Minireview

Nuclear transport and transcriptional regulation

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Abstract Studies over the past 10 years have provided major insights into the molecular mechanisms responsible for active transport of macromolecules in and out of the nucleus. Nucleocytoplasmic transport pathways correspond to active and signal-mediated processes that involve substrates, adaptors and receptors. Regulation of both nuclear import and nuclear export is mainly exerted at the level of transport complex formation and has emerged as one of the most efficient mechanisms to adapt gene expression to the cell environment by restricting the access of transcriptional regulators to their target genes.

Key words: Nuclear import; Nuclear export; Regulation; Transcription

1. Introduction

One of the definitive features of eukaryotic cells is that DNA is contained within a specialized compartment, the nucleus, separated from the rest of the cell by a membranous nuclear envelope. This compartmentalization results in the physical separation of transcription and translation. In particular, proteins involved in the transcription and its regulation in response to distinct physiological conditions have to be expressed in the nucleus to fulfill their function. In this respect, nuclear import and export represent important regulatory checkpoints in the control of gene expression.

2. Nuclear transport mechanisms

2.1. Import mechanisms

Molecular exchanges between the nucleus and the cytoplasm occur through a specialized structure of the nuclear envelope, the nuclear pore complex (NPC). Polypeptides below 40 kDa can in principle freely diffuse into the nucleus through peripheral and central channels of the NPC although very few proteins do so. Proteins above 40–60 kDa are imported into the nucleus by a sequence-mediated process that involves the binding of the protein to the NPC, its translocation through the central channel of the pore and its release in the nucleoplasm [1].

Sequence-mediated nuclear import involves recognition of specific amino acid sequences called NLSs (for nuclear localization sequences) within the karyophilic protein by specific receptors, called karyopherins (Kaps) or importins which belong to a wide family of proteins conserved throughout evolution. Sequence similarity searches in yeast genome databases identified 13 potential Kaps and six of them, termed Kapβ1/Kap95p, Kapβ2/Kap104p, Kapβ3/Kap121p, Kapβ4/Kap124p, Sxm1p/Kap108p and Nmd5p, have been actually implicated in the nuclear import of various substrates. Some of their homologues have been identified in higher eukaryotes but there are still orphan Kaps and imported substrates in both yeast and metazoans [2].

First identified in the SV40 large T antigen and in the *Xenopus* nucleoplasmin, lysine-rich NLSs or basic NLSs are present in a large number of proteins. The basic NLS receptor is Kapβ1 (also called importin β or, in yeast, Kap95p), which functions as a dimer with Kapα (Kap60p or importin α), an adaptor protein providing the basic NLS binding site. In metazoans, as many as five different Kapα have been reported and can be expressed in a tissue- and stimulus-dependent manner. Kapβ1 also imports some proteins through direct binding or acts as a heterodimer with an adaptor called snurportin to import snRNPs. Another well characterized NLS is the M9 sequence in the vertebrate mRNA binding protein hnRNPA1 that directly binds to Kapβ2/transportin. Similarly, the yeast homologue of Kapβ2, Kap104p, is responsible for the nuclear import of mRNA binding proteins [2].

Members of the Kapβ family share domains allowing interaction with some nucleoporins. The nuclear import substrate/receptor complex first docks at cytoplasmic fibrils extending from the NPC prior to translocation through the NPC. This poorly understood step involves two other proteins, p10 or NTF2, whose role in the different translocation steps is not clearly established, and a small GTPase of the Ras superfamily, Ran. Ran is a very abundant protein (0.3–1% of total proteins) that plays a key role in nucleocytoplasmic transport (see also below for nuclear export). Like other GTPases, Ran exists in GDP- and GTP-bound states. However, RCC1, the Ran GDP exchange factor (RanGEF), is exclusively nuclear whereas the Ran GTPase activating protein (RanGAP) as well as the co-stimulatory factors RanBP1 and RanBP2 are cytoplasmic. This is thought to provide a gradient of Ran within the cell with GTP-Ran in the nucleus and GDP-Ran in the cytoplasm (Fig. 1A). Once the NPC is crossed, binding of Kapβ to GTP-Ran on the nucleoplasmic side of the NPC triggers dissociation of the import substrate/receptor interaction and release of the import substrate into the nucleus (Fig. 1B). Ran therefore ensures directionality of the transport by allowing dissociation of the nuclear import substrate/receptor interaction in the nucleus. After nuclear import, Kaps (receptors and eventual adaptors) are recycled back to the cytoplasm and are available for a new round of nuclear import [3]. Translocation of cargoless Kapβ is a Ran-independent process and some proteins, such as the viral protein Vpr,

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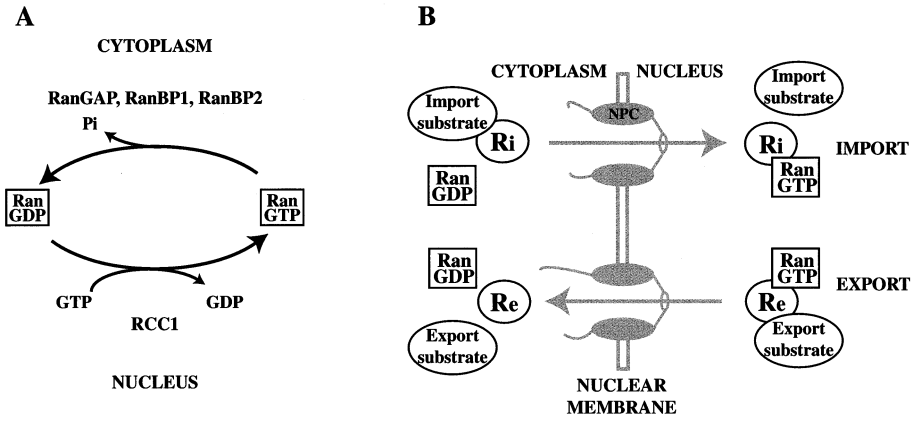

Fig. 1. A: The Ran GTPase cycle. The Ran GDP exchange factor RCC1 (Regulator of Chromosome Condensation 1) is exclusively expressed in the nucleus whereas the Ran GTPase activating protein RanGAP and the costimulatory Ran binding proteins RanBP1 and 2 are cytoplasmic. B: General model of nucleocytoplasmic transport. Nuclear import receptors (Ri) bind import substrates in the absence of RanGTP (in the cytoplasm) and dissociate from them in the presence of RanGTP (in the nucleus) whereas nuclear export receptors (Re) bind export substrates in the presence of RanGTP (in the nucleus) and release them after GTP hydrolysis (in the cytoplasm).

seem to be imported in a receptor- and Ran-independent manner possibly due to similarities to Kapβ and binding to the same nucleoporins [4].

2.2. Export mechanisms

More than 40 years ago, nuclear transplantation experiments demonstrated that some proteins migrate (shuttle) between the nucleus and the cytoplasm of amebas. Shuttling proteins in others organisms have more recently been identified by antibody microinjection and interspecies heterokaryon formation assays [1]. Export of a nuclear protein is primarily limited by the extent of its interaction with other nuclear components. For example, a non-shuttling protein such as lamin B2 can be converted into a shuttling protein by introducing mutations that impair its ability to polymerize at the nuclear membrane [5].

Three major groups of proteins can be distinguished from their export kinetics in interspecies heterokaryons or nuclear microinjection assays. The first group, represented by some major nucleolar proteins, nucleolin and B23/No38, is transported slowly from the nucleus to the cytoplasm (12–24 h) [5], but the role and mechanism of this pathway are still unknown. The second group of proteins displays faster nuclear export kinetic (4 h in the heterokaryon assay) and is illustrated by the glucocorticoid receptor (GR) [6]. This transport pathway is an ATP-dependent and cytosol-independent process that involves tyrosine phosphorylation [7]. Proteins from the third group are exported very efficiently out of the nucleus (45 min for the HIV-1 Rev protein [8]) and this pathway involves specific nuclear export signals (NES) present in the export substrate. The M9 sequence of hnRNP A1 confers ability of both nuclear import and export to this protein suggesting that kapβ2/transportin could also be involved in nuclear export of hnRNP A1 [9]. Another NES identified in an increasing number of cellular or viral proteins is a leucine-rich sequence in which leucine residues are critical for targeting proteins out of the nucleus. Two receptors responsible for nuclear protein export or exportins have been identified so far. CRM1 specifically interacts with leucine-rich NES [10–13] whereas CAS 1 mediates nuclear export of importin α whose NES is unknown [14]. Both exportins are related to the Kapβ family and are able to interact with NPCs and Ran-GTP. Exportins bind their substrates in a RanGTP-dependent way [11,14] but GTP hydrolysis is not required for translocation [15] (Fig. 1B). However, in the cytoplasm, stimulation of GTP hydrolysis on Ran by concerted action of RanGAP, RanBP1 and RanBP2 is thought to trigger dissociation of the exported protein from its receptor that is subsequently recycled back to the nucleus.

3. Nuclear transport and transcriptional regulation

Regulating the access of a transcriptional regulator to the nuclear compartment has been proved over the past years to represent a common way to switch transcription on or off. Considering the receptor-mediated nuclear transport mechanism, regulation could occur at various steps. However, a number of studies point out that regulation of nuclear transport is mainly exerted at the level of the transport substrate rather than at the level of the transport machinery. The different ways reported so far to regulate nuclear import and export of transcriptional regulators are summarized in Fig. 2.

3.1. Cytoplasmic or nuclear anchoring

In the first situation, the transport sequence of the transcriptional regulator is accessible to the nuclear transport receptor but the substrate is anchored in the cytoplasm or in the nucleus by a mechanism that overrides the presence of any NLS or NES. In particular, nuclear import of transmembrane proteins depends on cleavage of the cytosolic tail that can be subsequently transported into the nucleus. Under normal conditions, the entire sterol regulatory element binding protein (SREBP) is inserted in the endoplasmic reticulum membrane. Sterol depletion triggers a SREBP cleavage activating protein (SCAP)-dependent proteolytic cleavage of the NH_2-terminal domain of SREBPs that contains a basic NLS and a transcriptional activator region (Fig. 2a). In the nucleus, this domain promotes expression of genes encoding enzymes involved in the cholesterol biosynthetic pathway [16]. Cytoplasmic anchoring has also been observed for non-membranous proteins such as the thermosensitive $p53^{Val135}$ that interacts with the vimentin cytoskeleton at the restrictive tem-

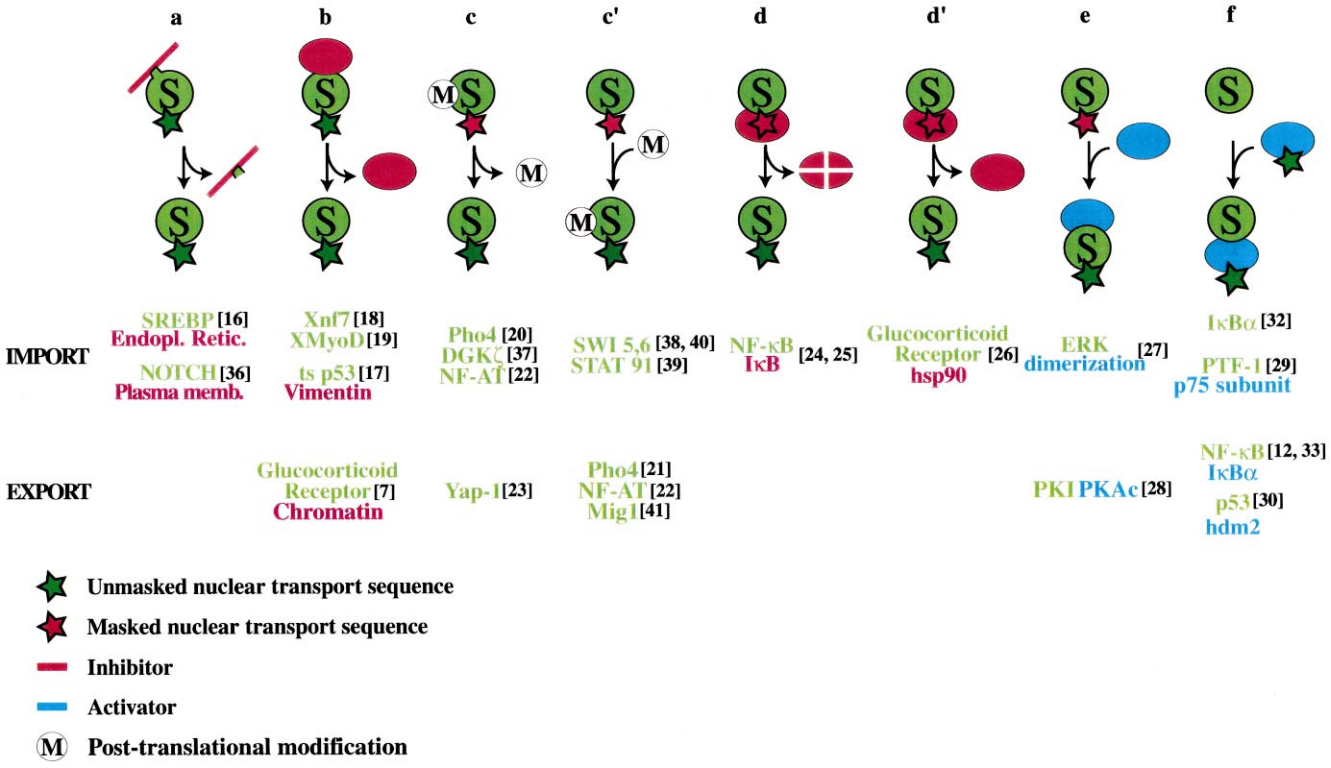

Fig. 2. Different ways to regulate the accessibility of a transport substrate (S) to the transport machinery. Access can be regulated by the cytoplasmic or nuclear anchoring of S to a membrane (a) or to another structure (b). Alternatively, availability of the nuclear transport sequence of S can be regulated by post-translational modification (c and c′), association with an inhibitor (d or d′) or an activator (e) protein or interaction with a protein that carries the transport sequence (f). Numbers in square brackets refer to references; refs. [36–41] only described here.

perature and accumulates in the nucleus at the permissive temperature [17] or the *Xenopus* factors xnf7 and XMyoD, which are retained in the cytoplasm by an unknown mechanism [18,19] (Fig. 2b).

Nuclear export of transcription regulators can also be prevented by their anchorage on nuclear components, particularly on chromatin. In the presence of hormone, glucocorticoid receptor (GR)associates with its specific promoter targets. Upon hormone withdrawal, a rapid release of glucocorticoid receptor from chromatin is observed that is followed by the nuclear export of GR back to the cytoplasm, where it can respond to a secondary hormone challenge [7] (Fig. 2b).

3.2. Masking/unmasking of the transport sequence

In other cases, the nuclear transport sequence is masked either as a consequence of a conformational change or by an interacting partner until a modification unmasks it. Sequence masking/unmasking can be achieved by post-translational modification of the transport substrate itself, can involve the degradation or dissociation of an inhibitory protein or, in contrast, can be due to association with another protein.

The most frequent post-translational modifications reported so far to regulate nuclear transport sequence/receptor interaction are dephosphorylation and phosphorylation and a large number of examples illustrates this major regulation route (Fig. 2c,c′). A well characterized dephosphorylation-dependent NLS unmasking coupled to phosphorylation-dependent NES unmasking is illustrated by the regulation of the yeast transcription factor Pho4. In the absence of phosphate in the growth medium, Pho4 is underphosphorylated and directly interacts with its nuclear import receptor, Kap121p/Kapβ3 [20]. When expressed in the nucleus, Pho4 activates transcription of phosphate-responsive genes. In the presence of phosphate in the growth medium, nuclear Pho4 becomes phosphorylated by the cyclin/cyclin-dependent kinase pair Pho80-Pho85. This modified form of Pho4 is then able to interact with nuclear export receptor Msn5 and is efficiently exported back to the cytoplasm where its phosphorylation inhibits the interaction with the import receptor [21]. Dephosphorylation/phosphorylation thus provides a nuclear import and export switch that tightly regulates the subcellular localization and consequently the transcriptional activity of Pho4 as a function of extracellular phosphate concentration. The nucleocytoplasmic distribution of the transcription factor NF-AT is regulated following a similar scheme. In resting T cells, NF-AT resides in the cytoplasm but upon TCR and CD28 coreceptor engagement, elevation of intracellular calcium triggers activation of the phosphatase calcineurin that dephosphorylates NF-AT resulting in its NLS unmasking and subsequent nuclear import. Upon return of calcium to resting levels, nuclear NF-AT is phosphorylated by glycogen synthase kinase-3 allowing the NES to be exposed and NF-AT is thus exported out of the nucleus through its interaction with CRM1 [22]. In contrast, oxidation of the yeast AP-1-like transcription factor Yap1p prevents its nuclear export by masking the NES [23]. Yap1p is therefore maintained in the nucleus where it activates genes involved in the stress response.

When a nuclear transport sequence is masked through an interaction with an inhibitory factor, its exposure can be en-

sured by proteolysis of the inhibitor (Fig. 2d). The best characterized example of such a regulation concerns the NF-κB transcription factor. In most unstimulated cells, NF-κB interact with inhibitory molecules called IκBs that directly mask NF-κB NLSs [24]. The stimulation of cells with cytokines or various environmental stresses leads to the phosphorylation of IκBs. For most inducers, phosphorylation occurs on conserved serines and targets IκBs for ubiquitination and degradation by the proteasome. IκB-free NF-κB then interacts with the basic NLS receptor and is imported into the nucleus where it activates responsive genes [25].

Dissociation of the transport substrate/inhibitor complex can also lead to NLS or NES unmasking. The conformational change that triggers dissociation from the inhibitory molecule can be due either to post-translational modifications or to the binding of a different transport substrate partner. In the case of the glucocorticoid receptor, hormone binding to its receptor triggers dissociation of the inhibitory hsp90 and NLS unmasking [26] (Fig. 2d′). The nuclear transport substrate can also interact with an activator protein that unmasks the NLS or the NES (Fig. 2e). This is illustrated by the mammalian ERK2 MAP kinase that translocates into the nucleus upon phosphorylation and homodimerization [27]. PKI, a specific inhibitor of the catalytic subunit (C) of the cAMP-dependent protein kinase, contains an NES only exposed when PKI binds to C thus limiting the kinase activity of C in the nuclear compartment [28].

3.3. *Piggy-back*

Finally, some transcriptional regulators do not contain any NLS or NES but their interaction with specific partners bearing nuclear transport sequences results in their nuclear import or export by a mechanism called piggy-back (Fig. 3f). This is illustrated by the nuclear import of the pancreatic transcription factor-1 (PTF-1) whereby the p75 subunit directs the cytoplasmic form of PTF-1 (p48/p64 heterodimer) to the nucleus [29]. Similarly, p53 is exported from the nucleus to the cytoplasm through its interaction with hdm2 which contains an NES. This nuclear transport is required for the proteasome-dependent degradation of p53 which occurs in the cytoplasm [30]. The piggy-back mechanism provides the cell with a supplementary regulation level since modulation of both the transport sequence and the interaction between the transport substrate and the NLS- or NES-bearing protein can be achieved.

4. Regulation of NF-κB-dependent transcription

Over the past few years, transcriptional activity of NF-κB has been shown to be tightly regulated by nucleocytoplasmic

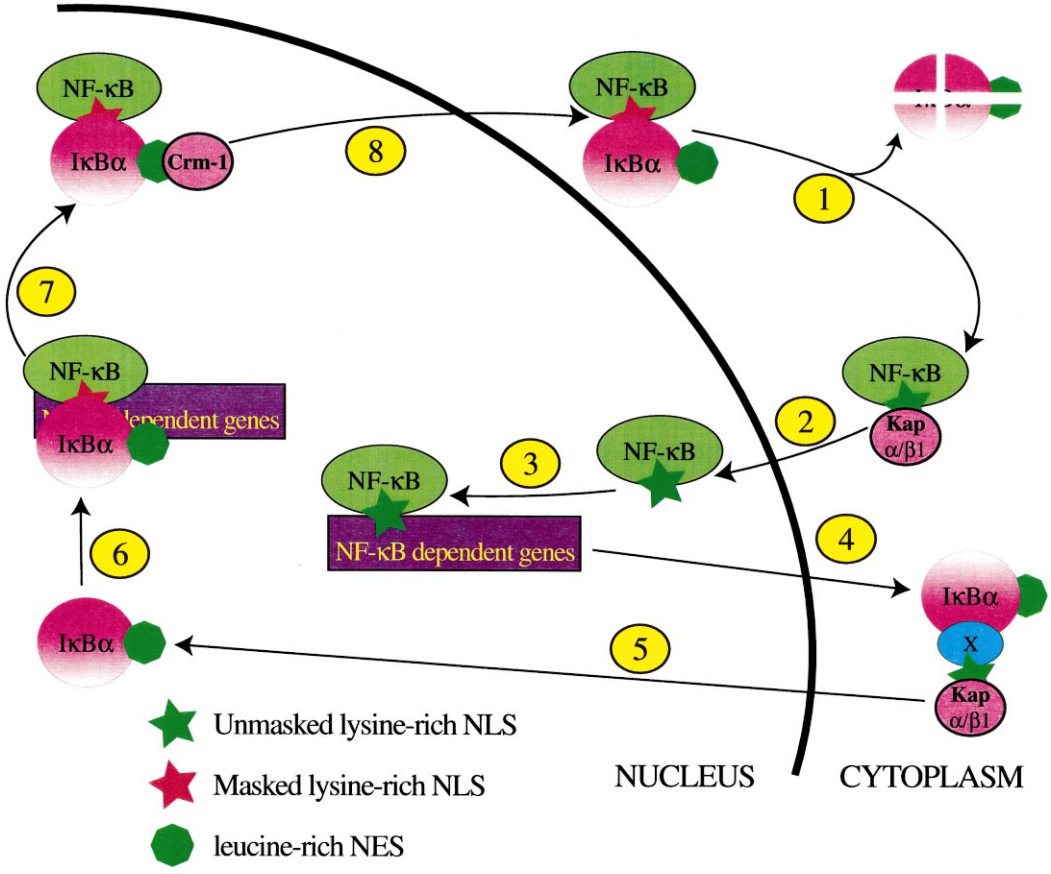

Fig. 3. Regulation of NF-κB-dependent transcription. In unstimulated cells, interaction with IκB masks NF-κB NLS. Stimulation of cells leads to the degradation of IκBs (1) and nuclear translocation of NF-κB (2). In the nucleus, NF-κB activates the transcription of responsive genes (3) and in particular the gene encoding IκBα (4). Neosynthesized IκBα is transported to the nucleus by a piggy-back mechanism involving an unknown protein (X) that carries a lysine-rich NLS (5). In the nucleus, IκBα interacts with NF-κB (6) and terminates NF-κB-dependent transcription by inhibiting NF-κB/DNA interaction (7) and by exporting NF-κB out of the nucleus,through a leucine-rich NES within IκBα (8).

trafficking. NF-κB transcription factors are involved in the control of apoptotic, inflammatory, immune as well as viral responses and share a Rel homology domain that contains a lysine-rich NLS and a DNA binding domain. In most unstimulated cells, interaction with inhibitory proteins, IκBs, masks NF-κB NLS and inhibits DNA binding in vitro. Stimulation of cells with pathogens, cytokines or environmental stresses activates a cascade of kinases leading to the phosphorylation of IκB and its subsequent ubiquitination and degradation by the proteasome (Fig. 3, step 1). Once IκB degraded, the NLS of NF-κB is unmasked and NF-κB proteins are transported in the nucleus (step 2) where they activate the transcription of responsive genes (step 3). In particular, NF-κB promotes the transcription of the gene encoding IκBα thus inducing a massive neosynthesis of IκBα [25] (step 4). Neosynthesized IκBα is transported into the nucleus by a piggy-back mechanism through interaction with an unknown protein (X) that carries a lysine-rich NLS [31,32] (step 5). In the nucleus, IκBα interacts with NF-κB (step 6) and terminates NF-κB-dependent transcription by inhibiting NF-κB/DNA interaction (step 7) and exporting NF-κB out of the nucleus. This latter function is ensured by a leucine-rich NES within IκBα [12,33] (step 8). In addition to its role in the postinductional repression of transcription, efficient export is also essential for maintaining a low level of IκBα in the nucleus and allowing NF-κB to be transcriptionally active upon cell stimulation [34]. Finally, another member of the IκB family, IκBβ, can also in some cases be imported into the nucleus where it interacts with NF-κB but without dissociating NF-κB from DNA and thus protects NF-κB from IκBα [35].

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