pith, cortex, and vascular cells of the seed [19], and an Arabidopsis study suggests a correlation between

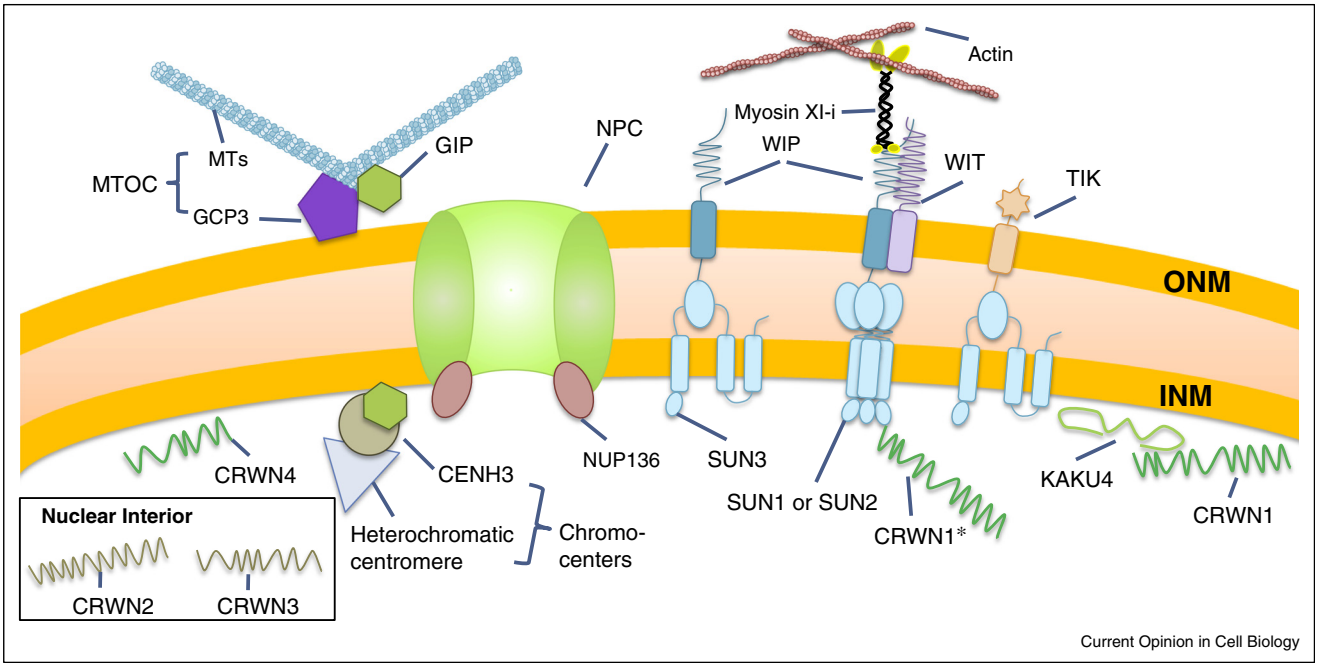
nuclear size and both low transcription rates and water loss in seeds [20]. Aside from seeds, *Craterostigma plantarum* (the 'resurrection plant') leaf nuclei were shown to shrink to about half size upon desiccation [17••]. Because osmotic stress has been shown to reduce nuclear size [21], seed or plant desiccation might provide an experimental system to dissect this aspect in a whole-tissue context.

Nuclear shape and plant lamin-like proteins

In humans, mice, and flies, lamin mutations cause aberrantly elongated nuclei in heart tissue [22]. In Hutchinson–Gilford Progeria Syndrome — a laminopathy — nuclei form blebs and protrusions from the nuclear surface, and have altered mechanical properties [22,23]. Lamin B overexpression causes nuclear blebs and misshapen nuclei and additional intranuclear membranes, while Lamin B depletion reduces nuclear size [1].

Plant genomes do not encode homologs of the animal lamins, but a filamentous meshwork adjacent to the inner nuclear membrane (INM) can be observed by electron microscopy [24]. Nuclear matrix component protein 1 (NMCP1) and its Arabidopsis homologs CROWDED NUCLEI (CRWN) 1, CRWN2, CRWN3, and CRWN4 are currently the best candidates for lamin analogs in plants [25,26]. NMCP1 has a central long coiled-coil domain, short N-terminal and C-terminal non-helical domains, and a predicted nuclear localization signal (NLS) in the C-terminal domain. An anti-NMCP1 antibody decorates the nuclear envelope (NE) in both light and electron microscopy and the protein fractionates with the insoluble nucleoskeleton fraction [27•]. These features are similar to lamins, but NMCP1 is about twice the size of human lamin A and has no sequence similarity to lamins [28]. Arabidopsis CRWN1 and CRWN4 are located at the nuclear periphery while CRWN2 and CRWN3 are in the nuclear interior (Figure 2) [26,29••,30••]. Some *CRWN* mutant combinations lead to severely dwarfed plants with smaller cells, and a quadruple knockout mutant is lethal [30••]. At the cellular level, the most prominent *CRWN* mutant phenotype is a significant nuclear size reduction and increased nuclear circularity. *crwn2* or *crwn3* alone do not affect nuclear morphology, but enhance *crwn1* and *crwn4* mutant phenotypes. *CRWN* mutant nuclei also have fewer chromocenters, suggesting defects in heterochromatin organization. No changes in

Figure 2



Proteins that play a role in plant nuclear envelope (NE) shape determination. From left to right: CRWN1 and CRWN4 are found at the nuclear periphery, near the inner nuclear membrane (INM), while CRWN2 and CRWN3 are restricted to the interior of the nucleus [26,29••]. GCP3 interacting proteins (GIPs) are distributed throughout the NE and associated near the nuclear pore complex (NPC) at both the outer nuclear membrane (ONM) and INM [71]. At the ONM GIPs associate with microtubule organizing center (MTOC), whereas at the INM they localize with chromocenters [74]. Nup136, a plant-specific FG-repeat nucleoporin, is part of the NPC located near the INM [49]. WIP, an Arabidopsis KASH protein, located at the ONM binds to INM protein SUN3 (a mid-SUN protein) and SUN1 and SUN2 (C-terminal SUN proteins) [10••,41•,54]. WIPs also bind to the ONM-associated WIT proteins to form a complex with Myosin XI-i and actin filaments [43••]. SUN1 and SUN2 binds CRWN1, whereas SUN3 binds TIK, another plant KASH protein [37,41•]. TIK further binds to SUN1, SUN2 and SUN4, another mid-SUN protein (not shown) [41•]. KAKU4 is located near the INM and was found to be associated with CRWN1 [63••]. Additionally, mid-SUNs interact with each other as well as with the C-terminal SUN proteins (not shown) [41•]. *Interactions only supported by one type of experiment.

ploidy were observed [29••]. Overexpression of CRWN4-GFP leads to larger, more elongated nuclei in pavement cells, suggesting a tight correlation between CRWN protein abundance and nuclear size [29••].

Nuclear shape and LINC complexes

Linker of nucleoskeleton and cytoskeleton (LINC) complexes span the INM and the outer nuclear membrane (ONM), forming a nucleocytoplasmic protein bridge (reviewed in [31]). In the NE lumen, INM SUN proteins interact via their C-terminal SUN domains with the C-terminal tails of ONM KASH-domain proteins. Some SUN proteins interact with nuclear lamins. KASH proteins interact directly or indirectly with actin, microtubules or intermediate filaments. Animal LINC complexes have been connected to a variety of developmental events that involve nuclear movement and nuclear positioning (reviewed in [1,31]). In human fibroblasts a dominant-negative KASH fragment eliminates the sensitivity of nuclear shape to substrate rigidity, and this effect could be mimicked by myosin inhibition, suggesting that nuclear shape is modulated by a connection of LINC complexes to actinomyosin tension [32]. Overexpression of different fragments of the giant KASH-domain protein Nesprin-2 either increases or decreases nuclear size and the interaction of Nesprin-2 with Nesprin-3 is involved in this size regulation [33].

SUN proteins are conserved between animals and plants, while plant KASH proteins are distinct from their animal counterparts [10••,34,35, reviewed in 36]. Arabidopsis and maize SUN1 and SUN2 are C-terminal SUN domain proteins, and form LINC complexes with plant KASH proteins [10••,34,35]. There is also evidence that SUN1 and SUN2 may bind CRWN1 *in planta* [37]. Trichomes and root hair nuclei of *sun1* null mutants are spherical, while *sun2* mutants have wildtype-like elongated nuclei in the same cell types [38]. In a *sun1 sun2* severe knockdown mutant, root hair nuclei are spherical, and never elongate during development [39,40•]. Arabidopsis also has three mid-SUNs — SUN3, SUN4, and SUN5 — characterized by multiple transmembrane domains and a SUN domain in the center of the protein [35,41•,42]. *sun3* mutant plants' root epidermal cell nuclei are more spherical than WT and lack membrane tails [41•]. Single *sun4* or *sun5* mutants have no phenotype, but a *sun4 sun5* double mutant has smaller nuclei [41•]. The maize mutant *desynaptic* has spherical nuclei in anthers, and while the locus has not been mapped, a likely candidate is the maize homolog of *SUN3* [42].

The most recently identified plant KASH protein is AtTIK, mutants of which have smaller nuclei than WT [41•]. The most extensively characterized Arabidopsis KASH proteins are WIP1, WIP2, and WIP3 [10••,36], which complex with another ONM protein family, WIT1 and WIT2, to form a SUN-WIP-WIT LINC complex (Figure 2) [10••]. *wit1* and *sun2* single mutants, as well as *wip1 wip2 wip3* (*wit123*) and *wit1 wit2* (*wit12*) null and *sun1 sun2* severe knockdown mutants result in spherical root hair and trichome nuclei [40•]. *wit12* further exhibits decreased velocity of nuclear movement in mature root hairs and defective high-light avoidance recovery in leaf mesophyll cells [43••]. *wit12* and *wip123* mutants, as well as expression of a dominant-negative SUN domain, also lead to reduced VN velocity in growing pollen tubes. In this case, however, no shape or size change of the VN was observed [38,44]. The pollen VN narrows during WT pollen tube germination and elongation. While treatment of *Galanthus nivalis* pollen tubes with the microtubule polymerization inhibitor colchicine does not affect this narrowing, treatment of *Lilium henryi* pollen tubes with the actin polymerization inhibitor cytochalasin D causes the VN to shorten considerably [45,46]. Together, the mutant and drug treatment data suggest that the WIT-WIP-SUN LINC complex at the VN acts via a microtubule-dependent mechanism to effect VN movement, and that additional factors that connect the VN to actin are involved in determining its elongated shape during WT development.

Nuclear shape and the cytoskeleton

During differentiation of mouse and human embryonic stem cells, a LINC complex-associated actin meshwork at the nuclear periphery called the perinuclear actin cap forms and progressively shapes the nucleus [47,48]. In HaCaT cells, actin depolymerization reduces nuclear size, MT depolymerization increases it, and a combination of both drugs decreases nuclear size, suggesting that actin plays a dominant role [33].

KAKU1 (*MyoXI-i*) was identified in a forward-genetic screen for nuclear shape mutants. MyoXI-i is a member of the plant-specific Myosin XI family, which has 13 members in Arabidopsis. Single mutants in all Myosin XIs were tested for nuclear shape and the *myoxi-i* mutant is the only one that leads to spherical nuclei. A MyoXI-i 'Δmotor' fragment fused to GFP decorates the NE. Interaction proteomics with this fragment identified the plant LINC complex component WIT2. The authors show that MyoXI-i Δmotor binds the closely related protein WIT1, which in turn binds the plant KASH protein WIP1, which interacts with INM proteins SUN1 and SUN2 [43••].

In a related set of experiments, Zhou *et al.* [40•] dissected this complex's components further. They established that: first, SUN1, but not SUN2 and WIT2, but not WIT1 are required for elongated nuclear shape in root epidermal cells; second, WIT2 interacts with WIP1, WIP2, and WIP3. MyoXI-i association with the NE is diminished in *sun1 sun2* severe knockdown and *wit2* mutants, but not *wit1* mutants. Together, these data imply that MyoXI-i is associated with the NE in root

epidermal cells through interactions with a WIT2-WIP-SUN1 complex, and that this complex is required — by an unknown mechanism — to establish the elongated nuclear shape of differentiated root and leaf epidermal cells.

Interestingly, *myoxi-i* exhibits nuclear movement phenotypes similar to *wit12*, including decreased nuclear movement in mature root hairs and defective high light avoidance recovery in mesophyll cells. However, no pollen tube nuclear movement defects have been reported. This suggests that the function of MyoXI-i as part of shape-controlling and movement-controlling LINC complexes is limited to the sporophyte.

Nuclear shape and the nuclear pore

Thirty Arabidopsis nucleoporins (Nups) have been identified through interactive proteomics, most of which are homologs of yeast or mammalian Nups [49]. However, one plant-specific FG-repeat Nup (Nup136) was also identified (also named Nup1 by [50]). In *nup136* mutants, leaf epidermal nuclei are more spherical than in WT and overexpression of Nup136 leads to elongated guard cell nuclei with membrane tails, and over-elongated nuclei in trichomes and leaf epidermal cells [51].

nup136 mutant plants have defects in flowering time, seed set, pollen germination, and mRNA nuclear export. Similar whole-plant and mRNA export phenotypes also occur in other Arabidopsis Nup mutants [52–55], but in these cases no altered nuclear morphology has been reported. This suggests that Nup136 plays an additional role. Consistently, the most drastic nuclear shape changes occur during Nup136 overexpression, but these plants have no whole-plant phenotypes.

Nucleocytoplasmic transport regulates nuclear size in both *Xenopus laevis* and *Tetrahymena*. In *Xenopus*, nuclear Lamin B is one of the important cargoes and the nuclear-growth defect of import-defective nuclei could be rescued by re-addition of recombinant Lamin B3 [56]. In *Tetrahymena*, import of histone H1 affects nuclear size, and different H1 isoforms are specifically imported into the micronucleus and the macronucleus [3]. Depletion of *Xenopus* Nup188, which is dispensable for NPC formation, increases nuclear size. This is caused by an accelerated translocation of integral membrane proteins through nuclear pore complexes (NPCs) [57]. No nuclear import studies have been performed in *nup136* mutants, and testing nuclear abundance and localization of CRWN proteins would be a logical next step.

Nup136 is proposed to be the functional analog of animal Nup153 [49,51]. Nup153 connects to the nuclear lamina in Drosophila and *Caenorhabditis elegans* and controls spatial distribution of NPCs [58–60]. No correlation between NPC numbers and nuclear size or shape has been found in opisthokonts [61,62], but it would nevertheless be valuable to investigate NPC abundance and distribution in *nup136* mutants. In addition, it would be interesting to establish if NUP136 interacts with CRWN1 or CRWN4, because such an interaction would suggest that a structure similar to the pore-lamina connection in animals has also evolved in plants.

Forward genetic screens for nuclear shape mutants

An EMS mutant screen for altered nuclear shape identified several *KAKU* genes, among them *KAKU1* (*MyoXI-i*; discussed above), *KAKU4* and *KAKU2* (*CRWN1*). KAKU4 is a plant-unique protein with a predicted NLS, no predicted transmembrane domain, and no other domain similarities other than a potential long alpha-helical domain [63••,64]. KAKU4 is located at the INM, where it interacts with CRWN1. *kaku4* and *crwn1* mutants have smooth and spherical nuclei, unlike the *kaku1* mutant nuclei, which are spherical and irregularly invaginated. KAKU4 overexpression leads to deformation and over-proliferation of the NE, including substantial invaginations, additional ring-like NE structures inside the nucleus, stacked NE without nuclear pores, and extra-nuclear membrane whorls [63••]. KAKU4 overexpression in a *crwn1* mutant leads to similar effects including ring and bleb-like structures as well as long KAKU4-GFP-labeled 'tails' extruding from the spherical nuclei, suggesting that the KAKU4-based membrane deformation does not require CRWN1. Nevertheless, co-overexpression of KAKU4 and CRWN1 had the most striking effect, leading to numerous spherical structures filling the nucleus. These data suggest that proteins located at the NE could play a role in regulation of the amount of NE membranes present around chromatin. It would be interesting to determine the identity of the additional membrane in the KAKU4 overexpressing mutants — could a loss of distinction between NE and ER identity result in additional ER membrane accumulating around the chromatin? Anderson and Hetzer [65] showed that overexpression of the ER tubule-forming proteins inhibited NE formation and nuclear expansion, suggesting that the transition from membrane tubules to sheets is rate-limiting for nuclear assembly in mammalian cells.

Several opisthokont INM proteins are also implicated in nuclear shape regulation. Depletion of lamin B receptor (LBR) causes hypo-lobulated nuclei, a truncated lamina-associated polypeptide 2 isoform β (Lap2β) fragment reduces nuclear size and depletion of *C. elegans* Ankyrin repeat and LEM domain-containing protein 2 homolog (LEM4) causes misshapen, multilobed nuclei [1]. The INM-associated protein complement appears to be not deeply conserved, and most opisthokont INM-associated proteins have no structural plant homologs [38]. Thus, KAKU4 is an exciting candidate for an INM protein

activity possibly functionally, but not structurally, conserved across supergroups.

Nuclear shape and mitosis

The events that re-form the NE at the end of open mitosis can be important to establish normal interphase nuclear morphology [66]. For example, depletion of the chromatin-binding protein developmental pluripotency associated 2 (Dppa2) from *Xenopus* egg extracts leads to the formation of small, misshapen nuclei, probably through defects in the regulation of microtubule polymerization during NE reformation [67].

In plants, which lack centrosomes, the NE plays a role as the microtubule organizing center (MTOC) at the onset of mitosis. Gamma-tubulin complexes (γ-TuCs) are recruited to the NE and contribute to the establishment of the mitotic spindle [68,69]. One of the γ-TuC core subunits, γ-TuC protein 3 (GCP3), binds GCP3 interacting proteins 1 and 2 (GIP1 and GIP2), two 8 kD alpha-helical proteins [70•]. *GIP* homologues were first seen in humans and, more recently, in *Schizosaccharomyces pombe* where they are known as mitotic spindle-organizing protein 1 (MZT1) [70•,71,72]. Arabidopsis GIP1 and GIP2 co-localize with both chromocenters at the INM and MTs at the ONM [73], and are required for centromere architecture. In their absence, recruitment of critical centromere proteins is altered and centromere assembly and cohesion are affected [74]. *gip1 gip2* double mutants have a variety of developmental defects, including germination and flowering defects, corkscrew-like root growth, short hypocotyls and bulging, irregularly shaped root meristem cells [70•].

Root tip cells of *gip1 gip2* double mutants have massively enlarged nuclei with distorted shapes, a loss of circularity, and an increase in ploidy. In addition, there are lobulated and highly dented nuclei in various differentiated cells [71]. NPC density was increased and the distribution of the NE marker Arabidopsis SUN1 was altered, suggesting an overall disturbance of NE composition.

Conclusions

Like in animals, proteins involved in regulating nuclear shape and size in plants center at the NE (Figure 2 and Table 1). Some players, such as the SUN proteins, are highly conserved, while in other cases functional analogs with little or no sequence or structural similarities have evolved. At least two independent shape-determining pathways have been dissected: one involving a WIT2-WIP-SUN1 LINC complex and leading to spherical nuclei with invaginated membranes, and a second involving the lamin-like protein CRWN1, leading to spherical nuclei with a smooth, minimal NE. This supports a model of nuclei being shaped both by cytoplasmic forces transferred to the NE and by nucleoplasmic filaments formed under the NE. It also suggests that disruption of some pathways leads to loss of control over the balance between nuclear volume and NE membrane abundance, while in other cases, this balance remains under control.

While not all nuclear size and shape mutants have been tested for nuclear movement, there are interesting correlations of loss of nuclear elongation and reduced velocity of movement (Table 1) (reviewed in [75]). Though this elongated shape does not occur in all plant cells with mobile nuclei, it is tempting to speculate that the elongated shape

Box 1 Glossary

Guard cells — pairs of cells that form the stomata located in the epidermis of aerial plant tissues. Stomata regulate gas exchange between the plant and the atmosphere.

Pavement cell — highly interlobed epidermal cells of leaves and cotyledons.

Trichome — large, unicellular hairs located in the epidermal layer of plant tissues.

Root hair — tubular outgrowth of a trichoblast, a hair-forming cell on the epidermis of a plant root.

Pollen — microgametophyte containing the male gametes in flowering plants. The pollen grain contains three nuclei; the vegetative nucleus and two sperm cell nuclei.

Sporophyte — the diploid phase of the plant life cycle, encompassing the aerial and subterranean tissues of higher plants, with the exception of the male and female gametophytes (pollen and ovules).

Gametophyte — the haploid phase of the plant life cycle. In higher plants, this encompasses pollen and ovules.

Meristem — tissue in plants containing undifferentiated cells that are areas of rapid growth in development. Meristems exist in both the root and shoot.

Cotyledon — the embryonic leaves of developing plants.

Hypocotyl — the developing stem in the plant embryo.

CRWN genes — short for *CROWDED NUCLEI*, originally described as *LITTLE NUCLEI* (LINC) genes; Arabidopsis homologs of *NMCP1* genes.

SUN — Sad1p/UNC-84.

KASH — Klarsicht, ANC-1, SyneHomology.

Cytochalasin D — actin depolymerizing agent.

Cytochalasin B — actin depolymerizing agent.

Latrunculin B — blocks actin polymerization.

N-ethylmaleimide — blocks actin binding.

Taxol — blocks microtubule depolymerization.

Oryzalin — depolymerizes microtubules.

Vinblastine — prevents microtubule polymerization.

Colchicine — prevents microtubule polymerization.

Arabidopsis gene nomenclature: typically a gene name is abbreviated by a 3-letter code; uppercase: protein; italicized uppercase: gene; italicized lowercase: mutant allele. Mutant allele names are used to refer to plant genotypes. For example, an Arabidopsis plant line containing a mutation in the *CRWN1* gene (which encodes the CRWN1 protein) would be referred to as *crwn1*.

Table 1

Mutants and treatments that affect nuclear shape and movement in plants

Protein	Organism(s)	Function	Nuclear shape	Nuclear movement
CRWN1/4; NMCPs	*A. thaliana, Allium cepa; Daucus carota*	Plant lamin-like proteins at the nuclear periphery	*crwn1; crwn4*: SSN in trichomes and leaf epidermal cells [26,29••,30••]	
CRWN2/3	*A. thaliana*	Plant lamin-like proteins in the nucleoplasm	*crwn1 crwn2; crwn1 crwn3*: enhanced SSN phenotype in trichomes and leaf epidermal cells [26,29••,30••]	
KAKU4	*A. thaliana*	Nuclear periphery-localized protein of unknown function	*kaku4*: SSN in leaf epidermal and root cells. KAKU4 OX: invaginated nuclei and ring-like structures [63••]	
SUN1/2	*A. thaliana*	C-terminal SUN domain proteins; INM components of plant LINC complexes	*sun1-KO sun2-KD*: SpN in trichomes, leaf epidermal cells. Spherical, invaginated nuclei in root hairs. Elonglated shape of pollen VN not affected [10••,38,39,40•,44].	A dominant-negative SUN2 fragment leads to reduced velocity of VN migration through the pollen tube [38]
SUN3/4/5	*A. thaliana, Z. mays*	Mid-SUN domain proteins; localized to the INM	*sun3*: SpN in root epidermal cells. *sun4 sun5*: small nuclei in root epidermal cells [41•]	
GIP1/2/3	*A. thaliana*	Localized to microtubule organizing centers near the ONM and chromocenters near the INM	*gip1 gip2*: enlarged, lobulated nuclei. Increased nuclear pore size and density [70•,71,73]	
WIP1/2/3	*A. thaliana*	Plant KASH proteins; ONM components of plant LINC complexes	*wip1 wip2 wip3*: SpN in trichomes, leaf epidermal cells and root hairs. Elonglated shape of pollen VN not affected [10••,44]	*wip1 wip2 wip3*: reversed VN/SN nuclear order in growing pollen tubes [44]
WIT1/2	*A. thaliana*	ONM protein that connects RanGAP and Myosin XI-I to the SUN/WIP LINC complex	*wit2; wit1 wit2*: SpN in trichomes and leaf epidermal cells. Spherical, invaginated nuclei in root hairs. Elonglated shape of pollen VN not affected [10••,40•,43••,44]	*wit1 wit2*: reduced velocity of VN through the pollen tube. *wit1 wit2*: delays recovery after high-light avoidance movement of leaf epidermal nuclei [43••,44]
TIK	*A. thaliana*	Plant KASH protein localized to the NE	*tik*: decrease in nuclear size in root epidermal cells [41•]	
Myosin XI-i	*A. thaliana*	Myosin XI family member; associates with the SUN/WIP LINC complex	*myosin xi-i*: SpN in root epidermal cells [43••]	*myosin xi-i*: delays recovery after high-light avoidance movement of leaf epidermal nuclei [43••]
Nup136	*A. thaliana*	Nucleoporin; FG-repeat protein of the nuclear pore complex	*nup136*: SpN in leaf epidermal cells. NUP136 OX: EN in guard cells, and exacerbated EN in trichomes and leaf epidermal cells [49,51]	

Filament	Organism(s)	Treatment(s)	Nuclear shape	Nuclear movement
Actin	*L. henryi; A. thaliana*	Cytochalasin D, Cytochalasin B, Latrunculin B, N-ethylmaleimide	Disruption of the actin network leads to a decrease in pollen tube VN length [11,46]	Disruption of the actin network leads to a reduction or elimination of root hair nuclear movement [11,75]
Microtubules	*L. henryi*; *A. thaliana; Medicago truncatula; Nicotiana tabacum; Nicotiana alata*	Taxol, Oryzalin, Vinblastine, Colchicine	Disruption of the microtubule network had no effect in root cells and pollen tubes [11,45]	Disruption of the microtubule network increased nuclear distance from the root hair tip in Medicago, but not Arabidopsis. In pollen tubes, disruption of the microtubule network reversed VN/SN order and stunted nuclear movement [11,45,75]

Abbreviations: SSN, small, spherical nuclei; SpN, spherical nuclei; VN, vegetative nucleus; SN, sperm cell nuclei; EN, elongated nuclei; OX, overexpression. Protein names belonging to a single family have been abbreviated to save space, such as SUN3/4/5 = SUN3, SUN4, and SUN5.

is energetically advantageous for nuclear movement through a dense cytoplasm, and that movement is decelerated in such mutants that do not allow for the most streamlined shape to form. An alternative model could be that the forces of movement themselves cause the elongated shape. This, however, seems less likely, because isolated nuclei retain their elongated shape *in vitro* [29••].

There is a need for a better mechanistic understanding of nuclear size and shape regulation, including dissecting lipid homeostasis at the NE, connections to chromatin organization, the connection between shape and movement, and the biomechanics of the NE. Plants appear to tolerate several drastic nuclear shape and size changes without obvious deleterious effects on growth and development and might thus emerge as a robust model system to dissect the underlying molecular mechanisms that are currently unknown in any system.

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References and recommended reading

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- • of special interest
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