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REVIEW

The Molecular Mechanisms of Messenger RNA Nuclear Export

Mikiko C. Siomi

Institute for Genome Research, University of Tokushima, 3-18-15 Kuramoto, Tokushima 770-8503, Japan

ABSTRACT. **In eukaryotic cells, the nuclear membrane creates a barrier between the nucleus and the cytoplasm. Whereas RNA synthesis occurs in the nucleus, they mostly function in the cytoplasm; thus export of RNA molecules from the nucleus to the cytoplasm is indispensable for normal function of the cells. The molecular mechanisms involved in each kind of cellular RNA export is gradually understood. The focus of this review will be mRNA export. mRNAs are multiformed. In order to ensure that this variety of mRNA molecules are all exported, cells are probably equipped with multiple export pathways. A number of proteins is predicted to be involved in mRNA export. Ascertaining which proteins play crucial roles in the pathways is the key point in the study of mRNA export.**

Key words: nuclear transport/RNA export/WRNA/RNA-binding protein soluble nuclear export factors/RNA processing

In eukaryotic cells, genomes, the templates for general transcription, are sequestered within the nucleus and this compartment is the only place in cells devoted to the synthesis of RNA molecules. After transcription, the RNA molecules undergo a variety of posttranscriptional processing steps. The majority of RNAs including transfer RNA, rinsbosomal RNA, small nuclear RNA and messenger RNA, are exported to the cytoplasm. Nuclear transport occurs through nuclear pore complexes (NPCs) located across the nuclear envelope. A nuclear pore complex is an assembly of up to one hundred different proteins termed nucleoporins (Nups)(Ohno, 1998; Yang, 1998; Stoffler, 1999). While relatively small molecules such as ions and proteins smaller than 40 kDa can diffuse through the nuclear pores, macromolecules including RNAs traverse the NPC by an active, temperature-dependent manner.

Active transport both in and out of the nucleus requires soluble nuclear transport receptors, which first bind specific sequences (nuclear import or export signals) within the proteins to be transported (cargos) and then transport them in concert with Nups (for reviews see Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998; Nakielny and Dreyfuss, 1999). Directionality of nuclear transport is provided by a small GTPase Ran (Dahlberg and Lund, 1998). In the nucleus Ran exists mostly as the GTP-bound form and causes dissociation of cargos from the nuclear import factors upon translocation across the nuclear membrane (Görlich *et al*., 1996; Rexach and Blobel, 1995). In the case of nuclear export, however, RanGTP is required in efficient binding of nuclear export factors to the molecules to be exported (Arts *et al*., 1998a; Fornerod *et al*., 1997; Kutay *et al*., 1997). Cytoplasmically localized RanGAP and RanBP1 facilitate hydrolysis of Ran-bound GTP to GDP. The exchange of RanGTP to RanGDP causes dissociation of the exported cargos from the nuclear export factors upon translocation to the cytoplasm (Görlich, 1998; Kehlenbach *et al*., 1999; Kutay *et al*., 1997). Whether hydrolysis of RanGTP is the energy source for the nuclear transport is still questionable (Englmeier *et al*., 1999; Izaurralde *et al*., 1997b; Kose *et al*., 1997; Nakielny and Dreyfuss, 1998; Ribbeck *et al*., 1999; Richards *et al*., 1997; Schwoeble *et al*., 1998) and needs to be investigated further.

Among all the nuclear import factors so far identified, the best known is importin β (Görlich *et al*., 1995). This protein associates with the cargos through a 60 kDa protein termed importin α, which directly recognizes the nuclear import signals within the cargos. The majority of the nuclear transport factors show some but significant similarities to importin β and they are known as members of the importin β family (Nakielny and Dreyfuss, 1999 and references therein). For the active nuclear import, a large number of factors have been identified and well characterized and it has also been revealed that some import factors do not belong to the importin β family (Ribbeck *et al*., 1998; Smith *et al*., 1998). In contrast to the nuclear import, studies in the nuclear export, especially of RNA molecules, seem to be somewhat behind. Even so, the molecular mechanisms of U snRNA and tRNA export have been becoming clear as results of the recent in-

Institute for Genome Research, University of Tokushima, 3-18-15 Kuramoto, Tokushima 770-8503, Japan.
Tel: +81–88–633–9456, Fax: +81–88–633–9451
E-mail: siomim@genome.tokushima-u.ac.jp

tensive investigation (for reviews see Cullen, 2000; Strasser and Hurt, 2000). The factors identified for U snRNA and tRNA export are exportin 1/CRM1 (Fornerod *et al*., 1997) and exportin-t (Arts *et al*., 1998a; Kutay *et al*., 1998; Sarkar and Hopper, 1998), respectively. Although both belong to the importin β family, their binding styles to the cargos differ from each other. While exportin-t can bind tRNA directly (Arts *et al*., 1998b; Lipowsky *et al*., 1999; Lund and Dahlberg, 1998), exportin 1 associates with U snRNA only through particular proteins, cap-binding complex (CBC) and a novel protein termed PHAX (Izaurralde *et al*., 1995; Ohno *et al*., 2000).

Despite intensive investigation, the molecular mechanisms of mRNA export have not become completely clear yet. However, recent studies on mRNA export revealed a number of RNA-binding proteins which fall under the category of trans-acting mRNA export factors. Interestingly some proteins among them act as importin β does; namely showing the ability to bind both the cargos and NPCs and to directly mediate mRNA export, although they certainly do not belong to the importin β family. In this review the soluble factors which play critical roles in mRNA export, mainly in the process occurring within the nucleus of vertebrate cells, will be focused and described.

RNA processing and mRNA export

In eukaryotic cells, RNA synthesis is mostly carried out by RNA polymerase I, II, or III. RNA molecules transcribed by RNA polymerase II can be divided into two groups; snRNA (small nuclear RNA) and the major products of the enzyme, hnRNA (heterogeneous nuclear RNA). The precursors of actually translatable mRNAs, pre-mRNAs, belong to the latter group. Upon transcription, various kinds of RNA-binding proteins interact with pre-mRNAs and a variety of posttranscriptional processing events take places. Three major processing events are 5' capping, removal of introns, and polyadenylation to the 3' end. After the events, pre-mRNAs become to be matured mRNAs and then are exported from the nucleus to the cytoplasm for the expression of genetic information.

Important links between splicing and export have recently emerged (Kataoka *et al*., 2000; Zhou *et al*., 2000). Apparently, mRNPs that have undergone intron-removal event have a different structure and at least one protein composition than mRNPs containing identical RNA that was not produced by splicing. Some of the proteins associated specifically with spliced mRNPs are mediators of mRNA export and are discussed in more detail below. Suffice to say here, the process of splicing appears to result in the loading of mRNA expor factors. This provides one way to couple splicing and export such that RNA molecules that have not passed through the splicing machinery do not exit the nucleus.

Both the 5' cap structure and the 3' poly (A) tail of mRNAs can influence the rate of mRNA export but neither is essential for the process (Eckner *et al*., 1991; Hamm and Mattaj, 1990; Jarmolowski *et al*., 1994; Sun *et al*., 1992). In the case of U snRNA, however, the same 5' cap structure is known to be necessary for its export. As briefly described above, the cap-binding complex (CBC) comprising CBP80 and CBP20 first binds to the m^7G cap and then a novel 55 kDa protein termed PHAX interacts to the complex, which in turn is recognized by exportin 1 and finally the export complex comes into existence (Ohno *et al*., 2000). It is very interesting that the requirement of an identical structure (i.e. 5' cap) differs in the case of mRNA and U snRNA export (Hamm and Mattaj, 1990; Izaurralde *et al*., 1995; Jarmolowski *et al*., 1994). It has been shown under immunoelectron microscopy that CBP20 at least accompanies mRNAs known as Balbiani ring in *Chironomus tentans* salivary glands while they are exported through the nuclear pores to the cytoplasm (Visa *et al*., 1996b). Therefore, it is not difficult to speculate that the contribution of the 5' cap/CBC complex to the mRNA export could be greater than we know at this date.

hnRNP proteins

A subset of specific proteins which bind nascent pre-mRNAs upon transcription are known as heterogeneous ribonucleoprotein (hnRNP proteins) (Dreyfuss *et al*., 1993). The hnRNP proteins comprise about 20 major proteins and form higher order structures on pre-mRNAs that are refer to as hnRNP complexes (Krecic and Swanson, 1999). It is widely believed that the hnRNPs are the structural entities that serve as substrates for the processing reactions that generate mature mRNAs. Although the subcellular localization shown by immunostaining is predominantly nuclear, more than several hnRNP proteins such as hnRNP A1 continuously shuttle between the nucleus and the cytoplasm (Krecic and Swanson 1999; Pinol-Roma and Dreyfuss, 1991). Other hnRNP proteins are retained in the nucleus once they are imported to the nucleus after translation in the cytoplasm (Nakielny and Dreyfuss, 1996).

The shuttling hnRNP proteins, especially hnRNP A1, are most likely to play key roles in the mRNA export (Fig. 1). The reasons are following; first, hnRNP A1 is a nuclear RNA-binding protein that shuttles between the nucleus and the cytoplasm. Second, hnRNP A1 is shown to bind mRNAs not only in the nucleus but also in the cytoplasm (Pinol-Roma and Dreyfuss, 1992) and at last an A1-like protein in *C. tentans* has been shown under immunoelectron microscopy to translocate through the nuclear pores in association with Balbiani ring (Daneholt, 1999; Visa *et al*., 1996a). It could be thought that hnRNP A1 was just carried (piggy-backed) to the cytoplasm by binding to mRNA molecules, but it became very unlikely since a nuclear export signal (NES) with no detectable RNA-binding activity was identified within the sequence of hnRNP A1 (Michael *et al*., 1995). The NES of hnRNP A1 corresponds to the same re-

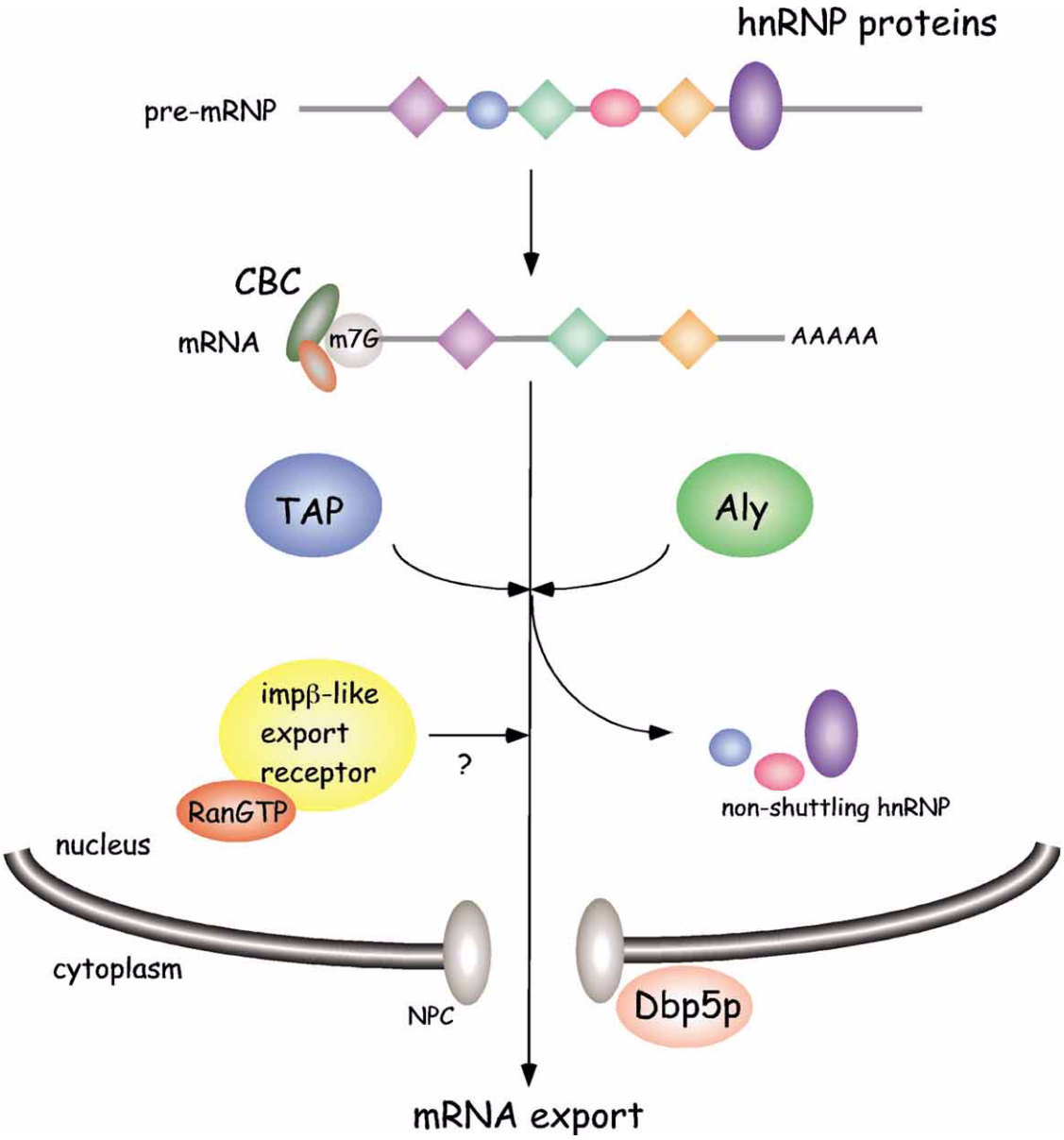
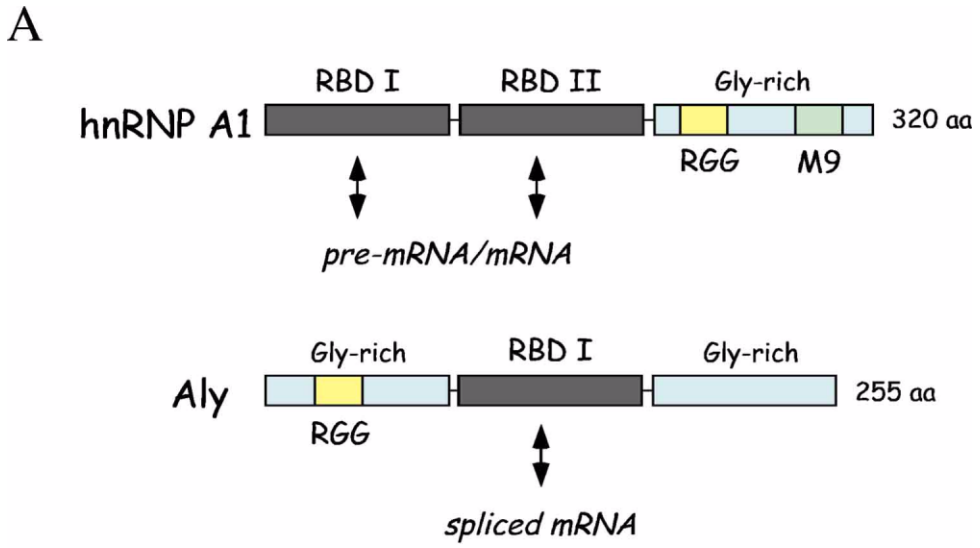


Fig. 1. Various trans-acting proteins for mRNA export in cells. pre-mRNAs transcribed by RNA polymerase II are bound with a number of RNA-binding proteins including hnRNP proteins and hnRNP complexes are formed on the nascent transcripts. After a variety of RNA processings occur, pre-mRNAs become matured mRNAs and some proteins identified recently as trans-acting factors such as TAP and Aly are recruited to the mRNP complexes. Nonshuttling hnRNP proteins which likely function in retaining intron-containing pre-mRNAs in the nucleus are released and as the result the export of mRNP complexes takes place. During translocation through the NPCs, Dbp5p may work on the RNA molecules in the mRNP complexes to unwind them and /or to release from the complexes in the cytoplasm. Whether proteins belonging to importin β family are involved in mRNA export is not known yet. CBC may also be involved in mRNA export as trans-acting factors since CBP20 has been shown to accompany to Balbiani ring transcripts in *C. tentance*.

gion identified as the nuclear import signal (NLS), a 38-amino acid domain termed M9 (Siomi and Dreyfuss, 1995; Weighardt *et al*., 1995). An importin β family member, transportin (TRN), mediates the nuclear import of hnRNP A1 while it binds the M9 region specifically (Pollard *et al*., 1996). As is the case for the import pathway mediated with importin β, hnRNP A1/TRN complexes become dissociated by binding of RanGTP to TRN in the nucleus upon translocation (Izaurralde *et al*., 1997a; Siomi *et al*., 1997). TRN in the nucleus then seems to remain binding to RanGTP untill TRN itself returns to the cytoplasm, meaning that although M9 acts as both the NLS and the NES of A1, TRN is unlikely to be also the export factor of A1. Despite further investigation, the export factor for hnRNP A1 has not been identi-

fied yet.

The other data which supports that hnRNP A1 is one of the mRNA export factors is that nuclear microinjections of A1 in *Xenopus* oocytes inhibit mRNA export (Izaurralde *et al*., 1997a). When A1 lacking M9 was injected instead, though, the inhibition of the mRNA export was not observed. This inhibition was most likely triggered by M9 titrating the endogeneous factors needed for the mRNA export, supporting the idea that M9 is one of the export signals crucial for the mRNA export in living cells. For references,



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Aly      1 MADKMDMSLDDIIKLNRSQRGGRGGGRGRGRAGSQGGRGGAVQAAARVNRGGGPMRNRPA
REF2     1 MADKMDMSLDDIIKLNRNQR------------------------RVNRGGGPRRNRPA
Yra1     1 MSANLDKSLDEIIGSNKAGS--------------------------NRARVGGTRGNGPR
Y14      1 MADVLDLHEAGGEDFAMDED----------------------------GDGSIHKLKEKA

Aly     61 IARGAAGGGRNRPAPYSRPKQLPDKWQHDLFDSGFGGGAGVETGGKLLVSNLDFGVSDAD
REF2    35 IAR----GGRNRPAPYSRPKPLPDKWQHDLFDSGCGGGEGVETGAKLLVSNLDFGVSDAD
Yra1    35 RVG-KQVGSQRRSLPNRRGPIRKNTRAPPNAVARVAKLLDTTREVKVNVEGLPRDIKQDA
Y14     33 KKR---KGRGLGSEEGSR-ARMREDYDSVEQDGDEPGPQRSVEGWILFVTGVHEEATEED

Aly    121 IQELFA-EFGTLKKAAVHYDRS-GRSLGTADVHFERKADALKAMKQYNGVPLDGRPMNIQ
REF2    91 IQELFA-EFGTLKKAAVDYDRS-GRSLGTADVHFERRADALKAMKQYKGVPLDGRPMDIQ
Yra1   102 VREFFASQVGGVQRVLLSYNER-GQSTGMANITFKNGELARRAVERFNGSPIDGGRSRLR
Y14     89 IHDKFA-EYGEIKNIHLNLDRRTGYLKGYTLVEYETYKEAQAAMEGLNGQDLMGQPISVD

Aly    179 LVTSQIDTQRRPAQSINRGGMTRNRGSGGFGGGGTRRGTRGGSRGRGRGTGRNSKQQ---
REF2   149 LVASQIDPQRRPAQSGNRGGMTRSRGSGGFGG-------RG-SQGRGRGTGRNSKQQQ--
Yra1   161 LN-LIVDPNQRPVKSLADRIKAMPQ-KGGNAPRPVK---RGPNRKAAMAKSQNKPKREKP
Y14    148 WC-----FVRGPPK--------------GKGKR------RG-GRRRSRSPDR--------

Aly    236 --LSAEELDAQLDAYNARMDTS
REF2   199 --LSAEELDAQLDAYNARMDTS
Yra1   216 AKKSLEDLDKEMADYFEKK---
Y14        ----------------------
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Fig. 2. (A) Schematic drawing of the structures of hnRNP A1 and Aly. The RBDs and the other regions rich in glycine in both proteins are shown by black and blue boxes, respectively. The RGG box are highlighted in yellow. M9 identified as the NLS and NES of hnRNP A1, to which TRN specifically interacts and mediates the nuclear import of hnRNP A1, is shown by a green box. hnRNP A1 is known to bind RNAs through the RBDs and hence the RBD in Aly is likely responsible for the RNA-binding activity of the protein. (B) The amino acid sequence alignment of Aly, REF2, Yra1p and Y14. The region corresponding to an RBD in Aly is shown with a gray line on the top of the sequence. Amino acid residues in REF2, Yra1p, and Y14 identical or very similar to those in Aly (including residues in Aly) are highlighted in gray.

the yeast homolog of hnRNP A1 is Npl3p. *NPL3* was found in a genetic screen for poly(A)$^+$RNA export and hence it is suggested that Npl3p plays an critical role in mRNA export pathway (Strasser and Hurt, 1999). These data also strongly support the hnRNP A1 function in mRNA export.

Among nonshuttling proteins, the best-characterized is hnRNP C1. A nuclear retention signal (NRS) has been identified within the protein (Nakielny and Dreyfuss, 1996). It has been demonstrated that the NRS can override an NES identified within shuttling hnRNP proteins like M9. The data led a scenario that pre-mRNA associated with both NES-containing and NRS-containing proteins is retained in the nucleus until the NRS-containing proteins are released from the complex. As a matter of fact, hnRNP C1 is known to preferably bind intron-like sequences (for a review see Dreyfuss *et al*., 1996) and some insect hnRNP proteins, likely the functional homologs of nonshuttling human hnRNP proteins, are released from mRNP complexes just before mRNA export (Sun *et al*., 1998). This may be one of the mechanism that ensures only fully matured mRNA molecules are exported.

TAP

Nuclear export of intron-containing mRNA is indispensable for retroviral gene expression and replication. In studies of the molecular mechanisms in type D retroviruses such as the simian retrovirus type1 (SRV-1), the constitutive transport element (CTE) was identified in the viral RNA genome (Bray *et al*., 1994). CTE is a structured RNA element that induces unspliced viral mRNA export in infected cells. Functionally, CTE is very similar to the Rev response element (RRE) identified in the mRNA of human immunodeficiency virus-1 (HIV-1) (Pollard and Malim, 1998). In the case of RRE, the molecular mechanism of how the unspliced RRE-containing mRNA is exported in infected cells has been well characterized and it is clear that a virally encoded RNA-binding protein, Rev, promotes the export while it binds RRE directly (Pollard and Malim, 1998). Rev is known to contain a leu-rich NES, with which the export factor, exportin 1, interacts subsequently and mediates the export of the complex. It has been demonstrated that the RRE in the HIV mRNA can be functionally replaced by the CTE export signal from SRV-1 (Bray *et al*., 1994; Zolotukhin *et al*., 1994). This strongly suggests that a cellular protein binds the CTE specifically and plays a similar role to the viral Rev protein. TAP was identified as such a cellular protein (Braun *et al*., 1999; Gruter *et al*., 1998; Kang and Cullen, 1999). Upon further investigation, TAP is now known to continuously shuttle between the nucleus and the cytoplasm and to directly interact with some nucleoporins (Bachi *et al*., 2000; Kang and Cullen, 1999; Katahira *et al*., 1999). These characteristics of TAP raised an interesting possibility that in contrast to Rev, TAP-mediated unspliced mRNA export does not depend on any importin β family member.

Mex67p is the yeast ortholog of TAP. Mex67p was identified in a genetic screen as a protein essential for the export of poly(A)$^+$RNA in yeast (Segref *et al*., 1997). Like TAP, Mex67p also binds poly(A)$^+$RNA and interacts with NPCs. Involvement of a protein termed Mtr2p seems to be critical for both interactions (Santos-Rosa *et al*., 1998). Although the sequence at the amino acid level is not homologous, p15 was identified in vertebrates as a protein functionally equivalent to Mtr2p (Katahira *et al*., 1999). The combination of TAP and p15 could rescue the viability of yeast cells lacking the function of Mex67p and Mtr2p, suggesting that Tap plays a critical role in cellular mRNA export (Katahira *et al*., 1999). It has also been demonstrated that in *Xenopus* oocytes exogeneously injected TAP could overcome the inhibition of nonviral mRNA export caused by excess amounts of CTE, which in addition supports the idea of TAP involvement to the cellular mRNA export (Fig. 1)(Gruter *et al*., 1998; Pasquinelli *et al*., 1997). In vertebrate cells, however, p15 is demonstrated to be not essential for NPC binding and shuttling of TAP and more curiously for the CTE-containing RNA export mediated by TAP (Bachi *et al*., 2000; Kang and Cullen, 1999). At least, p15 can form a ternary complex with TAP and CTE, suggesting that p15 plays an important but unknown role in the mRNA export. Studies on the structural properties of TAP have revealed that a domain near the carboxyl terminus is the NPC-docking domain and functions as both the NLS and NES of TAP. It is also reported that TAP contains an additional NLS which interacts with TRN, the nuclear import factor originally identified for hnRNP A1 (see above)(Bachi *et al*., 2000; Bear *et al*., 1999; Truant *et al*., 1999). Tap may choose one of the signals to suit the occasion depending on, for example, the concentration of TAP demanded in the nucleus.

Aly and Y14

In order to identify Mex67p-interacting proteins other than Mtr2p, synthetic lethal screening was carried out and a protein termed Yra1p was discovered as Mex67p-binding protein (Strasser and Hurt, 2000). Yra1p was originally reported as a novel nuclear protein with RNA annealing activity in yeast (Portman *et al*., 1997). It has been reported that in mouse there are at least two Yra1p orthologs termed REF1 and REF2 (RNA and export factor binding proteins)(Stutz *et al*., 2000). REF1 is also known as Aly, which was originally reported as a novel context-dependent transcriptional coactivator of two proteins (LEF-1 and AML-1) in the T-cell receptor alpha enhancer complex (Bruhn *et al*., 1997). Recent studies on mRNA export revealed that Aly is recruited to mRNP complexes generated by splicing but does not associate with artificially produced intron-less mRNP (Zhou *et al*., 2000). Aly seems to be recruited during splicing assembly and consistent with this, the protein co-

localizes with splicing factors in the nucleus. Aly also meets another criterion to be a trans-acting protein participating in mRNA export; namely it shuttles between the nucleus and the cytoplasm and more importantly, it specifically stimulates mRNA export in Xenopus oocytes. In conclusion, Aly possibly functions as a regulator to ensure that only spliced mRNAs are efficiently exported and as an intermediary between the two events, splicing and mRNA export (Fig. 1).

Aly (REF1) was originally described as an hnRNP-like protein (Stutz *et al*., 2000), because it is a nuclear RNA-binding protein containing an RNA-binding domain (RBD) often observed in hnRNP proteins (Fig. 2A). However, it was clearly shown that Aly is not likely so, since it is not an H-complex (an assembled complex on pre-mRNA molecules upon transcription) component while all hnRNP proteins are found in the complex (Zhou *et al*., 2000). Structurally, though, hnRNP A1 and Aly show significant homology; i.e. they both contain not only RBD(s) but also an RGG box, the remaining regions of the proteins are rich in glycine, and even the sizes of the proteins are similar to each other. Recently and coincidentally, novel proteins quite homologous to Aly (Fig. 2B), have been reported, one of which is Y14 (Kataoka *et al*., 2000), a protein identified as an interacting protein to RanBP5, an importin β-like import factor (Deane *et al*., 1997; Jakel and Görlich 1998; Rout *et al*., 1997). Y14 shows interesting similarities to Aly; namely Y14 is also a nuclear RNA-binding protein with one RBD, shuttles between the nucleus and the cytoplasm, is localized within nuclear speckles, and finally is associated preferentially with spliced mRNAs. Nevertheless the cellular function of Y14 seems not to completely overlap with that of Aly. Whether all the proteins similar to Aly can be thought of as members of one family or what determines among those proteins to function as mRNA export factor are the questions needed to be addressed in this area. Considering that Aly is only recruited to mRNP complexes generated by splicing but not to artificially produced intron-less mRNP, the protein is likely associated with export machinery through protein-protein interactions. Which region in Aly is responsible for its specific recruitment to the machinery is one of the interesting questions to be answered.

Other potential factors

Several other proteins have been reported that might play important roles in the mRNA export. One example is Rae1 (Brown, 1995; Bharathi, 1997). In *S. pombe*, Rae1 is essential for poly(A)$^+$RNA export and this deficiency can be complemented by the human Rae1. Human Rae1 meets the criteria to be a mRNA export factor; Rae1 shuttles between the nucleus and the cytoplasm and predominantly associates with NPCs (Kraemer and Blobel, 1997; Pritchard, 1999). It has also been demonstrated to associate with poly(A)$^+$ RNA in cells. However, the role played by Rae1 in the mRNA export is not clear yet. Dbp5p is also known as a protein required for the mRNA export (Tseng, 1998; Schmitt, 1999; Snay-Hodge, 1998). Dbp5p contains a DAED box and therefore is likely with RNA helicase activity. Dbp5p is predominantly localized at the cytoplasmic side of the NPCs, although it is known to shuttle between the nucleus and the cytoplasm. In fact, Dbp5p was reported to interact with Yra1p (Schmitt *et al*., 1999), the Aly yeast ortholog, which is known to be localized in the nucleus in yeast cells (Portman *et al*., 1997). Because of the probable RNA helicase activity of Dbp5p, the predicted function of this protein is unwinding of RNA molecules during and/or shortly after the translocation through the nuclear pores or removing proteins from mRNP complexes in the cytoplasm.

Perspective

None of the proteins described in this review as trans-acting mRNA export factors belong to the importin β family of nuclear transport factors. Is any one of the importin β family members in fact necessary for mRNA export (Fig. 1)? As described above, tRNA export is mediated by exportin-t belonging to the importin β family. It has been reported that under the condition where RanGTP was depleted from the nucleus, tRNA export was completely blocked and the block was relieved by supplement of RanGTP (Izaurralde *et al*., 1997b). The results were quite reasonable since exportin-t interacts with tRNA molecules only in the presence of RanGTP. Under the same conditions mRNA molecules behaved similarly, except that the inhibition caused by depletion of RanGTP from the nucleus was partial and a portion of the mRNA molecules were still exported to the cytoplasm. All things considered, in the case of mRNA export it is possible that molecule(s) belonging to the importin β family are also involved and play certain roles to some extent. Compared to tRNA and U snRNA, mRNA represents a much more complex group of cargo molecules. Therefore, it is easy to imagine that mRNAs are exported by multiple mechanisms. The intricacy certainly is the main hindrance to the study of mRNA export. Needless to say, identifying and understanding the factors and mechanisms underlying mRNA export is of great consequence. Emergence of novel RNA export factors is clearly to be expected and this will surely reveal unanticipated aspects of control of gene expression.

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