

PŘÍRODOVĚDECKÁ FAKULTA UNIVERSITY KARLOVY V PRAZE

KATEDRA EXPERIMENTÁLNÍ BIOLOGIE ROSTLIN

BAKALÁŘSKÁ PRÁCE

ROLE A REGULACE JADERNÉHO TRANSPORTU U ROSTLIN
ROLE AND REGULATION OF NUCLEAR TRANSPORT IN PLANTS

Pavel Bokvaj

Školitel: RNDr. Kateřina Schwarzerová, Ph.D.

2009/2010

Prohlašuji, že jsem tuto práci vypracoval samostatně s použitím uvedené literatury a pod vedením vedoucí bakalářské práce.

Pavel Bokvaj

Abstract

The nucleocytoplasmic transport represents a crucial checkpoint of all signal pathways leading to the gene expression, the cell cycle maintenance, and RNA export processes. It is mediated by nuclear pore complexes (NPC) anchored in double nuclear membrane. The NPC structure and the basic architecture of the transport regulations are evolutionarily highly conserved across eukaryotic kingdoms; however, some significant differences and specifics exist in plants. In this thesis, I describe the contemporary level of our knowledge of the nucleocytoplasmic transport regulation and its actors *in animalia* generally and *in planta* in detail.

Key words

Arabidopsis thaliana, nuclear pore complex, nucleocytoplasmic transport, nucleoporin, nucleus

Abstrakt

Transport mezi jádrem a cytoplasmou představuje důležitý regulační bod všech drah vedoucích k expresi genů, kontrole buněčného cyklu a exportu RNA. Je zprostředkován komplexem jaderného póru (NPC), které jsou ukotvené v dvojité jaderné membráně. Struktura jaderného póru a základní způsoby regulace transportu jsou v eukaryotických organismech evolučně vysoce konzervované, avšak v rostlinných buňkách lze nalézt některé významné rozdíly. V této práci shrnuji současné poznatky o regulaci transportu mezi jádrem a cytoplasmou. Zvláštní pozornost věnuji těmto procesům v rostlinných buňkách.

Contents

1. Introduction	6
2. The nuclear pore complex	7
2.1 The animal and the yeast NPC	8
2.2 Plant NPC and its comparison to animal NPC	10
2.3 The FG-network and cargo translocation	11
3. Actors of nucleocytoplasmic transport	11
3.1 Nuclear localization sequences.....	12
3.3 Karyopherins	13
3.3.1 Importins	14
3.3.2 Exportins	14
3.4 Ran GTPase and other regulatory proteins.....	14
3.5 Nucleoporins	16
4. Mechanisms of nucleocytoplasmic transport in animal cells	17
4.1 Ran-dependent import to the nucleus	17
4.2 Ran-dependent export from the nucleus	21
4.3 Ran-independent transport	22
5. The regulation of nucleocytoplasmic transport	23
5.1 Hiding the substrate NLS/NES from recognition by importin/exportin	23
5.2 Regulation by changing the strength of binding of importin/exportin to the NLS/NES.....	23
5.3 Regulation by co-transport and changing the binding sites of importin/exportin ..	24
5.4 Regulation by retention in the nucleus or cytoplasm	24
5.5 Regulation by availability of importins and exportins.....	25
5.6 Regulation by changing the variety of nucleoporins	25
6. Nucleocytoplasmic transport mechanism differences between animals and plants	25
7. Specific pathways <i>in planta</i> involving nucleocytoplasmic transport	27
7.1 Nucleoporins in plant-microbe interactions	27

7.2 Hormone signaling	27
7.3 Cold-stress resistance.....	28
7.4 Flowering time regulation	28
7.5 mRNA export	28
8. Methods and approaches used in nucleocytoplasmic transport studies.....	29
9. Conclusion	29
10. Acknowledgements	31
11. References	32

Glossary:

CGC:	Central gated channel of nuclear pore complex.
FG-Nups	Nucleoporins with phenylalanine-glycine repeats tails.
NPC:	Nuclear pore complex.
NTF2:	Nuclear transport factor 2, the acceptor of RanGDP/cargo import complex in nucleoplasm.
Nups:	Nucleoporins.
RanBP1:	Activator of RanGAP1.
RanBP2:	Nucleoporin 358 serves as docking site for RanGAP1; present at cytoplasmic face of nuclear pore complex.
RanBP3:	Activator of RanGEF.
RanGAP:	Soluble, exclusively cytoplasmic RanGTPase activating protein; increases GTPase activity of Ran.
RanGAP1u:	SUMOylated RanGAP1, bound to RanBP2.
RanGEF:	Ran guanine nucleotide exchange factor; increases Ran exchange rate of GDP for GTP in nucleus. RanGEF is also called RCC1.

1. Introduction

During the evolution of life, eukaryotic and some prokaryotic cells have shown that compartmentalization may be a beneficial means of organisation of the cell. Complex eukaryotic organisms usually possess larger coding regions in their genomes than organisms with simpler cell construction, and operate with larger number of different proteins and RNAs during their life cycle. The large amount of genes and proteins implies the use of more sophisticated regulations and signaling pathways when “housekeeping”, reproducing and interacting with environment and other cells. For these regulations, it is useful to divide the cell into spatially and functionally different compartments.

The nucleus is one of these compartments. It separates replication and transcription from translation processes. The nuclear membrane represents a checkpoint of many signal pathways, mainly those of transcription. It provides a strictly controlled, enabled or prohibited exchange of material between cytoplasm and nucleoplasm through the nuclear membrane via nuclear pore complexes (NPC). Mainly, proteins regulating and executing transcription are imported, and mRNAs and small regulatory RNAs are exported. Also, the nuclear membrane serves as a scaffold for DNA bound to lamins, and finally, it protects the genome from pathogens and chemical damage.

Due to more intense research in animal cells than in plant cells in the past, our current knowledge of nucleocytoplasmic transport in plants is smaller. Recently, valuable papers reviewing nucleocytoplasmic transport have been published; for example Sorokin et al. (2007) reviews nucleocytoplasmic transport in yeast and animals, and Xu, Meier and Brkljacic (2007 and 2009) review new features of nucleocytoplasmic transport in plants.

In this thesis, I briefly describe structures and mechanisms of nucleocytoplasmic transport at the contemporary level of understanding and its regulation and role in the cell, with respect to plant-specific structures.

2. The nuclear pore complex

The nuclear pore complex (NPC) is a very large, 40-125 MDa protein complex connecting inner nuclear membrane (IM) and outer nuclear membrane (OM) of eukaryotes. NPC size, composition and morphology vary across the plant, fungal and animal kingdoms. It consists of nucleoporins (Nups), which are usually present in large numbers in one NPC. These proteins have structural, regulatory and cargo complex docking/movement functions.

The yeast NPC is constructed of about 30 different nucleoporins, and in the human NPC, about 20 nucleoporins have been identified so far (Stoffler et al. 1999). Nucleoporins forming NPC are present in multiple copies and the amount of nucleoporin copies is dividable by 8 due to the symmetry of the NPC. The total number of nucleoporins is estimated to be 500-1000 per NPC (Cronshaw et al., 2002). Nucleoporins can be divided into three morphological categories – 1) FG nucleoporins with phenylalanine (F)-glycine (G) repeats and hydrophobic linkers, 2) nucleoporins with WD (tryptophan-aspartate) repeats and a β -propeller motif (Cronshaw et al., 2002; Devon et al., 2004), 3) transmembrane nucleoporins, which fasten NPCs to the nuclear membrane.

In general, the NPC has a circular, 8-fold symmetry with three main substructures – central core with central gated channel (CGC), 50 nm long cytoplasmic fibrils and a nuclear basket with 100 nm long fibrils, which merge in a basket-like structure. The NPC diameter ranges from 95 nm (yeast; Kiseleva et al., 2004) to 120 nm (animals; Goldberg and Allen, 1996); the plant NPC has a diameter of about 105 nm (Fišerová et al., 2009).

The NPC allows molecules smaller than ~30 kDa to pass through by passive diffusion; larger molecules require nuclear localization sequence (NLS) to be imported into the nucleus or nuclear export sequence (NES) to be exported out from the nucleus.

2.1 The animal and the yeast NPC

Consistent with the fact that the yeast NPC is simpler than the animal NPC, the yeast NPC mass has been estimated by scanning transmission electron microscopy to be only 55-66 MDa (Yang et al., 1998) and the animal NPC mass to be 125 MDa.

Based on the scanning electron microscopy approach, the NPC of animal cells (*Xenopus laevis* oocyte) can be divided into following parts (Figure 1): the plug (transporter) (12 MDa), heavy ring (32 +/- 5 MDa), light ring (21 +/- 4 MDa), plug-spoke complex (66 +/- 8 MDa) and spoke complex (52 +/- 3 MDa) (Reichelt et al., 1990). Generally, 125 MDa for animal cells' NPC and ~60 MDa for yeast cells' NPC is the highest estimate of the NPC mass. However, it is proposed that the scanning electron microscopy specimens contained transport factors and transported cargo proteins entrapped in the studied NPCs during fixation. Consequently, other studies suggest significantly smaller NPC mass – 60 MDa for rat liver cells and 40 MDa for yeast cells (Rout et al., 2000).

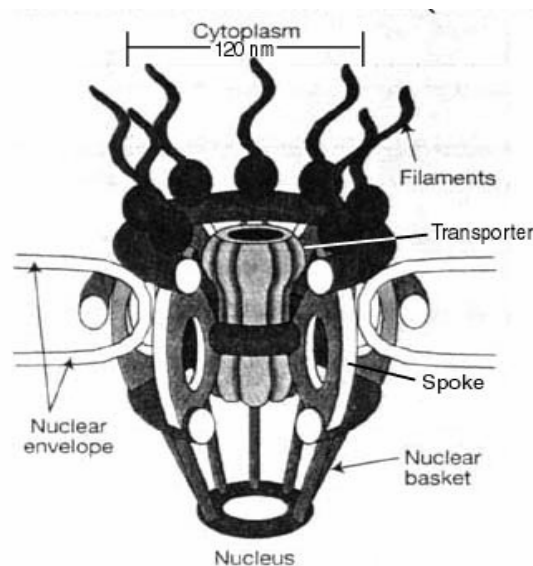


Figure 1. The nuclear pore complex. (from <http://course.1.winona.edu>)

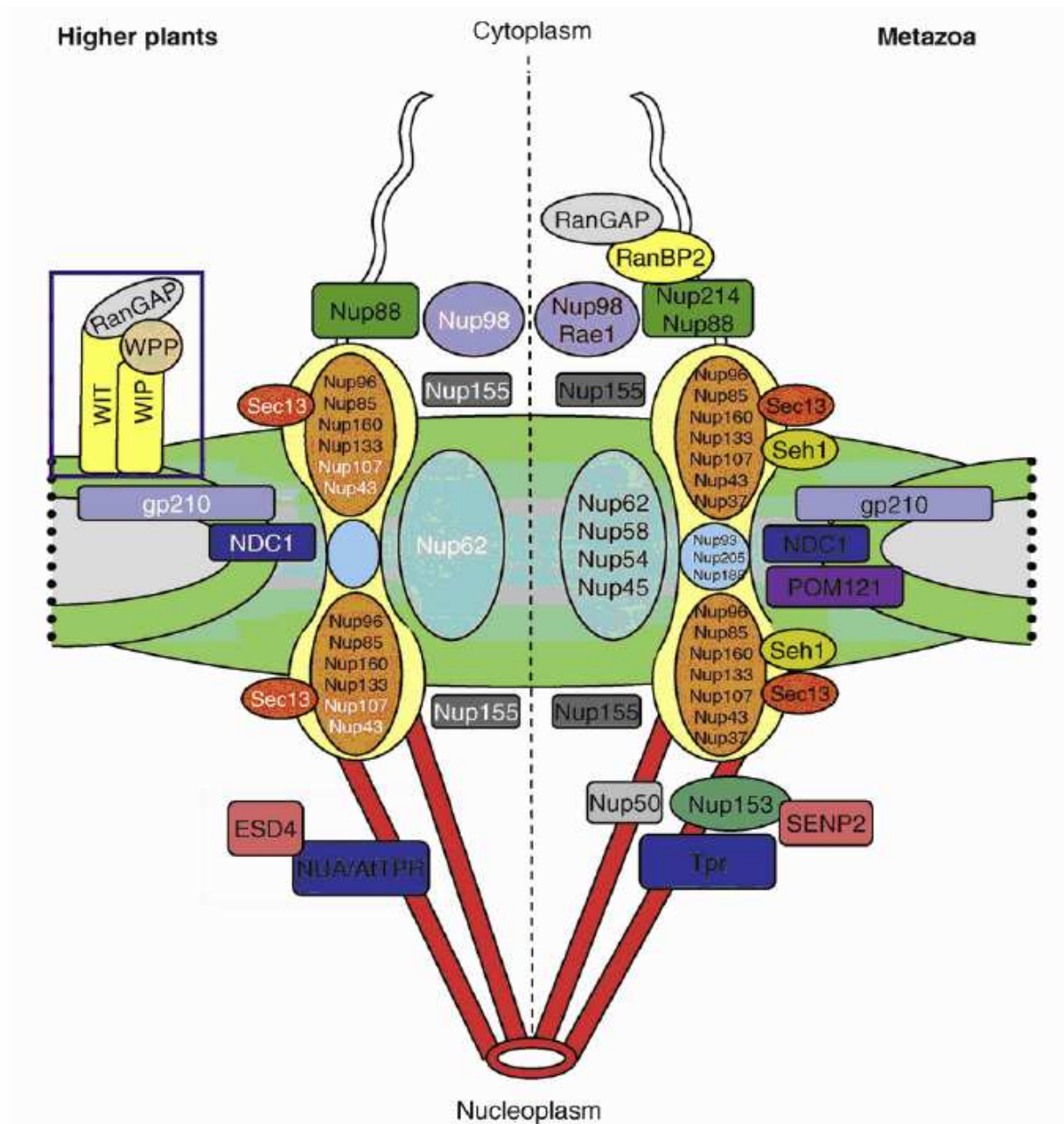


Figure 2. The comparison of plant and animal nuclear pore complex; except for the WIP/WIT/WPP/RanGAP1 and NUA(AtTPR)/ESD4 complex (shown in blue boxes) all localization and association information for plant Nups is deduced from their mammalian counterparts. Plant Nups identified solely by sequence similarity to their animal counterparts are shown in white. Unit colors indicate homologous complexes, based either on sequence or on functional similarity (WIP/WIT and RanBP2). (modified, from Current opinion in plant biology, Meier and Brkljacic, 2009)

2.2 Plant NPC and the comparison with animal NPC

The localization and function of RanGAP represents the best described difference between animal and plant NPC. Besides this, the function of most nucleoporins is unknown in both animal and plant cells thus other functional differences between animal and plant cells remain hidden. For nucleoporin composition of the animal and plant NPC, see Figure 2.

Recent findings discovered many structural details of the plant NPC using an in-lens feSEM (Fišerová et al., 2009). Firstly, significant morphological differences between the NPCs of the plant cells in different phases of the cell cycle were found (Figure 3a). The nuclei of 3-day old and 10-day old BY-2 cells (*Nicotiana tabacum*) show different content of particular NPC categories (Figure 3b). Categories I and II resemble animal NPC intermediate structures in *Xenopus* and *Drosophila*, and it is suggested that categories III and IV represent mature, fully active NPCs. It is also suggested that the different NPC types transport different cargos. It is important to note that cytoplasmic filaments of the NPC have not been observed in each NPC, meaning these filaments are either difficult to preserve or that not each plant NPC contain the cytoplasmic filaments *in vivo*.

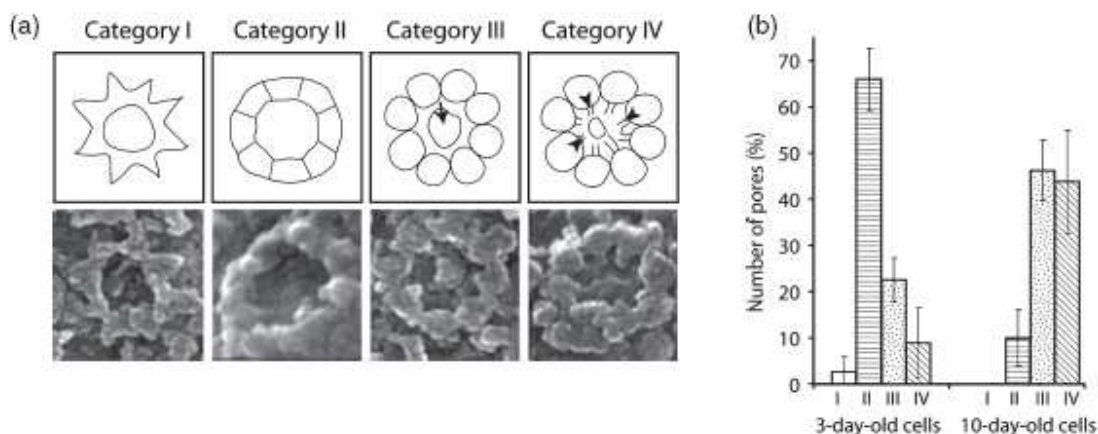


Figure 3. a) Morphological categories of NPCs in 3-day and 10-day old BY-2 cells observed by an in-lens feSEM. b) Different content of particular NPC categories in 3-day and 10-day old BY-2 cells (reproduced from Fišerová et al., 2009).

Secondly, a filament-like structure under the nuclear envelope has been found. This is an important discovery since the organization of the nuclear envelope of plants is very poorly understood. These filaments are 5-13 nm thick and interconnect the NPCs. The structure of these plant nuclear filaments resembles of animal nuclear lamina, therefore it was named “plamina”. At last, it was shown that NPCs of BY-2 cells are not placed randomly in the nuclear membrane but form 5-30 NPC-long rows (Fišerová et al., 2009).

2.3 The FG-network and cargo translocation

When a cargo is transported through an NPC, it interacts with FG-Nups of CGC. The way of translocation of cargo complex through the CGC is not clear at all. There are two dominant models attempting to describe the dynamics of the process. The first model, selective phase model (Ribbeck and Gorlich, 2001) suggests weak hydrophobic interactions between FG domains, resulting in sieve-like physical barrier inside the CGC. The transport complex has to “melt through” this hydrogel (Frey and Gorlich, 2007). Another model, Brownian affinity model (Rout et al., 2000) describes Brownian-like movement of the cargo complex through the FG-Nups network of CGC while peripheral structures would function as docking sites for the cargo complex to help overcome the energetic demands of the CGC. There are other models, the affinity gradient model for instance (Ben-Efraim and Gerace, 2001).

FG-Nups may also serve as a regulatory point due to selective binding of the cargo complex to different FG domains.

3. Actors of nucleocytoplasmic transport

The nucleocytoplasmic transport of proteins of a mass higher than ~30 kDa (cargo) requires NLS or NES in the amino acid sequence of the cargo protein. Nucleoporins of the NPC represent a “scaffold” for all the events happening during the

translocation of cargo. Most of the cargo proteins need to be chaperoned through the NPC by karyopherins (importins and exportins) during their transport. The transport also requires energy in certain steps of the procedure. This energy is provided by small GTPase protein Ran (Ras-related nuclear protein), which belongs to Ras (Rat sarcoma) protein family. Some proteins may require additional specific factors not related to karyopherins or RanGTP.

3.1 Nuclear localization sequences

A NLS is an amino acid sequence recognized by importins- α . This sequence does not have to be continuous; it often contains spacers of amino acids not recognized by importins or can be located in distant parts of the protein. A protein can have more than one NLS. There are two main groups of NLSs – classic NLS (cNLS) and non-classic NLS.

cNLSs consist of positively charged amino acids, eventually interrupted by a spacer. For example, nucleoplasmin, one of the first proteins shown to be transported to the nucleus, has a NLS that consists of two clusters of positively charged amino acids interrupted by a spacer (positively charged amino acids are highlighted in bold): **KR**(10AA)-**KKKL**. Large T antigen (SV40 virus protein) NLS is represented by a simple repeat of positively charged amino acids **PKKKRKV** (Jans et al., 2000). These NLSs are very conservative and frequent. For example in plants, maize protein Rab28 NLS has an amino acid sequence of **QPRRP** (Niogret et al., 1996).

Non-classic NLSs are recognized by importins- α (also called karyopherins- α) or directly by importin- β (also called p97 or karyopherin- β , see chapter 3.3.1) and they comprise a much more diverse group than classic NLSs. For example, the yeast repressor $\alpha 2$ protein NLS contains polar amino acids interspersed with non-polar ones (polar amino acids are underlined): VRILESWFAKNIENPYLDT (Hall et al., 1990). In contrast, the c-Myc protein NLS has a positively charged amino acid cluster with proline and aspartate on the boundaries: **PAAKRVKLD** (Dang and Lee, 1988).

3.2 Nuclear export sequences

Hydrophobic leucine-rich NES with 3 or 4 hydrophobic aminoacids belongs to the most common NESs. The hydrophobic NES is found in nucleocytoplasmic proteins of all eukaryotes, for example the HIV protein Rev NES (LPPLERLTL) (Fischer et al., 1995) or human TFIIIA protein NES (LSTVAVLTL) (Guddat et al., 1990). These hydrophobic NESes are recognized by exportins (see chapter 3.3.2), especially by exportin Crm1, which is able to transport substrates with or without other transport factors in the presence of RanGTP (Paraskeva et al., 1999).

Some proteins without the classic hydrophobic NES are exported by other exportins. For instance, karyopherin Msn5p exports protein Pho4, which does not possess a hydrophobic NES (Komeili and O'Shea, 1999).

Analogically to imported proteins, exported proteins often have more than one NES. Our laboratory identified 3 NESs in the molecule of β -tubulin: LQLERINV, ICFRTLKL and LNSDLRKLAV (Schwarzerová et al., 2006).

3.3 Karyopherins

In the history of nucleocytoplasmic research, proteins have been named regarding their nuclear import or nuclear export regulatory functions – importins or exportins. However, it has been shown that many exportins have nuclear import function as well and vice versa, therefore a general name “karyopherin” was established. Karyopherin is literally a “transporter related to the nucleus”, thus RanGTP/GDP has to be considered karyopherin as well. In this work, I use the old-fashioned name importin/exportin as much as possible for better comprehensibility of the text.

3.3.1 Importins

Importins comprise two families – importins- α and importins- β . For the import of a cargo, importins- α bind the cNLS of the cargo. Importins- β 1 bind cargos with non-classic NLSs or an importin- α /cNLS cargo complex. This selective affinity of different importins to NLSs is the main difference in the shuttling of cNLS and non-cNLS proteins. It has been shown that importins- β can adopt a number of different conformations, thus they can bind various different cargos including diverse importin- α /cNLS cargo complexes (Cingolani et al., 2002). At the same time, importins- β interact with FG-repeats of the NPC (Radu et al., 1995; Görlich et al., 1994), functioning as a linker between the NPC and the cargo complex.

3.3.2 Exportins

One of the most important exportins is Crm1, which recognizes classical, hydrophobic NESs. Crm1 also exports RanBP1 from the nucleus, thus maintaining the proper concentrations of RanBP1 in the nucleus and the cytoplasm. Apart from proteins, also various RNAs are exported from the nucleus. Exportin-t is responsible for export of tRNA and exportin-5 exports tRNA as well as microRNA recognizing a part of its structure as NES (Zeng and Cullen, 2004; Kim, 2004).

Export of mRNA proceeds without exportins, using TAP/NXF1 (nuclear export factor) proteins (Kang and Cullen, 1999; Herold et al., 2000).

A notable exportin CAS (Cse1p) exports importin- β , thus recycling this import factor back to cytoplasm (Künzler and Hurt, 1998).

3.4 RanGTPase and other regulatory proteins

RanGTPase is 25 kDa large protein which binds GTP and hydrolyzes it very weakly. Number of other regulatory proteins that regulate (increase or decrease) the rate

of GTP hydrolysis or GDP exchange for GTP on Ran exist. Therefore, rich possibilities of various regulations exist, which help to control the function of RanGTPase.

In order to explain nucleocytoplasmic transport mechanisms, it is necessary to describe these Ran-regulating proteins. All proteins that bind Ran contain specific Ran-binding domains (RanBD). The Ran-regulating proteins are: 1) RanGAP1 (RanGTPase activating protein) and its activator – RanBP1 (Ran binding protein); 2) RanGEF (Ran guanine nucleotide exchange factor) and its activator RanBP3. Whereas RanGAP1 is present in the cytoplasm or on the outer nuclear membrane, RanGEF interacts with chromatin and is localized solely in the nucleus. The most important consequence of RanGAP1 localization in the cytoplasm and RanGEF in the nucleus is that GDP form of Ran is dominantly present in the cytoplasm, whilst concentration of GTP form of Ran is very high in the nucleus. When an import cargo complex with RanGTP is docked at the cytoplasmic face of NPC, the GTP of Ran is hydrolyzed by RanGAP1 very quickly, while after crossing the central gated channel of the NPC, the GDP of Ran is exchanged for GTP immediately by RanGEF. The crucial fact is that NTF2 (see chapter 4.1), the transporter of the cargo complex from cytoplasm to the nucleus, binds the cargo complex with RanGDP only and has no affinity to RanGTP. On the contrary to the import to the nucleus, exportins bind cargoes with RanGTP only. Therefore, the selective affinity of Ran-binding proteins to various forms of Ran together with the gradient of RanGTP and RanGDP across the nuclear membrane ensure the unidirectionality of import and export processes.

RanGAP1 enhances the Ran GTP hydrolysing activity 10000 times or 100000 times, if RanBP1 is present (Bischoff et al., 1995). RanGAP1 is present in the cytoplasm only, and its SUMOylation (a conjugate with SUMO-1 protein) ensures RanGAP1 interaction with fibrils of the cytoplasmic side of the NPC through its binding to Nup358 (RanBP2) (Mahajan et al., 1997).

RanBP1 shuttles between the cytoplasm and the nucleus and is predominantly localized to the cytoplasm due to its active export from nucleus by Crm1 (Künzler et al., 2000). Besides enhancing the RanGAP1 activity, it raises accessibility of the complex cargo-RanGTP to RanGAP1.

RanGEF (also called RCC1) is present in the nucleus in a complex with histones H2A and H2B. It increases the exchange rate of the GDP for GTP on Ran 10 000 times, thus providing the restoration of RanGTP pool (Bischoff and Postingsl, 1991).

RanBP3 is localized mainly in the nucleus and binds with RanGDP to the RanGEF, boosting its activity 10 times (Nemergut et al., 2002).

Regarding the type of interactions with Ran, Ran binding proteins can be categorized in three groups: 1) proteins with about 150 amino acids long N-terminal sequences which interact with RanGTP but not with RanGDP and inhibit nucleotide exchange and/or hydrolysis – RanBP5, RanBP7, RanBP8, importin- β 1, exportin-t, Crm1, and CAS; 2) proteins which bind RanGTP at a different site – RanBP1, RanBP2 and RanBP3. These proteins inhibit nucleotide exchange but not GTP hydrolysis (Görlich et al., 1997; Dingwall et al., 1995); 3) Ran binding proteins containing other unique Ran binding sequences, diverse from those of the first and the second group. For example, transportin-1 probably belongs to this group (Pollard et al., 1996).

It is important to note that proteins of the first and of the second group do not compete for Ran, thus they can form ternary complexes with Ran together (Deane et al., 1997).

Members of the first two groups participate in translocation of proteins with NLS or NES in complexes with importin- β while not interacting with the cargo directly. Proteins of the third group interact with the cargo directly.

3.5 Nucleoporins

Nucleoporins, components of the NPC, are the last group of proteins involved in the nucleocytoplasmic transport I take as important to mention. The NPC is composed of approximately 30 different nucleoporins, of which some are still poorly described, especially in plants. Nucleoporins directly related to the nucleocytoplasmic transport and plant-specific pathways are described in detail in chapters 4 and 7, respectively. These are Nup62, Nup153, Nup214, Nup358 and Nup 85, Nup 96, Nup133, Nup160.

4. Mechanisms of nucleocytoplasmic transport in animal cells

Naturally, there are plenty of translocation modifications for particular proteins. The most common model include RanGTP as a necessary element, although Ran-independent transport is described as well.

As to passive diffusion of small molecules and ions, there is high controversy about this topic. It is not certain whether passive diffusion and active nucleocytoplasmic transport are spatially separated processes or not. Evidence exists for both ways (Kramer et al., 2007, Keminer and Peters, 1999, Fišerová and Goldberg, 2010)

4.1 Ran-dependent import to the nucleus

Firstly, importin- α binds to the cNLS-containing protein. The importin- α /cargo complex is then connected with the NPC by importin- β . In case of non-classic NLS proteins, there is just one main difference – the NLS is recognized directly by importin- β .

After the assembly of importin- α /cNLS-protein/importin- β , the complex binds to Nup358 (RanBP2), which is a part of cytoplasmic fibrils (Yokoyama and Hayashi, 1995). RanGAP1 binds to Nup358 too. In a free form, importin- β prevents connection of RanGTP to RanGAP1, thus unbound RanGTP does not hydrolyze its GTP (Floer and Blobel, 1996).

Consequently, the importin- α /cNLS-protein/importin- β /RanGTP cargo complex approaches the central gated channel (CGC) of the NPC.

It is suggested that the distance of 40 nm from the cytoplasmic fibril to the CGC may be overcome by bending of the fibril (Panté and Aebi, 1996). After GTP hydrolysis by RanGAP1, the cargo complex is released in proximity of the CGC. The journey through the CGC is still shrouded by many questions. An actual simplified concept is as follows: after the release from the fibril, the cargo complex binds to Nup p62 and NTF2

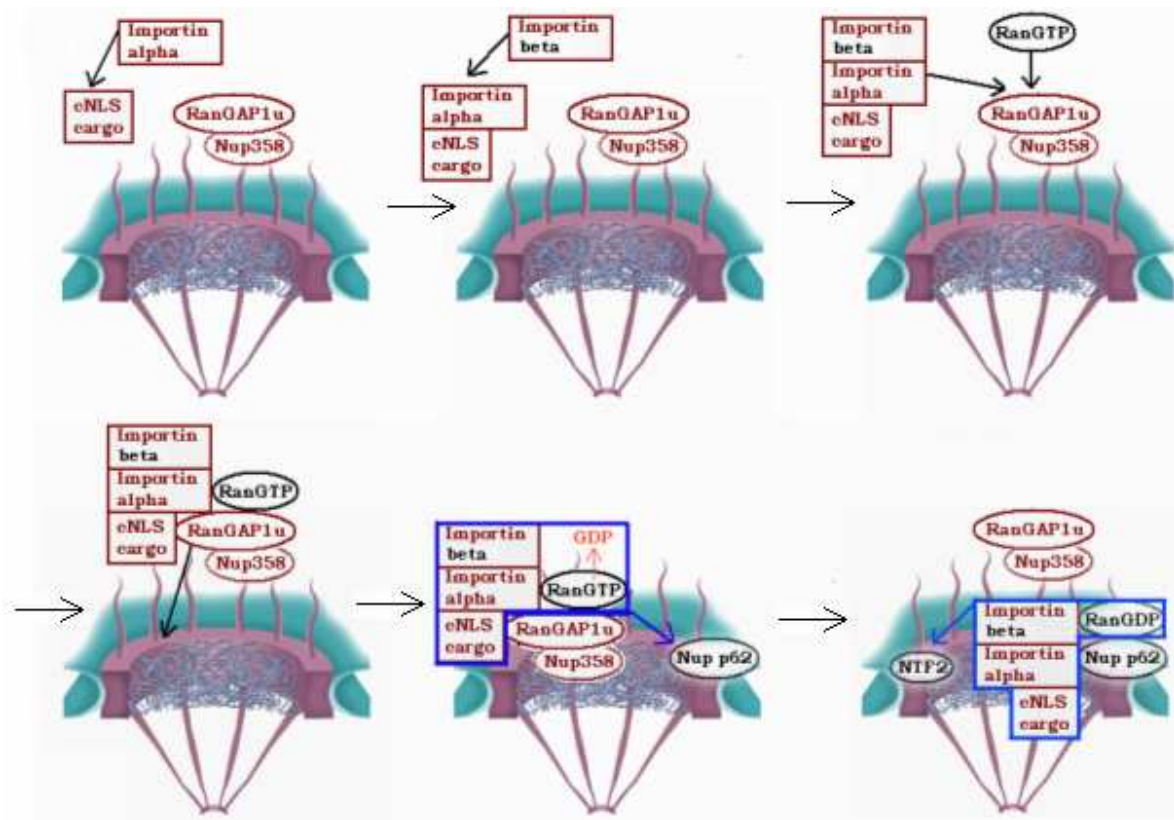


Figure 4. Docking of cNLS cargo complex to the NPC.

After the assembly of the importin- α /cNLS cargo/importin- β complex in cytoplasm, the cargo complex and RanGTP binds to RanGAP1u bound to Nup358. Consequently, cytoplasmic NPC fibril with the cargo complex probably bends towards the CGC (central gated channel) of the NPC. Then hydrolysis of GTP on Ran takes place, the cargo complex is released from the fibril and binds to Nup p62 and to NTF2 which transports the cargo complex via CGC. (modified, from <http://stke.sciencemag.org>)

(nuclear transport factor) protein on the cytoplasmic face of the CGC (Paschal and Gerace, 1995); NTF2 is a small homodimer protein which binds to RanGDP only, not to RanGTP. As a result, unidirectionality of RanGDP-cargo complex transport to the nucleus is maintained, because RanGDP changes to RanGTP immediately after reaching the nuclear space. When reaching the nucleoplasm after crossing the CGC, the cargo complex binds to Nup153, where it breaks up (Shah et al., 1998, figure 4). To describe the mechanism of translocation of the cargo complex through the network of FG Nups of the CGC, more research is needed; for further details see chapter 2.3.

Disassembly of the importin- α /cNLS protein/importin- β complex has three stages: 1) Nup153 binds importin- β of the cargo complex by FG repeats. The connection of importin- β with FG repeats is broken, when GTP and a soluble factor, most probably RanGTP, is present. 2) RanGTP, being in the nucleus in high concentration, binds to importin- β of the cargo complex causing it to be released from the complex (Floer et al., 1997). 3) The release of importin- β renders importin- α /cNLS protein complex unstable, thus the cargo cNLS protein is released to the nucleoplasm. Like Nup153, Tpr protein binds to the complex too (Shah et al., 1998). Its role is still unclear but recent studies show that Tpr binds importin- β complexes, suggesting it plays a direct role in import and/or recycling of importin- β (Ben-Efraim et al., 2009). It is probable that Tpr keeps importin- β in proximity to the NPC. This is consistent with the fact importin- β is not present in the nucleoplasm after crossing the NPC (Görlich et al., 1995; see Figure 5 for scheme).

At least two aspects remain unclear: it has not been shown when NTF2 leaves the transport complex – if it is released right after reaching nucleoplasmic face of the NPC or after destabilization of the complex by RanGTP. Further, it remains to elucidate when RanGDP is released from the transport complex. It is not clear if the complex with RanGDP breaks up by action of another Ran molecule with bound GTP, or if the RanGDP of the complex is changed to GTP form, thus destabilizing the complex.

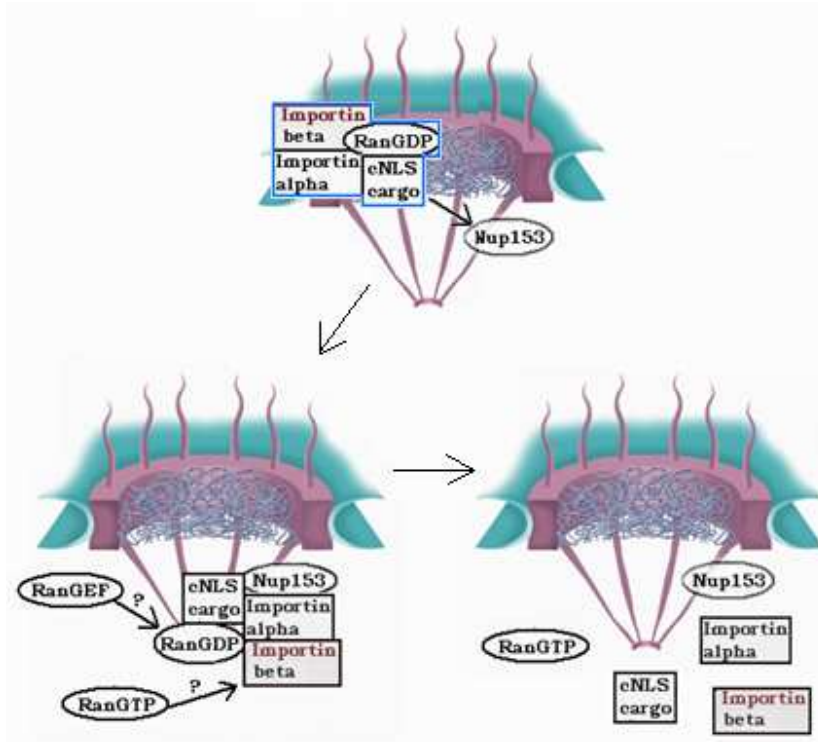


Figure 5. Release of the cNLS cargo to the nucleoplasm.

It is not clear whether NTF2 is still a part of the complex at this moment; NTF2 is not shown. After passing through the central gated channel of the NPC, the transport complex binds to Nup153 where it undergoes disassembly by RanGTP. It is not known if this RanGTP comes from the nucleoplasm or is created of the RanGDP of the transport complex due to nuclear RanGEF. Tpr protein, which binds importin- β is not shown, its role remains unclear (modified, taken from <http://stke.sciencemag.org>).

4.2 Ran-dependent export from the nucleus

The nuclear export has been described poorly so far. It is not known how the export complex travels through the central gated channel of the NPC and how the export process is terminated.

The export of proteins with NES out from the nucleus requires RanGTP and exportin. The association of exportin with RanGTP is broken only if RanGTP hydrolyzes its GTP. Thus, RanGAP1, the Ran GTPase activity stimulating protein located at the cytoplasmic side, should be involved. RanBP1 and RanBP3 are also implicated – RanBP1 raises accessibility of RanGTP/exportin/NES cargo complex for RanGAP1 outside the nucleus and RanBP3 enhances RanGEF nucleotide exchange activity during nuclear import (Nemergut et al., 2002) and raises the affinity of exportin to RanGTP and proteins with hydrophobic NES in the nucleus during nuclear export (Lindsay et al., 2001). The transition through the central gated channel of the pore may proceed by low affinity associations of the exportin with FG repeats of the NPC (Ribbeck and Görlich, 2001).

Afterwards, the complex breaks up by RanGTP hydrolysis stimulated by RanGAP1 (Paraskeva et al., 1999) or by release of RanGTP from the transport complex (Kehlenbach et al., 1999). RanGDP returns to the nucleus with NTF2, and RanBP3 is imported back to the nucleus by importin- α 3 (Welch et al., 1999). NTF2 can freely travel between the cytoplasm and the nucleus due to free diffusion, because it is only a 28 kDa large protein. NXT, NTF2-related export protein (also called p15), is also involved in nuclear export. It binds to the export complex in the nucleoplasm and facilitates the final substrate release from the transport complex in the cytoplasm (Black et al., 2001). As well as in import, Tpr protein plays a direct role in nuclear export; it has been shown that Tpr binds to CRM1 in export complex with NES cargo (Ben-Efraim et al., 2009).

Both import and export factors have to be “recycled” back to the cytoplasm/nucleus after the transport procedure. For example, recycling of import factors proceeds either by binding RanGTP, which is exported from nucleus, or by functioning as an export factor. For instance, importin 13 imports proteins Pax6, RBM8

and hUbc9 to the nucleus while exports protein eIF1A from it (Mingot et al., 2001). In yeast, importin- α is exported from the nucleus in a complex with RanGTP and Cse1p protein, a homologue of human CAS (cellular apoptosis susceptibility protein) (Künzler and Hurt, 1998).

4.3 Ran-independent transport

Ran-independent transport has been described for importin- α , importin- β 1, exportin-t, Crm1, transportin-1, hnRNP K, β -catenin, protein kinase A inhibitor (PKI), calmodulin, TIAR, STAT family proteins and many other proteins.

STAT proteins (signal transducers and activators of transcription) normally undergo a Ran-dependent translocation between the nucleus and the cytoplasm (Meyer and Vinkemeier, 2004), but this translocation may be Ran- and exportin- independent, without any additional factors or energy, because STAT1 can directly interact with FG-nucleoporins Nup153 and Nup214 (Marg et al., 2004).

In vitro, β -catenin does not need any additional factors to be transported and it is inhibited if importin- β 1 is present. Hence, binding sites for β -catenin and importin- β on the NPC probably overlap. The transport of β -catenin is also NES-independent (Fagotto et al., 1998).

RNA-binding proteins TIAR and TIA-1 possess three RNA recognition motifs (RRM), which are also responsible for nuclear import and export. RRM2 is needed for import of these proteins and RRM3 is required for their export. The export is Ran-independent, while the import is Ran-dependent (Zhang et al., 2005).

In the case of glucocorticoid receptor, a Ran-dependent, but Crm1-independent transport alternative was revealed - calreticulin binds to the NES of the glucocorticoid receptor in the nucleus and exports it in complex with RanGTP, substituting Crm1 (Holaska et al., 2002).

5. The regulation of nucleocytoplasmic transport

Since there are a huge amount of different interactors in nucleocytoplasmic transport, a vast number of various regulations of the transport exist. Many proteins have their unique regulations or additional regulatory functions; therefore I describe the general means of regulations only.

5.1 Hiding the substrate NLS/NES from recognition by importin/exportin

The hiding of NLS/NES motifs is the most widespread mechanism of nucleocytoplasmic regulation. The mechanism is based on NLS or NES decreased accessibility for importin/exportin factor due to unfit conformation of the importin/exportin binding site or due to unsuitable electrical charge of the site.

Hiding or unveiling of the NLS/NES is caused by a conformational change due to binding of another protein, by phosphorylation or due to binding of other non-protein ligand. For example, human integrase interactor 1 (INI1) has its NES masked by C-terminus of the protein (Craig et al., 2002).

5.2 Regulation by changing the strength of binding of importin/exportin to the NLS/NES

Nucleocytoplasmic transport can be regulated by different affinity of importin/exportin to NLS/NES. An example of such regulation may be the SV40 virus large T antigen. After phosphorylation by CKII kinase at Ser111, which lies in proximity of the NLS, the affinity of the importin- α /importin- β 1 complex to the T-antigen grows 100 times (Hübner et al., 1997).

5.3 Regulation by co-transport and changing the binding sites of importin/exportin

Each importin/exportin binds many different substrates. Also, many cargo proteins bind other cargo proteins to get in or out from the nucleus (Leslie et al., 2004). This is consistent with the fact that some proteins have to be in exact ratio in order to regulate a cell process. This can also be achieved by transport of two or more different proteins by one importin/exportin at once. Such type of transport was described for histones H2A and H2B and their chaperone Nap1p (nucleosome assembly protein) by importin Kap114p (Mosammamarast et al., 2002). Kap114p has four overlapping substrate binding sites for H2A, H2B, Nap1p and Sua7p as well as binding sites for RanGTP and nucleoporins. Kap114p containing both H2A and H2B and Sua7p cannot be formed, while Kap114p with both Sua7p and Nap1p was found during the experiments. However, when the Kap114p/Sua7p/Nap1p complexes were present, the Kap114p/H2A/H2B/Sua7p complexes have been assembled (Hodges et al., 2005). This may have two reasons: either H2A/H2B conformation is changed due to connection with Kap114p/Sua7p/Nap1p complex or upon binding one substrate, the karyopherin conformational change could create another binding site for other cargo.

5.4 Regulation by retention in the nucleus or cytoplasm

The nucleocytoplasmic transport of a molecule can be prohibited or enabled if the molecule is kept in the cytoplasm or in the nucleus. For example, the tumor suppressor p53 seems to be retained in cytoplasm by Parc protein (Parkin-like ubiquitin ligase) – when Parc is suppressed, the p53 is imported to the nucleus (Nikolaev et al., 2003).

Cytoplasmic or nuclear retention can be regulated by phosphorylation. The chicken anemia virus protein 3 (apoptin) is normally nuclear, because its NES is disabled by phosphorylated Thr108. The mutation of the Thr108 renders the site unable to be phosphorylated and consequently, apoptin concentration in the nucleus is significantly reduced (Poon et al., 2005).

5.5 Regulation by availability of importins and exportins

The level of expression of any nucleocytoplasmic transport regulator influences thus regulates the transport. The expression of transport regulators is often tissue-specific. For instance, importin- α genes expression in mouse is different across the tissues – importin- α mSRP1 is present mainly in brain and cerebellum, whereas mPendulin importin- α levels are high in thymus and spleen (Prieve et al., 1996). Importin- α hSRP1 γ represents 1% of all the proteins in skeletal muscle myocytes, but is absent in heart, kidney and spleen (Nachury et al., 1997).

5.6 Regulation by changing the variety of nucleoporins

Nucleoporins have different specificity or affinity to various importins and exportins (Allen et al., 2001) or to the cargoes directly; also, the contents of the NPC is diverse across the tissues (Fan et al., 1996). For example, nucleoporin Nup BS-63, a splice variant of Nup358, interacts with Ran, importin- β 2 and the chromatin remodeling factor aF10, but it is present in germinal cells of testis only (Cai et al., 2002).

6. Nucleocytoplasmic transport mechanism differences between animals and plants

The nucleocytoplasmic transport mechanisms are highly conserved in both, but differences do exist. There is not much known about plant nucleocytoplasmic transport uniques. The best described known difference between animals and plants is plant RanGAP1, which shows a plant-specific interaction with nuclear membrane, which is substantially different from its animal counterpart.

6.1 Plant RanGAP1

Ran is present mostly in the GTP form in the nucleus due to nuclear RanGEF activity and in the GDP form in the cytoplasm because of cytoplasmic RanGAP activity (Izaurre et al., 1997). In order to maintain the RanGDP pool in the cytoplasm in close proximity to the NPC, the RanGAP1 has to be localized close to the NPC. This is provided by SUMOylation on C-terminus of vertebrate RanGAP1; SUMOylated RanGAP1 (RanGAP1u) binds to Nup358 in cytoplasmic fibrils of the NPC.

It is notable that *Saccharomyces cerevisiae* RanGAP does not concentrate near the nuclear envelope and it lacks the SUMOylation domain (Hopper et al., 1990; Melchior et al., 1993). The *Aspergillus nidulans* RanGAP is not associated with the nuclear envelope either (de Souza et al., 2004). The dispensability of element concentrating the RanGAP at the nuclear envelope might be caused by higher concentration of RanGTP in general, or by smaller cell size, which would imply lower diffusion of RanGTP out from the nuclear periphery.

Unlike vertebrate RanGAP1, *Arabidopsis* RanGAP1 (AtRanGAP1), although localized on the nuclear periphery, does not have the C-terminal sequence, which can be SUMOylated. Thus, the protein cannot be tethered to Nup358 in a vertebrate fashion. As mentioned above, the plant NPC was not found to possess Nup358 (RanBP2).

However, AtRanGAP1 contains unique plant-specific WPP domain (with Trp-Pro-Pro motif) at the N-terminus, which serves for tethering AtRanGAP1 to the nuclear membrane. An interactor with WPP was found; the protein was named WPP-domain interacting protein 1 (WIP1). It binds to the N-terminal WPP domain of AtRanGAP1 via its C-terminal domain. Two homologues of WIP1 in *Arabidopsis* were discovered – WIP2 and WIP3. All the WIP proteins have, apart from the WPP-interacting domains, C-terminal transmembrane tails (TMD), which are necessary for the connection with the nuclear envelope (Meier, 2000).

7. Specific pathways *in planta* involving nucleocytoplasmic transport

7.1 Nucleoporins in plant-microbe interactions

An *Arabidopsis* homolog of mammalian Nup96, a part of the Nup107-160 complex, is important for the NPC assembly (Zhang and Li, 2005; Walther et al., 2003). This nucleoporin, the product of MOS3 gene, seems to be important for pathogen defence of the plant, because plants with *mos3* mutation are more susceptible to some pathogens like *Pseudomonas syringae*. Proteins of which nucleocytoplasmic transport might be affected by Nup96, are NPR1 (disease resistance protein), PAD4 (protein arginine deiminase), bZIP10 (basic leucine zipper transcription factor) and EDS1 (enhanced disease susceptibility protein) (Wiermer et al., 2007).

In *Lotus japonicus* and *Medicago truncatula*, two nucleoporins related to mycorrhiza have been found – Nup133 and Nup85, both part of the Nup107-160 complex. Loss-of-function mutants of these two nucleoporins have a temperature sensitive deficiency in mycorrhizal colonization and nodule formation, and their seed production is reduced (Kanamori et al., 2006; Saito et al., 2007).

7.2 Hormone signaling

AXR1 is a subunit of RUB-activating protein, which is a part of the pathway, which leads to auxin-dependent degradation of the Aux/IAA repressors. Two suppressors of AXR1 have been found – SAR1 and SAR3, homologues of animal Nup160 and Nup96. Apart from influencing the AXR1, these nucleoporins also play a role in import of AXR3 (auxin transcriptional repressor) to the nucleus (Parry et al., 2006).

7.3 Cold-stress resistance

The defect in *Arabidopsis* Nup160 renders plants more susceptible to cold stress and disrupts acquired freezing tolerance. The ICE1 cold response regulator is not affected and it is possible that the lowered cold stress resistance is caused by abnormal mRNA export as described below (Chun-Hai Dong et al., 2006).

7.4 Flowering time regulation

All the plant nucleoporin mutants mentioned (disrupted Nup85, Nup96, Nup133 and Nup160) flower early and have other defects like impaired stamens, stunted shape and abnormal transition from juvenile to adult. A very early-flowering phenotype is observed in NUA (nuclear pore anchor) mutant. NUA is an inner nuclear envelope protein associated with nuclear basket of the NPC. NUA (also called AtTpr) is homologous to human Tpr and yeast Mlp1/Mlp2 (Xu et al, 2007 /76/). When mutated, NUA suppresses the expression of the FLC floral repressor (Jacob et al., 2007).

7.5 mRNA export

The mRNA export from the plant nucleus has been described very poorly so far. The only available information is that the described mutants of nucleoporins of the Nup107-Nup160 complex accumulate mRNA in the nucleus, as well as NUA protein, *los4-1* (low expression of osmotically responsive genes) and *los4-2* mutants do (the above mentioned references and Gong and Dong, 2005; Dong et al., 2006; Xu et al., 2007 /80/). Also it is known that mRNA export and quality control of the exported RNAs is related to SUMOylation.

8. Methods and approaches used in nucleocytoplasmic transport studies

Methods used in studies of the nucleocytoplasmic transport differ by the choice of the structure examined.

The transport itself is usually studied by observation of molecules involved in the transport tagged by a fluorescent tag like fluorescein or fusion with GFP. For instance, mRNA export defects have been determined by exposing the cells to oligo(dT) probes tagged by fluorescein. These probes bind to poly(A) ends of the mRNAs thus making them visible using fluorescence microscopy. The consequent comparison of wild type cells with the cells containing mutation in mRNA export effector shows significant difference in fluorescein signal in the nucleus and the cytoplasm (Gong and Dong, 2005).

Another factor used in the study of transport mechanisms is wheat germ agglutinin (WGA, lectin) and leptomycin B. WGA binds to N-acetyl glycosylated nucleoporins and inhibits them, therefore, WGA-sensitive transport is dependent on N-acetyl glycosylated nucleoporins. Leptomycin B inhibits specifically exportin Crm1 (Kudo et al., 1999). Thus, in order to determine if the export of a molecule is dependent on Crm1, the use of leptomycin B may be sufficient.

The 3D structure of the NPC is studied using electron microscopy, more precise cryo-electron microscopy and X-ray crystallography. The most advanced approach is cryo-electron tomography, which requires less purified material.

9. Conclusion

The role of the nucleocytoplasmic transport in plants is obvious – it is a checkpoint in signaling pathways, which usually lead to the gene expression or it represents a quality-control of exported messenger RNAs and other RNAs.

The regulation of nucleocytoplasmic transport is a less clear topic. There are many common regulation patterns operating in cells, but there are plenty unique, uncategorizable regulations as well. Therefore, it is complicated to describe “regulation”

of such a huge and complicated signaling pathway checkpoint as of the nucleocytoplasmic transport.

Contemporary biology of the plant nucleocytoplasmic transport is clearly still in its childhood. Fortunately, recently there is a stronger interest in plants too. As the human population grows (and surely will grow) and tries harder to fight with the growing need for food, I think we can expect a higher influx of resources to the biology of the plants. I am convinced that even the beginning of efforts to colonize extraterrestrial planets will bring a significant contribution to the biology of the plant cell, because plants provide us with food, oxygen, materials, as well as beautiful visual percepts and many other essentials.

The study of the nucleocytoplasmic transport also enriches the available evolutionary data by new homologous structures across the kingdoms. I take import to mention the evolutionary relationships because science is subjectively devaluated by the orthodox creationism, which still exists in a considerable portion of the world and has a significant impact on the view of science.

10. Acknowledgements

I kindly thank my supervisor RNDr. Kateřina Schwarzerová, Ph.D. for patience with my endless questions, her kind and professional attitude and for all the support.

I thank my friend Julie Fishman for partial proofreading of the English text.

I thank my parents for all the support and help with informational and linguistic consistency of the text.

11. References

- Allen NPC, Huang L, Burlingame A, Rexach M (2001) Proteomic analysis of nucleoporin interacting proteins. *J Biol Chem* 276(31): 29268-29274
- Ben-Efraim I, Frosst PD, Gerace L (2009) Karyopherin binding interactions and nuclear import mechanism of nuclear pore complex protein Tpr. *BMC Cell Biol* 10: 74
- Ben-Efraim I, Gerace L (2001) Gradient of increasing affinity of importin β for nucleoporins along the pathway of nuclear import. *J Cell Biol* 152, 411–417
- Bischoff FR, Krebber H, Smirnova E, Dong W, Ponstingl H (1995) Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. *EMBO J* 14(4): 705-715
- Bischoff FR, Ponstingl H (1991) Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. *Nature* 354(6348): 80-82
- Black BE, Holaska JM, Lévesque L, Ossareh-Nazari B, Gwizdek C, Dargemont C, Paschal BM (2001) NXT1 is necessary for the terminal step of Crm1-mediated nuclear export. *J Cell Biol* 152(1): 141-155
- Cai Y, Gao Y, Sheng Q, Miao S, Cui X, Wang L, Zong S, Koide SS (2002) Characterization and potential function of a novel testis-specific nucleoporin BS-63. *Mol Reprod Dev* 61: 126-134
- Cingolani G, Bednenko J, Gillespie MT, Gerace L (2002) Molecular basis for the recognition of a nonclassical nuclear localization signal by importin beta. *Mol Cell* 10(6): 1345-1353.
- Craig E, Zhang Z-K, Davies KP, Ganjam VK (2002) A masked NES in INI1/hSNF5 mediates hCRM1-dependent nuclear export: implications for tumorigenesis. *EMBO J* 21: 31-42
- Cronshaw JM, Krutchinsky AN, Zhang W, Chait BT, Matunis MJ (2002) Proteomic analysis of the mammalian nuclear pore complex. *J Cell Biol* 158(5): 915-27
- Dang CV, Lee WM (1988) Identification of the human c-myc protein nuclear translocation signal. *Mol Cell Biol.* 8(10):4048-4054
- Deane E, Schäfer W, Zimmermann H-P, Mueller L, Görlich D, Prehn S, Ponstingl H, Bischoff FR (1997) Ran-binding protein 5 (RanBP5) is related to the nuclear transport factor importin-beta but interacts differently with RanBP1. *Mol Cell Biol* 17(9): 5087-5096

Dingwall C, Kandels-Lewis S, Séraphin B (1995) A family of Ran binding proteins that includes nucleoporins. *Proc Natl Acad Sci USA* 92(16): 7525-7529

Dong CH, Hu X, Tang W, Zheng X, Kim YS, Lee BH, Zhu JK (2006) A putative Arabidopsis nucleoporin, AtNUP160, is critical for RNA export and required for plant tolerance to cold stress. *Mol Cell Biol* 26(24): 9533-9543

Fagotto F, Glück U, Gumbiner BM (1998) Nuclear localization signal-independent and importin/karyopherin-independent nuclear import of beta-catenin. *Curr Biol* 8(4): 181-190

Fan F, Liu C-P, Korobova O, Heyting C, Offenberger HH, Trump G, Arnheim N (1997) cDNA cloning and characterization of Npap60: a novel rat nuclear pore-associated protein with an unusual subcellular localization during male germ cell differentiation. *Genomics* 40: 444-453

Fischer U, Huber J, Boelens WC, Mattaj IW, Lührmann R (1995) The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell* 82: 475-483

Fišerová J, Goldberg MW (2010) Nucleocytoplasmic transport in yeast: a few roles for many actors. *Biochem Soc Trans* 38: 273-277

Fišerová J, Kiseleva E, Goldberg MW (2009) Nuclear envelope and nuclear pore complex structure and organization in tobacco BY-2 cells. *Plant J* 59(2): 243-255

Floer M, Blobel G, Rexach M (1997) Disassembly of RanGTP-Karyopherin beta complex, an intermediate in nuclear protein import. *J Biol Chem* 272(31): 19538-19546

Frey S, Gorlich D (2007) A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes. *Cell* 130, 512–523

Goldberg MW, Allen TD (1996) The nuclear pore complex and lamina: three-dimensional structures and interactions determined by field emission in-lens scanning electron microscopy. *J Mol Biol* 257, 848–865

Gong Z, Dong CH, Lee H, Zhu J, Xiong L, Gong D, Stevenson B, Zhu JK (2005) A DEAD box RNA helicase is essential for mRNA export and important for development and stress responses in Arabidopsis. *Plant Cell* 17(1): 256-267

Görlich D, Dabrowski M, Bischoff FR, Kutay U, Bork P, Hartmann E, Prehn S, Izaurralde E (1997) A novel class of RanGTP binding proteins. *J Cell Biol* 138(1): 65-80

Görlich D, Kostka S, Kraft R, Dingwall C, Laskey RA, Hartmann E, Prehn Siegfried (1995) Two different subunits of importin cooperate to recognize nuclear localization signals and bind them to the nuclear envelope. *Curr Biol* 5: 353-392

Görlich D, Prehn S, Laskey RA, Hartmann E (1994) Isolation of a protein that is essential for the first step of nuclear protein import. *Cell* 79: 767-778

Guddat U, Bakken AH, Pieler T (1990) Protein-mediated nuclear export of RNA: 5S rRNA containing small RNPs in xenopus oocytes. *Cell* 60(4): 619-628

Hall MN, Craik C, Hiraoka Y (1990) Homeodomain of yeast repressor $\alpha 2$ contains a nuclear localization signal. *Proc Natl Acad Sci USA* 87: 6954-6958

Herold A, Suyama M, Rodrigues JP, Braun IC, Kutay U, Carmo-Fonseca M, Bork P, Izaurralde E (2000) TAP (NXF1) belongs to a multigene family of putative RNA export factors with a conserved modular architecture. *Mol Cell Biol* 20(23): 8996-9008

Hodges JL, Leslie JH, Mosammaparast N, Guo Y, Shabanowits J, Hunt DF, Pemberton LF (2005) Nuclear import of TFIIB is mediated by Kap114p, a karyopherin with multiple cargo-binding domains. *Mol Biol Cell* 16: 3200-3210

Holaska JM, Black BE, Rastinejad F, Paschal BM (2002) Ca^{2+} -dependent nuclear export mediated by calreticulin. *Mol Cell Biol* 22(17): 6286-6297

Hopper AK, Traglia HM, Dunst RW (1990) The yeast RNAI gene product necessary for RNA processing is located in the cytosol and apparently excluded from the nucleus. *J Cell Biol* 111: 309-321

Hübner S, Xiao C-Y, Jans DA (1997) The protein kinase CK2 site (Ser111/112) enhances recognition of the simian virus 40 large T-antigen nuclear localization sequence by importin. *J Biol Chem* 272(27): 17191-17195

Izaurralde E, Kutay U, von Kobbe C, Mattaj IW, Görlich D (1997) The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J* 16(21): 6535-6547

Jacob Y, Mongkolsiriwatana C, Veley KM, Kim SY, Michaels SD (2007) The nuclear pore protein AtTPR is required for RNA homeostasis, flowering time, and auxin signaling.

Plant Physiol 144(3): 1383-1390

Jans DA, Xiao C-Y, Lam MHC (2000) Nuclear targeting signal recognition: a key control point in nuclear transport? *Bioessays* 22: 532-544

Kanamori N, Madsen LH, Radutoiu S, Frantescu M, Quistgaard EM, Miwa H, Downie JA, James EK, Felle HH, Haaning LL, Jensen TH, Sato S, Nakamura Y, Tabata S, Sandal N, Stougaard J (2006) A nucleoporin is required for induction of Ca^{2+} spiking in legume nodule development and essential for rhizobial and fungal symbiosis. *Proc Natl Acad Sci USA* 103(2): 359-364

- Kang Y, Cullen BR (1999) The human Tap protein is a nuclear mRNA export factor that contains novel RNA-binding and nucleocytoplasmic transport sequences. *Genes Dev* 13(9): 1126-1139
- Keminer O, Peters R (1999) Permeability of single nuclear pores. *Biophys J* 77, 217–228
- Kim VN (2004) MicroRNA precursors in motion: exportin-5 mediates their nuclear export. *Trends Cell Biol* 14(4): 156-159
- Kiseleva E, Allen TD, Rutherford S, Bucci M, Wentz SR, Goldberg MW (2004) Yeast nuclear pore complexes have a cytoplasmic ring and internal filaments. *J Struct Biol* 145, 272–288
- Komeili A, O'Shea EK (1999) Roles of phosphorylation sites in regulating activity of the transcription factor Pho4. *Science* 284(5416): 977-980
- Kramer A, Ludwig Y, Shahin V, Oberleithner H (2007) A pathway separate from the central channel through the nuclear pore complex for inorganic ions and small macromolecules. *J Biol Chem* 282, 31437–31443
- Kudo N, Matsumori N, Taoka H, Fujiwara D, Schreiner EP, Wolff B, Yoshida M, Horinouchi S (1999) Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. *Proc Natl Acad Sci USA* 96(16): 9112-9117
- Künzler M, Gerstberger T, Stutz F, Bischoff FR, Hurt E (2000) Yeast Ran-binding protein 1 (Yrb1) shuttles between the nucleus and cytoplasm and is exported from the nucleus via a CRM1 (XPO1)-dependent pathway. *Mol Cell Biol* 20(12): 4295-4308
- Künzler M, Hurt EC (1998) Cse1p functions as the nuclear export receptor for importin alpha in yeast. *FEBS Lett* 433(3): 185-190
- Leslie DM, Zhang W, Timney BL, Chait BT, Rout MP, Wozniak RW, Aitchison JD (2004) Characterization of karyopherin cargoes reveals unique mechanisms of Kap121p-mediated nuclear import. *Mol Cell Biol* 24(19) 8487–8503
- Lindsay ME, Holaska JM, Welch K, Paschal BM, Macara IG (2001) Ran-binding protein 3 is a cofactor for Crm1-mediated nuclear protein export. *J Cell Biol* 153(7): 1391-1402
- Mahajan R, Delphin C, Guan T, Gerace L, Melchior F (1997) A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 88: 97-107

- Marg A, Shan Y, Meyer T, Meissner T, Brandenburg M, Vinkemeier U (2004) Nucleocytoplasmic shuttling by nucleoporins Nup153 and Nup214 and CRM1-dependent nuclear export control the subcellular distribution of latent Stat1. *J Cell Biol* 165(6): 823-833
- Meier I (2000) A novel link between ran signal transduction and nuclear envelope proteins in plants. *Plant Physiol* 124(4): 1507-1510
- Meier I, Brkljacic J (2009) The nuclear pore and plant development. *Curr Opin Plant Biol* 12: 87-95
- Melchior F, Paschal B, Evans J, Gerace L (1993) Inhibition of nuclear protein import by nonhydrolyzable analogues of GTP and identification of the small GTPase Ran/TC4 as an essential transport factor. *J Cell Biol* 123(6 Pt 2): 1649-1659
- Meyer T, Vinkemeier U (2004) Nucleocytoplasmic shuttling of STAT transcription factors. *Eur J Biochem* 271(23-24): 4606-4612
- Mingot J-M, Kostka S, Kraft R, Hartmann E, Görlich D (2001) Importin 13: a novel mediator of nuclear import and export. *EMBO J* 20(14): 3685-3694
- Mosammaparast N, Guo Y, Shabanowitz J, Hunt DF, Pemberton LF (2002) Pathways mediating the nuclear import of histones H3 and H4 in yeast. *J Biol Chem* 277, 862-868
- Nachury MV, Ryder UW, Lamond AI, Weis K (1997) Cloning and characterization of hSRP1g, a tissue-specific nuclear transport factor. *Proc Natl Acad Sci USA* 95: 582-587
- Nemergut ME, Lindsay ME, Brownawell AM, Macara IG (2002) Ran-binding protein 3 links Crm1 to the Ran guanine nucleotide exchange factor. *J Biol Chem* 277(20): 17385-17388
- Nikolaev AY, Li M, Puskas N, Qin J, Gu W (2003) Parc: a cytoplasmic anchor for p53. *Cell* 112: 29-40
- Niogret MF, Culiáñez-Macià FA, Goday A, Mar Albà M, Pagès M (1996) Expression and cellular localization of rab28 mRNA and Rab28 protein during maize embryogenesis. *Plant J.* 9(4): 549-557
- Panté N, Aebi U (1996) Sequential binding of import ligands to distinct nucleopore regions during their nuclear import. *Science* 273(5282): 1729-1732
- Paraskeva E, Izaurralde E, Bischoff FR, Huber J, Kutay U, Hartmann E, Lührmann R, Görlich D (1999) CRM1-mediated Recycling of Snurportin 1 to the Cytoplasm. *J Cell Biol* 145(2): 255-264

Parry G, Ward S, Cernac A, Dharmasiri S, Estelle M (2006) The Arabidopsis SUPPRESSOR OF AUXIN RESISTANCE proteins are nucleoporins with an important role in hormone signaling and development. *Plant Cell* 18(7): 1590-1603

Paschal BM, Gerace L (1995) Identification of NTF2, a cytosolic factor for nuclear import that interacts with nuclear pore complex protein p62. *J Cell Biol* 129(4): 925-937

Pollard VW, Michael WM, Nakielny S, Siomi MC, Wang F, Dreyfuss G (1996) A novel receptor-mediated nuclear protein import pathway. *Cell* 86: 985-994

Poon IK, Oro C, Dias MM, Zhang J, Jans DA (2005) Apoptin nuclear accumulation is modulated by a CRM1-recognized nuclear export signal that is active in normal but not in tumor cells. *Cancer Res* 65(16):7059-7064

Prieve MG, Guttridge KL, Munguia JE, Waterman ML (1996) The nuclear localization signal of lymphoid enhancer factor-1 is recognized by two differentially expressed Srp1-nuclear localization sequence receptor proteins. *J Biol Chem* 271(13): 7654-7658.

Radu A, Blobel G, Moore MS (1995) Identification of a protein complex that is required for nuclear protein import and mediates docking of import substrate to distinct nucleoporins. *Proc Natl Acad Sci USA* 92(5): 1769-1773

Ribbeck K, Görlich D (2001) Kinetic analysis of translocation through nuclear pore complexes. *EMBO J* 20(6): 1320-1330

Rout MP, Aitchison JD, Suprpto A, Hjertaas K, Zhao Y, Chait BT (2000) The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J Cell Biol* 148(4): 635-651

Saito K, Yoshikawa M, Yano K, Miwa H, Uchida H, Asamizu E, Sato S, Tabata S, Imaizumi-Anraku H, Umehara Y, Kouchi H, Murooka Y, Szczyglowski K, Downie JA, Parniske M, Hayashi M, Kawaguchi M (2007) Nucleoporin85 is required for calcium spiking, fungal and bacterial symbioses, and seed production in *Lotus japonicus*. *Plant Cell* 19(2): 610-624

Schwarzerová K, Petrášek J, Panigrahi KCS, Zelenková S, Opatrný Z, Nick P (2006) Intranuclear accumulation of plant tubulin in response to low temperature. *Protoplasma* 227: 185-196

Shah S, Tugendreich S, Forbes D (1998) Major binding sites for the nuclear import receptor are the internal nucleoporin Nup153 and the adjacent nuclear filament protein Tpr. *J Cell Biol* 141(1) 31-49

Sorokin AV, Kim ER, Ovchinnikov LP (2007) Nucleocytoplasmic transport of proteins. *Biochemistry (Mosc)* 72(13): 1439-1457

de Souza CP, Osmani AH, Hashmi SB, Osmani SA (2004) Partial nuclear pore complex disassembly during closed mitosis in *Aspergillus nidulans*. *Curr Biol* 14(22):1973-1984

Stoffler D, Fahrenkrog B, Aebi U (1999) The nuclear pore complex: from molecular architecture to functional dynamics. *Curr Opin Cell Biol* 11(3): 391-401

Walther TC, Alves A, Pickersgill H, Loiodice I, Hetzer M, Galy V, Hülsmann BB, Köcher T, Wilm M, Allen T, Mattaj IW, Doye V (2003) The conserved Nup107-160 complex is critical for nuclear pore complex assembly. *Cell* 113(2): 195-206

Welch K, Franke J, Köhler M, Macara IG (1999) RanBP3 contains an unusual nuclear localization signal that is imported preferentially by importin- α 3. *Mol Cell Biol* 19(12): 8400-8411

Wiermer M, Palma K, Zhang Y, Li X (2007) Should I stay or should I go? Nucleocytoplasmic trafficking in plant innate immunity. *Cell Microbiol* 9(8): 1880-1890

Xu XM, Meier I (2007) The nuclear pore comes to the fore. *Trends Plant Sci* 13(1): 20-27

Xu XM, Rose A, Meier I (2007) NUA Activities at the plant nuclear pore. *Plant Signal Behav* 2(6): 553-555

Xu XM, Rose A, Muthuswamy S, Jeong SY, Venkatakrisnan S, Zhao Q, Meier I (2007) Nuclear pore anchor, the Arabidopsis homolog of Tpr/Mlp1/Mlp2/Megator, is involved in mRNA export and SUMO homeostasis and affects diverse aspects of plant development. *Plant Cell* 19: 1537-1548

Yang Q, Rout MP, Akey CW (1998) Three-dimensional architecture of the isolated yeast nuclear pore complex: functional and evolutionary implications. *Mol Cell* 1(2): 223-234

Yokoyama N, Hayashi N, Seki T, Panté N, Ohba T, Nishii K, Kuma K, Hayashida T, Miyata T, Aebi U et al. (1995) A giant nucleopore protein that binds Ran/TC4. *Nature* 376(6536): 184-188

Zeng Y, Cullen BR (2004) Structural requirements for pre-microRNA binding and nuclear export by Exportin 5. *Nucleic Acids Res* 32(16): 4776-4785

Zhang T, Delestienne N, Huez G, Krays V, Gueydan C (2005) Identification of the sequence determinants mediating the nucleo-cytoplasmic shuttling of TIAR and TIA-1 RNA-binding proteins. *J Cell Sci* 118(Pt 23): 5453-5463

Zhang Y, Li X (2005) A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by suppressor of npr1-1, constitutive 1. *Plant Cell* 17: 1306-1316