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***Note added in proof:**

AXELROD, J. *et al.* (1996) *Science* 271, 1826–1832 have recently reported that DISHEVELLED antagonizes NOTCH activity in a WG-dependent manner, identifying another branch-point in the WG pathway.

Signal-mediated nuclear export pathways of proteins and RNAs

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Although it has been known for several years that most nuclear-encoded RNAs and some proteins can be exported from the nucleus to the cytoplasm, the molecular mechanisms of these transport processes have been poorly understood. Recently, signals that can induce the rapid and active nuclear export of macromolecules have been identified in the HIV-1 Rev protein, the inhibitor of cAMP-dependent protein kinase (PKI) and the hnRNP A1 protein. Thus, nuclear export appears to be mechanistically similar to nuclear import in that it requires specific signal-receptor systems.

The nuclear envelope encloses most of the cellular DNA and divides the process of gene expression – transcription and maturation of RNA molecules occur mostly in the nucleus, whereas translation occurs in the cytoplasm. To integrate these different activities, the cell must employ an elaborate and specific transport system that allows regulated bi-directional transport of a plethora of different macromolecules between the nucleus and the cytoplasm. This nucleocytoplasmic trafficking of molecules

occurs through the nuclear pore complex (NPC)¹. NPCs form gated channels in the nuclear envelope that allow passive diffusion of small molecules with apparent molecular masses of less than 40 kDa. Larger molecules, such as most proteins, RNAs and RNA-protein complexes (RNPs), however, need to be translocated through the NPC by a process that requires energy. Some of the components and requirements for the nuclear import of proteins have been identified recently. Most proteins that are imported into the nucleus contain short basic peptide sequences that function as nuclear-localization sequences (NLSs)¹. In the cytoplasm, the NLS is recognized by the NLS receptor complex importin $\alpha\beta$, which docks the NLS-containing substrate to the cytoplasmic side of the NPC. Subsequently, the docked complex is translocated across the NPC into the nucleus by a process that requires multiple interactions with components of the pore and the hydrolysis of GTP by the Ran GTPase^{1,2}.

Recent work has demonstrated that some proteins not only enter the nucleus but also can be re-exported to the cytoplasm. Moreover, most nuclear-encoded RNAs, such as mRNAs, tRNAs, U snRNAs and ribosomal RNAs, are exported post-transcriptionally to the cytoplasm, probably in the form of RNPs. The export of all these different RNA species is energy dependent and factor mediated. Competition experiments in *Xenopus laevis* oocytes indicated that each RNA class requires specific as well as common factors for its export³. However, the nature of these factors and how they facilitate export are largely unknown. In particular, until recently, it was not clear whether specific signals mediate the export of proteins and RNAs or whether the export of these molecules is a default process that is regulated only by nuclear retention. Here, we summarize recent findings that demonstrated the existence of specific nuclear-export signals (NESs) in the RNA-binding proteins hnRNP A1 and HIV-1 Rev, as well as in the inhibitor of cAMP-dependent protein kinase (PKI). These findings support a view that NESs of RNA-binding proteins provide the crucial recognition as well as the nuclear-export capacity for protein-based transport of most, if not all, cellular RNA.

An NES in hnRNP A1

Transport of mRNA from the nucleus to the cytoplasm is integrated with the processing of RNA in that removal of introns from pre-mRNAs is normally a prerequisite for export because binding of spliceosomal factors actively retains pre-mRNAs in the nucleus⁴. Throughout their nuclear residency, pre-mRNA/mRNA molecules are associated with a set of about 20 abundant RNA-binding proteins collectively termed heterogeneous nuclear ribonucleoprotein complex proteins (hnRNP proteins)⁵. Several lines of evidence are consistent with the idea that, after splicing, mRNAs are transported to the cytoplasm by virtue of their association with hnRNP proteins. This evidence includes the fact that some hnRNP proteins, for example hnRNP A1, shuttle rapidly and continuously between the nucleus and cytoplasm and are bound in both compartments to mRNA (Ref. 6). Furthermore, immunoelectron microscopy experiments have demonstrated directly that an A1-like protein is associated with a specific mRNA during translocation through the NPC into the cytoplasm⁷. Although these data demonstrate that some hnRNP proteins move with mRNA during nuclear export, they cannot distinguish between a cargo or carrier role in the export process. However, recent studies on the signal within A1 that mediates its shuttling lend considerable support to a carrier role for these proteins.

Subcellular transport of A1 is determined by a 38-amino-acid sequence, termed M9, located near the C-terminus of the protein (Fig. 1). M9 was originally identified as a unique class of NLS, as it functions as a transport signal in nuclear import but bears no sequence similarity to the classical NLSs contained within the SV40 large T antigen or nucleoplasmin⁸. Heterokaryon shuttling experiments have recently shown that M9 is also a positively acting, transferable NES, as fusion of M9 to a protein that is normally retained in the nucleus activates its nuclear export. M9, therefore, defines a novel class of transport signal in that it has the capacity to mediate both nuclear import and export of heterologous proteins⁹. Limited mutational analysis indicates that the M9 NLS and NES cannot be separated, as mutations that block NLS activity also abolish NES activity. Assuming that these signals are in fact contiguous, an interesting problem arises as to how the import and export activities are regulated to give efficient A1 trafficking – i.e. export in the context of mRNPs and import as free protein. A simple solution to this problem could be the existence of two related M9 receptors with similar M9-binding domains yet different subcellular localizations. The nuclear envelope would then serve to compartmentalize the activities of these factors in such a way that the nuclear form mediates export to the cytoplasm and the cytoplasmic form mediates import into the nucleus. Alternatively, a single receptor may utilize compartment-specific accessory factors to accomplish directionality.

Other hnRNP proteins, including A2 and B1, also contain M9 domains. It is attractive, therefore, to speculate that nuclear mRNA molecules are exported to the cytoplasm by virtue of their association with M9-bearing proteins. However, M9 is probably not the

M9

A1 268–305	NQSSNFGPMKGGNFGGRSS--GPYGGGGQYFAKPRNQ--GGY
A2/B1 296–335	QQPSNYGPMKSGNFGGSRNMGGPYGGG--NYGPGGSGGSGGY

Leucine-rich

PKI 37–46	LALK-LAGLDI
HIV Rev 73–81	LPPLERLTL
TFIIIA 330–338	LPVLENLTL
Visna Rev 106–114	MVGMENLTL
EIAV Rev 34–51	GPLESGQWCRVL-RQSLPE

FIGURE 1

Sequences of nuclear-export signals (NESs). The top part of the figure shows the M9 sequence near the C-terminus of hnRNP A1 that functions as both a nuclear-localization sequence (NLS) and NES, and a related sequence in hnRNPs A2 and B1. The bottom part of the figure lists the leucine-rich NESs of viral Rev proteins, the inhibitor of cAMP-dependent protein kinase (PKI) and transcription factor TFIIIA of *Bufo americanus*.

only NES contained within transported mRNPs; other shuttling hnRNP proteins, for example E, I and K, do not contain M9-like sequences and may therefore contain distinct NES types. Preliminary data demonstrating a unique NES in hnRNP K support this view. Involvement of multiple protein-based NESs in mRNA export may indeed be necessary for transport of this large and structurally diverse class of RNA molecules.

HIV-1 Rev is an RNA-export factor that contains an NES

The human immunodeficiency virus type 1 (HIV-1) encodes a regulatory protein, termed Rev, that is essential for the expression of viral structural proteins¹⁰. These proteins are encoded by a set of incompletely spliced viral mRNAs (equivalent to intron-containing mRNAs) that are sequestered in the cell nucleus in the absence of Rev. Rev induces the export and hence the translation of these intron-containing viral mRNAs upon binding to their Rev-response element (RRE). These findings, together with the observation that, like hnRNP A1, Rev shuttles between the nucleus and the cytoplasm¹¹, suggested that Rev may be a nuclear export factor for RNA.

Two essential domains for the functions of Rev have been defined by mutational analysis. The N-terminal region contains sequences that are required for RRE binding, nuclear localization and multimerization of Rev, while the C-terminus contains an ~10-amino-acid leucine-rich sequence termed the activation domain. The activation domain, which is dispensable for RRE binding, has been suggested to interact with a cellular factor and mediate the transport of the protein with its associated, unspliced RRE-containing viral mRNAs to the cytoplasm¹⁰.

There has been considerable controversy as to the mechanism of action of Rev – specifically whether

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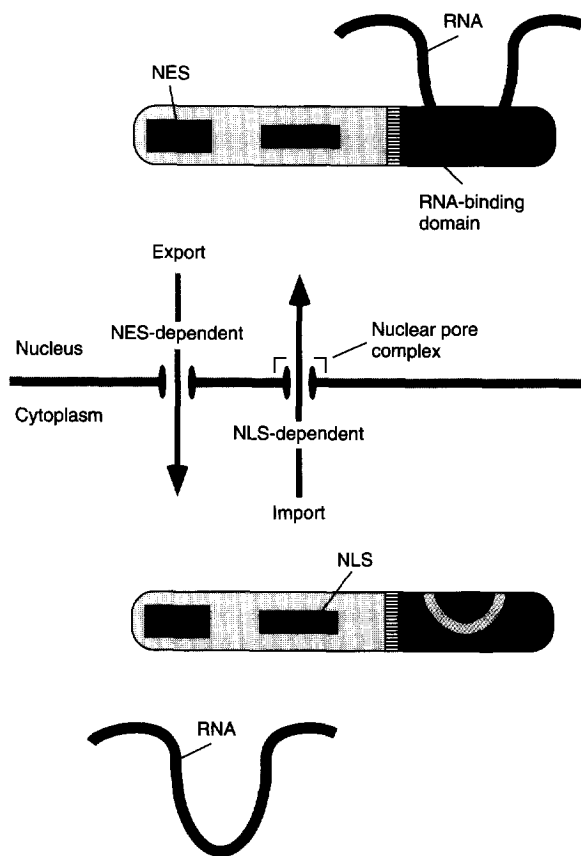


FIGURE 2

A general model of protein-mediated RNA export from the nucleus to the cytoplasm. RNA-binding proteins mediate export of nuclear RNA to the cytoplasm by virtue of the nuclear-export signals (NESs) contained within them. Shuttling proteins such as hnRNP A1 and HIV-1 Rev associate with their cognate RNAs in the nucleus, and the protein-based NES then promotes transport of the RNA-protein complex (RNP) to the cytoplasm. Once in the cytoplasm, the RNA dissociates from the transporting RNA-binding protein, which is then rapidly reimported into the nucleus by means of its nuclear-localization sequence (NLS) to begin another transport cycle. NLSs and NESs can be separate signals, as in HIV-1 Rev, or they can be interdigitated, as in hnRNP A1.

Rev induces export by releasing the viral mRNA from its nuclear retention (i.e. by stripping retention factors from the viral mRNA) or by directly targeting the viral mRNA to a cellular export pathway¹⁰. Evidence for the latter mechanism has been provided by microinjection experiments in *Xenopus* oocytes, which demonstrated that Rev induces the rapid nuclear export of a variety of RRE-containing RNAs, even if they lack introns¹². Rev-mediated export could be saturated by peptides comprising the Rev activation domain coupled to bovine serum albumin (BSA). The activation domain alone is sufficient, therefore, to interact with and titrate out an essential export factor. Surprisingly, activation domain peptides were found to be necessary and sufficient to induce rapid nuclear export of the coupled BSA. These experiments defined, therefore, the activation domain of Rev as an NES that accesses a specific nuclear export pathway^{13,14} (Fig. 1). Other complex retroviruses [e.g. human T-cell

leukaemia virus type I (HTLV-I), Visna virus and equine infectious anaemia virus (EIAV)] encode proteins that are required for the export of their unspliced mRNAs and are thus functionally equivalent to Rev¹⁰. Most, if not all, of these viral proteins contain an NES and are likely to function by a mechanism similar to that demonstrated for the Rev NES^{13,15}.

Using a microinjection strategy similar to that described above for Rev, Wen and co-workers identified a leucine-rich NES in PKI that is very similar to the Rev NES and, therefore, probably accesses the same export pathway¹⁴ (Fig. 1). However, unlike hnRNP A1 and Rev, PKI does not seem to bind to RNA. Rather, PKI induces, upon association, the rapid nuclear export of the catalytic subunit of cAMP-dependent protein kinase. Thus, NESs may be required not only to target RNAs from the nucleus to the cytoplasm but also to mediate the export of a variety of other macromolecules, including proteins.

NES-interacting proteins

While factors that interact with the NES of hnRNP A1 are not yet known, proteins that bind specifically to the NES of Rev, and may therefore represent the export receptor, have been identified recently in yeast and mammalian cells¹⁶⁻¹⁹. In the yeast two-hybrid system, two different but related nuclear proteins have been shown to interact specifically with the Rev NES: the yeast Rev-interacting protein (Rip) and the mammalian Rev/Rex activation domain binding protein (Rab; also called hRip). A hallmark of both proteins is that they contain a large number of FG dipeptide repeats, which are also frequently found in certain NPC proteins (nucleoporins). Overexpression of Rab enhances Rev-mediated export, indicating that Rab is involved in this export process. By UV-crosslinking techniques, the eukaryotic initiation factor 5A (eIF-5A) has also been shown to bind specifically to the Rev NES (Ref. 20). eIF-5A mutants that retain their NES-binding activity inhibited Rev-mediated export in a *trans*-dominant fashion, indicating an involvement of this factor in Rev-mediated export as well. Currently, it is premature to propose a detailed model of how these NES-interacting factors may mediate Rev-induced export since definitive proof for a direct function of any of them has yet to be obtained.

A unified model for nuclear export of RNAs

The findings that the RNA-binding proteins hnRNP A1 and Rev contain positively acting NESs suggest that not only proteins but also many, and possibly all, RNA species that are exported from the nucleus exit by virtue of specific interactions with NES-containing proteins (Fig. 2). Saturation of Rev-mediated export by NES peptides coupled to BSA also inhibits the export of 5S rRNA and spliceosomal U snRNAs¹³. This implies that 5S rRNA and U snRNAs bind prior to their nuclear export to proteins that have a Rev/PKI-type (leucine-rich) NES. Indeed, transcription factor IIIA (TFIIIA), which associates with 5S rRNA in the nucleus, contains a sequence very similar to the Rev/PKI NES that can also function as an NES^{13,21}. It will be interesting to test whether

TFIIIA mediates the export of 5S rRNA in a manner analogous to Rev-mediated viral mRNA export. A cap-binding complex (CBC) consisting of two proteins, CBP 20 and CBP 80, has recently been shown to be directly involved in U snRNA nuclear export²². It remains to be shown whether CBC also contains an NES that mediates U snRNA export and acts, therefore, in a way similar to Rev. Alternatively, CBC could interact with additional factors that contain NESs. Export of cellular mRNA, tRNA and ribosomal RNAs in the form of preribosomal complexes does not compete with Rev-mediated export, suggesting that these molecules are exported by distinct NES(s)¹³. Interestingly, the hnRNP A1 NES, which is thought to participate in nuclear export of cellular mRNA, has no sequence similarity to the Rev/PKI NES, and appears to access a different export pathway. Therefore, it seems that at least two, and probably even more, distinct signal types exist for mediating nuclear export of RNAs and proteins.

Are the import and export processes mechanistically similar?

Although it is too soon to propose a comprehensive model for NES-mediated export, some interesting mechanistic parallels to nuclear import of macromolecules are apparent. For instance, both the import and export processes are energy dependent and mediated by relatively short, positively acting signals that access specific transport pathways. One of the putative Rev cofactors may act like the import receptor complex importin $\alpha\beta$ in recognizing the transport signal and targeting the cargo to the NPC. Thus, recognition of a signal on the transport cargo by a receptor seems to be a common step in nuclear transport. Furthermore, it is possible that translocation through the NPC in both directions occurs by similar mechanisms. Translocation of proteins from the cytoplasm to the nucleus has been suggested to occur via repeated interactions with nucleoporins that contain FXFG repeats^{2,23}. Similarly, the repeated association of leucine-rich NES-containing proteins or RNA-protein complexes with Rip/Rab-type nucleoporins could account for translocation from the nucleoplasm to the cytoplasm.

While at least some of the signal-recognizing molecules for import and export are likely to differ, it is possible that other factors are common for transport in both directions across the NPC. One candidate that could fulfil such a dual role may be the Ran GTPase. Ran is an essential translocation factor for protein import and genetic evidence has been obtained in yeast that it may also be involved in the export of mRNAs and U snRNAs^{1,3,24}. However, unlike the case for protein import, so far there is no indication that Ran is involved in the translocation step of export. Ran may act, therefore, at a step prior to docking onto the NPC, probably at the release of RNA from nuclear retention sites or by transferring RNA from its site in the nucleus to the NPC. Given the similarities described above between import and export, it will be of great interest to analyse whether the processes are actually interconnected and whether additional factors are shared by both transport processes.

Future prospects

The results discussed here have shown that the export of some proteins and RNAs is dependent on protein-based NESs. Moreover, they suggest that export of many, and probably all, RNA molecules may be accomplished by RNA-binding proteins that bear NESs (Fig. 2). Identification of additional proteins directly involved in the export of cellular and/or viral RNA species will help to clarify this issue. Furthermore, with the identification of additional cofactor(s) for nuclear export, the detailed mechanism of nuclear export should soon be uncovered. An additional area of future research will certainly be analysis of the mechanism by which NES-containing proteins are removed from RNA after nuclear export to become available for another transport cycle.

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