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RNA TRANSPORT

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ABSTRACT

RNA molecules synthesized in the nucleus are transported to their sites of function throughout the eukaryotic cell by specific transport pathways. This review focuses on transport of messenger RNA, small nuclear RNA, ribosomal RNA, and transfer RNA between the nucleus and the cytoplasm. The general molecular mechanisms involved in nucleocytoplasmic transport of RNA are only beginning to be understood. However, during the past few years, substantial progress has been made. A major theme that emerges from recent studies of RNA transport is that specific signals mediate the transport of each class of RNA, and these signals are provided largely by the specific proteins with which each RNA is associated.

INTRODUCTION

Most eukaryotic RNAs are produced in the nucleus by RNA polymerase I, II, or III. The RNA molecules undergo a variety of posttranscriptional processing events, after which they are transported to their sites of function throughout the cell. Clearly, the subcellular locations of each type of RNA—namely messenger RNA (mRNA), small nuclear RNA (snRNA), ribosomal RNA (rRNA), and transfer RNA (tRNA)—are critical to the normal functions of the cell. A few RNAs are retained in the nucleus, and may be targeted to subnuclear domains, but the majority of RNAs are transported to the cytoplasm. Some RNAs need to be reimported to the nucleus for their function, for example U snRNA and 5S rRNA.

The exchange of macromolecules between the nucleus and the cytoplasm occurs mostly, if not exclusively, through nuclear pore complexes (NPCs). Each

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NPC is a large structure comprised of upwards of 100 different proteins that assemble to form a pore across the nuclear envelope. The NPC allows the free diffusion of ions, metabolites, and small proteins, but large molecules, in contrast, traverse the NPC by an active, energy-dependent mechanism (Rout & Wente 1994, Davis 1995). The best understood active transport process is protein import to the nucleus, which can be reproduced in vitro (Adam et al 1990). Protein import is mediated by specific sequences in the protein, termed nuclear localization sequences (NLSs) (Boulikas 1993). The NLS is recognized in the cytoplasm by a specific receptor, termed importin, karyopherin, or NLS receptor. Translocation into the nucleus requires several other factors, including a GTPase cycle (Sweet & Gerace 1995, Görlich & Mattaj 1996). While the molecular mechanism of import of proteins to the nucleus has been characterized in some detail, our understanding of RNA transport across the NPC has lagged behind considerably, because of the difficulty of analyzing RNA export from the nucleus. Despite this limitation, some general principles of RNA export have been established. All cellular RNA species exit the nucleus through NPCs by an active, mediated mechanism (Stevens & Swift 1966, Zasloff 1983, Dworetzky & Feldherr 1988, Featherstone et al 1988, Khanna-Gupta & Ware 1989, Bataillé et al 1990, Guddat et al 1990, Mehlin et al 1992, Mehlin & Daneholt 1993, Jarmolowski et al 1994). Recently, it has become clear that RNA transport through the NPC is mediated by proteins associated with the RNA molecules, and that these proteins, like proteins that are actively imported to the nucleus, utilize signal-dependent, receptor-mediated pathways to transport RNA molecules. The export pathway for each class is unique: Class-specific factors are used (Jarmolowski et al 1994, Pokrywka & Goldfarb 1995). However, the pathways likely merge at some point, so they may also utilize common factors.

This review focuses on transport of RNA molecules between the nucleus and the cytoplasm. We discuss what is understood about the transport mechanism of each class of RNA, and then consider some common themes and perspectives.

Experimental Approaches

Unlike transcription, RNA splicing, and nuclear import, RNA export from the nucleus to the cytoplasm has not been reproduced in an in vitro system. Most information on the molecular mechanisms of RNA trafficking between the nucleus and the cytoplasm has, so far, been obtained from studies in *Xenopus laevis* oocytes. The large size of the oocyte ($\sim$1 μl, or up to 200,000 times the size of a HeLa cell) facilitates the microinjection of a large amount of radiolabeled RNA into either the nucleus or the cytoplasm. The subcellular distribution of the injected RNA can be directly visualized by manually dissecting the nucleus from the cytoplasm and analyzing the RNA by gel electrophoresis (e.g. Zasloff

et al 1982a). Although the oocyte system offers several attractive features for the analysis of RNA transport, the oocyte is a highly specialized cell type. Therefore, results obtained from *X. laevis* oocyte studies may not always be relevant to RNA transport in other cell types.

Some information on the molecular mechanisms of RNA trafficking, in particular mRNA trafficking, has been provided by the analysis of *Saccharomyces cerevisiae* mutants. In this approach, yeast cells are usually randomly mutagenized, and mutants displaying defects in mRNA localization are selected. Poly(A)$^+$ RNA (i.e. mRNA) is detected by in situ hybridization to a fluorescently tagged oligo (dT) probe. In wild-type cells, the majority of the poly(A)$^+$ RNA is in the cytoplasm. When mRNA export is defective, poly(A)$^+$ RNA accumulates in the nucleus. Genes required for RNA export are identified by screening temperature-sensitive mutant strains for those that accumulate poly(A)$^+$ RNA in the nucleus at the restrictive temperature (Kadowaki et al 1992, 1994a; Amberg et al 1992).

In the following sections, we integrate the information obtained from both of these approaches to the study of RNA transport.

TRANSPORT OF mRNA

Nuclear Export of mRNA

VISUALIZATION OF NUCLEAR EXPORT OF mRNA Balbiani ring transcripts in *Chironomus tentans* salivary glands have been particularly useful for studying mRNA export at the ultrastructural level. This RNA is very large, ~40 kb, and abundant in gland cells, which allows it to be easily identified by electron microscopy. Moreover, Balbiani ring pre-mRNA undergoes minimal splicing, allowing the transcripts to be followed from their site of synthesis, through the nucleoplasm to the NPC (reviewed in Mehlin & Daneholt 1993). The protein-coated transcript released from chromatin appears as a ribbon that is bent into an asymmetric ring-like structure 50 nm in diameter. Of the four domains of this structure that can be distinguished by electron microscopy, one can be identified that contains the 5′ end of the mRNA, and another that contains the 3′ end (Skoglund et al 1986). During translocation through the NPC, the mRNA-protein complex undergoes a dramatic reorganization, which appears to occur in ordered steps (Stevens & Swift 1966; Mehlin et al 1991, 1992). During the first step of RNA-protein particle translocation, the complex associates with fibrous material extending from the rim of the NPC into the nucleoplasm. It then orients itself in front of the NPC; the domain containing the 5′ end faces the opening of the pore, and the domain containing the 3′ end is closely aposed to the rim of the pore. Finally, the ring structure of the transcript is gradually relaxed and translocated through the pore, with the 5′ end in the

lead. Thus, several temporally and spatially coordinated events, all of which appear to involve contacts between the RNA-protein complex and the NPC, lead to the translocation of Balbiani ring mRNA through the NPC. Although in the absence of other examples to draw on, it cannot yet be established that these ordered structural rearrangements leading to translocation are typical for all transcripts, Balbiani ring RNA currently serves as a useful model for the analysis of mRNA export at the ultrastructural level.

PROCESSING EVENTS THAT INFLUENCE NUCLEAR EXPORT 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 (for reviews, see Banerjee 1980, Moore et al 1993, Keller 1995, Shuman 1995, Adams et al 1996). The relationship between processing and export has been addressed in many studies. As discussed below, these studies have shown that splicing signals and introns are, in general, *cis*-acting nuclear retention elements, whereas the 5′ cap and 3′ end appear to enhance the rate of mRNA export.

Splicing signals and introns Pre-mRNA molecules bearing splice sites are largely retained in the nucleus. The block in cytoplasmic accumulation of pre-mRNA is overcome when splicing complex formation is disturbed by mutating either the splicing signals or the *trans*-acting factors required early in the formation of splicing complexes (e.g. U1 snRNA). These observations led to the spliceosome retention hypothesis, which predicts that certain splicing factors can function as negative *trans*-acting factors in mRNA export (Legrain & Rosbash 1989, Chang & Sharp 1989, Hamm & Mattaj 1990). It is unclear how retention of pre-mRNA is accomplished. Certain splicing factors may interact with nuclear structures and thereby hold the RNA in the nucleus, or the spliceosome may prevent interaction of the RNA with export factors.

Removal of introns by splicing does not, in most cases, appear to be essential for export of RNA; for example, overexpression of an intron-containing gene in yeast results in the appearance of pre-mRNA in the cytoplasm. This suggests that an interaction of the pre-mRNA with retention factors is saturable, allowing the pre-mRNA to leave the nucleus (Legrain & Rosbash 1989). Furthermore, some mechanism must exist to allow export of alternatively spliced (i.e. intron-containing) transcripts. It is currently unclear whether alternatively spliced mRNAs are exported by a pathway similar to that which exports fully spliced transcripts, or whether a specialized mechanism ensures that alternatively spliced mRNAs can leave the nucleus (see section on Viral Regulation of mRNA Export, Rev and Rex Proteins).

Although splicing is believed to influence export indirectly by a retention mechanism, the export of some mRNAs appears to be directly dependent on splicing. For example, β-globin mRNA is not exported to the cytoplasm when expressed from intronless transfected DNA (Collis et al 1990). The process of splicing appears to convert the mRNA from an export-incompetent to an export-competent form. It is unknown why splicing is apparently necessary for efficient export of these transcripts but not for those encoded by other intron-containing genes, such as cellular thymidine kinase, or for those encoded by natural intronless genes, such as α-interferon and c-*jun* (Nagata et al 1980, Gross et al 1987, Hattori et al 1988).

Recently, two naturally occurring intronless genes, herpes simplex virus thymidine kinase and hepatitis B virus S transcripts, were each found to contain an element that when fused to the intron-dependent β-globin gene, allowed efficient cytoplasmic accumulation of β-globin RNA in the absence of splicing (Liu & Mertz 1995, Huang & Yen 1995). These viral RNA sequences may function as *cis*-acting RNA export elements.

Cap structure and 3′ end Mattaj and coworkers have demonstrated a correlation between the export rate of a transcript and its 5′ cap structure. Transcripts synthesized in vitro with a trimethylguanosine $m^{2,2,7}G$ (m_3G) cap or an adenosine (A) cap (uncapped transcripts cannot be analyzed because they are degraded in cells) were found to be exported from *X. laevis* oocyte nuclei more slowly than an mRNA synthesized with an m^7G cap (Hamm & Mattaj 1990, Jarmolowski et al 1994). The m^7G cap structure can therefore enhance the rate of mRNA export, but it does not appear to be essential. Several observations indicate that the m^7G cap structure is neither necessary nor sufficient for mRNA export. For example, *Caenorhabditis elegans* mRNAs processed by *trans*-splicing acquire a trimethylated cap and are still translated in, and therefore transported to, the cytoplasm (Liou & Blumenthal 1990, Van Doren & Hirsh 1990), and transfected histone genes lacking their normal 3′ end produce an m^7G-capped mRNA that does not leave the nucleus (Sun et al 1992).

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 (Ohno et al 1990, Kataoka et al 1994, Izaurralde et al 1994; see also the section on U snRNA Export). Although it is not certain whether CBC mediates the effect of the cap on mRNA export, Visa et al (1996b) have shown by immunolocalization that CBP20 accompanies Balbiani ring mRNA out of the nucleus.

Several studies suggest that the 3′ poly(A) tail and the histone 3′ end stem-loop structure can stimulate mRNA export, but that they are not an absolute

requirement for transport (Eckner et al 1991, Sun et al 1992, Jarmalowski et al 1994). Neither is a 3′ poly(A) tail alone sufficient to export an mRNA, since some poly(A)$^+$ RNA molecules never leave the nucleus (for example, Xist RNA and omega-n RNA; Brockdorff et al 1992, Brown et al 1992, Hogan et al 1994).

In summary, although the m^{7}G cap and 3′ end may influence the rate of transport, the role these structures play in mRNA export is unclear. Since neither structure is essential, other RNA sequence elements yet to be identified may be important for mRNA export.

TRANS-ACTING mRNA EXPORT FACTORS This section describes evidence for the involvement of *trans*-acting protein factors in mRNA export, which is summarized in Figure 1. These potential *trans*-acting factors include hnRNP proteins, NPC proteins, components of a GTPase cycle, and various yeast proteins identified in poly(A)$^+$ RNA export mutants.

hnRNP proteins From the moment pre-mRNA emerges from the transcription complex and throughout its lifetime in the nucleus, pre-mRNA/mRNA probably never exists as free RNA, but rather is associated with proteins (reviewed in Dreyfuss et al 1993; see the section on Visualization of Nuclear Export of mRNA). These pre-mRNA/mRNA-binding proteins comprise the abundant heterogeneous nuclear RNA-binding proteins (hnRNP proteins) and other proteins involved in transcription and pre-mRNA processing (Piñol-Roma et al 1988; reviewed in Dreyfuss et al 1993).

The hnRNP proteins, which have been most extensively characterized in human cells, comprise a group of about 20 major proteins that associate with nascent pre-mRNA, and participate in the processing reactions that generate mature mRNA (Munroe & Dong 1992, Mayeda & Krainer 1992, Dreyfuss et al 1993, Portman & Dreyfuss 1994, Yang et al 1994, Cáceres et al 1994). All transcripts appear to be associated with most of the hnRNP proteins, but the stoichiometry varies, so the protein constellation that assembles on each transcript is probably unique (Piñol-Roma et al 1989, Matunis et al 1993).

Several hnRNP proteins, for example, hnRNP A1 and hnRNP K, although predominantly nuclear, shuttle continuously between the nucleus and the cytoplasm. While in the cytoplasm, hnRNP A1 is associated with mRNA, suggesting that shuttling hnRNP proteins accompany mRNA from the point of its emergence from the transcription machinery, through the nucleoplasm to the NPC, and during translocation of the RNP complex through the NPC (Piñol-Roma & Dreyfuss 1992, 1993). This suggestion is now supported by the recent observation that an insect A1-like hnRNP protein in *Chironomus tentans*, Ct-hrp36, translocates through the NPC associated with RNA (Visa et al 1996a). The question of whether mRNA is carrying, or is being carried by, the shuttling hnRNP proteins has been addressed with the identification of a

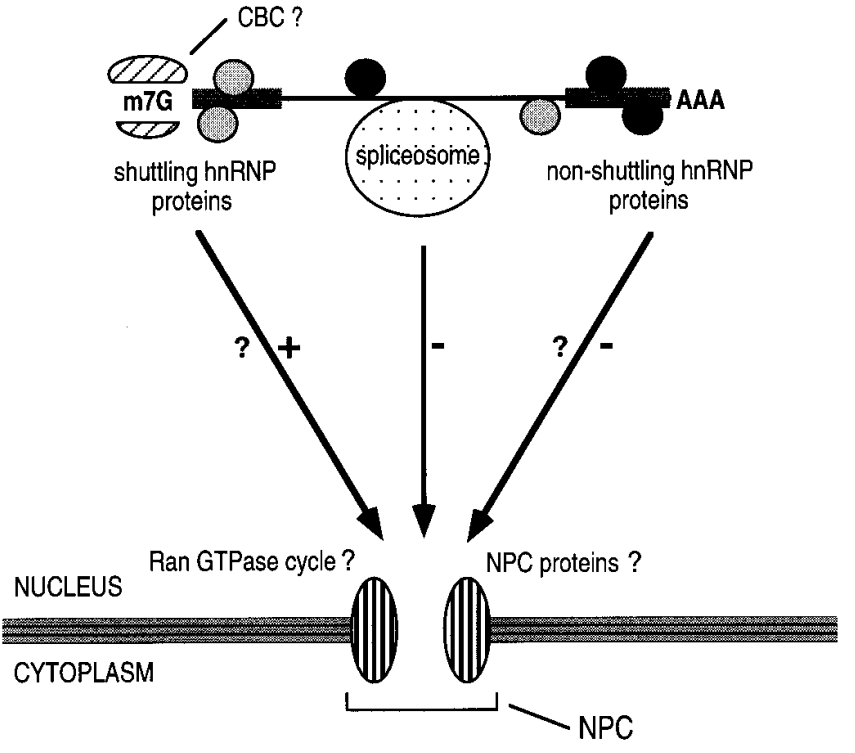


Figure 1 Potential *trans*-acting factors for mRNA nuclear export. Messenger RNA is transcribed in the nucleus by RNA polymerase II as a precursor with an m^7G cap, and, in the majority of higher eukaryotic transcripts, introns and a 3′ poly(A) tail. Evidence indicates that shuttling hnRNP proteins mediate export of mRNA from the nucleus to the cytoplasm. Interaction with spliceosomes and nonshuttling hnRNP proteins prevents mRNA export. The roles of the cap-binding complex (CBC), the Ran GTPase cycle, and individual nuclear pore complex (NPC) proteins are less clear. See text for details.

protein nuclear export signal (NES) within hnRNP A1 (Siomi & Dreyfuss 1995, Michael et al 1995). The A1 NES (a 38–amino acid segment of the protein termed M9) is not involved in RNA binding and, when fused to a protein that is normally restricted to the nucleus, is capable of promoting its export to the cytoplasm in a temperature-sensitive manner (Michael et al 1995). Together, these observations support the model that hnRNP A1, and probably other shuttling hnRNP proteins, mediate, via their NESs, nuclear export of cellular mRNA.

While the shuttling hnRNP proteins appear to remain associated with mRNA until it reaches the cytoplasm, nonshuttling hnRNP proteins, such as hnRNP C1

and hnRNP U, do not leave the nucleus. Recently, a nuclear retention sequence (NRS) has been identified in the hnRNP C proteins (Nakielny & Dreyfuss 1996). The NRS is capable of overriding hnRNP protein NESs, suggesting that pre-mRNA/mRNA that is associated with both NES-bearing and NRS-bearing hnRNP proteins cannot leave the nucleus. It is therefore an attractive possibility that nonshuttling NRS-containing hnRNP proteins prevent transcripts that have not been fully processed from exiting the nucleus to the cytoplasm. Nonshuttling hnRNP proteins are removed from mRNA prior to, or during, mRNA export. This removal is likely to be critical to allow mRNA to be exported, and may be an important regulatory step in mRNA transport.

After translocation through the NPC as components of the RNP complex, shuttling hnRNP proteins must dissociate from the mRNA and be reimported to the nucleus. Import of hnRNP A1 is mediated by the same region of the protein (M9) that mediates A1 export (Siomi & Dreyfuss 1995). It is not yet clear whether the import and export signals in A1 are one and the same, although so far, mutations within M9 that abolish import also prevent export (Michael et al 1995). Interestingly, nuclear import of A1, and of the majority of other shuttling hnRNP proteins, is dependent upon ongoing RNA polymerase II transcription, which suggests that import of these candidate *trans*-acting mRNA export factors is coupled to the nuclear content of exportable mRNA (Piñol-Roma & Dreyfuss 1991, 1993; Michael et al 1996). Import of proteins bearing classical basic NLSs does not depend on transcription, indicating that the import mechanism of shuttling hnRNP proteins is fundamentally different from that of basic NLS-bearing proteins.

Nuclear pore complex proteins At least nine yeast NPC proteins have been identified that, when they bear a temperature-sensitive mutation or when they are deleted, cause poly(A)$^+$ RNA to accumulate in the nucleus. These include Rat2p/Nup120p, Rat3p/Nup133p, Nup100p, Nup49p, Nup116p, Nup1p, Rat10p/Nup145p, Nup82p, and Rat7p/Nup159p (reviewed in Schneiter et al 1995, Maquat 1996). Fragments of two of these proteins, Nup145p and Nup116p, are capable of binding RNA homopolymers in vitro, leading to the suggestion that these NPC proteins interact with RNA molecules being exported, or with a putative RNA component of the NPC (Fabre et al 1994).

For most of these genes, disruption results in phenotypes additional to that of nuclear poly(A)$^+$ RNA accumulation, for example, defective protein import and abnormalities in NPC morphology (reviewed in Schneiter et al 1995, Maquat 1996). Also, in a number of cases, only a small proportion of the mutant cells show poly(A)$^+$ RNA accumulation. Although it may well be that some of these NPC proteins are not only involved in mRNA export, but also function in other processes (such as protein import and NPC assembly), a direct role for

these yeast NPC proteins in mRNA export cannot yet be assigned. One possible exception is Rat7p/Nup159p. Identified in a screen for genes required for mRNA export, a temperature-sensitive mutation causes a rapid accumulation of poly(A)$^+$ RNA in the nucleus at the nonpermissive temperature (Gorsch et al 1995). No protein import defect could be detected, and although NPC morphology is abnormal, this phenotype is apparent at the permissive temperature, when mRNA export appears normal. Further characterization of Rat7p/Nup159p should establish whether it plays a direct role in mRNA transport.

GTPase cycle Import of proteins to the nucleus has been found to be dependent upon a GTPase cycle comprising a GTPase (termed Ran in higher eukaryotes, spi 1 in fission yeast, and GSP1 and 2/CNR1 and 2 in budding yeast), a guanine nucleotide exchange factor (termed RCC1 in higher eukaryotes, pim 1 in fission yeast, and PRP20/MTR1/SRM1 in budding yeast), and a GTPase-activating protein (termed RanGAP1 in higher eukaryotes and rna1 in fission and budding yeasts). This GTPase cycle may also play a role in mRNA export (Dasso 1993, Melchior et al 1993, Moore & Blobel 1993, Tachibana et al 1994, Schlenstedt et al 1995, Sweet & Gerace 1995, Tartakoff & Schneiter 1995, Sazer 1996). Mutation of the genes encoding the GTPase cycle components in yeast or mammalian cells causes poly(A)$^+$ RNA to accumulate in the nucleus. However, mutation of these genes results in a variety of defects in addition to a block in mRNA export, for example, abnormalities in nuclear morphology (Aebi et al 1990), mRNA transcription initiation and 3′ end formation (Forrester et al 1992), rRNA and tRNA maturation (Kadowaki et al 1993), and pre-mRNA splicing (Vijayraghavan et al 1989). A direct role for the GTPase cycle components in mRNA export remains to be demonstrated.

Other potential trans-acting factors In addition to NPC proteins and Ran GTPase cycle proteins, yeast genetic screens have identified a number of other genes, defects in which result in inhibition of mRNA export (Schneiter et al 1995, Maquat 1996). The proteins encoded include Rat1p, an exonuclease (Amberg et al 1992), Mtr2p, a novel nuclear protein (Kadowaki et al 1994b); Mas3p, a heat shock transcription factor (Kadowaki et al 1994a); Rpa190p, a subunit of RNA polymerase I (Schneiter et al 1995); Rae1p, a novel protein with β-transducin repeats (Brown et al 1995); Mtr3p, a novel nucleolar protein (Kadowaki et al 1995); and Mtr4p, a novel protein with sequence motifs characteristic of DEAD-box proteins (Liang et al 1996). For all the mutants that have been analyzed, defects additional to that in mRNA export have been described. These frequently include abnormal nucleolar morphology and deficiences in rRNA processing. It is currently unclear whether the mRNA export defect is primary or secondary to the other effects of the mutations, and/or whether the gene products are multifunctional.

REGULATION OF mRNA EXPORT: VIRAL REGULATION Studies of viral pre-mRNA processing and transport have uncovered the first clear examples of regulated RNA export (reviewed in Izaurralde & Mattaj 1992, 1995; Elliott et al 1994; Krug 1993). A number of viral proteins, or protein complexes, are currently known to influence the nuclear export of viral and cellular RNA molecules. The best understood of these are human immunodeficiency virus 1 (HIV-1) Rev protein, human T cell leukemia virus 1 (HTLV-1) Rex protein, adenovirus early region 1B 55-kDa protein/early region 4 34-kDa protein complex (E1B 55-kDa /E4 34-kDa), and M1 matrix protein and nonstructural protein 1 (NS1) of the influenza virus.

Rev and Rex proteins Rev and Rex proteins induce the nuclear export of partially spliced or unspliced transcripts encoding structural proteins of HIV-1 and HTLV-1, respectively (reviewed in Cullen & Malim 1991; Cullen 1992, 1995). In the absence of Rev or Rex, these transcripts are retained in the nucleus until they are either spliced or degraded (Malim et al 1989b, Felber et al 1989, Emerman et al 1989). Since Rev and Rex are functional equivalents (Hope et al 1991, Weichselbraun et al 1992), the following discussion focuses on Rev, the more extensively characterized of the two proteins.

The mechanism of action of Rev in viral mRNA export has been controversial. It has been suggested that Rev promotes the export indirectly by dissociating splicing factors, directly by activating transport, or by a combination of these two mechanisms (Cullen & Malim 1991, Cullen 1992). As outlined below, several lines of evidence have shown that Rev can directly promote export, although it is unclear whether it also functions in part by dissociating splicing factors (Chang & Sharp 1989, Lu et al 1990, Kjems et al 1991, Luo et al 1994, Stutz & Rosbash 1994).

Rev, a small protein of 116 amino acids, binds via an arginine-rich region to a complex 234-nucleotide RNA stem-loop structure in the partially spliced/unspliced viral transcripts, termed the Rev response element (RRE). This Rev-RRE interaction is essential for Rev function (Zapp & Green 1989, Daly et al 1989). Rev shuttles between the nucleus and the cytoplasm, an activity that requires another domain critical for Rev function, termed the activation domain (Malim et al 1989a, 1991; Mermer et al 1990; Venkatesh & Chinnadurai 1990; Malim & Cullen 1991; Daly et al 1993; Meyer & Malim 1994; Kalland et al 1994; Wolff et al 1995). The leucine-rich activation domain (sequence: LPPLERLTL) is capable of promoting nuclear export of heterologous proteins in a saturable and temperature-sensitive manner, and it can function in the absence of the RRE (Wen et al 1995, Fischer et al 1995, Meyer et al 1996).

Evidence that Rev directly promotes RNA export came from microinjection studies in *X. laevis* oocytes. Rev protein coinjected with RRE-containing

pre-mRNA into oocyte nuclei induces export of the RNA independently of splicing (Fischer et al 1994b). Rev is therefore capable of carrying an RRE-containing RNA molecule from the nucleus to the cytoplasm.

These findings together showed that the leucine-rich Rev activation domain is an NES and that an RNA can be transported to the cytoplasm by associating with an NES-containing protein.

The effect of Rev on RNA export is evident in the absence of any other viral protein, suggesting that Rev promotes export by interacting with cellular factors. Several cellular proteins that interact with Rev and may mediate Rev function have recently been described, including eukaryotic initiation factor 5A, human Rev interacting protein or Rev/Rex activation domain binding protein (hRIP/Rab) and yeast Rev interacting protein (Rip1p) (Fritz et al 1995, Bogerd et al 1995, Stutz et al 1995, Bevec et al 1996). Rip1p and hRIP/Rab are distantly related, and both proteins contain phenylalanine-glycine (FG) repeats and other sequence repeats, all of which are characteristic of NPC proteins. It is still unclear if either hRIP/Rab or Rip1p are bona fide components of NPCs. The Rev interaction domain of hRIP/Rab and Rip1p lies within the FG repeat-containing region of the proteins (Fritz et al 1995, Stutz et al 1995). Rev can interact with a subset of yeast NPC proteins, all of which contain FG repeats, and one of which is Rat7p/Nup159p, that has been identified as a potential cellular mRNA export factor (Stutz et al 1995, Gorsch et al 1995; see the section on Trans-Acting mRNA Export Factors, NPC proteins). A model that arises from this information is that translocation of Rev from the nucleus to the cytoplasm is mediated by sequential interactions between Rev and FG repeat proteins in the nucleoplasm and the NPC.

Saturation of the Rev export pathway, by nuclear injection of bovine serum albumin (BSA) coupled to peptides comprising the Rev activation domain, has no effect on the export of coinjected mRNA, and saturation of mRNA transport does not interfere with Rev-mediated RNA export (Fischer et al 1995). Thus, Rev accesses an export pathway that is mechanistically different from the cellular mRNA export pathway, even though Rev exports RNA molecules belonging to the mRNA class. The reason for this redirection of RRE-containing mRNAs by Rev is still unclear, but the Rev NES, in contrast to NESs that may mediate cellular mRNA export, may direct the RNA to an export pathway that can override nuclear retention of intron-containing pre-mRNA molecules.

The export of tRNA and rRNA (in the form of ribosomes) is also unaffected under conditions that saturate Rev-mediated export, indicating that these RNAs, like cellular mRNA, use export pathways that are different from that used by Rev. Under the same conditions, however, export of U snRNA and 5S rRNA is inhibited. These observations indicate that at least one limiting factor for Rev-mediated transport is shared by the cellular 5S rRNA and U snRNA export

pathways (Fischer et al 1995). It has therefore been proposed that export of 5S rRNA and U snRNA is mediated by proteins that harbor an NES that is functionally equivalent to the NES of Rev (see the sections on U snRNA and 5S rRNA Transport, below).

Adenovirus early region 1B 55-kDa protein/early region 4 34-kDa protein complex A compex of two adenovirus proteins, E1B 55-kDa and E4 34-kDa, facilitates the cytoplasmic accumulation of late viral mRNAs and blocks the cytoplasmic accumulation of host cell mRNAs. In mutant viruses lacking functional E1B or E4 protein, reduced levels of late viral mRNAs accumulate in the cytoplasm, and cytoplasmic accumulation of host cell mRNAs is normal (Babiss & Ginsberg 1984, Babiss et al 1985, Halbert et al 1985, Pilder et al 1986). Also, in the absence of the E1B protein, the nuclear distribution of viral transcripts is altered (Pilder et al 1986, Leppard & Shenk 1989). The molecular mechanisms that underlie the effects of the E1B/E4 protein complex are not understood, but it has been suggested that the complex recruits a factor that is essential for cellular mRNA export to viral replication/transcription centers, thereby enhancing viral mRNA transport and at the same time inhibiting cellular mRNA export (Ornelles & Shenk 1991).

Influenza virus NS1 and M1 proteins Influenza virus encodes two proteins that appear to regulate export of RNA molecules from the nucleus at different stages during the production of viral particles (reviewed in Krug 1993, Whittaker et al 1996). The NS1 protein affects cytoplasmic accumulation of viral protein-coding transcripts, whereas M1 protein regulates transport of mature viral ribonucleoproteins (vRNPs).

Influenza virus proteins are synthesized in two phases, early and late, and the switch between these two phases appears to be regulated at the level of mRNA export from the nucleus (Shapiro et al 1987). The NS1 protein mediates this regulation by inhibiting the export of late protein transcripts until the appropriate time. The molecular mechanisms involved are unknown, although NS1 has been shown to bind the poly(A) tails of all RNA molecules tested (Qiu & Krug 1994, Qian et al 1994). NS1 protein function must be inactivated to allow export of late protein transcripts, and this appears to be mediated by post-translational modifications, since protein kinase and methyltransferase inhibitors block export of late protein transcripts (Kurokawa et al 1990, Martin & Helenius 1991, Vogel et al 1994). NS1 also indirectly inhibits transport of intron-containing viral and cellular transcripts by inhibiting pre-mRNA splicing (Lu et al 1994, Fortes et al 1994).

A prerequisite for the production of virus particles in the cytoplasm is export of vRNPs, which assemble in the nucleus. Export of vRNPs is dependent

on nuclear-localized M1 protein (Fraser 1967, Martin & Helenius 1991). The mechanism by which M1 functions has not yet been characterized, although studies of the transport properties of vRNPs of a virus bearing a temperature-sensitive transport mutation in M1 have been informative (Whittaker et al 1995, Ye et al 1995). At the nonpermissive temperature, most of the mutant M1 protein appears to be restricted to the nucleus, and the vRNPs are exported without binding detectable M1 protein. This suggests that M1 facilitates vRNP export not by associating with them and accompanying them to the cytoplasm, but rather by allowing their release from a nuclear retention mechanism. Viral RNP components that mediate export of the released vRNPs have not yet been identified.

REGULATION OF mRNA EXPORT: CELLULAR REGULATION Clear examples of regulated nuclear export of cellular mRNA have yet to be described at the mechanistic level. However, several reports suggest that like viruses, cells regulate nucleocytoplasmic mRNA transport. For example, heat-shocked yeasts accumulate mRNA in their nuclei, but can selectively export mRNA-encoding heat shock proteins (Saavedra et al 1996), and overexpression of eukaryotic initiation factor 4E appears to alter the nucleocytoplasmic distribution of cyclin D mRNA (Rousseau et al 1996). In addition, it is of interest that the export of unspliced RNA of two simple retroviruses, Mason-Pfizer monkey virus (MPMV) and Rous sarcoma virus (RSV), is mediated by *cis*-acting RNA elements, which can function in the absence of any viral protein (Bray et al 1994, Ogert et al 1996). Furthermore, these RNA elements, termed constitutive transport elements (CTEs), can substitute for RRE RNA/Rev protein to induce nuclear export of unspliced HIV transcripts, and the MPMV CTE can promote export of a cellular intron-containing RNA that is normally retained in the nucleus (Bray et al 1994, Ogert et al 1996). These observations suggest that a cellular protein(s) can mediate the effects of these viral CTEs. It will be of considerable interest to identify this factor, since it may play some role in regulating export of alternatively spliced cellular transcripts.

Nuclear Import of Viral RNA

Many RNA viruses, including HIV-1 and influenza virus, replicate their genomes in the nucleus of the infected cell. In nondividing cells, import of these nucleic acids has been shown to require ATP and to be protein mediated. HIV nucleic acid import is mediated by viral matrix protein (MA) and viral protein R (Vpr). MA contains a classical basic NLS and facilitates viral nucleic acid import by utilizing the cellular classical NLS protein import pathway (Bukrinsky et al 1993, von Schwedler et al 1994). The sequence elements within Vpr required for nuclear localization have not been delineated (Heinzinger et al

1994). Influenza virus RNA import appears to be mediated by viral NP, which like HIV MA, utilizes the cellular classical NLS import machinery (O'Neill et al 1995, Whittaker et al 1996). In an in vitro import assay, viral RNA is imported only when NP is present, indicating that this RNA is transported as a specific RNP (O'Neill et al 1995).

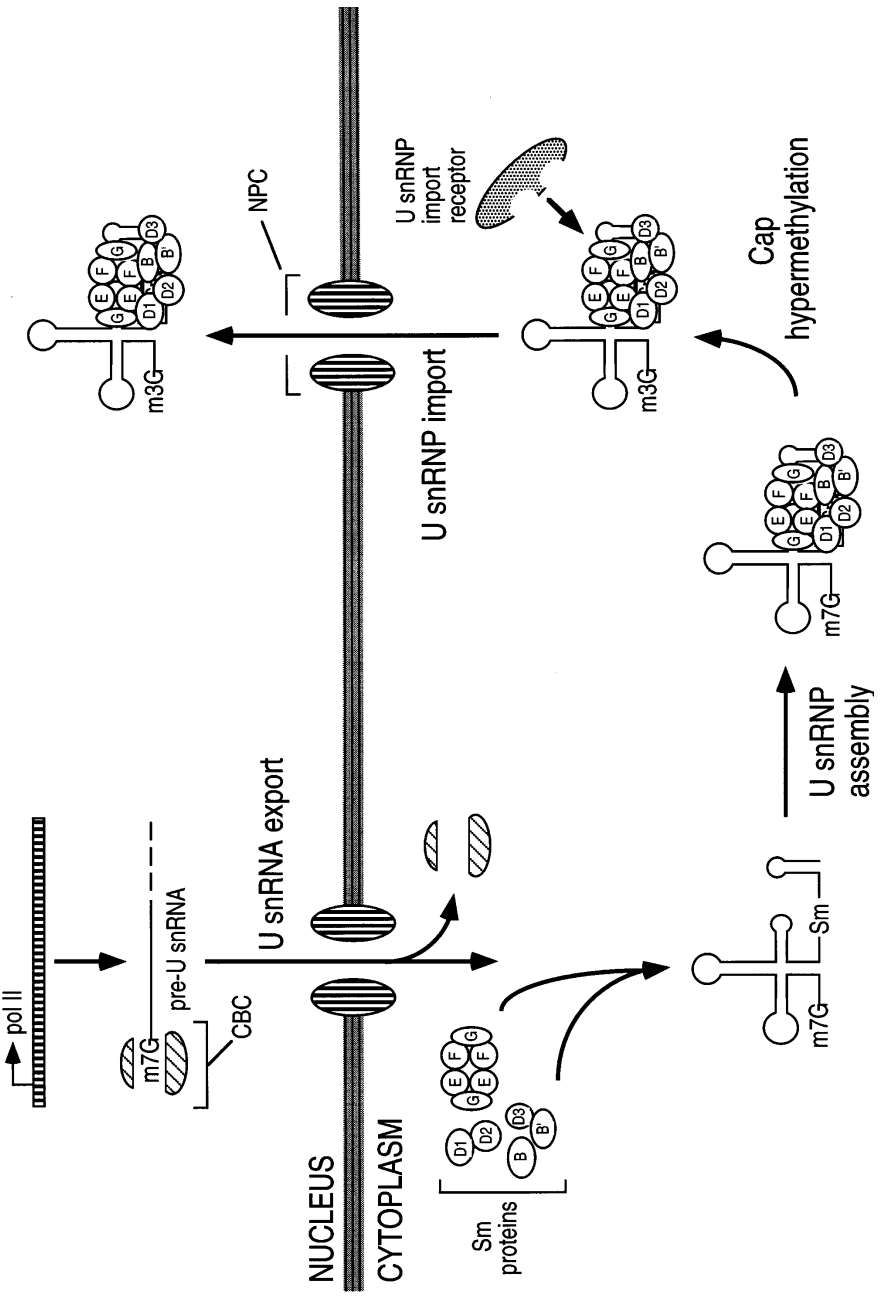
TRANSPORT OF U snRNAs

Overview of U snRNA Transport

The snRNPs U1, U2, U4/6, and U5 are RNA-protein complexes and are part of the spliceosome in which pre-mRNA processing takes place. Each snRNP complex consists of one (U1, U2, and U5) or two (U4/6) snRNAs, a common set of proteins (the Sm proteins B, B′, D1, D2, D3, E, F, and G), and proteins that are specific to each U snRNP (Lührmann et al 1990).

All spliceosomal U snRNAs, with the exception of U6, undergo bidirectional transport across the nuclear envelope as part of their maturation pathway from precursor transcripts to functional nuclear U snRNP complexes (Figure 2) (DeRobertis 1983, Mattaj 1988, Izaurralde & Mattaj 1992). The snRNAs U1-U5 are synthesized in the nucleus by RNA polymerase II and acquire a 5′-terminal m^7G cap cotranscriptionally (Reddy & Bush 1988). In contrast, the U snRNP proteins are synthesized and stored in the cytoplasm and do not migrate on their own into the nucleus (DeRobertis 1983, Mattaj & DeRobertis 1985). Instead, the newly transcribed snRNAs are exported from the nucleus to the cytoplasm, where they assemble with the Sm proteins to form the Sm core of U snRNP—a common structure of these particles. Thereafter, the m^7G cap is hypermethylated to form the m_3G cap structure, and the U snRNP is then imported into the nucleus (Figure 2) (Mattaj 1986, 1988). When and where the specific proteins are incorporated into the snRNP particles is unknown.

Figure 2 Schematic drawing of the U snRNP biogenesis cycle in oocytes as exemplified by U1 snRNP. The snRNA is transcribed in the nucleus by RNA polymerase II as a precursor with an m^7G cap and in some cases a 3′-terminal extension. The RNA is then transported to the cytoplasm by virtue of binding to the nuclear CBC, and possibly by other factors that are so far unknown. In the cytoplasm the snRNA associates with the Sm proteins B, B′, D1, D2, D3, E, F, and G, which form, together with the RNA, the Sm core domain. The m^7G cap is then hypermethylated to form the m_3G cap, and this is dependent on the prior formation of the Sm core domain. Eventually, the U snRNP particle is transported to the nucleus, which requires interaction of the m_3G cap and the Sm core domain with a U snRNP-specific import receptor. The time and place of association of U snRNP-specific proteins are in most cases unclear and are therefore not included in the figure.


pol II
m7G
pre-U snRNA
CBC
U snRNA export
NUCLEUS
CYTOPLASM
Sm proteins
Sm
m7G
U snRNP assembly
m7G
Cap hypermethylation
m3G
U snRNP import
U snRNP import receptor
NPC
m3G

Nuclear Export of U snRNA

ROLE OF *CIS*-ACTING STRUCTURES IN NUCLEAR EXPORT OF U snRNAs All spliceosomal snRNAs appear to exit the nucleus via a common export pathway, because nuclear injection of large amounts of any one particular snRNA interferes with the export of all the others. This suggests that common structures in all spliceosomal snRNAs contribute to their export (Jarmolowski et al 1994). In search for such structures that may mediate snRNA nuclear export, Mattaj and coworkers identified the m^7G cap structure as one important signal element (Hamm & Mattaj 1990). This observation led to the purification of a nuclear m^7G cap-binding complex (CBC) (Ohno et al 1990; Izaurralde et al 1992, 1994, 1995; Kataoka et al 1994, 1995). CBC consists of two proteins, CBP80 and CBP20, whose properties suggest a role in export. Antibodies against CBP20 interfere with CBC binding to the cap, and specifically inhibit snRNA export, thereby demonstrating a direct involvement of CBC in the nuclear export of spliceosomal snRNA (Izaurralde et al 1995). The mechanism by which CBC induces U snRNA nuclear export is still unclear. One possibility is that the CBC contains an NES that allows access to a specific export pathway. Alternatively, CBC may interact with cellular components that contain NESs.

The m^7G cap alone, however, is insufficient for mediating the export of snRNAs. Other sequences that influence the export of U1 snRNA have been localized to the 5′-terminal 124 nucleotides as well as to the 3′-terminal stem/loop of the RNA (Terns et al 1993).

TRANS-ACTING U snRNA EXPORT FACTORS *GTPase cycle* Evidence suggests an involvement of the Ran GTPase cycle in nuclear export of spliceosomal U snRNAs (see also the mRNA *trans*-acting export factors section). In the Chinese hamster cell line tsBN2, whose mutant RCC1 is unstable at 40°C, nuclear export of spliceosomal snRNAs is inhibited (Cheng et al 1995). Under the same conditions, U3 snoRNA targeting to the nucleoli is also inhibited. Since, unlike spliceosomal U snRNAs, U3 snoRNA is not exported to the cytoplasm, the Ran GTPase cycle may be generally required to enable the intranuclear movement of certain classes of RNPs.

Nuclear Import of U snRNA

U snRNAs exported from the nucleus assemble in the cytoplasm into U snRNPs before returning to the nucleus. Because neither the snRNA nor the snRNP proteins alone are imported independently of each other, the functionally active NLS is generated during assembly of the U snRNP particle.

NUCLEAR IMPORT OF U snRNA IN *X. LAEVIS* OOCYTES Microinjection studies in *X. laevis* oocytes showed that the assembly of the common Sm proteins with

the snRNA is an essential step in the formation of the snRNP NLS. Mutant U snRNAs lacking the Sm protein binding site (Sm site) fail to accumulate in the oocyte nucleus, whereas binding sites for the specific proteins are dispensible for import (Mattaj & DeRobertis 1985, Mattaj 1986).

Because cap hypermethylation is also dependent on the assembly of the common proteins on the Sm site of the U snRNA, the possibility of whether the m_3G cap may also contribute to the formation of a functional NLS was tested. U1 snRNA carrying either no cap or the artificial adenosine (A) cap assembles in the cytoplasm with the common proteins but fails to localize to the nucleus (Fischer & Lührmann 1990, Hamm et al 1990). In addition, the m_3GpppG cap dinucleotide specifically inhibits nuclear import of U snRNAs (Fischer & Lührmann 1990, Fischer et al 1991, Michaud & Goldfarb 1991). Thus, the m_3G cap has an essential signaling role in nuclear import of these RNAs. However, the m_3G cap alone is not sufficient for import of U snRNPs. Another signal on the Sm core has been defined that, together with the m_3G cap, is necessary and sufficient for nuclear import (Fischer et al 1993). Not all U snRNAs, however, have the same m_3G cap requirement for their nuclear import. For example, U4 and U5 snRNAs, or an artificial RNA that contains only the Sm site flanked by two stem loops, do not absolutely require the m_3G cap for import, since they can enter the nucleus with an A cap. However, in these cases import is considerably slower than that of m_3G-capped snRNAs (Fischer et al 1991, 1993; Jarmolowski & Mattaj 1993).

The mechanism by which the m_3G cap and the signal on the Sm core domain cooperate to create the fully active composite NLS is not yet clear. In particular, it is unclear whether one import receptor recognizes both signals or whether two different receptors are required that recognize the cap and the Sm core separately. Another possibility is that the m_3G cap induces a conformational change in the U snRNP particle and thereby exposes a preexisting signal on the Sm core domain.

Competition experiments have been carried out in order to determine whether the nuclear import pathways of classical NLS-containing proteins and U snRNPs share common features. These experiments indicated that protein and U snRNP import are mediated by different rate-limiting factors (Michaud & Goldfarb 1991, 1992; Fischer et al 1991, 1993, 1994a; van Zee at al 1993). However, it is unknown whether proteins and U snRNAs use entirely different import pathways or whether their pathways converge at some point and therefore share some common factors.

NUCLEAR IMPORT OF U snRNAs IN SOMATIC CELLS In somatic cells, import of microinjected U1 snRNPs is not strictly dependent on the m_3G cap. However, transport is accelerated by the presence of an m_3G cap. The Sm core domain, in

contrast, is necessary and sufficient for nuclear import. The same signal requirement is observed in different cell types, including *X. laevis* somatic cells (Fischer et al 1994a). The development of an in vitro transport system that faithfully mimics in vivo snRNA import has provided further insights into mechanistic aspects of U snRNA import. As in vivo, the Sm core domain is required for nuclear import of U1 and U2 snRNPs in vitro. However, the m_3G cap dependence varies, depending on the source of cytosol. When the assay is provided with *X. laevis* oocyte cytosol, U1 and U2 snRNP nuclear import is strictly dependent on the m_3G cap. In contrast, somatic cell cytosol supports the import of ApppG-capped U snRNPs. Therefore, cell type-specific rather than species-specific differences in the transport machinery apparently account for the differential m_3G cap requirement for nuclear import of U1 snRNPs in *X. laevis* oocytes and somatic cells (Fischer et al 1994a, Marshallsay & Lührmann 1994).

TRANSPORT OF 5S rRNA

5S rRNA is a component of large ribosomal subunits. Synthesis of 5S rRNA differs from that of the other rRNAs (18S, 28S, and 5.8S) by two general criteria. First, it is transcribed by RNA polymerase III, as opposed to RNA polymerase I. Second, the 5S rRNA genes are not included in the rDNA repeats that produce the nucleolar organizer regions; consequently 5S rRNA is synthesized at sites in the nucleoplasm distinct from nucleoli (Warner 1990, Scheer & Weisenberger 1994).

Much of the analysis of 5S rRNA transport has been accomplished by using *X. laevis* oocytes as a model system, but as explained below, the oocyte pathway is highly specialized and more complicated than the somatic cell pathway. Therefore, we discuss the oocyte experiments first, followed by what is known about the pathway in somatic cells, and attempt to highlight general features that relate to both cell types.

Overview of 5S rRNA Transport in X. laevis Oocytes

X. laevis contains two types of 5S rRNA genes, the oocyte and the somatic, which are differentially expressed during development and differ by only six nucleotide substitutions (Wolffe & Brown 1988). During oogenesis, oocyte-type 5S rRNA is expressed before the other ribosomal components (Mairy & Denis 1971) and migrates to the cytoplasm, where it is found in one of two storage particles, the 7S and 42S RNPs. The 42S particle is a mixture of 5S rRNA, tRNAs, and several proteins, while the 7S particle is composed of a 1:1 ratio of 5S rRNA and the transcription factor TFIIIA (reviewed in Tafuri & Wolffe 1993). This composition remains the case until the onset of vitellogenesis, when synthesis of the other ribosomal components commences. At this

stage the concentration of the storage particles decreases (Dixon & Ford 1982), and 5S rRNA rapidly localizes to the nucleoli of the oocyte nucleus, where it becomes incorporated into large ribosomal subunits (Allison et al 1991). These subunits are then exported to their site of function in the cytoplasm. Thus, the oocyte 5S rRNA pathway is unique in that two distinct nuclear export events occur and can be summarized as follows: transcription in the nucleus, export to the cytoplasm as a 7S or 42S storage particle, nuclear import, nucleolar localization, and re-export in the context of the large ribosomal subunit. Perhaps because of this complex, bidirectional transport pathway, 5S rRNA is the only RNA examined for which nuclear export cannot be completely saturated (Jarmolowski et al 1994).

NUCLEAR EXPORT OF 5S rRNA IN XENPOUS OOCYTES *Trans-acting factors* Three proteins are known to specifically bind to 5S rRNA: the La protein, transcription factor IIIA (TFIIIA), and ribosomal protein L5. La protein, which transiently interacts with all pol III products, functions in pol III transcription termination (Rinke & Steitz 1982, Gottlieb & Steitz 1989). TFIIIA binds both to the 5S rRNA gene, where it facilitates transcription, and to 5S rRNA itself (Honda & Roeder 1980). The other known 5S rRNA-binding protein is ribosomal protein L5 (Chan et al 1987).

Pieler and colleagues first examined the relationship between 5S rRNA export and protein factors by immunoprecipitation of defined 5S RNP complexes from the nucleus and cytoplasm of fractionated oocytes (Guddat et al 1990). These experiments revealed that the La-5S RNP is confined to the nucleus at all times, thus excluding a role for La in nuclear export of 5S rRNA, and that RNP complexes containing either TFIIIA or L5 form initially in the nucleus and then migrate to the cytoplasm. Additionally, using 5S rRNA mutants, they found that as long as a given 5S mutant can interact with either TFIIIA or L5, then export to the cytoplasm occurs. However, mutants that no longer bind either protein are retained in the nucleus. These studies demonstrate that both TFIIIA and L5 have the capacity to export 5S rRNA to the cytoplasm.

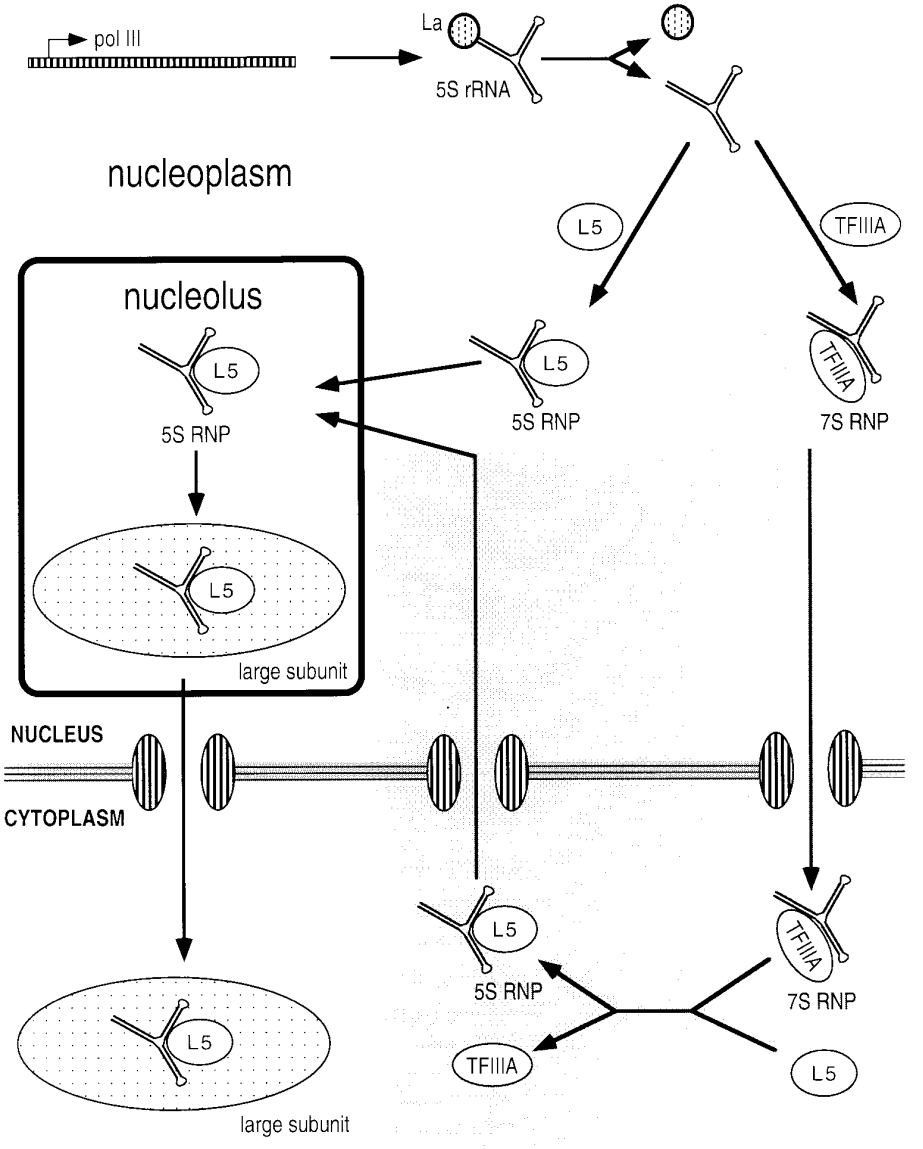
NUCLEAR IMPORT OF 5S rRNA IN *X. LAEVIS* OOCYTES Nuclear import of 5S rRNA is similar to import of karyophilic proteins in that it is temperature- and ATP-dependent and occurs through NPCs (Allison et al 1993). Analysis of 5S rRNA nuclear import in oocytes reveals that L5 plays a significant role in this process. Allison and colleagues have shown that during vitellogenesis, when production of ribosomal proteins begins, 5S rRNA is exchanged from the cytoplasmic 7S storage particle onto L5 and then enters the nucleus (Allison et al 1991, 1993). Additionally, it has been shown that 5S rRNA mutants are only imported into the nucleus if they retain L5-binding activity (Rudt & Pieler 1996).

A MODEL FOR 5S rRNA TRANSPORT IN OOCYTES The 5S rRNA transport pathway in *X. laevis* oocytes is quite complicated, principally because of the oocyte's strategy for stockpiling ribosomes within the egg for later use during embryogenesis. The picture that emerges *en toto* is that, in previtellogenic oocytes, oocyte-type 5S rRNA is transcribed, transiently associates with La protein, and is then bound by TFIIIA to form the 7S RNP. This RNP is then exported to the cytoplasm, where it exists as a storage particle until synthesis of other ribosomal components begins. Consistent with a role for TFIIIA in 5S rRNA export is the fact that the protein contains an NES similar to the Rev/PKI-type of NES and that the Rev NES conjugated to BSA efficiently competes for export of 5S rRNA (Fischer et al 1995, Fridell et al 1996). The storage particles remain in the cytoplasm until L5 becomes available and displaces TFIIIA to form the 5S RNP. This RNP is then rapidly imported into the nucleus, becomes concentrated within the nucleoli, and is assembled into large ribosomal subunits that are then exported to the cytoplasm. This model, summarized in Figure 3, can account for the fact that both TFIIIA and L5 mediate export of 5S rRNA.

5S rRNA Transport in Somatic Cells

The 5S rRNA transport pathway is simpler in somatic cells than in *X. laevis* oocytes. Using HeLa cells, Steitz and coworkers showed that after a transient, posttranscriptional association with La protein, 5S rRNA immediately binds L5 to form a particle that is a precursor to assembly of 5S rRNA into nascent ribosomal subunits within the nucleoli (Steitz et al 1988). Therefore nascent

Figure 3 Intracellular trafficking of 5S rRNA. 5S rRNA is transcribed in the nucleoplasm by RNA polymerase III and then briefly associates with La antigen, which functions in transcription termination. At this point the pathway as it is known in *X. laevis* oocytes appears to differ from the pathway that is operational in somatic cells. The area shaded in gray depicts the *X. laevis* oocyte-specific portion of the pathway: 5S rRNA is bound by TFIIIA, which also functions in its transcription, to form a 7S RNP that is then rapidly exported to the cytoplasm, where it is sequestered as the 7S storage particle. As the level of L5 accumulates in the oocyte, an exchange reaction occurs in which 5S rRNA is transferred to L5 to form the 5S RNP. Free TFIIIA, which is now competent for nuclear import, then presumably returns to the nucleus. The 5S RNP is rapidly imported into the nucleus and is subsequently concentrated within nucleoli, where it is assembled into nascent large ribosomal subunits. After complete assembly, the large ribosomal subunits are exported to the cytoplasm, where they function in translation. In somatic cells, which do not stockpile ribosomes for use later in development, the pathway is less complex. After disassociation from La, 5S rRNA immediately interacts with the L5 to form the 5S rRNP in the nucleoplasm. The RNP then localizes directly to the nucleolus, where it is incorporated into nascent large ribosomal subunits and then exported to the cytoplasm.


pol III
La
5S rRNA
nucleoplasm
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TFIIIA
nucleolus
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5S RNP
L5
5S RNP
TFIIIA
7S RNP
L5
large subunit
NUCLEUS
CYTOPLASM
L5
5S RNP
TFIIIA
7S RNP
L5
TFIIIA
L5
large subunit

5S rRNA does not shuttle out of and back into the nucleus in somatic cells but instead accumulates directly in the nucleoli as a complex with L5. Consistent with these findings is the fact that, despite considerable effort, the 5S rRNA-containing storage particles have not been found in the cytoplasm of somatic cells (e.g. see Honda & Roeder 1980).

What is common between the two pathways is the fact that transcription of the 5S genes occurs in the nucleoplasm and that the L5-5S RNP forms prior to ribosomal subunit assembly in the nucleolus, a point which prompted Steitz et al (1988) to suggest that L5 functions in part to deliver 5S rRNA to the nucleolus. In *X. laevis* this delivery pathway can begin in the cytoplasm, whereas in somatic cells the pathway is confined to within the nucleus (summarized in Figure 3). Recent work on the domain structure of L5 supports this model, as it was found that the 5S rRNA-binding and nucleolar localization domains are separable within the protein. Furthermore, L5 mutants that maintain 5S rRNA-binding activity cannot properly localize to the nucleolus when the nucleolar localization domain is interrupted, suggesting that an intact nucleolar localization domain of L5 is required for efficient accumulation of the L5-5S RNP within the nucleolus (Michael & Dreyfuss 1996). Once targeted to the nucleolus, the L5-5S RNP is probably assembled into nascent ribosomal subunits that are then exported to the cytoplasm. Therefore, the problem of 5S rRNA export in somatic cells can be reduced to the problem of ribosomal subunit export.

RIBOSOMAL RNA TRANSPORT

All rRNAs except 5S rRNA—namely 18S, 28S, and 5.8S rRNAs—are transcribed as a common precursor that is completely processed and assembled into ribosomal subunits, all within the nucleolus (Warner 1990, Scheer & Weisenberger 1994, Shaw & Jordan 1995). Therefore, nuclear export of these rRNAs occurs in the context of ribosomal subunits. Beyond the fact that ribosomal subunit export is a unidirectional, saturable process that requires energy and occurs through NPCs (Khanna-Gupta & Ware 1989, Bataillé et al 1990), very little is known about export of ribosomes.

TRANSPORT OF tRNA

Nuclear Export

PROCESSING EVENTS THAT INFLUENCE NUCLEAR EXPORT Transfer RNA is transcribed in the nucleus by RNA polymerase III, and like RNA polymerase II–transcribed mRNA, it is produced as a precursor tRNA. Pre-tRNA molecules are processed in the nucleus before transport to their functional location in the cytoplasm, although they are further modified in the cytoplasm. Nuclear tRNA

processing reactions comprise excision of 5′ and 3′ terminal sequences, addition of CCA to the 3′ end, base modifications, and for some tRNA molecules, removal of introns and base editing (reviewed in Deutscher 1995; Altman et al 1993, 1995; Westaway & Abelson 1995; Björk 1995).

Only tRNA molecules with mature termini and without introns are detected in the cytoplasm of *X. laevis* oocytes, suggesting that 5′ and 3′ terminal extensions and intron sequences prevent pre-tRNA export (Melton et al 1980, Zasloff et al 1982a; reviewed in Westaway & Abelson 1995). It has been suggested that the process of splicing is coupled to transport through the NPC, because in yeast, components of the splicing process (an endonuclease and a ligase) appear to be localized in close apposition to NPCs, and mutations in certain NPC proteins result in accumulation of unspliced tRNA (Peebles et al 1983, Clark & Abelson 1987, Sharma et al 1996; reviewed in Westaway & Abelson 1995). However, the block in pre-tRNA splicing in NPC protein mutants may be secondary to a defect in the nuclear import of a tRNA splicing factor. In addition, microinjection of large amounts of pre-tRNA into the nucleus of *X. laevis* oocytes results in the appearance of unspliced pre-tRNA in the cytoplasm, suggesting that tRNA splicing is not coupled to the export process (Boelens et al 1995).

CIS-ACTING AND *TRANS*-ACTING tRNA EXPORT FACTORS Zasloff and coworkers defined the critical regions of human tRNAmet for nuclear export in *X. laevis* oocytes. Although point mutations in many regions of the molecule perturb transport, the most drastic effects are caused by mutations in the most highly conserved regions of tRNA, the D and T stem loops (Santos & Zasloff 1981, Zasloff et al 1982a,b, Tobian et al 1985). The most critical regions for transport also appear to be the most important regions for tRNA transcription, processing, and ribosome binding (see discussion of Tobian et al 1985). All transport mutants described so far are also defective in processing, suggesting that a processing factor also serves as an export factor or, more likely, that the same structural features of the tRNA molecule are recognized by both processing and transport machineries (Zasloff et al 1982a, Tobian et al 1985).

Different tRNA species competitively inhibit the export of each other when microinjected into *X. laevis* oocyte nuclei, demonstrating that different tRNAs share a common rate-limiting factor(s), which likely recognizes the highly conserved stem loops D and T (Zasloff 1983). The glycolytic enzyme glyceraldehyde 3 phosphate dehydrogenase has been identified as a factor that binds to wild-type tRNAs but not to transport-defective mutants, although a function for this enzyme in tRNA export has not been described (Singh & Green 1993). It has recently been suggested that some transport-defective mutant tRNAs may be defective, not because they fail to interact with proteins of the transport machinery, but rather because they are retained in the nucleus by interaction with

nuclear components (Boelens et al 1995). This is unlikely to be the case for all transport-defective tRNA mutants, so some of them should be of use in the identification of tRNA export factors.

THEMES AND PERSPECTIVES

A common theme that emerges from the mechanistic information on nucleocytoplasmic trafficking of RNA is that transport of possibly all RNA classes, in both directions across the NPC, is mediated largely by signals on proteins that are associated with the RNA (Figure 4). Thus, mRNA export is most likely mediated, at least in part, by NESs of the shuttling hnRNP proteins such as the M9 NES of hnRNP A1; U snRNA export is mediated, at least in part, by CBC (whose NES remains to be identified); and 5S rRNA export is mediated by L5 (whose NES remains to be identified) and TFIIIA (which bears a leucine-rich NES similar to that of HIV-1 Rev protein) (Figure 4). The factors that mediate rRNA and tRNA export are still obscure.

Nuclear import of U snRNAs, 5S rRNA (in oocytes), and viral RNAs is also largely protein mediated. The nuclear import signal of U snRNAs is a composite signal consisting of proteins that associate with the RNA and the 5′ terminal m_3G cap. This signal accesses a different import pathway than that used by classical NLS-containing proteins. In contrast, import of 5S rRNA in oocytes appears

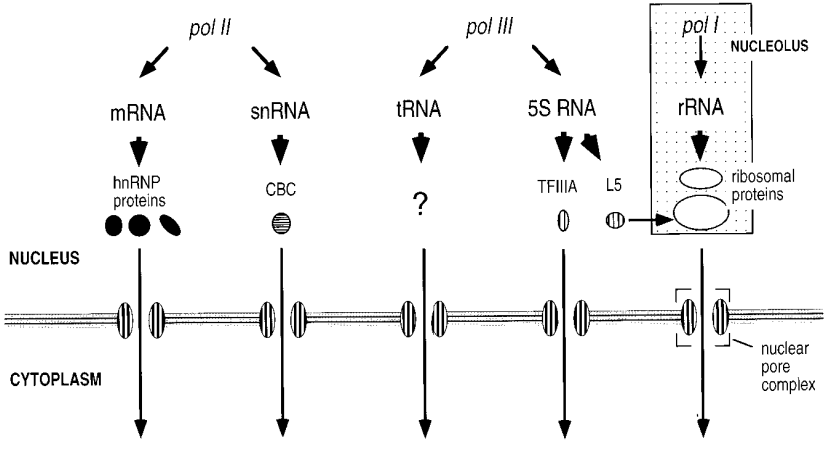

Figure 4 RNA export from the nucleus into the cytoplasm is mediated by RNA-binding proteins. Proteins that specifically interact with each class of RNA carry the signals for RNA transport to the cytoplasm. Messenger RNA export is most likely mediated by shuttling hnRNP proteins; U snRNA export is mediated by the cap-binding complex (CBC); and 5S rRNA export is mediated by TFIIIA and L5. Factors involved in tRNA and rRNA transport remain to be identified.

to be mediated exclusively by the L5 protein via the classical NLS pathway. Viral RNA import is also, in some cases, mediated by proteins that utilize the cellular classical NLS import pathway (HIV-1 MA and influenza virus NP) or by proteins whose import pathway has not yet been characterized (HIV-1 Vpr).

In most cases, RNA-binding proteins dictate the transport pathway taken by a particular RNA molecule. However, the sequence of the RNA molecule, in turn, determines the complement of proteins with which it will interact, so the pathway taken is ultimately specified by RNA sequence.

This review has focused on transport of RNA molecules between the nucleus and the cytoplasm. However, once exported to the cytoplasm, some mRNAs (and probably other classes of RNA that function in the cytoplasm) are transported and anchored to very precise subcytoplasmic locations. This targeting is essential in processes as diverse as development of the embryo and neuronal cell function, and the molecular mechanisms appear to follow a principle similar to that of nucleocytoplasmic transport of RNA molecules. Transport and anchoring of mRNAs within the cytoplasm is also mediated by proteins that interact with the RNA (reviewed in Ding & Lipshitz 1993, Wilhelm & Vale 1993, Singer 1993, Okita et al 1994, Stebbings et al 1995, St Johnston 1995a,b, Micklem 1995, Steward 1995). This basic principle of RNA transport is likely to be of considerable generality.

Our understanding of cellular RNA transport pathways is at an early and exciting stage. For most RNA classes, known mediators/effectors of transport are few, but for mRNA export, at least, potential mediators are plentiful, and a number of these may become bona fide export factors with more extensive investigation. Immediate questions, such as the number and diversity of protein NESs and protein NLSs that mediate RNA export and import, seem quite tractable. Transport of each type of RNA may very likely involve both pathway-specific components and components that are shared with other pathways. The identification of points of pathway convergence should also be possible. In addition, regulatory mechanisms of mRNA export, as exemplified by viruses, have yet to be characterized for cellular transcripts. However, with progress in our understanding of viral regulation of mRNA export, cellular regulatory mechanisms (for example, those required for export of alternatively spliced transcripts) are expected to be uncovered.

Because the detailed molecular mechanisms by which RNA molecules move from their site of synthesis to the NPCs, and by which they are translocated through the NPCs, remain mysterious, we are currently left to speculation. We do know that there are energy-consuming steps, which may include intranuclear transport of RNA and, most likely, translocation through the NPC. Maybe translocation is motor protein driven, which would certainly account for part of the energy requirement. If the Ran GTPase cycle is directly involved in RNA

export, this will also account for some of the energy consumption. It will be fascinating to learn how directionality is imposed on RNA-protein complexes as they move through the NPC, and how shuttling components of the transport machineries that move with them through the NPC are recycled.

A major tenet of RNA transport, that it is mediated by proteins associated with the RNA, has been firmly established. With this understanding, a more detailed knowledge of these fundamental transport pathways is expected in the near future.

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