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A conserved family of nuclear export receptors mediates the exit of messenger RNA to the cytoplasm

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Abstract. The distinguishing feature of eukaryotic cells is the segregation of RNA biogenesis and DNA replication in the nucleus, separate from the cytoplasmic machinery for protein synthesis. As a consequence, messenger RNAs (mRNAs) and all cytoplasmic RNAs from nuclear origin need to be transported from their site of synthesis in the nucleus to their final cytoplasmic destination. Nuclear export occurs through nuclear pore complexes (NPCs) and is mediated by saturable transport receptors, which shuttle between the nucleus and cytoplasm. The past years have seen great progress in the characterization of the mRNA export pathway and the identification of proteins involved in this process. A novel family of nuclear export receptors (the NXF family), distinct from the well-characterized family of importin β-like proteins, has been implicated in the export of mRNA to the cytoplasm.

Key words. CTE; nuclear export; NXF; NXT; p15; TAP.

Introduction

Macromolecular traffic between the nucleus and cytoplasm occurs through NPCs, large proteinaceous structures that form aqueous channels across the nuclear envelope [1, 2]. NPCs are composed of multiple copies of up to ~30–50 distinct polypeptides called nucleoporins. Nucleoporins are characterized by the presence of protein/protein interaction domains and often contain clusters of multiple phenylalanine-glycine (FG)-dipeptide repeats [1–3]. It has been proposed that the FG-repeats of nucleoporins provide docking sites during translocation of macromolecules through the central channel of the pore [1–3].

Nuclear transport is mediated by saturable transport receptors that shuttle between the nucleus and the cytoplasm [4–6]. Transport receptors recognize and bind nuclear localization signals (NLSs) or nuclear export signals (NESs) in the transported molecules. Following binding, transport receptors dock their cargoes to the NPC and facilitate their translocation through the pore. After delivering their cargoes, the receptors are recycled to initiate additional rounds of transport.

The vast majority of known transport receptors are members of a large family of RanGTP-binding proteins exhibiting limited sequence similarity with the Ran binding domain of importin-β, and have been termed importins/exportins or karyopherins. Binding of Ran to these receptors regulates their interactions with their cargoes (or substrates) and with nucleoporins [4–6]. During export, the binding of RanGTP to the receptor is required for interaction of the receptor with its cargo and probably for the binding of the receptor to the pore. The opposite situation exists during import: free nuclear RanGTP is thought to trigger the release of the cargo on the nucleoplasmic side of the pore after translocation [4–6].

Messenger RNAs (mRNAs) exist in the cell in dynamic association with multiple distinct proteins and are then exported from the nucleus as large ribonucleoprotein complexes (mRNPs). The most striking example of this

principle are the Balbiani Ring (BR) mRNPs of *Chironomus tentans* which are composed of an RNA molecule of 35–40 kb in length associated with about 500 protein molecules [7, 8]. Among the most abundant RNA-binding proteins associated to heterogeneous (in size) nuclear mRNPs are the hnRNP proteins. There are about 20 different hnRNP proteins in higher eukaryotes (hnRNP A to hnRNP U) [9].

Nuclear export of cellular mRNPs is highly selective, because only fully processed mRNAs are normally exported. Incompletely spliced precursor mRNAs (pre-mRNAs) and excised introns are retained within the nucleus. Studies in yeast and in vertebrate cells suggest that nuclear retention of pre-mRNAs occurs by virtue of the association with spliceosomal factors [10–12]. Electron microscopy studies on the export of BR mRNPs have shown that several proteins associated with the mRNP in the nucleoplasm are selectively removed before or during translocation across the NPC [8]. Together, this implies that the mRNP export pathway may include steps at which specific retention factors are removed, by an as yet unknown mechanism.

Proteins implicated in the export of cellular mRNPs are distinct from the nuclear transport receptors of the importin β-like family and include several nucleoporins and RNA-binding proteins. In addition, the RNA helicase Dbp5 and the NPC-associated proteins RAE1/Gle2p and TAP/Mex67p [4–6] play a role in this process. RAE1/Gle2p and Mex67p were originally identified in yeast, in searches for mutants that accumulate mRNAs within the nucleus or in screens for genetic interactions with nucleoporins. Although these proteins do not exhibit sequence similarities, they have both been implicated in mRNA export. Mex67p is essential for mRNA export in *Saccharomyces cerevisiae,* whereas RAE1 (also known as Gle2p) is essential for mRNA export in *Schizosaccharomyces pombe* [13–15]. The vertebrate homologues of Mex67p and RAE1/Gle2p, TAP and hRAE1, respectively, have also been implicated in the export of cellular mRNA [16–20].

Viral subversion of mRNA nuclear export factors

Viruses have been an important model system for the definition and functional dissection of the various nuclear export pathways encoded by their host cells. In particular, retroviruses have evolved mechanisms to promote the efficient export of unspliced forms of viral RNA [21]. This is essential for viral replication, as the full-length, unspliced viral transcript represents the genomic RNA that will be assembled into progeny virions in the cytoplasm of the host cell. In complex retroviruses such as HIV-1 (human immunodeficiency virus-type 1), export of unspliced viral RNAs occurs by the interaction of the viral protein Rev with an RNA sequence present in the viral transcripts, the Rev responsive element (RRE). Rev binds to the RRE through an RNA binding domain. A second domain called the Rev activation domain functions as a NES recognized by CRM1 (also known as exportin-1), a member of the importin β-like family [4–6, 22].

Simple retroviruses do not encode a Rev-like trans-acting protein, and export of their unspliced RNA relies on the interaction of cis-acting RNA elements with cellular factors. In simian type D retroviruses (SRV-1 and -2, simian retroviruses 1 and 2; and MPMV, Mazon Pfizer monkey virus), this element is referred to as CTE, the constitutive transport element, because its interaction with cellular factors results in constitutive export of unspliced viral RNA [23, 24]. In 1998 we identified TAP as the cellular factor recruited by the CTE of simian type D retroviruses to promote nuclear export of their genomic RNA [25]. In *Xenopus laevis* oocytes, titration of TAP with an excess of CTE RNA prevents cellular mRNAs from leaving the nucleus [25–27]. This and the observation that TAP is the vertebrate homologue of Mex67p suggested that TAP could be an essential mediator of cellular mRNA nuclear export in higher eukaryotes.

TAP: a non-β-like nuclear export receptor for mRNA

Investigation of the mechanism by which TAP mediates the nuclear export of cellular mRNA and of CTE-bearing RNAs led us to the hypothesis that TAP may function as an mRNA nuclear export receptor distinct from the importin β-like family of Ran-binding proteins [28]. Indeed, as mentioned above, nuclear transport receptors are shuttling molecules that bridge the interaction between transport cargoes and the NPC. Therefore, transport receptors are expected to interact directly with components of the NPC, to recognize and bind (directly or indirectly) their cargoes, and to shuttle between nucleus and cytoplasm. Several lines of evidence suggest that TAP fulfils these functions (see below). However, further studies are required to understand fully the mechanism of TAP action during the export process.

TAP belongs to a multigene family of putative RNA export factors with a conserved modular architecture

Recently, we have shown that TAP and its yeast orthologue Mex67p belong to an evolutionarily conserved family of proteins having more than one member in higher eukaryotes [18]. There are two TAP homologues in *Caenorhabditis elegans* (Ce), four in *Drosophila melanogaster* (Dm) and six putative homologues in human (Hs); these proteins were termed NXFs (nuclear export factors) [18]. Phylogenetic analysis of the protein sequences indicate that the multiplication of *nxf* genes has occurred independently in different lineages [18].

TAP has a modular domain organization consisting of a noncanonical RNP-type RNA-binding domain (RBD), four leucine-rich repeats (LRRs), a middle domain showing significant sequence similarity to nuclear transport factor 2 (the NTF2-like domain) and a C-terminal ubiquitin associated (UBA)-like domain (fig. 1) [18, 29, 30]. The crystal structure of the RBD and the LRRs of TAP have been determined at 3.15 and 2.90 Å resolution [30]. The predicted folding of the NTF2-like domain and of the UBA domain of TAP is based on the known three-dimensional structures of NTF2 and UBA domains [29].

Comparison of the amino acid sequences of the NXF proteins in all species including yeast indicates that the overall domain organization of the protein family has been evolutionarily conserved (fig. 2), although not all domains are always present. The RBD is conserved in most NXF proteins but may be absent in yeast Mex67p. The LRRs and the NTF2-like domain are present in all members of the family, with the exception of Dm NXF4, which lacks the NTF2-like domain. The UBA domain is absent in Dm NXF3, Dm NXF4, Ce NXF2 and Hs NXF3 and NXF5 (fig. 2) [18].

Direct evidence for a role of NXF proteins in mRNP export has been obtained for *S. cerevisiae* and *Schiz pombe* Mex67p [13, 31], for the *Caen. elegans* and *Drosophila* NXF1 [32 and unpublished] and for human TAP [18–20]. Furthermore, vertebrate TAP has been directly implicated in the export of simian type D retroviral RNAs bearing the CTE [25]. The observation that at least five members of the NXF family are implicated in mRNA export together with the conservation of their structural organization suggest that NXFs are likely to participate in the export of cellular mRNA to the cytoplasm, although a different function from export cannot currently be excluded for some of these proteins. The diversification of NXF proteins in higher eukaryotes as compared with yeast may reflect a greater substrate complexity or, alternatively, tissue-specific requirements. Consistent with this latter possibility is the observation that human NXF5 is mainly expressed in brain, and interruption of the *nxf5* gene by a chromosomal inversion has been associated with one case of male mental retardation [33].

Binding of TAP to the CTE RNA

The CTE folds into an extended RNA stem-loop structure [23, 24], comprising two conserved internal loops, A and B, and an AAGA bulge adjacent to loop A. The loops are arranged in mirror symmetry on the RNA element (fig. 3, frame). The internal loops represent duplicated binding sites for TAP. Indeed, one single loop is sufficient for TAP binding in vivo and in vitro [25, 35], although (depending on the context in which the CTE is inserted), one single loop is not sufficient to promote CTE-dependent export [23–25, 28, 34, 35].

The minimal CTE binding domain of TAP includes the RBD and the LRRs [30, 34, 35]. The RBD functions as the general RNA-binding portion of the fragment, whereas the LRR domain is required in cis to the RBD domain for specific binding to the CTE RNA [30]. The two independent domains have similar structural and biochemical properties to the spliceosomal proteins U2B″ and U2A′, which bind cooperatively to the U2 snRNA small nuclear RNA. U2B″ contains the RBD but it is not able to specifically recognize the U2 snRNA. The U2A′ protein consist of five tandem leucine rich repeats, and is required in trans to U2B″ for specific binding to U2 snRNA. Although, the RBD and LRR domain of TAP bind cooperatively to the CTE RNA and show structural homology with the spliceosomal proteins U2B″ and U2A′, the mode of macromolecular recognition of TAP with the CTE RNA, and the spliceosomal U2B″-U2A′ complex with the U2 snRNA is not conserved [30].

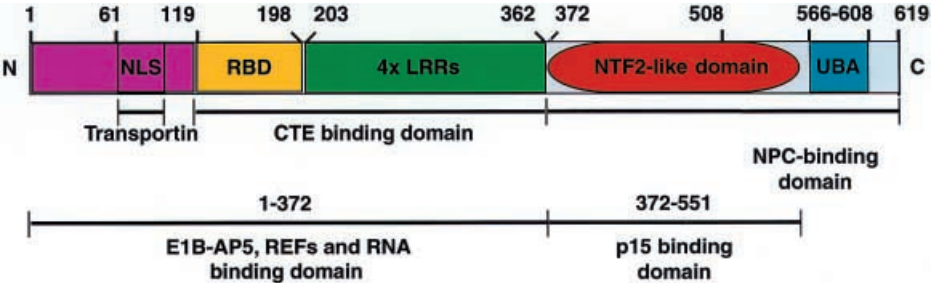

Figure 1. Domain organization of human TAP protein. TAP domains defined by our studies are indicated [18, 28–30]. The N-terminal domain consist of an RNP-type RNA binding domain (RBD, yellow), four leucine-rich repeats (4xLRRs, green) and a less conserved region upstream of the RBD (purple). This N-terminal domain includes the minimal CTE-binding fragment (residues 102–372) and exhibits general RNA binding affinity. This domain also binds to several mRNP-associated proteins such as E1B-AP5 and REF/Aly [28, 36] and bears TAP's NLS, which is recognised by transportin [28]. The NTF2-like domain of TAP (red) interacts with p15. The C-terminal domain of TAP comprises a UBA-like domain (cyan), and interacts with the FG-repeat domain of multiple nucleoporins [28]. Numbers indicate the position in the amino acid sequence.

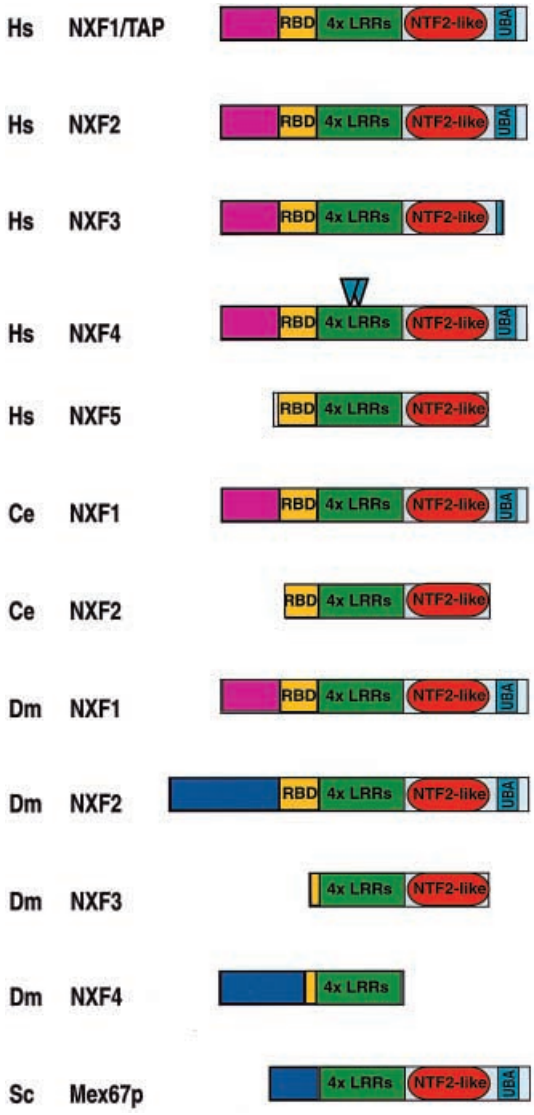

Figure 2. Domain organization of NXF proteins. Domains are colored as follows: purple, less conserved N-terminal portion found only in human homologues, Ce NXF1 and Dm NXF1; yellow, RBD domain; green, leucine-rich repeats; red, NTF2-like domain; gray, linkers upstream and downstream of NTF2-like domain; cyan, UBA-like domain. Protein sequences having no similarity to human TAP or to any other sequence in the database are colored in dark blue. The cyan triangles above the LRRs of NXF4 represent frameshifts; thus, NXF4 may be a pseudogene. Abbreviations: Hs, human; Ce, *C. elegans*; Dm, *D. melanogaster*; Sc, *S. cerevisiae*.

mRNP-associated proteins may act as adaptors between TAP and cellular mRNA

The mode of interaction of TAP with cellular mRNPs remains to be established, but is certainly different from that with the CTE RNA for the following reasons. First, most cellular mRNAs do not contain sequences similar to those present in the CTE. Second, TAP affinity for mRNAs (i.e. DHFR mRNA) in vitro is approximately three orders of magnitude lower than its affinity for the CTE RNA [34]. Third, while an excess of CTE RNA saturates export of mRNAs, an excess of mRNA does not interfere with CTE export [26, 27]. Based on these observations, we proposed that the CTE bypasses several steps in the mRNA export pathway and interacts directly with TAP, whereas cellular mRNAs may recruit TAP via protein-protein interactions [20, 25, 26, 34]. In agreement with this hypothesis we have found that TAP interacts in vitro with several mRNP-associated proteins, including E1B-AP5 and RAE1 [28], and members of an evolutionary conserved family of hnRNP-like proteins, the REF proteins (fig. 3) [36, 37]. Apart from these, other RNA-binding proteins may act as adaptors between TAP and cellular mRNPs. In particular, the splicing coactivator SRm160, the acute myeloid leukemia-associated protein DEK, RNPS1 and Y14, together with REFs, are components of a 335-kDa protein complex deposited by the spliceosome 20–24 nucleotides upstream of a splice junction [38] (fig. 3). These proteins, either individually or as a complex, bind mRNA in a splicing-dependent, but sequence-independent way, and it has been suggested that they facilitate the recruitment of TAP to mRNA after splicing [38–42]. This implies that splicing guarantees the recruitment of TAP partners, and hence of TAP, in a sequence-independent manner and provides a rationale for the observation that introns facilitate the export of some specific mRNAs [43 and refs therein].

Binding of TAP to E1B-AP5 and REFs is mediated by its N-terminal domain (residues 1–372, fig. 1) [28, 36]. Sequences upstream of the RBD are implicated in these interactions, whereas the LRRs are not strictly required [28, 36 and unpublished]. Interestingly, these sequences are the least conserved among the NXF proteins [18]. Thus, these sequences may confer different binding specificities to the different members of the NXF family. For instance, human NXF2 and NXF3 both interact with E1B-AP5, but only NXF2 binds REF proteins [18].

The NTF2-like domain of metazoan NXF proteins heterodimerizes with p15

The NTF2-like domain of metazoan NXFs mediates binding to a protein known as p15 or NXT, which is also related to NTF2 [19, 29, 44], but whereas NTF2 forms homodimers, p15 heterodimerizes with the NTF2-like domain of NXF proteins [29]. There is only one *p15* gene in *D. melanogaster* and *Caen. elegans* and two in available human genomic sequences (*p15-1* and *p15-2*) [18], suggesting that one p15 protein may heterodimerize with various NXF proteins. Indeed, we have shown that both human p15 proteins bind TAP, NXF2, NXF3 and NXF5 [18, 33]. The NTF2-like domain also occurs in *Schiz. pombe* and *S. cerevisiae* Mex67p, although there is no obvious p15 homologue encoded by the yeast genome [29,

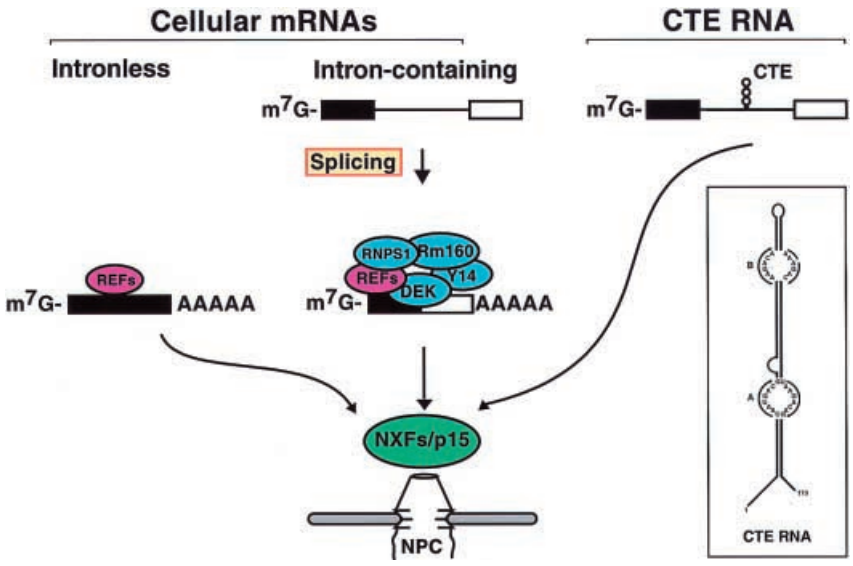

Figure 3. The mRNA export pathway. The proteins REFs (Aly/Yra1p), SRm160, DEK, Y14 and RNPS1 preferentially associate with spliced mRNAs, and may facilitate the recruitment of TAP/p15 heterodimers to mRNP complexes [38–42]. Note that only REFs have been shown to be involved in mRNP export [36, 37, 42, 50]. REFs also facilitate the export of intronless mRNAs [50]. The CTE bypasses several steps of the mRNA export pathway and interacts directly with TAP. RNA-binding proteins may be recycled back to the nucleus or may remain associated to the mRNP in the cytoplasm.

44]. In *S. cerevisiae* Mex67p, this domain is implicated in the interaction with a protein known as Mtr2p [45]. Prediction of Mtr2p secondary structure and the observation that coexpression of human TAP and p15 in *S. cerevisiae* partially restores growth of the otherwise lethal *mex67/mtr2* double knockout suggest that Mtr2p might be a p15 functional analogue [19, 29].

The UBA-like domain of NXF proteins is critical for their interaction with nucleoporins

In HeLa cells a fraction of TAP colocalizes with NPC markers at the nuclear envelope [19, 28, 46]. Using immunoelectron microscopy, we have localized TAP to both the cytoplasmic and nucleoplasmic faces of the NPC, suggesting that TAP interacts in vivo with multiple components of the pore [28]. Consistently, TAP binds to the FG-repeat domains of multiple nucleoporins in vitro [19, 28]. Binding of TAP to nucleoporin-FG-repeat domains is direct and mediated by its C-terminal NPC binding domain (fig. 1) [19, 28, 46]. This domain is necessary and sufficient for the localization of TAP to the nuclear rim [28, 46]. Furthermore, this domain is a potent inhibitor of multiple nuclear export pathways indicating that, in vivo, it competes with other transport mediators for binding sites at the NPC [28].

The NPC binding domain of NXF proteins comprises the UBA-like domain and part of the NTF2-like domain, but p15 binding by TAP is not necessary for nucleoporin interaction [28]. The UBA-like domain on its own (fragment 567–619) is not sufficient to localize TAP at the nuclear rim in vivo, but single amino acid changes in a conserved loop of this domain (NWD at positions 593–595 in human TAP) severely impair binding of TAP, Hs NXF2 and Ce NXF1 to nucleoporins in vitro and in vivo [18, 29, 32]. Conversely, Ce NXF2 and human NXF3 and NXF5, which lack the UBA-like domain, do not localize to the nuclear rim [18, 32, 33]. This suggests that high-affinity binding to nucleoporins requires both the UBA-like domain and at least part of the NTF2-like domain. The UBA-like domain is conserved in yeast Mex67p [29], but only the *S. cerevisiae* protein has been shown to interact directly with nucleoporins [47]. As shown for the metazoan proteins, *S. cerevisiae* Mex67p lacking the UBA-like domain no longer localizes to the nuclear envelope [45], indicating that the mode of interaction of yeast and metazoan NXF proteins with nucleoporins has been conserved.

TAP shuttles independently of mRNA traffic

TAP is a shuttling protein (fig. 4) [46]. As with other shuttling RNA binding proteins such as hnRNP A1 [48], poly(A)-binding protein II [49], and REFs [50], TAP shuttling was observed in the presence of the transcription inhibitor actinomycin D, indicating that TAP shuttling is independent of mRNA trafficking [unpublished]. One explanation for the shuttling of the RNA-free proteins is that binding to RNA may be required for their efficient release from import receptors in the nu-

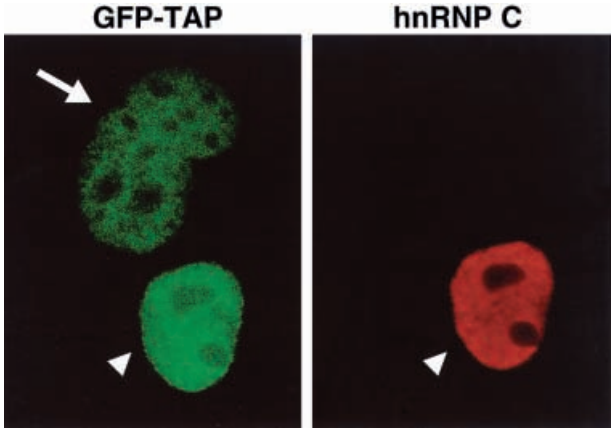

Figure 4. TAP is a shuttling protein. The shuttling of human TAP was monitored in human-mouse heterokaryons produced by polyethylene glycol-induced fusion of HeLa and mouse 3T3 cells. HeLa cells expressing GFP-TAP were cocultured with 3T3 cells and treated for 3 h with 20 μg/ml of the translational inhibitor emetine. After PEG-induced fusion, the resulting heterokaryons were further incubated in medium containing the same inhibitor for 1 h. The resulting heterokaryons were double-labeled with antibodies recognizing only human hnRNP C. hnRNP C does not shuttle and is retained in the human nucleus (arrowheads), whereas GFP-TAP is transported from the human into the mouse nucleus (arrow) (courtesy of Maria-Carmo Fonseca).

cleus. In the absence of RNA binding, these proteins may not dissociate efficiently from their import receptor and may engage in futile import/export cycles. This may provide a mechanism for regulating the levels and/or timing of the availability of RNA-binding proteins and of RNA-export factors in the nucleus [51].

Role of TAP domains in mRNA nuclear export

The role of the individual TAP domains in the export of cellular mRNA has been analyzed in cultured cells and in *Xenopus* oocytes [20]. In both systems, only the full-length protein efficiently stimulated export of a variety of mRNA export cargoes. The RBD was dispensable for mRNA export stimulation by TAP, whereas the LRRs and the NTF2-like domain were essential [20]. The first 60 amino-terminal residues of TAP contributed substantially to, but were not strictly required for, TAP-mediated export of cellular mRNA. Similarly, the NPC-binding domain of TAP contributed substantially to its export function, although a low but significant export activity was measured when this domain was deleted. Indeed, in the presence of p15, a mutant form of TAP lacking the NPC binding domain exhibited about 10% of the export activity of wild-type TAP. In *S. cerevisiae*, deletion of the UBA-like domain of Mex67p resulted in a thermosensitive growth phenotype and accumulation of polyadenylated RNAs within the nucleus, indicating that the UBA domain of Mex67p is also required, but not essential, for efficient mRNA nuclear export [45]. Overexpression of Mtr2p compensated for the lack of the UBA domain and restored growth but not the nuclear envelope localization of the protein [45]. Similarly, as mentioned above, NXF proteins lacking the UBA-like domain do not localize at the nuclear rim when coexpressed with p15 [18 and unpublished]. Thus, in vivo Mex67p and TAP lacking the NPC-binding domain have a residual export activity in the presence of Mtr2p or p15 and may still be able to interact transiently with nucleoporins, although at equilibrium they are no longer localized at the nuclear rim. The NTF2-like domain might mediate transient binding to nucleoporins when the UBA domain is not present [20, 45].

Distinct requirements for TAP-mediated export of cellular and viral mRNA

The requirement of TAP domains for TAP-mediated export of cellular mRNA or of CTE-bearing RNAs is different. The RBD is dispensable for TAP-mediated export of mRNA but essential for specific binding to the CTE RNA and therefore for TAP-mediated export of CTE-containing substrates [18, 30, 34]. Conversely, the first 60 amino acids of TAP have an important role in mRNA export stimulation, but are not required for TAP-mediated export of CTE-bearing substrates [20, 34, 35].

The requirements of the NTF2-like and the UBA domains for CTE export depend on the RNA molecule on which the CTE has been inserted. In *Xenopus* oocytes TAP-mediated export of CTE-bearing intron lariats is independent of these domains [34], whereas export of a chimeric RNA in which U6 sequences have been fused to the CTE requires the UBA domain but not the NTF2-like domain [28]. The U6 small nuclear RNA is not exported from the nucleus unless it is fused to the CTE. In quail cells, TAP-mediated export of an inefficiently spliced pre-mRNA carrying the CTE in the intron is abolished by mutations or deletions of the UBA domain [18, 53] but is only reduced by mutations preventing p15 binding [53].

Only the LRRs are essential for export of both cellular mRNA and CTE-bearing RNAs, but their role in these processes is different. Indeed, by introducing mutations in phylogenetically conserved residues exposed on the surface of the LRRs, we showed that some mutations impaired mRNA export stimulation by TAP but not CTE-dependent export [20]. Thus, it is likely that the LRR domain of TAP binds one or more unidentified cellular ligands that are bypassed by the CTE, but it is essential for export of cellular mRNA. This provides further support to the hypothesis that the mode of interaction of TAP with cellular mRNA is different from that with the CTE RNA and that the CTE subverts TAP from its normal cellular function [20, 28, 34].

Perspectives

Several lines of evidence indicate that metazoan TAP (NXF1) and *S. cerevisiae* Mex67p exhibit the expected properties of an mRNA nuclear export receptor. These proteins shuttle between the nucleus and cytoplasm and associate with cellular mRNPs either directly by binding to the mRNA or indirectly, through the interaction with hnRNP-like proteins. Following binding, these proteins can bridge the interaction between the mRNP export cargoes and the NPC through their NPC binding domains. Moreover, TAP competes with the importin β-like family of transport receptors for binding sites at the NPC, suggesting that the interactions between NXFs and the FG-repeat domains of nucleoporins are analogous to those described between nucleoporins and the transport receptors of the importin β-like family. Together these data suggest that TAP and members of the NXF family represent a novel class of nuclear export receptors mediating the export of mRNAs.

A number of issues, however, remain unanswered. The mechanism by which NXF proteins mediate directional transport of mRNP cargoes across the NPC is unknown. After translocation through the NPC, export receptors are dissociated from their cargoes and are recycled back to the nucleus to initiate additional rounds of export. Human TAP is imported into the nucleus by transportin [28], and the nuclear uptake of other NXF proteins is likely to be mediated by members of the importin β-like family. How NXF proteins release their cargoes before being reimported into the nucleus is unknown. It has been proposed that Dbp5, a DEAD-box RNA helicase essential for mRNA export, plays an important role in disrupting mRNA/protein interactions as the mRNP emerges from the NPC [54–56], but direct evidence is missing. Also, how the interactions between NXF proteins and the NPC are regulated remains to be elucidated. Other unclear aspects of mRNP export are the mechanism of nuclear retention and selective removal of retention factors prior to NPC translocation. Finally, the mechanism for movement of large mRNPs through the central channel of the pore remains a challenge for the foreseeable future.

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