

# Crp79p, Like Mex67p, Is an Auxiliary mRNA Export Factor in *Schizosaccharomyces pombe*

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The export of mRNA from the nucleus to the cytoplasm involves interactions of proteins with mRNA and the nuclear pore complex. We isolated Crp79p, a novel mRNA export factor from the same synthetic lethal screen that led to the identification of spMex67p in *Schizosaccharomyces pombe*. Crp79p is a 710-amino-acid-long protein that contains three RNA recognition motif domains in tandem and a distinct C-terminus. Fused to green fluorescent protein (GFP), Crp79p localizes to the cytoplasm. Like Mex67p, Crp79-GFP binds poly(A)<sup>+</sup> RNA in vivo, shuttles between the nucleus and the cytoplasm, and contains a nuclear export activity at the C-terminus that is Crm1p-independent. All of these properties are essential for Crp79p to promote mRNA export. Crp79p import into the nucleus depends on the Ran system. A domain of spMex67p previously identified as having a nuclear export activity can functionally substitute for the nuclear export activity at the C-terminus of Crp79p. Although both Crp79p and spMex67p function to export mRNA, Crp79p does not substitute for all of spMex67p functions and probably is not a functional homologue of spMex67p. We propose that Crp79p is a nonessential mRNA export carrier in *S. pombe*.

## INTRODUCTION

A large number of proteins associate with the unspliced and mature mRNA in the nucleus to perform a variety of functions (Izaurrealde and Adam, 1998; Stutz and Rosbash, 1998; Nakielny and Dreyfuss, 1999; Conti and Izaurrealde, 2001; Zenklusen and Stutz, 2001). The mature mRNA, bound to some of these proteins as ribonucleoprotein (mRNP) complexes, is exported through the nuclear pore. Genetic and biochemical studies in yeast, mammalian, and viral systems

have identified a large number of proteins that are thought to be involved in the export of mRNA (Stutz and Rosbash, 1998; Nakielny and Dreyfuss, 1999). These export factors include heterogeneous RNA-associated nuclear proteins (e.g., human hnRNP A1, or *Saccharomyces cerevisiae* Npl3) (Michael *et al.*, 1995; Singleton *et al.*, 1995; Lee *et al.*, 1996), RNA export factor (REF) class of proteins (e.g., Yra1/ALY/REF) (Lee *et al.*, 1996; Strasser and Hurt, 2000; Stutz *et al.*, 2000; Rodrigues *et al.*, 2001), splicing-related factors (e.g., UAP56/Sub2/HEL, U2AF) (Gatfield *et al.*, 2001; Strasser and Hurt, 2001; Zolotukhin *et al.*, 2002), exon-junction proteins (e.g., Y14) (Le Hir *et al.*, 2000; Kim *et al.*, 2001), putative carriers of mRNA (e.g., Rev, Tap/Mex67) (Segref *et al.*, 1997; Kang and Cullen, 1999; Katahira *et al.*, 1999; Yoon *et al.*, 2000), and finally, components of the nuclear pore complex (NPC) (Yang *et al.*, 1998; Strambio-de-Castillia *et al.*, 1999). In addition, protein import-export pathways are probably indirectly involved in mRNA export processes (Dasso and Pu, 1998). A few proteins, like Mex67 or Tap, that function as export carriers of mRNA characteristically shuttle, associate with the substrate mRNA directly or via adapter proteins like REFs, and connect the mRNP complexes to the nuclear pore proteins to promote nuclear mRNA export (Conti and Izaurrealde, 2001; Zenklusen and Stutz, 2001). The carrier-

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Abbreviations used: GFP, green fluorescent protein; Gr, glucocorticoid receptor; mRNP, ribonucleoprotein; NES, nuclear export signal; NLS, nuclear localization signal; NPC, nuclear pore complex; REF, RNA export factor; RRM, RNA recognition motif; *ts*, temperature-sensitive.

mediated export of mRNA functionally separates the carriers from noncarrier export factors that may be passively exported along with mRNA.

Several potential mRNA carriers have been identified and their mechanism of function studied (Stutz and Rosbash, 1998; Nakielny and Dreyfuss, 1999). One prototypic carrier is the Rev protein of HIV-1. It exports viral transcripts containing an RNA sequence called the Rev-responsive element (Stutz and Rosbash, 1998). Rev binds to the Rev-responsive element and uses a nuclear export signal (NES) to export the mRNA cargo (Malim *et al.*, 1989; Fischer *et al.*, 1995; Pollard and Malim, 1998). The Rev NES is a short leucine-rich peptide that interacts with its export receptor Crm1p (Xpo1 in *S. cerevisiae*), a member of the importin- $\beta$  class of import/export receptors (Fornerod *et al.*, 1997; Stade *et al.*, 1997). Crm1p directs the export of NES-containing proteins along with their cargo mRNA by interacting with the NPC (Stutz and Rosbash, 1998).

scMex67p of *S. cerevisiae*, spMex67p of *S. pombe*, and their human homologue Tap are non-importin- $\beta$  type mRNA carriers. scMEX67 is an essential gene, and at nonpermissive temperatures, its temperature-sensitive (*ts*) mutants accumulate poly(A)<sup>+</sup>RNA in the nucleus (Segref *et al.*, 1997). scMex67p binds to poly(A)<sup>+</sup> RNA, to REFs, and to the GLFG regions of Nup116p and Nup100p (Segref *et al.*, 1997; Strasser and Hurt, 2000; Strasser *et al.*, 2000; Strawn *et al.*, 2001). In contrast, spmex67 is not essential, and its involvement in poly(A)<sup>+</sup> RNA export is revealed only by its synthetic lethality with a mutation in *rae1*, an essential gene required for mRNA export in *S. pombe* (Brown *et al.*, 1995; Yoon *et al.*, 2000). spMex67p binds poly(A)<sup>+</sup> RNA, shuttles between the nucleus and cytoplasm, and functions to export mRNA that is dependent on a nuclear export activity present within its middle domain (Yoon *et al.*, 2000). Recent *in vitro* binding experiments suggest that the splicing factor UAP56/Sub2p interacts with the REF protein Aly/Yra1 and that Tap/Mex67p can displace UAP56/Sub2p from Aly/Yra1 (Luo *et al.*, 2001; Strasser and Hurt, 2001). Thus, Tap/Mex67 may interact with the mRNP via Aly/Yra1. Conversely, Tap/Mex67p independently or with cofactors p15/Mtr2p can interact with the NPC to target the mRNP complexes to the nuclear pores (Bachi *et al.*, 2000; Strasser *et al.*, 2000; Strawn *et al.*, 2001).

In *S. pombe*, Rael1p is an essential protein that is required for export of mRNA and cell cycle progression (Brown *et al.*, 1995). At the restrictive temperature, the *ts* mutant *rae1-167* rapidly accumulates poly(A)<sup>+</sup> RNA in the nucleus (Brown *et al.*, 1995). The *rae1-167* allele is also synthetically lethal with *nup184-1* mutation. Nup184p is a nonessential nucleoporin in *S. pombe* that does not contain degenerate FG repeats. Its orthologue in *S. cerevisiae*, Nup188p, has been implicated in nuclear membrane biogenesis (Nehrbass *et al.*, 1996; Zabel *et al.*, 1996).

In this work, we describe the isolation and functional characterization of a novel cytoplasmic protein, Crp79p, which, like spMex67p, complements the synthetic lethality of a *rae1-16 nup184-1* double mutation by preventing accumulation of poly(A)<sup>+</sup> RNA in the nucleus. We show that like spMex67p, Crp79p shuttles between the nucleus and the cytoplasm, can be UV cross-linked to poly(A)<sup>+</sup> RNA *in vivo*, and mediates export of mRNA via a nuclear export activity present at the C-terminus. We argue that Crp79p can func-

tion as a carrier in transporting mRNA from the nucleus to the cytoplasm.

## MATERIALS AND METHODS

### Strains and Culture

The basic genetic and cell culture techniques used have been described (Moreno *et al.*, 1991; Alfa *et al.*, 1993). The strains used are wild-type 217: *h<sup>-</sup> leu1-32 ura4-d18*; SL27: *h<sup>-</sup> leu1-32 ura4-d18 rae1-167 nup184-1/pREP81X-rae1<sup>+</sup>* (Whalen *et al.*, 1999); *h<sup>-</sup> rae1-167* (Brown *et al.*, 1995); *h<sup>-</sup> rae1-167 pim1-d1* (this work); *pim1-d1* (Demeter *et al.*, 1995); *h<sup>-</sup> Δcrp::ura4 leu1-32 ura4-d18*; *h<sup>-</sup> Δcrp::ura4 Δmex::kan leu1-32 ura4-d18* (this work); and *h<sup>-</sup> rae1-167 npp106-1/pREP81X-rae1<sup>+</sup> leu1-32 ura4-d18* (Yoon *et al.*, 1997). Appropriately supplemented-essential minimal media EMM medium was used to express genes from the pREP plasmids containing the *nmt* promoter (Maudrell, 1993). The *nmt* promoter was repressed by the addition of 10  $\mu$ M thiamine in EMM medium (Forsburg, 1993).

### Isolation of *crp79*

SL27 (*rae1-167 nup184-1/pREP81X-rae1<sup>+</sup>*) cells were transformed with a library of partially digested genomic DNA constructed at the *SalI* site of pUR18 (Berbet *et al.*, 1992), and Ura<sup>+</sup> colonies were isolated that could grow in the presence of thiamine at 28°C. After elimination of the *rae1<sup>+</sup>* clones by virtue of their ability to confer growth of SL27 cells at 36°C in the presence of thiamine, plasmids were rescued and retransformed into the SL27 strain. Analysis of the plasmids showed that eight different genes had been isolated. One clone, p27-9, encoded several open reading frames with significant similarity to the RNA recognition motif (RRM) found in RNA binding proteins. Using reverse transcription and PCR, we isolated a cDNA whose sequences revealed the presence of three introns within the gene of *crp79*.

### Construction of Crp79p-GFP Fusion Proteins

The full-length Crp79p-GFP fusion in pAB3 was constructed by introduction of a *KpnI* site at the termination codon of the *crp79* gene in p27-9, followed by insertion of a *KpnI-SacI* fragment bearing the gene for the green fluorescent protein (GFP) (S65T). Except for pAB58, the truncated derivatives of pAB3 shown in Figure 2 and pAG116 in Figure 5 were constructed by introduction of a *BglIII* site at the end points of the deletion. The C-terminal deletion (pAB58) was made by introduction of a *KpnI* site at the deletion endpoint, and the *crp79* open reading frame was fused in frame to GFP as in pAB3. The point mutant Crp79p(1-122, F74V) (pAB106F74V) was made by PCR mutagenesis of two overlapping fragments containing the F74V mutation. These fragments were used as templates in a crossover PCR to produce the full-length construct that was cloned into pAB86.

Plasmids for the HeLa export were constructed with pXRGG as the source plasmid (Love *et al.*, 1998), into which a polylinker was introduced at the *XhoI-BglIII* sites. The new plasmid, pAG112, was used to clone various PCR fragments as *XhoI-BglIII* fragments.

Plasmids pAG177-pAG188 were made from pAG116 by replacing its *BglIII-KpnI* fragment, followed by insertion of a polylinker at the unique *KpnI* site to generate pAG177. pAG179 and pAG180 were made by placing wild-type or mutant Rev-NES sequence at the polylinker between the *XhoI* and *BglIII* sites of pAG177. These sequences were generated by PCR using pXRGG as the template and wild-type or mutant oligonucleotide primers. pAG188 was constructed similarly by placing a DNA fragment encoding for the nuclear export activity (435-509 aa) of spMex67p. pAG178 was constructed by replacing the *BglIII-KpnI* fragment of pAG116 with a *BglIII-KpnI* fragment containing 477-694 aa of Crp79p. Plasmids for UV cross-linking experiments were constructed by fusing the RRM

domains to the C-terminus of GST in a pREP3X-vector. For the full-length Crp79p, a GFP fusion was used.

### Construction of *crp79* Null and the Genomic Integration of *crp79-gfp*

The full-length *crp79* insert from p27-9 was cloned into pSK<sup>+</sup>, and a deletion between amino acids (aa) 125 and 651 with a *Hind*III site placed at the deletion junction was constructed. A *Hind*III fragment bearing *ura4*<sup>+</sup> was placed in the deletion junction. This plasmid was cut with *Pst*I and *Xho*I, and the *crp79::ura4* fragment was gel-purified and transformed into the SP286 diploid strain. *ura*<sup>+</sup> transformants were selected and screened by Southern blotting for the disruption of one of the *crp79* loci. Analysis of 12 tetrads showed a 2:2 segregation of *ura4*<sup>+</sup>, and the *crp79::ura4* colonies grew as well as *crp79*<sup>+</sup> colonies. For chromosomal integration of the full-length *crp79p-gfp* fusion, the *ars1* sequence was removed from pAB3 by digestion with *Aat*II and *Nhe*I. The resulting plasmid was linearized by digestion with *Age*I and transformed into JBP217. Stable *ura4*<sup>+</sup> transformants were selected and screened for integration at the *crp79* locus by Southern blotting.

### Spot Assay for Growth

Single transformants were first patched on EMM-agar plates in the absence of thiamine (-B1) with appropriate auxotrophic selection and then grown into 10 ml liquid medium until the stationary phase (3 d). Tenfold dilutions were plated in the presence or absence of thiamine (final concentration of B1 was 10  $\mu$ M) on EMM plates without leucine and/or uracil as appropriate.

### In Situ Hybridization

In situ hybridization was performed as described previously (Amberg *et al.*, 1992) with the following modifications. Oligo dT<sub>50</sub> carrying an  $\alpha$ -digoxigenin at the 3' end was used as the hybridization probe. FITC-antidigoxigenin or rhodamine-antidigoxigenin, as appropriate, was used for detecting the hybridization probe by fluorescence microscopy. 4',6'-Diamidino-2-phenylindole (DAPI) was used for observing the DNA.

### UV Cross-Linking

Plasmids expressing full-length Crp79p-GFP (pAB3) or C-terminal GST fusions of RRM1, RRM (F74V) mutant, RRM2, and RRM3 were introduced into wild-type strains. The methods for UV cross-linking, mRNA isolation, and Western blotting were as described previously (Yoon *et al.*, 2000). Monoclonal anti-GST, anti-GFP antibodies were used for detection of the fusion proteins using the Renaissance kit (NEN, Boston, MA).

### Heterologous Nuclear Export Assay in HeLa Cells

Export activity of various regions of Crp79p were measured with a heterologous assay described previously (Love *et al.*, 1998). Briefly, a vector containing glucocorticoid receptor (Gr)-GFP fusions with specific protein segments of interest was used to transfect HeLa cells. In the presence of 2  $\mu$ M corticosterone, the GFP fusion protein translocated from the cytoplasm into the nucleus. One hour after incubation with hormone, cells were washed extensively, and the export of proteins was assayed in presence of cycloheximide (10  $\mu$ g/ml) by monitoring depletion of the fusion protein in the nucleus and its redistribution into the cytoplasm for 1 h at 37°C. As a positive control for export, a fusion of spMex67p-NES-Gr-GFP was used.

## RESULTS

### Isolation of *crp79* as a Multicopy Suppressor of *rae1-167 nup184-1* Synthetic Lethality

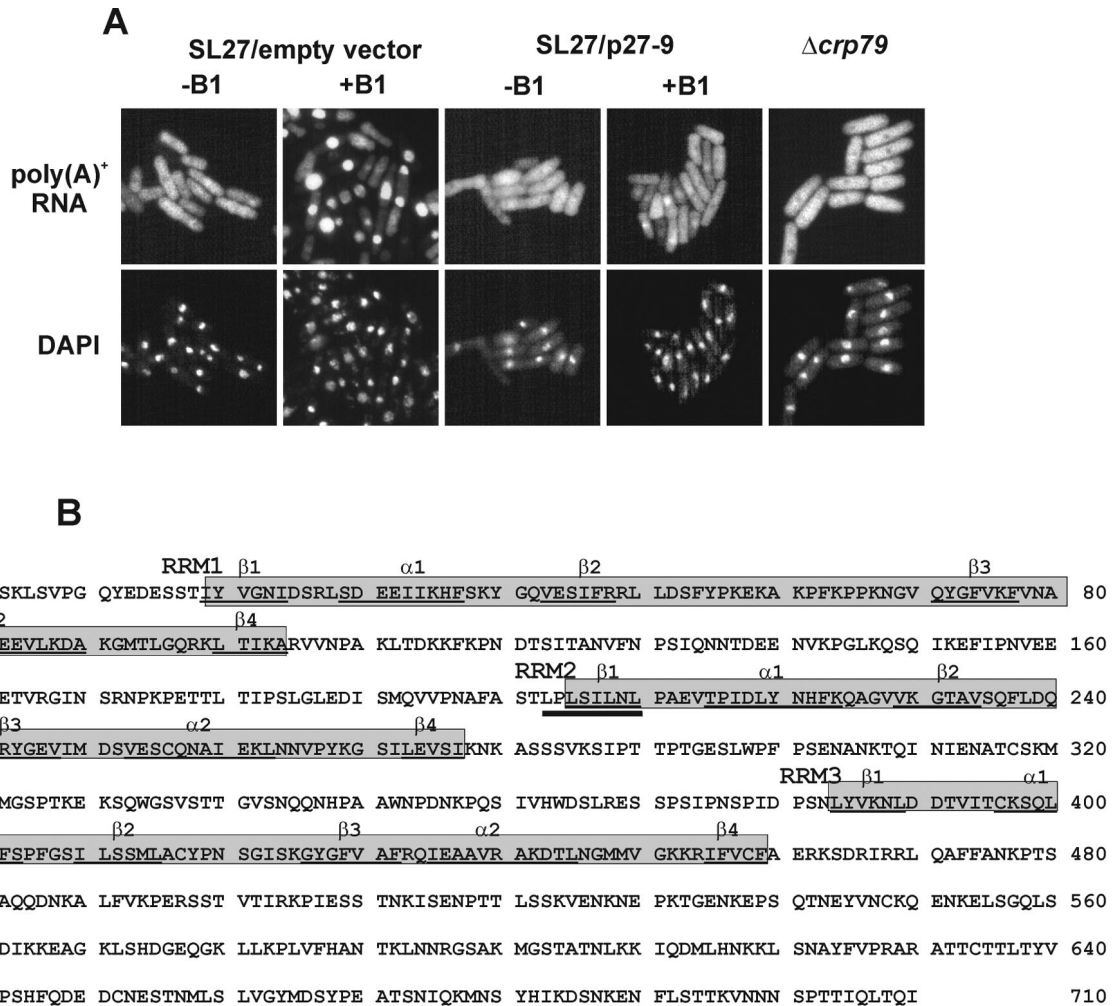
The *S. pombe* SL27 (*rae1-167 nup184-1/pREP81X-rae1*) is a synthetic lethal strain that contains the *ts rae1-167* mutation in Rae1p and a mutation *nup184-1* in Nup184p (Whalen *et al.*, 1999). In this article, *rae1-167 nup184-1/pREP81X-rae1* cells are referred to as SL27 cells for the purpose of brevity. The SL27 strain is kept viable by expression of *rae1* gene from a pREP81X plasmid under the control of a weak, thiamine-repressible, *nmt1* promoter. In the absence of thiamine (-B1), Rae1p is synthesized, and the SL27 cells grow normally. In the presence of thiamine (+B1), the expression of Rae1p is repressed, resulting in inhibition of growth. The SL27 cells accumulate poly(A)<sup>+</sup> RNA in the nucleus under synthetic lethal conditions 12–18 h after addition of thiamine to the medium.

Previously, by screening an *S. pombe* genomic library, we isolated *spmex67* as a multicopy suppressor of growth and mRNA export defects of the SL27 strain (Yoon *et al.*, 2000). Using the same strategy, we isolated another multicopy suppressor plasmid, p27-9, that suppressed both growth (data not shown) and the mRNA export defects of SL27 under synthetic lethal conditions (+B1, 18 h) (compare +B1 panels for empty vector vs. p27-9 in Figure 1A).

Sequence analyses of p27-9 and its inserts of cDNA revealed a single gene with three introns encoding a 710-aa protein of predicted molecular weight ~79 kDa (Figure 1B) (GenBank accession No. AF432874). We named this protein Crp79p (cytoplasmic RRM containing protein of 79 kDa) to indicate its cytoplasmic localization in the wild-type strain (see below). A strain with a deletion of *crp79* ( $\Delta$ *crp79::ura4*) grew normally at temperatures between 18 and 37°C (data not shown) without any detectable mRNA export defects (Figure 1A). Thus, *crp79* is a nonessential gene, and loss of the protein has no significant impact on mRNA export in *S. pombe*.

The HMMER program in the Pfam database identified three RRMs in Crp79p located sequentially from the N-terminus, with designations of RRM1, RRM2, and RRM3 (shaded in Figure 1B). All three RRMs carry the conserved hexapeptide RNP2 (L/I F/Y V/I G/K N/L L) and the octapeptide RNP1 (K/R G F/Y G/A FVX F/Y) RNA binding consensus within the predicted  $\beta\alpha\beta\alpha\beta$  fold (underlined in Figure 1B) (Burd and Dreyfuss, 1994a, 1994b). RRM2 also contains a leucine-rich, Rev-NES-like motif (LxLxxLxL) between residues 203 and 210. This is located within the RNP2 region (residues above thick underline in Figure 1B). In contrast, the C-terminus of Crp79p (from 460 to 710 aa) apparently lacked significant homology to any known protein or structural motif in the databases. BLASTP searches conducted with Crp79p showed similarities with other poly(A)<sup>+</sup> RNA binding proteins; however, these were limited to the RRM1 and RRM3 domains only (Burd and Dreyfuss, 1994a). Recently, from a search for genes of *S. pombe* induced in meiosis, the same gene was isolated independently and named *meu5* (Accession No. AB020594) (Watanabe *et al.*, 2001).



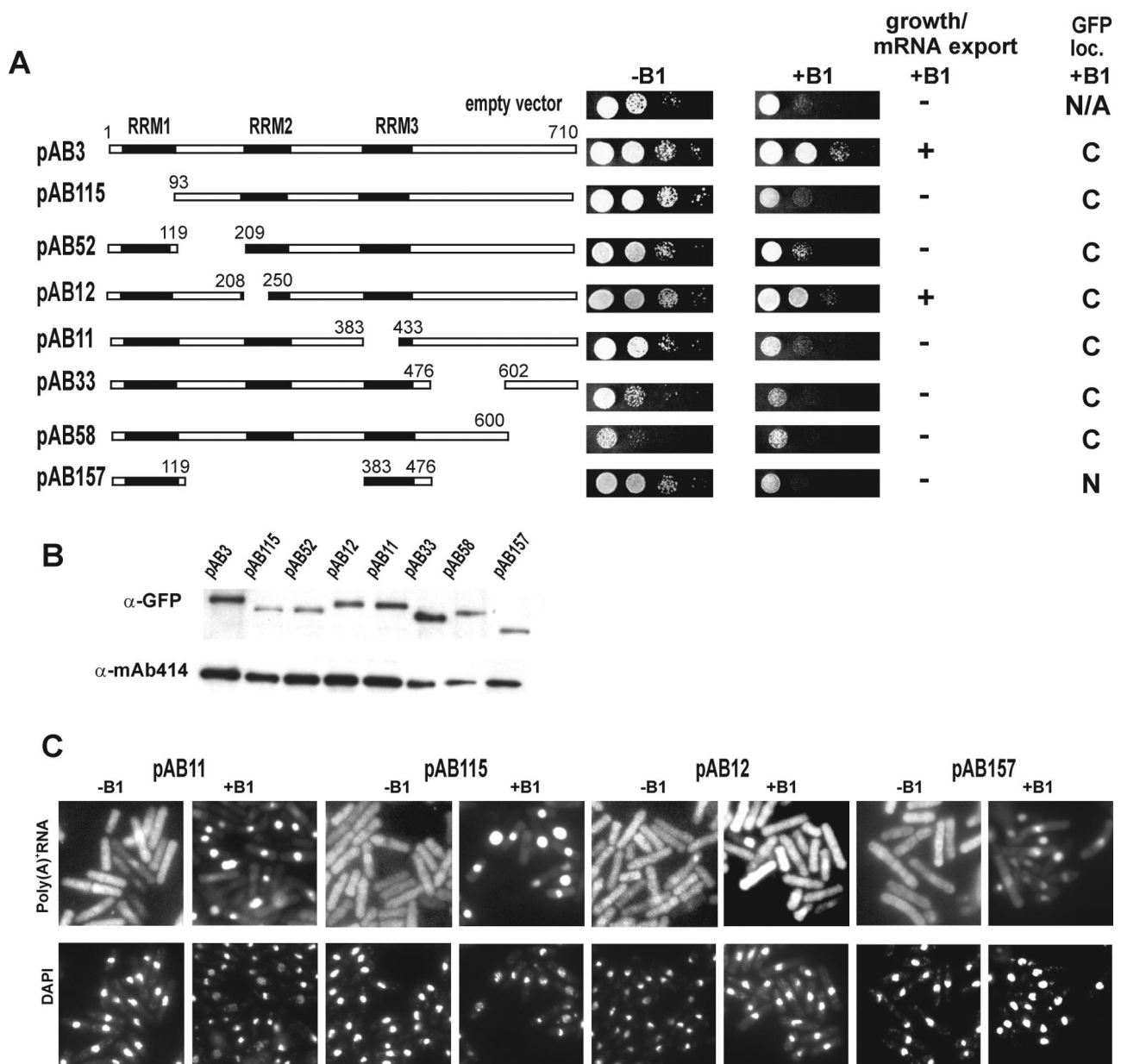


**Figure 1.** Suppression of the mRNA export defect in SL27 (*rae1-167 nup184-1*/pRXE81X-*rae1*) cells. Poly(A)<sup>+</sup> RNA localization in SL27 cells and in cells with the  $\Delta$ *crp79* mutation. (A) Poly(A)<sup>+</sup> RNA was detected in  $\Delta$ *crp79* cells grown in EMM medium. The SL27 cells carrying an empty vector or a plasmid (p27-9) expressing Crp79p were grown in EMM to midlog phase expressing *rae1* (absence, -B1) or without expression of *rae1* (18 h in presence of 10  $\mu$ M B1). Coincident DAPI staining is shown at bottom. (B) aa sequence and structural organization of Crp79p of *S. pombe*. The sequence of Crp79p has been deposited in the GenBank data base with accession No. AF432874. Shaded boxes indicate the three RRM domains. The secondary structure predictions of  $\alpha$ -helix and  $\beta$ -sheets are indicated and underlined (Burd and Dreyfuss, 1994a). The dark underline indicates the position of the leucine-rich NES.

### Common Functional Determinants within Crp79p-GFP Required for Growth and mRNA Export

The genomic copy of *crp79* was fused at the C-terminus to a gene fragment encoding the GFP. Crp79-GFP was fully functional and complemented growth (pAB3 in Figure 2A) and mRNA export (data not shown) phenotypes of *rae1-167 nup184-1* (SL27) cells when *rae1* expression was repressed (+B1). We undertook a systematic deletion analysis of the three RRM domains of Crp79-GFP, including the regions between the RRMs and the unique C-terminus, to determine which parts of the protein are necessary for its function. SL27 cells were transformed with plasmids carrying deletions of Crp79-GFP, and the ability of the transformants to grow in the presence (-B1) or absence (+B1) of *rae1* expression was compared (Figure 2A). Complementation was

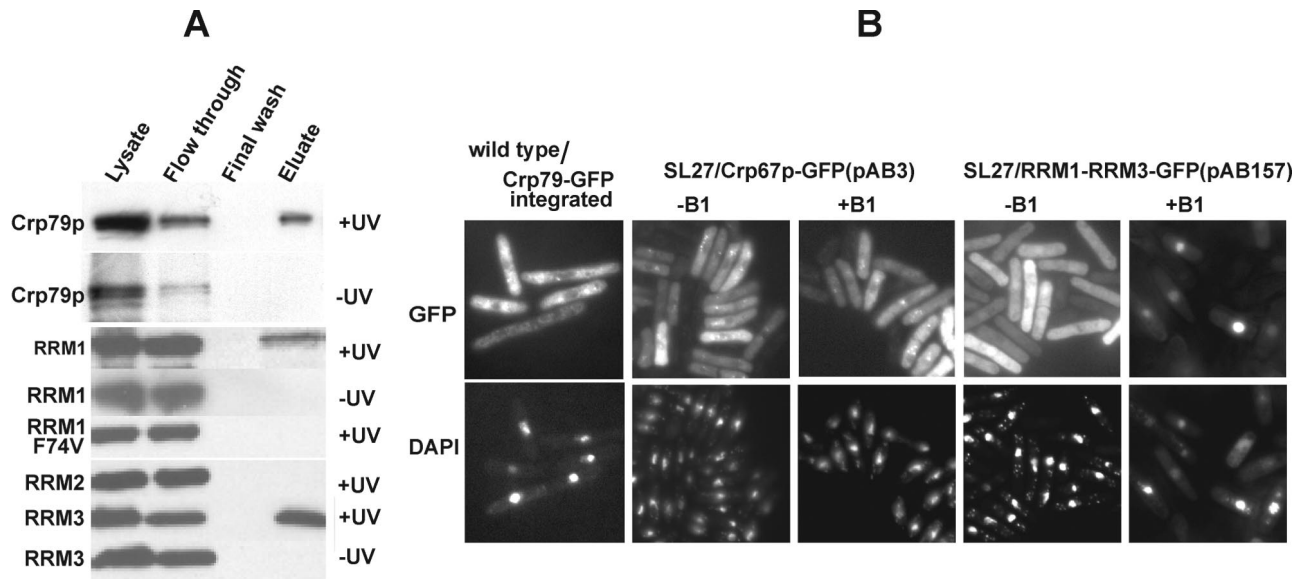
achieved by the full-length version of Crp79-GFP (pAB3) or partially by a deletion in RRM2 (pAB12) (Figure 2A). However, deletions in RRM1 (pAB115), RRM3 (pAB11), or in the region between RRM1 and RRM2 (pAB52) failed to complement. A C-terminal deletion between 601 and 710 aa (pAB58) or between 477 and 601 aa (pAB33) also failed to rescue the growth of *rae1-167 nup184-1* (SL27) cells. In the latter two cases, however, the transformants grew poorly in the absence of B1 also, indicating some dominant negative effects on cell growth. Another deletion construct (pAB157) that contained RRM 1 and RRM3 also failed to complement growth. The various deletion constructs or the full-length Crp79p-GFP expressed adequate amounts of intact protein (Figure 2B). Thus, the inability to complement growth by the truncated proteins was unlikely, because of an insufficient



**Figure 2.** Growth and mRNA export of SL27 cells. (A) Complementation of synthetic lethality of *rae1-167 nup184-1*/pREP81X-*rae1* (SL27) by Crp79p-GFP and its deletions. A schematic diagram of the regions surrounding the deletions along with deletion endpoints is shown (for details of plasmid construction, see MATERIALS AND METHODS). Growth of SL27 cells was monitored for 4 d with (-B1) and without (+B1) expression of *rae1*. Ability and inability to restore growth and mRNA export to SL27 cells under conditions of synthetic lethality is indicated and summarized by (+) and (-). The GFP loc. indicates cellular localization of relevant fusion protein; C, cytoplasm; N, nucleus; N/A, not applicable. (B) Determination of steady-state protein levels of Crp79p-GFP and deletion derivatives in SL27 cells grown when expression of *rae1* was repressed (in presence of B1). Top, Western blot analysis using a monoclonal anti-GFP antibody. Bottom, Control for protein loading by Western blot for a protein that reacts with NPC-specific mAb414 mAb. (C) Poly(A)<sup>+</sup> RNA was detected in SL27 cells carrying pAB11, pAB12, pAB115, and pAB157 (see Figure 2A for deletion endpoints) grown in EMM to midlog phase in absence (-B1) or 18 h in presence of thiamine (10  $\mu$ M). Coincident DAPI staining is shown at bottom.

quantity of the functional proteins. Taken together, these results suggest that RRM2 is the only dispensable region in Crp79p for complementation of the synthetic lethality because of the combination of *rae1-167* and *nup184-1* mutations.

We tested whether Crp79-GFP and its various deletions could suppress mRNA export defects of SL27 cells when the expression of *rae1* was repressed. For this, the total poly(A)<sup>+</sup> RNA was detected by in situ hybridization in SL27 cells grown in the absence or in the presence of B1 for 18 h. Figure



**Figure 3.** UV cross-linking and localization of Crp79p-GFP. (A) Crp79-GFP and N-terminal GST-fusions of RRM1, RRM1 F74V, RRM2, and RRM3 were expressed from plasmids under the control of the genomic *crp79* promoter or a heterologous (*rae1*) promoter, respectively. UV cross-linked (+UV) or uncross-linked (–UV) samples were prepared. The protein cross-linked to poly(A)<sup>+</sup> RNA were detected by Western blot analysis using polyclonal anti-GST antibody. (B) Cells in which Crp79-GFP was integrated at its genomic locus were tested for GFP signal. For cellular localization of Crp79-GFP (pAB3) or a deletion carrying RRM1 and RRM2 fused to GFP (pAG157) in SL27 cells, transformants were grown with or without *rae1* expression (–B1 or 18 h in presence of B1), and the proteins were visualized by GFP fluorescence. Coincident DAPI staining is shown at bottom.

2C shows in situ hybridizations in SL27 cells transformed with various deletion derivatives of Crp79-GFP. Like Crp79p (p27–9, shown in Figure 1A), deletion in RRM2 (pAB12) was able to suppress the nuclear accumulation of mRNA of SL27 cells depleted of Rae1p compared with cells containing an empty vector (compare Figures 2C and 1A). In contrast, cells expressing deletions that removed RRM1 (pAB115) or RRM3 (pAB11) exhibited extensive mRNA accumulation in the nucleus when *rae1* expression was repressed (Figure 2C). These were fusions that were also unable to suppress the growth defect of SL27 cells under synthetic lethal conditions (Figure 2A). Similarly, the deletion that combined RRM1 and RRM3 (pAB157) showed mRNA accumulation in the nucleus (Figure 2C). Other deletions that were unable to rescue synthetic lethality (e.g., pAB52, pAB33, and pAB58) also did not rescue the mRNA export defects (not shown; data summarized in Figure 2A). Therefore, deletions of Crp79-GFP that complemented the growth defect also mitigated the mRNA export defect of SL27 cells.

A multicopy plasmid carrying *spmex67* was shown previously to suppress the growth and mRNA export defects of *rae1–167 nup184–1* or *rae1–167 npp106–1* synthetic lethal mutants (Yoon *et al.*, 1997, 2000). The same plasmid suppresses the *ts* phenotype of the *rae1–167* mutation, albeit at the lower restrictive temperature of 32°C (Yoon *et al.*, 2000). However, multicopy *crp79* was unable to suppress either the synthetic lethality of *rae1–167 npp106* mutant or the *ts* phenotype of *rae1–167* at 32°C (data not shown). In addition, Crp79p expression was also unable to suppress the growth and mRNA export defect associated with *rae1–167 Δmex67* syn-

thetic lethality (data not shown). Thus, Crp79p does not substitute for and is not a functional homologue of *spMex67p*. We tested whether  $\Delta crp79$  and  $\Delta mex67$  were synthetically lethal when combined. The double-null mutant cells grew normally and showed no detectable mRNA export defect (data not shown).

#### UV Cross-Linking of Crp79-GFP, RRM1, or RRM3 to poly(A)<sup>+</sup> RNA In Vivo

The ability of Crp79-GFP to be cross-linked to RNA was tested by isolating poly(A)<sup>+</sup> RNA from UV-treated or untreated SL27 cells transformed with the plasmid pAB3 expressing the full-length Crp79-GFP fusion protein. Figure 3A shows that Crp79-GFP was cross-linked to poly(A)<sup>+</sup> RNA in vivo. We also tested the individual RRM domains for their ability to be UV cross-linked to poly(A)<sup>+</sup> RNA. Figure 3A also shows the results of a UV cross-linking experiment with cells expressing wild-type RRM1, RRM2, and RRM3 and an RRM1 mutant, F74V, that carried a conserved phenylalanine to valine mutation known to abolish both specific and nonspecific binding to poly(A)<sup>+</sup> RNA in RRM-containing proteins (Deardorff and Sachs, 1997). We found that individually RRM1 or RRM3 UV cross-linked to poly(A)<sup>+</sup> RNA in vivo. Moreover, as expected, the RRM1 with the F74V mutation showed no detectable UV cross-linking to poly(A)<sup>+</sup> RNA. However, the RRM2 domain failed to show any cross-linking to poly(A)<sup>+</sup> RNA. Thus, although a deletion of either RRM in the context of the whole protein led to complete loss of mRNA export/growth complementation



functions, individually the domains RRM1 and RRM3 cross-linked RNA. These data also suggest that proteins with the C-terminal deletions that retain the RRM domains (pAB33 or pAB58) are expected to bind RNA, and as such, their inability to complement growth or mRNA export defects may reside in other aspects of Crp79p function.

### *Crp79-GFP Localizes in the Cytoplasm*

In wild-type cells, Crp79p-GFP expressed from an integrated chromosomal locus (Figure 3B) or from multicopy plasmids (pAB3) (not shown) was found to be located predominantly in the cytoplasm. In most cells, some of the fusion protein was concentrated in the cytoplasm as dots or speckles. When the dots were photo-bleached, they reappeared (data not shown), suggesting that they are probably dynamic in nature. In SL27 cells, Crp79-GFP (pAB3) localized predominantly to the cytoplasm in the presence or absence of *rae1* expression (Figure 3B). Moreover, with the exception of RRM1-RRM3 fused to GFP (pAG157), all other deletion derivatives of Crp79p-GFP were also located in the cytoplasm, irrespective of whether *rae1* was expressed (see GFP localization +B1 data summary in Figure 2A). RRM1-RRM3 fusion to GFP (pAG157) in the presence of *rae1* expression was located in the cytoplasm but accumulated in the nucleus under synthetic lethal conditions (Figure 3B). Thus, the RRM domains (pAB157) can be imported into the nucleus.

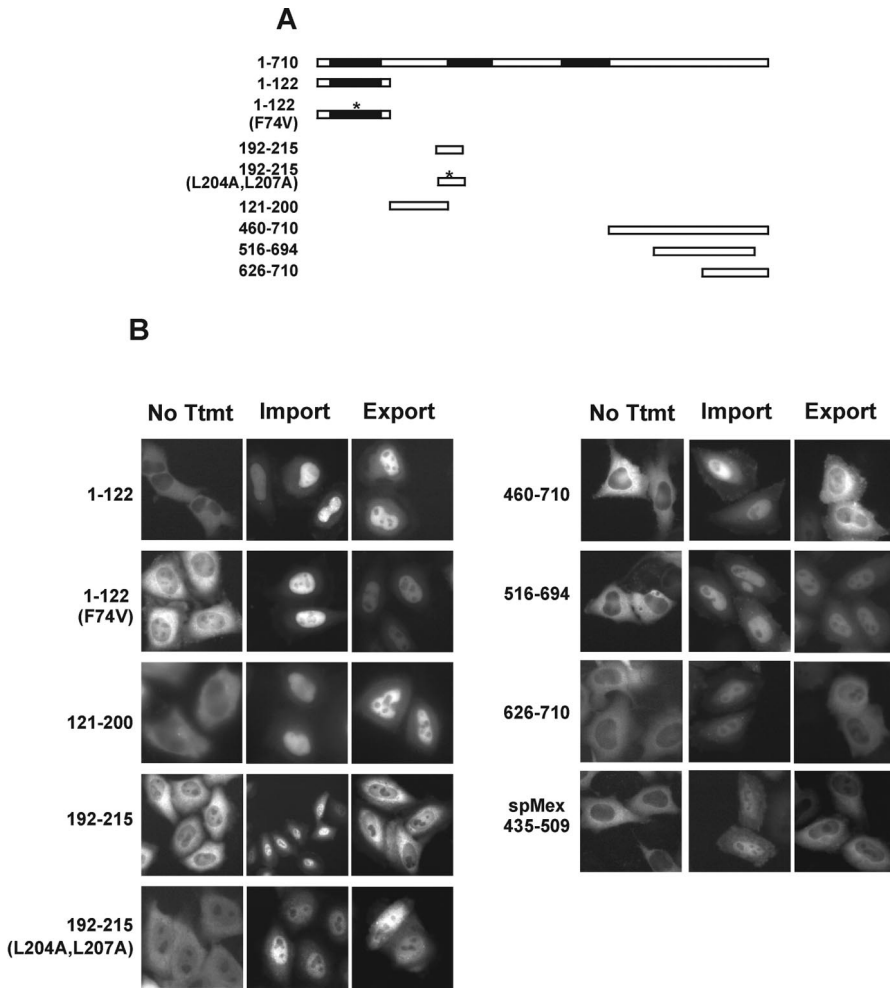
### *Identification of a Nuclear Export Activity at the C-Terminus of Crp79p*

The above results raised the possibility that Crp79p may also be imported into the nucleus, but in the steady state, most of the protein is located in the cytoplasm. This led us to investigate whether there is an NES within Crp79p that could direct its export from the nucleus. Alternatively, there could also be a region within the protein that contains a nuclear export property by contributing to an interaction with an adapter protein(s) that contains the proper export signal. We used a heterologous nuclear export assay developed in HeLa cells to scan the entire protein sequence for nuclear export activities (Love *et al.*, 1998; Yoon *et al.*, 2000). Briefly, in this assay, DNA fragments corresponding to the protein segments of interest are fused to a Gr-GFP chimera. HeLa cells are transfected with plasmids expressing various Gr-GFP fusions. Untreated cells show the Gr-GFP fusion in the cytoplasm (no Tmt samples in Figure 4). In the presence of micromolar concentrations of the steroid hormone, the fusion proteins are imported rapidly (~30 min) into the nucleus (Import). When the hormone is washed off, the fusion protein can exit the nucleus (within 60 min) if the protein segment encodes an NES or an activity (Export). A previously characterized nuclear export activity from spMex67 (435–509 aa) was used as a positive control in the export assay (Yoon *et al.*, 2000). Using this assay, we found that RRM1 (1–122 aa), RRM1 (F74V), and sequences between RRM1 and RRM2 (120–200 aa) were deficient in promoting export when fused to a version of Gr-GFP in the export assay (Figure 4A and 4B). A leucine-rich Rev-like signal sequence present within RRM2 (201–214 aa) (Figure 1B) showed an NES activity, and mutations of the two internal leucine residues to alanine abolished its export activity (Fig-

ure 4, A and B). However, when this activity was compared with the HIV-Rev NES, its relative nuclear export activity was weak (data not shown). A small amount of Gr-GFP fusion of RRM3 localized to the nucleus in the absence of the steroid (No Tmt). Treatment with the steroid hormone resulted in further increase of the fusion protein in the nucleus. This nuclear accumulation was unaffected upon removal of the hormone (data not shown). The C-terminal region from 460–710 aa demonstrated a strong nuclear export activity (Figure 4B). This export activity was insensitive to leptomycin B, suggesting that the Crm1p pathway most likely was not involved in its export (data not shown). A smaller region from position 626 to 710 also showed a significant amount of export activity. When a large number of cells were compared, the activity of the 460–710 aa was slightly higher than the 626–710 aa region. However, the 662–710 aa and the 516–694 aa fragments failed to show any detectable export activity (data not shown and Figure 4B). We conclude that there is a minimal nuclear export activity contained within aa 626 and 710, whereas the region between residues 460 and 620 may have a stimulatory role. It is not known at this time whether the export activity is encoded directly by an NES sequence within this region or whether it is a result of an interaction with another NES-containing protein.

### *mRNA Export by Crp79p Requires Its C-Terminal Nuclear Export Activity*

Irrespective of the nature of the export activity, its presence provoked us to consider the possibility that the protein could be exported from the nucleus via its nuclear export activity and in the process carry mRNA out of the nucleus. Such a process may underlie the rescue of the synthetic lethal phenotype of *rae1-167 nup184-1* (SL27) cells by ensuring nuclear export of essential mRNA. To test this hypothesis, we first made a GFP fusion that retained RNA binding (RRM1 and RRM3) but was devoid of any nuclear export activity by combining 1–208 aa with 383–460 aa of Crp79p (see Figures 1B and 4B). (This construct was different from the pAB157 used in Figure 2, which also contained a version of RRM1-RRM3-GFP fusion.) The ability of this fusion protein to support growth and mRNA export in *rae1-167 nup184-1* cells under the synthetic lethal condition was examined. The resulting GFP fusion (pAG177) was unable to complement growth and the mRNA export defect of *rae1-167 nup184-1* (SL27) synthetic lethal cells (Figure 5, A and C). The fusion protein was localized in the cytoplasm when *rae1* was expressed (–B1) but accumulated in the nucleus when expression of *rae1* was repressed (+B1) (Figure 5D; compare –B1 with +B1 panels). Next, we examined the effect of attaching the C-terminal region containing the nuclear export activity onto the RNA-binding segment in pAG177 on the complementation of growth and mRNA export defect of SL27 cells. For this, a region containing the full nuclear export activity (460–710 aa) or a truncated, export-deficient region (460–694 aa) was inserted into pAG177 to obtain RRM1-RRM3-NES (460–710)-GFP (pAG116) or RRM1-RRM3-NES (460–694)-GFP (pAG178) fusions (Figure 5A). As seen in Figure 5, A and C, cellular growth and significant rescue of mRNA export was achieved in cells carrying the pAG116 but not the pAG178 plasmid. These results suggest that the ability to comple-



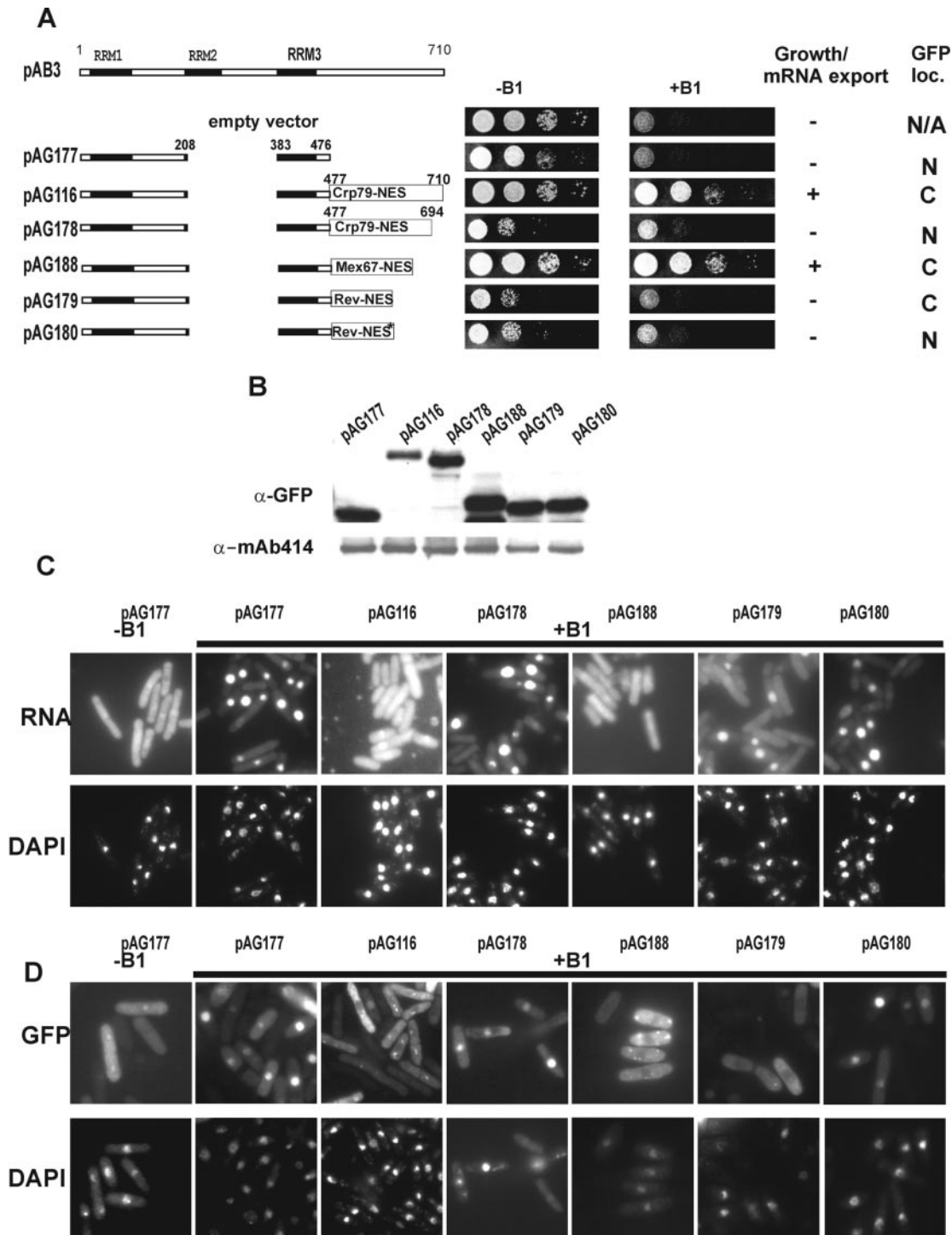
**Figure 4.** Identification of nuclear export activities in Crp79p using a heterologous assay for protein export. (A) A schematic map of different deletions used in the nuclear export assay in B is indicated. (B) The regions of Crp79p as indicated were expressed as hormone-inducible Gr-GFP chimera proteins in HeLa cells. Import: treatment with 2  $\mu$ M corticosteroid for 1 h at 37°C. Export: cells were washed three times with phosphate-buffered saline to remove hormone and incubated for an additional hour at 37°C in the presence of cycloheximide (10  $\mu$ g/ml). spMex67(NES)-Gr-GFP was used as a positive control for the assay (Yoon *et al.*, 2000).

ment the growth of *rae1-167 nup184-1* (SL27) synthetic lethal strain and the rescue of the mRNA export defects probably depended on the presence of a functional nuclear export activity as well as an ability to bind RNA. Consistent with the complementation properties, the GFP fusion expressed from pAG116 accumulated in the cytoplasm, whereas that from pAG178 was seen to be located in the nucleus under the conditions of synthetic lethality (Figure 5D). The cytoplasmic localization of the NES-GFP fusion in the absence of *rae1* could be the result of a steady-state localization of a dynamic protein that shuttles. These results raised the possibility that when the RRM domains are fused to a nuclear export activity, the resulting protein may be able to function as an mRNA export carrier by using the RRM domain to bind the cargo RNA and transporting them during its own export.

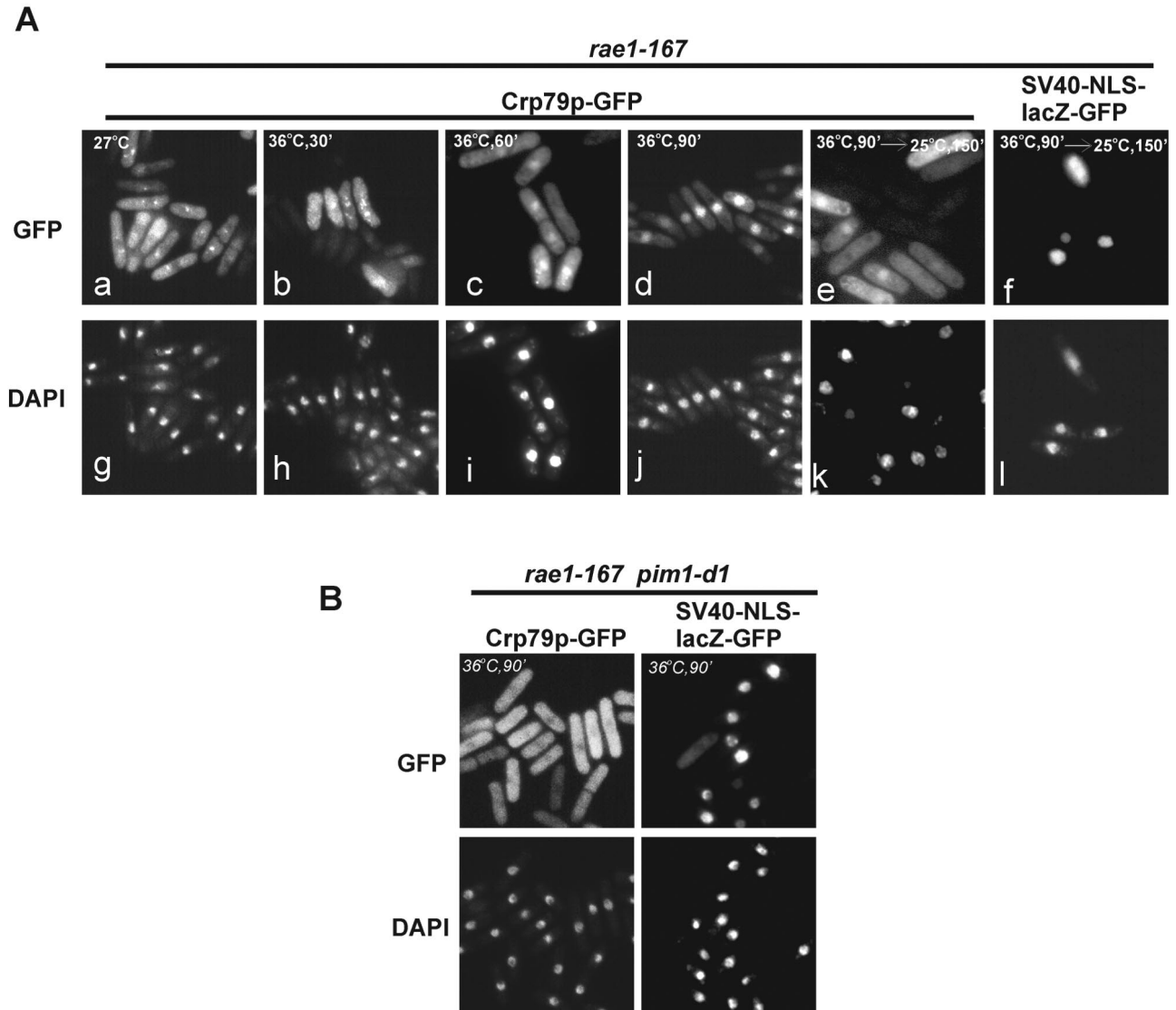
It is possible that the C-terminal region with nuclear export activity has additional functions that are necessary for mRNA export. We directly tested whether the export activity of Crp79p could be functionally substituted by a heterologous nuclear export function from spMex67p or the leucine-rich HIV-Rev NES (and its mutant) (Fischer *et al.*, 1995; Yoon *et al.*, 2000). By inserting the spMex67 export

activity region (435–509 aa), the Rev-NES, or its mutant version into the RRM1-RRM3-GFP fusion (pAG177), we constructed the plasmids pAG188, pAG179, and pAG180, respectively (Figure 5A). RRM1-RRM3 fused to the spMex67 nuclear export activity could suppress the synthetic lethality as well as the mRNA export defects of *rae1-167 nup184-1* (SL27) cells (Figure 5, A and C). In cells expressing the Rev-NES fusion (pAG179), slightly reduced accumulation of poly(A)<sup>+</sup> RNA in the nucleus and increased levels in the cytoplasm were observed compared with *rae1-167 nup184-1* (SL27) cells that expressed the mutant version of Rev-NES (pAG180) under similar conditions (Figure 5C). However, no clear growth complementation was achieved by either wild-type or the mutant Rev-NES GFP fusions. Fusion proteins containing either the spMex67p or Rev-NES both localized in the cytoplasm under the conditions of synthetic lethality (Figure 5D). In contrast, the fusion protein containing the mutant Rev-NES was located predominantly in the nucleus. The steady-state level of various proteins bearing nuclear export activity was comparable (Figure 5B). The ability of the spMex67-NES to functionally replace the nuclear export function of Crp79p suggests that mRNA export functions of the latter were indeed mediated by a nuclear





**Figure 5.** Nuclear export activity–dependent suppression of growth and mRNA export defects of SL27 cells. (A) Schematic diagram of various GFP fusions. The coordinates for the spMex67 are aa 439–505 (Yoon *et al.*, 2000). The Rev-NES sequence is 23 aa long, containing LPPLERLTL (wild-type) or LPPAERATL (mutant) peptide sequence (Love *et al.*, 1998). Growth of *rae1*–167 *nup184*–1 cells was monitored for 4 d, expressing different GFP fusion constructs with or without *rae1* expression (–B1 and +B1, respectively). + indicates ability to complement, and – indicates inability to complement under synthetic lethal conditions. The GFP loc. indicates cellular localization of relevant Crp79p-GFP fusion; C, cytoplasm; N, nucleus; and N/A, not applicable. (B) Steady-state levels of GFP fusion proteins used in A following a procedure similar to that used in Figure 2B. (C) Poly(A)<sup>+</sup> RNA localization of various GFP fusions in A as indicated in SL27 cells after 18 h of growth in presence of B1 (10  $\mu$ M) are shown. (D) Localization of the GFP fusions in A as indicated in the absence (–B1) or 18 h after addition of B1 to the media. Coincident DAPI staining is shown at bottom.



**Figure 6.** Shuttling of Crp79p-GFP and Ran-dependent import. (A) Shuttling of Crp79p-GFP fusion in *rae1-167* cells. The nuclear accumulation of Crp79p-GFP in cells with *rae1-167* mutation at 27°C (a) and after shift to 36°C for 30', 60', and 90' (b–d) are indicated. Incubation of cells at 36°C for 90 min followed by additional incubation for 150 min at a permissive temperature of 25°C is shown in e. Localization of SV40-NLS-lacZ-GFP was used as a control; cells were incubated at 36°C followed by incubation at 25°C for 150 min (f). Coincident DAPI staining for all samples are shown at bottom (g–i). (B) Localization of Crp79p-GFP and SV40-NLS-lacZ-GFP in *rae1-167 pim1-d1* strain after simultaneous inactivation of both proteins for 90 min at 36°C are shown. Coincident DAPI staining is shown below.

export activity-dependent mechanism. Because the RRM1-RRM3-Rev-NES-GFP (pAG179) fusion was located in the cytoplasm in a Rev-NES-dependent manner, these results suggest that it also was capable of exporting mRNA, although the strong nuclear accumulation of poly(A)<sup>+</sup> RNA may mean that the magnitude of mRNA export was not significant.

#### *Crp79p-GFP Shuttles between the Nucleus and the Cytoplasm*

For Crp79p to function as an mRNA export carrier, it would be expected to shuttle between the nucleus and the cyto-

plasm. The nuclear localization of a GFP fusion consisting of RRM1 and RRM3 alone (pAG157) under the conditions of synthetic lethality already suggested this possibility (Figure 3B). To test directly whether shuttling occurred, we looked for genetic backgrounds in which Crp79p-GFP accumulated in the nucleus. After inactivation of Rae1p (36°C, *rae1-167* mutation), Crp79p-GFP translocated from the cytoplasm into the nucleus within 90 min (inactivation for 60 min resulted in only a partial nuclear accumulation) (Figure 6, compare panels b, c, and d). It was previously shown that under similar conditions, a strong nuclear accumulation of poly(A)<sup>+</sup> RNA took <30 min (Brown *et al.*, 1995). Thus,

Crp79p-GFP was capable of entering into the nucleus, but it took considerably more time to accumulate in the nucleus than poly(A)<sup>+</sup> RNA.

To determine whether Crp79p-GFP was capable of exiting the nucleus, the fusion protein was imported into the nucleus as above (Figure 6A, panel d). These cells were then returned to the permissive temperature of 25°C for 2.5 h in the presence of cycloheximide to prevent new protein synthesis. Results in Figure 6A (compare panels d and e) show that Crp79p-GFP was able to exit the nucleus after a shift back to the permissive temperature. As a control for nuclear envelope integrity, an SV40-(NLS)-GFP-lacZ reporter protein remained in the nucleus throughout the experiment (Figure 6A, panel f). The nuclear import and export of Crp79p together show that the protein could shuttle between the nucleus and the cytoplasm. Crp79p-GFP fusions that possess the C-terminal nuclear export activity (Figure 2A) similarly could shuttle between the nucleus and cytoplasm (data not shown).

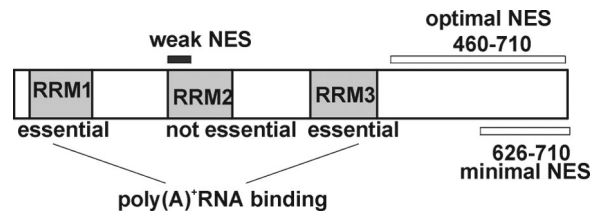
### Nuclear Import of Crp79p Is Ran-Dependent

In our search for an import signal within Crp79p, no short sequences within the RRM domains was identified that could direct nuclear import of a lacZ-GFP fusion protein (data not shown). Most proteins that are imported into the nucleus either carry or bind to adapter protein(s) that contain a nuclear localization signal (NLS) for interaction with members of the  $\beta$ -importin-type import receptors. Typically, the import receptors require the functioning of the Ran system (Dasso and Pu, 1998). We examined whether the import of Crp79p-GFP into the nucleus depends on the Ran-GTPase switch system. For this, the Crp79-GFP fusion was expressed in a *rae1-167 pim1-d1* strain, encoding *ts* mutations for both Rae1p and RCC1p proteins (Demeter *et al.*, 1995). The latter is a guanine nucleotide exchange factor, and its *ts* mutation in *S. cerevisiae* confers defects in the nuclear import of proteins at restrictive temperatures (Demeter *et al.*, 1995). After simultaneous inactivation of *rae1-167* and *pim1-d1* at 36°C for 90 min, Crp79p-GFP was found to be located predominantly in the cytoplasm (Figure 6B). In some cells, a hollow nucleus could be detected. Because cells carrying the *pim1* mutation may show disintegration of the nuclear envelope, we ensured the nuclear envelope integrity by using a reporter SV40-NLS-LacZ-GFP that remained nuclear during and after the inactivation treatment (Demeter *et al.*, 1995). Only ~10% of total cells showed a diffused localization of the reporter, suggesting that most cells maintained nuclear envelope integrity during the course of the experiment (Figure 6B). We conclude that the inability to import Crp79p-GFP is caused by a loss of RCC1 function, and thus, its import depends on the Ran system.

## DISCUSSION

### Crp79p Is an spMex67p-like mRNA Export Carrier

In this article, we describe the isolation and functional characterization of a novel poly(A)<sup>+</sup> RNA binding protein from *S. pombe*, Crp79p, that can function in the nuclear export of mRNA. A schematic depicting the functional domains of Crp79p is shown in Figure 7. Like spMex67p, Crp79p was



**Figure 7.** Schematic summarizes the functional domains of Crp79p. A weak leucine-rich NES present in RRM2 is indicated, and the coordinates of the minimal and optimal nuclear export activity (NES) as identified in HeLa cells are indicated.

isolated as a multicopy suppressor of growth and mRNA export defects of the SL27 strain, suggesting that both support the export of similar mRNA cargo. spMex67p and Crp79p share some features. Both can UV cross-link poly(A)<sup>+</sup> RNA in vivo and shuttle between the nucleus and the cytoplasm, and neither is essential for mRNA export in normal cells. Their simultaneous loss in  $\Delta$ *crp79*  $\Delta$ *mex67* cells resulted in no apparent growth or mRNA export defects, suggesting functional redundancies of both proteins in *S. pombe*. Nonetheless, both Crp79p and spMex67p can function as auxiliary mRNA export carriers by using distinct, functionally essential nuclear export activities. Both properties of poly(A)<sup>+</sup> RNA binding and nuclear export activity of Crp79p are required for exporting mRNA. Therefore, Crp79p may actually function directly as a carrier of mRNA.

### The Mode of Substrate Recognition of Crp79p May Be Different from That of spMex67p

Despite functional similarity, there was very little structural homology between Crp79p and spMex67p. spMex67p contains a single RNP-type RNA recognition motif and can bind mRNA, but by comparison with scMEX67p/Tap, it is likely that spMex67p uses adapter proteins, such as Yra1, to capture mRNA indirectly (Strasser *et al.*, 2000; Zhou *et al.*, 2000). In contrast, Crp79p, like the poly(A)-binding proteins, contains three RRM domains, two of which (RRM1 and RRM3) were essential for its function. The RRM-type motif is used directly for binding substrate RNA, as shown in the case of the U1A protein to U1 snRNA (Scherly *et al.*, 1989). Although the role of Yra1-like proteins cannot be ruled out, it seems likely that Crp79p interacts directly with specific transcripts via its multiple RRM domains. Precedence for such a direct mode of substrate recognition is well known for Rev, a protein that binds an RNA sequence called Rev-responsive element present on HIV-1 transcripts (Pollard and Malim, 1998).

### The mRNA Export Function of Crp79p Depends on Its C-Terminal Nuclear Export Activity

Several experimental observations suggest that nuclear export activity present at the C-terminus of Crp79p is necessary for mRNA export in SL27 cells under synthetic lethal conditions. First, the steady-state localization of Crp79p in cytoplasm in the presence of B1 correlates directly to the rescue of the mRNA export defect, whereas the nuclear localization of RRM1-RRM3-GFP fusion (pAG177) is con-



temporaneous with nuclear accumulation of mRNA. The simplest interpretation of these results is that nuclear export activity-dependent export of the protein directly exports bound mRNA. Second, nuclear export activity of spMex67p could substitute for that of Crp79p in shuttling as well as mRNA export functions of Crp79p. Third, after inactivation of Rae1p, RRM domains carrying the C-terminal nuclear export activity took 90 min, whereas the RRM domains without it took only 30 min, to accumulate in the nucleus. These results suggest that 1) Crp79p was still shuttling when mRNA accumulated in the nucleus, 2) Crp79p was probably exporting some mRNA during its exit from the nucleus, and 3) the nuclear exit of Crp79p is probably independent of Rae1p.

Interestingly, in some cases the presence of RRMs plus a nuclear export activity was not sufficient to rescue growth and mRNA export defects. Some deletion derivatives of Crp79p-GFP (Figure 2A) were unable to rescue the growth and mRNA export defects of *rae1-167 nup184-1* (SL27) cells under synthetic lethal conditions, although they presumably are capable of binding RNA, contain a nuclear export activity, and shuttle between the nucleus and cytoplasm. Because all of these deletion derivatives were localized predominantly in the cytoplasm under the conditions of synthetic lethality, one possibility is that they exported some RNA but that the magnitude of mRNA export was too low and/or export of specific transcripts was affected by the internal deletions.

#### **How Does Crp79p Interact with the NPC?**

The current understanding of scMex67/Tap function in the export of mRNA suggests that they bridge mRNP complexes directly to the nuclear pores (Conti and Izaurralde, 2001; Zenklusen and Stutz, 2001). The C-subterminal regions of both scMex67 and Tap interact directly with the FG repeats of nuclear pore proteins directly or in the presence of Mtr2/p15 (Bachi *et al.*, 2000; Strasser *et al.*, 2000; Strawn *et al.*, 2001). In the case of Rev, it has a distinct NES that is recognized by its receptor, Crm1p (Fornerod *et al.*, 1997; Stade *et al.*, 1997). The latter interacts with pore factors to navigate Rev-associated mRNA through the pores. It is not clear whether scMex67/Tap and Rev follow some common steps or whether they operate in completely separate pathways. At this point, it is not clear whether the C-terminus of Crp79p interacts directly with NPC proteins or binds to a karyopherin that, in turn, directs the Crp79p-mRNA complex out of the nucleus.

#### **Does Crp79p Possess an NES or an Interaction Domain?**

Two nuclear export activities were identified within Crp79p by use of HeLa cells in a heterologous assay of nuclear export (Figure 7). One activity was a leucine-rich NES (Figure 4B) within RRM2 that was not essential for the mRNA export function of Crp79p (see pAB12 in Figure 2A). The second nuclear export activity was present at the C-terminus (626–710 aa), was essential for Crp79p function, and was functionally conserved in human cells. However, the nuclear export activity at the C-terminus required an additional stimulatory region between aa 460 and 625 for full activity. Interestingly, in the context of *S. pombe*, the minimal nuclear

export (626–710 aa) activity was sufficient for the export of the fusion protein (data not shown), but complementation of the mRNA export defect required a larger segment (460–710 aa) (see pAB33 in Figure 2, A and C). The nuclear export activity of the C-terminus may be complex, containing more than one activity or signal region. A small deletion could damage the export function by deleting one or more such noncontiguous elements. Alternatively, there could be one or more adapter proteins that interact with the C-terminal region, such that a larger section of the protein is needed for optimal function. We are currently investigating these possibilities.

#### **The Nature of the RRM Domains**

By analogy with other hnRNP-like proteins, the RRM domains of Crp79p may function by binding to RNA. Specifically, RRM1 and RRM3 each could independently bind poly(A)<sup>+</sup> RNA, as determined by UV cross-linking experiments. Both RRM1 and RRM3 are essential for complementation of growth and mRNA export defect of *rae1-167 nup184-1* cells. Most hnRNP proteins contain several RRM domains, and these may be required for cooperative binding, and/or they may have specificity for RNA sequences or structure (Burd and Dreyfuss, 1994b). Interestingly, the *rae1-167* inactivation experiments suggest that RRM1 and RRM3, when fused to GFP, could shuttle between the nucleus and the cytoplasm (data not shown). Apparently, these domains do not possess a nuclear export activity, and their export from the nucleus is probably the result of binding RRM domains to poly(A)<sup>+</sup> RNA that is exported by some other mechanism. And no discrete sequence with the RRMs was detected that could lead to import of lacZ-GFP fusions (data not shown). Nevertheless, the Ran-dependency of Crp79p import suggests the involvement of an importin- $\beta$  type receptor.

#### **Redundancy and Conservation of mRNA Export Pathways**

Both *spmex67* and *crp79* are nonessential genes, so the mRNA export functions of spMex67p and Crp79p are redundant. In contrast, the orthologous *S. cerevisiae* MEX67 gene is essential for both growth and mRNA export. Moreover, both *S. cerevisiae* and humans lack a protein closely related to Crp79p. These observations suggest that each organism has a specific set of mRNA export carrier proteins that potentially use different export pathways. Despite this variation, the mechanism of export within a particular pathway is likely to be conserved. For example, both scMex67p and human Tap bind to FG-repeat nuclear pore proteins. Similarly, although an orthologue for Crp79p has not been found, its C-terminus can function efficiently to direct nuclear export in HeLa cells, suggesting that a similar export pathway may exist in human cells.

When different pathways are present, each export pathway may acquire some substrate specificity but not completely lose its ability to export other substrates. This redundancy would lead to the inability to detect an mRNA export defect when only one of the carrier proteins is defective. Substrate specificity would also play a role in the ability of a carrier protein gene to be a multicopy suppressor when different mRNA export pathways are defective. In this

study, leucine-rich Rev NES or its likeness in RRM2 could not substitute for the C-terminal nuclear export functions of Crp79p, suggesting that mere export of the protein by any pathway would not constitute concurrent mRNA export. However, the Mex67p nuclear export activity could substitute for the C-terminal export functions of Crp79p, and the suppression pattern of the Crp79p-Mex67p fusion (pAG188) (ability to suppress *rae1-167 nup184-1* but not *rae1-167* at 32°C; A.G.T. and R.D., unpublished observations) was that of Crp79p and not of Mex67p. Presumably, the Mex67p export activity directs export of Crp79p along the same export pathway as it does when functioning to export Mex67p, or both export pathways have common steps. This suggests that the substrates that interact with Crp79p and Mex67p are different, with Mex67p able to export substrates when Rae1p is defective that Crp79p cannot. It is interesting to note in this regard that *crp79* expression increases during meiosis (Watanabe *et al.*, 2001). Given the redundancy in mRNA export pathways and the ability of multicopy level of expression of proteins to export less preferred substrates, defining the specificity of each individual carrier protein will require the identification of all of the carriers and examination of their specificities when expressed in a single copy.

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