Crossing the borders
Poly(A)-binding proteins working on both sides of the fence

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The addition of a 3′ poly(A) tail is a pre-requisite for the maturation of the majority of eukaryotic transcripts. In most eukaryotic species, RNA poly(A) tails are bound by two important poly(A)-binding proteins (PABPs): PABPC1 and PABPN1 that localize to the cytoplasm and the nucleus, respectively. Such steady state localization for PABPN1 and PABPC1 led to a model whereby PABPN1-bound nuclear mRNAs are remodeled during or after nuclear export so that PABPN1 is replaced by PABPC1 to allow robust cap-dependent translation in the cytoplasm. Here we discuss evidence that challenge the view in which PABPN1 and PABPC1 function solely in the nucleus and cytoplasm, respectively. We discuss accumulating evidence that support nuclear roles for PABPC1 in mRNA biogenesis as well as cytoplasmic roles for PABPN1 in translational control. Because 3′ poly(A) tails can also act as a degradation mark via the exosome complex of 3′-5′ exonucleases, we also discuss recent results that involve the nuclear PABP in posttranscriptional gene regulation.

Introduction: Current View of mRNA 3′ End Processing and Nuclear Export

Transcription of protein-coding genes by RNA polymerase II (Pol II) generates mRNA precursors that are co-transcriptionally matured. Co-transcriptional maturation of pre-mRNAs is achieved by the recruitment of several protein complexes via the carboxy-terminal domain (CTD) of RNA Pol II. The recruited complexes are subsequently transferred onto nascent transcripts for the addition of a 5′ cap structure, the removal of introns by the spliceosome, and the addition of a 3′ poly(A) tail. Studies during the past decade have shed light on evolutionarily conserved quality control systems that monitor the accuracy of these maturation events before an mRNA is exported through the nuclear pore complex, thereby improving the fidelity of gene expression. Interestingly, evidence now converge toward the nature of the poly(A) tail as a critical mean of discriminating between normal and aberrant mRNAs.

Polyadenylation of mRNA precursors is a two-step process. An endonucleolytic cleavage first generates a free hydroxyl group at the 3′ end of the pre-mRNA. The second step is the addition of a poly(A) tail to the 3′ end of the nascent transcript. Species-specific cis-acting elements located upstream and downstream of the cleavage site are responsible for the recruitment of a multi-subunit cleavage and polyadenylation complex. As part of this complex, the evolutionarily conserved poly(A) polymerase (PAP) catalyzes the addition of adenosine residues that will become the poly(A) tail. Interestingly, the average poly(A) tail length is also species-specific: mRNAs from S. cerevisiae, S. pombe and humans have about 70, 40 and 250 adenosyl residues, respectively. As yet, however, the molecular mechanism of poly(A) tail length control in these various species is not clearly understood. Nevertheless, a common theme for poly(A) tails of all eukaryotic species is their recognition by
contact between CPSF and PAP. However, when poly(A) tails exceed ∼250 adenosine residues, additional PABPN1 can no longer be accommodated in such a spherical structure, which results in the loss of interaction between CPSF and PAP, leading to poor PAP processivity.

Ultimately, nascent mRNAs exit the nucleus to be translated in the cytoplasm. Similar to capping, splicing and 3' end processing, several mRNA export factors are recruited during transcription to allow efficient nucleo-cytoplasmic transport of mRNAs. Biochemical evidence indicates that transit of the mRNP through the NPC involves remodeling of several proteins present on the mRNA; yet, the extent of mRNP remodelling is poorly understood and remains an active area of research. According to the steady state distribution of PABPN1 and PABPC1 to the nucleus and the cytoplasm, respectively, it has been proposed that PABPC1 substitutes for PABPN1 after nuclear export of the mRNA. Consistent with such a model, electron microscopic studies using the Balbiani ring (BR) mRNA of the insect *Chironomus tentans* demonstrated mRNA-associated PABPN1 during docking of the BR mRNA at the nuclear pore, but little PABPN1 was present on the cytoplasmic side of the nuclear envelope. These data, together with the steady state localization of PABPN1 and PABPC1, supported a model (see Fig. 1A) in which an exchange reaction between nuclear and cytosolic PABPs occurs to allow efficient cap-dependent translation via the formation of a close-loop structure mediated by interactions between the translation initiation complex and PABPC1. Despite the established steady state localization of these poly(A)-binding proteins, PABPN1 and PABPC1 have both been shown to be shuttling proteins as discussed below.

**Nuclear Role of PABPC1**

The transport of macromolecules between the nucleus and the cytosol is generally accomplished by different complexes of poly(A)-binding proteins (PABPs). Two main PABPs are found in most eukaryotic cells: PABPN1 and PABPC1 present in the nucleus and the cytosol, respectively. In the nucleus, PABPN1 is thought to function during the polyadenylation step of mRNA synthesis. Accordingly, efficient RNA polyadenylation only necessitates 3 components in vitro: the cleavage and polyadenylation specificity factor (CPSF), PABPN1 and PAP. Separately, CPSF and PABPN1 modestly stimulate PAP processivity; however, a rapid and processive polyadenylation reaction occurs when both CPSF and PABPN1 are combined with PAP. Such evidence led to a tethering model in which CPSF and PABPN1 cooperate in the recruitment and stabilization of PAP to its substrate RNA. Notably, when CPSF, PABPN1, and PAP are from mammalian sources, the average poly(A) tail length using such in vitro polyadenylation assays is about 250-nucleotides, similar to mRNA poly(A) tails found in human cells. This suggested that poly(A) tail length control in humans is mediated by ending processive elongation via destabilization of the ternary complex. In this model, formation of a spherical poly(A) tail-PABPN1 complex ensures...
receptor proteins. The importins convey cargoes from the cytosol to the nucleus, whereas exportins carry cargoes from the nucleus to the cytosol. A well-established and evolutionarily conserved exportin is Xpo1/Crm1. This receptor specifically binds to cargoes that contain a leucine-rich nuclear export signal (NES). Similarly to its mammalian ortholog PABPC1, Pab1 of S. cerevisiae localizes to the cytosol at steady state. However, experiments using the yeast xpo1-1 strain, which expresses a temperature-sensitive allele of xpo1 that is defective for NES-mediated protein export, demonstrate that Pab1 accumulates in the nucleus at the nonpermissive temperature. Consistent with the ability of Pab1 to shuttle between nucleus and cytoplasm, Pab1 was shown to associate with Xpo1 and with the nuclear import receptor Kap108/Sxm1. Notably, the nuclear accumulation of Pab1 was markedly increased when the xpo1-1 allele was combined with a temperature-sensitive version of the mRNA export factor Mex67. Pab1 therefore appears to actively enter the nucleus and exit via two transports pathways: an mRNA-dependent and -independent pathway. Importantly, evidence also support the presence of PABPC1 in the nucleus of mammalian cells. First, fractionation experiments using different human cell types indicate the presence of PABPC1 in the nucleus. Furthermore, cell biological experiments demonstrate that PABPC1 actively shuttles between the cytoplasm and nucleus.

What is the functional significance for the presence of the cytosolic PABP in the nucleus? Biochemical and genetic evidence in budding yeast tend to support a role for Pab1 in mRNA biogenesis. Accordingly, mRNA processing and export defects are seen in cells in which the nuclear localization signal, and therefore Pab1’s ability to be imported into the nucleus, was perturbed. Furthermore, yeast cells lacking Pab1 expression accumulate hyperadenylated RNAs in the nucleus. Interestingly, a similar phenotype consisting of hyperadenylated transcripts retained near their transcription site was previously reported in different mRNA export mutants of budding yeast. The hyperadenylated mRNAs that accumulate in the nucleus of cells lacking Pab1 are most likely targeted for nuclear surveillance by the exosome, as deletion of rrp6, which encodes a subunit of the nuclear exosome, releases those transcripts for nuclear export. Further evidence supporting a functional role of Pab1 in mRNA biogenesis is the accumulation of polyadenylated mRNAs in the nucleus of pan2 and pan3 deletion mutants, which code for two subunits of a deadenylase complex (PAN) recruited by Pab1. Because the PAN complex is thought to participate in the last step of mRNA biogenesis, these results suggest that Pab1 is involved in the final step of 3’ end processing before mRNA export.

To date, no functional evidence has yet been reported to support a nuclear role for PABPC1 in mammals. However, several observations suggest that PABPC1 is implicated in nuclear events of mRNA biogenesis. RNA immunoprecipitation experiments indicate a poly(A) tail-dependent association of PABPC1 with unspliced pre-mRNAs. Furthermore, the canonical poly(A)-polymerase copurifies with PABPC1, suggesting the presence of PABPC1 in the vicinity of the 3’ end processing complex. Consistently, a proteomic study that characterized the protein content of the mRNA 3’ processing complex identified several peptides corresponding to PABPC1. Surprisingly, on the basis of the number of peptides identified by mass spectrometry analysis of the purified complex, the stoichiometry of PABPC1 in this complex appears to be greater than PABPN1.

The aforementioned evidence using yeast and human cells strongly suggest that the evolutionarily conserved cytosolic PABP is likely to be involved in nuclear events associated to mRNA biogenesis. The nucleo-cytoplasmic shuttling and the involvement of Pab1/PABPC1 in 3’ end processing/mRNA export therefore challenge the traditional view of PABPC1 functions that are limited to the cytosol (Fig. 1B).

**Cytosolic Function of PABPN1**

PABPN1 functions in the polyadenylation step of mRNA synthesis, consistent with its nuclear localization. Yet, electron micrographs detect PABPN1 in the cytosol. Accordingly, studies indicate that PABPN1 and its fission yeast ortholog, Pab2, shuttle between the nucleus and the cytoplasm. Recent experiments using fission yeast also indicate that a significant fraction of Pab2 cosediments with poly-somes. Consistently, a proteomic analysis of Pab2-associated proteins identified peptides from cytoplasmic translation factors and several ribosomal proteins. Although the functional significance of the association between Pab2 and translating mRNAs remains unclear, these data indicate that a fraction of the nuclear PABP is associated with cytoplasmic transcripts in fission yeast. Conversely, experimental evidence in mammals does not support the association between PABPN1 and actively translating mRNAs.

Specifically, PABPN1 is thought to be restricted to a pioneer round of translation that detects aberrant mRNAs with nonsense mutations. Perhaps the strongest evidence to support a function for PABPN1 in the cytosol comes from studies in the fruitfly, Drosophila melanogaster. In this organism, specific mRNAs are translationally regulated during development via cytoplasmic polyadenylation, which activates translation. Interestingly, mutations in the gene encoding the Drosophila PABPN1 ortholog, pabp2, cause poly(A) tail extension for specific mRNAs regulated by cytoplasmic polyadenylation, thereby increasing the level of the proteins encoded by these transcripts. Although the physical association between PABP2 and the regulated cytosolic transcripts was not demonstrated in this study, PABP2 copurifies with the cytoplasmic polyadenylation factor, Orb, thus providing support for a direct role of PABP2 in such cytoplasmic control of gene expression. Consistently, PABP2 localizes to the cytosol of oocytes and early embryos where these mRNAs are regulated. Therefore, although the mechanism by which Drosophila PABP2 shortens poly(A) tails of these cytosolic transcripts remains unclear, PABP2 appears to play an essential cytoplasmic function during early Drosophila development.
An Unexpected Role for the Nuclear PABP in the Control of Noncoding RNA

As discussed above, in vitro biochemical studies provided significant insights about the mechanism by which human PABPN1 functions in RNA polyadenylation. Nonetheless, the precise function of PABPN1 in human cells remains poorly understood as few studies have been performed using mammalian cells. One of the few evidence supporting the role of PABPN1 in nuclear polyadenylation in human cells comes from studies using the influenza virus NS1 protein that was reported to inhibit the stimulating activity of PABPN1 on polyadenylation in vitro. Consistently, β-actin mRNAs with short poly(A) tails are produced following influenza virus infection, whereas β-actin transcripts with normal poly(A) tail length are detected after infection with an influenza virus isolate that encodes a truncated version of NS1. Although these aforementioned evidence are mostly indirect, more recent gene silencing experiments using primary mouse cells support the role of PABPN1 as a general mRNA polyadenylation factor. The fact that the hypoadenylation phenotype of PABPN1-depleted cells did not significantly alter the expression levels of poly(A)-containing mRNAs is intriguing, however, given the important role of the 3′ end of mRNAs in mRNA export. This study also shows drastic accumulation of polyadenylated RNA in the nucleus of PABPN1-depleted cells, suggesting a role for PABPN1 in mRNA export. Interestingly, this robust mRNA export defect was not accompanied by mRNA hyperadenylation, a phenotype that was previously associated with mRNA export defects in yeast, fruit flies and mammalian cells.

In Drosophila, specific pabp2 mutants also show shorter poly(A) tails using total RNA or individual mRNAs. Although this remains unclear, this hyperadenylation phenotype is assumed to be linked to PABP2’s nuclear role since an hyperadenylation phenotype is associated with the cytosolic function of Drosophila PABP2 (discussed above). The budding yeast *Saccharomyces cerevisiae* has also been a valuable system to study the mechanism of 3′ end processing and poly(A) tail synthesis. Although *S. cerevisiae* appears to lack an homolog of PABPN1, the cytosolic PABP of budding yeast, Pab1, was shown to function during mRNA 3′ end processing, as discussed above. Moreover, another nuclear RNA-binding protein with specificity to poly(A), Nab2, contributes to the regulation of poly(A) tail synthesis in this organism. The recent identification of Pab2, the *S. pombe* ortholog of PABPN1, is interesting and should provide powerful biochemical and genetic tools to study the cellular function of the nuclear PABP. Notably, pab2-null cells are viable and display hyperadenylated RNAs, suggesting that factors other than Pab2 can stimulate PAP processivity in fission yeast. Recent microarrays experiments revealed that the abundance of most mRNAs is unaffected by the deletion of pab2 and that 3′ end decision and mRNA poly(A) tails are similar to normal cells. Unexpectedly the deletion of *pab2* led to the accumulation of polyadenylated small nucleolar RNAs (snoRNAs), noncoding RNAs that assemble into ribonucleoprotein particles (snoRNPs) and that function in the processing and modification of pre-ribosomal RNAs. Paradoxically, mature forms of snoRNAs are not polyadenylated. Yet, recent findings suggest that polyadenylation is involved in 3′-end maturation of snoRNA precursors by the exosome complex of 3′-5′ exonucleases. Accordingly, the nuclear exosome was identified as the machinery that functions with Pab2 to promote processing of polyadenylated snoRNAs uncovering the key role of a PABP in the processing of noncoding RNAs. In fission yeast, therefore, Pab2 does not appear to be required for general mRNA polyadenylation, but rather functions in a polyadenylation-dependent processing pathway together with the exosome. It will be of great interest to determine whether this novel function identified for Pab2 is conserved in humans given the significant similarity between Pab2 and PABPN1. Noteworthy is a recent study reporting the involvement of human PABPN1 in a viral-mediated polyadenylation-dependent RNA decay pathway, possibly similar to the role of *S. pombe* Pab2 in polyadenylation-dependent RNA processing.

Conclusions

Given the aforementioned evidence that support cytoplasmic functions for PABPN1 and nuclear roles for PABPC1, we believe that a simple exchange model in which poly(A)-bound PABPN1 in the nucleus is replaced by PABPC1 in the cytoplasm is unlikely. A plausible model (see Fig. 1B) that is consistent with the results discussed herein is one in which PABPC1 and PABPN1 are bound to the same mRNA poly(A) tail, but that the PABPN1:PABPC1 stoichiometry may change in the nucleus and the cytoplasm given the different concentration of these PABPs in each of these cellular compartments. Although PABPN1 and PABPC1 were highlighted in our discussions, it is likely that other RNA-binding proteins with affinity to poly(A) tracts also play important roles in gene expression in the nucleus and cytoplasm. Finally, polyadenylation has recently emerged as a molecular mark to distinguish between normal, immature and aberrant transcripts. We therefore envisage that such polyadenylation-dependent quality control systems will unveil previously uncharacterized roles for PABPs in gene regulation.

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