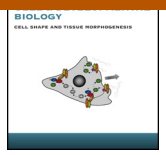
Seminars in Cell & Developmental Biology 32 (2014) 47–54



Seminars in Cell & Developmental Biology

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



Review

# Mechanisms of mRNA export

Petra Björk[1], Lars Wieslander*

*Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, SE-106 91 Stockholm, Sweden*

ARTICLE INFO

*Article history:*
Available online 9 May 2014

*Keywords:*
Gene expression
Pre-mRNA processing
mRNA
mRNP export

ABSTRACT

Release of properly processed and assembled mRNPs from the actively transcribing genes, movement of the mRNPs through the interchromatin and interaction with the Nuclear Pore Complexes, leading to cytoplasmic export, are essential steps of eukaryotic gene expression. Here, we review these intranuclear gene expression steps.



**Contents**

1. Introduction ..... 47
2. Formation of pre-mRNPs/mRNPs at the gene ..... 49
   2.1. Pre-mRNA and mRNA binding proteins ..... 49
   2.2. RNA-folding and RNA-protein assembly ..... 49
3. mRNP release from the gene ..... 49
4. Movement of mRNPs through the interchromatin and export competence ..... 50
   4.1. Restricted diffusion in the interchromatin ..... 50
   4.2. When is the mRNP completely export competent? ..... 50
   4.3. Acquisition of export receptors ..... 50
   4.4. Export receptors and elements in the mRNA ..... 50
   4.5. Initiation and completion of splicing ..... 51
5. Docking at the Nuclear Pore Complex ..... 51
   5.1. NPC composition and structure ..... 51
   5.2. Productive and non-productive mRNP–NPC interactions ..... 51
   5.3. Active gene–NPC interactions in yeast ..... 51
6. Translocation through the Nuclear Pore Complex ..... 52
   6.1. Entrance of the mRNP into and passage through the central channel ..... 52
   6.2. Directionality of translocation and release of the mRNP ..... 52
7. An alternative export pathway ..... 52
8. Conclusions ..... 52
   Acknowledgements ..... 52
   References ..... 52

## 1. Introduction

Gene expression in eukaryotic cells requires coordinated nuclear processes (Fig. 1A). These processes include synthesis and maturation of pre-mRNAs, assembly of pre-mRNA–protein complexes (pre-mRNPs), followed by transport through the interchromatin space, docking at and export through the Nuclear Pore

* Corresponding author. Tel.: +46 8 161720.
*E-mail addresses:* Petra.Bjork@su.se (P. Björk), Lars.Wieslander@su.se (L. Wieslander).
[1] Tel.: +46 8 161720.

http://dx.doi.org/10.1016/j.semcdb.2014.04.027

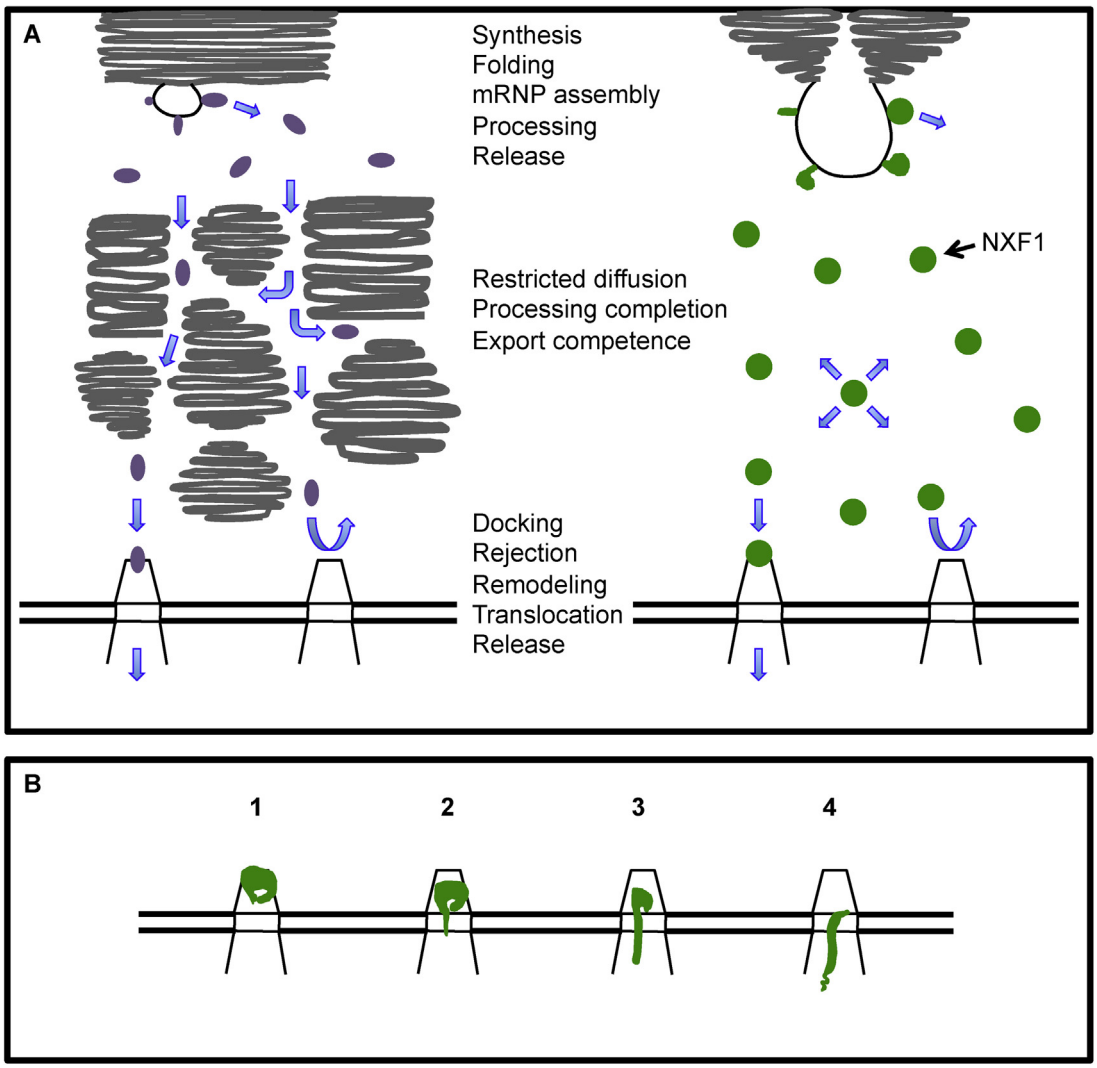



**Fig. 1.** From the gene to the cytoplasm. (A) Schematic views on diploid nuclei (left) and polytene nuclei (right). In diploid nuclei, transcribing genes with pre-mRNPs are found in perichromatin regions. The released mRNPs (violet ovals) move by restricted diffusion inside interchromatin channels (arrows). These channels form a network that opens in the nuclear periphery close to NPCs (two NPCs are depicted at the bottom of the figure). Movement inside channels may influence directionality and efficiency of the transport from gene to NPC. The mRNPs often are rejected at the NPCs (bent arrow). In polytene nuclei of *C. tentans* salivary gland cells, the BR mRNPs (green circles) are released from the gene locus into a large continuous volume of interchromatin and become part of an mRNP pool. The BR mRNPs move in all directions. NXF1 is recruited in the interchromatin. Eventually the BR mRNPs interact with the NPCs. Also here, the majority of interactions lead to rejection of the BR mRNPs (bent arrow). Fully export competent BR mRNPs are translocated through the NPC central channel. The processes occurring at the different nuclear compartments are indicated. Folding of the nascent transcript, assembly into RNA–protein complexes and processing into mRNPs take place at the gene loci. BR mRNPs are released from the gene locus and recruit NXF1, the major export receptor, in the interchromatin. (B) Schematic view of the docking and translocation of BR mRNPs through the NPC (70). The export competent BR mRNP first interacts with the basket of the NPC (1). The BR mRNP then moves to the center of the NPC (2). The BR mRNP is rearranged into a ribbon-like structure that is translocated through the central channel with the 5′-end first, but retain contact with the nuclear ring (3). At the cytoplasmic face, the BR mRNP unfolds into a thinner mRNP fiber (4).

Complexes (NPC) and release of the mRNP into the cytoplasm. Pre-mRNA processing results in extensive remodeling of the pre-mRNAs themselves, as a consequence of capping, splicing, 3′-end cleavage and polyadenylation and in specific cases editing. All of these processing events require specific interactions of molecular machineries with the pre-mRNAs and all of them take place largely cotranscriptionally. The active gene locus thus constitutes a local environment where essential steps of gene expression occur [1]. In addition to physical changes of the transcript itself, the pre-mRNA and subsequently the mRNA bind a multitude of different RNA-binding proteins and additional, associated proteins. Many of these proteins become part of the pre-mRNP already at the gene and therefore, the unit that is released from the gene is an mRNA–protein complex (mRNP). Downstream processes of gene expression mainly involve the protein components of the mRNPs. Knowledge about eukaryotic gene expression is therefore highly dependent upon studies of the formation and behavior of mRNPs. In particular, we need knowledge about endogenous, gene specific mRNPs in intact cells, but this knowledge is difficult to achieve because of experimental limitations. In this review, we will highlight one biological system, the Balbiani ring (BR) genes in the salivary glands of the dipteran *Chironomus tentans* (*C. tentans*) and their pre-mRNPs/mRNPs [2,3]. The reason is that these genes express huge pre-mRNPs/mRNPs in large quantities in polytene nuclei. These features make it possible to, in intact cells, analyze the formation of individual BR pre-mRNPs during transcription, cotranscriptional processing, movement of the BR mRNPs inside the interchromatin space, docking at the NPCs, passage through the NPCs and subsequent release into the cytoplasm. A uniquely detailed spatial and temporal view of the pathway from the gene to the cytoplasm is therefore possible to obtain for the BR genes.

In this review, we focus on the exit of mRNPs from the gene, movement through the interchromatin and export to the cytoplasm. We will use the polytene nuclei and the BR mRNPs as an illustrative example, but also relate to other specific experimental systems. Together, knowledge obtained from favorable experimental biological systems will hopefully provide an up to date view of intranuclear processes of central importance for eukaryotic gene expression in general and especially for mRNP export.

## 2. Formation of pre-mRNPs/mRNPs at the gene

### 2.1. Pre-mRNA and mRNA binding proteins

EM images of active BR genes in situ [2] as well as of lampbrush chromosome loops [4], show that the nascent transcripts form RNA–protein complexes continuously during transcription. Also in perichromatin regions in diploid interphase nuclei, nascent transcripts form RNA–protein complexes [5]. Structural studies of the elongating RNA polymerase II have demonstrated that the nascent transcript passes through a channel in the polymerase [6]. Very rapidly after emerging from the polymerase the transcript is presumably accessible for various proteins that can interact with the transcript. Interactions between pre-mRNPs, the RNA polymerase II elongation complex and processing machineries take place and result in coordination, efficiency and regulation [7]. All subsequent nuclear gene expression steps thus take place in the context of a pre-mRNP/mRNP [8].

In the cell, a large number of proteins exist that contain domains capable of binding to RNA, often combined with other type of domains that give the proteins various specific functions. RNA-binding proteins are involved in all gene expression events. The large repertoire of RNA-binding proteins exists as a result of the combination of domain organization, achieved by alternative splicing, tissue specific expression and protein modifications [9].

Pre-mRNPs/mRNPs contain both universal and unique proteins. In addition, proteins can be present in different amounts. For example; cap-binding proteins CBP20 and CBP80, as well as PABN1 (polyadenylate binding protein1), are parts of presumably all mRNPs. Exon Junction Complex (EJC) components are present in mRNPs containing exon–exon junctions. Several proteins, such as SR proteins and hnRNP proteins have varying degrees of sequence binding specificity and it has been shown that different pre-mRNPs/mRNPs contain different combinations and amounts of such proteins [10]. So far, exact measurements of the composition of endogenous gene specific pre-mRNPs/mRNPs are lacking largely because of experimental difficulties to purify individual pre-mRNPs/mRNPs. Analyses of the protein composition of mRNPs, in vitro assembled in HeLa cell extracts, indicate that a large number of proteins are present in mRNPs and that splicing influences the composition [11]. Immuno-EM studies have located a number of different protein components in the BR pre-mRNPs/mRNPs.

### 2.2. RNA-folding and RNA-protein assembly

Pre-mRNA has an intrinsic property to fold, presumably influenced by evolution to favor functionally productive folds, although a great number of possible folds are most likely almost as energetically stable. Folding of the pre-mRNA starts during transcription [12] and can be functionally highly important. The transcription rate can influence how the transcript folds by restricting or enlarging the available secondary structure options [13,14]. It is also possible that the RNA fold can influence the rate of transcription [15]. During splicing, external snRNAs guide the local fold at the exon–intron borders to optimize and direct the transesterification reactions of splicing. Splicing signals, for example intronic polypyrimidine tracts, may be hidden in RNA secondary structures and influence splicing efficiency and location [16]. Incorrect folding or slow folding may be involved in kinetic proofreading of splicing [17].

Equally important during splicing is the influence on RNA folding by various RNA-binding proteins.

We need to learn more about the folding of pre-mRNAs/mRNAs. Also detailed knowledge about the structure of the RNA–protein assembly within gene specific pre-mRNPs/mRNPs is required. RNA-binding proteins influence the folding of the pre-mRNA. EM observations of nascent transcripts, present in perichromatin, have revealed fibrillar and granular structures corresponding to pre-mRNPs [5]. During transcription, the BR pre-mRNAs initially assemble into RNA–protein fibers and at a distinct length from the transcription start site (about 8 kb), folds into a globular structure, finally reaching a diameter of about 50 nm [2]. The substructure of the BR pre-mRNP/mRNP seems to be a 7 nm in diameter RNA–protein fiber [18]. In yeast, isolated mRNPs, studied by EM have a thickness of 5–7 nm [19]. It is possible that compaction of the BR pre-mRNP into a globular complex depends on interactions between EJC core components and SR proteins as suggested for human endogenous mRNPs [20]. EJC core components and SR proteins are present in the BR pre-mRNPs when the pre-mRNA has reached the critical length where a more compact folding takes place. Although the BR mRNP structure can be dynamic, as shown during transport through NPCs [21], the globular overall morphology remains intact during movement within the interchromatin.

Measurements of the diffusion coefficients for RNAs of different lengths, in vitro assembled into mRNPs, demonstrated that the RNPs adopted compact shapes [22]. Images of purified mRNPs from yeast showed that they had compact, elongated, ribbon-like structures [19]. Longer RNAs were present in longer structures but it was suggested that the mRNPs had a common core structure. Considering that mRNPs from different genes have different lengths, different numbers of exon–exon junctions with recruited EJCs and different sequences influencing which RNA-binding proteins are associated with the mRNPs, it is likely that variants of mRNP structures exist. Pre-mRNPs from the BR1 and BR2 genes have very similar morphology. These genes are also very similar as to length, exon–intron organization and sequence. In contrast, the shorter BR3 gene has a different exon–intron organization and sequence, and the BR3 pre-mRNPs are different [23].

From a theoretical point of view, several consequences of assembly into compact pre-mRNPs/mRNPs have been put forward. Packaging could influence processing events by influencing the proximity of RNA sites in space or by hiding/exposing specific sites. It is well known that certain hnRNP proteins influence alternative splicing by competing with SRFS1 for pre-mRNA binding [24]. Interaction between proteins included in different processing complexes, but brought close in space are known to occur and may coordinate different processes [7]. Packaging could also influence physical parameters during transcription and transport through the interchromatin.

## 3. mRNP release from the gene

Cotranscriptional pre-mRNP assembly and processing events such as capping and splicing are important steps in preparing the mRNP for export. Correct 3′-end formation is a critical step in reaching export competence because it is required for release of the mRNP from the gene. 3′-End processing is dependent on RNA polymerase II CTD Ser2 phosphorylation, because this enhances binding of 3′-end processing factors [25], and on polyadenylation signals in the mRNA. Recognition of the poly(A) site and cleavage most often occur before transcription termination. Excision of the

terminal intron and 3′-end processing are coupled through interaction between spliceosome components and 3′-end processing factors [26], but either can come before the other. In the BR1 gene, 3′-end cleavage, initial polyadenylation (about 20 As) and excision of the final intron take place at the gene [27].

In yeast, there is a clear coupling between 3′-end formation and mRNP export [28,29]. Polyadenylation is followed by binding of poly(A) binding protein Nab2, that together with Yra1 recruit the mRNA export receptor Mex67. In addition, Yra1 can be recruited through an interaction with Pcf11, a component of the 3′-end machinery. Pcf11 then transfers Yra1 to the TREX (transcription-export) complex with the aid of the Sub2 helicase.

In yeast, the THO complex and Sub2 are needed for efficient polyadenylation by Pap1 and may be involved in coordinating polyadenylation, dissociation of the polyadenylation machinery and release of the mRNP from the gene. If this does not happen, polyadenylation by TRAMP may occur, followed by exosome degradation, probably at the gene loci. This may also happen in higher eukaryotes since retention at gene loci also occur upon defects in 3′-end processing [30].

These observations underline that pre-mRNPs are assembled and processed at the gene loci and that interaction between different processes, culminating in 3′-end processing, optimize release of correct mRNPs into the interchromatin. In yeast, Mex67 appears to be recruited in a transcription- and 3′-end processing dependent manner. In metazoans, recruitment of the corresponding export receptor NXF1 seems more to be dependent on splicing and addition of the CBC.

## 4. Movement of mRNPs through the interchromatin and export competence

### 4.1. Restricted diffusion in the interchromatin

Upon release from the gene, mRNPs enter the interchromatin space. In polytene nuclei of *C. tentans*, BR mRNPs move away from the gene in all directions [31]. The movement of individual BR mRNPs inside interchromatin has been followed in intact nuclei, showing that they move by restricted diffusion. The BR mRNPs move in a discontinuous manner [32]. No distinct diffusion coefficient described the movement. Instead, trajectories of individual BR mRNPs showed discontinuous movement where the BR mRNPs repeatedly were hindered by and also interacted with non-chromatin structures in the interchromatin [33]. EM has described direct contacts between BR mRNPs and interchromatin structures [34], but the functional implications of such interactions are not known.

The interchromatin in polytene nuclei makes up a large volume that is uninterrupted by chromatin. This presumably influences the behavior of the BR mRNPs compared to mRNPs in diploid nuclei. In diploid nuclei, detailed EM studies have demonstrated that the interchromatin takes up almost 50% of the nuclear volume and that it forms a network of interchromatin channels running between chromatin and sometimes opening up in the periphery, close to NPCs [35,36]. It is likely that the movement of mRNPs in diploid nuclei is highly influenced by the chromatin/interchromatin organization [22]. This leads to restricted diffusion of mRNPs inside interchromatin channels, possibly leading to directionality of the mRNP movements toward the nuclear periphery.

Studies of mRNP movement in diploid nuclei have shown that the mRNPs can move with diffusion coefficients up to 10 $\mu m^2/s$, presumably in interchromatin channels, although also being retarded for unknown reasons, possibly by interaction with chromatin structures [37].

### 4.2. When is the mRNP completely export competent?

In several aspects, mRNPs appear to be export competent as they are released from the gene. BR mRNAs obtain a Cap Binding Complex (CBC) early during transcription [38]. The introns are excised almost completely at the gene locus. The excision of some introns is however completed in the interchromatin [39,40]. Polyadenylation is initiated at the gene locus where the first approximately 20 As are added and polyadenylation is completed in the interchromatin [27]. The BR mRNPs reach their compact, globular structure at the gene and most proteins present in the BR mRNPs are added at the gene locus.

As the BR mRNPs are released from the gene, they become part of an interchromatin pool of BR mRNPs. This pool is turned over in about 2 h [31]. Our current view is that when BR mRNPs enter the interchromatin space, they move around by diffusion. At some point, they will come into contact with the basket of the NPCs and if export competent and the NPC is not occupied, they will be exported into the cytoplasm. An important aspect then is how and where the BR mRNPs become fully export competent.

### 4.3. Acquisition of export receptors

For export, mRNPs require export receptors and there are two major export receptors described, CRM1 and NXF1. The karyopherin CRM1 is utilized for some mRNAs, in addition to for example ribosomal RNAs and uridine-rich small nuclear ribonucleotide particles (U snRNAs). CRM1 binds its cargo when it also binds GTP-bound Ran GTPase. CRM1 binds to a nuclear export signal (NES) in an adapter protein. Several different adapter proteins have been identified. CRM1, and its cofactor Ran, are part of the BR mRNPs [41]. However, leptomycin B treatment showed that a NES-CRM1-RanGTP complex is not necessary for BR mRNP export. This suggests that other export receptors also are involved. BR mRNPs indeed contain NXF1 as discussed below.

The main mRNA export receptor is NXF1/NXT1 heterodimer. NXF1 binds to mRNPs via different adapter proteins. The TREX complex component Aly/REF (Yra1 in yeast) is one such adapter. Aly/REF (Yra1) is recruited to mRNPs in different ways. Early, it was reported that Aly/REF (Yra1) could be recruited by the EJC [42]. In yeast, Aly/REF (Yra1) can be recruited during 3′-end processing through interaction with Pcf11, a component of the 3′-end cleavage machinery [43]. Another important recruitment pathway appears to be splicing dependent and mediated by CBP80 [44]. Also dephosphorylated SR proteins serve as NXF1 adapters [45,46].

NXF1 is present throughout the nucleus in HeLa cells [47] but it could not be detected at transcription sites [48]. It has not been demonstrated where in the nucleus NXF1 associates with endogenous mRNPs in diploid nuclei, but BR mRNPs bind NXF1 in the interchromatin of polytene nuclei [49]. It has been shown that a CBC is added to BR pre-mRNPs early during transcription [38] and recently it was demonstrated that the EJC core components, all bind to BR pre-mRNPs cotranscriptionally [49]. In addition, 3′-end processing takes place at the BR gene loci [27]. It has also been shown that Aly/REF (Yra1) is present in BR pre-mRNPs/mRNPs at the gene [50]. Several different SR proteins are also present in BR pre-mRNPs after completed splicing at the gene [10] and therefore most likely in a hypophosphorylated state. The known adapters for NXF1 are thus part of the BR mRNPs already at the gene loci. In spite of this, NXF1 is not recruited to the BR mRNPs until in the interchromatin [49].

### 4.4. Export receptors and elements in the mRNA

Export of mRNPs can be influenced by elements within the mRNA itself. Rev, a NES-containing protein binds to the HIV-1

Rev Response Element and CRM1 subsequently binds to Rev. In type D retroviruses, RNAs contain a so-called CTE-element that is directly bound by NXF1. Specific classes of mRNAs in vertebrates, for example those that encode mitochondrion-targeted or secretory proteins, can contain RNA-elements important for export [51]. Other examples are the presence of specific sequence elements in the untranslated regions of mRNAs. AU-rich elements in the 3′ UTRs bind human antigen R (HuR) that indirectly recruit CRM1 [52]. Sequences in UTRs of heat-shock proteins are involved in export during heat-shock stress [53].

### 4.5. Initiation and completion of splicing

Early studies of individual genes demonstrated cotranscriptional splicing [39,54]. Global studies have since shown that the majority of splicing is indeed cotranscriptional [55,56]. Splicing can be initiated cotranscriptionally and completed in the interchromatin [40]. Individual introns can be excised post-transcriptionally if they have impaired splice signals or if they are subject to alternative splicing [57]. It then appears that individual introns within a pre-mRNA can be excised posttranscriptionally while neighboring introns are excised cotranscriptionally. Splicing factors are largely stored in interchromatin granule clusters, seen as speckles in the fluorescence microscope and it is believed that the splicing factors are recruited to nascent transcripts on active genes [58]. Nascent RNA [59,60] and active spliceosomes [61], are present around speckles. Speckles may be sites of posttranscriptional splicing [61,62].

## 5. Docking at the Nuclear Pore Complex

### 5.1. NPC composition and structure

The majority of mRNPs leave the nucleus through the Nuclear Pore Complexes (NPC). The composition and structure of the NPC is well known [63]. In vertebrates, the NPC is an approximately 125 MDa macromolecular complex. In yeast, it has about half the molecular weight, although the structure is evolutionary conserved. Approximately 30 different proteins called nucleoporins (Nups) make up the NPC. At least 8 copies of each nucleoporin, are present in a NPC that has an eight-fold rotational symmetry in the plane of the membrane. Transmembrane nucleoporins anchor the NPC in the nuclear membrane, structural nucleoporins, embedded in the NPC, provide a scaffold for other nucleoporins and the FG-nucleoporins are rich in phenylalanine–glycine (FG)-repeats. The FG-nucleoporins bind directly to export receptors and contribute to translocation of the export cargos. They are also part of the permeability barrier for non-specific transport. The FG-nucleoporins cover the surface of the central channel from the nuclear to the cytoplasmic side. There are two groups of FG-nucleoporins: the symmetrical nucleoporins that are present on both sides of the NPC and the asymmetrical FG-nucleoporins that are localized exclusively on one side of the NPC [64]. The NPC has a nucleoplasmic region that consists of a nuclear ring, and a basket including long filaments. In the central part spanning the membrane, the central spoke region forms the translocation channel. Finally, a cytoplasmic ring and long filaments make up the cytoplasmic region [65].

Functional specialization of NPCs has been reported. For example, myogenic and neuronal differentiation requires a changed NPC composition [66] and in yeast, the nucleolus is close to a region of the nuclear membrane where the NPCs lack the proteins Mlp1 and Mlp2 [67].

### 5.2. Productive and non-productive mRNP–NPC interactions

The mRNP is targeted to the NPC by direct interactions between export factors and basket components. In yeast, Mex67 interacts with Nup1 and Nup2, and Nab2, a Mex67 adapter, interacts with Mlp1. The FG-repeats of Nup1 and Nup2 are proposed to be the first binding sites for the mRNP to the NPC. Imaging of individual BR mRNPs in intact *C. tentans* cells [68] and of individual mRNPs in HeLa cells [69], have demonstrated that mRNPs often associate with the basket but then return to the interchromatin. This seems to happen in about 60–75% of the cases. In agreement, export inhibition has shown that interactions between mRNPs and NPCs do not require export [22]. It is possible that such unproductive associations between baskets and mRNPs can be due to the lack of export receptors as suggested for the BR mRNPs not containing NXF1 and therefore are not fully export competent [49]. Other explanations, such as NPCs being fully loaded and already engaged in transport, are possible. EM analyses have shown that the BR mRNPs first attach to the nucleoplasmatic fibers of the NPC, still having its globular structure. It then moves to the center of the NPC and finally docks at the entrance of the central channel [70]. In vivo studies have identified a rate-limiting step at the basket [68]. In vivo imaging in mouse cells has also shown that the rate-limiting steps for mRNP transport are access to and release from the NPC [71]. At the nuclear side, the rate-limiting step might be due to the mRNA quality control and surveillance mechanisms. At the cytoplasmic side, the rate-limiting step is proposed to be due to the mRNP remodeling to promote directionality. Translocation through the channel is much faster than diffusion through the nucleus [22].

In yeast, a quality control step has been identified upon docking. Unspliced or malformed mRNPs are identified and degraded. Several proteins have been suggested to be involved, for example, Mlp1, Mlp2, Nup60, Pml39, endonuclease Swt1 and protease Ulp1 [72,73].

Docking to the basket of the NPC induces mRNP rearrangement, including dissociation of proteins from the mRNP [74,75]. BR mRNPs change conformation and a number of proteins have been shown to leave the BR mRNPs at the NPCs, for example Aly/REF (Yra1) and HEL/UAP56. So far, one protein, RAE1 (Gle2p), has been observed to transiently associate with the BR mRNP at the NPC [76]. RAE1 has been shown to interact with mRNPs and nucleoporins.

### 5.3. Active gene–NPC interactions in yeast

In higher eukaryotes, active genes often reside in the interior of the nucleus. However, at the nuclear periphery there are euchromatic regions associated with NPCs. This may reflect interchromatin channels opening close to NPCs. In budding yeast, several highly transcribed genes and some inducible genes are recruited to the nuclear periphery when transcription is activated. A reversible tethering of these active genes to NPCs has been described, involving NPC interactions at different stages of gene expression [77]. These contacts with components of the NPC, occurring during activation of transcription, leads to formation of gene loops. NPC components also interact with Mex67 and Sus1, bound to the RNA pol II during transcription elongation. Also 3′-end processing may be coupled to NPCs through interactions with the export complex TREX-2 and Mex67. In mammals, a TREX-2 complex analogs complex, GANP is associated with transcription and it has been suggested that it mediates transport of mRNPs from active genes to the NPCs [78], but has also been described to be stably associated with the NPC [79].

## 6. Translocation through the Nuclear Pore Complex

### 6.1. Entrance of the mRNP into and passage through the central channel

The NPC channel allows ions and molecules smaller than 40 kDa to pass passively by diffusion, but larger molecules and molecules that move against a concentration gradient require facilitated transport. Translocation of mRNPs through NPCs has been measured to take between 180 and 500 ms.

Real-time visualization of mRNPs has shown that the mRNPs are restructured and unfolded during translocation [22]. EM 3D reconstruction has shown very clearly that during passage through the NPC channel (Fig. 1B), the large BR mRNP is unfolded into a ribbon-like, extended shape, about 25 nm in diameter, and that the 5′-end of the mRNP leads the way through the channel, while the 3′-end remains in contact with the nuclear ring [70]. During translocation, the 3′-end of the BR mRNP rotates, probably as a result of feeding of the ribbon into the channel. The connection to the nuclear ring remains until the 5′-end reaches the cytoplasmic side and subsequently the 5′-end is unfolded into a thin RNP fiber.

NXF1/NXT1 promotes first docking of the mRNP to the basket and then translocation through the NPC channel via sequential interactions with FG-nucleoporins. Specific domains in NXF1 are involved in the interaction with the nucleoporins [80] and NXT1 regulates the affinity of NXF1 for the FG-repeats [81,82]. Also structural nucleoporins have been implicated in the translocation process [83].

Several models exist for the barrier function of FG-repeats and for how export receptors mediate translocation through the barrier. These include the “selective phase partitioning model” in which export receptors can disrupt a FG-repeat meshwork, the “oily spaghetti model” in which successive associations and dissociations between export receptors and FG-repeats allows diffusion through the NPC channel and the “virtual gating model” in which stepwise binding of export receptors to FG-repeats overcomes the entropic barrier formed by the FG meshwork. Distinctly different FG-nucleoporin requirements exist for protein transport mediated by karyopherin versus mRNP export mediated by NXF1 [64]. It appears that mRNPs are translocated along the periphery of the central channel as opposed to small molecules that passively diffuse through the central part [69].

### 6.2. Directionality of translocation and release of the mRNP

Direct measurements have demonstrated that mRNPs can move back and forth within the central channel [71]. Directionality of export appears to be due to NPC-associated factors at the cytoplasmic side of the NPC. In yeast, Nup42 and Nup159, FG-nucleoporins that are components of the cytoplasmic filaments, participate in the dissociation of the mRNP from the NPC. Nup42 binds Gle1 and its cofactor inositol hexakisphosphate ($IP_6$). Gle1–$IP_6$ interacts with the DEAD-box helicase Dbp5 and promotes binding of ATP. Dbp5-ATP can then associate with and, in an ATP-dependent manner, remodel the mRNP [84]. During the conformational change of the mRNP, some factors will be released, for example Mex67 and Nab2. As a consequence, the mRNP will irreversibly leave the NPC. Nup159 has been shown to interact with Dbp5-ADP and participate in the Dbp5 nucleotide cycle. Mex67 and Nab2 are recycled back to the nucleus and can be used in new rounds of mRNP export. As suggested from EM images of BR mRNPs exiting from the NPCs, initiation of translation can occur immediately at the cytoplasmic side of the NPC, even before the entire mRNP has been translocated [21].

Some mRNPs are transported to specific cytoplasmic locations. It is furthermore possible that NPCs influence the cytoplasmic localization of some mRNPs. This is exemplified in *C. reinhardtii*, asymmetrically positioned NPCs are important for flagellar biogenesis [85] and in yeast, where Nup60 is required for export and localization of ASH1 mRNA to the bud tip [86].

## 7. An alternative export pathway

In addition to the export pathway through NPCs, it has been known for some time that herpes simplex virus nucleocapsid exits the nucleus by disrupting the nuclear lamina and then bud into the perinuclear space, before fusing with the outer nuclear membrane [87]. Recently it has been shown, by studying Wnt signaling in neuromuscular junction formation in Drosophila, that assemblies of large mRNPs can be exported from the nucleus by nuclear membrane budding [88]. These mRNPs are transported to the neuromuscular junctions for localized translation.

## 8. Conclusions

Gene expression in eukaryotes is a multistep process. During transcription at the gene, the pre-mRNA is immediately folded and assembled with numerous proteins and processing machineries. Cotranscriptional capping, splicing and 3′-end processing shapes the mRNP and ensures that many requirements for downstream steps of gene expression are fulfilled at the gene. Upon release from the gene, the mRNP moves by restricted diffusion. Splicing and polyadenylation may be completed and export receptor NXF1/Mex67 is recruited. At the Nuclear Pore Complex, the mRNPs are often rejected. If accepted for translocation, conformational and compositional changes take place. Directionality in the translocation process is achieved by removal of export receptors at the cytoplasmic face of the NPC. In this review, focus is on the release of the mRNP from the gene, movement through the interchromatin and interaction with the NPC. Further knowledge about these steps in gene expression will require analyses of individual, endogenous mRNPs with a focus on the dynamic formation of the export competent mRNP and the interactions between all involved components in space and time inside the intact cell nucleus.

## Acknowledgements

This work was supported by the Swedish Research Council. We apologize to all colleagues whose work could not be cited because of lack of space.

## References

[1] Perales R, Bentley DL. Cotranscriptionality: the transcription elongation complex as a nexus for nuclear transactions. Mol Cell 2009;36:178–91.
[2] Daneholt B. Assembly and transport of a premessenger RNP particle. Proc Natl Acad Sci U S A 2001;98:7012–7.
[3] Björk P, Wieslander L. Nucleocytoplasmic mRNP export is an integral part of mRNP biogenesis. Chromosoma 2011;120:23–38.
[4] Kaufmann R, Cremer C, Gall JG. Superresolution imaging of transcription units on newt lampbrush chromosomes. Chromosome Res 2012;20:1009–15.
[5] Fakan S, van Driel R. The perichromatin region: a functional compartment in the nucleus that determines large-scale chromatin folding. Semin Cell Dev Biol 2007;18:676–81.
[6] Kornberg RD. The molecular basis of eukaryotic transcription. Proc Natl Acad Sci U S A 2007;104:12955–61.
[7] Bentley DL. Coupling mRNA processing with transcription in time and space. Nat Rev Genet 2014;15:163–75.
[8] Müller-McNicoll M, Neugebauer KM. How cells get the message: dynamic assembly and function of mRNA–protein complexes. Nat Rev Gen 2013;14:275–87.
[9] Glisovic T, Bachorik JL, Yong J, Dreyfuss G. RNA-binding proteins and post-transcriptional gene regulation. FEBS Lett 2008;582:1977–86.
[10] Björk P, Jin S-B, Zhao J, Singh OP, Persson J-O, Hellman U, et al. Specific combinations of SR proteins associate with single pre-messenger RNAs in vivo and contribute different function. J Cell Biol 2009;184:555–68.

[11] Merz C, Urlaub U, Will CL, Lührmann R. Protein composition of human mRNPs spliced in vitro and differential requirements for mRNP protein recruitment. RNA 2007;13:116–28.
[12] Lai D, Proctor JR, Meyer IM. On the importance of cotranscriptional RNA structure formation. RNA 2013;19:1461–73.
[13] Pan T, Sosnick T. RNA folding during transcription. Annu Rev Biophys Biomol Struct 2006;35:161–75.
[14] Zhao P, Zhang W, Chen SJ. Cotranscriptional folding kinetics of ribonucleic acid secondary structures. J Chem Phys 2011;135:245101.
[15] Zmaft B, Bintu L, Ishbashi T, Bustamante C. Nascent RNA structure modulates the transcriptional dynamics of RNA polymerases. Proc Natl Acad Sci U S A 2012;109:8948–53.
[16] Vargas D, Shah K, Batish M, Levandovski M, Sinha S, Marras SAE, et al. Single-molecule imaging of transcriptionally coupled and uncoupled splicing. Cell 2011;147:1054–65.
[17] Egecioglu DE, Chanfreau G. Proofreading and spellchecking: a two-tier strategy for pre-mRNA splicing quality control. RNA 2011;17:383–9.
[18] Lönnroth A, Alexciev K, Mehlin H, Wurtz T, Skoglund U, Daneholt B. Demonstration of a 7-nm RNP fiber as the basic structural element in a premessenger RNP particle. Exp Cell Res 1992;199:292–6.
[19] Batisse J, Batisse C, Budd A, Böttcher B, Hurt E. Purification of nuclear poly(A)-binding protein Nab2 reveals association with the yeast transcriptome and a messenger ribonucleoprotein core structure. J Biol Chem 2009;284:34911–7.
[20] Singh G, Kucukural A, Cenik C, Leszyk JD, Shaffer SA, Weng Z, et al. The cellular EJC interactome reveals higher-order mRNP structure and an EJC-SR protein nexus. Cell 2012;151:750–64.
[21] Mehlin H, Daneholt B, Skoglund U. Translocation of specific premessenger ribonucleoprotein particle through the nuclear pore studied with electron microscope tomography. Cell 1992;69:605–13.
[22] Mor A, Suliman S, Ben-Yishay R, Yunger S, Brody Y, Shav-Tal Y. Dynamics of single mRNP nucleocytoplasmic transport and export through the nuclear pore in living cells. Nat Cell Biol 2010;12:543–54.
[23] Wetterberg I, Zhao J, Masich S, Wieslander L, Skoglund U. In situ transcription and splicing in the Balbiani ring 3 gene. EMBO J 2001;20:2564–74.
[24] Mayeda A, Krainer AR. Regulation of alternative pre-mRNA splicing by hnRNPA1 and splicing factor SF2. Cell 1992;68:365–75.
[25] Licatalosi DD, Geiger G, Minet M, Schroeder S, Cilli K, McNeil JB, et al. Functional interaction of yeast pre-mRNA 3′ end processing factors with RNA polymerase II. Mol Cell 2002;9:1101–11.
[26] Martinson HG. An active role for splicing in 3′-end formation. Wiley Interdiscip Rev RNA 2011;2:459–70.
[27] Baurén G, Belikov S, Wieslander L. Transcriptional termination in the Balbiani ring 1 gene is closely coupled to the 3′-end formation and excision of the 3′-terminal intron. Genes Dev 1998;12:2759–69.
[28] Schmid M, Jensen TH. Quality control of mRNP in the nucleus. Chromosoma 2008;117:419–29.
[29] Qu X, Lykke-Andersen S, Nasser T, Saguez C, Bertrand E, Jensen TH, et al. Assembly of an export-competent mRNP is needed for efficient release of the 3′-end processing complex after polyadenylation. Mol Cell Biol 2009;29:5327–38.
[30] de Almeida SF, Garcia-Sacristan A, Custódio N, Carmo-Fonseca M. A link between nuclear RNA surveillance, the human exosome and RNA polymerase II transcription termination. Nucl Acids Res 2010;38:8015–26.
[31] Singh OP, Björkroth B, Masich S, Wieslander L, Daneholt B. The intranuclear movement of Balbiani ring premessenger ribonucleoprotein particles. Exp Cell Res 1999;251:135–46.
[32] Siebrasse P, Veith R, Dobay A, Leonhardt H, Daneholt B, Kubitscheck U. Discontinous movement of mRNP particles in nucleoplasmic regions devoid of chromatin. Proc Natl Acad Sci U S A 2008;105:20291–6.
[33] Veith R, Sorkalla T, Baumgart E, Anzt J, Häberlein H, Tyagi S, et al. Balbiani ring mRNPs diffuse through and bind to clusters of large intranuclear molecular structures. Biophys J 2010;99:2676–85.
[34] Miralles F, Ofverstedt LG, Sabri N, Aissouni Y, Hellman U, Skoglund U, et al. Electron tomography reveals posttranscriptional binding of pre-mRNPs to specific fibers in the nucleoplasm. J Cell Biol 2000;148:271–82.
[35] Schermelleh L, Carlton PM, Haase S, Shao L, Winoto L, Kner P, et al. Subdifraction multicolor imaging of the nuclear periphery with 3D structured illumination microscopy. Science 2008;320:1332–6.
[36] Rouquette J, Genoud C, Vazguez-Nin GH, Kraus B, Cremer T, Fakan S. Revealing the high-resolution three-dimensional network of chromatin and interchromatin space: a novel electron microscopic approach to reconstructing nuclear architecture. Chromosome Res 2009;17:801–10.
[37] Politz JC, Browne ES, Wolf DE, Pederson T. Intranuclear diffusion and hybridization state of oligonucleotides measured by fluorescence correlation spectroscopy in living cells. Proc Natl Acad Sci U S A 1998;95:6043–8.
[38] Visa N, Izaurralde E, Ferreira J, Daneholt B, Mattaj IW. A nuclear cap-binding complex binds Balbiani ring pre-mRNA cotranscriptionally and accompanies the ribonucleoprotein particle during nuclear export. J Cell Biol 1996;133:5–14.
[39] Baurén G, Wieslander L. Splicing of Balbiani ring 1 gene pre-mRNA occurs simultaneously with transcription. Cell 1994;76:183–92.
[40] Wetterberg I, Baurén G, Wieslander L. The intranuclear site of excision of each intron in Balbiani ring 3 pre-mRNA is influenced by the time remaining to transcription termination and different excision efficiencies for the various introns. RNA 1996;2:641–51.
[41] Zhao J, Jin S-B, Wieslander L. CRM1 and Ran are present but a NES–CRM1–RanGTP complex is not required in Balbiani ring mRNP particles from the gene to the cytoplasm. J Cell Sci 2004;117:1553–66.
[42] Kataoka N, Diem MD, Kim VN, Yong J, Dreyfuss G. Magoh, a human homolog of *Drosophila mago* nashi protein, is a component of the splicing-dependent exon–exon junction complex. EMBO J 2001;20:6424–33.
[43] Johnson SA, Cubberly G, Bentley DL. Cotranscriptional recruitment of the mRNA export factor Yra1 by direct interaction with the 3′ end processing factor Pcf11. Mol Cell 2009;33:215–26.
[44] Cheng H, Dufu K, Lee CS, Hsu JL, Dias A, Reed R. Human mRNA export machinery recruited to the 5′ end of mRNA. Cell 2006;127:1389–400.
[45] Huang Y, Steitz JA. Splicing factors SRp20 and 9G8 promote the nucleocytoplasmic export of mRNA. Mol Cell 2001;7:899–905.
[46] Huang Y, Gattoni R, Stévenin J, Steitz JA. SR splicing factors serve as adapter proteins for TAP-dependent mRNA export. Mol Cell 2003;11:837–43.
[47] Teng IF, Wilson SA. Mapping interactions between mRNA export factors in living cells. PLoS ONE 2013;8(6):e67676.
[48] Custódio N, Carvalho C, Condado I, Antoniou M, Blencowe BJ, Carmo-Fonseca M. In vivo recruitment of exon junction complex proteins to transcription sites in mammalian cell nuclei. RNA 2004;10:622–33.
[49] Björk P, Persson JO, Wieslander L. Binding of the exon–junction complex and NXF1 to endogenous pre-mRNP/mRNP in vivo, in preparation.
[50] Kiesler E, Miralles F, Visa N. HEL/UAP56 binds cotranscriptionally to the Balbiani ring pre-mRNA in an intron-independent manner and accompanies the BR mRNP to the nuclear pore. Curr Biol 2002;12:859–62.
[51] Palazzo AF, Akef A. Nuclear export as a key arbiter of mRNA identity in eukaryotes. Biochem Biophys Acta 2012;1819:566–77.
[52] Brennan CM, Gallouzi IE, Steitz JA. Protein ligands to HuR modulate interaction with target mRNAs in vivo. J Cell Biol 2000;151:1–14.
[53] Saavedra CA, Tung KS, Amberg DC, Hopper AK, Cole CN. Regulation of mRNA export in response to stress in *Saccharomyces cerevisiae*. Genes Dev 1996;10:1608–10.
[54] Beyer AL, Bouton AH, Miller Jr OL. Correlation of hnRNP structure and nascent transcript cleavage. Cell 1981;26:155–65.
[55] Han J, Ji X, Wang D, Fu X-D. Pre-mRNA splicing: where and when in the nucleus. Trends Cell Biol 2011;21:336–43.
[56] Brugiolo M, Herzel L, Neugebauer KM. Counting on cotranscriptional splicing. F1000 Prime Rep 2013;5:9–14.
[57] Vargas D, Shah K, Batish M, Levandovski M, Sinha S, Marras SAE, et al. Single-molecule imaging of transcriptionally coupled and uncoupled splicing. Cell 2011;147:1054–60.
[58] Lamond AI, Spector DL. Nuclear speckles: a model for nuclear organelles. Nat Rev Mol Cell Biol 2003;4:605–12.
[59] Spector DL, Fu XD, Maniatis T. Associations between distinct pre-mRNA splicing components and the cell nucleus. EMBO J 1991;10:3467–81.
[60] Fakan S, Leser G, Martin TE. Ultrastructural distribution of nuclear ribonucleoproteins as visualized by immunocytochemistry on thin sections. J Cell Biol 1984;98:358–63.
[61] Girard C, Will CL, Peng J, Makarov EM, Kastner B, Lemm I, et al. Post-transcriptional spliceosomes are retained in nuclear speckles until splicing completion. Nat Commun 2012;3:1–12.
[62] Dias AP, Dufu K, Lei H, Reed R. A role for TREX components in the release of spliced mRNA from nuclear speckle domains. Nat Commun 2010;1:97.
[63] Aitchison JD, Rout MP. The yeast nuclear pore complex and transport through it. Genetics 2012;190:855–83.
[64] Terry LJ, Wente SR. Flexible gates: dynamic topologies and functions for FG nucleoporins in nucleocytoplasmic transport. Eukaryot Cell 2009;12:1814–27.
[65] Fahrenkrog B, Aebi U. The nuclear pore complex: nucleocytolasmic transport and beyond. Nat Rev Mol Cell Biol 2003;4:757–66.
[66] D'Angelo MA, Gomez-Cavazos JS, Mei A, Lackner DH, Hetzer MW. A change in nuclear pore complex composition regulates cell differentiation. Dev Cell 2012;22:446–58.
[67] Galy V, Gadal O, Fromont-Racine M, Romano A, Jacquier A, Nehrbass U. Nuclear retention of unspliced mRNAs in yeast is mediated by perinuclear Mlp1. Cell 2004;116:63–73.
[68] Siebrasse JP, Kaminski T, Kubitsheck U. Nuclear export of single native mRNA molecules observed by light sheet fluorescence microscopy. Proc Natl Acad Sci U S A 2012;109:9426–31.
[69] Ma J, Liu Z, Michelotti N, Pitchiaya S, Veerapeneni R, Androsavich JR, et al. High-resolution three-dimensional mapping of mRNA export through the nuclear pore. Nat Commun 2013;4:2414.
[70] Mehlin H, Daneholt B, Skoglund U. Structural interaction between the nuclear pore complex and a specific translocating RNP particle. J Cell Biol 1995;129:1205–16.
[71] Grünwald D, Singer RH. In vivo imaging of labeled endogenous actin mRNA during nucleocytoplasmic transport. Nature 2010;467:604–9.
[72] Vinciguerra P, Iglesias N, Camblong J, Zenklusen D, Stutz F. Perinuclear Mlp proteins downregulate gene expression in response to a defect in mRNA export. EMBO J 2005;24:813–23.
[73] Nino CA, Hérissant L, Babour A, Dargemont C. mRNA nuclear export in yeast. Chem Rev 2013;113:8523–45.
[74] Strässer K, Hurt E. Yra1p a conserved nuclear RNA-binding protein, interacts directly with Mex67p and is required for mRNA export. EMBO J 2000;19:410–20.
[75] Stutz F, Bachi A, Doerks T, Braun IC, Séraphin B, Wilm M, et al. REF, an evolutionary conserved family of hnRNP-like proteins, interacts with TAP/Mex67p and participates in mRNA nuclear export. RNA 2000;6:638–50.
[76] Sabri N, Visa N. The Ct-RAE1 protein interacts with Balbiani ring RNP particles at the nuclear pore. RNA 2000;6:1597–609.

[77] Dieppois G, Stutz F. Connecting the transcription site to the nuclear pore: a multi-tether process that regulates gene expression. J Cell Sci 2010;123:1989–99.
[78] Jani D, Lutz S, Hurt E, Laskey RA, Stewart M, Wickramasinghe VO. Functional and structural characterization of the mammalian TREX-2 complex that links transcription with nuclear messenger RNA export. Nucl Acids Res 2012;40:4562–73.
[79] Umlauf D, Bonnet J, Waharte F, Fournier M, Stierle M, Fisher B, et al. The human TREX-2 complex is stably associated with the nuclear pore basket. J Cell Sci 2013;126:2656–67.
[80] Fribourg S, Braun IC, Izaurralde E, Conti E. Structural basis for the recognition of a nucleoporin FG repeat by the NTF2-like domain of the TAP/Mex67 mRNA nuclear export factor. Mol Cell 2001;8:645–56.
[81] Lévesque L, Guzik B, Guan T, Coyle J, Black BE, Rekosh D, et al. J Biol Chem 2001;276:44953–62.
[82] Wiegand HL, Coburn GA, Zeng Y, Kang Y, Bogerd HP, Cullen BR. Formation of TAP/NXT1 heterodimers activates Tap-dependent nuclear mRNA export by enhancing recruitment to nuclear pore complexes. Mol Cell Biol 2002;22:245–56.
[83] Siniossoglou S, Wimmer C, Rieger M, Dove V, Tekotte H, Weise C, et al. A novel complex of nucleoporins, which includes Sec13p and Sec13p homolog, is essential for normal nuclear pores. Cell 1996;84:265–75.
[84] Folkmann AW, Noble KN, Cole CN, Wente SR. Dbp5, Gle1-IP6 and Nup159: a working model for mRNA export. Nucleus 2011;2:540–8.
[85] Powrie EA, Zenklusen D, Singer RH. A nucleoporin Nup60p, affects the nuclear and cytoplasmic localization of ASH1 mRNA in *S. cerevisiae*. RNA 2011;17:134–44.
[86] Colón-Ramos DA, Salisbury JL, Sanders MA, Shenoy SM, Singer RH, Garcia-Blanco MA. Asymmetric distribution of nuclear pore complexes and the cytoplasmic localization of beta2-tubulin mRNA in *Chlamydomonas reinhardtii*. Dev Cell 2003;4:941–52.
[87] Lee CP, Chen MR. Escape of herpesviruses from the nucleus. Rev Med Virol 2010;20:214–30.
[88] Speese SD, Ashley J, Jokhi V, Nunnari J, Barria R, Li Y, et al. Nuclear envelope budding enables large ribonucleoprotein particle export during synaptic Wnt signaling. Cell 2012;149:832–46.