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REVIEW

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Nuclear import and export of proteins in plants: a tool for the regulation of signalling

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Keywords *Arabidopsis* (signal transduction) · Nuclear export/import · Nucleo-cytoplasmic partitioning · Protein (nuclear export/import) · Signal transduction

Abbreviations CPRF: common plant regulatory factor · GFP: green fluorescent protein · GUS: β-glucuronidase · IBB: Importin β-binding · LMB: leptomycin B · NES: nuclear export signal · NLS: nuclear localisation signal · NPC: nuclear pore complex

Introduction

Compartmentation is a characteristic feature of eukaryotic cells. It provides the possibility of concentrating specific sets of macromolecules and metabolites and separating cellular processes from one another, thereby creating specific micro-environments within the cell. Moreover, it allows control of the traffic of macromolecules and regulation of the transduction of signals between the individual compartments of a cell. Two key cellular processes are separated by the nuclear envelope: transcription takes place in the nucleus whereas proteins are synthesised in the cytoplasm. This implies that many different classes of macromolecule must be exchanged constantly and at high rates between the nucleus and the cytoplasm in order to ensure proper regulation and course of events of these processes in a living cell. Since the nuclear envelope provides a diffusion barrier for macromolecules, a complex transport machinery has evolved that is highly conserved from yeast to plants and mammals. This review introduces the current view of the regulatory mechanisms of well-investigated nuclear transport pathways that have emerged from work in animals and yeast, summarises work on nucleo-cytoplasmic transport in plants, and focuses on the importance of nuclear transport for the regulation of various signal-transduction pathways in plants.

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Nuclear transport of macromolecules is receptor-mediated

After the molecular characterisation of the first nuclear import receptor in 1994 the existence of a gene family encoding nuclear transport receptors was discovered (Görlich et al. 1994, 1995, 1997). These receptors are able to interact with components of the nuclear pore (nucleoporins), with the regulatory GTPase Ran in its GTP-bound form, and they shuttle continuously between the nucleus and the cytoplasm. Members of this nuclear transport receptor family are 90–130 kDa in size and share an amino-terminal Ran-GTP-binding domain, which shows limited sequence conservation. They bind very specifically to the different classes of macromolecule that have to be transported across the nuclear envelope. In general, the presence of nuclear transport receptors guarantees the efficiency and the high rates of exchange of macromolecules between the nucleus and the cytoplasm. The specificity of the transport processes is ensured by the variety of receptors that recognise different substrates (for reviews, see Corbett and Silver 1997; Mattaj and Englmeier 1998; Görlich and Kutay 1999). Only recently have kinetic experiments of nuclear transport processes revealed maximal rates in the order of 10^3 translocation events per second per nuclear pore (Ribbeck and Görlich 2001).

Transport processes across the nuclear envelope take place at gigantic proteinaceous pores called nuclear pore complexes (NPCs), which are embedded in the nuclear envelope and provide the gates between the nucleus and the cytoplasm. In vertebrates, the estimated molecular mass of the NPC is 125 MDa, in *Saccharomyces cerevisiae* about 60 MDa. NPCs have a

highly conserved architecture and show an 8-fold rotational symmetry, which includes the cytoplasmic fibrils, the central gated channel, spokes, rings, and the nuclear basket (for review, see Stoffler et al. 1999). Genetic and immuno-electron microscopy studies have revealed the function and the localisation of various nucleoporins. Some of them provide important contacts for proteins belonging to the soluble transport machinery (Fornerod et al. 1997a; Stoffler et al. 1999; Ryan and Wente 2000). However, the classical view of the NPC as a stable assembly of the transport apparatus is changing, since recent findings have shown that some nucleoporins are mobile and not permanently associated with the NPC (Nakielny et al. 1999; Zolotukhin and Felber 1999).

Nuclear import of proteins

Nuclear import is signal-mediated, like the other nuclear transport processes. The classical nuclear localisation signal (NLS) was first characterised on the simian virus (SV40) large T-antigen and consists of a short stretch of basic amino acid residues, mostly lysines. This kind of signal, which is also referred to as a monopartite NLS, is necessary and sufficient to label a protein for rapid transfer to, and accumulation in, the nucleus. In addition, it is a permanent and transferable signal. A variant of the monopartite NLS is the bipartite signal, which consists of two short stretches of basic residues separated by a short spacer. The first step in the nuclear import of a karyophilic protein that contains a classical basic monopartite (SV40-like) or bipartite NLS is its specific binding to the cytoplasmic NLS receptor Importin α (also called Karyopherin α or Kap α; Görlich et al. 1994). However, Importin α is not the nuclear transport receptor itself. The import receptor that is able to interact with the NPC is Importin β (also called Karyopherin β or Kap β; Görlich et al. 1995). Importin α serves as an adapter protein that bridges the interaction of classical NLS-containing proteins with Importin β (Fig. 1A). After the co-operative formation of a complex consisting of Importins α and β and the NLS cargo protein in the cytoplasm, this triple import complex docks as an integrated whole to the cytoplasmic side of the NPC via Importin β. Following translocation through the nuclear pore, a process that is still under investigation, the import complex is dissociated in the nucleus by the action of Ran-GTP (see Fig. 1A). Since the binding sites for Importin α and for Ran-GTP on Importin β overlap and the concentration of Ran-GTP is high in the nucleus, the competition between the two proteins for Importin β leads to the displacement of Importin α from Importin β and to the formation of a complex of Importin β with Ran-GTP (Izaurralde et al. 1997; Kutay et al. 1997a). As a result, the NLS-containing cargo protein is released into the nucleoplasm and Importin β is recycled back to the cytoplasm in a complex with Ran-GTP. There, the GTP on Ran is hydrolysed, which results in the dissociation of the complex of Importin β and Ran. Importin β is then ready for a new round of nuclear import (see Fig. 1A and below). Importin α also has to be recycled back to the cytoplasm, which is accomplished by a specific export receptor termed CAS (cellular apoptosis susceptibility protein). CAS is also a member of the Importin β family of nuclear transport receptors and was originally believed to be implicated in apoptosis and cell proliferation before it was characterised as the nuclear export receptor for Importin α, like its homologue from yeast, Cse1p (Kutay et al. 1997b; Hood and Silver 1998; Künzler and Hurt 1998; Solsbacher et al. 1998).

Importin α interacts with Importin β by means of its arginine-rich amino terminus, which is called the Importin β-binding (IBB) domain and may constitute an archaic nuclear import signal (Görlich et al. 1996a; Weis et al. 1996). Proteins that are imported into the nucleus by direct interaction with Importin β contain such an IBB-like domain. It is characterised by stretches of positively charged residues, but is considerably larger than a classical basic NLS and its localisation is more restricted. For optimal interaction with Importin β this domain is localised at the amino terminus of the cargo protein (Görlich et al. 1996a). Accordingly, one reason for the existence of an adapter protein for Importin β-dependent nuclear import may be its ability to recognise smaller signals that are less conserved and may be localised almost anywhere on the surface of a karyophilic protein.

There are also many other proteins that are imported into the nucleus and that neither contain an IBB-like domain nor a classical basic NLS (for review, see Görlich and Kutay 1999). Nuclear import of these proteins depends on other receptors, which in most cases are also members of the Importin β family. A well-investigated example of a nuclear import pathway that is different from that of Importin β depends on the nuclear import receptor Transportin 1 (Pollard et al. 1996). This Importin β-like protein recognises a nuclear import signal that is completely different from the classical basic NLS and is found on some heterogeneous nuclear ribonucleoproteins (hnRNPs). These proteins are implicated in pre-mRNA splicing and/or mRNA export out of the nucleus. Some hnRNPs are confined to the nucleus whereas others are exported from the nucleus in a complex with mRNA, like hnRNP A1 (Pinol-Roma and Dreyfuss 1992). In the cytoplasm, hnRNP A1 is stripped off the RNA and is recycled to the nucleus by direct interaction with Transportin 1 (Pollard et al. 1996; Bonifaci et al. 1997). The signal that is recognised by the import receptor Transportin 1 is called the M9 domain and is located in the carboxy terminus of hnRNP A1 (Michael et al. 1995; Siomi et al. 1998). When fused to a heterologous protein, the M9 domain confers rapid nucleo-cytoplasmic shuttling to this protein, because this signal contains nuclear import and nuclear export activities. Attempts to separate the two activities by mutational analysis failed, since

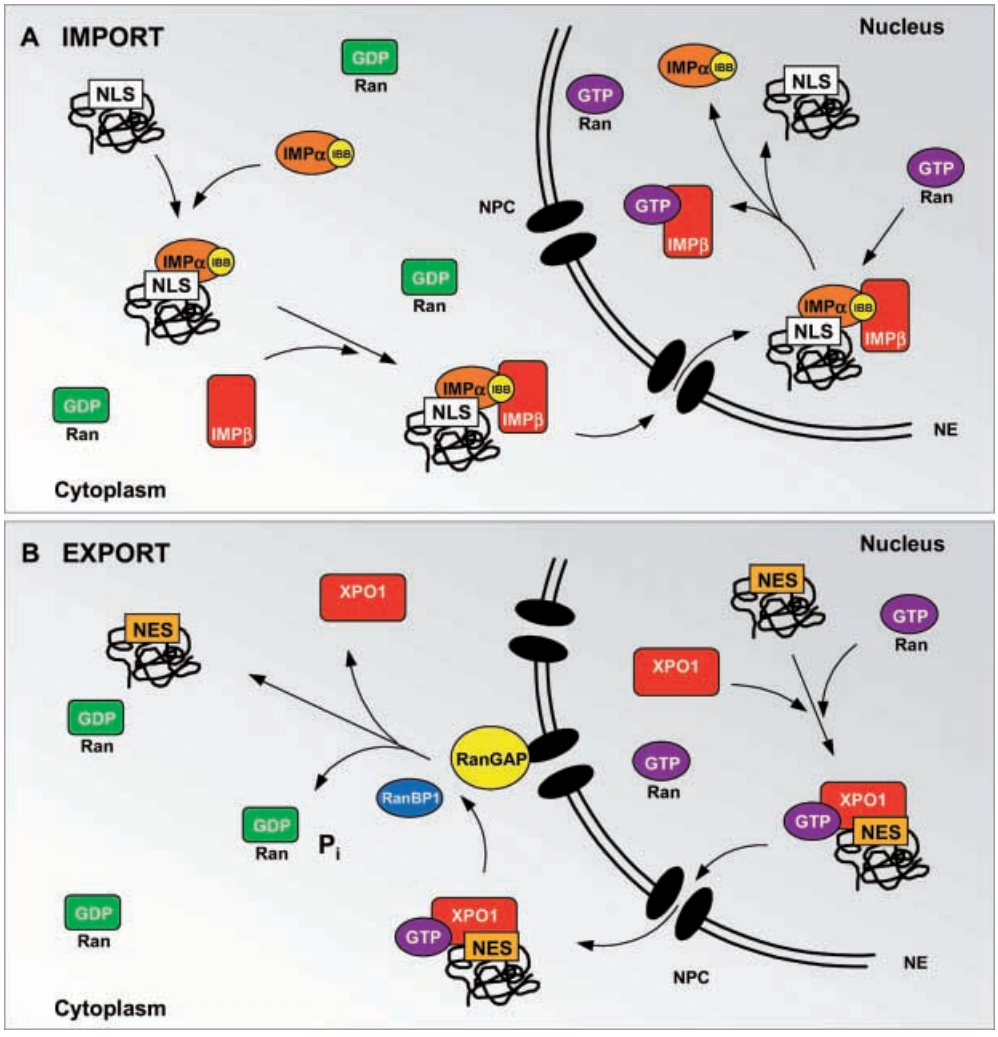

Fig. 1A, B Comparison of nuclear import and export of proteins. **A** Nuclear import of NLS-containing proteins is initiated by the specific recognition of the NLS of a karyophilic protein by the NLS receptor Importin α (*IMPα*) in the cytoplasm. The nuclear import receptor Importin β (*IMPβ*) binds co-operatively to the Importin α/cargo protein complex in the absence of Ran-GTP, since the concentration of Ran-GTP is low in the cytoplasm. The triple import complex then docks to the nuclear pore via Importin β and, after translocation into the nucleus, it is dissociated by the action of Ran-GTP, which is present in high concentrations in the nucleus. This leads to the release of Importin α and the import cargo protein into the nucleoplasm. **B** Nuclear export of proteins that contain a leucine-rich NES is accomplished by the export receptor Exportin 1 (*XPO1*), which directly and specifically binds to the NES and to Ran-GTP in a co-operative manner in the nucleoplasm. After translocation of this triple export complex through the NPC into the cytoplasm, the co-ordinated action of the cytosolic proteins RanBP1 and RanGAP leads to the hydrolysis of GTP on Ran and to the dissociation of the export complex, resulting in the release of the export cargo in the cytoplasm. *IBB* Importin β-binding domain, *NE* nuclear envelope, *NPC* nuclear pore complex, *RanBP1* Ran-binding protein 1, *RanGAP* Ran-specific GTPase-activating protein, *Pi* inorganic phosphate

mutations that inhibited the import activity also blocked the export, and vice versa (Michael et al. 1995; Bogerd et al. 1999). The nuclear export receptor for M9 domain-containing proteins has not been identified to date. The import receptor Transportin 1, however, cannot be responsible for both transport directions, since complexes of Transportin 1 and M9-containing cargo proteins are dissociated by Ran-GTP (Siomi et al. 1997; Bogerd et al. 1999).

Nuclear export of proteins

Short signals conferring rapid nuclear export of proteins were first discovered in the HIV protein Rev and in the inhibitor PKI of the cAMP-dependent protein kinase (PKA) in humans (Fischer et al. 1995; Wen et al. 1995). These nuclear export signals (NES) were characterised as a short peptide sequence with a specific spacing of leucine or other long-chain hydrophobic amino acid residues. In 1997, the receptor that specifically recognises these leucine-rich NESs was identified and functionally characterised (Fornerod et al. 1997b; Fukuda et al. 1997; Ossareh-Nazari et al. 1997; Stade et al. 1997). It turned out that the protein had been identified before as Crm1p (chromosome region maintenance protein 1) in *Schizosaccharomyces pombe* mutants showing deformed chromosome domains (Adachi and Yanagida

1989). Due to its function as a nuclear export receptor for proteins in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Drosophila*, *Xenopus*, humans, and plants (Haasen et al. 1999a; Callanan et al. 2000; Collier et al. 2000; Fasken et al. 2000) it was renamed Exportin 1 (XPO1). It also belongs to the Importin β family of nuclear transport receptors and, like all members of this protein family, XPO1 contains an amino-terminal Ran-binding domain. In contrast to Importin β, however, the binding of Ran-GTP to XPO1 does not dissociate the cargo substrate. XPO1 binds NES-containing proteins directly and in a co-operative manner with Ran-GTP in the nucleus (see Fig. 1B). This triple export complex then docks to the nucleoplasmic side of the NPC and is subsequently translocated to the cytoplasm (Fornerod et al. 1997a; Neville et al. 1997). There, the co-ordinated action of two cytosolic regulatory proteins (Bischoff et al. 1995; Bischoff and Görlich 1997), namely the Ran-binding protein 1 (RanBP1) and the GTPase-activating protein for Ran (RanGAP1), leads to the hydrolysis of GTP on Ran, which results in the dissociation of the entire export complex. The export cargo is released into the cytoplasm, and the hydrolysis of GTP on Ran renders this step irreversible. The export receptor XPO1 is recycled to the nucleus due to its ability to interact with nucleoporins and is ready for a new export cycle (Fig. 1B).

Although this is the best-investigated nuclear export pathway, there are other signals for nuclear export of macromolecules that are recognised by different export receptors (for review, see Görlich and Kutay 1999). An example that has already been mentioned is human CAS and *Saccharomyces cerevisiae* Cse1p, which were characterised as nuclear export receptors for Importin α (Kutay et al. 1997b; Hood and Silver 1998; Künzler and Hurt 1998; Solsbacher et al. 1998). Interestingly, XPO1 is also implicated in the export of an import adapter, similar to CAS, which recycles the import adapter Importin α to the cytoplasm. Snurportin1 is a 45 kDa protein that contains an amino-terminal Importin β-binding domain and serves as an adapter protein mediating nuclear import, in the same way as Importin α. However, Snurportin1 specifically binds to m_3G-capped small nuclear ribonucleoprotein particles (snRNPs) in the cytoplasm to initiate their nuclear import (Huber et al. 1998). After the translocation into the nucleus and the dissociation from the cargo and Importin β, Snurportin1 is recycled to the cytoplasm by XPO1. However, Snurportin1 does not contain a short leucine-rich NES, but a rather large domain that is involved in the interaction with XPO1 (Paraskeva et al. 1999).

Directionality of nuclear transport processes is regulated by a G protein

The GTPase Ran (Ras-related nuclear protein) is the key regulator of all nuclear transport processes mediated by members of the Importin β receptor family. It is found in the nucleus and in the cytoplasm of mammalian and plant cells (Bischoff and Ponstingl 1991a; Merkle et al. 1994). Like all G proteins, Ran can exist in two stable conformations, in which Ran is associated either with GDP or with GTP. The intrinsic enzymatic activities of Ran for the nucleotide exchange reaction and for the GTP hydrolysis reaction are very low. These properties of Ran create a bona fide molecular switch. In addition, Ran is exceptional within the superfamily of small G proteins, since it is the only Ras-related GTPase which is soluble and which shuttles continuously between two cellular compartments (Moore and Blobel 1993).

The GTPase cycle of Ran is distributed asymmetrically between the nucleus and the cytoplasm (Izaurralde et al. 1997; see Fig. 2), because the proteins that regulate the Ran GTPase cycle show different localisations. The GTPase-activating protein that is specific for Ran (RanGAP1) is localised in the cytoplasm (Matunis et al. 1996; Mahajan et al. 1997). Together with Ran-binding protein 1 (RanBP1), which is also restricted to the cytoplasm and acts as a co-activator for RanGAP1 (Bischoff et al. 1995; Bischoff and Görlich 1997), this protein ensures that concentrations of Ran-GTP are very low in the cytoplasm. A part of the RanGAP1 pool in mammalian cells was shown to be modified by a ubiquitin-like polypeptide, which is essential for RanGAP function. The modification by SUMO1 (small ubiquitin-like modifier 1) results in the association of RanGAP1 with the cytoplasmic side of the NPC (Matunis et al. 1996; Mahajan et al. 1997). The other regulatory protein of the Ran GTPase cycle, the Ran-specific guanine nucleotide-exchange factor (RanGEF), is confined to the nucleus. In humans, this protein is called RCC1 (regulator of chromosome condensation 1) and is associated with chromatin (Bischoff and Ponstingl 1991b). As a result, levels of Ran-GTP are high in the nucleus.

The asymmetric distribution of the regulatory proteins RanGAP1/RanBP1 and RanGEF predicts a steep gradient of Ran-GTP across the nuclear envelope. In a number of elegant experiments it was demonstrated that this gradient of Ran-GTP is maintained in the cell and that it is used to regulate the directionality of transport processes between the nucleus and the cytoplasm. Nuclear transport receptors that belong to the Importin β family of proteins are able to interact with Ran-GTP. These proteins interact with Ran-GTP in a way that either promotes their association with, or dissociation from, their substrates, depending on whether they are export receptors or import receptors (Görlich et al. 1996b; Izaurralde et al. 1997). Importins, like Importin β and Transportin 1, bind their substrates only in the absence of Ran-GTP, whereas exportins, like XPO1 and CAS, bind to their substrates only in presence of, and co-operatively with, Ran-GTP. This explains why import complexes only form in the cytoplasm and dissociate in the nucleus, whereas export receptors bind to their cargo only in the nucleus and release it in the

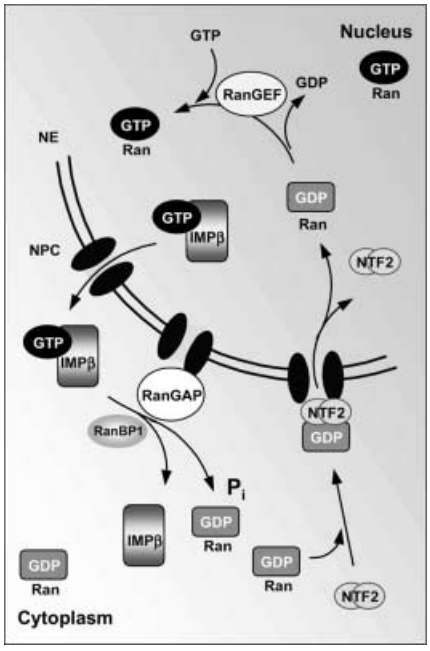

Fig. 2 The GTPase cycle of Ran during interphase. Ran-GTP is exported from the nucleus in a complex with nuclear import factors that are recycled back to the cytoplasm, like with Importin β (*IMPβ*), or as a component of export complexes (see Fig. 1B). Once in the cytoplasm, these complexes are rapidly dissociated by the concerted action of RanBP1 and RanGAP. These regulatory proteins are confined to the cytoplasm and ensure low Ran-GTP levels by catalysing the hydrolysis of GTP on Ran. Nuclear import of Ran is accomplished by nuclear transport factor 2 (*NTF2*). This import receptor specifically binds to Ran in its GDP-bound form in the cytoplasm. In the nucleoplasm, Ran-GDP is rapidly converted to Ran-GTP by RanGEF, which is a chromatin-associated protein. The result is a steep gradient of Ran-GTP across the nuclear envelope: concentrations of Ran-GTP are high in the nucleus and very low in the cytoplasm. *NE* Nuclear envelope, *NPC* nuclear pore complex, *RanBP1* Ran-binding protein 1, *RanGAP* Ran-specific GTPase-activating protein, *Pi* inorganic phosphate, *RanGEF* Ran-specific guanine nucleotide exchange factor

cytoplasm. The importance of the Ran-GTP gradient for the directionality of nuclear transport was directly demonstrated by experiments that disrupted the Ran-GTP gradient, e.g. by micro-injection of RanGAP1 into the nucleus (Izaurralde et al. 1997). Such experiments resulted in the inhibition of transport processes. The direction of nuclear transport through the NPC could even be inverted in vitro by experimentally inverting the Ran-GTP gradient (Nachury and Weis 1999). Again, this clearly demonstrated that the gradient of Ran-GTP across the nuclear envelope defines the identity of the two compartments with respect to nuclear transport.

Recycling of Ran

As mentioned above and shown in Fig. 1, Ran-GTP is exported from the nucleus in a complex with Importin β-like transport receptors during each and every transport cycle, either as part of a triple export complex or during recycling of an import receptor back to the cytoplasm. With regard to the high rates of nuclear transport reactions, this would quickly result in depletion of Ran from the nucleus. Therefore, the nuclear transport factor 2 (NTF2) compensates for the export of Ran-GTP by catalysing the nuclear import of Ran-GDP (Moore and Blobel 1994; Ribbeck et al. 1998; Smith et al. 1998). NTF2, which does not belong to the Importin β receptor family, specifically binds Ran-GDP in the cytoplasm and, besides its function as a nuclear import receptor for Ran, also acts as a GDP dissociation inhibitor of Ran (Yamada et al. 1998). A summary of the Ran GTPase cycle and the nuclear import and export of Ran is given in Fig. 2. Hydrolysis of GTP on Ran in the cytoplasm is thought to ensure completeness of the Ran GTPase cycle and recycling of all transport factors rather than to directly provide energy to the transport processes. It was shown that GTP hydrolysis on Ran is not necessary for the translocation process across the NPC per se (Nakielny and Dreyfuss 1997; Ribbeck et al. 1999).

Importin β-like transport receptors and the import receptor for Ran, NTF2, are not the only classes of Ran-binding proteins. A third class is represented by RanBP1, which contains a Ran-GTP binding domain that is entirely different from that of the Importin β-like proteins (Beddow et al. 1995). Proteins harbouring this type of Ran-binding domain do not contain GTPase activity on their own, but are able to increase the rate of RanGAP-catalysed GTP hydrolysis on Ran considerably (see above). Another class of Ran-binding domain was found to reside on some nucleoporins, where zinc finger domains act as targets for the binding of Ran-GDP (Nakielny et al. 1999; Yaseen and Blobel 1999). Interestingly, RanBP2/Nup358 is a nucleoporin that, besides this novel type of Ran-binding domain, also contains four RanBP1-like Ran-binding domains. This extremely large nucleoporin is located on the cytoplasmic fibrils of the NPC (Wu et al. 1995; Yokoyama et al. 1995). The fraction of RanGAP1 that is modified by the addition of SUMO1 is also found at the cytoplasmic side of the NPC, in close interaction with RanBP2 (Matunis et al. 1996; Mahajan et al. 1997; Saitoh et al. 1997)). Therefore, with its RanBP1-like domains, RanBP2 could also provide the co-activation activity for RanGAP1-catalysed GTP hydrolysis on Ran. Finally, an NTF2-related export protein 1 (NXT1) was identified recently as a nuclear transport factor that continuously shuttles between the nucleus and the cytoplasm (Black et al. 1999). In contrast to NTF2, NXT1 binds Ran-GTP and regulates both XPO1-dependent and XPO1-independent nuclear export processes (Black et al. 2001; Ossareh-Nazari et al. 2000). In this way, NXT1 may also contribute to nuclear export of Ran.

Functions of the GTPase Ran and of Importins α and β during mitosis

Interestingly, Ran is not only the key regulator of the directionality of nuclear transport processes during

interphase, but is also involved in the regulation of spindle assembly during mitosis in mammalian cells. The high local concentration of Ran-GTP provided by the chromatin-bound RanGEF activity of RCC1 is necessary for spindle formation (Carazo-Salas et al. 1999; Kalab et al. 1999; Ohba et al. 1999; Wilde and Zheng 1999). Recently it has been demonstrated that Importin α and Importin β bind and thereby inactivate a microtubuli-associated protein and a protein that is essential for aster-promoting activity, respectively (Gruss et al. 2001; Nachury et al. 2001; Wiese et al. 2001). Upon the action of Ran-GTP, which is present in high concentrations in close proximity to the chromosomes, these proteins are released to promote the formation of spindle microtubuli. The mitotic role of Ran is thus largely mediated by Importin α and Importin β, which inhibit spindle formation by sequestering essential protein factors, and Ran-GTP generally functions by locally releasing protein cargoes from nuclear transport factors, which also serve to regulate spindle formation during mitosis.

Vertebrates and higher plants undergo open mitosis, which implies that the nuclear envelope has to be reassembled at the end of mitosis by fusion of nuclear envelope vesicles around the decondensing chromatin. Nuclear envelope formation was shown to depend on the generation of Ran-GTP by RCC1 and on GTP hydrolysis on Ran (Hetzer et al. 2000; Zhang and Clarke 2000a). Nuclear envelope formation could even be induced independently of the presence of chromatin by providing beads that were coated with Ran in *Xenopus* egg extracts (Zhang and Clarke 2000a). The GTPase cycle of Ran, brought about by RanGAP and RCC1, is necessary for this activity and controls both the recruitment of nuclear envelope vesicles and their fusion to re-assemble the nuclear envelope (Zhang and Clarke 2000b).

Regulation of signalling by regulating nuclear transport

The role of nuclear transport in the regulation of transcription

The localisation of transcription and translation in two different compartments of the eukaryotic cell provides a link between nuclear transport and the regulation of transcription. Besides the activity of a transcription factor that may be subject to regulation, changes in the localisation may be also used as a molecular switch in response to intra- and extracellular signals that affect growth, development, or differentiation. Both regulatory mechanisms affect the half-life of a transcription-factor activity in the nucleus, which is crucial for the regulation of transcription, and very often both mechanisms are operational at the same time.

Until recently, changes in the nucleo-cytoplasmic localisation of a protein were considered to reflect changes in the rate of import, only. However, it is now well established that the steady-state level of a protein within the nucleus may also be controlled by the regulation of nuclear export. The regulatory potential of nuclear export is similar to that of nuclear import. In both cases, the first step in nuclear transport is the specific recognition of a signal sequence by the receptor protein. Therefore, the rate of transport in either direction may be regulated by interference with the binding of the receptor protein to the signal sequence of the cargo protein (Fig. 3). This may occur by modifications such as phosphorylation, by shielding of the NLS or NES as a result of a conformational change of the protein (intramolecular masking), by interaction with another protein (intermolecular masking), or by anchoring the protein to a fixed structure in one compartment (cytoplasmic or nuclear retention). One transport step, either nuclear import or nuclear export, may be regulated and the other may be default, or both may be regulated, resulting in a binary switch mechanism for the control of localisation. To make this picture even more complex, not only the transcription factors themselves but also interacting proteins that modulate their activity may be subject to regulated transport into or out of the nucleus in response to a stimulus. The diverse possibilities regarding the regulation of nuclear transport in combination with the regulation of the activities of transcription factors provide a multifaceted network of mechanisms for the control of signalling.

A third factor that may influence the half-life of a protein in a specific cellular compartment or its status of activity is protein degradation (Fig. 3). Protein degradation may be also regulated and constitutes an important regulatory tool in several signal-transduction chains (for reviews, see Callis and Vierstra 2000; Karniol and Chamovitz 2000).

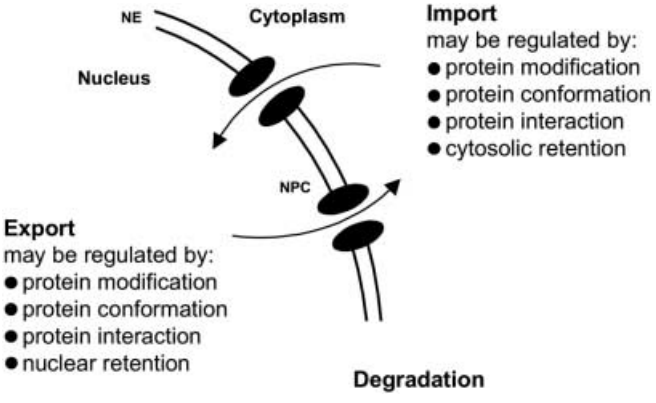

Fig. 3 Factors that influence the nucleo-cytoplasmic localisation of a protein. The steady state of the localisation of a protein may be changed by regulating the rate of its nuclear import or export. In addition, protein degradation may influence the half-life of a protein in a cellular compartment. Degradation may occur in the cytoplasm and in the nucleus. If a protein is not a substrate for nuclear export, the steady state of its localisation may be controlled by regulating the rate of nuclear import and degradation. *NE* Nuclear envelope, *NPC* nuclear pore complex

Transcriptional regulation by localisation – examples from animals and yeast

In many cases, transcription is controlled on several levels and by more than one mechanism, resulting in a partially redundant network of regulatory switches (for review, see Kaffman and ÓShea 1999; Yoneda 2000). This principle of redundancy and the regulatory potential of nuclear import and export are illustrated by the following examples of transcriptional control by Pho4 in yeast, by NF-AT4, a member of the family of nuclear activators in T-cells, and by NF-κB in mammals.

When yeast cells are grown in phosphate-rich medium, the transcription factor Pho4, which is involved in the phosphate-starvation response, is phosphorylated by the cyclin-dependent cyclin kinase (CDK) complex Pho85-Pho80 and is localised in the cytoplasm. Under conditions where phosphate is limiting, the CDK inhibitor Pho81 inactivates Pho85-Pho80, Pho4 is de-phosphorylated and is imported into the nucleus to activate the transcription of phosphate-responsive genes together with Pho2. Pho4 is imported by Pse1 and is exported by Msn5, which both are members of the Importin β family (Kaffman et al. 1998a, b). Interestingly, a mechanism involving phosphorylation at four different residues regulates the function of Pho4 at three different levels. Phosphorylation on two sites is essential for the interaction with Msn5 and hence for nuclear export, and phosphorylation of a third residue within the nuclear localisation signal of Pho4 reduces its affinity for the import receptor Pse1 and inhibits nuclear import. Finally, phosphorylation at a fourth position inhibits the transcriptional activity of Pho4 by preventing the interaction with Pho2 (Komeili and ÓShea 1999). In summary, the four phosphorylation sites have distinct and independent roles and control the activity of Pho4 by regulating nuclear localisation and interaction with Pho2.

The NF-AT transcription factors comprise a small gene family consisting of four members. Their nucleo-cytoplasmic localisation is regulated in response to intracellular calcium levels. In resting cells, NF-AT4 resides in the cytoplasm because its NLS is masked by phosphorylation of critical serine residues in the amino-terminal regulatory domain. Elevated levels of intracellular calcium during stimulation of T-cells induce the activation of the phosphatase calcineurin, which dephosphorylates these residues, and the subsequent exposure of the NLS results in nuclear import of NF-AT4 (Zhu et al. 1998). Upon decrease of intracellular calcium levels, NF-AT4 is re-localised to the cytoplasm. This re-localisation is dependent on nuclear export by Xpo1/Crm1, and is accompanied by the re-phosphorylation of the regulatory domain of NF-AT (Shibasaki et al. 1996; Zhu and McKeon 1999). Nuclear import, however, is not sufficient for the activation of target genes by NF-AT4, but also requires masking of two NESs by calcineurin. Calcineurin is co-imported into the nucleus with NF-AT and stays bound to high-affinity sites that overlap with the NESs. When calcium levels drop, calcineurin dissociates from NF-AT4, which results in the exposure of its NESs and rapid nuclear export by Xpo1/Crm1 (Zhu and McKeon 1999). Differential phosphorylation and the mutually exclusive binding of calcineurin and Xpo1/Crm1 thus constitute a strictly regulated mechanism for the calcium-sensitive nucleo-cytoplasmic shuttling of NF-AT4.

NF-κB is a transcription factor that functions in immune and inflammatory responses and in the regulation of apoptosis. In unstimulated cells, NF-κB pre-exists in the cytoplasm as a heterodimer (p65/p50) that is bound to its inhibitor IκB. The best-characterised IκB protein is IκBα, which is able to inactivate NF-κB by interfering with DNA binding and by masking the carboxy-terminal NLS (Beg et al. 1992; Ganchi et al. 1992). Activation of a kinase by different environmental stimuli leads to the phosphorylation of IκBα, resulting in the rapid degradation of IκBα and in the unmasking of the NLS of NF-κB (Chen et al. 1995; Scherer et al. 1995; Traenckner et al. 1995). Subsequent import of NF-κB into the nucleus allows the activation of target genes. Besides various other genes, NF-κB also activates the transcription of IκBα and thereby regulates its own activity in a feedback loop (Sun et al. 1993; Cheng et al. 1994; Chiao et al. 1994). Newly synthesised IκBα enters the nucleus, binds to and thereby inhibits NF-κB, and mediates its export to re-establish a cytoplasmic pool of NF-κB/IκBα complexes (Zabel and Baeuerle 1990; Arenzana-Seisdedos et al. 1995, 1997). IκBα continuously shuttles between the nucleus and the cytoplasm as a consequence of its nuclear export due to the presence of an NES in its amino terminus that is recognised by Xpo1/Crm1 (Johnson et al. 1999; Huang et al. 2000) and its nuclear import, which is still under investigation. IκBα functions as a molecular switch of NF-κB activity that controls its cytoplasmic localisation by its ability to block nuclear import and to promote nuclear export, and controls its transcriptional activity by inhibiting DNA binding of NF-κB.

Nucleo-cytoplasmic trafficking in plants

Plant transport factors and plant-specific features of nuclear transport

Like in vertebrates, many different Importin α proteins have been described in *Arabidopsis thaliana*. Six cDNAs encoding different proteins have been reported so far, which probably originate from four different genes (see Table 1). A computer search using all known *Arabidopsis* Importin α protein sequences against the complete genome at The Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org) revealed the presence of even more genes encoding Importin α-like proteins. Altogether, eight genes and one pseudogene were found in the *Arabidopsis* genome (summarised in Table 1 and Fig. 4). The protein sequences of

Table 1 Genes encoding Importin α-like proteins in *Arabidopsis*. Database accessions and references of all known *Arabidopsis* cDNAs and corresponding genes that encode Importin α-like proteins. Additional putative genes encoding novel Importin α-like proteins were identified by a computer search using all known *Arabidopsis* Importin α protein sequences against the *Arabidopsis* genome

Designation	Accession numbers		References	Chromosome
	Genomic clones[a]	cDNAs		
AtIMPa1	AC023912	AF077528 Y15224	Smith et al. 1997 Schledz et al. 1998	3
AtKap alpha	AC023912	U69533[b]	Ballas and Citovsky 1997	3
At-IMP alpha	AC023912	L81172[b]	Smith and Raikhel 1996	3
AtIMPa2	Z97340	Y14615	Schledz et al. 1998	4
AtIMPa3	AL161493	Y15225 Y09511	Schledz et al. 1998 Nemeth et al. 1998	4
AtIMPa4	AC003114	Y14616	Schledz et al. 1998	1
AtIMPa-like 5	AB016872	[c]		5
AtIMPa-like 6	AC022521	[c]		1
AtIMPa-like 7	AC011620	[c]		3
AtIMPa-like 8[d]	AB015478	[c]		5
	AC069144		Pseudogene	1

[a]The accession of only one bacterial artificial chromosome is given, even when there were more annotations in the database for a specific gene
[b]These mRNAs most probably originate from the AtIMPa1 gene by differential splicing within the distal part of the pre-mRNA, as judged by comparison of sequence identity between the deduced proteins and the cDNAs
[c]AtIMPa-like 5–8 were deduced from genomic sequences. Their expression and their cDNA sequences were not confirmed experimentally
[d]Predicted protein lacks IBB domain

AtIMPa-like5–8 were deduced from the genomic sequences and therefore still lack experimental verification, whereas the expression of the other genes was confirmed experimentally. With the exception of AtIMPa-like8, which lacks an IBB domain in its present annotation, all deduced proteins show the typical architecture of Importin α proteins (Fig. 4A): an amino-terminal IBB domain that is rich in arginine residues and that is responsible for the interaction with Importin β (Görlich et al. 1996a; Weis et al. 1996), armadillo (ARM) repeats that make up the core of the protein and contain the NLS-binding pockets (Conti et al. 1998), and a carboxy-terminal acidic patch that is implicated in the interaction with the export receptor CAS (Herold et al. 1998). The alignment shows that the eight deduced *Arabidopsis* Importin α proteins are very similar (Fig. 4B). The identity values range from 59% (AtIMPa-like8) to 86% (AtIMPa2), as compared to AtIMPa1. The question of why there are so many Importin α proteins has been tackled in humans, in which seven different Importin α proteins have been described (Köhler et al. 1999). The authors found tissue-specific expression of some isoforms, but most of them were expressed within the same tissues. There was no difference between the Importin α isoforms in binding to the import receptor Importin β and to the export receptor CAS. However, differential substrate affinities were discovered when two substrates were offered simultaneously in in vitro import assays (Köhler et al. 1999). The newly discovered role of Importin α in the control of spindle assembly during mitosis (Gruss et al. 2001) may also account for the existence of many different Importin α proteins. In plants, AtIMPa1 was characterised by binding to three different import signals in vitro, namely a classical monopartite NLS, a basic bipartite NLS, and a Matα-like signal (Smith et al. 1997). In addition, AtKap alpha was found to interact with the *Agrobacterium* VirD2 protein (Ballas and Citovsky 1997), AtIMPa3 (also termed ATHKAP2) interacts with the WD-40 repeat-containing protein PRL1 (Nemeth et al. 1998), and rice Importin α was shown to interact with NLS-containing proteins and to confer nuclear import in co-operative manner with Importin β from rice (Jiang et al. 1998a, b).

To date plant Importin β homologues have been characterised only in rice, which contains two highly similar proteins (Jiang et al. 1998b; Matsuki et al. 1998). The rice proteins interact with Ran-GTP in a way that excludes binding of Importin α, as described in animals (Izaurralde et al. 1997; Kutay et al. 1997a). Although many Importin β-like proteins are found in the *Arabidopsis* genome, only one has been characterised so far. In *Arabidopsis*, the nuclear export receptor XPO1 that specifically binds to leucine-rich NESs has been functionally analysed (Haasen et al. 1999a, b). The *Arabidopsis* protein shares 42–50% identity to its functional human, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* homologues, interacts with *Arabidopsis* Ran and with NESs of plant and human proteins including the HIV protein Rev. In addition, the interaction of XPO1 with NES-containing proteins and the export activity in planta is sensitive to the cytotoxin leptomycin B (LMB). Export activity within a plant cell was demonstrated in vivo using an assay system that employs green fluorescent protein (GFP) fusion proteins, which also revealed that the Rev NES is fully functional in plants. This finding revealed the high conservation of this nuclear export pathway between animals, yeast, and plants (Haasen et al. 1999a).

Genes encoding the GTPase Ran have been isolated from several plant species, including five genes from tobacco and three genes from *Arabidopsis* (for review,

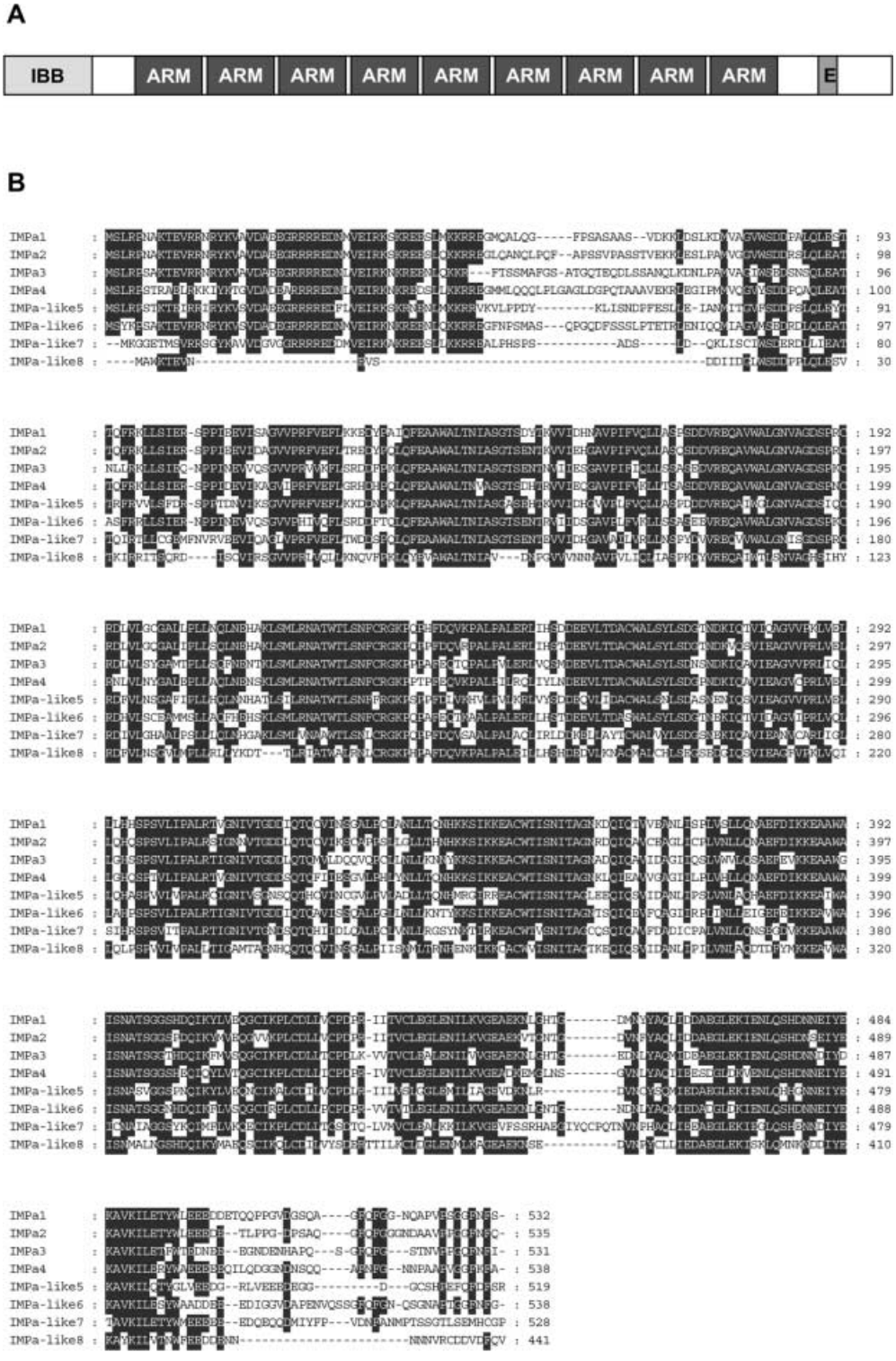

Fig. 4A, B Importin α-like proteins from *Arabidopsis thaliana*. **A** General structure of Importin α proteins: *IBB* Importin β-binding domain, *ARM* armadillo repeats, *E* glutamate-rich acidic patch. **B** Alignment of Importin α-like proteins deduced from cDNA and genomic sequences. The alignment was performed in CLUSTAL W 1.5 (Thomson et al. 1994) and boxed with GeneDoc (http://www.psc.edu/biomed/genedoc). The *shading* indicates amino acid residues that are identical in at least five deduced proteins

see Merkle and Nagy 1997). All three *Arabidopsis Ran* genes are located on chromosome 5, and *AtRan1* and *AtRan2* are arranged in tandem repeat (Haizel et al. 1997; accession numbers of genomic clones: AF296836, AB010071). *Arabidopsis,* tomato, and tobacco Ran proteins are functional in *Schizosaccharomyces pombe*, since over-expression of a plant Ran protein suppresses the phenotype of the temperature-sensitive mutant *pim1-46* (Ach and Gruissem 1994; Merkle et al. 1994; Haizel et al. 1997). Three genes encoding the Ran-specific regulator RanBP1 have been isolated from *Arabidopsis*, two of them by a yeast two-hybrid screen with a Ran mutant that is permanently blocked in its GTP-bound form (Xia et al. 1996; Haizel et al. 1997).

Plant proteins that are homologous to RanGEF and RanGAP have not been functionally characterised to date. However, putative plant RanGAPs, one from rice and from alfalfa and two from *Arabidopsis*, are annotated in the databases. The deduced plant proteins show highest similarity to human RanGAP1 in the middle part of the protein. Interestingly, the putative plant RanGAPs share a domain with MAF1, a possible nuclear envelope-associated protein from *Arabidopsis* and from several other plant species (Meier 2000). This domain, termed the WPP domain, is located at the amino terminus of plant RanGAPs and is not present in the human protein, whereas in MAF1 the WPP domain occupies almost the entire protein (Meier 2000). MAF1 is a small protein consisting of 123 amino acid residues, which is localised at the nuclear rim and interacts with a protein called matrix attachment region (MAR)-binding filament-like protein 1 (MFP1; Gindullis et al. 1999). MFP1 is also localised at the nuclear envelope, but in contrast to lamins from animals and yeast, contains an extensive coiled-coil domain and two hydrophobic, putative transmembrane domains within the amino terminus (Meier et al. 1996; Gindullis and Meier 1999). Since MAF1 binds to the coiled-coil domain of MFP1 with its WPP domain, Meier (2000) hypothesised that plant RanGAPs might also interact with MFP1. In the light of the recently discovered role of Ran, RanGAP, and RanGEF in the re-assembly of the nuclear envelope after mitosis (Hetzer et al. 2000; Zhang and Clarke 2000a, b), a possible recruitment of plant RanGAPs to nuclear envelope vesicles via MFP1 and a regulation of this interaction by the competing activity for MFP1 binding by MAF1 is an attractive hypothesis to be analysed in the future.

Although the analysis of nuclear transport factors and nuclear import and export in plants confirmed that the basic processes are highly conserved between organisms, there are some plant-specific features. The development of in vitro nuclear import systems for plants revealed that nuclear import is not inhibited at 4 °C, as in animal cells (Hicks et al. 1996; Merkle et al. 1996). In addition, the lectin wheat germ agglutinin (WGA) does not block this process in plants, which is in contrast to animal systems. This could be due to the fact that plant nucleoporins show a different glycosylation pattern as compared to animals (Heese-Peck et al. 1995). However, Yamamoto and Deng (1999) reported recently that rice Importin α1a was able to functionally replace mouse Importin α in digitonin-permeabilised HeLa cells in an in vitro import assay. This substitution was sufficient to almost completely release the WGA block on the nuclear import of NLS-containing proteins in permeabilised HeLa cells. This finding links the differential effect of this lectin in animal and plant nuclear import directly to Importin α instead of to the NPC. Another feature of the plant in vitro nuclear import systems is that they cannot be depleted of endogenous nuclear transport factors in order to render nuclear import experiments dependent on the addition of cytosolic factors (Hicks et al. 1996; Merkle et al. 1996). The depletion of transport factors in the animal in vitro import systems during permeabilisation has allowed biochemical approaches and greatly enhanced the characterisation of essential transport factors (Görlich et al. 1994). However, a recent report demonstrated beautifully that plant nuclei can contain extensive grooves and invaginations (Collings et al. 2000). This finding may provide a possible explanation of why nuclear transport factors are effectively trapped during the preparation of in vitro nuclear import systems.

Interestingly, At-IMPα, one of the *Arabidopsis* Importin α-like proteins, was reported to be able to function as a nuclear import receptor without binding to Importin β (Hübner et al. 1999). In addition, Importin α has been co-localised with elements of the cytoskeleton in plant cells, suggesting that these structural elements are implicated in nuclear import (Smith and Raikhel 1998). It is not known to date, whether the latter finding is unique to plants or whether this property of Importin α is also shared with other organisms.

Plant nucleoporins have not been identified so far. However, in our laboratory, an *Arabidopsis* protein has recently been characterised as the functional homologue of Nup214/CAN on the basis of its interaction with the *Arabidopsis* export receptor XPO1, its localisation at the nuclear envelope as revealed by indirect immune localisation, and by its sequence similarity to the mammalian protein (D. Haasen and T. Merkle, unpublished results). Research on plant NPCs has been reviewed by Heese-Peck and Raikhel (1998), and the functional domains of the plant endoplasmic reticulum and its continuity with the nuclear envelope has been extensively reviewed by Staehelin (1997).

While the basic transport apparatus seems to be conserved between animals, yeast, and plants, investigation of the role(s) of nuclear transport in specific signalling pathways has revealed plant-specific aspects of regulation. The following paragraphs give examples in which regulated nucleo-cytoplasmic partitioning of signalling proteins is part of a control mechanism within a plant signal-transduction pathway.

Photoreceptors

Light is the most important environmental and developmental factor for plants. Besides being exploited as an energy source in photosynthesis, light controls many developmental and physiological responses from germination to flowering and seed maturation. Plants are capable of sensing the quality, quantity, duration, and the direction of light. For this they have evolved at least three different photoreceptor systems: red/far red-reversible phytochromes (phy), blue/UV-A receptors, and UV-B receptors (for review, see Briggs and Olney 2001). While UV-B receptors have been characterised by action spectroscopy only (Wellmann 1983), phytochromes and blue/UV-A photoreceptors have also been

identified molecularly (Ahmad and Cashmore 1993; Lin et al. 1996; Mathews and Sharrock 1997; Hoffman et al. 1996; Christie et al. 1998).

Many phytochrome-controlled responses involve the activation or repression of transcription of specific genes as an early step of signal transduction (for review, see Nagy and Schäfer 2000). Nuclear localisation of phytochromes themselves, however, was first demonstrated by Sakamoto and Nagatani (1996). Before, it was generally assumed that phytochromes were localised exclusively in the cytoplasm. Sakamoto and Nagatani (1996) demonstrated an increase in immunologically detectable phytochrome in nuclei purified from red-light-irradiated plant tissues as compared to nuclei from dark-grown plants. In addition, transgenic plants that expressed fusion proteins consisting of fragments of phyB with the β-glucuronidase (GUS) reporter showed nuclear accumulation of the reporter protein. Further detailed studies revealed that fusion proteins of the photoreceptors phyA and phyB with the green fluorescent protein (GFP) in transgenic plants show a light-dependent nuclear localisation with the characteristics of phyA- and phyB-dependent photoresponses. Transgenic plants that express phyB-GFP fusion proteins showed light-dependent nuclear accumulation of the green fluorescence, whereas it was restricted to the cytoplasm in etiolated seedlings or in dark-adapted plants (Kircher et al. 1999a; Yamaguchi et al. 1999). Kircher et al. (1999a) demonstrated that nuclear import of phyB-GFP in transgenic tobacco plants is red-light dependent and red/far red reversible and thus shows a low-fluence response (LFR) that is typical of phyB-dependent photoresponses. In contrast, in transgenic tobacco plants that express phyA-GFP fusion proteins, nuclear accumulation of the green fluorescence could be initiated by pulses of red light and far-red light as well as by continuous irradiation with far-red light (Kircher et al. 1999a). These are the characteristics of a very low-fluence response (VLFR) and of a high-irradiance response (HIR), respectively, both of which are typical of phyA-dependent photoresponses (Nagy and Schäfer 2000). The functionality of the phyB and phyA fusion proteins was demonstrated by their ability to complement the phenotypes of *phyB* and *phyA Arabidopsis* mutants, respectively (Yamaguchi et al. 1999; Kim et al. 2000). Strong support for a nuclear function for phytochromes after their light-induced nucleo-cytoplasmic shift is also provided by the isolation of several nuclear proteins that directly interact with these photoreceptors (for review, see Quail 2000).

Further analysis suggests a cytosolic retention mechanism for phytochromes in the dark, where they exist in their physiologically inactive P_r forms. The light-dependent nuclear import of phytochromes depends on the conformational change of the protein from the P_r form in the dark to the P_{fr} form in the light, since a GFP fusion protein containing a mutant phyB protein that is unable to bind the chromophore stays almost exclusively in the cytosol, regardless of the light regime (Kircher et al. 1999a). In addition, a fusion of the carboxy-terminal half of phyB with GFP is permanently localised in the nucleus (Sakamoto and Nagatani 1996). This shows that the carboxy terminus of phyB contains at least one functional NLS and that the amino terminus plays an important role in the cytosolic retention of the protein in the dark (Nagy et al. 2000). Other strong evidence for a cytosolic retention mechanism for phytochromes in the dark stems from experiments employing fusions of additional NLSs to the phyB-GFP protein, which did not result in light-independent nuclear localisation of the green fluorescence (Nagy et al. 2000).

Nuclear accumulation of phyA-GFP and phyB-GFP fusion proteins result in a speckled pattern instead of a uniform distribution of the green fluorescence within the nucleus (Kircher et al. 1999a; Yamaguchi et al. 1999). This may indicate a concentration of the photoreceptors in specialised sub-domains or bodies of the plant nucleus. The physiological relevance of this finding is currently under investigation. Another question that has not been answered yet is what happens to the phytochromes once they are photo-activated and imported into the nucleus. Reversion by far-red light of the nuclear accumulation of phyB-GFP initiated the loss of the speckled green fluorescence from the nucleus (Gil et al. 2000). In principle, three possibilities exist to switch off a signalling molecule in the nucleus: (i) inactivation by modification or by interaction with a ligand, (ii) degradation, or (iii) nuclear export. While degradation of phytochrome has been described (for review, see Callis and Vierstra 2000), little is known about whether or not this also happens in the nucleus. If the "light-stable" phyB could interact with a nuclear export receptor, at least a portion of the phytochrome pool in a cell would then show light-dependent nucleo-cytoplasmic shuttling, which could be an important regulatory switch in phytochrome signalling.

The blue-light photoreceptors are not as well characterised as the phytochromes to date. Phototropin is a membrane-associated protein and is implicated in phototropism (Christie et al. 1998), whereas the cryptochromes CRY1 and CRY2 are soluble and have partially overlapping functions in inhibiting extension growth of the hypocotyl and in promoting the expression of chalcone synthase (for review, see Batschauer 1998). Transient transformation experiments using *Arabidopsis* CRY1 fused to GFP show a nuclear accumulation of the green fluorescence in the dark (Cashmore et al. 1999). However, light-dependent cytosolic localisation of a carboxy-terminal fragment of CRY1 fused to the GUS reporter in transgenic *Arabidopsis* plants also suggests a light-dependent nucleo-cytoplasmic partitioning of the photoreceptor (Yang et al. 2000). Studies using *Arabidopsis* CRY2 fused to GFP or GUS in transgenic plants that constitutively express these proteins show a nuclear localisation of reporter-gene activity (Guo et al. 1999; Kleiner et al. 1999). In the fern *Adiantum capillus-veneris*, five genes encoding cryptochromes were identified, and two of them were

shown to localise in the nucleus (Imaizumi et al. 2000). The authors presented evidence for an influence of light on the nucleo-cytoplasmic distribution of these photoreceptors. Mas et al. (2000) showed that *Arabidopsis* CRY2 forms speckles in the nucleus after irradiation with blue light, like phyB after irradiation with red light, and that CRY2 and phyB directly interact in these nuclear speckles. Taken together, there is evidence that the cryptochromes may also show light-dependent nucleo-cytoplasmic partitioning.

Transcriptional regulators involved in light signalling

After the absorption of light, phytochromes trigger many signal transduction cascades in the cytoplasm, in addition to the recently discovered nuclear function(s) after their light-dependent shift into the nucleus. The activation of transcription factors in the cytoplasm belongs to the cytosolic effects of P_{fr}. It involves the nuclear translocation of a signalling molecule that is part of a phytochrome-dependent signal-transduction pathway. Light-dependent nuclear translocation of a group of plant basic leucine-zipper (bZIP) transcription factors was first demonstrated by analysing cellular fractions of parsley cells (Harter et al. 1994). In addition, the authors showed that the nuclear translocation of bZIP transcription factors depends also on their phosphorylation status. These bZIP transcription factors were termed CPRFs (common plant regulatory factors), bind to G-box promoter elements containing the ACGT core motif, and play a regulatory role in the expression of light-responsive genes like the chalcone synthase gene in parsley (Weisshaar et al. 1991; Armstrong et al. 1992). Further studies on the nucleo-cytoplasmic partitioning of several members of the CPRF family revealed that CPRF2 is localised predominantly in the cytoplasm in the dark (Kircher et al. 1999b). In contrast, the nuclear localisation of CPRF1 and CPRF4 was not influenced by light. The re-localisation of CPRF2 to the nucleus can be induced by a red-light treatment and is, at least partially, red/far red reversible, which indicated the involvement of phytochrome in the light-regulated nuclear shift of CPRF2 (Kircher et al. 1999b). Furthermore, Wellmer et al. (1999) demonstrated that CPRF2 is phosphorylated within its carboxy terminus by a cytosolic serine kinase in a phytochrome-dependent manner. This modification did not alter the DNA binding activity of the transcription factor. Together with the finding that CPRF2 is a component of a high-molecular-weight complex in the cytoplasm in darkness (Wellmer et al. 1999), it suggested a phosphorylation-induced release of CPRF2 from a cytosolic complex that prevents the import of CPRF2 in darkness. Analysis of the localisation of fragments of CPRF2 fused to GFP by transient expression in parsley protoplasts identified the amino terminus that is responsible for the inhibition of nuclear import in the dark. In addition, the amino-terminal domain could be fused to a different CPRF protein and conferred cytosolic localisation to the normally nuclear protein (Kircher et al. 1999b). In all, the data provide strong evidence that the light-regulated nuclear shift of CPRF2 is accomplished by a phytochrome-triggered release of the bZIP transcription factor from cytosolic intermolecular masking or retention by a phosphorylation mechanism. However, as with the phytochromes, it is not known at present whether CPRF2 is inactivated, degraded, or exported out of the nucleus to switch off signalling.

Another bZIP transcription factor was identified that showed a different nucleo-cytoplasmic partitioning in darkness and in the light. The localisation of *Arabidopsis* GBF2, however, is regulated by blue light (Terzaghi et al. 1997). Several *Arabidopsis* GBF transcription factors were assayed individually as GUS fusion proteins. In contrast to GBF1 and GBF4, whose localisations were not significantly influenced by light, GBF2 showed about 50% nuclear localisation in the dark, whereas more than 80% was found in the nucleus in cells cultured under blue light. Irradiation with red light had no effect on the nucleo-cytoplasmic partitioning of GBF2 (Terzaghi et al. 1997). To date, it is not known which blue-light receptor is responsible for the shift of GBF2 towards a predominantly nuclear localisation, and by which mechanism the re-localisation of GBF2 is accomplished. Nonetheless, a light-induced nuclear shift of the transcription factor GBF2 could be an important step in blue-light signalling since members of the GBF family are important for the expression of light-regulated genes (Schindler et al. 1992a, b), and it provides an interesting parallel to the phytochrome-induced nuclear translocation of CPRF2.

The photomorphogenic repressor protein CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1) contains three well-defined domains: an amino-terminal zinc-binding RING finger domain, a coiled-coil domain that may be important in the formation of protein interactions, and a carboxy-terminal WD-40 repeat domain (Deng et al. 1992; von Arnim and Deng, 1993). COP1 is localised in the nucleus during seedling germination in darkness and functions as a repressor of photomorphogenesis by suppressing the expression of light-inducible transcripts in the nucleus (Deng et al. 1991; von Arnim and Deng 1994). Regulation of COP1 activity is negatively controlled by light and involves a light-dependent re-localisation of the protein into the cytoplasm. As a result, COP1 levels are drastically reduced in nuclei of hypocotyl cells that are transferred to light as compared to those that are kept in darkness. In root cells, which do not undergo photomorphogenesis, COP1 levels are constitutively high in the nuclei (von Arnim et al. 1997; Osterlund and Deng 1998). Detailed analysis of the localisation and the physiological effects of COP1 fragments and mutants revealed discrete domains that mediate light-responsive nuclear and cytoplasmic localisation of the protein. A bipartite NLS that is responsible for nuclear localisation of COP1 was located within the core domain, and an amino-terminal

domain including parts of the RING domain and parts of the coiled-coil domain was identified that conferred cytoplasmic retention (Stacey et al. 1999, 2000). Recently, a novel Importin α homologue from rice that preferentially interacts with COP1 has been isolated (Jiang et al. 2001). In addition, a motif that mediates the targeting of COP1 to sub-nuclear foci has been described, which overlaps the cytosolic retention signal and the coiled-coil domain (Stacey and von Arnim 1999). How the switch between the cytosolic and the nuclear localisation of the COP1 protein is accomplished at the molecular level is under investigation. COP1 acts as a transcriptional regulator by interaction with other regulatory proteins in the nucleus, similar to the COP1-interactive proteins 4 and 7 (CIP4, Yamamoto et al. 2001; CIP7, Yamamoto et al. 1998) or with the bZIP transcription factor HY5, which is constitutively nuclear, binds to G-box motifs within promoters of several light-inducible genes, and is necessary for their optimal expression (Oyama et al. 1997; Ang et al. 1998; Chattopadhyay 1998a, b). Recently, it was shown that the nuclear interaction of COP1 and HY5 leads to the degradation of HY5, which is thereby negatively regulated by COP1 at the level of protein stability (Osterlund et al. 2000).

Nuclear transport and plant pathogens

Plants respond to pathogen attack by a variety of defence reactions including the transcriptional activation of specific genes and the accumulation of phytoalexins and pathogen-related proteins (for review, see Scheel 1998). Cultured parsley cells have retained most of the defence responses that are elicited upon the infection of parsley plants with the fungus *Phytophthora sojae*, and therefore serve as a model system (Dangl et al. 1987). Oligopeptide fragments of 13 or 25 amino acid residues in length that contain sequence motifs of a 42-kDa fungal glycoprotein are sufficient to replace the glycoprotein as elicitor. An elicitor peptide specifically binds to a target at the plasma membrane, suggesting a receptor-initiated signal transduction cascade that leads to the transient activation of plant defence genes and to the accumulation of phytoalexins (Nürnberger et al. 1994). Among the early responses to elicitor treatment are calcium-dependent transient changes in protein phosphorylation (Dietrich et al. 1990). Ligterink et al. (1997) isolated a parsley cDNA that encodes a protein with high similarity to mitogen-activated protein (MAP) kinases from other plants that was responsible for the kinase activity that could be detected within 5 min after elicitor treatment. The protein was therefore designated elicitor-responsive MAP (ERM) kinase. Interestingly, the ERM kinase not only shows a very rapid induction of its activity after elicitor treatment, but also a rapid elicitor-induced nuclear translocation (Ligterink et al. 1997). Using indirect immune localisation, the authors demonstrated nuclear accumulation of ERM kinase in parsley cells within 5 min of the addition of elicitor. Since no NLS was identified on ERM kinase, Ligterink et al. (1997) hypothesise that the kinase may enter the nucleus in a complex with another protein. The elicitor-induced nuclear translocation of ERM kinase may provide a link from cytosolic signal transduction to nuclear events in the defence response. A number of elicitor-responsive genes, *cis*-regulatory elements, and *trans*-acting factors have been identified in parsley (Rushton et al. 1996; Eulgem et al. 1999). Although experimental evidence is still lacking to date, the nuclear import of ERM kinase and the subsequent phosphorylation of target proteins leading to changes in transcriptional activity is an attractive hypothesis for future work.

Heat-shock response and nucleo-cytoplasmic partitioning

Plants and other organisms respond to an increase in temperature with the expression of heat-shock proteins (HSPs). It is generally assumed that the presence of HSPs that have been synthesised in response to a sub-lethal heat shock protect the cells from a second, otherwise lethal shock. This phenomenon is termed acquired thermotolerance (Li and Werb 1982; for review, see Schöffl et al. 1998). The expression of HSP genes is primarily controlled at the transcriptional level. The key regulators of the heat-shock response are the heat-shock transcription factors (HSFs) that bind to heat-shock elements (HSEs) in the promoters of HSP genes. The modular structure of HSFs is conserved throughout the eukaryotic kingdom. HSFs contain an amino-terminal DNA-binding domain consisting of a helix-turn-helix motif, followed by a heptad repeat domain that is implicated in HSF oligomerisation, an NLS, and a carboxy-terminal domain (Wu 1995). Plant HSFs may be constitutively expressed or may themselves be induced by a heat-stress treatment (Prändl et al. 1998; Heerklotz et al. 2001). Moreover, two classes of HSF exist in plants, which are discriminated by a short peptide insertion within the heptad repeat domain that is present in class A HSFs, but not in HSFs belonging to class B or in HSFs from other organisms (Schöffl et al. 1998). The two classes of plant HSF also contain different carboxy-terminal domains. Two or more acidic peptide motifs are found in class-A HSFs, which are essential for transcriptional activation (Döring et al. 2000), whereas the carboxy-terminal domains of class-B HSFs are neutral or basic (Czarnecka-Verner et al. 2000).

Tomato HsfA1, which is constitutively expressed, is distributed between the nucleus and the cytoplasm at normal temperature and accumulates in the nucleus upon heat stress. In contrast, tomato HsfA2 is a heat-stress-inducible protein that is excluded from the nucleus under control or heat-stress conditions when expressed on its own in plant or Chinese hamster ovary cells (Lyck et al. 1997; Heerklotz et al. 2001). Co-expression of both

proteins results in a co-localisation of HsfA1 and HsfA2 at normal temperature and upon heat stress. Thus, interaction with HsfA1 is essential for nuclear import of HsfA2 (Scharf et al. 1998). Heerklotz et al. (2001) identified an NES at the very carboxy terminus of HsfA2 that is responsible for its nuclear exclusion and that confers cytoplasmic localisation when fused to the otherwise nuclear HsfB1. The dominant cytoplasmic localisation of HsfA2 was sensitive to LMB, indicating that XPO1 is likely to mediate its nuclear export. Upon the addition of LMB, HsfA2 accumulated in the nucleus at control temperature, due to its functional NLS (Heerklotz et al. 2001). However, nuclear accumulation of HsfA2 was not observed in presence of LMB under heat-stress conditions. Thus, in addition to nuclear export, this finding provides evidence for a shielding of the NLS due to a temperature-dependent conformational transition of HsfA2 that also contributes to its cytoplasmic localisation. In contrast, nuclear accumulation in the presence of LMB was observed for a mutant HsfA2 protein lacking the heptad repeat domain that is necessary for hetero-oligomerisation. In addition, HsfA2 is also nuclear when co-expressed with HsfA1 under control or heat-stress conditions, even in the presence of LMB (Heerklotz et al. 2001). The authors conclude that HsfA2 is a shuttling protein whose nucleo-cytoplasmic partitioning is regulated by the balance of nuclear import and nuclear export. Furthermore, the localisation of HsfA2 is influenced by the interaction with HsfA1 and provides a control level for the regulation of the heat-shock response, since nuclear translocation of HsfA2 results in an increased expression of an HSF-dependent reporter gene.

Future prospects

Only few nuclear transport pathways have been elucidated in plants so far. In addition, many of the nuclear transport factors that are known from animals and yeast have now been identified in the *Arabidopsis* genome on the basis of sequence similarity but are not yet functionally characterised. Clearly, the completion of the sequence of the *Arabidopsis* genome will greatly facilitate the identification of new transport factors and structural proteins, such as plant nucleoporins. Research on plant nuclear transport pathways is important not only in its own right, but also for understanding the communication between the nucleus and the cytoplasm, and the regulation of signalling that involves transcriptional control of genes. During the past 10 years, work on animals and yeast has uncovered a panoply of different regulatory mechanisms that employ control of nuclear trafficking of proteins to regulate signal-transduction pathways, and has widened our view of the regulatory potential of nuclear transport processes. Recent findings in plant signal-transduction research leave no doubts that plants also take advantage of this regulatory potential and that there is much more to discover.

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