doi:10.1016/j.jmb.2006.02.041 J. Mol. Biol. (2006) 358, 997–1009

JMB

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# Stimulated Expression of mRNAs in Activated T Cells Depends on a Functional CRM1 Nuclear Export Pathway

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In metazoans, the nuclear export of bulk mRNAs is mediated by the export receptor TAP, together with its binding partner p15. A number of viral mRNAs, including the unspliced and partially spliced mRNA species of the human immunodeficiency virus (HIV), however, use an alternative export route *via* the importin β-related export receptor CRM1. This raises the question of whether a subset of cellular mRNAs might be exported by CRM1 as well. To identify such mRNAs, we performed a systematic screen in different cell lines, using representational difference analyses of cDNA (cDNA-RDA). In HeLa and Cl-4 cells no cellular transcripts could be identified as exported *via* CRM1. In contrast, we found a number of CRM1-dependent mRNAs in Jurkat T cells, most of which are induced during a T cell response. One of the identified gene products, the dendritic cell marker CD83, was analyzed in detail. CD83 expression depends on a functional CRM1 pathway in activated Jurkat T cells as well as in a heterologous expression system, independent of activation. Our results point to an important role of the CRM1-dependent export pathway for the expression of CD83 and other genes under conditions of T cell activation.



*Keywords:* RNA export; CRM1; T cell activation; representational difference analysis; CD83

## Introduction

Nuclear export of different classes of RNA is mediated by different factors.[1] In recent years, transport pathways and transport factors for all classes of RNA have been identified. RNAs are always transported as RNA–protein complexes. Hence, it is not surprising that RNA transport is governed by similar principles as nuclear protein transport.[2–4] Indeed, most of the transport receptors involved in nuclear RNA export belong to the well-characterized family of importin β-like proteins, also referred to as karyopherins, which mediate nuclear transport of proteins in both directions. Importin β, the prototype of this family, is the transport receptor for nuclear import of proteins containing a "classic" nuclear localization signal. Examples for importin β-like proteins involved in RNA transport are the export receptors exportin-t for tRNA,[5,6] exportin-5 for microRNA precursors[7,8] and CRM1 for U snRNAs[9,10] and ribosomal subunits.[11,12]

mRNA appears to be the only major species of RNA that takes an export route independent of importin β-like proteins.[13] The transport factors TAP/NXF1 and p15/NXT1 are required for export of the bulk of cellular mRNA,[14,15] and their depletion or inactivation leads to nuclear accumulation of poly(A)$^+$ mRNA.[16–19] Additional factors are involved in the formation of export complexes, as TAP and p15 themselves do not bind to cellular mRNAs. A picture is emerging where mRNA export is tightly coupled to mRNA transcription, splicing, and also 3′ end formation (for a review, see Reed & Hurt[20]).

CRM1 is the major cellular transport receptor for export of proteins out of the nucleus.[10,21–24] CRM1

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Abbreviations used: HIV, human immunodeficiency virus; LMB, leptomycin B; NES, nuclear export signal; RDA, representational difference analysis; GFP, green fluorescent protein.

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interacts with so-called leucine-rich nuclear export signals (NESs), which were originally identified in the inhibitor of the cAMP-dependent protein kinase (PKI[25]) and the Rev-protein of the human immunodeficiency virus (HIV[26]). HIV-Rev serves as an adapter protein for CRM1-dependent export of unspliced and partially spliced viral mRNAs. This raises the question of whether CRM1 may also function in cellular mRNA export. Indeed, some cellular transcripts have been identified that seem to use CRM1 as an export receptor. These include the transcripts for interferon-α1,[27] the proto-oncogene c-fos,[28] and the cyclooxygenase COX-2.[29] Interestingly, the TAP-related protein NXF3, which is mainly expressed in testis, contains a leucine-rich NES which mediates its interaction with CRM1 and has the ability to export mRNA.[30] Hence, NXF3 may serve as a tissue-specific adapter protein for CRM1-dependent transport of specific mRNA molecules.

Here, we used cDNA representational difference analysis (cDNA-RDA)[31] to systematically search for cellular RNA species that are exported *via* the CRM1-pathway. This method is based on the subtractive hybridization of two cDNA populations and subsequent amplification of sequences enriched in one of the two populations. In stimulated T cells, we identified a number of mRNAs whose cytoplasmic concentration is strongly reduced in the presence of the CRM1-inhibitor leptomycin B (LMB) or upon transfection of cells with a fragment of the nucleoporin Nup214 (CANc), which is known to inhibit CRM1-dependent transport. For one of the identified clones, the dendritic cell marker CD83, we observed a specific reduction in the expression level in a heterologous expression system, when the CRM1 pathway was inhibited. Our results point to an important role of the nuclear export receptor CRM1 in the expression of a subset of genes under conditions of cellular activation or differentiation.

## Results

It has been suggested that a certain percentage of cellular mRNA molecules are exported *via* the CRM1-pathway.[32] Here, we devised a screen that allows the identification of such mRNA species. RNA molecules exported out of the nucleus in a CRM1-dependent fashion will be depleted from the cytoplasm upon inhibition of the CRM1 pathway. We used LMB for inhibition of CRM1-mediated nuclear export. LMB is a fungal metabolite that covalently modifies CRM1,[33] thereby inhibiting CRM1-dependent export.[10,34] Inhibition by LMB is highly specific, as CRM1 is its only cellular target.[33]

In our experimental approach, we compared either cytoplasmic or nuclear RNA populations of cells treated with or without LMB. We used cDNA-RDA for the comparison, a PCR-based method that has been used for the analysis of differential gene expression in various tissues and cell lines.[31] Briefly, cDNA derived from RNA from two populations is cut with the endonuclease DpnII and ligated to linker oligonucleotides. After PCR amplification, the resulting representations (i.e. cDNAs representing the original mRNA pool) are again digested with DpnII to remove the original oligonucleotide. A second oligonucleotide is then ligated to the representation that is supposed to contain the sequences of interest at higher concentrations (the "*tester*"). The *tester* is then hybridized with an excess of the other representation (the competitor or "*driver*"), which lacks the new oligonucleotide ends. Subsequent amplification with the second oligonucleotide as primer results in exponential enrichment of *tester-tester* hybrids, leading to difference product 1 (DP1). The procedure of exchanging the linker oligonucleotide in the difference products, hybridization to increasing amounts of *driver* and PCR amplification is repeated to obtain DP2, DP3 etc. With this procedure, sequences are obtained that are enriched in the *tester* representation and therefore also in the original RNA population. These sequences are then cloned into an appropriate vector for further analysis.

### Validation of the method: isolation of HIV-env and rRNA sequences

To demonstrate the validity of our screening method, we set out to identify an mRNA species known to be exported by CRM1. Cl-4 cells expressing the HIV-env mRNA in an HIV-Rev (i.e. CRM1)-dependent fashion,[35] were incubated with or without LMB for 24 h and nuclear and cytoplasmic fractions were prepared. The quality of the subcellular fractionation was assessed by Northern blotting, using a probe that detects U6 snRNA, an RNA species that is expected not to leave the nucleus[36] (data not shown). For experiments using nuclear RNA as starting material, plus-LMB representations were used as *tester*, as these were expected to contain sequences that are enriched in the nucleus upon CRM1-inhibition. For experiments using cytoplasmic RNA, the plus-LMB representations were now used as *driver*, as CRM1-dependent RNAs should be depleted from the cytoplasm in the presence of the drug.

Figure 1 shows a typical example of the first difference products (DP1–DP3), obtained after one, two or three rounds of subtractive hybridization and subsequent amplification. Cytoplasmic RNA from Cl-4 cells incubated with or without LMB was used as starting material. DP1 usually consists of a complex smear,[37] whereas distinct bands in DP2 and DP3 may correspond to specific cDNAs that are amplified during the procedure. We cloned the entire difference products DP2 and DP3 into a plasmid vector. From each experiment about 400 colonies were transferred to membranes for further screening analyses and selected clones were sequenced.

The CRM1-dependent HIV-env sequence expressed in Cl-4 cells was detected in ~1% of the clones from an RDA with cytoplasmic Cl-4