Structure and function of mRNA export adaptors

Matthew J. Walsh, Guillaume M. Hautbergue and Stuart A. Wilson
Department of Molecular Biology and Biotechnology, The University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, U.K.

Abstract
The mRNA export adaptors provide an important link between multiple nuclear mRNA processing events and the mRNA export receptor TAP/NXF1/Mex67p. They are recruited to mRNA through transcriptional and post-transcriptional events, integrating this information to licence mRNA for export. Subsequently they hand mRNA over to TAP and switch TAP to a higher-affinity RNA-binding state, ensuring its stable association with mRNA destined for export. Here we discuss the structure and function of adaptors and how they are recruited to mRNA.

Introduction
Eukaryotic cells produce mRNA in the nucleus through a series of events including 5′ capping, 3′-end processing and splicing which are coupled with transcription. Once these processes are complete, mRNA is exported from the nucleus to the cytoplasm where it can be translated to generate proteins. A key protein involved in mRNA export is TAP/NXF1 (Mex67p in yeast). TAP forms a heterodimer with p15 and binds nucleoporins via C-terminal UBA-like and central domains [1]. Normally unspliced mRNAs are prevented from leaving the nucleus. However, when TAP is artificially tethered to pre-mRNA [2] or bound directly via the viral CTE (constitutive transport element), nuclear retention of unspliced mRNAs is overcome [3]. Clearly, export of such partially processed mRNAs is not desirable for the majority of cellular mRNAs and there are mechanisms in place to ensure appropriate loading of TAP/Mex67p on to mRNA. Mex67p is initially recruited to sites of transcription by interaction with ubiquitylated Hrp1p but this is RNA independent [4]. The main proteins integrating the signals provided by mRNA processing and triggering TAP loading are the export adaptors. The term ‘adaptor’ was coined because these proteins, which bind RNA with high affinity, were thought to serve as a bridge between mRNA and TAP. This idea arose from the intrinsically low RNA-binding activity observed for recombinant TAP [5]. However, experiments have shown that the binding of RNA and TAP to adaptors is mutually exclusive. TAP binding to an adaptor–RNA complex triggers transfer of the mRNA directly to TAP, leaving the adaptor free of mRNA but bound to TAP. TAP uses an N-terminal arginine-rich motif to bind RNA and in the presence of a bound adaptor, binds RNA with approx. 4-fold higher affinity [6]. Therefore adaptors stimulate initial recruitment of TAP to the mRNP (messenger ribonucleoprotein) and the subsequent stable direct interaction of TAP with mRNA, which is required for efficient nuclear export of mRNA. Interestingly, overexpression of TAP-p15 overrides normal nuclear retention mechanisms and stimulates export of pre-mRNA which is normally retained in the nucleus [7]. The high levels of TAP–p15 presumably compensate for the low RNA-binding affinity in the absence of a suitably loaded adaptor protein, allowing export. In the cytoplasm, TAP plays a role in translation when bound to the CTE [8], although whether it is involved in the translation of cellular mRNA is unclear. Ultimately TAP dissociates from mRNA to be recycled to the nucleus and, in yeast, DBP5p, which is conserved in mammals, is involved in the displacement of Mex67p [9].

The adaptor protein family
The canonical adaptor is REF (RNA-binding and export factor) in metazoans (Yra1p in yeast). In Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster and mouse there are multiple REF genes, whereas in humans there is a single gene named REF/ALY/THOC4. Knockout of Yra1 in yeast leads to an mRNA export block which can, however, be complemented by overexpression of Yra2p [10]. In contrast, knockdown of multiple REF genes in C. elegans [11] and D. melanogaster [12] has no significant impact on global mRNA export. Even in human cells, knockdown of REF only leads to a partial block in mRNA export [13]. These results suggest the existence of other adaptors and there may be redundancy of action. Consistent with this, the shuttling SR (serine- and arginine-rich) proteins 9G8, SRp20 and ASF/SF2 all bind TAP directly and function as adaptors [14]. These proteins bind specific elements in mRNA such as exonic splice enhancers and trigger recruitment of TAP to mRNAs harbouring these sequences. Since RNA and TAP binding are also mutually exclusive for SR proteins [6,15] this means that on TAP recruitment they are unlikely to remain directly bound to the mRNA, rather they will remain associated with the mRNP via TAP. The SR proteins undergo a cycle of dephosphorylation in the nucleus during splicing followed by cytoplasmic rephosphorylation. TAP

Key words: ALY, mRNA export, NXF1, RNA-binding and export factor (REF), serine- and arginine-rich protein (SR protein), TAP.

Abbreviations used: CTD, C-terminal domain; CTE, constitutive transport element; FMRP, Fragile X mental retardation protein; mRNP, messenger ribonucleoprotein; Pol II, RNA polymerase II; REF, RNA-binding and export factor; RNA recognition motif; SR, serine- and arginine-rich.

1To whom correspondence should be addressed (email stuart.wilson@sheffield.ac.uk).

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binds the hypophosphorylated form of SR proteins [16,17]. This leads to a model in which SR proteins interact with TAP post-splicing in the nucleus and the TAP:SR protein complex is destabilized in the cytoplasm. The destabilization of the TAP:SR protein complex, which will revert TAP to a low-affinity RNA-binding state might also prime removal of TAP from cytoplasmic mRNA by Dbp5. The third type of general adaptor is heterodimer U2AF, which binds TAP via the U2AF35 subunit. Overexpression of U2AF65 subunit leads to enhanced recruitment of TAP to the mRNP and increased export of a reporter mRNA [18]. FMRP (Fragile X mental retardation protein) binds both TAP and its parologue, NXXF2 [19–21] and may stimulate export of FMRP target mRNAs. Recently, THOC5 has been identified as a co-adaptor for mRNA export which binds simultaneously with REF to TAP and stimulates export of specific mRNAs [13]. THOC5 is part of TREX and therefore TREX has multiple subunits which bind TAP (REF, THOC5 and HP1R). While adaptors can remodel TAP so that it binds RNA with high affinity, it is not yet clear whether co-adaptors share this activity. Also, it is uncertain whether THOC5 binds RNA and TAP simultaneously as was originally expected of REF or whether it is also displaced from mRNA on binding TAP, remaining associated with the mRNP through TAP. Several other proteins bind TAP including E1B-AP5 [22], DDX3 [23] and a number of proteins initially identified in a genome wide RNAi (RNA interference) screen for mRNA export factors [24]. However, it is not clear yet whether any of these proteins function as adaptors or co-adaptors. In yeast, Yra2p and the SR-like Npl3p also bind Mex67p. In common with metazoan SR proteins, Npl3p binds Mex67p in the dephosphorylated state and this is promoted by the phosphatase Gic7p [25]. Therefore in yeast there also appear to be multiple factors which bind Mex67p and contribute to its recruitment to the mRNP.

Structure and function of adaptors

The structures for relevant domains of ALY, REF2-I, ASF/SF2, 9G8 and SRp20 mRNA export adaptors have been determined using NMR [2,5,26,27] (Figures 1A and 1B). A common feature of these structures is an RRM (RNA recognition motif) flanked by unstructured regions. For REF2-I, the RRM is flanked by two unstructured regions, with an N-terminal transient helix. The N-terminal domain including the helix interacts transiently with the RRM in the free state (Figure 1B). Both N- and C-terminal domains carry arginine-rich regions which bind RNA and TAP, although the interaction between the N-terminal domain and TAP is stronger [6]. The interaction of RNA with REF also involves weak interactions with loops 1 and 5 of the RRM and similarly TAP binds the surface of the RRM weakly [27] (Figures 1A and 1B). Despite the weak interaction observed between the REF RRM and TAP in vitro, this interaction is important for a stable REF-TAP interaction in vivo and for REF function in mRNA export [6]. A minimal 21 amino acid arginine-rich peptide has been identified in REF2-I which is necessary and sufficient for TAP binding and within this peptide several arginine residues are essential for the interaction (Figure 1). The arginine residues which bind TAP are also implicated in RNA binding which in combination with the overlapping binding sites in the RRM, probably explains why RNA and TAP binding to REF are mutually exclusive [6]. Intriguingly, REF also has the effect of stimulating the RNA-binding activity of TAP, once bound to TAP. The mRNA-binding domain of TAP resides between amino acids 60–119 which are predicted to be unstructured. This region is encompassed within the minimal REF-binding domain of TAP (amino acids 1–198). Therefore it is possible that on binding REF, the RNA-binding domain of TAP adopts a more constrained structure capable of binding RNA with higher affinity. The NMR structures have also been determined for relevant regions of SRp20, 9G8 and ASF/SF2. For 9G8 and SRp20, RNA binding principally involves the RRM and the structure for SRp20 bound to RNA has been determined [2]. For both these proteins, TAP binding involves an arginine-rich peptide which lies immediately C-terminal of the RRM and adjacent to an extended unstructured C-terminal RS domain rich in serine and arginine residues. ASF/SF2 is a two-RRM domain protein connected by a glycine- and arginine-rich linker and the TAP interaction with ASF/SF2 requires arginine residues within the linker. The ASF/SF2 RRM2 structure has been determined and shows an atypical RRM fold with a considerably extended loop 5 region. Moreover, this RRM interacts with RNA in an unusual way, involving important interactions with helix alpha 1, not previously observed with the RRM fold [15]. A striking feature of the TAP-binding peptide found in adaptors is its low level of complexity. Why bind this peptide in SR proteins and not the extended C-terminal domain rich in arginine residues? Answers may lie in the fact that the TAP-binding peptide is immediately adjacent to the RRM, which for REF and 9G8 have been shown to contribute to the overall interaction with TAP.

mRNA association/dissociation of adaptors

Yra1p directly binds Sub2p [28] and this interaction is conserved in humans (REF-binding UAP56) [29], similarly U2AF binds UAP56 [30] whereas there is no evidence for an interaction between SR proteins and UAP56. Sub2p/UAP56 play an essential role in mRNA export and their loss leads to a profound export block. The Yra1p–Sub2p complex assembles with the multisubunit THO complex to create the TREX complex, which is conserved [31]. THO associates with the transcriptional machinery, leading to the view that a THO–Sub2p complex would recruit Yra1p to genes undergoing transcription to create TREX. Surprisingly though, Sub2p is not required for this process [32]. Instead the Pcf11p subunit of the 3′-end processing complex CF1A, which directly binds the CTD (C-terminal domain) of the large subunit of Pol II (RNA polymerase II), interacts with Yra1p and stimulates its recruitment to transcribing genes. As well as providing a link to the transcription machinery, Pcf11p connects adaptors and
the 3′-end processing machinery. Efficient 3′-end processing is essential for mRNA export and it is noteworthy that Yra1p binds the same region of Pcf11p as another component of the 3′-end processing machinery, Clp1p. Therefore Yra1p might regulate 3′-end processing by preventing recruitment of Clp1p to this complex until the appropriate time [33]. Furthermore, exchange of Clp1p for Yra1p on Pcf11p might be coupled with loading Yra1p onto the nascent mRNA by interaction with the Sub2p/UAP56 component of TREX (Figure 2). Consistent with this, loss of Sub2p leads to polyadenylation defects [34] and accumulation of a stalled export intermediate [35]. While the Pcf11p–Yra1p interaction is observed with the human counterparts, a number of other factors influence REF recruitment in human cells. First, the histone chaperone Spt6 binds Ser2-phosphorylated CTD of Pol II directly. In turn Spt6 binds Iws1 which recruits REF to Spt6-responsive genes [36]. Intriguingly, another histone chaperone Nap1p, is recruited to genes via Yra1p [37]. This suggests the possibility that nucleosome reassembly in the wake of transcribing Pol II may be co-ordinated in part by export factors bound to the nascent mRNA produced. A more general factor involved in adaptor loading is the
nuclear cap-binding complex. REF is preferentially loaded on to mRNAs which are capped and binds very close to the 5′ end, whereas other components of TREX appear to bind slightly further away from the 5′ cap. The ability of REF to bind near the 5′ cap is probably governed by its interaction with CBP80 [38]. The recruitment of TREX to intron-containing pre-mRNAs is also dependent on splicing [39], although REF is recruited to intronless mRNAs and promotes their export [40]. Once REF is associated with mRNA, an mRNP remodelling event occurs as described above in which TAP is recruited to the mRNP. This has three main consequences; first, UAP56 is displaced from REF by TAP; secondly mRNA is handed over from REF to TAP and thirdly REF remains bound to TAP and enhances its RNA-binding affinity (Figure 2) [6]. The exchange of Sub2p for Mex67p bound to Yra1p may be stimulated by the TREX-2 complex [41] which also plays a role in positioning active genes close to the nuclear pore [42].

Importantly, once TAP has been recruited to an adaptor–mRNP complex, the adaptor is likely to remain bound to the mRNP via TAP and not via a direct interaction with the mRNA [6]. This arrangement may predispose adaptors to be readily dissociated from the mRNP. Dissociation of SR proteins from the mRNP is coupled with their phosphorylation status. In contrast, results from both Chironomus tentans [43] and yeast [9] indicate that REF/Yra1p dissociates from the mRNP immediately prior to or during its export through the nuclear pore. Mlp1 and Mlp2, which interact with Yra1p and reside on the nuclear side of the pore may be involved [44] and Tom1 ubiquitylates Yra1p [45], which may be coupled with its displacement from Mex67p. Following adaptor dissociation from TAP/Mex67p, they are predicted to revert to a low-affinity RNA-binding conformation, priming them for cytoplasmic displacement from the mRNA by Dbp5p (Figure 2).

Summary

mRNA must undergo multiple processing events in the nucleus before it is licensed for export to the cytoplasm. Export adaptors and co-adaptors integrate information feeding in from multiple nuclear mRNA processing events, including splicing, capping and polyadenylation and decode this to determine whether or not mRNA is ready for export. Therefore adaptors play a central role in the basic mechanisms governing eukaryotic gene expression. Evidence is emerging that adaptors and co-adaptors interact with specific mRNAs potentially providing different pathways for mRNA export. This opens up the possibility of regulated mRNA export for different classes of mRNA, which might be a genome-wide means of controlling gene expression.

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References

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