Biochimica et Biophysica Acta 1819 (2012) 578–592

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Review

Structural basis for the assembly and disassembly of mRNA nuclear export complexes ☆

Eugene Valkov, Jack C. Dean, Divyang Jani, Sonja I. Kuhlmann, Murray Stewart *

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK

ARTICLE INFO

Article history:
Received 22 November 2011
Received in revised form 26 January 2012
Accepted 17 February 2012
Available online 2 March 2012

Keywords:
mRNA nuclear export
Nuclear export factor
NXF1
Mex67
Dbp5
DEAD-box helicase

ABSTRACT

Most of the individual components of the nuclear elements of the gene expression pathway have been identified and high-resolution structural information is becoming available for many of them. Information is also starting to become available on the larger complexes they form and is beginning to give clues about how the dynamics of their interactions generate function. Although the translocation of export-competent messenger ribonucleoprotein particles (mRNPs) through the nuclear pore transport channel that is mediated by interactions with nuclear pore proteins (nucleoporins) is relatively well understood, the precise molecular mechanisms underlying the assembly of export-competent mRNPs in the nucleus and their Dbp5-mediated disassembly in the cytoplasm is less well defined. Considerable information has been obtained on the structure of Dbp5 in its different nucleotide-bound states and in complex with Gle1 or Nup159/NUP214. Although the precise manner by which the Dbp5 ATPase cycle is coupled to mRNP remodelling remains to be established, current models capture many key details of this process. The formation of export-competent mRNPs in the nucleus remains an elusive component of this pathway and the precise nature of the remodelling that generates these mRNPs as well as detailed understanding of the molecular mechanisms by which this step is integrated with the transcriptional, splicing and polyadenylation machinery by the TREX and TREX-2 complexes remain obscure. This article is part of a Special Issue entitled: Nuclear Transport and RNA Processing.

1. Introduction

1.1. Overview of mRNA nuclear export

The separation of transcription and translation in eukaryotes enables nascent mRNA to be processed in a number of ways in the nucleus before it is translated in the cytoplasm. In addition to 5′ capping, splicing, and cleavage/polyadenylation, the spectrum of proteins bound to the mRNA also undergoes a series of transformations before the resulting mRNP is exported to the cytoplasm. Although often treated as separate features of the gene expression pathway, it is emerging that these steps are integrated to a considerable extent so that the pathway, or at least its nuclear section, is better thought of as a concerted production line rather than as a series of isolated processes. It is crucial for the nuclear phase of the pathway to be completed before translation can begin. Consequently, the nuclear envelope is critical in preventing the ribosomes from encountering the mRNA until processing has been completed. It is only when this stage is reached that the mRNA is finally exported through the nuclear pores (NPCs) to the cytoplasm. This export is mediated by specific transport factors such as Mex67:Mtr2 in yeast or the corresponding NXF:NXT family in metazoans.

2. Nuclear export of mRNA as a thermal ratchet

Nuclear pores (NPCs) mediate the exchange of macromolecules between the nuclear and cytoplasmic compartments and have a central transport channel that is lined with nuclear pore proteins (nucleoporins or “nups”) that have characteristic phenylalanine–glycine (FG) repeating sequence motifs. These FG-nucleoporins form a dense meshwork that functions as a barrier to the movement of macromolecules of Mr greater than ~40 kDa. Although overall the export of mRNA to the cytoplasm through the NPCs is an active process, it is powered indirectly through a thermal ratchet mechanism [1–3]. There are three key phases to this process: first, an export-competent mRNP is formed in the nucleus by attaching transport factors to the mRNA; second, these transport factors or carrier proteins are able to overcome the NPC barrier function and thus enable the mRNP to diffuse back and forth between the nucleus and cytoplasm through the NPC transport channel. Third, nett transport is achieved by the disassembly of the export-competent mRNP in the cytoplasm, removing the transport factors, which prevents return of the mRNP though the NPCs to the nucleus. Both, the generation of an export-competent mRNP in the nucleus

☆ This article is part of a Special Issue entitled: Nuclear Transport and RNA Processing.

* Corresponding author. Tel.: +44 1223 402463; fax: +44 1223 213556.
E-mail address: ms@mrc-lmb.cam.ac.uk (M. Stewart).

1874-9399/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbagrm.2012.02.017

and its dissociation in the cytoplasm, are mediated by specific remodelling of the mRNP that results in the attachment and detachment of the transport factors at the appropriate place (reviewed in [1,4–8]). The movement of an export-competent mRNP back and forth through the NPC central transport channel is mediated by thermal motion (diffusion), albeit in the confined environment of the FG-nucleoporin meshwork, and it is the remodelling of the mRNP both before and after the passage that rectifies this thermal motion and results in a nett overall movement of the mRNA from the nucleus to the cytoplasm [1–3]. Thus, although nuclear transport is an active process, the movement of the material through the pore transport channel does not require metabolic energy. Instead, metabolic energy is required for the assembly of export-competent mRNPs in the nucleus and for their disassembly in their cytoplasm [1–3]. Both the assembly and disassembly of export-competent mRNPs is facilitated by factors associated with the pores themselves. This review will concentrate on the structures of the components involved in these processes, principally the transport factors and their interaction with NPC proteins (nucleoporins); the factors involved in the remodelling steps associated with the formation and dissociation of export-competent complexes; and the factors involved in integrating mRNA nuclear export with earlier steps in the gene expression pathway. Although many of the key components and mechanisms involved in the nuclear export of mRNA are broadly conserved from yeast to mammalian cells, the nomenclature employed in different systems is not. Table 1 shows the principal yeast components of these pathways together with their corresponding metazoan homologues.

3. Mex67:Mtr2 — the principal mRNA export factor in budding yeast

The principal mRNA nuclear export factor Mex67:(TAP or NXF1 in metazoans) is one of the better-characterised constituents of the mRNA export machinery and can be readily cross-linked to poly(A)$^+$ RNA *in vivo* [9]. Moreover, the thermosensitive *mex67-5* allele shows rapid poly(A)$^+$ RNA accumulation in the nucleus at the restrictive temperature coupled with the encoded protein becoming mislocalised to the cytoplasm [10,11]. Both Mex67 and TAP/NXF1 are members of the NXF protein family and contain an N-terminal RNA recognition motif (RRM) followed by a leucine-rich region (LRR), then a domain homologous to the transport factor NTF2, and finally a C-terminal motif related to ubiquitin-associated (UBA) domains (Fig. 1A). The NTF2-like domains of Mex67 and TAP/NXF1 heterodimerise with Mtr2 and p15/NXT1, respectively, two small proteins of unrelated sequence but similar structure and function [12–14]. Mex67:Mtr2 binds mRNPs that constitute a significant proportion of all transcriptional events in yeast [15] and can also bind to NPCs [16]. The association of Mex67 with Mtr2 is crucial for nuclear mRNA export and this heterodimer shuttles between the nucleus and the cytoplasm [14,17].

The NTF2-like and UBA domains of Mex67 interact with nucleoporin (nup) phenylalanine–glycine (FG) repeats that line the NPC channel. They probably compete with karyopherins for binding to FG-nucleoporins, because both types of receptor use the same binding sites for translocation through the NPCs [16,18,19]. The structure of the human TAP:p15 heterodimer complex bound to an FG repeat closely resembles the NTF2 homodimer that mediates the nuclear import of RanGDP [20]. However, two nucleoporin-binding sites are required for TAP-mediated directional export of mRNA, which can be provided by the wild-type protein or by two NTF2-like domains or two UBA-like domains in tandem [18]. For a TAP variant in which the NTF2-like domain was replaced by a second copy of the UBA domain, mRNA export was independent of p15 [18]. Over-expression of human TAP:p15 in yeast partially restores the growth of the lethal *mex67/mtr2* double knockout [13,21], underscoring the high degree of conservation of the mRNA export machinery. The crystal structure of the yeast Mex67:Mtr2 NTF2-like complex (Fig. 1) showed that Mtr2 is a novel member of the NTF2-like family and that its association with Mex67 resembles that of the TAP:p15 complex [22]. However, unlike TAP, Mex67 only binds FG-nucleoporin repeats efficiently when complexed to Mtr2 [19]. In addition, Mtr2 associates with Nup85, a subunit of the Nup84 complex that is crucial for nuclear mRNA export [14], and both Mex67 and Mtr2 interact with the NPC-associated protein Sac3 [23,24].

Despite its functional importance, the Mex67:mRNA interaction appears to be weak and non-specific, so that additional adaptor proteins are required to facilitate this association [13,14]. Recruitment of Mex67 to nascent pre-mRNAs is facilitated by the RNA-binding adaptor, Yra1 [25]. Although Yra1 involvement in mRNA export is conserved throughout eukaryotes, it is not always essential in metazoans [26–28]. Following the recruitment of Mex67:Mtr2 by Yra1, mRNPs are thought to be remodelled, after which Yra1 appears to dissociate from the mRNP before it is exported [29]. Initial observations that Yra1 binds only a portion of the yeast transcriptome [15] have led to genetic investigations in model metazoans that have identified additional adaptors that assist in mediating the interaction with mRNA [26,30]. In yeast, Mex67 may be also recruited to the mRNP by the SR-like protein Npl3 [31]. The association of Mex67:Mtr2 with Npl3 is tightly regulated at the post-translational level by phosphorylation, thus providing a possible means of checkpoint control and trapping of mRNA in the cytoplasm [31–33].

3.1. The structural framework of TAP:p15 and Mex67:Mtr2 complexes

P15 and the p15-interacting domain of TAP are both based on a highly curved β-sheet flanked by α-helices, forming a conical fold analogous to that of NTF2 [20]. Despite their low sequence identity, both p15 and the NTF2-like TAP domain are structurally homologous (Fig. 1C). However, there is a 33-residue loop inserted after the first β-strand of TAP, which contains two short helical segments whose overall spatial orientation is dictated through interactions with conserved residues (Fig. 1C). The TAP and p15 NTF2-like domains form a compact heterodimer (Fig. 1A) similar to the NTF2:NTF2 homodimer with the two NTF2-like domains opposing each other across the convex faces of their β-sheets. The interacting surfaces include hydrophilic residues, especially in p15, which are only weakly conserved and interact indirectly via solvent molecules, suggesting a degree of conformational plasticity at the interface. However, the interface is strengthened considerably by contacts involving the insertion loop of TAP. A salt bridge between Asp482 (TAP) and Arg134 (p15) is highly conserved. Unlike its structural equivalent in NTF2, this salt bridge is not symmetric in the TAP:p15 heterodimer, where Asp76 (p15) interacts instead with Arg440 in the TAP insertion loop, with additional stability provided by main chain contacts

Table 1
Components of the mRNA nuclear export machinery in yeast and their metazoan homologues.

Protein		*Function*
Yeast	*Metazoa*	
Mex67	NXF1 (TAP)	Principal mRNA export factor. Binds Mtr2
Mtr2	NXT1 (P15)	Binds NTF2-like domain of Mex67
Yra1	ALY (REF, THOC4)	Adaptor that links Mex67 to mRNA
Sub2	UAP56 (DDX39)	DEAD-box helicase that remodels mRNPs prior to nuclear export
Dbp5	DDX19	DEAD-box helicase that disassembles export complex
Gle1	GLE1	Activator of Dbp5 DEAD-box helicase
Nup159	NUP214 (CAIN, CAN)	Nucleoporin to which Gle1 binds
Sac3	GANP	Scaffold for TREX-2 complex
Sus1	ENY2	Component of TREX-2 and SAGA complexes
Cdc31	Centrin-2/3	Component of TREX-2 complex