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REVIEWS

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Organization and Regulation of Nucleocytoplasmic Transport

S. P. Chumakov[a, b] and V. S. Prassolov[a]

[a] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 119991 Russia; e-mail: prasolov@eimb.ru*

[b] *Department of Molecular Genetics, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA*

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Abstract—Separation of DNA replication and transcription, which occur in the nucleus, from protein synthesis, which occurs in the cytoplasm, allows a more precise regulation of these processes. Selective exchange of macromolecules between the two compartments is mediated by proteins of the nuclear pore complex (NPC). Receptor proteins of the karyopherin family interact with NPC components and transfer their cargos between the nucleus and cytoplasm. Nucleocytoplasmic transport pathways are regulated at multiple levels by modulating the expression or function of individual cargoes, transport receptors, or the transport channel. The regulatory levels have increasingly broad effects on the transport pathways and affect a wide range of processes from gene expression to development and differentiation.

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Key words: nuclear pore, intracellular transport of macromolecules, karyopherins, nucleoporins, nuclear transport regulation, Ran-dependent transport

INTRODUCTION

Since eukaryotic genetic material is localized in a separate compartment (the nucleus), DNA replication and transcription are spatially separated from protein synthesis, which occurs in the cytoplasm. The spatial separation provides the cell with additional opportunities to finely regulate the processes and, on the other hand, requires the highly selective exchange of many macromolecules between the nucleus and cytoplasm. Exchange is due to a variety of receptor transport proteins, which interact with components of the nuclear pore complex (NPC) to transport bound proteins between the two compartments. Each of the receptors is involved in its specific transport pathway, transferring a certain set of substrates (cargoes). The state of a substrate also plays an important role in the transfer, since a cargo must have necessary signal sequences to properly bind to its receptor. The majority of cell signaling pathways are involved in signal transduction between the nucleus and cytoplasm. Thus, detailed knowledge of the organization and regulation of nucleocytoplasmic transport is essential for the understanding of cell metabolism and functional activity.

Macromolecular exchange between the nucleus and cytoplasm is mediated by nuclear pores. Selective transport of molecules is due to the NPC. Nuclear pores were first identified in 1950, when the nuclear membrane was examined by electron microscopy [1]. Further studies showed that holes in the nuclear membrane are occupied by the NPC, whose structure is evolutionarily conserved [2]. The NPC is a protein complex of 40–60 MDa that has sevenfold symmetry and consists of more than 30 proteins known as nucleoporins (Nup).

The spatial structure of the NPC was established by tomographic reconstruction. The NPC consists of a cylindrical central framework, eight microfilaments attached to it at the cytoplasmic side, and a nuclear basket of eight filaments, which are attached to the framework at the nuclear side and are distally bound with each other. The central part of the NPC has a channel, which resembles a sand glass in shape and has a diameter of approximately 45 nm in the narrowest region (Fig. 1) [3].

The cytoplasmic filaments of the NPC are approximately 35 nm in length; the nuclear filaments, which form the nuclear basket, are 60 nm. The length of the NPC including the central framework of 50 nm is approximately 150 nm; its outer diameter is 125 nm [4]. NPC components slightly vary in size among organisms but the general structure and proportion are the same [5].

The NPC number on the nuclear membrane broadly varies depending on the cell size and activity. One yeast cell has approximately 200 NPCs, while actively proliferating human cells have an NPC density of 10–20 NPCs/μm^2, corresponding to 2000–5000 NPCs per nucleus. Mature oocytes of the spurtoed frog *Xenopus* have 60 NPCs/μm^2, corresponding to 5×10^7 NPCs per cell [6]. The NPC density increases as the cell progresses through the cell cycle [7].

One NPC transmits 1000 molecules per second on average; i.e., at least ten molecules simultaneously

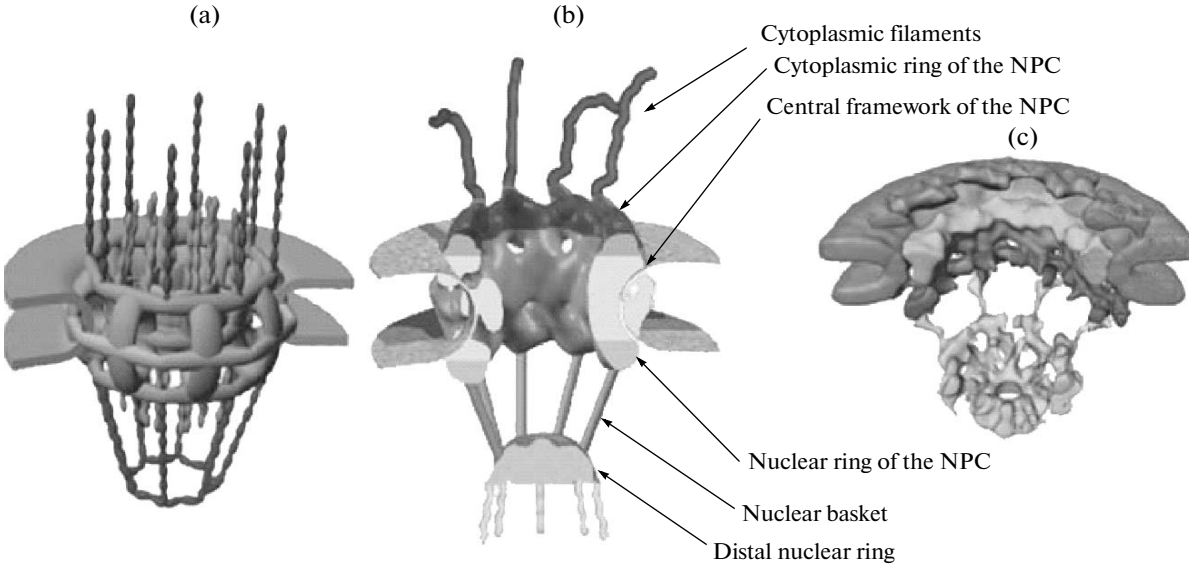

Fig. 1. Models of the NPC structure: (a) an averaged model of the NPC structure, (b) a NPC model based on the reconstruction of complexes embedded in amorphous ice, (c) a reconstruction based on the results of cryoelectron microscopy of intact *Dictyostelium discoideum* nuclei.

pass through one nuclear pore. Proteins of up to 39 nm can pass through the nuclear pore. Proteins of less than 30–40 kDa freely diffuse through the nuclear pore, while larger proteins (40–100 kDa) must have a nuclear localization signal (NLS) or a nuclear export signal (NES) to be transferred from the cytoplasm into the nucleus or backwards. The signals are bound by transport receptors, which are capable of passing through the NPC both alone and in complex with another protein [8].

KARYOPHERINS AND THEIR ROLE IN THE ORGANIZATION OF NUCLEOCYTOPLASMIC TRANSPORT

Highly efficient (more efficient than simple diffusion) transfer of a protein of any size into the nucleus requires the NPC-mediated active transport mechanisms [9]. This transport depends on either affinity of the target protein for NPC components or its complexation with the receptor proteins that have such affinity. Kariopherins, which are the largest class of transport receptors, include importins, exportins, and transportins, which are involved in both nuclear import and export. Importin-β (karyopherin 95 in yeasts) is one of the best-studied karyopherins. This protein binds with cargo molecules through the adaptor protein importin-α (karyopherin 60 in yeasts) and is capable of interacting with several Nup proteins [10].

According to a typical mechanism of interactions of karyopherins with a target protein and Nup, a short motif (NLS or NES) is recognized in the target protein and is bound by karyopherins or intermediate adaptor proteins, which then interact with karyopherins. The resulting protein complex may interact with the Nup proteins contained in the NPC to enter the nucleus or leave it. Proteins of the same size that lack a NLS/NES are incapable of passing across the NPC barrier.

Energy for transportation of complexes through the NPC is generated by a high gradient of the Ran GTPase between the nucleus and cytoplasm. Nuclear RanGTP is capable of releasing the target protein from an imported complex upon binding with karyopherins. Exportins occurring in the nucleus interact with their targets in the presence of RanGTP, which is involved in transferring the complex through the NPC into the cytoplasm, where RanGTP is hydrolyzed to disassemble the transport complex and to replenish the cytoplasmic RanGDP pool [11].

Proteins of the karyopherin family are involved in the majority of specific nucleocytoplasmic transport pathways. There are at least 20 karyopherins in human cells and 14 in yeast cells [12]. Target proteins that are transferred by karyopherins into or out of the nucleus usually have an NLS or NES. The main, or classical, NLS structure was established in studies of the NLS of the SV40 large T antigen. This NLS consists of one or two positively charged amino acid clusters, which are connected via a neutral linker [6]. Further studies revealed many NLSs that lack this classical structure. Functional activity of an NLS may be regulated via posttranslational modification or signal masking as a result of conformational changes.

The classical NLS binds with the adaptor protein karyopherin-α (importin-α), which forms a heterodimer with importin-β. In turn, importin-β ensures

nuclear import [13]. NLS binding is due to a karyopherin-α region that contains ten ARM repeats, each consisting of 40 amino acid residues forming three α-helices. The region assumes a superhelical structure, which has a shallow groove, which accommodates a NLS. An NLS is accommodated in the acceptor pocket of karyopherin-α owing to its certain charge and hydrophobicity. Binding with karyopherin-α, one NLS contacts several ARM repeats [14, 15].

Most proteins of the karyopherin-β family bind with a target protein directly. The primary structures of NLSs recognizable by karyopherin-β are far more diverse than the structures of NLSs interacting with importin-α. An NLS often has a consensus sequence of several positively charged amino acid residues. Such sequences were found in the NLSs of histones, ribosomal proteins, and certain RNA-binding proteins. In some cases, a minimal functional NLS is rather large. For instance, the M9 NLS consists of 38 amino acid residues, is enriched in glycine, and includes only a few positively charged residues. Even longer NLSs are known, indicating that a certain spatial structure of the karyopherin-binding region plays an important role in complex formation [16]. Karyopherins that mediate nuclear export recognize NESs [9, 11]. Minimal functional NESs have been identified for several proteins. A moderately conserved short motif with three or four hydrophobic amino acid residues (LPPLERLTL in the Rev NES) has been studied in most detail to date. This NES is utilized in all eukaryotes and serves as a binding site for the karyopherin Crm1. The NES was found in at least 75 proteins, including many transcription factors, cell cycle regulators, and virus proteins, such as Rev and proteinase K inhibitor of the human immunodeficiency virus type 1 (HIV-1), where this signal was identified for the first time. Like importin-β1, Crm1 is capable of transferring complexes through the NPC, binding its target via an adaptor protein [18].

Certain proteins have nonhydrophobic NESs. Yeast Msn5p is the best known karyopherin that binds to such sites [12]. This transportin is capable of mediating both nuclear export and import of various proteins [19]. Export of all known targets of Msn5p is induced by their phosphorylation, indicating that a phosphate group is contained in the NES or that phosphorylation indirectly affects the recognition of the NES by a receptor [20].

Some exportins utilize other karyopherins as targets. For instance, CAS exports karyopherin-α from the nucleus, thus, bringing it back into the cytoplasm [21]. In addition to proteins, certain RNAs are subject to karyopherin-dependent export. At least two exportins, exportin-t and exportin 5, are capable of directly binding RNA. Exportin-t exports tRNA from the nucleus by binding to a tRNA structural element acting as a signal. In higher eukaryotes, exportin 5 transfers microRNA precursors from the nucleus upon recognizing their hairpin structure with a protruding 3' end [22].

The main properties that allow karyopherins to ensure efficient nuclear transport are their capabilities of binding with a target protein (directly or through an adaptor) and interacting with Nup or RanGTP [12]. Almost all karyopherins known in humans and yeasts are involved in either export or import. Two proteins (human importin 15 and yeast Msn5p) are exceptions, having both exporting and importing activities. Known importins are more numerous than exportins. For instance, ten importing, two exporting, and one universal karyopherins were identified in yeast cells. Karyopherin targets are far more numerous than karyopherins. A clear view of the situation is just emerging, since karyopherins recognize the localization signals having low structural homology (Table 1).

All karyopherins are similar in molecular weight (96–146 kDa), charge, and domain structure. A Ran-binding domain is usually at the N end, a Nup-binding domain is in the center, and a target protein-binding domain is at the C end. Structural studies of four different karyopherins showed that a karyopherin molecule contains approximately 20 HEAT repeats, each consisting of 40 amino acid residues that form two antiparallel α-helices linked by a turn. The repeats stack together to form superhelical arches at both ends of the protein [23]. One karyopherin may form different complexes with different target proteins; i.e., the karyopherin molecule has many binding sites, each recognizing a certain motif. In addition, karyopherins may change their conformation depending on the target protein [24]. This circumstance explains why one karyopherin transfers several proteins lacking homologous regions [25].

RAN GTPase AND RAN-DEPENDENT TRANSPORT

Both importins and exportins bind with RanGTP. Importins bind with RanGTP with high affinity, and the target protein is consequently released from its complex with an importin. Exportins, whose function requires the formation of a ternary complex with RanGTP and a cargo, have low affinity for RanGTP when not bound with a target protein [9, 11]. A study of the atomic structure for the complexes of importin-β1 and karyopherin-β2 with RanGTP showed that RanGTP binds similarly with both receptors. RanGTP interacts with a concave on the N-terminal arch of importins, and its binding site has two spatially separate domains, one being closer to the N end of the arch, and the other closer to its C end [26]. The C-terminal domain that contacts RanGTP is a negatively charged loop between two HEAT domains. The same loop was found to bind with proteins transported by karyopherin-β2. This circumstance may explain why the target protein is released from its complex with importin once transferred into the nucleus (and in contact

Table 1. Known members of the human karyopherin-β family and their yeast orthologs

Human karyopherin-β	Yeast karyopherin-β orthologs	Transport target	
		human	yeast
Importin-β1	Karyopherin	Many targets; proteins containing the classical NLS is mediated by importin-α; that of nsnRNPU, by snurportin	Many targets; proteins containing the classical NLS is mediated by karyopherin-α
Karyopherin-β2	Karyopherin104	hnRNPA1, histones, ribosomal proteins	Nab2, Hrp1
Transportin SR1	Mtr10/Karyopherin 111	SR proteins	Npl3, Hrb1
TransportinSR2		HuR	
Importin 4	Karyopherin 123	Histones, ribosomal proteins	Histones, ribosomal proteins
Importin 5	Karyopherin 121	Histones, ribosomal proteins	Histones, ribosomal proteins, Pho4, others
Importin 9	Karyopherin 114	Histones, ribosomal proteins	TBP, histones, Nap1p
Importin 7	Nmd5/Karyopherin 119	RTC HIV, glucocorticoid receptor, ribosomal proteins	TFIIS, Hog 1, others
	SxmI/Karyopherin108		Lhp1, ribosomal proteins
	Karyopherin 122		TFIIA
		Exportins	
Crm1	Crm1	Proteins with a leucine-rich NES	Proteins with a leucine-rich NES
Exportin-t	Los1	tRNA	tRNA
CAS	Cse1	Karyopherin-α	Karyopherin-α
Exportin 4		eIF-5A	
Exportin 5		microRNA precursors	
Exportin 6		Profilin, actin	
Exportin 7		P50Rho-GAP, 14-3-38	
		Import–export	
Importin 13		Rbm8, Ubc9, Pax6 (import); eIF-1A (export)	
		Msn5	Pho4, several other phosphorylated proteins (import); replication complex A (export)
		Others	
RanBP6		Unknown	
RanBP17		Unknown	
		Karyopherin 120	Unknown

with RanGTP). Mutations of the C-terminal RanGTP-binding domain result in a loss of the association between karyopherin binding with RanGTP and the release of a cargo. Thus, a contact of RanGTP with this region may regulate the formation of the importin–cargo complex [23].

Structural studies of the exportin Cse1p in complex with RanGTP and its cargo karyopherin-α clearly demonstrate the difference in RanGTP binding between import and export complexes. Cse1p forms a superhelix with N- and C-terminal arches, which clamp around RanGTP on both sides. Simultaneously, Cse1p binds with karyopherin α and fixes it in a conformation that prevents cargo binding. Karyopherin α also forms bonds with RanGTP in this complex. A feature of Cse1p–RanGTP interaction is that the N-terminal arch alone can form a weak bond with RanGTP, but the complex becomes sufficiently stable only when RanGTP binds with both N- and C-terminal arches, which is conformationally possible only when Cse1p is bound with cargo. The resulting ternary complex resembles a tight spring, which stretches in the cytoplasm upon RanGTP hydrolysis to release the cargo karyopherin-α [27].

The exportin Crm1 is similar in several structural features to karyopherin-β2. Like the C-terminal RanGTP-binding site of karypherin-β2, Crm1 has a flexible loop in the middle of the polypeptide chain. The domain is thought to shield the binding site for target proteins and, simultaneously, to prevent Crm1 from forming a stable complex with RanGTP. However, when Crm1 binds with a target protein or RanGTP, the loop changes its position and stimulates the formation of a stable ternary complex. It is possible that conformational changes of a flexible loop underlie a general mechanism of the formation and dissociation of transport complexes [28]. At the same time, the exportin Cse1p lacks regions similar to the Crm1 flexible loop, indicating that the mechanism is not universal [27].

Structural studies of karyopherin-β1 in complex with the phenylalanine–glycine (FG) repeat-containing fragment of yeast Nup showed that the interactions between the two proteins are mostly hydrophobic and involve the phenylalanine residues of Nup. The karyopherin molecule forms two hydrophobic pockets: between HEAT repeats 5 and 6 and between repeats 6 and 7. The pockets capture the amino groups of the phenylalanine residues contained in the FG repeats of Nup [29]. A similar Nup-binding region occurs at the C end of karyopherin-β1 [30].

There is evidence that importing karyopherins have higher affinity for the Nup proteins located at the nuclear side of the NPC, while exporting karyopherins have higher affinity for cytoplasmic Nup proteins [31]. A deletion analysis of all FG-containing Nup proteins (with a yeast model) made it possible to construct NPCs having minimal sets of the FG domains. It was found by this means that asymmetrical FG domains (which occur exclusively at the cyptoplasmic or nuclear side of the NPC) have no functional role. The role of asymmetrical Nup proteins in nuclear transport is still unclear.

The main role in nuclear transport is ascribed to the Ran GTPase, which controls the formation and dissociation of transport complexes. Ran activity is strongly regulated by evolutionarily conserved proteins, which regulate both the intracellular distribution of Ran and the transition between the GTP- and GDP-associated forms [6]. Although Ran occurs predominantly in the nucleus, it is continuously delivered into the cytoplasm at a high rate (10^5 molecules per second), mostly as a component of export complexes [32]. Re-import of Ran is due to NTF2. NTF2 interacts with RanGDP, which is the main cytoplasmic form of Ran. The NTF2–RanGDP complex is transferred into the nucleus, which is due to the ability of NTF2 to interact with low affinity with FG-containing Nup proteins, as karyopherins do. The RanGDP transfer is unidirectional because RanGDP is rapidly converted to RanGTP on the nuclear surface of the NPC. Since NTF2 binds to the SwitchII region of RanGDP and this region has another conformation in RanGTP, the NTF2–RanGTP transport complex dissociates, and NTF2 is recycled into the cytoplasm [33] (Fig. 2).

RanGDP is converted to RanGTP in the nucleus, where Ran interacts with the Ran guanine exchange factor (RanGEF). RanGEF is associated with chromatin, occurs in the nucleus at approximately one copy per nucleosome, and directly interacts with nucleosomal histones H2A and H2B. RanGEF stimulates the exchange of GDP for GTP because a RanGEF loop (β-wedge) penetrates into Ran and releases GDP. Binding with RanGEF fixes Ran in the free form for a period long enough for Ran to bind GTP contained in the nucleoplasm [34]. Although no preference for GTP is observed for nucleotide exchange in the presence of RanGEF in vitro, Ran–GTP binding in vivo is more likely owing to a higher GTP : GDP ratio in the nucleoplasm.

RanGEF does not always occur in a chromatin-associated form. Its free form is detectable in the cell both in the interphase and during mitosis [35]. However, only chromatin-associated RanGEF is capable of stimulating GTP–GDP exchange in complex with Ran. The T42N Ran mutant, which is incapable of nucleotide exchange, leads to a fixation of free RanGEF on chromatin [35]. It is most likely that GTP binding to Ran is necessary for a detachment of the two proteins from chromatin. In addition, the association of such exchange complexes with chromatin plays an important role in mitosis, since a high local concentration of RanGTP is necessary for the proper assembly of microtubules in the vicinity of the chromosome surface [36].

High-affinity interactions between RanGTP and karyopherins leaving the nucleus take place both when cargoes are transported into the cytoplasm (with exportins) and when imported karyopherins are recycled into the cytoplasm. In either case, a RanGTP–karyopherin complex is moved onto the cytoplasmic surface of the NPC and then interacts with RanGAP, which substantially (approximately by five orders of magnitude in vitro) increases the originally weak GTPase activity of Ran. This interaction causes GTP hydrolysis to yield RanGDP. RanGDP in complex with a karyopherin is not fully accessible for RanGAP, and RanBP1 facilitates the RanGTP–RanGAP binding via either generating a RanBP1–RanGTP–karyopherin intermediate complex or releasing RanGTP from karyopherin [37].

The cytoplasmic localization of RanGAP and the nuclear localization of RanGEF underlie the mechanism that maintains a high gradient of RanGTP between the nucleus and cytoplasm [38]. A fluorescence resonance energy transfer (FRET) analysis showed that the difference between RanGTP concentrations in the nucleus and cytoplasm is more than two orders of magnitude [36], which agrees well with modeling results.

RanGTP plays an important role in nuclear transport. A high RanGTP concentration in the cytoplasm

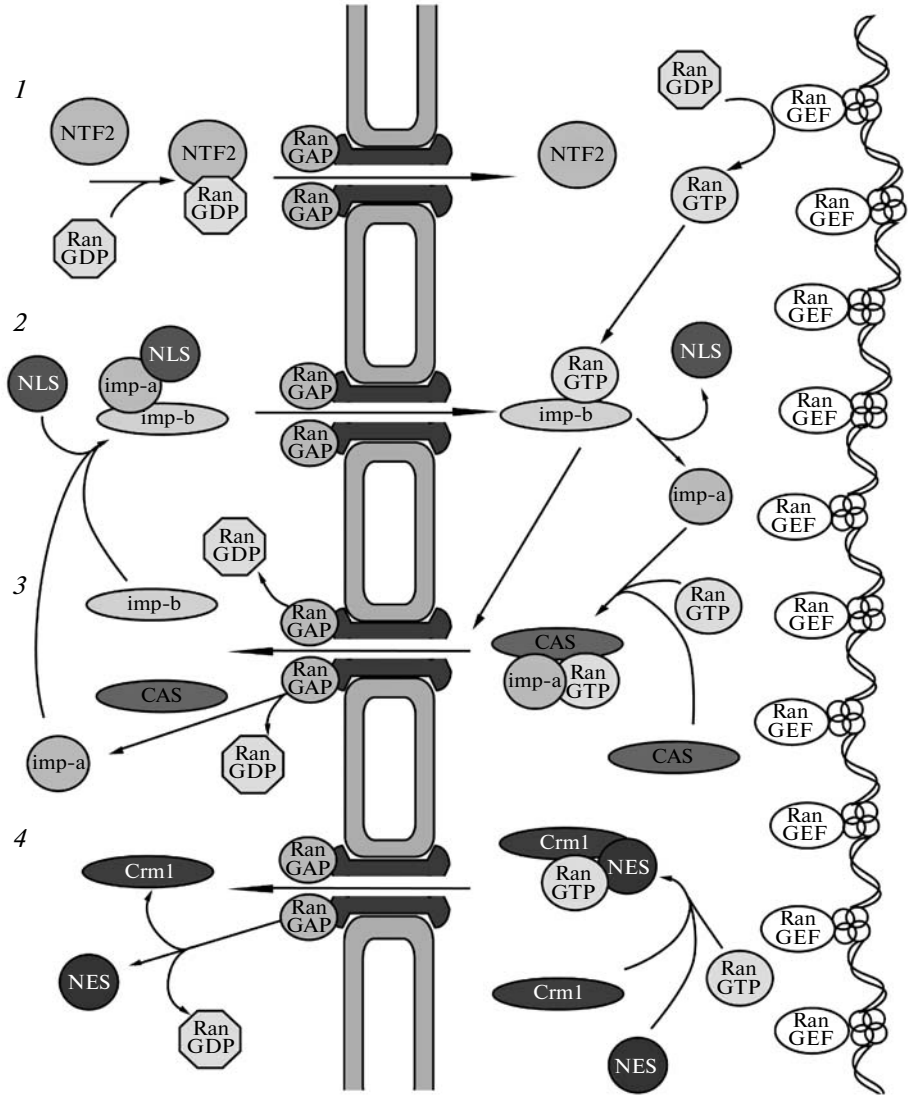

Fig. 2. Main nucleocytoplasmic transport pathways in eukaryotes: (*1*) nuclear import of RanGDP with the help of NTF2, (*2*) nuclear import of a NLS-containing protein by a karyopherin-α–importin-β heterodimer, (*3*) nuclear export and recycling of importin-β and CAS-mediated recycling of karyopherin-α, and (*4*) nuclear export of a NES-containing protein by the exportin Crm1.

would distort nuclear import, causing premature dissociation of import complexes. The cell has mechanisms to ensure the proper location of the Ran regulators. RanGEF-mediated exchange of GDP for GTP occurs exclusively in the nucleus owing to two import pathways [39]. RanGTP hydrolysis occurs in the cytoplasm, since RanGAP is too large to enter the nucleus through the NPC. RanGAP can be sumoylated (covalently bound with a protein of the SUMO family), and the resulting form has affinity for cytoplasmic Nup358 [40]. RanBP1 is small enough to enter the nucleus through the NPC via diffusion. However, RanBP1 has a NES recognizable by the exportin Crm1, which ensures continuous active export of RanBP1 appearing in the nucleus via diffusion into the cytoplasm.

RanGTP and karyopherins are the main means of nuclear transport. It should be noted, however, that the eukaryotic cell has several accessory factors that affect the transport efficiency. For instance, the efficiency of karyopherin-α-dependent nuclear import is improved upon Npap60 binding, which increases karyopherin-α affinity for importin-β1. Appearing in the nucleus, the Npap60–importin-β1–karyopherin-α–NLS-cargo complex is affected by RanGTP and CAS, which acts as an exportin to recycle karyopherin-α into the cytoplasm. The import complex is cleaved into two complexes, Npap60–importin-β1–RanGTP and CAS–karyopherin-α–RanGTP. A feature of the Npap60 effect is that Npap60 differently binds with importin-β1 during import. It is thought that Npap60 has a structure of a tri-stable switch [41]. RanBP3,

which is a Npap60 analog involved in export, is structurally similar to Npap60 and binds with the exportin Crm1 to increase its affinity for RanGTP and NES-containing proteins, thus improving the efficiency of Crm1-dependent export [42].

NUCLEOPORINS: THEIR STRUCTURE AND FUNCTIONS

The NPC consists of more than 30 different Nup proteins, each occurring in at least eight copies [43]. Some Nup proteins are predominantly included in the NPC, while some others shuttle between the nucleus and cytoplasm and are only associated with the NPC for a short while [44].

The FG repeats, which are found in one-third of all Nup proteins, are particularly important in the functioning of NPC. There are approximately 128 FG domains containing 3500 FG repeats in one NPC. FG domains are not distinctly structured and are thought to line the inner wall of the nuclear channel. All known transport receptors bind with FG repeats, which are essential for nuclear transport [11]. The interaction with a transport receptor involves mostly the phenylalanine ring of the core FG region; the ring binds with hydrophobic amino acid residues on the surface of a transport receptor [45]. Hydrophilic regions, which occur between individual FG motifs and account for a major part of an FG domain, allow several FG regions of one domain to bind with a receptor [46] and, presumably, are necessary for modulating the receptor binding (Table 2).

Structural studies of the FG domains in yeast Nup proteins by biophysical methods showed that the domains are unfolded and lack a distinct secondary structure in natural conditions [47]. Similar data were obtained for the FG domains of Nup proteins of other organisms [48]. As was revealed by electron microscopy, structures containing FG domains are flexible and are capable of moving along the NPC. Atomic force microscopy (AFM) showed that the human Nup153 FG domain (700 amino acid residues) occurs as a long (180 nm) unstructured sequence [49].

The spatial structure of the NPC changes dynamically, adapting to nuclear transport requirements. Two main types of NPC conformations were identified by cryoelectron microscopy. Conformations of one type are characterized by the cytoplasmic filaments extended towards the central channel of the NPC. The filaments apparently interact with a protein complex transferred though the NPC. Conformations of the other type have stretched unfolded cytoplasmic filaments [50].

The arrangement of certain functional domains of Nup changes in the course of nuclear transport. For instance, the C-terminal end of the FG domain of Nup214 moves onto the nuclear side of the NPC when polyadenylated RNA is introduced into the nucleus, while the N end remains associated with cytoplasmic fibrils [50]. The FG domain of Nup153 similarly moves along the NPC as cargoes are transported [51].

In addition to their role in nucleocytoplasmic transport, the FG domains may perform other functions. For instance, the FG repeats of the RRM domain of mouse Nup35 have a distinct secondary structure and do not interact with transport receptors. These domains interact with the Ndc1 transmembrane protein and, possibly, are involved in forming the central framework of the NPC [52].

Nup proteins may play a role in transcriptional regulation by interacting with active genes. This assumption is supported by the fact that NPCs are not regularly distributed through the nuclear membrane, but their distribution corresponds to the distribution of active chromatin within the nucleus. Such a distribution is necessary for a correct rearrangement of the chromosomes and nuclear envelope during mitosis and for mRNA export through specific NPCs in the interphase [53]. Further studies revealed that yeast genes whose transcription is increasing move to the periphery of the nucleus, which is due to their binding with certain Nup proteins [54].

In addition, several Nup proteins, such as Nup2p, bind to loci at the boundary between euchromatin and heterochromatin, thus preventing heterochromatin from spreading to transcriptionally active regions. As was revealed more recently, Nup2p binds to chromatin within a complex consisting of Nup2p, Nup60p (an element of the NPC nuclear basket), Prp20 (a yeast analog of RanGEF), and the Htz1 histone protein [55]. Htz1 is responsible for the prevention of heterochromatin spreading [56]. Nup2p interacts with the promoters of functionally active genes, and this interaction depends on transcriptional activators and the TATA box located 5' of them [57]. Thus, the NPC may serve as a multifunctional regulator of gene expression by distributing transcription activation signals and checking the quality of spliced mRNA.

Nup proteins can act as a platform for the attachment of various transport factors. For instance, Nup358, which is a component of the cytoplasmic fibrils of the NPC, provides a platform for the cleavage of transport complex and a subsequent recycling of transport factors. The Nup358–RanBP1 complex has four Ran-binding sites and a binding site for the sumoylated Ran activator (RanGAP1). To bind to this complex, RanGAP1 must be sumoylated, while RanBP1 has sumoylating activity. Acting in complex with the E2 ligase Ubc9 similarly to the E3 ligase, RanBP1 stabilizes RanGAP1 on the cytoplasmic fibrils [40]. RanGAP1 is capable of stimulating hydrolysis of RanGTP associated with export complexes, which is essential for nuclear export.

Some Nup proteins bind with proteins possessing desumoylating activity, such as SENP2 (yeast Ulp1) [58]. There is evidence that Ulp1 binds with Nup60p and Mlps located on the nuclear side of the NPC [59]. Other data indicate that Ulp1 is anchored on the NPC

Table 2. Main vertebrate Nup proteins and their yeast orthologs

Vertebrate Nup	Yeast Nup ortholog	NPC formation		Folding type	Possible function
		assembly stage	association with NPC		
Cytoplasmic side					
Nup358		Early	Unknown	FG	Structural and transport
Nup214	Nup159	Intermediate	Stable	β-p, SH, FG	Structural
CG1	Nup42	Unknown	Moderate	FG	Structural and transport
Nup88	Nup82	Intermediate	Stable	β-p, SH	Structural
Symmetrical					
Nup62 complex	Nsp1 complex	Intermediate	Moderate	FG, SH	Structural and transport
Nup107–160 complex	Nup84 complex	Early	Stable	β-p, α-h	Folding
Nup93 complex	Nic96 complex	Early	Moderate	β-p, α-h, FG	Structural and transport
Rae1/Gle2	Gle2	Unknown	Dynamic	β-p	Transport
Nup98	Nup100, Nup116, Nup145N	Unknown	Dynamic	FG, Nup98-like	Transport
Transmembrane					
Pom121		Early	Stable	TMH, FG	Structural
gp210	Pom152	Late	Dynamic	TMH	Transport
Ndc1	Ndc1	Unknown	Unknown	TMH	Structural
	Pom34	Unknown	Unknown	TMH	Structural
Nuclear side					
Nup50/Npap60	Nup2	Unknown	Dynamic	FG	Transport
Nup153	Nup1	Early	Dynamic	FG	Structural and transport
	Nup60	Unknown	Dynamic	FG	Transport
TPR	Mlp1 and 2	Late	Unknown	SH	Structural and transport

Note: β-p, β-propeller; α-h, α-helix; TMH, transmembrane helix; SH, superhelix.

Composition of nucleoporin complexes: Vertebrate complexes: Nup62 includes Nup62, Nup58, Nup54, and Nup45; Nup107–160 includes Nup160, Nup133, Nup107, Nup96, Nup75/85, Nup43, Nup37, Sec13, Seh1, and ALADIN; and Nup93 includes Nup205, Nup188, Nup155, Nup93, and Nup35/53. Yeast complexes: Nic96 includes Nic96, Nup192, Nup188, Nup170, Nup157, Nup59, and Nup53; Nsp1 includes Nsp1, Nup57, and Nup49; and Nup84 includes Nup145C, Nup133, Nup120, Nup85, Nup84, Sec13, and Seh1.

by NPC-associated karyopherins [58]. This possibly facilitates desumoylation of hnRNPs that were sumoylated on the cytoplasmic side of the NPC and then transferred into the nucleus. Yet this assumption disagrees with the data that Ubc9 occurs on the nuclear side of the NPC as well, possibly binding with Nup153p [59].

Apart from sumoylation, the DEAD-box helicase Dbp5 plays a substantial role in separating hnRNP from mRNA. Dbp5 also occurs on the cytoplasmic surface of the NPC in complex with Nup214 (yeast Nup159p). ATPase activity of Dbp5 depends on the binding of Gle1, an mRNA export factor capable of binding to the NPC, and is stimulated by inositol hexaphosphate (IP_6) [54]. In yeast cells, Dbp5 is bound with Mex67, which is an analog of TAP/NXF1. The level of Mex67-bound RNA is elevated in cell lines with mutant *dbp5*, implicating Dbp5 in the Mex67 recycling to the nucleus [60].

Thus, both karyopherin-dependent export and mRNA export depend on a protein stimulating NTPase activity (RanGAP1 in the case of Ran GTPase and Gle1/IP_6 in the case of ATPase activity of Dbp5). Moreover, the interaction of these activators with a substrate is determined by specific binding sites of cytoplasmic Nup proteins and is regulated by additional cofactors (sumoylation and IP_6).

HIERARCHIC REGULATION OF NUCLEAR TRANSPORT

Nuclear transport is regulated by several mechanisms, which are organized hierarchically. A flow of proteins transferred between the nucleus and cyto-

plasm changes in response to various signals, such as hormones, cytokines, and growth factors, as well as signals regulating the cell cycle, differentiation, and the immune response, and in stress. Modification of signal molecules via phosphorylation or dephosphorylation, which is the most clearly understood mechanism regulating nuclear transport, may involve many kinases and phosphatases [61]. Kinases and phosphatases are regulated by many cell signals, directly linking external signaling factors and intracellular signals with changes in the nuclear import or export of signal molecules, such as cell cycle regulators, kinases, and transcription factors. Many of these proteins have both NES and NLS, which allow a fine regulation of their intracellular localization by changing the efficiency of nuclear export or import [62].

REGULATION AT THE LEVEL OF INDIVIDUAL CARGO

Intramolecular Masking of the NLS or NES

One of the key steps of nuclear transport is the interaction of importins and exportins with the NLS or NES of a target protein. Changes in NLS or NES accessibility via inter- or intramolecular masking are one of the most common mechanisms modulating the efficiency of nuclear transport of a particular protein. Intramolecular masking occurs when the accessibility of the NLS or NES decreases as a result of conformational changes in the protein containing the given site. An example of such regulation is provided by the NF-κB p50 transcription factor. The factor occurs in the cytoplasm in the form of a p105 precursor, which has an NLS inaccessible for binding with importin α. During the immune response, phosphorylation and degradation of the C-terminal fragment of p105 unmasks the NLS, which then binds with importin-α to allow active transfer of NF-κB into the nucleus [63].

Conformational changes that result from disulfide bonding between the amino groups of cysteine residues in one protein may also mask or unmask the NES or NLS. For instance, a disulfide bond is formed in stress in the yeast transcription factor Yap1p between Cys598 and Cys620, which belong to a cysteine-rich region. An NES, which is in the same region, consequently becomes inaccessible for Xpo1p [64].

Intermolecular Masking of the NLS or NES

Such masking takes place when binding with another protein makes the localization signals inaccessible for transportins.

An example is provided by the NF-AT4 transcription factor. At a higher Ca^{2+} concentration, NF-AT4 binds with the Ca^{2+}-responsive phosphatase calcineurin, which masks the CRM1-binding NES of NF-AT4. When the Ca^{2+} concentration decreases, calcineurin dissociates from NF-AT4, the NES becomes accessible for CRM1, and CRM1 transfers NF-AT4 from the nucleus [65].

The nuclear localization of the p53 tumor suppressor is regulated by several mechanisms. One of these is p53 homotetramerization in the nucleus, which occurs in response to DNA damage [66] and masks the C-terminal NESs. It is essential for a nuclear export of p53 that the tetramer dissociate and the C-terminal NESs be unmasked [67].

Ligand binding may also mask the NLS or NES of a receptor. For instance, the NES of the androgen receptor is close to its ligand-binding domain. Upon binding with the ligand, the NES becomes inaccessible for Crm1, and nuclear export is suppressed until the ligand dissociates from the receptor [68]. An intermolecular masking of an NLS or NES is possible upon DNA or RNA binding. HIV-1 Rev, which transfers nonspliced virus mRNA from the nucleus into the cytoplasm, masks its own importin-β2-dependent NLS upon mRNA binding [69]. A release of mRNA restores the ability of Rev to bind with importin-β2, which then recycles Rev into the nucleus. Rev binds again with virus mRNA, this binding facilitates Rev dissociation from its complex with importin-β2, and a new round of mRNA nuclear export starts.

The yeast GAL4 transcription factor and human SRY chromatin-remodeling factor have DNA-binding domains that overlap their NLSs [70]. Binding to DNA prevents their association with importin-β2 and vice versa. It is possible that this mechanism is alternative to a RanGTP-mediated release of cargoes from transport complexes. The mechanism is effective when the local RanGTP concentration is too low or Ran activity is suppressed by high Ca^{2+} concentrations [71].

Modulation of Affinity for Karyopherins via Posttranscriptional Modification

Many various RNAs are expressed in the nucleus. To be exported from the nucleus, RNAs undergo posttranscriptional modification, which is necessary for successful interactions with proteins of the transport complex. For instance, tRNAs acquire the capability of binding with exportin-t at the last step of their maturation in the nucleus, and this capability ensures the export from the nucleus only for mature tRNAs. Several cell mRNAs are exported owing to *cis*-regulatory elements. In mouse cells, certain retroviral transcripts leave the nucleus owing to the constitutive transport element (CTE), which is capable of binding directly to the mNxf1 export receptor [9]. The mRNAs of several genes involved in the cell cycle control have a β1-untranslated region (UTR) that is recognized by the translation initiation factor eIF4E, which binds to the 3'-UTR and mediates the mNxf1-dependent export of these transcripts [72].

In addition to an NES, mRNA may contain an NLS, which provides for an additional mechanism of gene expression regulation. The 3'-UTR of the MSF

cytokinin gene contains a sequence that holds this mRNA in the nucleus. Further posttranscriptional modification in response to TGF-β1 activates the export of the MSF mRNA and MSF synthesis [73].

Signal elements may regulate the RNA nuclear import as well. For instance, an 8-nt sequence is necessary and sufficient for the nuclear import of the miR-29b microRNA [74].

The export of mature mRNAs and ribosomal subunits results from a series of standard modifications essential for the binding with export receptors. During maturation, mRNA interacts with various proteins to form mRNP. The composition of this complex changes in the course of mRNA splicing, capping, and polyadenylation. Mature mRNA is capable of interacting with the mNxf1–Nxt1 (yeast Mex67–Mtr2) transport complex, which is necessary for cytoplasmic export [75]. The mRNP transfer through the NPC depends on IP_6, thus allowing phospholipase C to regulate the mRNA nuclear export. Similar modifications necessary for the export from the nucleus occur in rRNA as well [75].

Modulation of Protein Affinity for Karyopherins via Posttranslational Modification

Phosphorylation of proteins at sites close to an NLS or NES is capable of not only masking these signals, but also improving protein affinity for importins and exportins. For instance, CK_2 kinase phosphorylation of the T antigen at Ser111/112, which are adjacent to the NLS, results in a 100-fold increase in NLS affinity for importin-α, and this leads to a 50-fold increase in the nuclear import of the T antigen [76]. Phosphorylation may affect the nuclear export as well. For instance, phosphorylation of Pho4 at Ser114 and Ser128 improves the recognition of its NES by the Msn5p exportin [77].

In addition to phosphorylation, target proteins may experience other types of posttranslational modification, such as methylation and ubiquitination, which are also capable of regulating the intracellular location of the protein. For instance, the proper nuclear import of RNA helicase A requires methylation of its NLS, and PTEN phosphatase does not efficiently enters the nucleus until monoubiquitinated at certain lysine residues [78].

Ubiquitinase UbcM2 appears in the nucleus only in an activated ubiquitinated form. This example supports the idea that the functional state of an enzyme may affect its intracellular localization [79].

Retention in the Cytoplasm or Nucleus

Another mechanism regulating nuclear transport is a binding of NLS- or NES-containing proteins with cytoplasmic or nuclear factors that anchor the bound proteins to retain them in the cytoplasm or nucleus. For instance, p53 is retained in the cytoplasm in the absence of stress signals owing to its binding with Parc ubiquitin ligase. Excess synthesis or inhibition of Parc modulates the cytoplasmic localization of p53 [80].

The HIV-1 transactivator Tat, the vascular growth factor angiogenin, and the interferon-dependent transcription factor IFi16 are retained in the nucleus via NLS-dependent binding with nuclear or nucleolar components. Angiogenin is small enough to enter the nucleus via passive diffusion, and its NLS does not interact with importins, but binds with nuclear proteins. As a result, angiogenin is retained in the nucleus, and its backward diffusion into the cytoplasm is prevented [81]. Such anchorage is due to NLS phosphorylation in some cases. For instance, affinity of the IFi16 NLS for nuclear proteins increases as a result of phosphorylation by the CK_2 kinase. In contrast to these two proteins, Tat has an NLS that has affinity for both nuclear and cytoplasmic anchoring proteins, and its affinity depends on protein modification [82].

REGULATION AT THE LEVEL OF TRANSPORT RECEPTORS

Modulation of the Expression of Components of the Nucleocytoplasmic Transport System

Different members of the importin family, especially importins-α, have affinity for different groups of transport targets in higher eukaryotic cells. Moreover, different transport complexes, for instance, β-katenins or STAT family proteins, pass through the NPC via different pathways, binding exclusively with the FG repeats of a certain set of Nup proteins [83]. Thus, the presence or absence of a particular Nup or karyopherin can determine whether a certain protein is transferred through the nuclear pore. A difference in the tissue distribution of transport proteins affects the efficiency of nuclear transport of the same proteins. An example is provided by the *Drosophila melanogaster* heat shock protein dHSF, which is transferred into the nucleus by importin-α3. Importin-α3 is absent in early embryo development, and, consequently, dHSF does not enter the nucleus [84].

Differences in the tissue distribution of importins α are especially clear in higher eukaryotes. For instance, importin α4 accounts for more than 1% of total protein in human striped muscle cells (i.e., its content is 100-fold higher than that of importin-α5) and is almost absent in heart, spleen, and kidney cells [85]. In contrast, importin-α1 is abundant in the heart, testis, skeletal muscle, and ovary, while importins-α3 and -α7 occur at a high content in the ovary and brain. The levels of mRNA expression in one tissue greatly vary among different importins-α; however, a high content of importin-α6 is only characteristic of ovarian cells [86].

Competition between different karyopherins-β for binding sites on the NPC surface may also be subject to regulation. The contents and composition of karyopherins-β and their targets change during the cell life,

affecting the efficiency of nucleocytoplasmic transport. A higher content of a particular karyopherin-β increases the transport efficiency of its targets [87], the saturation point differing between individual karyopherins-β. Since different transport receptors are capable of interacting with the same sites on the NPC surface, an excess of one karyopherin β may inhibit the transport of proteins associated with another karyopherins. For instance, the import in cultured cells is approximately tenfold lower than in systems in vitro, possibly, because artificially reconstructed systems lack the majority of competing karyopherins-β [87].

The regulation of nucleocytoplasmic transport pathways changes in certain diseases. Overproduction of karyopherin-β and karyopherin-α family proteins was observed in several colorectal, breast, and lung cancers. Deregulation of karyopherin-α2, which is frequent in melanoma and breast cancer cells, correlates with a low survival. It is still unclear which karyopherin α2-dependent proteins are redistributed to determine the high malignant potential of these tumors. A possible cause is that karyopherin-β1 is sequestered by overproduced truncated karyopherin-α2 to alter the karyopherin-α1-dependent import of p53 into the nucleus [88].

The antiviral response is similarly altered in cells infected with the Ebola virus or the avian influenza virus (SARS-CoV) [89].

Hodgkin's lymphomas are characterized by excess phosphorylation and degradation of I-κB, which results in an extremely high nuclear content of NF-κB p65 because the intermolecular masking of its NLS is deregulated [90].

Role of Importins in Gametogenesis

Gametogenesis is well understood and is known to depend, to a substantial extent, on the nonuniform expression of various importin-α genes. Each of the three *D. melanogaster* importins-α has its own expression pattern during spermatogenesis. Importin-α3 is synthesized predominantly in the postmeiotic phase, reaching its maximum during spermatid elongation. The level of importin-α1 remains low throughout the mitotic phase of spermatid development and is completely suppressed at spermatid elongation. Importin-α2 is actively synthesized in spermatogonia during the first four mitoses and during the two subsequent meioses. Flies with a mutant importin-α2 (imp-$\alpha2^{D14}$) have a low fertility. An elevated level of importins-α1 and -α3 in transgenic flies is capable of restoring the male fertility, indicating that these importins may functionally substitute importin-α2 to a certain extent during spermatogenesis. However, the fertility of imp-$\alpha2^{D14}$ mutant females is not restored at higher levels of other importins, suggesting a key role in oogenesis for importin-α2 [91].

Caenorhabditis elegans importins are synthesized differentially in different cells. Importins-α1 and -α2 occur mostly in germline cells, while importin-α3 is found in somatic cells as well. Suppression of importin -α3 synthesis via RNA interference blocks meiosis in pachytene. It seems that importins-α1 and -α2 are incapable of compensating for lack of importin-α3, indicating that importin-α3 is essential for successful meiosis. Inhibition of importin-α2 leads to aneuploidy, an improper chromatin organization during cell division, and an incomplete restoration of the nuclear membrane after meiosis [92].

In lower eukaryotes, different importins are critical at different stages of gametogenesis, indicating that the proper organization of nuclear transport is important for meiosis.

Role of Exportins in Development

An essential developmental role of Crm1-dependent nuclear export was demonstrated in experiments with leptomycin B, which acts as a specific inhibitor of Crm1. When nondifferentiated gonad explants from female mouse embryos were treated with leptomycin B, the SOX9 chromatin-remodeling factor was redistributed into the nucleus, as characteristic of nondifferentiated male gonads [93]. The SOX9 content in the nucleus increased, which was apparently related to a role of Crm1 in the export of SOX9 into the cytoplasm.

While Crm1 and importins-β2 and -β3 occur in all cells in *Drosophila*, exportin dCAS, which is responsible for a recycling of importin-α from the nucleus into the cytoplasm, is differentially expressed in different tissues. Importin-α synthesis is almost absent at the mid-blastodermal stage. Its level increases at subsequent stages, especially in embryonic nervous cells. Mutations of dCAS result in either a lethal phenotype or, in the case of hypomorphic dCAS, developmental defects of the nervous system. Such developmental defects are possibly caused by a lack of cytoplasmic importin-α3 and a consequent decrease in the concentration of the Notch-regulated Su9(H) protein in the nucleus [94].

REGULATION AT THE LEVEL OF NPC COMPONENTS

Developmental Roles of Nup and Other Modulators of Nuclear Transport

Nup proteins differ in affinity for importins and exportins, and, consequently, changes in the levels of certain Nup proteins may affect the efficiency of nuclear transport. Lack of Nup98B alters the nuclear import of all proteins possessing the classical NLS, except for the spliceosomal factor U1A [95].

Nup BS-63, which is functionally associated with the RanBP2/Nup358 complex, is synthesized exclusively in spermatids [96]. Nup BS-63 is capable of interacting with the aF10 chromatin-remodeling factor, which is contained in postmeiotic cells. Factors

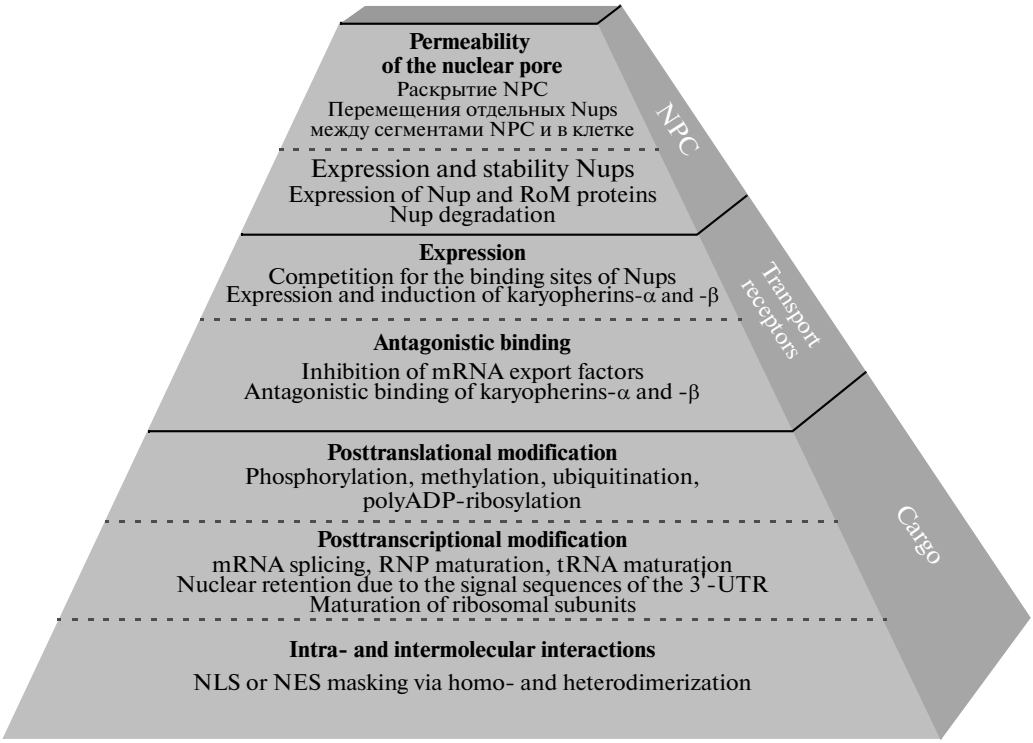

Fig. 3. Nucleocytoplasmic transport may be regulated at the levels of NPC components, transport receptors, or individual cargoes. The regulations of higher hierarchic levels exert broader and less specific effects on transport processes.

similar to aF10 may enter the nucleus via direct interactions with certain Nup proteins, as is the case with β-catenins and SMAD family proteins. Thus, Nup BS-63 possibly provides a specific binding site for aF10 in haploid sperms [97].

The NPC, a dynamically changing macromolecular complex, can mediate both passive transport and active transport of specific complexes. Some Nup proteins act as active components of a transport pathway.

Nup Role in Mitosis

During mitosis, the NPC presumably dissociates into several subcomplexes differing in Nup composition. A functional NPC is again reassembled from these subcomplexes in telophase [53].

In lower eukaryotes, whose mitosis proceeds without a disassembly of the nuclear membrane, certain Nup proteins (Gle2/Rae1 and Nup98 in *Aspergillus*) are separated from the NPC to improve its permeability [98].

In *Saccharomyces cerevisiae*, the NPC is not disassembled in mitosis even partly, but Nup53, which is bound with Nup170 in the interphase, is phosphorylated and transferred onto Nic96. As a result, a high-affinity binding site for karyopherin 121 is opened in Nup53, and karypherin 121-dependent nuclear transport is suppressed [99].

During mitosis, several Nup proteins (Nup107–160, Nup358, RanBP2, and Gle2/Rae1–Nup98) bind with the kinetochores and play a role in regulating the interactions between the kinetochores and microtubules, which are also necessary for the NPC formation after mitosis [100].

As was revealed in experiments with *Xenopus*, Gle2/Rae1 induces the assembly of microtubules into a mitotic spindle. According to other data, Gle2/Rae1–Nup98 inhibits the anaphase-promoting complex (APC) to delay anaphase [101].

Dynamic changes in the NPC composition affect the transmission of intracellular signals. The regulation of nuclear transport is usually modulated by changes in the karyopherin interactions with target proteins. In particular, conformational changes in a protein or posttranslational modification of sites in the vicinity of or within the NLS (or NES) may mask these signals. However, NPCs are possibly capable of serving several independent transport pathways at the same time [102]. If so, structural and functional changes in the NPC may provide for a fine regulation of the transmission of certain signals. This hypothesis is supported by the results of studying the independent changes in nuclear import of various proteins (Fig. 3). The NPC contains approximately 128 FG domains with several thousands of FG repeats [103]. The FG repeats harbor various binding sites for transport proteins. The multiplicity of FG-associated transport

pathways is evident from the fact that only some of the nuclear import pathways are altered in yeast cells with functionally defective FG-Nup nsp1-s5 [104].

Transport pathways that involve different karyopherins need different sets of FG domains [105]. This finding well correlates with in vitro data. Therefore, different pathways of transport through the NPC have a common mechanism, but utilize different karyopherin sites and may be regulated independently.

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