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Regulation of nucleocytoplasmic trafficking in plants

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The timing and position of molecular components within the cell are clearly important in the context of signal transduction. One challenge in attaining correct cellular positioning is the nuclear envelope, which separates the cell into two fundamentally different compartments. Molecular passaging from one to the other is highly selective due to the required recognition by the nucleocytoplasmic transport machinery. It is becoming increasingly clear that a highly diverse set of mechanisms have developed to allow environmental (biotic and abiotic) and endogenous signals to alter the nucleocytoplasmic partitioning of key molecules. In many cases this occurs by adjusting the access of the regulated species to the canonical import/export machinery. Recent studies are uncovering the sophistication and complexity of the processes that use the canonical transport machinery in the service of a diversity of signaling pathways.

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Introduction

Signal transduction cascades rely not only on post-translational activation and inactivation of signaling proteins, but also on their selective inclusion in and exclusion from the nucleus. Nucleocytoplasmic trafficking of proteins is a highly selective process (Box 1). There are numerous examples in animals and fungi for the importance of nucleocytoplasmic partitioning in signaling, beginning with the classic instance of the control of nuclear import of the stress-response transcription factor NF-kappaB. The mechanism here involves phosphorylation, heterodimer formation, and ubiquitination-dependent proteolysis, three mechanisms frequently found in nuclear import regulation [1].

While cargo phosphorylation is emerging as an important mechanism for nuclear import regulation in animals, there are many different effects phosphate addition or removal can have on nucleocytoplasmic partitioning. They include masking/activating an NLS and preventing/facilitating protein–protein interactions. A protein partner can either cause cytoplasmic retention, as in case of NF-kappaB, or be itself an NLS-containing protein that allows nuclear entry by a 'piggyback' mechanism. In some cases, phosphorylation enhances docking of a protein to the nuclear pore complex itself [2].

Nuclear import as part of signal transduction is recently also taking center stage in plant research. Several signaling mutants were found to be compromised in either the nuclear pore itself or components of the import apparatus, such as karyopherins or components of the Ran cycle (Box 1). Similarly, signaling proteins for a variety of pathways are now known that undergo regulated nuclear import. The effects of nuclear pore and nuclear transport machinery mutants on plant signaling pathways have been recently reviewed [3,4]. In this review, we focus on the regulation of nucleocytoplasmic trafficking through alterations of the cargo proteins.

Regulated nucleocytoplasmic trafficking in plants

In plants, a number of pathways are known that involve a step of regulated nucleocytoplasmic partitioning. They group loosely into four categories, namely (1) modification (e.g. phosphorylation) or conformational change that either masks or exposes an NLS or NES; (2) heterodimerization or heterocomplex formation based on modification that allows trafficking by a 'piggyback' mechanism; (3) cytoplasmic or nuclear retention by protein–protein interactions and its release by protein modification; (4) cytoplasmic retention by membrane association and its release by proteolysis. None of these mechanisms are mutually exclusive, for example phosphorylation could modify an NLS of a protein that then allows piggybacking of another factor. In the following we highlight some recent or notable examples of each of these mechanisms.

Protein modification/conformational change

Nuclear localization of phytochromes, once a controversial finding, is now known to occur through at least two distinct mechanisms. The simplest is the case of light-stable phytochrome B (phyB) which appears to enter the nucleus through a light-mediated change in conformation that unmasks an NLS that is obscured in part by the

Box 1 Mechanism of protein nucleocytoplasmic trafficking in eukaryotes.

The nuclear pore complex (NPC) is the gateway of macromolecular trafficking between nucleus and cytoplasm. It is a large multiprotein complex consisting of multiple units of 30 different nucleoporins (Nups) [63]. Together, they form a channel-like structure with eightfold symmetry. Karyopherins, also called importins and exportins are nuclear trafficking receptors and adaptors that bind to either a nuclear localization signal (NLS) or a nuclear export (exclusion) signal (NES) of a cargo protein. Nups that contain hydrophobic phenylalanine–glycine (FG) repeats enable access of the receptor–cargo complexes to the pore, possibly by forming a hydrogel with permeability properties that allow selective access of the transport complexes [64,65]. The small GTPase Ran is required for the directionality of nucleocytoplasmic trafficking. The presence of the Ran GTPase activating protein (RanGAP) in the cytoplasm and the Ran guanosine exchange factor (RCC1) in the nucleus results in a higher nuclear RanGTP concentration. The association of RanGTP with karyopherins of the importin family inside the nucleus triggers the release of imported cargo proteins, whereas it stabilizes the interaction between karyopherins of the exportin family and export cargos. The hydrolysis of RanGTP to RanGDP outside the nucleus triggers the release of RanGDP from exportins, thus dissociating export cargo complexes (Figure 1).

N-terminal bilin-binding domain [5]. In the dark, the N-terminal domain of the Pr form of phyB directly interacts with the C-terminal PAS-related domain, restricting NLS exposure. After red-light absorbance, structural changes are presumed to occur, opening up the dimer to the NLS-dependent import machinery (Figure 2a). Once inside the nucleus, phyB sublocalizes to at least two types of nuclear bodies, whose functions are variously related to protein turnover (e.g. certain PIF proteins) or are unknown [6]. Although other light-stable phytochromes (phyC, D and E) also localize to the nucleus in the light [7], it is unclear whether they are regulated in the same way.

An interesting example of regulated partitioning through reversible conformational change is that of the master-regulator of defense gene expression, NPR1. Innate immune responses are often associated with changes in cellular oxidative states, and NPR1 localization responds to these redox changes. In nonchallenged plants, NPR1 accumulates in the cytoplasm, where it oligomerizes via intermolecular disulfide bond formation. After pathogen infection, the concentration of salicylic acid increases, causing reduction of the disulfide bonds and release of NPR1 monomers, which can then localize to the nucleus [8]. Oligomerization of proteins by forming intermolecular disulfide bonds is unusual in a reductive environment like the cytoplasm. Tada *et al.* [9] have addressed this seeming paradox by showing that oligomerization is facilitated by *S*-nitrosylation — covalent attachment of NO to a reactive cysteine — by *S*-nitrosglutathione (GSNO). In turn, monomerization is facilitated by thioredoxins and the balance between these competing reactions can fine tune the nucleocytoplasmic partitioning of NPR1 [9] (Figure 2b).

Until recently a classic example of phosphorylation-based nucleocytoplasmic trafficking in plants was that of the cytokinin-relay AHP proteins [10]. The cytokinin receptors, a family of histidine kinases (AHKs), autophosphorylate upon cytokinin binding and transfer a phosphate to histidine phosphotransfer proteins (AHP). Phosphorylated AHPs were reported to preferentially accumulate in the nucleus after cytokinin treatment [10–12]. However, Punwani *et al.* have now probed further and have found that a phosphoinsensitive version of AHP2 shows no altered partitioning. Thus, cytokinin signaling seems to still involve the transfer of the phosphate signal from the cytoplasm to the nucleus via shuttling of phosphorylated AHPs, but likely without a change in overall nucleocytoplasmic partitioning [13].

Another candidate for phosphorylation-dependent nuclear import involved in cytokinin signaling is the cytokinin response factor (CRF). CRF accumulates in the nucleus in response to cytokinin, and this accumulation depends on the histidine kinases in the pathway. However, direct evidence that nuclear import of CRF is regulated by phosphorylation is still lacking [14].

Nuclear import by heterocomplex formation

Phosphorylation by a MAPK cascade is required for the nuclear import of the transcription factor VIP1 and — by a clever piggyback mechanism — the *Agrobacterium tumefaciens* T-DNA. The imported T-DNA strand appears in the cytoplasm in a complex with two Agrobacterium proteins, VirD2 and VirE2. Nuclear import of this complex is facilitated by the host protein VIP1 [15,16]. The MAPK MPK3 is induced by stress and phosphorylates VIP1, leading to nuclear import of phosphorylated VIP1 and induction of stress-response genes. The T-DNA thus exploits the plant's MAPK defense signaling to gain nuclear access via activated VIP1 [17].

An area that has seen much progress lately is the role of nucleocytoplasmic trafficking in plant–pathogen interactions [18]. This is on the basis of the increasing number of examples of soluble immune receptors — formerly considered cytoplasmic — now found partially or exclusively in the nucleus after pathogen exposure. Only some receptors have recognizable NLS motifs, and nuclear import is triggered by pathogen effectors. In several cases, the nuclear fraction was shown to be functional, for example when the receptor was fused to an NES and this has led to a reduction of the resistance response. Some examples demonstrate that both cytoplasmic and nuclear fractions can be required, presumably for different steps of the signal transduction chain [19–22].

An interesting expansion of this theme was described by Caplan *et al.* [23•]. Nuclear import of the tobacco TIR-NB-LRR immune receptor N was previously shown to be triggered by the viral effector p50 [24]. Caplan *et al.* [23•]

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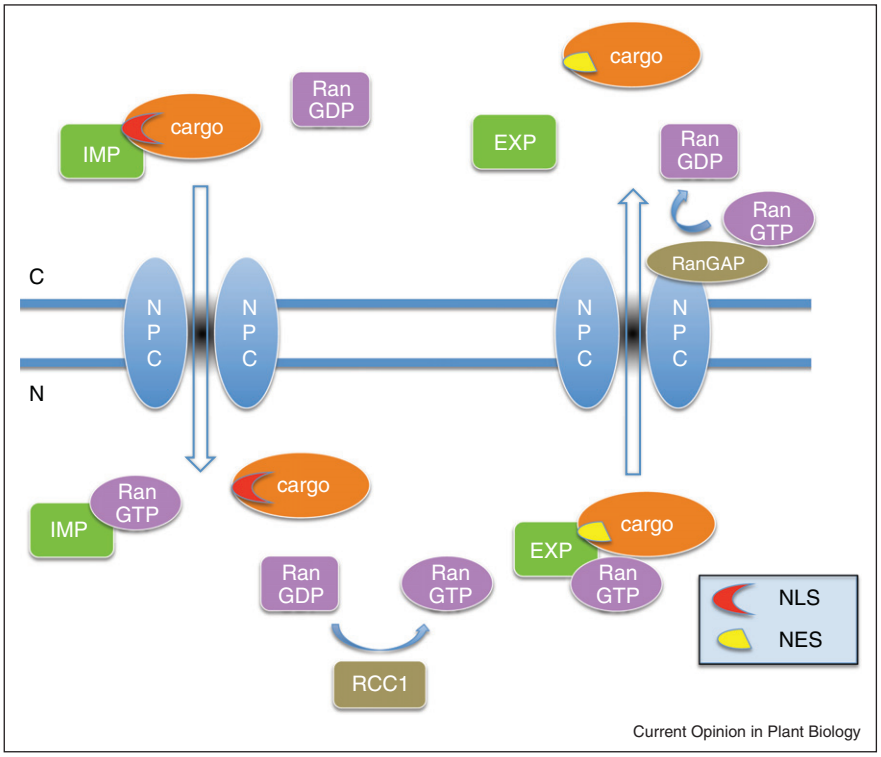
Figure 1



Simplified scheme of active protein nuclear import and export. The nuclear pore complex (NPC) provides a gateway between the cytoplasm and the nucleoplasm. Protein cargoes containing a nuclear localization signal (NLS) are bound in the cytoplasm by transport receptors of the karyopherin family (IMP). IMP often represents one import adaptor (importin α) that binds the NLS, and one import receptor (importin β) that binds importin α. IMP can traverse the NPC bidirectionally. Binding of RanGTP in the nucleus dissociates IMP from the protein cargo, trapping the cargo in the nucleus. The nuclear localization of the Ran guanosine exchange factor RCC1 provides a high nuclear RanGTP concentration. Proteins containing a nuclear export signal (NES) form a complex with a karyopherin-type export receptor (EXP) and RanGTP in the nucleus. In the cytoplasm, GTP hydrolysis of Ran is stimulated by nuclear-pore-associated Ran GTPase activating proteins (RanGAP). This dissociates the complex, trapping the export cargo in the cytoplasm. For a recent, more in-depth review on the mechanism of nucleocytoplasmic trafficking see [4].

show that this interaction is mediated by a chloroplast protein, N-receptor-interacting protein (NRIP1). In uninfected plants, NRIP1 is a chloroplast-localized functional rhodanese sulfur-transferase. In the presence of TMV p50, NRIP1 localizes both to the cytoplasm and nucleus, where it interacts with N and is required for N function (Figure 2c). The proposed mechanism is a diversion of NRIP1 from chloroplast import by p50 binding in the cytoplasm, followed by transport of the complex into the nucleus. The precise role of nuclear NRIP in the signaling is not yet known.

A similar situation has been found for RRS1, a soluble immune receptor required for full resistance against the bacterial wilt pathogen *Ralstoria solanacearum*. RRS1 binds the bacterial effector PopP2, leading to translocation of RRS1 and PopP2 to the nucleus [25]. Bernoux *et al.* [26•] have now shown that a normally vacuole-associated cysteine protease (RD19) is required for RRS1-based resistance and that upon PopP2 expression it appears in the nucleus, bound to the effector, and together with, but not bound to RRS1.

The precise role in pathogen defense of rerouting these cellular proteins to the nucleus is currently not known. It is possible that it is part of the pathogenicity itself. For example in case of RD19, disruption of vacuolar membranes might release the protease. The novel cellular location of the protein might then be recognized by the plant defense system. One might even speculate that the actual subcellular location change of the protein, and not a protein modification, could have a 'guardee' function [27].

In addition to the immune receptors discussed above, transcription factors involved in pathogen signaling can be involved in rerouting a cellular protein of presumably unrelated function. AtMYB30 is a transcription factor that acts as a positive regulator of the hypersensitive response. It interacts with a secreted phospholipase A alpha subunit ($AtPLA_2$-a) and mediates its relocalization from cytoplasmic vesicles to the nucleus. This leads to a repression of AtMYB30-based gene activation, thus dampening the hypersensitive response. These effects are independent of the enzymatic activity of $AtPLA_2$-a, suggesting a dual function of the protein [28].

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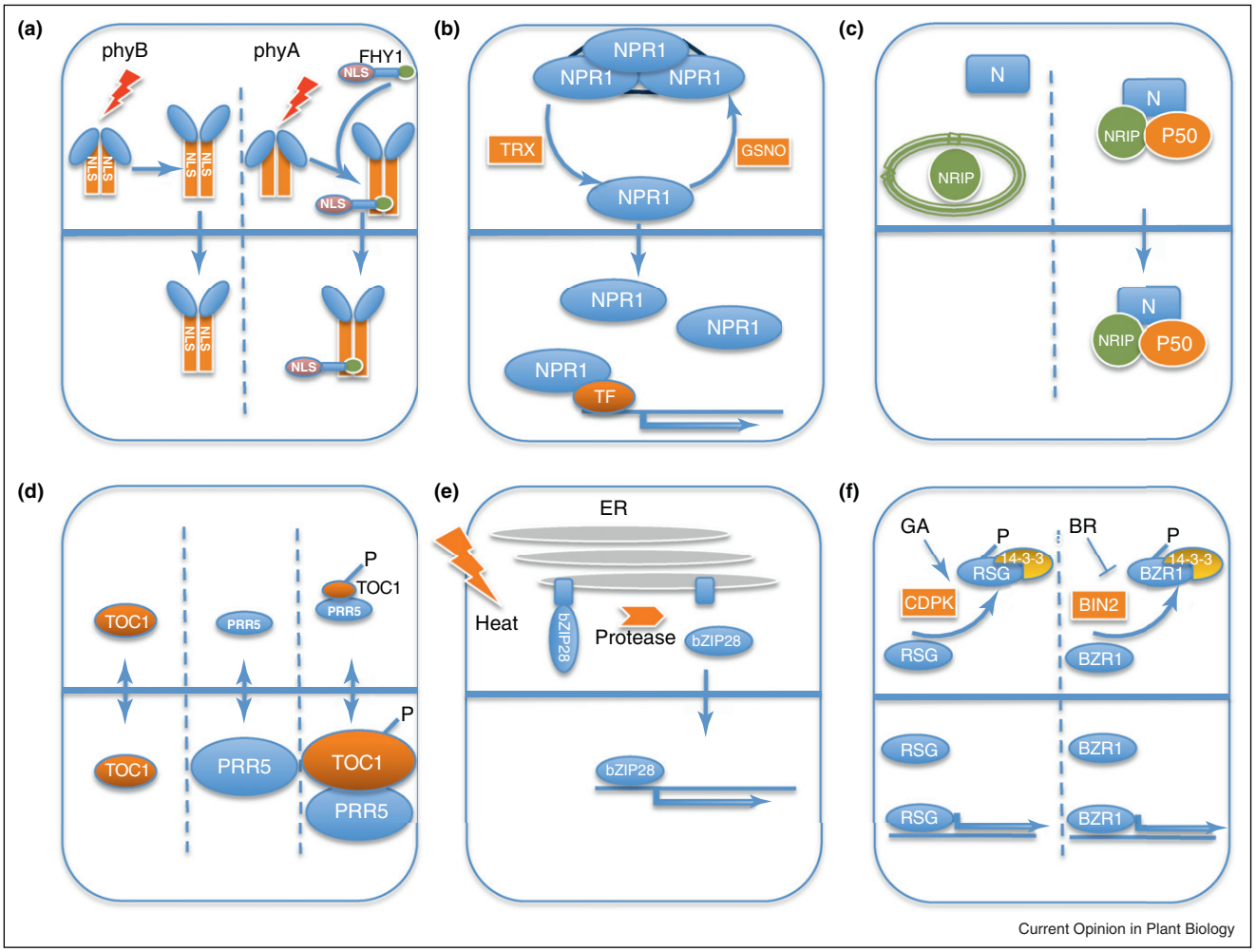
Figure 2



Select examples of regulated nucleocytoplasic trafficking of plant proteins. **(a)** Redox-mediated regulation. Thioredoxin (TRX)-mediated reduction of NPR1 complexes allows import of NPR1 monomers to the nucleus. Monomer oxidation by *S*-nitrosglutathione (GSNO) promotes oligomerization and nuclear exclusion. **(b)** Regulation by heterocomplex formation. Nucleocytoplasmic partitioning of circadian clock factor TOC1 is shifted toward the nucleus in the presence of PRR5. Heterocomplexing of the two increases TOC1 phosphorylation, but has no detectable effect on PRR5 partitioning or phosphorylation state. **(c)** Proposed nuclear import by diversion from chloroplast targeting. In the absence of the viral effector p50, NRIP is located in the chloroplast and the resistance protein N is located in the cytoplasm (left side of panel). In the presence of the viral effector p50, the chloroplast protein NRIP forms a complex with p50 and the resistance protein N in the cytoplasm, which enters the nucleus (right side of panel). **(d)** Regulation by proteolytic release from membrane association. Heat stress activates proteolytic cleavage of the ER-associated transcription factor bZIP28. Once soluble, bZIP28 is imported into the nucleus. **(e)** Phytochrome regulation by NLS exposure/association. Red light induces a conformational change in phyB, exposing an NLS leading to nuclear import (left side of panel). Red- light- induced association of FHY1 with phyA allows nuclear import of the complex via the FHY1 NLS (right side of panel). **(f)** Regulation by 14-3-3 interaction. GA initiates sequestration of RSG in the cytoplasm through CDPK1-dependent phosphorylation and subsequent binding to a 14-3-3 protein (left side of panel). BR initiates events that lead to de-phosphorylated BZR1 accumulation in the nucleus. Absent BR, BIN2 phosphorylation of BZR1 sequesters it in the cytoplasm through 14-3-3 binding, followed by proteolysis (right side of panel).

In contrast to the simple light-mediated unmasking of the phyB NLS (see above), phytochrome A (phyA) nuclear entry requires interaction with plant-specific cytosolic factors, FHY1 and/or FHL [6] (Figure 2a). Both proteins contain an NLS and NES at their N-termini and a phyA interaction domain at the C-termini and are able to homodimerize and heterodimerize through their C-termini [29]. Both are involved in trafficking phyA into the nucleus in a red-light-dependent manner, though FHY1 appears to be more important, due likely to higher expression levels [29,30]. The presence of both an NLS and NES in these proteins suggests they shuttle between the nucleus and cytosol [29], though current evidence is that phyA is partnered with them only for nuclear import. Supporting this notion is the recent finding that nuclear phyA is degraded more rapidly than

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cytoplasmic phyA, suggesting that FHY1-mediated import indirectly regulates phyA levels [31]. However, complicating the relationship, FHY1 is phosphorylated by phyA in a red-light-dependent manner [32]. It is not clear how FHY1 phosphorylation affects its interaction with phyA, but since phosphorylated FHY1 is more rapidly degraded this may provide for a phyA-mediated down-regulation of its own import [32].

In metazoan and fungal circadian clock systems, nucleo-cytoplasmic partitioning of core elements of the clock is central to circadian regulation. Phase-specific and compartment-specific phosphorylation are the best characterized mechanisms. In *Drosophila*, the cytosolic interaction between two core clock transcription regulators, PERIOD (PER) and TIMELESS (TIM) is essential in controlling the phosphorylation state, nuclear presence and activity of both proteins [33].

In the Arabidopsis clock, recent studies have identified a surprisingly similar mechanism. Two closely related circadian factors, TOC1 and PRR5, are part of a five-member family of pseudoresponse regulator (PRR) proteins that are themselves circadianly regulated [34]. TOC1 and PRR5 are phased with peak expression in the early evening and can heterodimerize *in vivo*. Fractionation experiments show a large proportion of PRR5 is nuclear at all times, independent of TOC1 levels. In contrast, partitioning of TOC1 is shifted toward the nucleus when PRR5 is overexpressed, or conversely, is more cytoplasmic in *prr5* mutants [35•] (Figure 2d). Both proteins possess a putative C-terminal NLS, and C-terminal deleted TOC1 (N-TOC1) is found exclusively in the cytosol. In the presence of PRR5, N-TOC1 becomes nuclear-localized, and a hyperphosphorylated form appears in both the cytosol and nucleus [35•]. Thus, PRR5 facilitates both nuclear accumulation and hyperphosphorylation of TOC1, which varies over the circadian cycle due, in part, to the oscillation of PRR5.

What remains unclear is whether the PRR5-mediated phosphorylation of TOC1 enhances TOC1 nuclear import, or whether the PRR5-TOC1 association alone is sufficient. Although other clock proteins are clearly phosphorylated throughout the circadian cycle [34,36], the relevance to their nucleocytoplasmic partitioning remains unclear. The LHY/CCA1-like protein LCL1 possesses demonstrably active nuclear export and nuclear localization signals [37], and it is likely that the close relatives, the myb transcription factors CCA1 and LHY, contain similar motifs.

Nuclear or cytoplasmic retention by heterocomplex formation

An interesting alternative to enhanced nuclear import through heterocomplex formation is increased nuclear retention through heterocomplex-based inhibition of nuclear export. This is the emerging model of the partitioning control of COP1, a RING-finger E3 ubiquitin ligase and central regulator of photomorphogenesis. In the dark, COP1 is enriched in the nucleus where it inhibits photomorphogenic development through proteolysis of light-regulatory transcription factors. Light inactivates COP1 in part through translocation to the cytosol [38]. COP1 possesses both an NLS and a cytoplasmic localization signal (CLS) which have been localized to discrete domains of the protein [39,40]. Although a wide array of COP1-interacting factors are known, a newly identified interaction between COP1 and the eight-subunit COP9 signalosome (CSN) now implicates CSN1 as the nucleus-sequestering factor in the dark. The CSN1-COP1 interaction occurs through a domain that overlaps with the COP1 CLS sequence, effectively blocking COP1 nuclear exclusion when the two are complexed [41•]. Interestingly, the COP1 NLS is still required for its nuclear localization, indicating that the role of CSN1 may be to simply eliminate the effectiveness of the resident COP1 CLS by shifting the competitive balance between the NLS and CLS of COP1 toward nuclear retention.

In case of the potato immune receptor Rx1 it is actually cytoplasmic retention that supports function. Rx1 confers extreme resistance to Potato virus X through interaction with the viral coat protein (CP). RanGAP (see Box 1) is an Rx-interacting protein that is required for this resistance. Rx is normally located both in the nucleus and in the cytoplasm but co-expression with RanGAP excludes Rx from the nucleus and potentiates Rx-mediated immune signaling [42••]. The cytoplasmic sequestering of Rx by RanGAP2 is independent of its GTPase activating function. Instead, the N-terminal nuclear envelope-targeting domain of RanGAP [43] is sufficient both for cytoplasmic sequestering and for enhancing the immune response, suggesting that the role of RanGAP is to increase the cytoplasmic pool of Rx. On the other hand, the nuclear pool is also required for the response [21], suggesting that both the cytoplasmic and the nuclear location of the receptor are important for function.

Low-temperature-dependent transposition (LTDT) is a well-known phenomenon of the Antirrhinum transposon *Tam3*, which transposes preferentially at low temperature. It was now shown that this is related to temperature-dependent nuclear import of the Tam3 transposase (*Tam3* TPase). *Tam3* TPase contains three functional NLSs that independently confer nuclear import to GFP. In addition, it contains a domain that inhibits nuclear import at high temperature (Nuclear Localization Inhibitory Domain, NLID). This effect is specific for Antirrhinum and does not occur in tobacco or onion cells, suggesting that one or more Antirrhinum factors are involved [44•]. Although the mechanism is still unclear, it might be a good candidate for temperature-regulated

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cytoplasmic retention by protein–protein interactions, involving the NLID. Temperature regulation of nucleocytoplasmic trafficking is an exciting emerging field. It has been known for a long time that plants are less resistant to pathogens at higher temperature. Zhu *et al.* [45•] have now used this observation for a genetic screen to identify mutants that are resistant at higher temperature. One mutant has a point mutation in the immune receptor SCN1. Introducing a similar mutation into the related immune receptor N also allowed N to confer immune resistance at higher temperature. Suppressors that regain temperature sensitivity show reduced nuclear localization of SNC1 and N, suggesting that impaired nuclear import is a reason for the temperature effect.

Cytoplasmic retention by membrane association and its release by proteolysis

A growing number of membrane-tethered transcription factors are found in Arabidopsis that are translocated to the nucleus based on a stimulus. Examples include transcription factors with a transmembrane domain (TMD) that is anchored either to the ER or to the plasma membrane.

Best characterized in this process are a group of bZIP proteins. Expression of bZIP28 in Arabidopsis is upregulated by heat and the null mutant has a heat-sensitive phenotype and an attentuated expression of the ER chaperone BiP2. Importantly, heat-stress induces the proteolytic release of bZIP28 from the ER membrane and its redistribution to the nucleus. An inducibly expressed truncated version of bZIP28 that lacks the TMD and the entire C-terminal domain was able to induce BiP2, indicating that this is the functional form of bZIP28 [46] (Figure 2e).

bZIP60 is related to bZIP28 and is involved in unfolded protein stress. It likewise translocates from the ER to the nucleus after proteolytic cleavage [47]. It is transcriptionally activated by tunicamycin and is cleaved off the membrane in response to tunicamycin or DTT. A truncated fragment not including the TMD activates tunicamycin-induced genes and translocates to the nucleus, suggesting that bZIP28 and bZIP60 follow very similar mechanisms for activation and nuclear translocation. A third example, bZIP17 is activated and relocated to the nucleus by high salt through cleavage off the ER by a subtilisin-like serine protease (S1P) [48].

Similar release mechanisms have been observed for plasma-membrane-associated proteins. The transcription factor NTL6 is proteolytically released from the plasma membrane by cold, enters the nucleus and activates a subset of pathogen response genes. Overexpression of the active form of NTL6 leads to enhanced disease resistance, suggesting a link between cold and pathogen response [49•]. Similarly, the membrane-bound transcription factor NTL8 mediates the salt regulation of seed germination via the GA pathway. NTL8 activity is regulated by controlled proteolytic release of the membrane-bound form, which is activated by high salinity [50].

These mechanisms resemble activation responses in animals [51]. Interestingly, the membrane-associated transcription factors so far known in plants are all activated by various abiotic stress conditions. This might indicate that their controlled proteolytic activation is a rapid response to fast environmental changes. In these examples, it will be important to identify the proteases responsible for the release from membrane attachment as well as to discover whether the transcription factors enter the nucleus after release simply by means of an NLS in their amino acid sequence, or whether there is an additional step involved in the cytoplasm.

Cytoplasmic retention by 14-3-3 binding

GA3, encoding ent-kaurene oxidase, acts early in the GA biosynthetic pathway. RSG (REPRESSION OF SHOOT GROWTH) is a basic leucine zipper (bZIP) transcription factor that activates *GA3* [52]. In the absence of GA, RSG distributes equally between the nucleus and the cytoplasm and part of this distribution is due to nuclear export [53]. After GA application, a rapid increase in intracellular Ca^{2+} sequesters RSG in the cytoplasm through CDPK1-dependent phosphorylation and subsequent binding to a 14-3-3 protein. This response results in a GA-mediated downregulation of its own biosynthesis [53–55] (Figure 2f).

A very similar mechanism has been uncovered for brassinosteroid signaling. Phosphorylation of the transcription factor Brassinazole-resistant 1 (BZR1) promotes binding to 14-3-3 proteins, causing cytoplasmic retention and/or nuclear export and inactivation of BZR1 [56–58] (Figure 2f). The closely related BZR2/BES1 is similarly regulated, and both family members undergo phosphorylation/dephosphorylation by GSK3-like kinases (e.g. BIN2) and the protein phosphatase BSU, respectively [59–61]. In addition to residues required for 14-3-3 binding, other phosphorylation sites on BES1 appear to be required for its nuclear export [59]. 14-3-3 proteins are emerging as regulators of many developmental processes [62] and phosphorylation-dependent binding to cytoplasmic 14-3-3 proteins might thus influence nucleocytoplasmic partitioning of more plant regulators than are currently known.

Summary and outlook

Increasingly, regulated nucleocytoplasmic transport is being recognized as a step in important plant signaling pathways, including hormone responses, responses to abiotic and biotic stresses, environmental changes, and endogenous regulatory systems like the circadian clock.

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In many cases, the first observation is a change in nucleocytoplasmic partitioning of a protein, either by cell fractionation or by microscopy. Altered partitioning is not sufficient proof of regulated nuclear import or export because other mechanisms, such as a change of protein stability in one compartment, may affect abundance. In most examples discussed above a specific molecular mechanism affecting import/export can be attributed to the change in nucleocytoplasmic partitioning of a given protein.

Future questions for these pathways include: What is the underlying basis of regulated nucleocytoplasmic partitioning? What is the upstream signaling chain that leads to the switch? And, what is the downstream signaling role of the partitioned protein(s)? Further investigations of the underlying bases might probe into whether they involve unmasking/addition of a canonical NLS or NES or if import/export is regulated by other transporters or by direct interaction with nuclear pore components. The upstream signaling steps are often unknown, but likely will involve discovery of the kinases, proteases, *etc.* that modify the cargo protein or its interactors. The functional role of the altered partitioning is another important aspect to investigate. Is the primary function in the nucleus, in the cytoplasm, or does the protein have a role in both compartments? Does upregulated nuclear import activate or dampen a response?

Finally, since nucleocytoplasmic trafficking is not an all-or-nothing response more quantitative methods are needed that can easily allow measuring or imaging of protein abundance, import and export rate and actual concentration changes. Photoactivatable fluorescent markers combined with real-time imaging should allow for this level of resolution. Trafficking-regulated proteins combined with such markers in semi-automated mutant screens could provide powerful tools to further unravel the regulatory components in the respective pathways.

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- • of special interest
- •• of outstanding interest

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