Cellular and viral mRNA export

Literature: Textbooks Alberts; Lodish; Pollard and Earnshaw

1. Erkmann JA, Kutay U.
Nuclear export of mRNA: from the site of transcription to the cytoplasm. Exp Cell Res. 2004 May 15;296(1):12-20.

2. Sandri-Goldin, RM
Viral Regulation of mRNA Export
Journal of Virology, May 2004, p. 4389-4396, Vol. 78, No. 9

3. Cullen BR.
Nuclear mRNA export: insights from virology.
Trends Biochem Sci. 2003 Aug;28(8):419-24.

4. Stutz F, Izaurralde E.
The interplay of nuclear mRNP assembly, mRNA surveillance and export.
Trends Cell Biol. 2003 Jun;13(6):319-27.

5. Köhler A, Hurt E.
Exporting RNA from the nucleus to the cytoplasm.
Nat Rev Mol Cell Biol. 2007 Oct;8(10):761-73. Review.

6. Katahira J
Nuclear export of messenger RNA
Genes. 2015 (6): 163-184.

1. Introduction

Cellular mRNAs are produced in the nucleus and must be exported to the cytoplasm to allow for their translation into proteins. Nuclear export of mRNAs occurs through nuclear pore complexes (NPCs). Export of mRNAs from the nucleus has turned out to constitute one of the most elaborate nuclear transport pathways. This is in part due to the complex nature of the transport cargo. Nuclear mRNAs exist as large RNA/protein complexes containing the cap binding proteins CBP20 and CBP80, general RNA binding proteins such as heterogeneous nuclear ribonucleoproteins (hnRNPs), splicing factors (for those mRNAs derived from spliced precursors) and other factors involved in pre-mRNA processing. Thus, the transport substrate recognized by the mRNA export machinery is the messenger ribonucleoprotein particle (mRNP) rather than the naked mRNA molecule.

2. mRNA export signals

Most mRNAs are transcribed as pre-mRNAs, which generally undergo three co- or posttranscriptional processing events: 5' capping, i.e. addition of a 7-monomethyl guanosine (m^7G) cap structure to the 5' end of the transcript; removal of introns by splicing; and polyadenylation at a defined site within the 3' untranslated region. But which of these events renders mRNA export competent and what is the nuclear

export signal of mRNAs?

Splicing signals and introns

Pre-mRNA molecules bearing splice sites are largely retained in the nucleus by the spliceosome.

Although splicing is believed to influence export indirectly by a retention mechanism, the export of some mRNAs appears to be directly dependent on splicing. The process of splicing appears to convert the mRNA from an export-incompetent to an export-competent form (see below – exon junction complex EJC).

Cap structure and 3' end

The cap accelerates export but is not absolutely required for mRNA export. Transcripts synthesized in vitro with a trimethylguanosine m 2,2,7 G (m 3G) cap or an adenosine (A) cap were found to be exported from *X. laevis* oocyte nuclei more slowly than an mRNA synthesized with an m7G cap. The m7G cap structure can therefore enhance the rate of mRNA export, but it does not appear to be essential.

The observation that the m 7G cap influences the rate of export of some mRNAs suggests that cap-binding proteins may be involved in export. A nuclear m 7G cap-binding complex (CBC) comprising two cap-binding proteins, CBP80 and CBP20, has been characterized to play a pivotal role in snRNA export by recruiting CRM1 to these RNAs (using PHAX as an adaptor).

Several studies suggest that the 3' poly(A) tail can stimulate mRNA export, but that it is not absolutely required for transport.

3. Export of cellular mRNAs

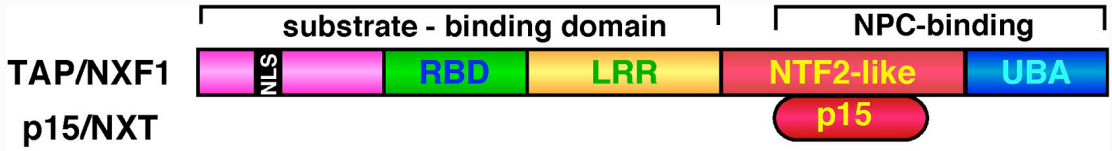
TAP/NXF1/Mex67p

A group of evolutionarily conserved proteins classified as nuclear export factors or NXFs is responsible for exporting the majority of cellular mRNAs and a subset of viral RNAs to the cytoplasm. TAP/NXFs bear no resemblance to prototypical nuclear transport receptors of the importin/ exportin family and lack the characteristic Ran-binding domain found in exportins.

The essential yeast protein Mex67p and human TAP, also called NXF1, are the two best-characterized NXFs. The conditional inactivation of yeast Mex67p leads to a rapid accumulation of poly(A)$^+$ mRNAs in the nucleus demonstrating that Mex67p is essential for mRNA export. A similar analysis using RNAi in *C.elegans* and *Drosophila* has demonstrated that depletion of NXF1 results in a rapid block in bulk poly(A)$^+$ mRNA export in both organisms.

Most NXF family members share the following functional properties: they associate with nuclear pores, have the ability to shuttle and require heterodimerization with p15 for efficient interaction with NPC components.

The human TAP protein can be divided into several functional sub-domains. The N-terminal region of TAP contains an RNA binding domain (RBD), followed by a leucine-rich repeat (LRR) which are needed for TAP-mediated export of viral mRNAs containing the constitutive transport element (see below). Also located in the N-terminal region of TAP is its nuclear localization signal, which is recognized by the import receptor transportin. In the C-terminal portion of TAP resides an NTF2-like domain, which is related to the nuclear transport factor 2 (NTF2), and an ubiquitin-associated (UBA) domain both involved in nucleoporin binding. The NTF2-like domain is required for TAP's heterodimerization with the export cofactor p15 (see below).



Mtr2p/p15

In yeast, Mex67p recruitment to nuclear pores requires the protein Mtr2p. The orthologous protein in humans, p15 (NXT1), is also essential for TAP association with nucleoporins and recruitment to NPCs *in vivo*. Although not related in primary sequence, both p15 and Mtr2p structurally resemble NTF2 and heterodimerize with the NTF-2 like domains in TAP and Mex67p, thereby helping to fold or expose the nucleoporin-binding site in this region of the export receptor.

Two nucleoporin binding sites in TAP have been shown to be optimal for mRNA export. In fact, the NTF2-like domain can be removed and substituted with a second UBA domain, yielding a TAP molecule with export activity similar to the wild-type protein independent of p15. Thus, the necessity for p15 in mRNA export is precluded by the artificial addition of an extra UBA domain.

Aly/REF/Yra1p

Although TAP is able to interact directly with RNA in a sequence nonspecific fashion through its non-canonical RNP domain, this region of TAP is not essential for mRNA export *in vivo*. This observation suggested that RNA binding proteins are needed to bridge the interaction between TAP and mRNA.

Using both genetic and biochemical approaches the essential protein Yra1p was identified as a Mex67p binding partner. The genetic and biochemical data combined suggest that Yra1p is an mRNA export adapter for Mex67p. Proteins homologous to Yra1p have been identified in higher eukaryotes and belong to an evolutionarily conserved protein family called **RNA export factor binding proteins or REFs**. Also metazoan REF (also called Aly) interacts directly with TAP.

Role of splicing and the exon-exon junction complex in mRNA export

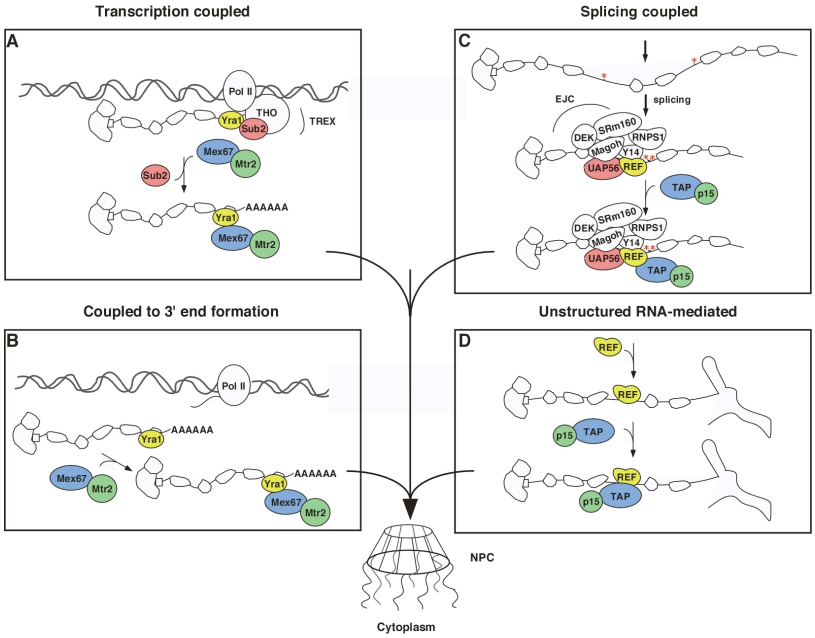
Export of some mRNAs in *Xenopus* oocytes is stimulated by splicing, suggesting that remodeling of the mRNP during splicing may render the mRNA capable of entering the export pathway. In vertebrates, a complex of proteins **(the exon-exon junction complex or EJC)** has been identified which is deposited on spliced mRNAs 20-24 nt upstream of exon-exon junctions.

REF is a component of the EJC. Thus, the formation of REF-containing EJCs on spliced mRNAs could be envisioned as an efficient means of ensuring the recruitment of TAP to spliced mRNAs. Supporting the involvement of the EJC in mRNA export was the observation that a 5' exon, too short to accommodate the EJC, resulted in inefficient export of the spliced mRNA in frog oocytes.

Aly, and perhaps the entire EJC, is recruited to the RNA by **UAP56/Sub2p**. While Aly is an adaptor that recruits TAP, Aly itself is recruited to mRNA by another factor, UAP56. UAP56 is a conserved DEAD-box helicase.

Note that the association of export adapters with the mRNA during splicing is only one of several mechanisms for ensuring the efficient recruitment of the export machinery to the mRNA. Features of an mRNA that influence the ability of an mRNA to recruit export factors independently of splicing and the EJC appear to include a critical mRNA length, mRNA sequence and RNA structure. Further, mRNA export factors are believed to be actively deposited on to the body of the mRNA during the processes of transcription and 3'end processing.

Components of the EJC have also been implicated in other posttranscriptional events such as mRNA localization in *Drosophila* or nonsense mediated decay (NMD) in human cells where the EJC helps to define exon boundaries and hence the position of premature termination codons (PTCs).



Principles of export factor recruitment to poly(A)+ mRNA in the cell nucleus.
(A) The THO complex, involved in transcription elongation, recruits Sub2p and Yra1p (together referred to as the transcription/export (TREX) complex) to mRNAs co-transcriptionally, as shown in yeast. (B) 3' end formation also contributes to Yra1p recruitment for both intronless and spliced mRNAs. (C) In vertebrates, a complex of proteins (the exon-exon junction complex or EJC) which contains Aly/REF is deposited on mRNAs upstream of exon-exon junctions. REF molecules present in EJCs subsequently recruit TAP/p15.

(D) Regions of unstructured RNA also support mRNA export factor recruitment. For example, U1 snRNA, normally a substrate for the export receptor Crm1p, can be redirected to the mRNA export pathway when harboring an inserted stretch of unstructured RNA greater than 200 nt in size. Asterisks indicate the position of the 5' and 3' splice sites.

Directionality of transport and dissociation of export factors

The directionality of transport reactions mediated by Ran-binding importins and exportins is well explained by the asymmetry of the RanGTPase system. mRNA export receptors of the NXF1 family do not interact with RanGTP. It is still unresolved how directionality in mRNA export is brought about and unknown whether nuclear pore passage of mRNPs is directly linked to NTP hydrolysis. Like for importin/exportin mediated translocation reactions, it would also be possible that energy is expended at another step in the process, such as in the cytoplasmic dissociation of mRNA export factors.

Clearly, mRNPs undergo considerable structural and compositional rearrangements on their way from the transcription site to the cytoplasm, but due to the complexity of the particle it is unknown which of these changes makes mRNP export unidirectional. Probably the most relevant question is how export factors are dissociated from the mRNP on the cytoplasmic side of the NPC. Dissociation might either be directly linked to the translocation reaction and assisted by NPC associated ATPases like e.g. Dbp5p or occur in the cytoplasm, e.g. in a pioneering round of translation. While Aly/REF presumably dissociates from mRNPs immediately after export by an unknown mechanism, another component of the EJC, the RNA binding protein Y14, is released from the mRNA during cytoplasmic translation suggesting that several mechanisms cooperate to remove export factors from mRNAs in the cytoplasm.

Table 1. Nomenclature of candidate nuclear mRNA export factors

Metazoan cells		
Common name	Alternative name	Yeast cells
Tap	Nxf1	Mex67p
Nxt	p15	Mtr2p
Aly	Ref	Yralp
UAP56	Hel	Sub2p

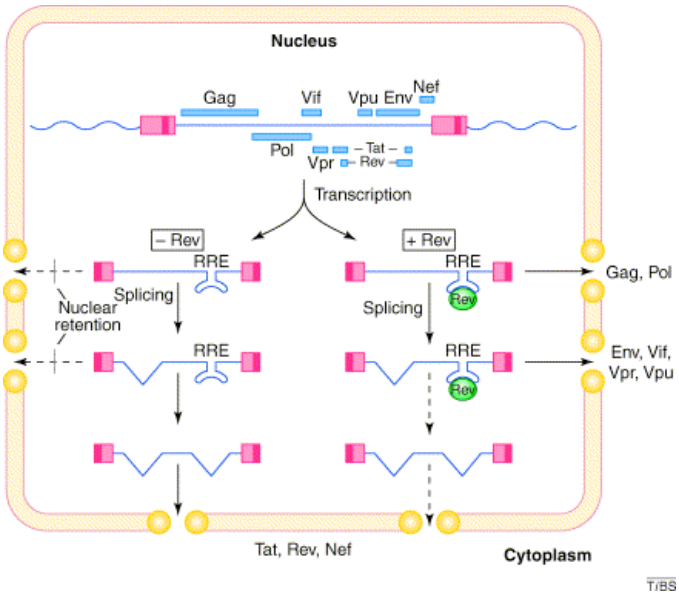
4. Viral mRNA export strategies

To maximize the production of progeny virions, several viruses have evolved mechanisms that promote the selective nuclear export of viral mRNA transcripts while, in some cases, inhibiting the export of cellular mRNAs. To achieve this goal, viruses have evolved regulatory proteins and *cis* -acting RNA elements that selectively interact with key cellular nuclear export factors.

Retroviruses are RNA viruses that replicate their genomic RNA through a DNA intermediate that is initially synthesized by reverse transcriptase. This DNA is then integrated in the host genome from which it can be transcribed by RNA polymerase II. The viral RNA undergoes processing, including splicing, like that of any other metazoan pre-mRNA. However, retroviruses must also express fully spliced, singly spliced, and unspliced versions of the same initial transcript. Unspliced RNAs serve both as transcripts for the translation of essential retroviral proteins and as genomic RNAs that are packaged into assembling virions.
Normally, unspliced cellular mRNAs are retained in the nucleus by the splicing machinery. This helps to ensure that translation occurs only on mature mRNAs. Retroviruses have evolved mechanisms to circumvent this nuclear retention and allow export of unspliced RNAs.

HIV (HIV RRE and Rev)

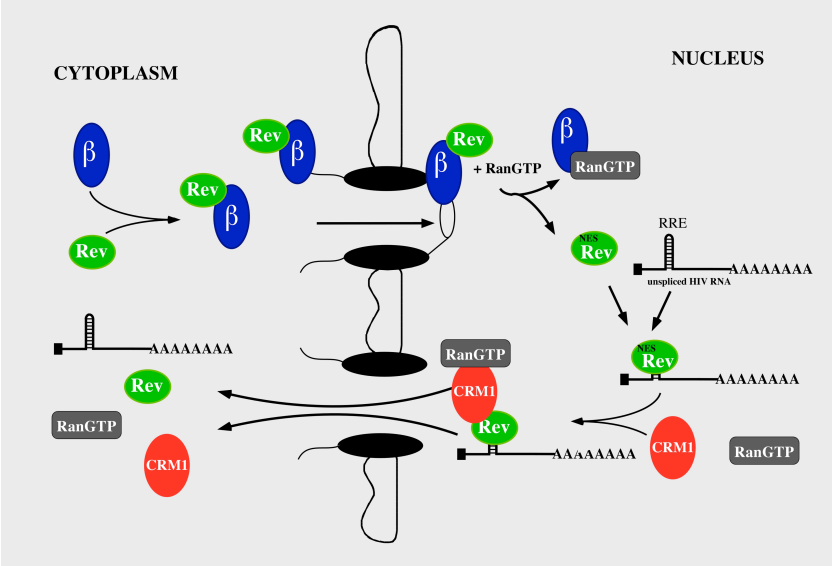
HIV type 1 (HIV-1) is a complex retrovirus or lentivirus, which has a total of nine genes that are expressed by alternative splicing of a single genome-length proviral transcript. This RNA is also used as RNA genome. HIV-1 replication requires the nuclear export of unspliced, singly spliced, and multiply spliced derivatives of the proviral transcript. Fully spliced mRNAs encode viral regulatory proteins; incompletely spliced mRNAs encode viral structural proteins, and unspliced RNA serves as genomic RNA and is packaged into virions.



Role of Rev in the HIV-1 life cycle.
HIV-1 replication requires the cytoplasmic expression of unspliced, singly spliced and fully spliced viral mRNAs. In the absence of Rev function, or early in the viral life cycle, only fully spliced viral mRNAs, encoding the regulatory proteins Tat, Nef and Rev itself, are exported from the nucleus and expressed. By contrast, incompletely spliced viral mRNAs, encoding primarily viral structural proteins, are retained in the nucleus by cellular proofreading proteins that also prevent the nuclear export of cellular pre-mRNAs. However, in the presence of Rev, these incompletely spliced viral mRNAs are exported and expressed due to the recruitment of Rev and its associated cellular cofactors to the *cis* -acting Rev response element (RRE) RNA target.
Taken from ref 3.

Early studies showed that the HIV Rev protein was absolutely required for expression of viral structural proteins encoded by incompletely spliced viral mRNAs. Nuclear export of these mRNAs depends upon the binding of multiple copies of Rev to a *cis*-acting, highly structured RNA target termed the **Rev response element (RRE)**.

A critical leucine-rich motif, initially termed the Rev activation domain, was found to be essential for Rev function. An important development in unraveling Rev function was the demonstration that this leucine-rich sequence served as a nuclear export signal (NES). NES sequences of the leucine-rich type were subsequently shown to bind CRM1, a nuclear export factor belonging to the importin/exportin family of nuclear transport receptors. Nuclear export of bound HIV-1 mRNAs is dependent on the interaction between Rev and CRM1 and requires CRM1 association with RanGTP.



MPMV (CTE and TAP)

Unlike lentiviruses, simple retroviruses do not encode *trans* -acting factors like Rev, yet these viruses also require the export of partly spliced and unspliced transcripts. Mason-Pfizer monkey virus uses a highly structured *cis* -acting RNA element that is sufficient for nuclear export of incompletely spliced viral mRNAs. This element was termed the **constitutive transport element (CTE)**.

TAP was isolated as the cellular cofactor interacting with the CTE and to promote the export of CTE-containing transcripts. That TAP/NXF1 was involved in cellular mRNA export was first demonstrated by nuclear injection of excess CTE into *Xenopus* oocytes, which competed with the export of cellular mRNAs but not of snRNAs, which use the CRM1 pathway. Unlike cellular mRNAs, which bind to TAP/NXF1 through export adaptor proteins such as Aly/REF, the CTE binds directly to TAP/NXF1.

HSV-1 (ICP27)

Herpes simplex virus type 1 (HSV-1) is a human DNA virus that expresses more than 80 transcripts during viral lytic infection. The unusual feature of HSV-1 transcripts is that the majority are intronless and thus do not interact with the splicing machinery. For this reason, nuclear retention of intron-containing mRNAs is not a problem for HSV-1, yet a lack of splicing still affects the efficiency of export of mRNAs because most HSV-1 mRNAs do not interact with splicing complexes and therefore do not acquire EJCs.

HSV-1 encodes a *trans* -acting protein that is involved in the export of viral mRNAs. This factor, termed ICP27, is a 512-amino-acid protein that shuttles between the nucleus and cytoplasm.
ICP27 is essential for viral replication. Among other activities, ICP27 inhibits host-cell gene expression by blocking the splicing and, hence, the nuclear export of cellular mRNAs. Moreover, it has been demonstrated that ICP27 directly interacts with Aly, and hence indirectly with the Tap–P15 heterodimer, to activate HSV mRNA nuclear export.

Summary Slide