ROLE OF CRM1 IN SIGNAL-MEDIATED NUCLEAR PROTEIN EXPORT

OSSAREH-NAZARI Batool[1], BACHELERIE Françoise[2] and DARGEMONT Catherine[3]

Institut Curie,CNRS UMR 144, 26 rue d'Ulm, 75248 Paris, France[1,3], Institut Pasteur, Unité d'Immunoligie Virale, 75015 Paris, France

Leucine-rich amino acid sequences responsible for efficient protein nuclear export (NES) have recently been identified in an increasing number of proteins, in particular, human immunodeficiency virus- type 1 Rev protein, the protein kinase A, and the inhibitor IkappaBα (IκBα). To analyze the molecular mechanisms govering NES-dependent nuclear protein export, we developed an assay that reconstitutes nuclear export *in vitro*. Hela cells were transiently transfected with cDNAs encoding fusion proteins consisting of Myc-tagged pyruvate kinase, wild-type or mutated IκBα NES, and SV40 large T antigen NLS (NLS-PK-NES and NLS-PK-NES mut, respectively). Cells were then treated with digitonin to permeabilize the plasma membrane without affecting the integrity of the nuclear envelope. Export of NLS-PK-NES and NLS-PK-NESmut were analyzed under different incubation conditions by indirect immunofluorescence and western blot. In this assay nuclear export was found to be NES, extracts and energy dependent. The replacement of total extracts by the recombinant proteins required for nuclear import (karyopherins, Ran/TC4, and p10) promoted the nuclear import of a karyophilic substrate, but did not induce the nuclear export of NLS-PK-NES, indicating that an essential component for nuclear export was provided by the total extracts. To identify this component, we used a drug, leptomycin B, shown to inhibit HIV Rev export *in vivo* . Leptomycin B was able to block NES-dependent protein export in the vitro assay, suggesting that this drug inhibits a component involved in nuclear export. In Schizosaccharomyces pombe, the target of leptomycin B is the CRM1 protein whose human homolog has recently been identified. CRM1 is a nuclear protein that shares sequence homology with karyopherin B familly. We cloned human CRM1 and found that in vitro translated CRM1 was able to bind specifically NES sequence. The CRM1-NES interaction was inhibited by leptomycin B in dose dependent manner. These data indicate that CRM1 acts as a NES receptor in protein nuclear export. Like the karyopherin B familly, CRM1 is able to bind Ran-GTP in a cooperative way with NES. By analogy with the nuclear import process, Ran or a Ran-binding protein may also regulate the interaction of CRM1-NES-containing protein complexes with the nuclear pore complex.

NUCLEAR IMPORT OF GLYCOCONJUGATES

DUVERGER Eric, MEUNIER Laurent, MAYER Roger, ROCHE Annie-Claude, MONSIGNY Michel

Glycobiologie, Centre de Biophysique Moléculaire, CNRS, Rue Charles Sadron, 45071 OrlEans, France

The nuclear import of proteins larger than Mr 40,000 into the nucleus is a highly selective processs (see for a review, G^rlich D. (1997), *Curr. Op. in Cell Biol.*, 9, 412-419), in many cases it is depending on a short sequence mainly containing basic amino acids, which is called nuclear localization signal (NLS). The nuclear import of NLS-bearing proteins is energy dependent, is inhibited by wheat germ agglutinin which binds *N*-acetyl-glucosamine-bearing proteins of the nuclear pore and, requires cytosolic factors sensitive to alkylation by *N*-ethylmaleimide. In addition, another import signal has been identified for instance, the M9 domain of hnRNPA1 protein (Mickaël *et al.* (1995), *Cell*, **83**, 415-422) does not compete with NLS-bearing proteins for import and is recognized by distinct receptors (Pollard *et al.* (1996), *Cell*, **88**, 985-994).

We showed that glycosylated proteins, which lack such a peptidic NLS, after their introduction into the cytosol by diffusion upon or permeabilization with digitonin, entered the nucleus in a sugar-dependent manner. Bovine serum albumin substituted with β-di-*N*-acetylchitobiosides or α-D-glucosides were efficiently transported from the cytosol to the nucleus in both a time and an ATP-dependent process. In contrast with the peptidic NLS based import, the nuclear import of neoglycoproteins neither required added cytosolic factors nor was sensitive to S-alkylation of thiol groups with *N*-ethylmaleimide. Moreover, upon incubation in the presence of a large excess of peptidic NLS-bearing proteins, the nuclear import of neoglycoproteins was not inhibited (Duverger *et al.* (1995), *J. Cell Sci.*, **108**, 1325-1332). More recently, we confirmed these results by showing that, upon microinjection into the cytosol, fluorescent neoglycoproteins were imported to the nucleus in a sugar dependent manner. These results suggest that sugars, thanks to intracellular lectins (see for a review, Roche A.C., Monsigny M. (1996), *Chemtracts Biochem. Mol. Biol.*, **6**, 188-201), can act as NLS and such revealed an alternative pathway to enter the nucleus. We are currently looking for naturales complexes glycosylated motifs which could be very efficiently involved in nuclear import.

CLUSTERS OF INTERCHROMATIN GRANULES AND REGULATION OF NUCLEO-CYTOPLASMIC TRANSPORT OF MESSENGER RNA AND RIBOSOMAL RNA

PUVION-DUTILLEUL Francine, BESSE Sylvie, PUVION Edmond

Laboratoire Organisation fonctionnelle du Noyau, UPR 9044 CNRS, 7 rue Guy Môquet, BP n°8, 94801 Villejuif Cedex, France

Clusters of interchromatin granules (IG) are present in the interchromatin space of eukaryotic cells. These structurally well-characterized RNP structures are neither transcription sites nor splicing sites but are considered to be involved indirectly in splicing because of their high content in spliceosome components. In order to ascertain the role of IG clusters in pre- and/or post-splicing events, we have undertaken at ultrastructural level the non-isotopic *in situ* hybridization detection of poly(A)$^+$ RNA and U1 and U2 snRNA and the immunogold detection of spliceosome proteins in normal cells and in cells showing higher level of transcriptional activity, such as DNA virus infected cells (herpes simplex virus type 1 (HSV-1) or adenovirus type 5), and transiently transfected cells (Us11 gene of HSV-1). The precise distribution of viral RNA and ribosomal RNA (rRNA) was investigated in infected and transfected cells by *in situ* hybridization of specific DNA probes. Infection and transfection were without visible effect on the content of IG clusters in spliceosome components. In contrast, detection of poly(A) tails was 3 times more intense than in normal cells. At least a part of the poly(A)$^+$ RNA detected in the IG clusters of infected and transfected cells was viral RNA as revealed by *in situ* hybridization of viral probes. An intriguing result was the presence of rRNA molecules within some IG clusters of infected and transfected cells in which the speed of migration of the ribosomal subunits toward the cytoplasm is known to be strongly reduced. In non-treated cells in which the export of the ribosomal subunits is rapid, the IG clusters were either entirely unlabeled or were decorated with only a few gold particles. Taken together these data reveal that the IG clusters, in addition to being accumulation sites of spliceosome components, contain, depending on the cellular model under study, different types of RNA including poly(A)$^+$ RNA, snRNA, rRNA and viral RNA. The results support our assumption that IG clusters might play a crucial role in the regulation of export of mRNA and rRNA from the nucleus to the cytoplasm. It remains to be determined whether these structures participate in some post-transcriptional sorting of the mature mRNA and ribosomal subunits awaiting their transfert to the cytoplasm or whether they are implicated in the recognition and decay of redundant or nonsens RNA.

NUCLEAR IMPORT OF IκBα

TURPIN[1] Pierre, HAY[2] Ron T., ARENZANA-SEISDEDOS[3] Fernando and DARGEMONT[1] Catherine

Institut Curie/ UMR144 75248 Paris[1], Institut Pasteur/ Immunologie Virale 75015 Paris[2] and University of St Andrews (Scotland)[3]

The regulation of numerous genes involved in immune and inflammatory responses relies upon NF-κB-dependent transcription. In unstimulated cells, this activity is prevented by the association of NF-κB with inhibitory proteins called IκB. IκB proteins mask the Nuclear Localization Sequence (NLS) of NF-κB and hold it in a cytoplasmic and inactive form. Our immunofluorescence data indicate that an IκB protein, IκBα, can be imported in the nucleus of HeLa cells. In this compartment, we have shown that IκBα down-regulates NF-κB-dependent transcription (Arenzana-Seisdedos F., Turpin P.H, Rodriguez M.S., Thomas D., Hay R.T., Virelizier J.L., Dargemont C. (1997), J. Cell Science, 110, 369-378). As IκBα does not contain any classical NLS, we were interested in the molecular mechanisms by which IκBα enters the nucleus. Its molecular weight (37 kDa) could allow IκBα to diffuse into the nucleus. However, using GFP fusion proteins, we showed that the ankyrin repeats of IκBα mediate its specific nuclear import. In a semi-acellular nuclear import system, the nuclear import of IκBα relies on the addition of cytoplasmic extracts and energy. Inhibition of the nuclear import of IκBα with GTPγS suggests the involvement of the GTPase Ran which is involved in many nuclear import processes. However, the whole "classical NLS" import machinery (NLS-receptor + Ran + p10) is not sufficient to drive the nuclear import of IκBα. This suggests that either additional factors are needed ("classical NLS"-bearing protein) or IκBα is imported through an alternative Ran-dependent nuclear import pathway.