

# SR Splicing Factors Serve as Adapter Proteins for TAP-Dependent mRNA Export

## Short Article

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### Summary

The only mammalian RNA binding adapter proteins known to partner with TAP/NXF1, the primary receptor for general mRNA export, are members of the REF family. We demonstrate that at least three shuttling SR (serine/arginine-rich) proteins interact with the same domain of TAP/NXF1 that binds REFs. Included are 9G8 and SRp20, previously shown to promote the export of intronless RNAs. A peptide derived from the N terminus of 9G8 inhibits the binding of both REF and SR proteins to TAP/NXF1 in vitro, and this finding argues for competitive interactions. In *Xenopus* oocytes, the N terminus of 9G8 exhibits a dominant-negative effect on mRNA export from the nucleus, while addition of excess TAP/NXF1 overcomes this inhibition. Thus, multiple adapters including SR proteins most likely cooperate to recruit multiple copies of TAP/NXF1 for efficient mRNA export.

### Introduction

The nuclear export of mRNA in vertebrate cells is a multi-step process. Messages destined for export are bound by export adapters that in turn interact with receptors, which mediate essential interactions with the nuclear pore complex (NPC). TAP/NXF1 (here referred to as TAP, Mex67p in yeast) is believed to be the major receptor for the export of bulk mRNAs to the cytoplasm (Izaurrealde, 2002; Lei and Silver, 2002; Reed and Hurt, 2002). Originally identified as a host factor that facilitates export of type D retroviral RNAs by binding directly to the constitutive transport element (CTE) of these messages (Bray et al., 1994; Grüter et al., 1998), TAP is thought to promote cellular mRNA export through interaction with RNA binding adapter proteins rather than by direct RNA binding (Straesser and Hurt, 2000; Stutz et al., 2000). To date, the only adapters identified for TAP are members of the highly conserved REF family (Aly in mice, Yra1p in *S. cerevisiae*) of RNA binding proteins (Rodrigues et al., 2001; Straesser and Hurt, 2000; Stutz et al., 2000; Zhou et al., 2000). Both TAP (Kang and Cullen, 1999) and Aly (Zhou et al., 2000) are nucleocytoplasmic shuttling proteins.

We previously reported that two members of the evolutionarily conserved SR (serine/arginine-rich) family of splicing factors, 9G8 and SRp20, specifically bind an intronless mRNA export element (Huang and Steitz, 2001). Importantly, these two proteins shuttle between the nucleus and the cytoplasm (Cáceres et al., 1998) and bind polyadenylated RNAs in both compartments of mammalian cells (Huang and Steitz, 2001), suggesting a role as adapters for mRNA export. In a search for their cognate export receptor(s), we unexpectedly found that not only 9G8 and SRp20, but also another shuttling SR protein, ASF/SF2 (Cáceres et al., 1998), interact directly with TAP. SR protein binding in vitro is to the same N-terminal region of TAP as that recognized by REF. In vivo support for TAP-SR interactions is provided by observations that injection of the N terminus of 9G8 (which interacts with TAP) into *Xenopus* oocyte nuclei blocks mRNA export, while coinjection of TAP relieves the inhibition. The identification of a second class of abundant RNA binding proteins that function as a link between mRNA and its dominant export receptor suggests that multiple adapters, including SR proteins, act to recruit multiple copies of TAP for efficient export.

### Results

#### Shuttling SR Proteins Selectively Bind TAP

Our original focus was the identification of the export receptor for 9G8. To determine whether TAP might function in this role in mammalian cells, Flag-tagged TAP (Flag-TAP), control Flag-tagged hnRNP A1 (Flag-A1), or Flag vector alone were transiently expressed in HEK-293 cells. Cell lysates were prepared and interacting proteins were analyzed by immunoprecipitation (IP) with anti-Flag antibodies in the presence of RNase A, followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. The expression level of Flag-TAP was significantly less than that of Flag-A1 (Figure 1A, bottom panel, compare lane 3 to lane 1). Yet, the amount of 9G8 associated with Flag-TAP was far greater than that with either Flag-A1 or Flag alone (upper panel, compare lane 3 to lanes 1 and 2). These results suggest that 9G8 interacts specifically with TAP in vivo. This interaction is not mediated by RNA, since the presence of RNase A (see Experimental Procedures) completely eliminated co-IP of hnRNP A1, which otherwise occurs because of RNA binding proteins associating with each other through RNA (data not shown). Also, the 9G8-TAP interaction is not likely to be mediated by other proteins, because purified recombinant versions of TAP and 9G8 interact (Figure 1B).

We next asked whether other SR proteins are also selectively bound by TAP in nuclear extracts. Glutathione beads pre-coated with GST or GST-TAP were incubated with HeLa cell nuclear extracts and selected proteins analyzed by Western blotting. In addition to 9G8, SRp20 and ASF/SF2 were bound by GST-TAP but not by GST alone (Figure 1C). In contrast, SC35, a non-shuttling SR protein (Cáceres et al., 1998), and HuR, a

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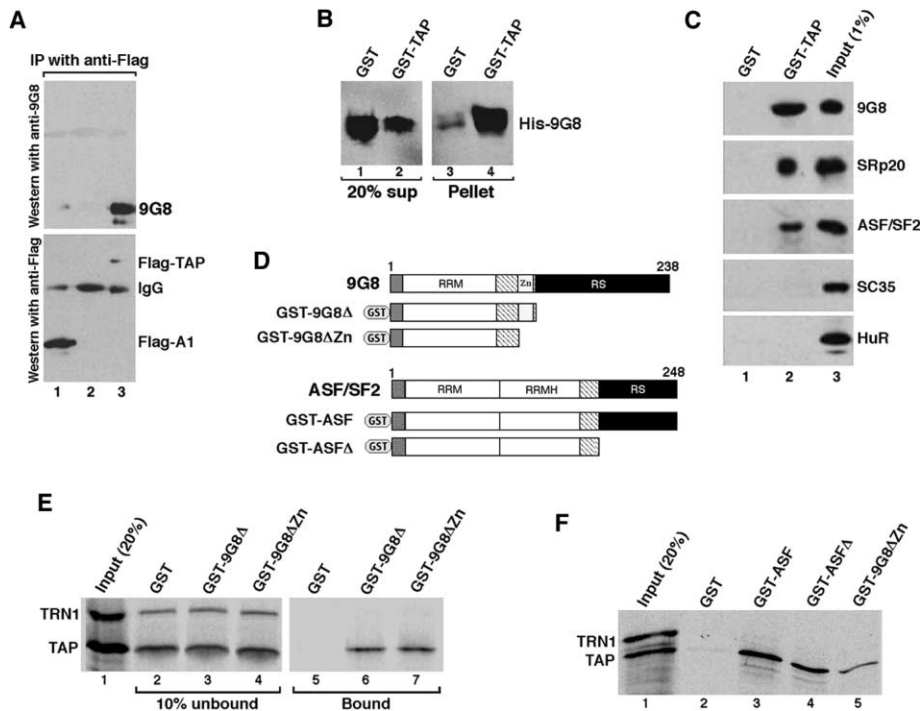


Figure 1. Shuttling SR Proteins Specifically Interact with TAP

(A) 9G8 binds Flag-TAP in transfected cells. After probing anti-Flag immunoprecipitates with anti-9G8, the upper blot was stripped and re-probed with anti-Flag (lower blot).  
 (B) Purified recombinant TAP and 9G8 interact. *E. coli*-expressed GST or GST-TAP prebound to beads were individually incubated with purified baculovirus-expressed histidine-tagged 9G8. Bound (lanes 3 and 4) and unbound (lanes 1 and 2) fractions were analyzed by Western blotting using anti-9G8.  
 (C) TAP interacts with shuttling SR proteins in nuclear extracts. GST or GST-TAP (lanes 1 and 2) pre-bound on beads were incubated with HeLa nuclear extract. Bound proteins and the nuclear extract (Input) were analyzed by Western blotting using antibodies indicated on the right.  
 (D) The fusion proteins used in (E) and (F).  
 (E) The indicated proteins prebound to beads were incubated with a mixture of <sup>35</sup>S-labeled TAP and TRN1 (lane 1). Bound (lanes 5–7) and unbound (lanes 2–4) fractions were analyzed.  
 (F) Bound proteins were analyzed as in (E).

shuttling RNA binding protein (Fan and Steitz, 1998), were not selected (Figure 1C, lane 2). We conclude that 9G8, SRp20, and ASF/SF2 all interact specifically with TAP in nuclear extracts.

### RS Domains Are Not Essential for TAP Interaction In Vitro

To identify the domain of 9G8 recognized by TAP, in vitro binding assays were performed. Because we were unable to obtain a fusion protein containing full-length 9G8 from *E. coli*, only 9G8 truncations were examined (Figure 1D). Glutathione beads pre-bound with GST, GST-9G8Δ (9G8 lacking the RS domain), or GST-9G8ΔZn (identical to 9G8Δ but with the Zn knuckle also removed) were incubated with a mixture of <sup>35</sup>S-labeled TAP and transportin1 (TRN1). TRN1 is a receptor for hnRNP A1 (Pollard et al., 1996) and serves as a negative control. Bound and unbound fractions were resolved and visualized by autoradiography. As shown in Figure 1E, lanes 6 and 7, both truncated versions of 9G8 bound TAP but not TRN1. Similar results were obtained with comparable GST-ASF fusion proteins (Figures 1D and 1F). Clearly, the RS domains of 9G8 and ASF/SF2 are not

essential for TAP interaction in vitro. We were unable to test SRp20 in these assays because we could not obtain soluble GST-SRp20 from *E. coli*.

### SR Proteins Interact with the Same Region of TAP as Recognized by REF

TAP can be structurally and functionally divided into three domains (Figure 2A, top) (Izaurralde, 2002). The substrate binding domain (aa 1–372) interacts with REF (Stutz et al., 2000); the NTF2-like domain binds the essential cofactor p15 (Bachi et al., 2000; Guzik et al., 2001; Katahira et al., 1999); and the UBA domain interacts with components of the NPC (Bachi et al., 2000). To determine which domain(s) of TAP bind the SR proteins, three <sup>35</sup>S-labeled TAP fragments (aa 1–362, 119–362, and 1–202, Figure 2A, top) were synthesized. Incubation with beads coated with GST-9G8Δ and GST-ASF but not with GST alone selected aa 1–202 and 1–362 of TAP, as did GST-REF (Figure 2B, lanes 7–9). We confirmed these interactions by showing that 9G8, SRp20, and ASF/SF2 in HeLa nuclear extract bind the N terminus (aa 1–231) of TAP (Figure 2C), while under the same conditions, hnRNPA1, SC35, and HuR (data not shown)

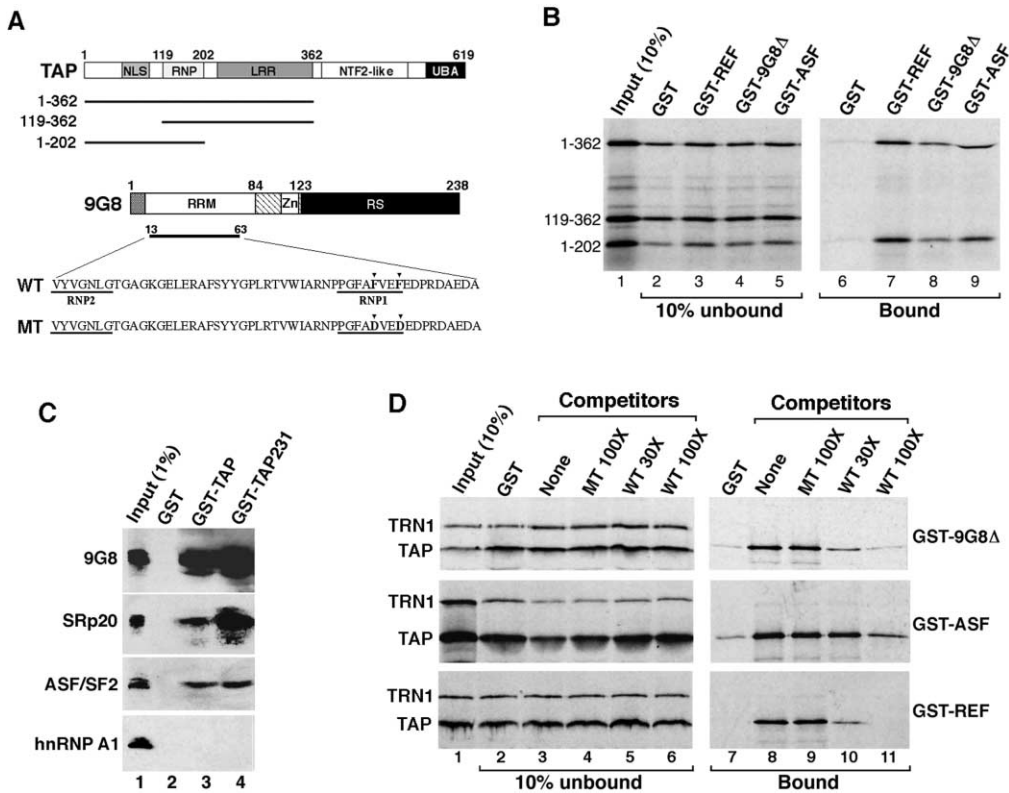


Figure 2. TAP Interacts with SR Proteins and REF via Its N Terminus In Vitro

(A) Top: TAP domain organization with the three fragments used in (B) depicted below. Bottom: 9G8 RRM peptides used in (D). Arrowheads mark the two amino acids mutated in the RNP1 motif.  
 (B) The indicated proteins were analyzed as in Figure 1E except that the input was a mixture of the three TAP fragments shown in (A).  
 (C) The N terminus of TAP interacts with shuttling SR proteins in nuclear extracts. The indicated proteins were incubated with HeLa nuclear extract and analyzed by Western blotting as in Figure 1C.  
 (D) The 9G8 RRM peptide competes for binding to TAP. Binding assays were performed as described in Figure 1E, but in the presence or absence of the peptides shown in (A). The molar excess of the peptides relative to the GST-fusion proteins are indicated at the top.

failed to bind. Since our anti-REF antibody crossreacted with GST (data not shown), we could not study REF-TAP interaction by Western blot analysis. We conclude that 9G8, ASF/SF2, and REF interact with aa 1–202 of TAP, a region significantly smaller than that reported previously for REF binding (Stutz et al., 2000).

To investigate these novel TAP interactions further, we designed two peptides (Figure 2A, bottom). WT corresponds to aa 13–63 from the N terminus of 9G8 (shown to interact with TAP in Figure 1E), whereas MT differs only in that the two conserved phenylalanines in the RNP1 motif are replaced with aspartic acids. The same mutations in the RRM of ASF/SF2 (Figure 1D) have been shown previously to reduce affinity for RNA in vitro (Cáceres and Krainer, 1993) and to abolish the ability of ASF/SF2 to shuttle (Cáceres et al., 1998). The TAP interaction assays (Figure 1E) were repeated in the presence or absence of the WT and MT peptides (Figure 2D). The WT peptide noticeably inhibited the binding of TAP to GST-9G8 $\Delta$ , GST-ASF, and GST-REF at 30-fold molar excess (compare lane 10 to lane 8) and reduced binding to background levels at 100-fold molar excess (lane 11). In contrast, the MT peptide exhibited no significant effect even at 100-fold excess (compare lane 9 to lane 8). These data indicate that 9G8, ASF/SF2, and REF all

compete for binding and support their interaction with the same region of TAP.

#### The N Terminus of 9G8 Blocks mRNA Export

Based on the peptide competition results, we suspected that an excess of the N terminus of 9G8 would exert a dominant-negative effect on mRNA export by sequestering TAP. To test this, GST, GST-9G8 $\Delta$ , and GST-9G8 MT were individually microinjected into *Xenopus* oocyte nuclei. GST-9G8 MT is identical to GST-9G8 $\Delta$  but with the two phenylalanines in the RNP1 motif replaced by aspartic acids (see Figure 2A, bottom). As expected, this protein exhibited lower affinity for TAP than GST-9G8 $\Delta$ , as assessed by 3-fold better competition by the WT peptide in in vitro assays (data not shown) like those in Figure 2D.

After 1 hr of incubation, the oocyte nuclei received a second injection of a mixture of labeled RNAs: AdML pre-mRNA and U1 and U6 snRNAs. U1 snRNA provides a negative control since it accesses an snRNA-specific export pathway (Jarmolowski et al., 1994), while U6 snRNA is exclusively nuclear and controls for accurate nuclear injection and fractionation (Jarmolowski et al., 1994). Three hours later, nuclear and cytoplasmic RNAs were isolated and analyzed. Regardless of which pro-

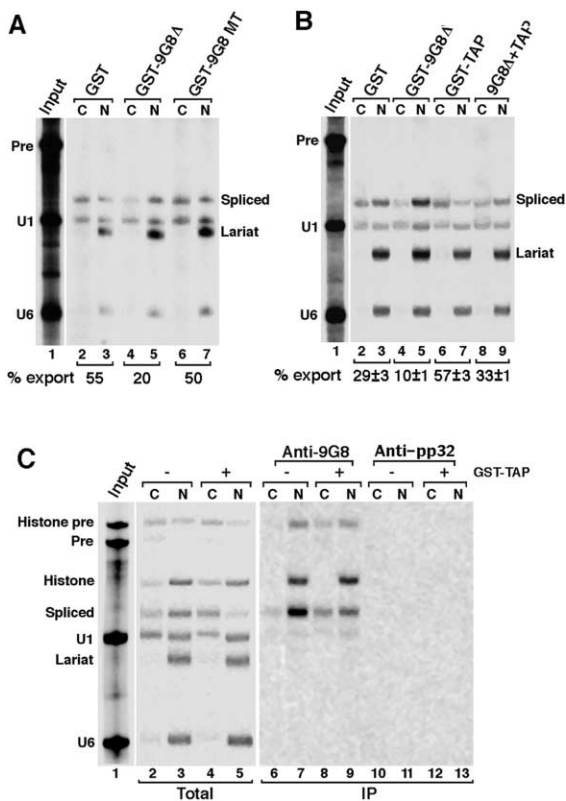


Figure 3. 9G8 Participates in Export by Recruiting TAP

(A) The N terminus of 9G8 is an export inhibitor. GST, GST-9G8Δ, or GST-9G8 MT (3 mg/ml) was injected into oocyte nuclei. The second nuclear injection was a mixture (Input) of labeled AdML pre-mRNA (Pre) (Zhou et al., 2000) and U1 and U6 snRNAs. For each group, eight oocytes were fractionated into nucleus (N) and cytoplasm (C) and one oocyte equivalent analyzed.

(B) TAP relieves the inhibition caused by GST-9G8Δ. GST (3 mg/ml), GST-9G8Δ (3 mg/ml), GST-TAP (0.3 mg/ml) plus GST (3 mg/ml), or GST-TAP (0.3 mg/ml) plus GST-9G8Δ (3 mg/ml) was injected into oocyte nuclei, followed by a second injection of the RNA mixture and analysis as in (A).

(C) 9G8 is associated with RNAs exported by TAP. GST (0.3 mg/ml, -) or GST-TAP (0.3 mg/ml, +) was injected into oocyte nuclei, followed by a second nuclear injection of a mixture (Input) of the histone pre-mRNA (Histone pre), AdML pre-mRNA (Pre), and U1 and U6 snRNAs. In lanes 2–5, one oocyte equivalent of nuclear and cytoplasmic RNAs was loaded on the gel. In lanes 6–13, six oocyte equivalents of nuclear and cytoplasmic fractions after IP with the indicated antibodies were loaded.

teins were injected, the AdML pre-mRNA (Pre) was efficiently spliced, indicated by its disappearance and the accumulation of the intron lariat in the nucleus (Figure 3A, lanes 3, 5, and 7).

Only 20% of the spliced AdML RNA (Spliced) was exported to the cytoplasm of oocytes preinjected with GST-9G8Δ (lanes 4 and 5). In contrast, the export of Spliced in oocytes preinjected with the control proteins GST (lanes 2 and 3) or GST-9G8 MT (lanes 6 and 7) was about 50%. This inhibition was mRNA specific since the export of U1 snRNA was not affected by GST-9G8Δ. Time-course analyses (data not shown) further revealed that the export block was constant over time and was not due to a secondary effect on splicing.

These results suggested that TAP is limiting in oocytes and that the N terminus of 9G8 blocks export by competing with TAP for binding to adapter proteins such as REF/Aly and SR proteins. In support of this hypothesis, injection of recombinant TAP resulted both in more efficient Spliced RNA export in the absence of GST-9G8Δ (Figure 3B, compare lanes 6 and 7 to lanes 2 and 3) and in relief of export inhibition in the presence of GST-9G8Δ (compare lanes 8 and 9 to 4 and 5). Injection of even higher doses of recombinant TAP failed to enhance export further (data not shown), presumably because of limited amounts of the essential TAP cofactor, p15, in oocytes (Braun et al., 2001; Guzik et al., 2001; Katahira et al., 1999). Injection of baculovirus-expressed and histidine-tagged full-length 9G8 had no effect on mRNA export (data not shown), likely because endogenous 9G8 is already present in excess.

### 9G8 Contributes to mRNA Export through Association with TAP

Inhibition of export by injection of the N terminus of 9G8 is consistent with a potential function for endogenous 9G8 in recruiting TAP to its bound mRNAs. If 9G8 is utilized as an export adapter, we would expect endogenous 9G8 to be associated with spliced RNAs exported by TAP. Oocyte nuclei were injected with GST or GST-TAP, followed by a second injection of a labeled mixture of an intronless histone-related pre-mRNA (Histone pre), the AdML pre-mRNA (Pre), and U1 and U6 snRNAs. Histone pre contains the 101 nt histone export element shown to bind 9G8 and SRp20 (Huang and Steitz, 2001; Huang et al., 1999). After incubating the oocytes for 3 hr, nuclear and cytoplasmic fractions were prepared and subjected to IP. In the absence of exogenous TAP, ~60% of Histone pre underwent 3'-end processing, and ~15% of these molecules (Histone) were exported to the cytoplasm. The AdML pre-mRNA (Pre) was completely spliced and ~30% of the spliced RNAs (Spliced) exported (Figure 3C, lanes 2 and 3). Exogenous TAP enhanced the export of Spliced about 3-fold and Histone about 2-fold (compare lanes 4 and 5 to lanes 2 and 3).

Significantly, the IP revealed that 9G8 is associated with processed mRNAs both in the nucleus and in the cytoplasm (lanes 6–9), while the control antibody did not IP any RNAs (lanes 10–13). The association is specific since intron lariats and U1 and U6 snRNAs were not appreciably precipitated. Importantly, elevated export by TAP led to an increased level of 9G8-associated spliced RNA in the cytoplasm (compare lane 8 to 6), suggesting that 9G8 actively recruits TAP and exits the nucleus associated with the mRNA.

We cannot explain the absence of 9G8 association with processed histone mRNA in the cytoplasm, but it is possible that this reflects differential dissociation of 9G8 from various RNA cargoes following export. Indeed, REF/Aly is likewise not detected in association with spliced mRNAs in the oocyte cytoplasm (Kim et al., 2001b). Binding of 9G8 to Histone pre containing the 101 nt element was predicted (lanes 8 and 9) (Huang and Steitz, 2001). The stimulated export of this precursor RNA in the presence of exogenous TAP (lanes 4 and 5) mimics previous observations of pre-mRNA and intron-

less mRNA export from oocyte nuclei when export factors are in excess (Zhou et al., 2000). Taken together, our results strongly suggest that 9G8 promotes the export of both intronless and spliced mRNAs by recruiting TAP.

## Discussion

Our data indicate that, like REF/Aly, shuttling SR proteins can recruit TAP to bound mRNAs, providing adapter function for export. Members of the SR protein family and the REF family all contain at least one RRM domain (Graveley, 2000; Stutz et al., 2000). Yet it is not this domain but rather the flanking regions of REFs that have been demonstrated to interact with TAP (Stutz et al., 2000). These regions exhibit no apparent sequence similarity to SR proteins (data not shown). However, the fact that SR proteins and REFs interact with the same region of TAP suggests that they share an unidentified common feature.

Our finding that shuttling SR proteins partner with TAP makes TAP an attractive candidate export receptor for SR proteins. Although the RS domains are not required for TAP interaction *in vitro* (Figures 1 and 2), it is possible that the phosphorylation status of the RS domains alters SR protein subcellular localization (Cáceres et al., 1998; Koizumi et al., 1999; Sanford and Bruzik, 2001) or interactions, thereby influencing TAP interactions *in vivo*. SC35 does not shuttle (Cáceres et al., 1998), nor does it bind TAP (Figure 1C), presumably because of association with interfering cellular factors (Cazalla et al., 2002).

The association of SR proteins with mRNAs occurs through the proteins' selective affinity for exonic splicing enhancers in pre-mRNAs (Graveley, 2000) and export elements in intronless mRNAs (Huang and Steitz, 2001). Cooperative binding with other SR proteins is also known to be important for mRNA association (Graveley, 2000), and splicing may remodel these complexes. In fact, SRp20 and REF/Aly are found in the exon junction complex (EJC) that stably associates with mRNAs after splicing and is thought to play important roles in nonsense-mediated decay as well as in mRNA export (Kim et al., 2001a, 2001b; Le Hir et al., 2001, 2000; Lykke-Andersen et al., 2001; Reichert et al., 2002). REF/Aly interacts with the splicing factor UAP56 (Sub2 in yeast) (Fleckner et al., 1997; Kistler and Guthrie, 2001) and is thought to be delivered to the EJC by UAP56 (Jensen et al., 2001; Luo et al., 2001; Straesser and Hurt, 2001). Although UAP56 (Sub2) is essential for general mRNA export (Gatfield et al., 2001; Jensen et al., 2001; Luo et al., 2001; Straesser and Hurt, 2001), its role in mRNA export may not be limited to recruiting REF/Aly to spliced mRNAs. Indeed, depletion of REF/Aly does not lead to significant nuclear accumulation of poly(A) RNAs in *Drosophila* cells (Gatfield and Izaurralde, 2002). We have tried but not succeeded in detecting interactions between human UAP56 and SR proteins (data not shown). Instead, SR proteins may be targeted to mRNAs via a transcription-coupled process (Cramer et al., 1999).

Since the nuclear exit of mRNAs involves numerous protein-protein interactions as the mRNP traverses the nuclear pore, it would not be surprising if multiple export adapters were required for efficient recruitment of multi-

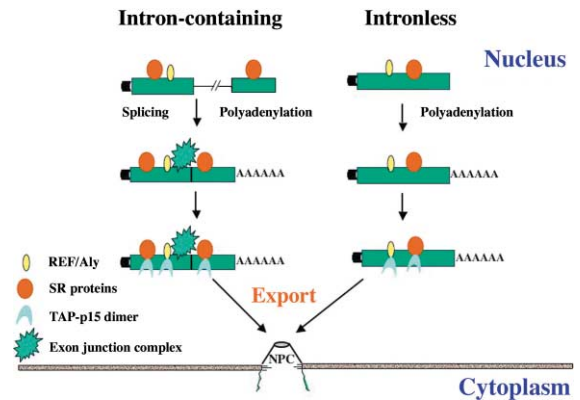


Figure 4. A Working Model for mRNA Nuclear Export

Left: SR proteins and REF/Aly bind pre-mRNA. Shuttling SR proteins remain bound to spliced mRNA. The EJC assembled upon splicing also contains SR proteins and REF/Aly (see text). TAP-p15 complexes are then recruited by SR proteins and REF/Aly. Right: SR proteins and REF/Aly bind intronless mRNAs in a splicing-independent fashion, followed by the cooperative recruitment of TAP-p15 complexes. In both cases, multiple adapter molecules interact with multiple TAP-p15 complexes to mediate export.

ple copies of TAP to each mRNA (Figure 4). Our results uncover another link between splicing and mRNA export, underscoring the extensive molecular interplay between the various steps in eukaryotic gene expression.

## Experimental Procedures

### Plasmid Constructs

The plasmids expressing Flag-TAP, Flag-A1, His-9G8, GST-9G8 $\Delta$ , GST-9G8 $\Delta$ Zn, GST-ASF, and GST-ASF $\Delta$  were described (Cavaloc et al., 1999, 1994; Lykke-Andersen et al., 2001). The plasmid encoding GST-9G8 MT was created by site-specific mutagenesis of the plasmid expressing GST-9G8 $\Delta$ . Plasmid pGEX-4T1-TAP encoding GST-TAP was made by J. Lykke-Andersen by subcloning the BamHI/NotI fragment of the plasmid expressing Flag-TAP into pGEX-4T1 (Pharmacia) at the corresponding sites. pGEX-4T1-TAP 1–231 used to express GST-TAP231 was prepared by J. Lykke-Andersen by digesting pGEX-4T1-TAP with StuI/NotI, treating with the Klenow fragment enzyme, and religating. The resulting construct expresses a truncated TAP containing the first 231 amino acids. Plasmid pGEX-4T1-REF2-1 encoding GST-REF (human REF2-1) was made by J. Lykke-Andersen by subcloning the BamHI/NotI fragments of the plasmid expressing Flag-REF2-1 (Lykke-Andersen et al., 2001) into pGEX-4T1 (Pharmacia) at the corresponding sites. The plasmid for Histone pre was created by inserting the 101 nt mouse histone H2a export element (Huang et al., 1999) into pBluescript at the EcoRV site, followed by insertion of a PCR fragment of the mouse histone H3.2 gene (nt 473–623, containing the histone 3'-end processing signal) at the HindIII/XhoI sites downstream of the 101 nt sequence.

### Antibodies

The anti-Flag M2 and anti-SRp20 antibodies were from Sigma and Zymed, respectively. The anti-hnRNP A1 antibody (Pixol-Roma and Dreyfuss, 1992) was a gift from Dr. G. Dreyfuss. The anti-pp32 (Brennan et al., 2000), anti-ASF/SF2 (Cavaloc et al., 1999), anti-HuR (Galoulzi and Steitz, 2001), and anti-SC35 (Cavaloc et al., 1999) antibodies were as described.

### Cell Transfection and Immunoprecipitation

Cell transfection and immunoprecipitation with the anti-Flag antibody was performed as described (Lykke-Andersen et al., 2001). All IP and *in vitro* binding assays were performed in the presence of 200  $\mu$ g/ml of RNase A. For the *in vitro* binding assays in Figures 1C and 2C, GST or the indicated GST-fusion proteins (10  $\mu$ g each)

pre-bound on 20  $\mu$ l of glutathione beads were incubated with HeLa nuclear extracts (40  $\mu$ l) (Dignam et al., 1983) in 3 ml of binding buffer (10 mM Tris-HCl [pH 8.0], 150 mM NaCl, 10% glycerol, 0.2% Triton X-100, 1 mM DTT, 0.25 mM PMSF, 1X protease inhibitor cocktail [Calbiochem], and 200  $\mu$ g/ml of RNase A) at 4°C for 2 hr. Beads were washed six times with binding buffer and bound proteins eluted with SDS sample buffer, followed by SDS-PAGE and Western blot analysis. In Figure 1B, 5  $\mu$ g of GST or GST-TAP pre-bound on 5  $\mu$ l of beads was mixed with 10  $\mu$ g of histidine-tagged 9G8 in 200  $\mu$ l of binding buffer and analyzed as above. In Figures 2 and 3, GST or the indicated GST-fusion proteins (5  $\mu$ g each) pre-bound on 5  $\mu$ l of beads were incubated with 5  $\mu$ l of an  $^{35}$ S-labeled mixture of TRN1 and TAP or of the three TAP fragments synthesized by the *TnT* coupled in vitro transcription and translation (Promega) in 200  $\mu$ l of binding buffer. Bound and unbound fractions were resolved and visualized by autoradiography. A plasmid expressing Flag-TAP (Lykke-Andersen et al., 2001) was used for making  $^{35}$ S-labeled TAP and the three TAP fragments. The plasmid for  $^{35}$ S-labeled TRN1 (Pollard et al., 1996) was from G. Dreyfuss.

#### **Xenopus Oocyte Injection and RNA Analysis**

Oocyte injection (13 nl/oocyte) and analyses were performed basically as described in Huang and Steitz (2001). In brief, proteins at the indicated concentrations were injected into oocyte nuclei, followed by a second nuclear injection of a  $^{32}$ P-labeled RNA mixture (150 cpm/oocyte) 1 hr later. After 3 hr incubation, oocytes were dissected, and nuclear and cytoplasmic RNAs analyzed by 8% denaturing PAGE. The quantitations in Figure 3B are an average of two independent experiments. The IP procedure was carried out as reported (Ohno et al., 2002); 12  $\mu$ g of affinity-purified anti-9G8 or 20  $\mu$ l of anti-pp32 was used per six oocytes. The injection and IP experiments were performed three times, and similar results were obtained.

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