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Signal-mediated nuclear export of proteins and RNAs

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The characterization of nuclear export signals (NESs) in a number of rapidly shuttling proteins revealed the occurrence of signal-dependent transport of proteins from the nucleus to the cytoplasm. Although passage of small proteins (<40kDa) through nuclear envelope pores can occur by passive diffusion, the import of larger proteins, as well as RNAs, is temperature- and energy-dependent. NES-mediated nuclear export, like nuclear import, is an active process and is much faster that the slow nuclear egress of proteins lacking an NES element. Analysis of the trafficking pathway of proteins shuttlling back and forth between the nucleus and the cytoplasm led to the identification of two types of specific signals for nuclear export. One is present in hnRNP A1 (heteronuclear ribonucleoprotein particles) and functions also as a nuclear import signal. The other one is a hydrophobic-rich short amino-acid sequence (usually leucine) of defined spacing and was originally described in the cAMP-dependent protein kinase A inhibitor (PKI). After appropriate stimulation and diffusion into the nucleus of the catalytic subunit of cAMP-dependent protein kinase (c-PKA), the binding of this subunit to PKI triggers rapid nuclear export of the complex (PKI/c-PKA).

Such a mechanism based on nucleocytoplasmic distribution changes also controls the regulation of NF-kappa B (NF-κB), which plays a key role in the activation of a wide variety of cellular genes. I kappa B alpha (IκBα) tightly regulates the transcriptional activity of NF-κB by retaining it in the cytoplasm in an inactive form. Exposure of cells to signals which activate NF-κB results in rapid degradation of IκBα followed rapidly by the appearance of newly synthesized IκBα in the nucleus. Concomitant to this accumulation in the nucleus, there is a progressive reduction of both NF-κB-DNA binding and NF-κB -dependent transcription along with a decrease in the amounts of nuclear NF-κB subunits. These findings are compatible with the notion that nuclear NF-κB interacting with newly synthesized IκBα would be exported from the nucleus. In support of this view, we reported that IκBα, when expressed in the nuclear compartment, not only abrogates NF-κB /DNA interactions and NF- κB -dependent transcription, but also transports NF-κB back to the cytoplasm. This function of IκBα is insured by a nuclear export sequence located in the C-terminal domain of IκBα which is homologous to the previously described NES found in PKI. Moreover, the ability of the IκBα -NES to mediate rapid nuclear export of linked proteins when microinjected into the nucleus of *Xenopus* oocyte demonstrates that this sequence is necessary and sufficient for the rapid export of proteins from the nucleus. Furthermore, in cells pulsed with TNF, a treatment which favors nuclear accumulation of newly cellular synthesized IκBα, expression of a reporter protein fused to IκBα NES motif, led to sustained accumulation of nuclear NF- κB lacking DNA-binding activity. This NES-mediated accumulation of inactive nuclear NF-κB is probably the consequence of an interference with the IkBa-mediated export of NF-κB. Thus, IκBα-dependent nuclear export may represent an important mechanism for the control of the expression of NF- κB -dependent genes.

Nucleocytoplasmic transport of genomic RNA is essential for the replication of retroviruses including Human immunodeficiency virus-1 (HIV-1). For HIV-1, this process is controlled by the HIV-1 shuttling protein Rev which contains a leucine-rich NES. Rev promotes specific transport of incompletely spliced and genomic viral RNAs to the cytoplasm through its RNA-binding activity and NES sequence via a cellular export pathway. Our goal was to see if

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nuclear IκBα could interfere with the Rev-NES-mediated export pathway of viral RNAs, thus inhibiting HIV-1 replication. We found that accumulation of IkBa in the nucleus inhibits viral replication by specifically blocking the expression of Rev-dependent genes. This effect is independent of the transcriptional inhibitory activity of IκBα and required a functional IκBα NES motif. Our findings indicate that IκBα and Rev compete for the same nuclear export pathway and suggest 1) that nuclear accumulation of IκBα, which would normally occur during physiological cell activation, may interfere with the export of viral RNA leading to non productive infection and 2) alternatively, that an ongoing HIV-1 infection may have deleterious consequences on IκBα - mediated export of NF-κB. The Rev NES can competively inhibit the export of 5S ribosomal RNA (TFIIIA-NES dependent) without affecting mRNA metabolism and can be functionally substituted by related NES motifs found in several cellular proteins (PKI, IκBα, Mitogen-activated protein kinase kinase (MAPKK) and Ran-binding protein 1 (RanBP1)). One can predict from this that saturation of the NES export pathway reflects the titration of a transport mediator common to this class of protein. The reported candidates for this function were the yeast protein Rip1 and the related human hRip/Rab proteins. Leucine-rich NES bind to both proteins and also interacts with Phe-Gly (FG)-repeats of several nucleoporins in two hybrids assays. In addition, Rip/Rab proteins were shown to contain multiple FG dipeptide motifs reminiscent of nucleoporins. However, direct binding between recombinant Rev and either purified Rip/Rab proteins or recombinant FG repeats has not been demonstrated.

A new perpective on the question emerged recently with the characterization of CRM1 (Chromosome maintenance region 1) as an essential mediator for nuclear export. Human CRM1 is an evolutionarily conserved protein that shares sequence similarities with the karyopherin beta family of proteins involved in the nuclear import pathway and was shown to interact with FG-repeat of nucleoporin CAN/nup214. We have shown that CRM1 form a complex with the leucine-rich NES, but not with the mutated, non-functional NES. This interaction is specifically inhibited by leptomycin B, a drug that prevents the function of the CRM1 protein in yeast. Leptomycin B was also suggested to inhibit the NES-mediated transport of Rev protein and ultimately the HIV-1 replication in human monocytes. This indicated that leptomycin B specifically inhibits the nuclear export of proteins which is mediated by the NES. To analyze the role of the CRM1-NES interaction in nuclear export, a transport assay based on semi-permeabilized cells was developed. In this NES-, cytosol-, and energy-dependent nuclear export reconstitution system, we have shown that leptomycin B specifically blocked export of NES-containing proteins. Our results and others data indicate that CRM1 protein can act as a receptor involved in NES-dependent nuclear export.

The energy-dependent nucleocytoplasmic transport proceeds through the requirement for the small GTPase Ran protein. CRM1 shares a sequence motif related to the Ran-GTP-binding site of Ran-GTP-binding proteins and both p10 and Ran GTP, but not Ran-dependent GTP hydrolysis appears to be required in NES-mediated protein export. By analogy with the nuclear import process, Ran or Ran-binding protein may also regulate the interaction of CRM1-NES-containing protein complexes with the nuclear pore complex (NPC) before translocation out of the nucleus. This model is supported by the recent observation that CRM1 binds to Ran GTP in a wild-type NES-dependent way and that this cooperative binding is sensitive to leptomycin B. Finally there is now compelling evidences drawing a picture where translocation through the NPC in both directions occurs by similar mechanisms. Identification of additional proteins directly involved in the NES-mediated export will help to explore this issue.