Specific Interaction between RNA Helicase A and Tap, Two Cellular Proteins That Bind to the Constitutive Transport Element of Type D Retrovirus*

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Constitutive transport element (CTE) facilitates retroviral RNA export by interacting with the cellular RNA export machinery. Two cellular proteins, RNA helicase A (RHA) and Tip-associated protein (Tap) were identified as binding to CTE and were proposed to function as CTE co-factors (1, 2). Here, we report that these two CTE-binding proteins interact with each other in vitro and in vivo. The in vitro binding of RHA to Tap is direct and independent of either CTE or the nuclear transport domain of RHA. The removal of the first 60 amino acids of Tap significantly diminishes the binding to RHA. The activity of this Tap mutant to enhance CTE-mediated gene expression is also markedly reduced. A transdominant mutant of Tap inhibited RHA-mediated up-regulation of CTE function in mammalian cells. The nuclear transport domain of RHA also interfered with Tap-mediated transactivation of the CTE function in quail cells, in which the function of CTE is dependent on the expression of a functional human Tap cDNA.

Retroviruses face the unique problem of having to export full-length, unspliced mRNA from the nucleus in order to propagate. Complex and simple retroviruses have evolved different systems to overcome this problem. Complex retroviruses encode a viral regulatory protein as the RNA exporter, whereas simple retroviruses utilize cellular RNA-exporting proteins. In both cases, the recognition of the viral unspliced RNA depends on cis-acting viral RNA motifs present in the virus genome but absent from the completely spliced subgenomic RNA.

HIV Rev is the prototypical viral RNA exporter. It contains a leucine-rich nuclear export signal (NES) and utilizes the nuclear export pathway mediated by cellular protein CRM-1 (3–7). Lencine-rich NES-containing proteins access the CRM-1 pathway through specific interactions among NES, RanGTP, and CRM-1 proteins. Such interactions can be disrupted by the drug leptomycin B (8). Not surprisingly, Rev/RRE-mediated HIV RNA export is leptomycin B-sensitive (8). Even though cellular mRNA export in mammalian cells appears to be different from the Rev/RRE pathway and thus CRM-1-independent (9, 10), studies in yeast suggest that the export of a subset of yeast mRNA may also utilize the CRM-1 pathway (11). A recent study in *Xenopus* oocytes also showed that LR-NES could compete for nuclear export of some cellular mRNA (12). These data suggest that the CRM-1 pathway is conserved from yeast to human, but in mammalian cells where greater amounts and more complex RNA/protein cargoes exist, it has evolved into a more specialized export pathway.

A well studied RNA export motif of the simple retroviruses is the constitutive transport element (CTE) of type D retroviruses (13–16). CTE is proposed to interact directly with cellular factor(s) to function and presumably utilize the export pathway of a subset of cellular mRNAs. Two CTE-binding proteins have been identified. The first candidate CTE co-factor is RNA helicase A (RHA), which binds to wild-type but not functionally impaired mutants of CTE. RHA also shuttles constantly between the cytoplasm and the nucleus, despite its steady-state nuclear localization (1). This shuttling ability was later shown to be conferred by a bidirectional nuclear transport domain (NTD) localized at the carboxyl terminus of the protein (17). The functional significance of CTE-RHA interaction is underscored by microinjection experiments in which injection of an anti-RHA antibody blocked CTE-mediated gene expression in human cells (18). Recently, RHA was shown to associate with pre-mRNA in vivo, suggesting a possible role in cellular mRNA processing/export (19).

Human Tap (herpesvirus saimiri Tip-associated protein) is identified as another CTE-binding protein (2, 20). Its yeast homologue, Mex67p, was previously shown to be involved in poly(A) RNA export in yeast. Mutation of Mex67p results in the nuclear accumulation of poly(A) RNA (21). Functional studies of both *Xenopus* oocytes and somatic cells further validate the role of Tap in the nuclear export of CTE-containing RNA. However, there are still conflicting reports regarding the minimal domains in Tap required to assist in CTE function (22–24) in vivo. Tap also binds to RNA nonspecifically in vitro and associates with a nucleoporin, Nup214, again suggesting a general role in cellular mRNA export (25, 26).

Here, we report the interaction between these two CTE-binding proteins, RHA and Tap. Interactions are detected in vivo by GST pull-down and in vitro by co-immunoprecipitation experiments. Binding domains are mapped to the amino termini of both proteins. The possible functional relevance of this interaction in CTE function is also addressed.

**EXPERIMENTAL PROCEDURES**

Plasmids—pGST-Tap-(1–619) and pGST-Tap-(61–619) were generous gifts from Dr. B. Felber. The GST-Tap carboxyl-terminal deletion mutants were constructed by direct digestion of GST-Tap-(1–619) with appropriate restriction enzymes and reclosing the parental plasmid by

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† The abbreviations used are: NES, nuclear export signal; HIV, human immunodeficiency virus; CTE, constitutive transport element; RHA, RNA helicase A; NTD, nuclear transport domain; Tap, Tip-associated protein; GST, glutathione S-transferase; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; CRM, chromosome region maintenance; RRE, Rev response element.
blunt-end ligation. The DNA fragments encoding amino acids 319–619 and 505–619 of Tap were amplified by PCR and cloned into pGEX-2T (Amersham Pharmacia Biotech) to make GST-Tap (319–619) and GST-Tap (505–619). pc-RHA, GFP-CTD, and PK-NTD were described previously (1, 17). Carboxyl-terminal deletions of RHA were constructed by digesting pcRHA with appropriate restriction enzymes and re-closing the parental plasmid by blunt-end ligation. The amino-terminal deletion plasmid pcRHA-(640–1269) was constructed by cloning the 2.0-kilobase Afl-II and XhoI fragment of pcRHA (encoding amino acids 640–1269 of RHA) back into pcDNA3 by blunt-end ligation. A consensus Kozak sequence and ATG start codon downstream of the AflII site facilitated the translation of the carboxyl-terminal half of RHA. The Tap plasmid pc-myc-Tap was constructed by cloning a BamHI/EcoRI fragment from pGST-Tap (1–619) containing the full-length Tap cDNA into the BamHI and EcoRI sites of Myc-NPc-TNLS (27). The coding sequence for Tap (61–619) was obtained by PCR and cloned into the BamHI/EcoRI sites of Myc-NPc-TNLS. Tap-X was constructed by digesting pc-myc-Tap with XhoI and re-closing the parental plasmid, thus removing the carboxyl-terminal sequence (amino acids 584–619) downstream of the XhoI site of Tap cDNA. Tap-X-NTD was constructed by cloning a PCR fragment encoding the NTD of RHA into the XhoI site of Tap-X. The XhoI site engineered onto the PCR fragment ensured that NTD was in frame with the rest of the Tap sequence. A stop codon was introduced after the NTD, pDM13sCTE, pDM13sCTE-antisense, and pSV-gagpol-MPMVCTE (gagpol-CTE) were all described previously (13, 28).

Cell Culture, Transfections, and Immunoprecipitation—Cos-1 cells, HeLa cells, and D-17 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Qc-3 cells were maintained in M199 medium containing 4% fetal calf serum and 1% chicken serum (29). Cos-1 and HeLa cells were transfected by conventional calcium phosphate precipitation method, and D-17 and Qc-3 cells were transfected with the Effectene transfection reagents (Qiagen). CAT assays, p24 assays, Western blotting, and heterokaryon assays were performed as described (17, 18, 28). Co-immunoprecipitation was done as follows: 293T cells were transfected with pc-myc-Tap, cells were collected and lysed in RIPA lysis buffer (50 mM Tris-Cl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS) 48 h post-transfection. The cell lysate was then incubated with normal rabbit serum-coated agarose beads for 1 h to block nonspecific interactions. The beads were removed by centrifugation, and the supernatant was then incubated with either a rabbit pre-immune serum or a rabbit polyclonal antibody against RHA for 1 h. Protein A/G beads (Santa Cruz Biotechnology) were then added, and the mixture was further incubated at room temperature for another 3 h and then washed four times with the lysis buffer. The precipitated proteins were separated on a 10% gel, and a monoclonal antibody, 9E10, was used to detect the myc-tagged Tap protein. The immunodepletion assay was done with the same cell lysate. After incubating with RHA antibody and protein A/G beads, the supernatant was analyzed by immunoblotting with the appropriate antibodies. The same cell lysate was also subject to RNA selection assays with biotinylated CTE RNA prepared by in vitro transcription. CTE RNA selected RHA and Myc-Tap from the cell extract simultaneously, suggesting that both proteins are in a complex with CTE RNA (Fig. 1C).

RESULTS

RHA Binds to Tap in Vitro and in Vivo—GST pull-down experiments were performed to test the interaction between RHA and Tap. A HeLa nuclear extract was used as a conventional source of RHA. Extracts were incubated with column-bound GST and GST-Tap to form protein complexes. After extensive washing, the bound proteins were separated by polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. RHA was identified by a polyclonal antibody on a Western blot. As shown in Fig. 1A, GST-Tap specifically pulled down RHA, whereas GST did not show any binding to RHA (lanes 4 and 7). Because both RHA and Tap are RNA-binding proteins, we treated the nuclear extracts with RNase before the binding reaction, to rule out the possibility that some cellular RNA was bridging the observed interaction. The RNase treatment did not affect the binding (lanes 11 and 13), suggesting that RNA does not play a bridging role. An interaction was also observed between GST-Tap and recombinant RHA protein (lane 16), indicating that the interaction is direct.

To test the in vivo interaction between Tap and RHA, co-immunoprecipitation experiments were performed. A myc-tagged form of Tap was expressed in 293T cells by transfecting the Tap expression plasmid pc-myc-Tap. Total cellular proteins were then subject to immunoprecipitation with an anti-RHA antibody. The precipitated proteins were separated, transferred to a polyvinylidene difluoride membrane, and probed with a monoclonal antibody 9E10, which recognizes the myc tag. Pre-immune rabbit serum was used as a negative control for the immunoprecipitation reaction. Myc-Tap was efficiently co-immunoprecipitated by the anti-RHA antibody, whereas the control serum did not produce any signal (Fig. 1B, left panel). It is noteworthy that this in vivo interaction was detected in the absence of any expression of CTE. Co-expression of CTE RNA with Tap did not enhance the binding (data not shown). To confirm that RHA and Tap are present in the same complex in vivo, an immunodepletion experiment was carried out to deplete RHA from the cell extracts containing the transfected Myc-Tap. Incubation with an anti-RHA antibody depleted 80% of RHA from the extract. (Fig. 1B, left). Similar depletion of Myc-Tap was also observed in the same extract (Fig. 1B, middle). In contrast, the level of a control cellular protein, MEK-1, was not affected (Fig. 1B, right). To demonstrate that CTE, Tap, and RHA can form a complex in vivo, the same cell lysate used for the co-immunoprecipitation and immunodepletion experiments was subject to RNA selection with biotinylated CTE RNA prepared by in vitro transcription. CTE RNA selected RHA and Myc-Tap from the cell extract simultaneously, suggesting that both proteins are in a complex with CTE RNA (Fig. 1C).

The Nuclear Transport Domain of RHA is Dispensable for Tap Binding—We next examined whether the NTD of RHA is required for the binding of RHA to Tap. RHA and deletion mutants of RHA were produced by in vitro transcription/translation and labeled with 35S. The proteins were then subjected to GST pull-down assays by GST-Tap. β-Galactosidase was also translated and used in the binding as a negative control. Like the endogenous RHA, the in vitro translated full-length RHA bound to GST-Tap. β-Galactosidase did not show any binding to GST-Tap under the same condition (Fig. 2A), demonstrating the specificity of the interaction. Next, truncation mutants of RHA with deletions from both the amino and the carboxyl termini of the protein were made and tested for binding to GST-Tap. As shown in Fig. 2B, the minimal Tap-binding domain on RHA is mapped to the amino terminus of the protein (amino acids 1–257), indicating that the carboxyl-terminal half of the protein, which contains the NTD, is not necessary for its binding to Tap.

The Amino-terminal Extension of Tap Contributes to Binding to RHA—The original Tap CDNA isolated by Yoon et al. (20) encoded 559 amino acids. Later, an additional sequence of 60
amino acids was found to reside upstream of this original cDNA. Both reverse transcriptase-PCR and Western analysis indicated that the longer cDNA represents the true full-length Tap cDNA (23, 24). We also used this longer version of Tap, designated Tap-(1–619), in our experiments for binding to RHA. To map the RHA-binding domain on Tap, we generated deletion mutants truncating from both ends of the Tap cDNA. These mutants were made as GST-fusion proteins and assayed for their abilities to pull down RHA from the HeLa nuclear extract. The Tap mutants containing the amino-terminal extension (amino acids 1–61) all produced significant degradation products upon purification from E. coli, whereas mutants lacking the first 61 amino acids were more stable. However, Tap-(1–619) is very stable when expressed in 293T cells (Fig. 1A), indicating the instability of the full-length Tap is result of a bacteria-specific degradation event, which was not investigated further. Despite the degradation in E. coli, we were able to obtain a sufficient amount of full-length recombinant Tap proteins to perform the GST pull-down experiments (Fig. 2C). The carboxyl terminus of Tap is dispensable for RHA binding. Although the amino-terminal 60-amino acid extension of Tap contributes to the binding, its removal did not completely abrogate RHA binding. Tap-(61–619) retained weak (about 20%) of the full-length Tap RHA binding (Fig. 2C, right panel). The amino-terminal half (amino acids 1–444) of the full-length Tap is sufficient to bind to RHA in vitro.

The NTD of RHA Exerts Transdominant Effects on Tap Function—To explore the possible cross-talk between RHA and Tap in assisting CTE function in vivo, we tested whether RHA or its mutants had any effects on CTE function in a system in which CTE function is dependent on Tap. In the Qcl-3 cell line, which is such a system, CTE-mediated gene expression is dependent on the co-expression of a functional cDNA of human Tap (24).

We first validated the requirement of human Tap for CTE function in these quail cells. The CAT gene expression from the CTE reporter construct pDM138CTE was low and comparable with that from the control plasmid pDM138CTE-antisense, in which CTE was expressed in the antisense orientation (Fig. 3A, left panel). In the canine cell line v-17, CAT expression from pDM138CTE was at least 10 time higher than from pDM138CTE-antisense (Fig. 3A, left panel). CTE also functioned very well in HeLa cells (Fig. 3A, left panel). We then co-transfected the Tap cDNA expression plasmid (pc-myc-Tap) with CTE reporter constructs. In Qcl-3 cells, Tap was found specifically to enhance CTE-mediated gene expression but had no effect on the CTE antisense reporter (Fig. 3A, right panel). However, in our hands, the transactivation effect of Tap was limited to 2–3-fold, in contrast to the >14-fold enhancement reported by Kang and Cullen (24) using essentially the same CAT reporter constructs. Varying the amount of co-transfected Tap plasmid from 50 to 500 ng did not raise the transactivation effect of Tap more than 3-fold (data not shown). The reason for this discrepancy is not clear at this time.

We continued to explore other reporter systems to obtain a higher level of transactivation by Tap on CTE in Qcl-3 cells. Using the HIV gag-pol-CTE reporter plasmid, pSV-gag-pol-MVCTE (13), we found that co-transfecting 100 ng of pg-myc-Tap resulted in a 7–10-fold increase in p24 expression from a functional CTE (Fig. 3B). Interestingly, Tap-(61–619) is only minimally functional in these experiments. An average of less than 2-fold increase of p24 expression was observed with this Tap...
mutant (Fig. 3B). Tap-(61–619) is as stable as Tap-(1–619) expressed in quail cells and more stable than Tap-(1–619) as bacterially expressed GST fusion proteins (Figs. 2C and 3C).

A bi-directional transport domain (NTD) was identified at the carboxyl terminus of RHA (17). When overexpressed in mammalian cells in which CTE is fully functional, NTD exerted a modest inhibitory effect on CTE-mediated reporter gene expression (data not shown). This low level of inhibition may be a result of the high endogenous level of RHA and/or Tap in these cells. We then tested the effect of NTD on CTE activity in Qcl-3 cells where CTE-mediated gene expression can be manipulated by controlling the amount of co-transfected Tap. As shown in Fig. 3D (upper left panel), in this system, excess NTD exerted a significant transdominant negative effect on CTE function. This inhibition was specific to CTE, as no effect was seen when a pCAT reporter or a Rev/RRE reporter system (pDM128) was used (Fig. 3D, middle and lower left panels). To control for possible promoter competitions, an irrelevant myc-tagged protein, NPc-TNLS, expressed from the same promoter was used in parallel experiments (Fig. 3C, right panels).

A transdominant Tap Mutant Inhibits RHA-mediated Up-regulation of CTE Function in Permissive Cells—We next examined whether a transdominant Tap mutant can interfere with the ability of RHA to up-regulate CTE function in permis-
sive cells (18). We deleted the carboxyl-terminal 36 amino acids of Tap and generated Tap-X. A similar mutant of Tap was reported to have a transdominant negative effect on wild-type Tap in Qcl-3 cells (24). Here, we show that Tap-X could also inhibit CTE function significantly in permissive cell lines such as D-17 and HeLa cells (Fig. 4A, compare CTE alone and with Tap-X). The up-regulation of CTE function by over-expression of RHA in HeLa cells was also completely abolished by co-transfecting an equal amount of Tap-X expression plasmid (Fig. 4A), suggesting that this Tap mutant interferes with RHA function in vivo. Again, no inhibition was seen with the pCAT reporter (Fig. 4B).

RNA Binding and Shuttling of Tap Is Not Sufficient for Its Function as a CTE Co-factor—A nuclear export signal of Tap was reported to reside at the carboxyl terminus of the Tap protein (24). However, this carboxyl-terminal domain was later shown to be important for the nuclear rim association and to interact with components of the nuclear pore complex (22, 25, 26), a distinct nuclear export domain was mapped to amino acids 83–110 of the protein (22). The inability of Tap-X to transactivate CTE in quail cells is thus probably a result of a defect in its interaction with the nuclear pore complex rather than the inability to partition or transport Tap to the nucleus.

Fig. 4. A transdominant mutant of Tap inhibits CTE function and RHA-mediated up-regulation of CTE function in mammalian cells. A, HeLa cells growing in 6-well plates were transfected with gagpol-CTE and various transactivator/inhibitor expression plasmids. RHA further increased the CTE-mediated p24 expression by 2.5-fold as reported previously (11). This up-regulation, as well as the basal CTE activity, was inhibited by Tap-X, a transdominant negative mutant of Tap. B, neither transactivation by RHA nor inhibition by Tap-X was observed when a control plasmid, pCAT, was used as the reporter.

FIG. 3. NTD of RHA inhibits Tap-mediated CTE function in Qcl-3 cells. A, left, CTE in the sense orientation failed to promote CAT expression in pDM138CTE in quail cells. D-17 and HeLa cells fully supported CTE function. Right, co-expression of full-length Tap with this CAT reporter specifically transactivated CTE-mediated CAT expression in Qcl-3 cells. The -folds of transactivation never exceeded 3 in all our experiments when pDM138CTE was used as the reporter. B, the first 60 amino acid residues of Tap are important for CTE-mediated HIV Gag expression in quail cells. C, proper expression of myc-tagged Tap and Tap-(61–691) in transfected Qcl-3 cells. D, the NTD of RHA specifically inhibited CTE-mediated HIV Gag expression in the presence of Tap. Qcl-3 cells and three different reporters were used. SV-gagpol-CTE was the CTE reporter, and pCAT served as the internal control for transfection efficiency variations and nonspecific inhibitions. A Rev/RRE reporter, pDM128, was also used. 100 ng of the Tap or Rev plasmid was co-transfected with the CTE reporter or the RRE reporter where indicated. Increasing amounts (100, 200, 400, and 500 ng) of PK-NTD or NPo-TNLS was transfected in all three groups to assess the specific, dosage-dependent inhibition of Tap-mediated CTE function in these quail cells.
than the lack of nuclear export per se. Because NTD contains a nuclear export signal and can be incorporated into the nuclear pore complex (Ref. 1; data not shown), we examined whether NTD could replace the carboxyl domain of Tap that is missing in Tap-X. To this end, we constructed an expression plasmid, Tap-X-NTD, in which NTD was fused to the carboxyl terminus of Tap-X. Proper expression of Tap, Tap-X, and Tap-X-NTD in transfected cells was verified by Western blot using an anti-Myc antibody that recognized the amino-terminal myc tag of all three proteins (Fig. 5A). Tap-X-NTD was then tested for its ability to transactivate the CTE function in Qcl-3 cells. Surprisingly, fusion of NTD to Tap-X not only did not rescue Tap function, it also further increased the inhibitory effect of Tap-X (Fig. 5, B and C). Tap-X typically inhibited the CTE activity by 50%, whereas Tap-X-NTD consistently inhibited more than 90% of the Tap-mediated CTE activity.

When the above experiments were repeated in the CTE-permissive cell line D-17, identical inhibition profiles were observed for Tap-X and Tap-X-NTD (Fig. 5D). In this cell line, the transactivation by human Tap is minimal because CTE is fully functional. We propose that Tap-X-NTD exerts a greater inhibitory effect by combining the inhibitory effect of Tap-X and NTD. Both Tap-X and Tap-X-NTD were able to shuttle in heterokaryon assays (Ref. 14; data not shown).

**DISCUSSION**

Post-transcriptional regulation is an important aspect of the tight control of retroviral gene expression. A unique feature of this process is the nuclear export of the unspliced retroviral mRNA, because normally only fully spliced mRNA can exit the nucleus. The CTE motif enables the type D retroviruses to access cellular RNA export pathways that normally would not be accessible to the unspliced RNA. In this study, we show that two CTE-binding proteins, RHA and Tap, can interact with each other and that a transdominant negative mutant of one interferes with the function of the other. The reciprocal inhibition by these mutants suggests that functional cross-talk exists between RHA and Tap in mediating CTE function in vivo.

The CTE export pathway overlaps with the cellular mRNA export pathway and is CRM-1-independent (9, 10). The NTD of RHA has also been shown to export through a leptomycin B-insensitive pathway, which is thus distinct from the CRM-1-dependent pathway (17). Similarly, the carboxyl-terminal domain of Tap did not interact with CRM-1 in vitro, and Tap-mediated transactivation of the CTE reporter gene was not inhibited by a nucleoporin mutant that inhibits the CRM-1 export pathway (24), indicating that Tap also utilizes an export receptor distinct from CRM-1. The fact that the RHA NTD is dispensable for Tap binding suggests that RHA is not “piggybacking” on Tap for export. The reverse cannot be proved or disproved at this time, as the real location of the nuclear export signal in Tap is still controversial (22–24). In any case, it is likely that Tap and RHA use similar or overlapping nuclear export pathways, as NTD can interfere with both RHA and Tap function in vivo. Because both Tap and RHA have been implicated in the nuclear export of subtypes of cellular mRNA, it is also possible that the RHA-Tap interaction contributes to the processing/export of these cellular mRNA.

The finding that the first 60 amino acids of Tap are required for optimal binding to RHA is interesting. This fragment was found to be dispensable for CTE binding both in vitro and in vivo. In Xenopus oocytes, Tap-(61–619) was sufficient to enhance CTE export from the nucleus when CTE was placed in an intron and exported as an excised intron lariat (2). However, the same investigators later reported that the domains of Tap required to stimulate CTE-mediated nuclear export of an excised intron lariat or of U6 RNA are very different (26). The data with U6-CTE is more consistent with data from somatic cells. Whether Tap-(61–619) is active in the U6-CTE system was not addressed in the more recent study. In the quail cell line Qcl-3, Tap-(61–619) was found to be less active than Tap-(1–619) in transactivation of CTE-mediated CAT gene expression, even though the CTE binding activities of the two proteins were comparable (24). In this report, we show that Tap-(61–619) is only marginally active in promoting CTE-mediated HIV.
Gag expression in quail cells. Because Tap-(61–619) retains full CTE binding, nuclear export, and nuclear pore association activities and is as stable as full-length Tap in quail cells, our data suggest that the defect of Tap-(61–619) to enhance CTE-mediated gene expression in quail cells results from its diminished binding to RNA.

We consistently observed a high level of transactivation of CTE function in quail cells when a CTE-Gag reporter (pSV-gagpol-CTE) was used instead of a CTE-CAT reporter (pDM138CTE). We also observed a very high background level of “leaky” CAT expression by the pDM138 reporter constructs in the quail cells, possibly contributing to the poor transactivation by Tap when these constructs were used.

Multiple RNA processing steps that can contribute to the function of CTE and its co-factors likely exist in vivo. Emerging evidence suggests that several aspects of RNA processing are tightly linked in vivo. Releasing RNA from the splicing complex, polyadenylation of the pre-mRNA, translocation across the nuclear pore complex, releasing the RNA exporter from the RNA-protein complex in the cytoplasm, and finally, recycling the exporter back to the nucleus are all related and possibly coupled steps of RNA processing after transcription and splicing. Recently, cis-acting RNA elements from three intron-less coupled steps of RNA processing after transcription and splicing in vivo are involved in the nuclear export of unspliced RNA.2

To note that CTE is not functional in yeast cells to promote the nuclear export of unspliced RNA.2 In vivo nonpermissive for CTE function, indicating that Tap is not required for Rev/RRE to function.

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REFERENCES

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