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REVIEW

Biogenesis of mRNPs: integrating different processes in the eukaryotic nucleus

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Abstract Transcription is a central function occurring in the nucleus of eukaryotic cells in coordination with other nuclear processes. During transcription, the nascent pre-mRNA associates with mRNA-binding proteins and undergoes a series of processing steps, resulting in export-competent mRNA ribonucleoprotein complexes (mRNPs) that are transported into the cytoplasm. Experimental evidence increasingly indicates that the different processing steps (5′-end capping, splicing, 3′-end cleavage) and mRNP export are connected to each other as well as to transcription, both functionally and physically. Here, we review the overall process of mRNP biogenesis with particular emphasis on the functional coupling of transcription with mRNP biogenesis and export and its relationship to nuclear organization.

Introduction

Gene expression involves multiple processes ranging from transcription to the proper processing of mRNAs, export, and translation (Fig. 1). Regulation of gene expression is fundamental to the development and survival of any organism. Transcription is a central cellular function that takes place in the nucleus of eukaryotic cells in coordination with other nuclear processes. It is thought to be predominantly regulated at the level of RNA polymerase recruitment to promoters, but increasing evidence suggests that transcription regulation at the level of elongation is also important. During transcription, the nascent pre-mRNA associates with RNA-binding proteins and undergoes a series of processing steps, resulting in export-competent mRNA ribonucleoprotein complexes (mRNPs) that are transported into the cytoplasm. The different processing steps (5′-end capping, splicing, 3′-end cleavage) and mRNP export are connected to each other and to transcription. The emerging picture is that cells have evolved sophisticated multi-protein complexes able to regulate gene activity at various steps of the transcription process and mRNP biogenesis in a dynamic, coordinated, and spatial manner. Excellent reviews have addressed how mRNA processing events are linked in the last few years (Bentley 2005; Buratowski 2005; Rosonina et al. 2006).

In addition, a number of recent studies point to a functional relationship between gene expression and nuclear organization of chromatin. It has been shown that certain genes can undergo dynamic recruitment to the nuclear periphery upon transcriptional activation, and it has been suggested that the nuclear pore complex (NPC) not only recruits the RNA processing and export machineries, but also participates in gene expression regulation (see Kohler and Hurt 2007). Here, we will review the multi-protein complexes that regulate gene activity at various stages of the transcription process and mRNP biogenesis, with particular emphasis on the functional coupling between transcription, mRNP biogenesis, and export as well as their relationship to nuclear organization. However, to facilitate a comprehensive reading, we will first introduce the most important aspects of the transcription cycle.

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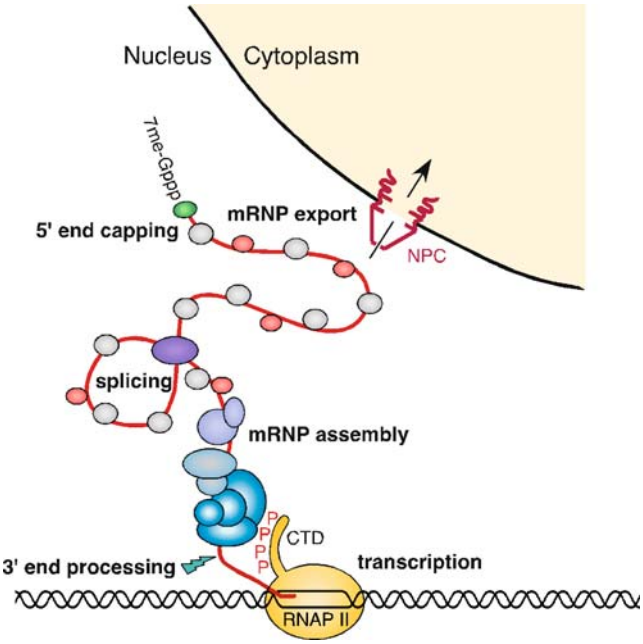

Fig. 1 Schematic view of the nuclear side of eukaryotic gene expression, from transcription to nuclear export. *NPC* Nuclear pore complex, *CTD* C-terminal domain of Rpb1, *RNAPII* RNA polymerase II

Transcription initiation and promoter escape

Transcription starts with the assembly of the preinitiation complex (PIC) at the promoter. The PIC consists of RNA polymerase II (RNAPII) and several auxiliary proteins known as the general transcription factors (GTFs), which position RNAPII near the transcription-start site and dictate the precise location of transcription initiation. Before starting RNA synthesis, ~11–15 bp of DNA get unwound to form an open complex allowing the single-stranded DNA template to enter the active site of RNAPII, an ATP-consuming process that requires TFIIH (Fig. 2a; Goodrich and Tjian 1994). During promoter clearance, a critical stage that is characterized by physical and functional instability of the transcription complex, the PIC is partially disassembled and some of the GTFs are released from the early transcription complex. The rate-limiting step of promoter clearance coincides with the transition to a so-called early elongation complex (EEC) after the addition of the eighth nucleotide (Hieb et al. 2006). Even though the exact mechanism of the transition between an initially transcribing complex to the more stable EEC is not clear yet, several lines of evidence indicate that the length of the nascent RNA is a crucial determinant of promoter clearance through its interactions with RNAPII and by triggering transcription-bubble collapse (reviewed in Saunders et al. 2006).

In vivo, promoter clearance coincides with the beginning of the phosphorylation cycle of the C-terminal domain (CTD) of Rpb1, the largest subunit of RNAPII. The CTD forms a mobile extension from the structural core of RNAPII (Cramer et al. 2001) and consists of heptapeptide repeats of the consensus sequence YSPTSPS, which can be phosphorylated at residues Ser2 and Ser5. This structure

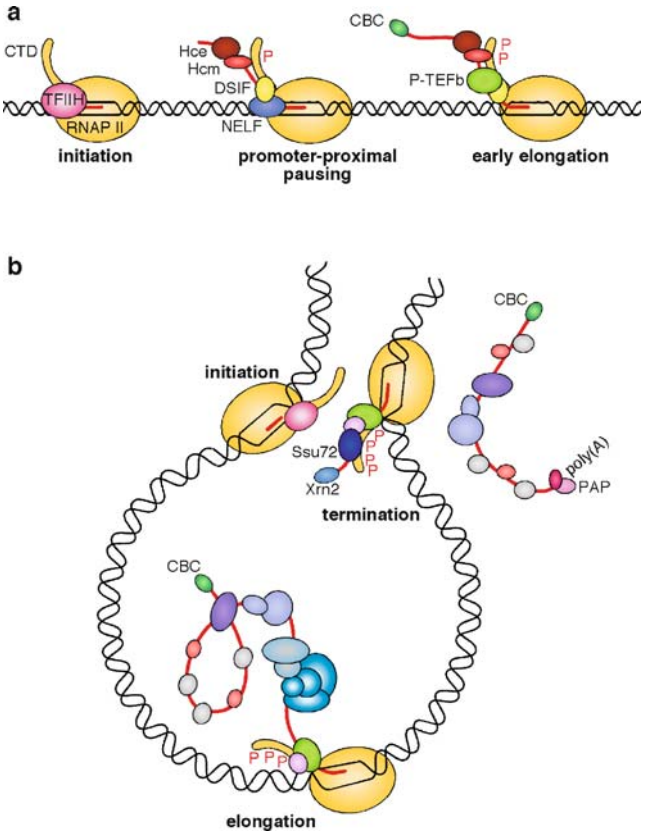

Fig. 2 **a** Simplified model of the first stages of transcription, from initiation to early elongation, as it may occur in metazoan cells. Transcription initiation requires unwinding of the template DNA by TFIIH. The CTD of RNAPII becomes phosphorylated on Ser5 by the Cdk7 subunit of TFIIH. Shortly after promoter clearance, transcription is paused by the joint action of the DSIF and NELF transcription elongation factors, allowing guanylyltransferase (*Hce*) and methyltransferase (*Hcm*) to cap the pre-mRNA 5′-end in two steps once it has emerged from the RNAPII. The cap-binding protein complex (*CBC*) is then loaded to the newly formed cap. Binding of the elongation factor P-TEFb leads to massive phosphorylation of CTD-Ser2 of RNAPII and to productive elongation. Escape from promoter-proximal pausing is tightly controlled and serves as a checkpoint to prevent extension of improperly capped RNAs. **b** Simplified model of the transcription cycle, from initiation to mRNP release and termination, which may induce a loop in the template DNA, as shown in yeast. During transcription elongation the CTD of RNAPII becomes highly phosphorylated at Ser2 and a number of factors are recruited to the elongating RNAPII. The mRNP gets assembled co-transcriptionally and the nascent mRNA eventually spliced. Termination is triggered by the release of the export-competent mRNP, which gets polyadenylated at its 3′-end by the action of poly(A) polymerase (*PAP*). The poly(A) tail is then bound and prevented from premature degradation by poly (A)-binding proteins. After release of the nascent mRNP, elongation pauses and the remaining uncapped 3′ transcript is degraded by the Xrn2 exonuclease. The highly phosphorylated CTD of the RNAPII is then dephosphorylated by Ssu72 and recycled to the promoter for a new round of elongation

serves as a scaffold for many specific factors, whose binding often depends on the phosphorylation pattern of the CTD, which changes during the transcription cycle and coordinates different steps of nuclear mRNA biogenesis. Although several kinases are capable of phosphorylating the CTD of RNAPII, Ser5 is mainly phosphorylated by the Kin28/Ctk7 subunits of TFIIH, while Ser2 is mainly phosphorylated by the Ctk1/Cdk9 subunit of the Ctk kinase complex/P-TEFb (positive transcription elongation factor-b; see below). CTD phosphorylation at Ser5 correlates with transcription initiation and early elongation, whereas Ser2 phosphorylation is associated with RNAPII farther away from the promoter. It has recently been shown that the CTD is also phosphorylated at Ser7 in a transcription-dependent manner in mammalian cells (Chapman et al. 2007; Egloff et al. 2007). Ser7 phosphorylation is specifically required for snRNA gene expression and constitutes an additional level of gene regulation. Nevertheless, its function in protein-coding gene expression is still unknown. Thus, the pattern of CTD phosphorylation is likely to be more complex and evidence exists suggesting that CTD phosphorylation might not be the same in all repeats (Chapman et al. 2007; reviewed in Phatnani and Greenleaf 2006).

From transcription initiation to elongation

Before becoming a fully productive elongation complex, the EEC undergoes continued adjustments, and this process is often accompanied by transcriptional pausing near the promoter (Fig. 2a), this pausing being an important step in the regulation of RNAPII transcription (reviewed in Saunders et al. 2006). The candidate pausing factors DSIF (5,6-dichloro-1-b-D-ribofuranosylbenzimidazole [DRB] sensitivity inducing factor) and NELF (negative elongation factor) are both required for the inhibition of transcription elongation by the nucleotide analog DRB in metazoan (Wada et al. 1998; Yamaguchi et al. 1999). DSIF is composed of the elongation factors Spt4 and Spt5, which are conserved from yeast to humans (Hartzog et al. 1998). NELF is a four-subunit complex that is conserved between mammals and *D. melanogaster*, though no homologues were found in *C. elegans*, *S. cerevisiae*, or *A. thaliana*. In *Drosophila*, DSIF and NELF were shown working together and causing promoter-proximal pausing (Wu et al. 2003). Genome-wide analyses of RNAPII occupancy across the *D. melanogaster* genome demonstrated that roughly 10–15% of all genes have elevated amount of RNAPII near their promoter (Muse et al. 2007; Zeitlinger et al. 2007), underscoring the importance of promoter-proximal pausing in the regulation of transcription.

Recent studies have challenged the once commonly held view that transcription is only regulated at the level of RNA polymerase recruitment to the promoter. It is now clear that transcription elongation is a dynamic and highly regulated stage of the transcription cycle capable of coordinating downstream events (reviewed in Bentley 2005; Saunders et al. 2006). Numerous factors have been identified that specifically target the elongation stage of transcription, whose function is to help the RNAPII to overcome difficulties arising from the complexity of its catalytic activity and obstacles intrinsic to its chromatinized DNA template. Transcriptional pausing occurs when the elongating complex is temporarily blocked, the nascent mRNA remaining aligned with the active site of the polymerase, in contrast to transcriptional arrest, which is caused by backtracking of RNAPII relative to the DNA template and leads to misalignment of the catalytic active site and 3′-OH of the nascent RNA transcript. Such pausing has been demonstrated for all three eukaryotic DNA-dependent RNA polymerases, as well as viral and prokaryotic RNA polymerases, and is thought to be a natural mode of transcriptional regulation.

Several factors are required for the efficient release of promoter-proximal paused RNAPII into productive elongation, after which RNAPII proceeds through the remainder of the gene. During escape from the pause the negative effects of Spt4–5/DSIF and NELF on RNAPII elongation are relieved by the action of the positive transcription elongation factor-b complex. P-TEFb phosphorylates Spt4–5/DSIF, NELF, as well as the RNAPII CTD (primarily at Ser2; reviewed in Peterlin and Price 2006). In *S. cerevisiae*, two homologues sum up the activities of P-TEFb: the Bur1/Bur2 and the Ctk kinase complexes (see Wood and Shilatifard 2006). After the transition to productive elongation, Spt4–5/DSIF remains associated with the elongation complex (Andrulis et al. 2000; Pokholok et al. 2002), concordantly with its positive role in transcription elongation (Rondón et al. 2003a; Yamada et al. 2006; and references therein). Other factors that modulate transcriptional pause, and thus, the rate of transcriptional elongation, include TFIIF, the ELL family, Elongins, Fcp1/FCP1, and Rad26/CSB, while release of arrested RNAPII complexes requires the Ppr2/TFIIS elongation factor (reviewed in Sims et al. 2004a).

In addition to transcriptional pause and arrest, the packaging of DNA into nucleosomes may represent a major block to transcription elongation. Current models of RNAPII elongation mechanisms through chromatin template involve both histone depletion and nucleosome mobilization by remodeling factors (reviewed in Li et al. 2007). Transcription elongation is coupled to the alteration of chromatin structure in the form of covalent histone modification including acetylation, methylation, phosphorylation, and ubiquitylation. The importance of chromatin during transcription and the role of remodeling factors, histone

chaperones, and histone modification complexes have been addressed in the last few years (reviewed in Hampsey and Reinberg 2003; Li et al. 2007; Reinberg and Sims 2006; Shilatifard 2006; Sims et al. 2004b; Svejstrup 2007).

Transcription termination

The final step in the transcription cycle is termination. At this stage, the mRNA is cleaved and transported to the cytoplasm. Transcription termination is intimately coupled to the maturation of mRNA 3′ ends (reviewed in Rosonina et al. 2006). Most nascent mRNAs are cleaved 20–30 bases downstream of a conserved polyadenylation signal, this endonucleolytic cut being achieved by the concerted action of several 3′ end mRNA processing factors. After cleavage of the nascent transcript, poly(A) polymerase adds a poly(A) tail to the created 3′-OH, which is then bound and protected from exonucleolytic degradation by poly(A)-binding proteins. CTD phosphorylation appears to play a crucial role in 3′-end maturation, as some yeast 3′-end mRNA processing factors preferentially bind Ser2-phosphorylated CTD heptads (Ahn et al. 2004; Licatalosi et al. 2002; Meinhart and Cramer 2004), while others bind to the phosphorylated CTD without a known preference for a particular phosphoisoform (reviewed in Buratowski 2005).

RNAPII transcription termination normally ensues shortly after transcript cleavage. Two different but non-mutually exclusive models for RNAPII transcription termination have been proposed and recently reviewed (Buratowski 2005; Rosonina et al. 2006). The ‘allosteric’ (or ‘anti-terminator’) model states that the transcription complex undergoes conformational changes upon passage of the polyadenylation signal leading to a decrease in processivity and subsequent dismantling. This model is supported by the ability of one cleavage factor to dismantle paused transcription elongation complexes (Zhang et al. 2005; Zhang and Gilmour 2006). The alternative ‘torpedo’ termination model proposes that exonucleolytic degradation of the uncapped 3′ transcript created by endonucleolytic cleavage at the poly(A) site somehow signals to the downstream RNAPII to terminate transcription. This model has been substantiated by the recent demonstration that the 5′–3′ exonuclease XRN2 in human and Rat1 in yeast are required for efficient termination (Kim et al. 2004b; Luo et al. 2006; West et al. 2004).

Structural features of actively transcribed genes: the looping model

Several 3′ mRNA processing factors associate with promoter regions, suggesting that transcription initiation and termination are interconnected and might influence each other’s efficiency. For example, Ssu72, the phosphatase responsible for CTD Ser5-P dephosphorylation (Krishnamurthy et al. 2004), interacts physically and functionally with the transcription initiation factor TFIIB and plays an essential role in 3′-end formation in the context of the cleavage/polyadenylation factor (CPF) complex (He et al. 2003; Steinmetz and Brow 2003). Furthermore, Ssu72 occupies both the promoter and terminator region of RNAPII-transcribed genes although it is absent from the ORF (Nedea et al. 2003). Similarly, the transcriptional coactivator Sub1 (PC4 in mammals) interacts with both the Pta1 subunit of CPF and TFIIB and enhances the recruitment of the Rna15 termination factor (CstF64 in mammals) to promoters (Calvo and Manley 2005; He et al. 2003), while the cleavage polyadenylation specificity factor is a component of TFIID (Dantonel et al. 1997). The explanation for these coincidences came from recent studies that revealed a physical association between promoters and poly(A) sites in yeast, suggesting that active genes exist in looped conformation (Ansari and Hampsey 2005; O’Sullivan et al. 2004; Singh and Hampsey 2007). The juxtaposition of promoter and terminator regions provides an explanation for the presence of 3′-end processing factors at the promoter of active genes and their interactions with GTFs. Furthermore, these studies showed that gene-loop formation depends on the CTD Ser5 kinase Kin28, the Ser5-P phosphatase Ssu72 and TFIIB. This suggests a provocative model in which the loops are formed after the initial round of transcription to promote transcription reinitiation by facilitating recycling of RNAPII from the terminator to the promoter (Fig. 2b). The recent findings that capping and 3′-end processing factors are found at both ends of genes in human open the possibility that gene loops might not be restricted to yeast (Glover-Cutter et al. 2008).

mRNP biogenesis: from mRNA capping to export

Nascent mRNAs assemble into RNA protein complexes and undergo a series of processing steps, which result in export-competent mRNPs. The association of mRNA-binding proteins with the nascent pre-mRNA occurs co-transcriptionally and directs RNA processing, export, sub-cellular localization, translation, and stability. A number of RNA-binding proteins remain associated with the RNA during export, accompanying the mRNP to the cytoplasm. Genome-wide analyses have shown that specific RNA-binding proteins associate with discrete subsets of mRNAs sharing functional attributes and that these associations help to coordinate mRNP biogenesis steps and might

influence expression (Hieronymus et al. 2004; Kim Guisbert et al. 2005).

RNA processing begins as the transcript starts emerging from the RNAPII. Thus, promoter-proximal pausing coincides with capping of the emerging nascent mRNA by methyl-guanylation at its 5′-end (Moteki and Price 2002; Rasmussen and Lis 1993). The capping reaction is carried out by two polypeptides in yeast, Cet1-Ceg1 (human Hce1 or HCE) carrying triphosphatase–guanylyltransferase activities and by Abd1 (human Hcm1) guanine-N7-methyltransferase (MTase), both of which have additional transcriptional functions in yeast (Myers et al. 2002; Schroeder et al. 2004). Various lines of experimental evidence suggests that promoter-proximal pausing might facilitate correct capping and acts as a checkpoint by ensuring that only correctly capped mRNAs get extended (Guiguen et al. 2007; Mandal et al. 2004; Pei et al. 2003). Co-transcriptional binding of the capping apparatus depends on Ser5 CTD phosphorylation, and capping proteins interact with Spt5, which stimulate their capping activity (reviewed in Bentley 2005). In yeast, Abd1 was shown to remain bound throughout the length of the gene, in contrast to Ceg1-Cet1, which is released within the first 500 bases (Komarnitsky et al. 2000; Schroeder et al. 2000). Recently, both human HCE and MTase were found located not only where capping takes place but also throughout the gene and at the 3′ flanking regions (Glover-Cutter et al. 2008). Although the function of these factors within genes and in their 3′ flanking region remains unclear, these results suggest that they might influence elongation, termination, and 3′-end processing.

Once the nascent pre-mRNA is capped, the nuclear cap-binding protein complex (CBC) binds co-transcriptionally to the monomethylated cap (Izaurralde et al. 1994). CBC seems to be the first protein to assemble on pre-mRNAs and is required for the subsequent steps of splicing, transcription termination, export, nuclear mRNA decay, translation, non-sense-mediated decay, and decapping (Cheng et al. 2006; Hosoda et al. 2005; Wong et al. 2007; and references therein).

Importantly, RNA processing must be completed before mRNAs can be exported, and improperly processed transcripts are retained within the nucleus. As a consequence, eukaryotic cells have evolved quality control mechanisms that prevent the export of suboptimal mRNPs and synthesis of dysfunctional proteins (reviewed in Sommer and Nehrbass 2005). The nuclear exosome, a protein complex of several 3′–5′ exonucleases, is responsible for the retention and degradation of aberrant transcripts at sites of transcription (reviewed in Saguez et al. 2005). Additional factors operate in quality control at the NPC, including the myosine-like poteins Mlp1 and Mlp2 and other associated nuclear envelope proteins (Galy et al. 2004).

The knowledge about the precise function of most of these proteins as well as the sequential succession of enzymatic steps governing mRNP biogenesis and export is poorer than that of transcription. For this reason, we will now focus on some representative factors whose functions link early events during mRNP assembly and export in close coordination with transcription.

The THO complex: connecting transcription and mRNA export

THO is a conserved eukaryotic nuclear complex that was first isolated in yeast as a four-protein complex composed of stoichiometric amounts of Tho2, Hpr1, Mft1, and Thp2 (Chávez et al. 2000). Mutations in THO confer a wide number of phenotypes including thermosensitivity, transcription impairment, hyper-recombination, mRNA export defects, and increased levels of exosome-dependent mRNA instability (see Aguilera 2005). THO interacts genetically with factors involved in different steps of mRNP biogenesis, from the early steps of transcription elongation (Chang et al. 1999; Rondón et al. 2003a), to transcription termination (Luna et al. 2005) and mRNA export (Jimeno et al. 2002). THO has also been purified in *Drosophila* and human cells and the complexes contain counterparts of the yeast subunits Tho2/Thoc2, Hpr1/Thoc1, as well as additional components such as Thoc5–Thoc7 (Rehwinkel et al. 2004; Strasser et al. 2002).

THO interacts physically and functionally with proteins involved in mRNA export: the Sub2/UAP56 RNA-dependent ATPase, the Yra1/REF1/Aly RNA-binding protein, and the conserved Tex1 protein. Together, these proteins form a bigger complex termed TREX (TRanscription-EXport complex; Fig. 3; Strasser et al. 2002). Nevertheless, despite the physical and genetic interaction between Sub2, Yra1 and THO, and the similar phenotypes conferred by their mutations (Fan et al. 2001; Jimeno et al. 2002), THO behaves as a stable and functional core independently of Sub2/UAP56 and Yra1/REF1/Aly in eukaryotic cells (reviewed in Aguilera 2005). Indeed, THO subunits can be purified by gel-filtration chromatography from yeast, *Drosophila*, and human cell extracts (Chávez et al. 2000; Guo et al. 2005; Masuda et al. 2005; Rehwinkel et al. 2004). These subunits are tightly bound, as they are present in purifications performed at high salt conditions. Under such conditions, however, Sub2/UAP56 and Yra1/REF1/Aly have been shown either not to co-purify with THO or to be present in substoichiometric amounts (Guo et al. 2005; Rehwinkel et al. 2004; Strasser et al. 2002). Consistently, inactivation of Sub2 does not affect the integrity of the THO complex in yeast, whereas null mutations in any gene encoding a THO

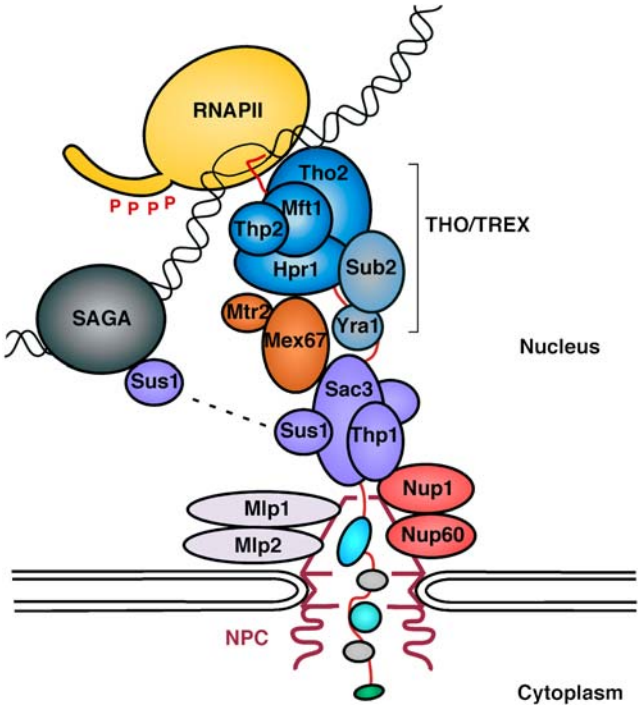

Fig. 3 Coupling between transcription and mRNP export: a role in gene recruitment to the nuclear periphery in *S. cerevisiae*. During transcription elongation, the THO complex may facilitate the loading of the Sub2 (metazoan UAP56) RNA-dependent ATPase and the Yra1 RNA-binding export factor (metazoan Aly/REF1) along the nascent mRNA molecule, generating a stable TREX complex. The mRNA export receptor Mex67 (metazoan TAP) may also be recruited to the nascent mRNP via its interaction with THO. Another protein complex, the Thp1–Sac3–Sus1–Cdc31, is associated with the nuclear pore by its interaction with Nup1 and plays a role in mRNP export in concert with Mex67. The Sus1 factor also interacts with the SAGA chromatin-remodeling complex and could be a bridge between transcription and mRNA export. The anchoring of active genes to the nuclear periphery seems to depend on interactions between the transcription and mRNP export machineries

subunit led to the dissociation of the whole THO complex and the degradation of its subunits (Huertas et al. 2006; Jimeno et al. 2002).

THO mutations lead to gene expression defects that are particularly evident for long and GC-rich DNA sequences (Chávez et al. 2000), as well as for repeat-containing genes (Voynov et al. 2006). Such defects reflect an impairment in transcription elongation as determined both in vivo and in vitro (Chávez et al. 2000; Mason and Struhl 2005; Rondón et al. 2003b). The current view is that THO could play a major role during transcription elongation in the formation of export-competent mRNPs. THO has been shown to be recruited to active chromatin and its pattern of crosslinking to overlap partially with RNAPII, since it binds strongly to coding regions of genes with a bias towards the 3′ end, and only weakly to promoters and regions downstream of polyadenylation sites (Kim et al. 2004a; Strasser et al. 2002; Zenklusen et al. 2002).

THO could participate in the co-transcriptional formation of export-competent mRNPs during transcription elongation by controlling the assembly of heterogeneous nuclear ribonucleoproteins (hnRNPs) onto the mRNA and avoiding the formation of suboptimal mRNPs. The observation that overexpression of the RNA-dependent ATPase Sub2 or the novel RNA-binding protein Tho1 suppresses both the transcription and RNA export defects of THO mutants (Fan et al. 2001; Jimeno et al. 2002, 2006) is consistent with this view. Excess of Sub2 or Tho1 could provide an alternative mechanism for optimal mRNP formation that would not depend on THO. The critical role of THO in the proper formation of an optimal export-competent mRNP during gene expression is further supported by the observation that, in THO mutants, the nascent mRNA itself mediates transcription impairment and contributes to genome instability (Huertas and Aguilera 2003). In *hpr1Δ* mutants, R-loops (in which the nascent RNA forms an RNA–DNA hybrid with the template DNA strand, the other strand remaining single-stranded) have been shown to be formed co-transcriptionally. The formation of such R-loops might lead to transcription defects and genome instability. R-loops are likely to become obstacles for the next elongating RNAPIIs, thus impairing transcription elongation. R-loops or mRNA–RNAPII–DNA tertiary structures can also obstruct replication, leading to genome instability (Wellinger et al. 2006). The relevance of mRNA processing enzymes in the prevention of R-loop formation may not be a unique characteristic of the THO complex, but a feature shared by a number of specific mRNP biogenesis factors. In fact, it has been shown that depletion of the *ASF/SF2* splicing factor in chicken DT40 cells and human HeLa cells lead to R-loop formation that triggers genome instability (Li and Manley 2005). Other specific proteins involved in mRNP biogenesis such as Sub2–Yra1, the Mex67–Mtr2 export factor, the Nab2 hnRNP or the NPC-associated Thp1–Sac3–Sus1–Cdc31 complex (see below) confer similar phenotypes in transcription, RNA export and genetic instability as THO/TREX mutants (Fischer et al. 2002; Gallardo et al. 2003; Jimeno et al. 2002). However, these properties are not shared by any mutant impairing mRNA biogenesis (Luna et al. 2005). The THO complex, therefore, may represent one protein complex working early at the interface transcription–mRNP formation. The possibility that THO may play a role as a transcriptional checkpoint that responds to improperly assembled export-competent mRNP particles by controlling transcription elongation is yet an open question (Huertas et al. 2006).

Sub2/UAP56 and Yra1/Aly: from TREX to RNA export

As mentioned earlier, THO was found to be associated with Sub2/UAP56 and to a lesser extent to Yra1/Aly in

yeast and humans in the so-called TREX complex (Strasser et al. 2002). The human UAP56 (56-kDa U2AF-associated protein) and its yeast ortholog Sub2 are conserved eukaryotic proteins of the DEAD/H box RNA helicase family (Linder 2006). Sub2/UAP56 was first implicated in splicing (Fleckner et al. 1997; Kistler and Guthrie 2001; Libri et al. 2001) and was found to be associated with the exon junction complex (EJC), a protein complex that binds −20 nts upstream of the exon–exon junction of spliced mRNAs (Le Hir et al. 2001). Later, different studies have provided evidence that Sub2/UAP56 has a functional role in the biogenesis of export-competent mRNP particles. Sub2/UAP56 was shown to interact with export factors such as Yra1/REF1/Aly and Mex67/TAP (Luo et al. 2001; Strasser and Hurt 2001) and to play an essential role in mRNA export, since inactivation or depletion of Sub2/UAP56 results in a strong nuclear accumulation of bulk mRNA (Gatfield et al. 2001; Strasser and Hurt 2001).

Yeast Yra1 was one of the first export factors shown to be recruited to genes in a transcription-dependent manner (Lei et al. 2001). This connection was further established when it was purified as a component of the conserved TREX complex (Strasser et al. 2002). Yra1 was initially identified on the basis of its potent RNA annealing activity in vitro. Together with its orthologs Aly and REF1, Yra1 belongs to an evolutionarily conserved family of hnRNP-like proteins characterized by an RNA-binding domain called REF (RNA and export-factor-binding proteins; Stutz et al. 2000) that acts as an adaptor in mRNA export by facilitating the recruitment of the receptor Mex67/TAP to cellular mRNPs (Strasser and Hurt 2001). Although Yra1 is an essential mRNA export factor in yeast, it is dispensable in other eukaryotes, raising the possibility of the existence of additional adaptors of mRNA export (reviewed in Rodriguez et al. 2004).

In yeast, TREX is recruited co-transcriptionally to chromatin, and Sub2 has been shown binding to both intron-less and intron-containing genes. Thus, it appears to regulate the association of RNA processing factors with both types of mRNAs (Abruzzi et al. 2004; Moore et al. 2006; Zenklusen et al. 2002). It is likely that Sub2 plays a general role as an RNA-dependent chaperone, according to its RNA-dependent ATPase activity (Shi et al. 2004). Sub2 and Yra1 are co-transcriptionally recruited to nascent transcripts via THO (Zenklusen et al. 2002). In metazoans, mRNP biogenesis is more complex, as most genes contain introns, and the mRNA export machinery is linked to the splicing machinery. Indeed, human UAP56 and Aly localize with EJC suggesting that these proteins are recruited to sites of active transcription in a splicing-dependent manner, and UAP56 mediates the association of Aly to THO (Custodio et al. 2004; Masuda et al. 2005). However, evidence supports additional mechanisms for the loading of these proteins. Thus, recruitment of TREX to the 5′ end of mRNAs may be mediated by the interaction of the cap-binding protein CBP80 with Aly (Cheng et al. 2006). In addition, other factors such as Spt6 could participate in the co-transcriptional recruitment of REF1/Aly and other mRNP biogenesis factors to the nascent mRNP, probably via interactions with the Ser2-P RNAPII (Yoh et al. 2007). It seems, therefore, that Yra1/Aly could serve as a bridge between RNA-binding proteins early during mRNP biogenesis, acting upstream during transcription and downstream at mRNA export.

The Mex67–Mtr2/TAP–p15 export factor

Mature and correctly packaged yeast mRNPs are transported into the cytoplasm with the help of the heterodimeric export receptor Mex67/Mtr2 (TAP/p15 in metazoan; Gruter et al. 1998; Segref et al. 1997). These mRNA export receptors shuttle between the nucleus and the cytoplasm and interact with nucleoporins facilitating mRNA transport through the nuclear pore. As Mex67/TAP shows low RNA affinity, its interaction with mRNAs is believed to be mediated by adaptors (reviewed in Kohler and Hurt 2007; Rodriguez et al. 2004). The yeast mRNA export receptor Mex67, as well as its metazoan counterpart TAP, both harbor an N-terminal domain that binds mRNPs via RNA-binding adaptors and a UBA domain in their C-terminus that participates in the interaction with FG nucleoporins, the Phe-Gly repeats containing nucleoporins essential for translocation. Different proteins have been proposed to act as adaptor of Mex67, the classical ones being Yra1 and the hnRNP Npl3. Npl3 is a SR (serine/arginine-rich)-like shuttling RNA-binding protein recruited co-transcriptionally to RNAPII-transcribed genes, which interacts directly with Mex67 and is essential for mRNA export in yeast (Gilbert and Guthrie 2004; Lei et al. 2001).

Recently, it has been shown that Mex67 is recruited to transcribed genes via its UBA domain and independently of RNA (Dieppois et al. 2006; Gwizdek et al. 2006). The UBA domain of Mex67 interacts with Hpr1, suggesting that this interaction protects Hpr1 from ubiquitin/proteasome-mediated degradation thereby coordinating the recruitment of the mRNA export machinery with transcription (Fig. 3). Therefore, RNA export adaptors and receptors appear to be recruited co-transcriptionally as shown for proteins involved in mRNP biogenesis. As discussed later, in *S. cerevisiae* some genes are recruited to the nuclear periphery when activated. Interestingly, however, the co-transcriptional recruitment of Mex67 has also been shown to contribute to the stable association of activated *GAL10* and *HSP104* genes with the nuclear periphery (Dieppois et al. 2006).

The Thp1–Sac3–Sus1–Cdc31 complex

Thp1–Sac3–Sus1–Cdc31 is a protein complex that was purified in the yeast *S. cerevisiae* and has a role in mRNP biogenesis and maintenance of genomic integrity (Fischer et al. 2002, 2004; Gallardo et al. 2003). It is located at the inner side of the NPC, binds to nucleoporins at the nuclear basket, and mediates the nuclear export of mRNPs in concert with the export receptor Mex67–Mtr2 (Fischer et al. 2002; Gallardo et al. 2003; Lei et al. 2003; Fig. 3). Thp1 and Sac3 are found in association with the centrin Cdc31 (Fischer et al. 2004; Gallardo et al. 2003), a protein acting in the duplication of microtubule-organizing centers, and with Sus1, a small protein recently identified as a novel component of the SAGA complex (Rodriguez-Navarro et al. 2004) (Thp1-Sac3-Sus1-Cdc31 complex). Sus1 seems to be important for both mRNA export and transcription initiation, although transcription initiation relies essentially on SAGA's activity. In addition, Sus1 interacts with Ubp8 and Sgf11, two SAGA subunits, and participates in histone H2B de-ubiquitylation and in the maintenance of normal H3 methylation levels (Kohler et al. 2006; Zhao et al. 2008). It has been proposed that Sus1 could act as a bridge linking transcription with the nuclear pore, facilitating therefore mRNP export. The Thp1–Sac3–Sus1–Cdc31 complex also participates in the anchoring of active genes to the nuclear periphery. Sus1, Sac3, and Ada2, another component of SAGA, are involved in the repositioning and subsequent confinement of dynamic motility of the *GAL1* locus to the nuclear periphery upon transcriptional activation (Cabal et al. 2006; Chekanova et al. 2008; Kurshakova et al. 2007). In this view, Sus1 could mediate the anchoring of genes to the nuclear periphery in a manner presumably independent of the nascent mRNA molecule.

Mutants of the Thp1–Sac3–Sus1–Cdc31 complex share similar transcription defects and hyper-recombination phenotypes as THO complex mutants (Gallardo et al. 2003). However, both complexes are currently seen as independent structural units located at different platforms in the cells, which act during mRNP assembly from the transcription elongation step to the nuclear pore and have an impact on genome instability. Both protein complexes are indeed different functional units. Not only have THO subunits not been found in purified Thp1–Sac3–Sus1–Cdc31 complexes (Fischer et al. 2002; Gallardo et al. 2003; Rodriguez-Navarro et al. 2004), but the phenotypes of each mutant are suppressed by different factors (Fan et al. 2001; Gallardo et al. 2003; Jimeno et al. 2002, 2006). Consistently, whereas the Thp1–Sac3–Sus1–Cdc31 complex is specifically found at the nuclear periphery, THO is found all over the nucleus.

Other mRNP biogenesis export factors

Other proteins with the ability to bind RNA, either directly or indirectly, play important roles in mRNP export; however, we will only mention two of them for their putative relationship with mRNP biogenesis. Nab2 is a shuttling poly(A)+ mRNA-binding protein that is required for both nuclear export and proper polyadenylation of mRNA transcripts (Anderson et al. 1993; Kelly et al. 2007). Interestingly, overexpression of Nab2 suppresses the transcription and the mRNA export defects of *thp1Δ* mutants (Gallardo et al. 2003). Also an N-terminal deletion of Nab2 (*nab2-1*) confers mRNA export and transcription defects, suggesting that this protein acts at the interface between transcription and mRNP biogenesis (Gallardo et al. 2003). It has been shown that Nab2 interacts physically and functionally with the nuclear pore platform of Mlp proteins and with other mRNP biogenesis factors; however, the putative role of this hnRNP in transcription is still unknown (Green et al. 2003; Vinciguerra et al. 2005).

Another relevant protein is Dbp5. It is an essential DEAD-box helicase that shuttles between the nucleus and cytoplasm and is recruited to mRNPs during transcription, performing a variety of functions in gene expression (reviewed in Cole and Scarcelli 2006). Recently, Dpb5 was shown to function as an RNP-remodeling factor required for displacement of Nab2 from RNA (Tran et al. 2007), suggesting that remodeling might be required for release of mRNPs from the NPC.

mRNP biogenesis and the nuclear organization

In the nucleus of eukaryotic cells, the genetic material is not randomly distributed but organized in different nuclear domains that contribute to the regulation of transcription levels and other processes of DNA metabolism. The nuclear periphery represents one of these domains and has been associated with repressed and silenced chromatin (reviewed in Schneider and Grosschedl 2007; Spector 2003). In *S. cerevisiae*, regions of transcriptionally silent chromatin such as telomeres and the mating-type loci associate with the nuclear envelope (reviewed in Akhtar and Gasser 2007). Telomeres are anchored at the nuclear periphery together with silencing factors, which promote and stabilize heterochromatin. In higher eukaryotes, gene-poor chromosomes position at the nuclear periphery whereas gene-rich chromosomes locate internally. This interaction between DNA and the nuclear periphery seems to be mediated by the nuclear lamina, a filament protein layer located just below the nuclear membrane that spans from NPC to NPC. The lamina is involved in the proper distribution and

maintenance of heterochromatin. A number of data suggest that proteins of the nuclear lamina regulate transcription by recruiting chromatin modifiers and transcription factors to the nuclear periphery (reviewed in Akhtar and Gasser 2007).

The nuclear periphery, however, could also serve as a nuclear activation domain. Several dynamically regulated genes are recruited to the nuclear periphery when activated. In *S. cerevisiae*, this repositioning effect has been observed for many highly expressed genes, such as those encoding proteins involved in metabolic pathways, heat shock proteins, and mating pheromone-induced genes (Abruzzi et al. 2006; Cabal et al. 2006; Casolari et al. 2004; Taddei et al. 2006). In higher eukaryotes, other examples exist, such as the β-globin locus during erythroid maturation in mouse (Ragoczy et al. 2006), and the activation of the dosage compensation complex in chromosome X of *Drosophila* (Mendjan et al. 2006).

In *S. cerevisiae*, the nuclear pore has been identified as the site to which activated genes are recruited. Genome-wide ChIP analysis has shown that many transcriptionally active genes physically interact with components of the nuclear pore and associated proteins (Casolari et al. 2004). The nuclear pore could be necessary for gene positioning, and indeed different studies have shown that the loss of some nuclear pore-associated factors blocks gene recruitment (Cabal et al. 2006; Dieppois et al. 2006).

Different models/mechanisms have been proposed to explain the peripheral location of activated genes. One of these mechanisms is based on interactions between transcriptional activators and nucleoporins. In support of this model, nucleoporin Nup2 has been mapped to promoter regions at *GAL1* and other genes, suggesting the existence of specific nucleoporin-promoter interactions that are independent of transcription (Schmid et al. 2006). It is possible that an initial NPC recruitment precedes transcription and can be regulated by transcription factors. According to this idea, the Rap1 transcription factor can activate transcription in yeast through its interaction with the nucleoporin Nup84 (Casolari et al. 2004; Menon et al. 2005; reviewed in Ahmed and Brickner 2007; Brown and Silver 2007). Genes, therefore, could shuttle to the pore and assemble the necessary transcriptional machinery there rather than in the nuclear interior. Another model suggests that transcription itself could promote gene repositioning to the nuclear periphery. Experiments of real-time confocal microscopy in yeast have shown that *GAL* genes are subject to a diffuse movement, but that the loci are confined to the nuclear periphery upon activation. This confinement has been shown to depend on Ada2, Sus1, Sac3, and Nup1 (Cabal et al. 2006). These data are consistent with the relevance of the promoter in gene recruitment, as Ada2 and Sus1 bind to *GAL1* promoters; however, there is evidence that transcriptional activation alone might not be sufficient for gene repositioning. Another proposal suggests that the nascent mRNA plays an important role in stable NPC binding. Thus, recruitment of active genes to the nuclear periphery has been shown to require 3′ untranslated regions, which are involved in efficient mRNA export and processing (Abruzzi et al. 2006). In addition, factors involved in mRNP biogenesis such as the myosin-like protein Mlp1, and the mRNA export factor Mex7 contributes to the nuclear pore anchoring of activated *GAL10* and *HSP104* genes (Dieppois et al. 2006).

Therefore, some reports suggest that the promoter is an important requisite for active gene repositioning whereas others suggest that the nascent transcript and mRNP machinery mediate gene recruitment. It is, thus, possible that the stable association of genes with the NPC could occur in two steps. First, a transient contact could be established by promoter-bound factors like SAGA and Sus1. Second, factors involved in the mRNA processing such as Mex67 and Mlp1 could stabilize this association (reviewed in Akhtar and Gasser 2007). In addition, the Thp1–Sac3–Sus1–Cdc31 complex has been recently shown to be required for the post-transcriptional retention of *GAL*-promoter-driven reporter genes at the nuclear periphery (Chekanova et al. 2008). The NPC association persists after transcriptional shut-off, and the RNA also remains tethered adjacent to the transcription site. Therefore, the scenario could be a complex network of mRNA gene and gene nuclear periphery tethering. Finally, an additional mechanism proposed to contribute to the recruitment, and/or subperipheral retention of genes at the nuclear envelope is transcription-associated chromatin remodeling (reviewed in Ahmed and Brickner 2007). In yeast, some active genes are retained at the nuclear periphery for several generations, and the retention and reactivation have been shown to require the histone variant H2AZ (Brickner et al. 2007).

Although all these models have been established from different experimental data obtained in *S. cerevisiae*, evidence opens the possibility that gene tethering to the nuclear periphery might also exist in some cases in other eukaryotes. Thus, the MLS (male-specific lethal) complex of *Drosophila* and humans, involved in up-regulation of X-linked genes, has been shown to co-purify with NPC and exosome components (Mendjan et al. 2006). Interestingly, upon inactivation of two NPC components, there is a reduction in the transcription of these X-linked genes, suggesting that the connection between NPC and gene activation might be a conserved mechanism in eukaryotes. Altogether, the data are in agreement with the "gene-gating" hypothesis (Blobel 1985), which postulates that the activation of non-randomly positioned genes in the nucleus is linked to specialized NPCs that in turn facilitate efficient mRNA export.

Concluding remarks and future perspectives

It is now clear that mRNP biogenesis is a complex and integrated nuclear process in which a number of different events are coordinated and coupled. Such events include transcription, pre-mRNA processing (5′-end capping, splicing, 3-end cleavage, and poly(A) addition), mRNP formation and export, as well as quality control. Coupling requires the participation of specific proteins working at the interface of these events. Nevertheless, even though our knowledge on mRNP biogenesis has increased notably in the last few years, we still know very little about the proteins mediating coupling and how this is really achieved. As technical approaches for nuclear gene expression studies have widened, knowledge of the connection between the transcription cycle, mRNP metabolism, and export has increased notably, but it has also raised new and unexpected questions. The recent observation that transcription of at least a subset of genes may be a process occurring at specific nuclear domains has changed our way of looking at transcription: from a mere biochemical reaction to a cellular perspective. How genes are targeted to particular domains, such as the nuclear periphery, and how this targeting regulates expression are among the many intriguing questions raised at this moment. The combination of molecular and cellular analyses as well as genome-wide approaches should provide answers to the actual questions on transcription and mRNP biogenesis and export. This will allow us to have a comprehensive understanding of nuclear metabolism and its impact on nuclear structure.

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