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Minireview

The rules and roles of nucleocytoplasmic shuttling proteins

Margarida Gama-Carvalho, Maria Carmo-Fonseca*

Institute of Histology and Embryology, Faculty of Medicine, University of Lisbon, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal

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Abstract The spatial separation of mRNA synthesis from translation, while providing eukaryotes with the possibility to achieve higher complexity through a more elaborate regulation of gene expression, has set the need for transport mechanisms through the nuclear envelope. In a simplistic view of nucleocytoplasmic transport, nuclear proteins are imported into the nucleus while RNAs are exported to the cytoplasm. The reality is, however, that transport of either proteins or RNAs across the nuclear envelope can be bi-directional. During the past years, an increasing number of proteins have been identified that shuttle continuously back and forth between the nucleus and the cytoplasm. The emerging picture is that shuttling proteins are key factors in conveying information on nuclear and cytoplasmic activities within the cell. **© 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.**

Key words: Nucleocytoplasmic transport; Nuclear import; Nuclear export; Shuttling protein

1. Introduction

Standard microscopic and biochemical methods impose an important constraint on localizing a protein to the nucleus or to the cytoplasm. Due to sensitivity limits, these methods only reveal the presence of a protein where its steady state concentration is above the detection threshold. Finding that a protein is exclusively detected in the nucleus by immunofluorescence or immunoblotting does not exclude the possibility that the protein transiently crosses the nuclear envelope and plays a role in the cytoplasm, or vice versa. Thus, appropriate assays had to be developed in order to specifically analyze nucleocytoplasmic shuttling (Fig. 1).

The first observations indicating that proteins can shuttle continuously between the nucleus and the cytoplasm date as far back as the 1950s [1] (see Fig. 1). However, it took approximately 30 years for the first shuttling protein, nucleolin, to be identified [2]. At present, the list of nucleocytoplasmic shuttling proteins includes transport receptors and adaptors [3,4], steroid hormone receptors ([5] and references therein), transcription factors [6], cell cycle regulators [7,8] and numerous RNA binding proteins [4,9].

Nucleolin is a slow shuttling protein, which is not completely retained in the nucleus and therefore 'leaks' to the cytoplasm [10]. Studies on the nucleocytoplasmic shuttling of nucleolin led to the proposal that all nuclear proteins have the ability to be exported from the nucleus. In the absence of specific intranuclear retention mechanisms, all nuclear proteins were predicted to shuttle [10,11]. More recently, however, novel classes of shuttling proteins were discovered that move in and out of the nucleus much faster than nucleolin. Indeed, while nucleolin takes about 24 h to be detected in a heterologous nucleus, shuttling of nucleocytoplasmic transport factors is detected within minutes. The rapid transport of fast shuttling proteins relies on the presence of conserved signal sequences recognized by specific receptors and adaptors, which efficiently translocate cargo through the nuclear envelope (for recent reviews see [4,3,12]). Shuttling proteins typically have both a nuclear localization signal (NLS), and a nuclear export signal (NES). Several types of signals have been identified that interact with distinct transport pathways. Some signals can be recognized by a variety of receptors and adaptors, and a given transport receptor can have distinct binding sites for different signals. In some cases, the same amino acid sequence confers both import and export activities.

A potential role of shuttling proteins in nucleocytoplasmic transport was first proposed by Goldstein [1]. At present, it is well established that shuttling proteins act not only as carriers of cargo in transit between the nucleus and the cytoplasm, but they also play an important role in relaying information between the two major cellular compartments.

2. Shuttling of nucleocytoplasmic transport receptors and adaptors

Transport between the nucleus and the cytoplasm takes place through the nuclear pore complex (NPC). In order to cross the NPC, each cargo must contain defined signal sequences, which are specifically recognized by transport receptors and adaptors (for recent reviews see [3,4,13,14]). Shortly after traversing the NPC, importins (import receptors) and exportins (export receptors) free their cargo and recycle, moving back to the other side of the nuclear envelope to start a new transport event (Fig. 2). Thus, transport receptors and adaptors are shuttling proteins that move rapidly back and forth across the NPC.

The majority of known transport receptors belong to the importin β super-family of RanGTP binding proteins. Cargo binding by these receptors may be direct or mediated by a specific adaptor [3]. Importin β, for instance, uses importin α as adaptor to mediate the import of proteins containing a

*Corresponding author. Fax: (351)-21-7951780.
E-mail: carmo.fonseca@fm.ul.pt

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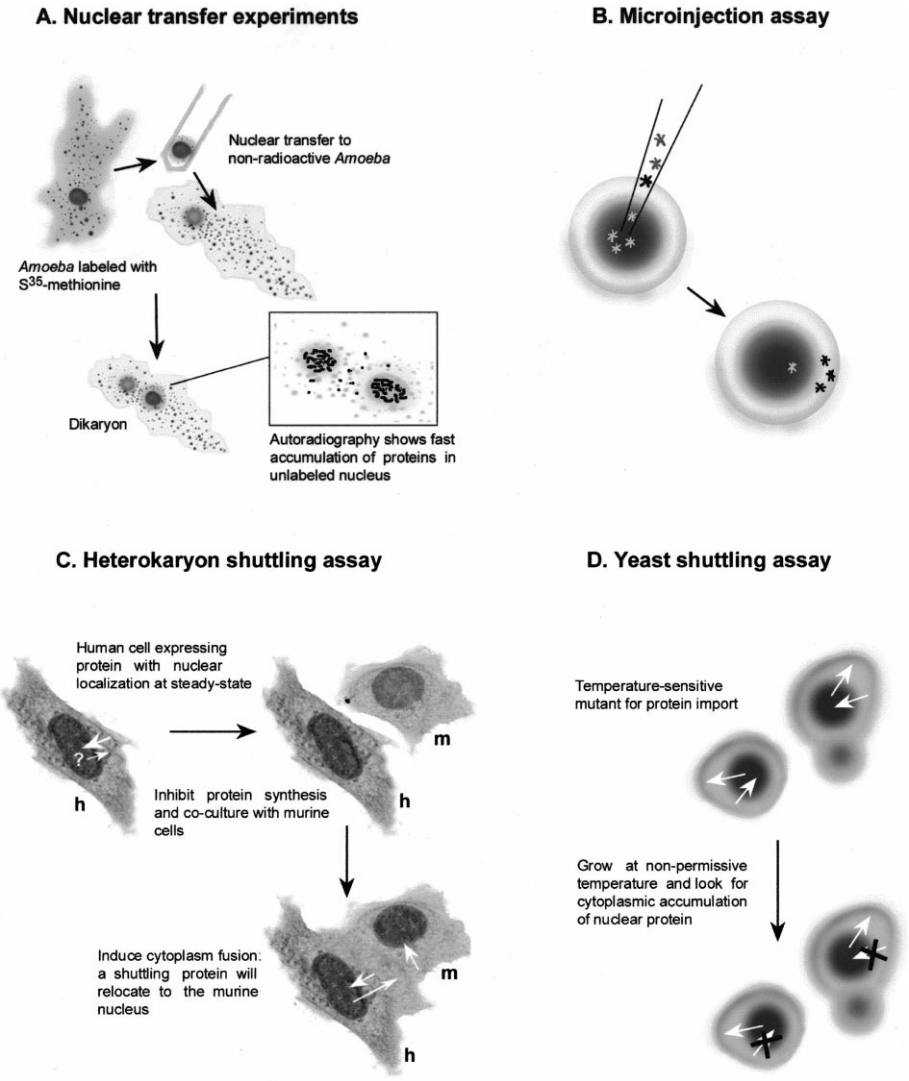

Fig. 1. Experimental approaches to identify shuttling proteins. A: Nuclear transfer experiments of *Amoeba* nuclei led Goldstein to postulate that proteins can shuttle between nucleus and cytoplasm [1]. In this assay, a radioactively labelled nucleus was grafted into an unlabelled *Amoeba*. At 4 h after the operation, the radioactive label had already left the grafted nucleus, and accumulated to a significant extent in the originally unlabelled nucleus, with very little labelling present in the cytoplasm. B: Two different shuttling assays use microinjection. *Xenopus* oocytes are most often used because, first, they are easy to inject, and, second, it is possible to manually separate nucleus and cytoplasm. Microinjection into the nucleus or into the cytoplasm of radioactively labelled proteins allows for a direct quantification of export and import rates. Alternatively, antibodies against the protein of interest are microinjected into the cytoplasm. In the case that the antigenic protein does not shuttle, the antibodies remain exclusively in the cytoplasm (because immunoglobulins do not have a NLS and are too big to diffuse through the NPC). In the case that the antigenic protein does shuttle, the antibody/antigen complex is imported into the nucleus (the assay is performed in the presence of protein synthesis inhibitors to rule out the possibility that antibody/antigen complexes form in the cytoplasm with newly synthesized protein). C: Interspecies heterokaryons are widely used to determine whether or not a nuclear protein shuttles. The basic principle is to monitor whether, in the presence of protein synthesis inhibitors, the protein originally present in the nucleus of one species appears in the nucleus of the other species. Monitoring protein migration can be performed using monoclonal antibodies that are specific for one of the two species. Alternatively, human cells (h) can be transfected with a construct encoding a tagged protein, and fused with mouse cells (m). The donor and receptor nuclei can be distinguished based on different chromatin staining patterns, or using an antibody specific for a human protein that does not shuttle. An important control consists in incubating the donor cells at 4°C. The protein will remain exclusively nuclear if its export is receptor-mediated; detection of the protein in the cytoplasm reflects passive diffusion through the NPC. D: A genetic approach may be performed in yeast cells, using temperature sensitive mutant strains with defects in protein import [59]. Under these conditions, a nuclear shuttling protein exits the nucleus and accumulates in the cytoplasm.

classical NLS. The same receptor, importin β, is involved in the import of snRNPs through a distinct adaptor, Snurportin-1. Importin β can also function without an adaptor in the import of some ribosomal proteins, the HIV-1 Rev and Tat proteins, and probably cyclin B1. Exportin 1/CRM1, the best characterized export receptor, may either directly recognize the leucine-rich NES sequence present in a protein cargo, or act through adaptor molecules [3]. For instance, export of snRNAs requires an adaptor, PHAX, which mediates interaction of the monomethyl cap structure with exportin 1 (Fig. 2B). Thus, adaptor molecules have an important role in determining cargo specificity. Transport of adaptors unbound to a cargo is reduced by the cooperative binding of cargo, adaptor and receptor molecules, and recycling of adaptor molecules also involves specific receptors.

The small GTPase Ran is a crucial element in nucleocyto-

plasmic transport events mediated by members of the importin β family (reviewed by [3,13]). Ran can switch between a GDP- and GTP-bound state and importin β-related transport receptors bind specifically to RanGTP. Ran regulates the binding of substrates (i.e. the cargoes) to the transport receptors. Importins bind to substrates in the absence of Ran, while export cargoes interact preferentially with the RanGTP-bound form of exportins. In the presence of RanGTP, importins release their cargo. Conversely, removal of RanGTP from an exportin results in displacement of the substrate. To ensure efficient transport of cargo and recycling of receptors, the relative concentration of RanGTP and RanGDP in the nucleus and in the cytoplasm is asymmetric. This is accomplished by concentrating the RanGTPase activating protein (RanGAP) in the cytoplasm, and the guanidine nucleotide exchange factor RCC1 (or RanGEF) in the nucleus. RanGTP is constantly exported from the nucleus in complexes with importins and exportins. As these complexes exit the nucleus, RanGAP localized at cytoplasmic filaments of the NPC removes RanGTP from the transport receptors. Ran is then reimported into the nucleus in order to replenish the nuclear stores and allow continued transport cycles. Thus, Ran shuttles continuously between nucleus and cytoplasm.

To date, only one major cellular transport pathway appears to be independent of the importin β family of transporters and Ran. This is the path responsible for export of spliced mRNAs to the cytoplasm [15]. Factors required for mRNA export, which are known to shuttle between nucleus and cytoplasm, include the essential yeast protein Mex67p and the corresponding human homologue TAP (more recently called NXF1), and yeast Gle2p (human RAE1) [3,4].

3. The role of shuttling proteins in signal transduction pathways

The eukaryotic cell takes advantage of the barrier established by the nuclear envelope to control access of transcriptional regulators to their target genes. A simple way to regulate transcription of a particular gene in response to a signalling pathway is to locate an activator protein to the cytoplasm, until a specific signal triggers its access to the nucleus. Conversely, genes may be activated in response to a signal that induces specific inhibitors to exit from the nucleus.

Although re-localization of a protein to the nucleus or to the cytoplasm in response to a signal does not necessarily imply that the protein shuttles continuously between nucleus and cytoplasm, it is becoming clear that most proteins whose activity is controlled by transport into different subcellular compartments are in fact shuttling molecules. Two of the most recently identified examples are histone deacetylases HDAC4 and HDAC5. Histone deacetylases remove acetyl

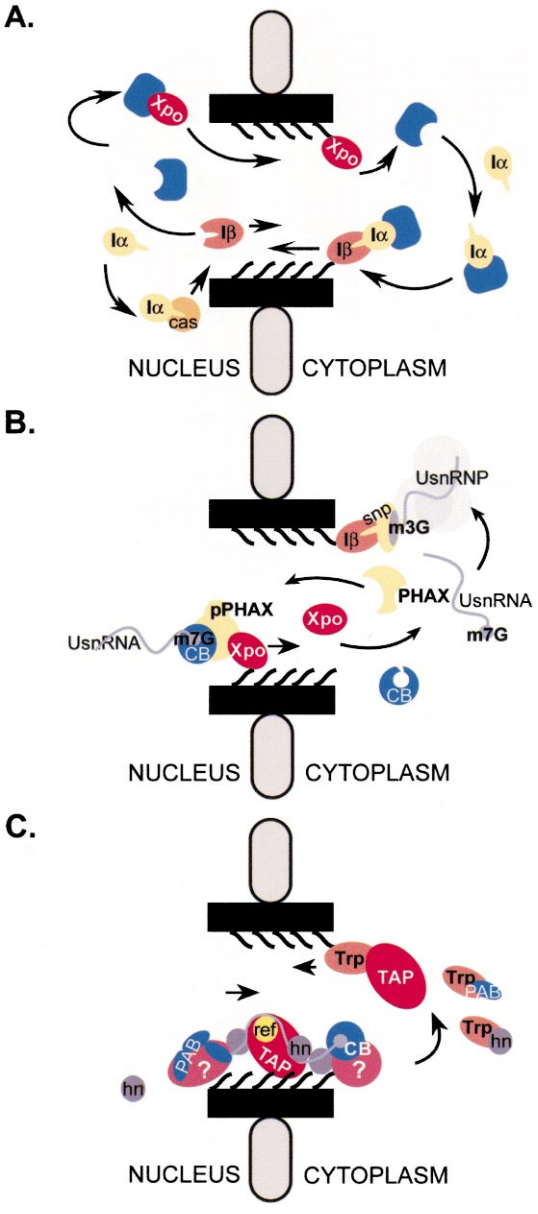

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Fig. 2. Transport receptors and adaptors. A: Receptors for classical NLS and NES-mediated transport. A shuttling protein (blue) with a classical NLS and a NES binds to importin α (Iα) in the cytoplasm. This will then form a complex with the receptor, importin β (Iβ), and RanGDP. The complex interacts with nucleoporins and translocates into the nucleus. The cargo is released, and importin β recycles back to the cytoplasm alone, while importin α requires an export receptor, CAS-1 (cas). Direct interaction of the NES on the cargo with the export receptor exportin 1/CRM1 (Xpo) results in export to the cytoplasm. As most transport receptors, after release of the cargo, exportin 1 recycles back to the nucleus by itself. B: Transport receptors of the importin β super-family use different adaptors for distinct cargoes. The export adaptor PHAX mediates interaction of UsnRNAs with exportin 1 [60]. Phosphorylated PHAX (pPHAX) cooperatively binds the Cap binding complex (CB) of UsnRNAs and exportin 1. In the cytoplasm, hydrolysis of RanGTP and dephosphorylation of PHAX will promote the release of the UsnRNA. After assembly with Sm proteins and modification of the cap structure, the resulting snRNPs are imported to the nucleus. The modified cap structure binds the adaptor Snurportin (snp) and importin β. C: Export of mRNA is mediated by a distinct family of export receptors. To date, the best characterized nuclear mRNA export factor is TAP(NXF1)/Mex67p. TAP interacts with nucleoporins (directly or through p15) and its recruitment to spliced mRNA is probably facilitated by REF/Aly. The hnRNP proteins (particularly hnRNP A1) are also thought to contribute to mRNA nuclear export. The 5′ cap structure, which binds the Cap binding complex (CB), is the lead as the mRNA crosses the NPC. However, there is still no evidence for a direct role of CBC in the translocation mechanism. It also remains to be determined whether PABP2 (PAB), which coats the 3′ end poly(A) tail, contributes to mRNA export. Interestingly, both TAP, hnRNP A1 and PABP2 make use of the import receptor transportin (Trp) to recycle back into the nucleus.

groups from histones bound to DNA, and HDAC4 was shown to shuttle into and out of the nucleus. During differentiation of skeletal muscle cells in culture, a calcium signal initiated at the cell membrane activates protein kinases that add phosphate groups to HDAC4 and HDAC5. The phosphorylated enzymes dissociate from MEF2, exposing their NES. Consequently, export of deacetylases out of the nucleus prevails over import. MEF2, a muscle-specific transcription regulator, is then free to activate the expression of muscle-specific genes [16].

Another example of proteins whose biological activity is regulated through nucleocytoplasmic shuttling is provided by the NFκB family of transcription factors. NFκB is normally composed of two subunits, p50 and p65, which work by turning on a set of anti-apoptotic genes. The heterodimer p50/p65 is usually detected predominantly in the cytoplasm, in a complex with an inhibitor protein called IκB. In response to appropriate stimuli, the IκB protein becomes phosphorylated and marked for degradation. As a consequence, NFκB accumulates in the nucleus, binds to target genes and activates a new program of gene expression. Although it was originally thought that the NFκB/IκB complex was held captive in the cytoplasm, recent results indicate that this complex shuttles continuously between the nucleus and the cytoplasm [17–20]. It is currently suggested that NFκB proteins locate predominantly to the nucleus or to the cytoplasm depending on a tightly regulated balance between import and export rates.

Regulated nucleocytoplasmic shuttling is also displayed by the NF-AT transcription factors. In unstimulated T cells, the NF-AT proteins are cytoplasmic, but they rapidly translocate into the nucleus in response to T cell receptor activation. Within the nucleus, NF-AT factors activate transcription of cytokine genes. Nuclear accumulation of NF-AT proteins is induced by the calcium-dependent phosphatase calcineurin, and requires continued calcium signalling. However, nuclear import is not sufficient to activate NF-AT target genes, because the proteins contain NESs recognized by the CRM1 exportin. In fact, calcineurin was also shown to mask the NESs present in NF-AT factors [21]. Cessation of the calcium-activated signal results in rapid rephosphorylation of NF-AT by casein kinase I and MEKK1, with consequent export to the cytoplasm [22]. Based on these data, the following model was proposed. In the absence of a regulatory mechanism, NF-AT would sequentially interact with importins and exportins, engaging in a futile cycling across the nuclear envelope that would prevent the functional interaction of NF-AT transcription factors with target genes. The NF-AT proteins (and presumably other shuttling proteins) avoid such futile cycling by coupling the activation of import signals to the suppression of export signals [21].

Shuttling in and out of the nucleus not only provides a mechanism to control signal-dependent access of proteins to nuclear targets, but also contributes to regulate the activity of proteins in the cytoplasm. In yeast, binding of pheromone to cell surface receptors activates a protein kinase (mitogen-activated protein kinase (MAPK)) cascade. Transmission of the pheromone signal to the kinase cascade in vivo requires proper localization of a scaffold protein, Ste5, to the plasma membrane. Ste5 shuttles constitutively between nucleus and cytoplasm, and its nuclear export is enhanced in the presence of pheromone [23]. Most important, blocking access of Ste5 to the nucleus impairs the ability of the protein to localize to the plasma membrane and to activate the pathway. It was therefore proposed that shuttling through the nucleus helps to prevent recruitment of cytoplasmic Ste5 to the membrane in the absence of pheromone [23].

Finally, it is noteworthy to highlight that also the kinases involved in signalling cascades shuttle between nucleus and cytoplasm. One example is the MAPK (or ERK), a ubiquitous component of signal transduction pathways in eukaryotes. In unstimulated cells, MAPK/ERK is predominantly cytoplasmic. Upon activation, the kinase translocates to the nucleus, where it phosphorylates nuclear targets. Import of MAPK/ERK to the nucleus depends on its own phosphorylation, which in turn promotes homodimerization of the protein ([24]). Exit of MAPK/ERK from the nucleus is mediated by MAPK kinase (MAPKK or MEK), which binds MAPK/ERK, contains NES and shuttles constantly between nucleus and cytoplasm [25].

4. Shuttling proteins control cell cycle progression and proliferation

During the past few years it has become well established that shuttling between nucleus and cytoplasm plays a critical role in the regulation of cell cycle progression and control of cellular proliferation. Mitotic events are normally initiated by the cyclin-dependent kinase Cdc2, which is activated by the protein phosphatase Cdc25. Cdc25 shuttles in and out of the nucleus [26,27]. In the presence of DNA damage, Cdc25 is phosphorylated and this creates a binding site for members of a family of small acidic proteins collectively called 14-3-3 proteins. Binding of 14-3-3 proteins markedly reduces the nuclear import rate of Cdc25, allowing nuclear export to predominate. As a result, Cdc25 is predominantly located to the cytoplasm, having no access to Cdc2 present in the nucleus. Thus, changing the relative rates of nuclear import and export of Cdc25 in response to DNA damage plays a critical role in preventing the onset of mitosis while DNA repair is under way.

Recently, members of the 14-3-3 family of signalling proteins were also found to interact with human TERT, the catalytic subunit of telomerase. Most immortal cells, including germ cells and cancers, contain active telomerase, which catalyzes de novo synthesis of telomeres. Telomerase activity is regulated by expression and post-translational modification of TERT. TERT contains a NES-like motif and shuttles between nucleus and cytoplasm. Possibly, 14-3-3 binding inhibits the interaction of the NES in TERT with the exportin CRM1. This would lead to accumulation of TERT in the nucleus and enhancement of telomerase activity on chromosomes [28].

Normal induction and coordination of M phase events in vertebrate cells involves localizing Cdc2-cyclin B1 to the nucleus during prophase. Cyclin B1 is a shuttling protein and its nuclear accumulation requires phosphorylation at specific residues. The polo-like kinase 1 was recently identified as a major kinase that phosphorylates cyclin B1 at G2/M transition and during M phase [29]. The polo-like kinase 1-mediated phosphorylation seems to be responsible for inactivating the NES of cyclin B1 rather then enhancing nuclear import of the protein. This strengthens the view that shuttling proteins can transiently accumulate either in the nucleus or in the cytoplasm depending on a tightly regulated balance between transport into and out of the nucleus.

Another shuttling protein, $p27^{Kip1}$, which inhibits Cdk2 complexes in resting cells, must be degraded at the G1/S transition of the cell cycle [30]. In order to be eliminated, $p27^{Kip1}$ must first be imported into the nucleus, where it is phosphorylated by cyclin E-Cdk2 kinase. Phosphorylated $p27^{Kip1}$ is then exported to the cytoplasm for degradation.

Coupling exit from the nucleus to cytoplasmic degradation is also used to control the activity of p53. The p53 protein is a transcription factor that functions in the nucleus to prevent the growth of abnormal or damaged cells through several mechanisms, the best understood of which are activation of cell cycle arrest and apoptosis. In normally dividing cells it is necessary to restrain p53 activity by transporting the protein out of the nucleus. The nucleocytoplasmic transport of p53 is tightly regulated by its interaction with the shuttling protein MDM2. Both MDM2 and p53 contain NESs, and it was recently suggested that ubiquitination of p53 may be necessary for its export, possibly by exposing the NES located in the C-terminus of the protein [31,32]. According to a current model, under normal conditions the activity of p53 may be regulated both by restraining import to the nucleus and by enhancing exit to the cytoplasm. In response to appropriate stress signals, nuclear import of p53 is activated while export is blocked. This model has major implications in understanding the biology of cancers that retain wild-type p53 (approximately 50%). In fact, in a significant number of tumors with wild-type p53 (particularly breast cancers and neuroblastomas) the protein appears to be inactive because it is predominantly localized to the cytoplasm.

Similarly to p53, BRCA1 is a tumor suppressor protein involved in transcriptional regulation and cellular responses to DNA damage. BRCA1 contains both NLS and NES and shuttles between nucleus and cytoplasm [33]. It remains to be established whether mutations in the *brca1* gene, which confer increased susceptibility to breast and ovarian cancers, have implications on the intracellular trafficking of the protein.

An additional tumor suppressor protein that was recently shown to shuttle between nucleus and cytoplasm is adenomatous polyposis coli (APC) protein [34,35]. Mutational inactivation of the APC protein occurs in most colorectal cancers. APC suppresses tumor progression by promoting cytoplasmic degradation of the oncogenic transcriptional activator β-catenin. The APC protein contains highly conserved NESs and shuttles continuously in and out of the nucleus. APC mutations associated with colon cancer are located adjacent to the NESs and affect exit of the protein from the nucleus. As a result, the APC-interacting protein β-catenin accumulates in the nucleus leading to the activation of transforming genes. Thus, the ability of APC to shuttle and remove β-catenin from the nucleus appears to be the basis for its tumor suppression function.

5. Shuttling proteins couple nuclear and cytoplasmic mRNA metabolism

Within the nucleus, nascent transcripts are packed with proteins forming ribonucleoprotein particles or RNPs. A significant proportion of proteins that bind to mRNA contain both import and export signals, and shuttle between nucleus and cytoplasm (reviewed in [4]). A subset of these protein belongs to the class of nucleocytoplasmic transport factors implicated in mRNA export. Others are splicing factors that participate in the regulation of alternative splicing. Some proteins persist associated with the mRNA and license it for export, while others have a role in cytoplasmic events such as mRNA localization, mRNA translation, and mRNA turnover (reviewed in [9]).

Piñol-Roma and Dreyfuss provided the first evidence that some heterogeneous nuclear RNP (hnRNP) proteins, which coat pre-mRNAs as they are synthesized, shuttle between nucleus and cytoplasm (reviewed by [36]). Shuttling hnRNP proteins include A1, I and K. HnRNP A1, the most extensively studied, contains a specific type of NES termed M9 [4]. Intriguingly, the nucleocytoplasmic distribution of shuttling hnRNPs A1 and I is coupled to transcriptional activity [37,38]. Under normal conditions, hnRNP A1 and I are exclusively detected in the nucleus, but after inhibition of transcription the proteins accumulate in the cytoplasm. A1 and I proteins do not contain classical NLSs. In contrast, hnRNPs C and U do not shuttle, localize in the nucleus independently of transcriptional activity and contain classical NLSs. Another member of the hnRNP family, the K protein, shuttles yet is transcription-independent and contains the classical NLS. Deletion of this NLS renders the K protein dependent on ongoing transcription for complete nuclear localization (see [39] and references therein). A possible interpretation for these results is that transcription inhibition selectively affects nuclear import of hnRNP proteins devoid of a classical NLS [39]. Alternatively, these proteins re-distribute to the cytoplasm when transcription is inhibited because they are no longer retained in the nucleus by nascent transcripts [40]. According to the latter hypothesis, shuttling is independent of transcriptional activity. Export to the cytoplasm of hnRNP proteins devoid of a classical NLS possibly occurs at a faster rate than import into the nucleus. In a transcriptionally inactive cell it is expected that the pool of free hnRNP proteins (i.e. proteins unbound to mRNA) will increase, and these would be rapidly exported to the cytoplasm. Presence of a classical NLS most likely increases the rate of import, counteracting the export activity. Consequently, a shuttling protein with such a 'dominant' NLS (like hnRNP K) would be exclusively detected in the nucleus, even in the presence of transcription inhibitors.

Additional proteins that coat mRNAs and shuttle in and out of the nucleus are poly(A) binding proteins. With only one known exception, all eukaryotic mRNAs contain a poly(A) tail at their 3′ end. In mammalian cells two distinct proteins, PABP1 and PABP2, bind to poly(A) tails. PABP1 is predominantly detected in the cytoplasm, where it is involved in mRNA stability and translation. PABP2, which is localized in the nucleus, binds with high affinity to nascent poly(A) tails, stimulating their extension and controlling their length. Both PABP1 and PABP2 shuttle between nucleus and cytoplasm [41–43], but precisely how PABP2 bound to poly(A) tails in the nucleus is replaced by PABP1 in the cytoplasm remains unknown. As 3′ end processing appears to be critical for efficient nuclear export [44], and transport of PABP2 to the cytoplasm persists in the absence of mRNA traffic, it has been proposed that shuttling of this protein may contribute to export of mRNA [43].

The 5′ cap structure of mRNAs also enhances the rate of mRNP export to the cytoplasm. This structure binds the Cap binding complex, CBC. In yeast, CBC interacts with Npl3p, an essential hnRNP-like protein with some similarity to SR

proteins. Both Npl3p and Cbp80p (a component of CBC) shuttle between nucleus and cytoplasm in a manner dependent on ongoing synthesis of RNA [45]. Possibly, CBC and Npl3p escort the mRNA across the NPC and play a role in cytoplasmic functions.

A role for shuttling proteins in coupling splicing to mRNA export has been recently demonstrated [46,47]. The shuttling protein REF1-I or Aly is a member of the REF family of evolutionary conserved hnRNP-like proteins (see [48] and references therein). The yeast homologue of Aly is the mRNA export factor Yra1p. Human REF, which associates with mRNAs in a splicing-dependent manner, is recruited during spliceosome assembly, and then becomes tightly associated with the spliced mRNP [46,49]. REF proteins bind RNA directly and interact with members of the NXF family of mRNA export factors including metazoan TAP and its yeast homologue, Mex67p. Thus, REF proteins are thought to act on mRNA export by recruiting TAP to mRNPs. Like Aly/REF, Y14 is a shuttling protein that associates preferentially with mRNAs produced by splicing but not with pre-mRNAs, introns, or mRNAs produced from intronless cDNAs [47]. Y14 and Aly define a novel intermediate in the pathway of gene expression: both proteins are recruited to spliced mRNPs, promoting export of the mRNA to the cytoplasm. Similarly to transport factors, Aly/REF and Y14 are expected to shuttle in order to recycle back into the nucleus after escorting the mRNA to the cytoplasm. However, Aly/REF has the ability to shuttle independently of mRNA export [48]. One explanation for the shuttling of RNA-free protein is that binding to RNA may be required for its efficient release from import receptors in the nucleus. In the absence of RNA binding, the protein may not dissociate efficiently from the import receptor and may engage in futile import/export cycles. This may provide a means for regulating the availability of mRNA export factors in the nucleus.

Contrasting to Aly/REF and Y14, which appear to be general mRNA binding proteins, mRNA-specific shuttling proteins also exist. For example, the mRNAs of many proto-oncogenes, cytokines and lymphokines are targeted for degradation by AU-rich elements located in their 3′ untranslated regions. A nucleocytoplasmic shuttling protein, termed HuR, binds selectively to these elements stabilizing the mRNAs [50]. Possibly, HuR binds to AU-rich element-containing mRNAs in the nucleus and accompanies them to the cytoplasm, providing protection against degradation. Stability of these RNAs is subject to regulation, and several lines of evidence suggest the involvement of signal transduction pathways. More recently, HuR was shown to interact with two shuttling phosphoproteins, pp32 and APRIL, that interact with the nuclear export receptor CRM1. Furthermore, the inhibition of CRM1 by leptomycin B leads to selective retention in the nucleus of c-*fos*, an AU-rich element-containing mRNA. This raises the possibility that particular mRNAs may have a dedicated export pathway regulated by specific shuttling proteins [51].

Additional mRNA binding proteins that shuttle between nucleus and cytoplasm include splicing factors and Upf proteins involved in nonsense-mediated decay (NMD) (reviewed by [9]). In NMD, aberrant transcripts containing premature stop codons are degraded [52]. Typically, premature termination codons are present in internal exons and it is currently thought that recognition of an intron downstream of a stop codon is critical to trigger NMD. Very recent data suggest that nuclear Upf proteins assemble in a complex at mRNA exon–exon junctions and trigger NMD in the cytoplasm when recognized downstream of a translation termination site [53].

Exonic sequences additionally contain binding sites for SR proteins, a family of essential pre-mRNA splicing factors. Some members of the SR protein family shuttle in and out of the nucleus, presumably because they remain bound to mRNAs in transit to the cytoplasm. In fact, stable binding to mRNA is essential for shuttling of these factors [54]. More recently, the U2 snRNP auxiliary factor (U2AF) was also shown to shuttle between nucleus and cytoplasm [55]. In contrast to SR proteins, U2AF binds to intronic sequences in the pre-mRNA and dissociates from the spliceosome during the splicing reaction. Thus, it appears unlikely that U2AF accompanies the mRNA to the cytoplasm. Supporting this view, shuttling of U2AF is independent of mRNA binding and continues in the absence of mRNA traffic [55].

Several possibilities have been envisaged to explain the shuttling of splicing factors. In the case of SR proteins, the association with spliced mRNPs could serve as a marker or guide to the transport machinery. Indeed, two shuttling SR proteins (SRp20 and 9G8) have recently been suggested to act as adaptors for the nuclear export of spliced cellular mRNAs [56]. Alternatively, SR proteins could remain bound to the mRNAs in the cytoplasm and play a role as regulators of mRNA stability or translation. In the case of U2AF, which exits the nucleus independently of mRNA, the possibility remains that this factor plays an as yet undetermined role in the cytoplasm. Most important, shuttling of both SR proteins and U2AF could provide a mechanism to control nuclear availability of splicing factors in response to external signals, thus allowing for a rapid tuning of splicing activity. Indeed, alternative splice decisions can be determined by changing the relative concentration of proteins that play antagonistic roles in the selection of splice sites [57]. A potential role of shuttling in regulation of alternative splicing is supported by the recent observation that cytoplasmic accumulation of the shuttling protein hnRNPA1 occurs in response to cellular stress and correlates with changes in alternative splicing [58].

6. Perspectives

Nucleocytoplasmic shuttling of proteins plays a major role in controlling gene expression. Although it was initially thought that regulatory factors are held captive in either the nucleus or the cytoplasm, until an appropriate signal triggers transport to the other side of the nuclear envelope, several lines of recent evidence indicate that most of these proteins are in fact constantly moving in and out of the nucleus. However, at steady state they accumulate predominantly in the nucleus or in the cytoplasm, depending on the balance between import and export rates. Compared to the model of retention versus transport, continuous shuttling may allow for a tighter regulation of protein activity. First, interaction of the shuttling protein with transport receptors most likely generates inactive complexes, preventing action in the absence of appropriate signals. Second, the existence of a permanent pool of the protein in both compartments favors a more rapid association to target molecules upon signal induction, without the delay associated with translocation across the nuclear envelope. Interfering with nucleocytoplasmic shuttling opens a

novel and exciting window to manipulate cellular commitments, and repercussions in cancer therapy are expected to follow the recent discovery that failure in import/export pathways is on the basis of tumor development.

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