

RNA Export: Searching for mRNA Identity

Dispatch

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Efficient eukaryotic gene expression hinges on the ability of mRNA to travel from the nucleus to its cytoplasmic destination. Recent work lends insight into features that allow diverse mRNAs to be recognized by shared export machinery.

In eukaryotic organisms, genomic DNA is housed within the nucleus, a compartment specialized to protect genomic content, ensure accurate DNA replication and facilitate fine-tuned gene regulation. Production of RNA within the nucleus necessitates a transport event in which RNAs transit through the nuclear pore complex and on to their destinations. Mattaj and colleagues [1] have recently reported results that advance our understanding of how the vast array of mRNAs are recognized as targets for export. This work defines a minimal stretch of unstructured RNA as an important determinant for mRNA export and further underscores the intimate connection between these recognition events, the export pathway and the RNA's downstream fate.

Although small nuclear (sn), messenger (m), transfer (t) and ribosomal (r) RNAs converge at the nuclear pore complex during nucleocytoplasmic transport, different classes of RNA have distinct requirements for export. This was first appreciated when cross-competition analysis of RNA export revealed that the limiting factors are not the same for each type of RNA: one class of RNA can saturate its own route of export, while leaving that of other RNA classes unperturbed [2,3]. Progress in the field has since led to a molecular understanding of some of the differences in export paths; in many cases these involve specialized soluble factors which recognize and transport specific RNA cargo. For instance, Exportin-t recognizes shared features of tRNAs [4,5], whereas a cap-binding complex, along with the adaptor PHAX, bridges U snRNAs to the export receptor Crm1/Exportin 1 [6].

The case of mRNA is unique in that we know several important players in the mRNA export machinery, but we are just beginning to understand how these factors recognize and load onto mature mRNA to facilitate its export. The challenge, for the researcher as well as the cell, lies in the diversity of mRNA. Surprisingly, structural features known to be shared by most mRNAs — such as a methylated cap structure at the 5' end and the poly(A) tail at the 3' end — are not critical determinants of export [2,7]. Many mRNAs also share a common step in their *biogenesis* and that is the process of splicing. Notably, splicing has been shown to lead to

deposition onto the RNA product of a complex of proteins, including a protein implicated in the process of mRNA export, REF/Aly [8,9].

Although the recruitment of export factors during splicing does not preclude a role for additional mechanisms, it does provide a satisfying explanation for the link between diverse mRNAs and a shared pathway of export. Currently, however, we do not have a clear explanation for how non-spliced mRNAs — from intronless genes — are recognized and channeled into a similar pathway (see Figure 1A). While transcription itself, as well as sequence-specific RNA recognition elements, can promote interaction between a particular mRNA and factors critical to mRNA export [10], there are indications that a more general point of entry into the mRNA export pathway exists. In particular, mRNAs engineered to be in mature form without any splicing events are exported when injected into the nuclei of *Xenopus* oocytes [11]. How would such unspliced mRNAs be distinguished from other RNA populations and selectively exported via the mRNA export route?

Ohno *et al.* [1] have taken on this intriguing question of how RNAs are discriminated by the machinery that drives different export paths and, in doing so, have gained insight into key aspects of mRNA recognition. Using a hybrid U1 snRNA, engineered to contain an insert consisting of an intron surrounded by minimal exonic flanking sequences (see Figure 1B), the authors were able to demonstrate that the process of splicing switches export machinery recognition of U1. Rather than following a route to the cytoplasm that relies on interaction between Crm1 and a nuclear export signal (NES), hybrid U1 RNAs that are spliced exit the nucleus via a route that has hallmark features of mRNA export. Similar results were found using hybrid tRNA molecules, indicating a dominant effect of splicing on export pathway choice.

The connection between splicing and deposition of a specific complex of proteins led to the prediction that the U1 ribonucleoprotein complex might be altered following passage through the pre-mRNA splicing machinery. This indeed was found to be the case. Y14 and REF/Aly, two members of the exon-exon junction complex, were found to associate with spliced versions of U1. Conversely, association with the U snRNA-specific adaptor PHAX was somewhat impaired when U1 had been spliced. When splicing was globally prevented by depletion of endogenous U1 and U2 snRNAs, export of the unspliced hybrid U1 transcript was inefficient. Although an export-competent complex was not formed, some recruitment of mRNA-specific proteins had taken place. Splicing-arrested hybrid U1 was found to associate with Y14, whereas PHAX association was severely impaired. Thus, the mere presence of an intron prevented or displaced PHAX binding, while promoting association with the mRNA adaptor protein

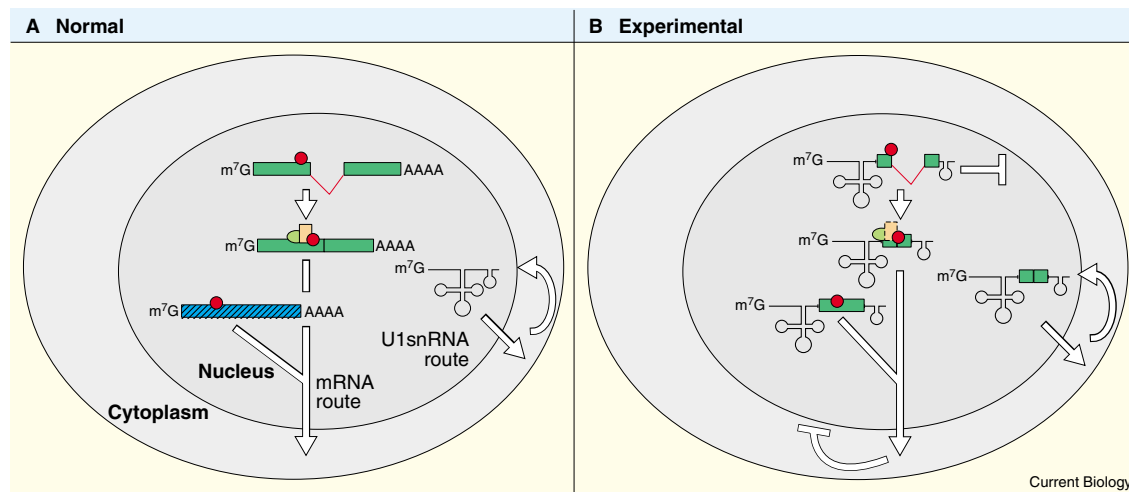


Figure 1.

(A) Under normal conditions, mRNA is typically generated from a precursor containing one or more introns. However, 5–8% of genes encoding mRNAs are estimated to lack introns [16]. Spliced (green box) and unspliced (hatched blue box) mRNA have been demonstrated to share at least some pathway components [11,17,18], although certain mRNAs also have alternative means of transport [19]. As depicted, the process of splicing leads to deposition of a group of proteins [8,9]. One member of this complex, Y14 (red circle), is predominantly tracked in the study by Ohno *et al.* [1]. Types of RNA that have been shown to co-immunoprecipitate with Y14, albeit with different efficiencies, are indicated [1,8,20]). A different set of soluble factors conveys U1 to the cytoplasm [6], functionally defining a distinct export route through nuclear pore complexes. (B) Experimental conditions designed to define features important to recognition of mRNA for export [1] are illustrated. Insertion of either an intron or a short unstructured stretch of mRNA from an exon into the U1 transcript resulted in a hybrid U1 RNA that was exported via a pathway with functional attributes of the mRNA export path. Y14 was found to bind to each of these modified versions of U1. However, when splicing was arrested, the unspliced form of U1 was not exported. The hybrid nature of this transcript disrupted recognition by the U snRNA export machinery; transport via the mRNA export route likely did not occur either due to active retention by early steps in intron recognition or to insufficient insert length for recognition as an mRNA identity element. Splicing was found to impact cytoplasmic fate as well as the route of export: U1 generated by splicing did not relocalize to the nucleus, whereas its normal counterpart, as well as a hybrid U1 with the same residual flanking sequences inserted in the primary transcript, are imported following cytoplasmic maturation.

Y14. This occurred either because of early (but non-productive) events of intron recognition by the splicing machinery, or simply as a result of the insertion of an extended non-structured stretch of RNA within the context of U1.

The notion that splicing itself is not necessarily essential to altering recognition of the hybrid U1 by mRNA adaptor proteins was further reinforced when relatively short (300–350 nucleotide) stretches of exonic sequences were inserted into the U1 transcript. These sequences not only promoted preferential association with Y14, they too circumvented stable association with PHAX. Surprisingly, these unstructured sequences also served to route the hybrid U1 into an mRNA export path and could do so independent of their orientation in the hybrid molecule. These results operationally define an mRNA identity element as a short — approximately 300 nucleotide — unstructured stretch of RNA.

It is increasingly clear that early steps in RNA biogenesis influence the efficiency of downstream events [12], and here too this paradigm proved true. When U1 transcripts were generated by a process of splicing and consequently exported in conjunction with mRNA export machinery components, the resulting snRNA had an altered fate [1]. Rather than completing a cytoplasmic maturation cycle that culminates in relocalization to the nucleus, spliced U1 snRNA

remained in the cytoplasm, although some maturation steps had occurred. These observations suggest that mRNA export factors, which persist in their association with RNA following remodeling of the ribonucleoprotein export complex, play an active role in directing downstream events. This is consistent with recent findings that implicate Y14 and its partner, Mago Nashi, in directing mRNA localization within the cytoplasm [13–15].

The elegant approach of using hybrid RNA transcripts taken by Mattaj and colleagues [1] has led to new insight into the requirements for building functional RNA export complexes. The concept that splicing profoundly impacts ribonucleoprotein (RNP) complex formation has been reinforced and indeed found to exert a dominant effect on the route taken by RNAs through the nuclear pore complex. Further clues as to how diverse mRNAs are recognized have also been revealed by the observation that non-structured, non-sequence-specific stretches of RNA can serve as mRNA recognition elements. Although a very general feature of RNA is implicated, among RNA export cargo such an element is typical only of mRNA and is thus compatible with selectivity of the mRNA export path. These identity elements would, additionally, be found in immature precursors of mRNA and in excised introns, underscoring the importance of retention mechanisms.

These results serve to focus our attention on new questions. For instance, what is the key cognate factor (or factors) that recognizes this element and links the RNA to the transport machinery? Many candidates exist, including certain hnRNP proteins, members of the exon-exon junction complex, mRNA export factors such as Gle1, Gle2 and Dbp5, as well as nuclear pore proteins themselves. The answer to this question will also allow for a better molecular understanding of the length requirement for this element. Additional questions arise concerning how recognition of this mRNA identity element is coordinated with factor recruitment directed by other means, such as transcription-coupled mechanisms, splicing-coupled mechanisms and specific *cis*-elements. And, does this identity element serve primarily as a 'back-up route' for mRNAs encoded by intronless genes or, more likely, as part of a broader mRNA signature that is important even when splicing has taken place? Answers to these questions will provide information critical to understanding both mRNA export and, ultimately, efficient gene expression.

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