Perspective

Messenger RNA Transport and HIV *rev* Regulation

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TRANSCRIPTIONALLY ACTIVE GENES ARE DISPERSED THROUGHOUT the nucleus of interphase cells, and the newly synthesized RNAs must be transported from these sites to the cytoplasm. Given the dimensions of the nucleus and the density of macromolecules in the nucleus, the underlying mechanism for mRNA transport is likely a vectorially driven active process. In contrast to the situation in the cytoplasm where several molecular transport processes have been characterized, the locomotion responsible for mRNA transport between the site of transcription and the nuclear pores remains unknown.

RNAs transcribed from different genes by RNA polymerase II (pre-mRNA) emerge from the nucleus at different rates. Not only are nuclear pre-mRNAs modified by capping, methylation, splicing, and polyadenylation before transport, they also interact with several distinct RNA binding proteins to form heterogeneous nuclear ribonucleoprotein complexes. These are processes unique to pre-mRNA and specify a pathway of RNA synthesis that must integrate with the transport process. For example, pre-mRNA splicing, an event that joins together protein coding sequences (exons) by precisely removing the intervening sequences (introns), involves the formation of the multi-component complex, the spliceosome (*1*). Several known constituents to the spliceosome have been visualized in discrete regions within the nucleoplasm (*2*). This localized distribution of spliceosomal components would limit intranuclear diffusion of pre-mRNAs and provide a framework for an ordered pathway for mRNA transport. In fact, recent in situ hybridization experiments have shown that the intranuclear movement of transcripts from individual genes may be confined to specific channels (*3*). The only described infrastructure for nuclear RNA is the poorly defined nuclear matrix, and there are evidences suggesting an association of spliceosome-bound pre-mRNA with the matrix (*4*). Such an interaction may be the target of regulation by an E1B gene product of adenovirus, which seems to enhance viral mRNA transport during late infection (*5*).

U1 and U2 small nuclear ribonucleoproteins (snRNPs) recognize sequences at the 5′ splice site (*s.s.*) and the 3′ *s.s.* and branch site, respectively, and facilitate formation of the complete spliceosome, which also contains U4/6 and U5 snRNPs. Within spliceosome, the pre-mRNA is processed in two steps: cleavage at the 5′ *s.s.* by formation of a lariat RNA containing a branch and then cleavage and ligation at the 3′ *s.s.* resulting in joining of the two exons. For many genes, deletion of either the 5′ or 3′ *s.s.* disrupts the synthesis of cytoplasmic RNA (*6*). The vast majority of mutant RNAs, which do not complete splicing, are retained and destroyed in the nucleus. Such findings have indicated that introns contain "forbidden sequences" that prevented the transport of unspliced RNA into the cytoplasm. The possibility that sequences forming the splice sites confer nuclear retention of unspliced RNA seemed paradoxical because of the common occurrence of nonconstitutive introns, which undergo splicing in a tissue-specific or temporally regulated manner. For example, two alternatively spliced mRNAs are generated from the T antigen gene of SV40. The larger of these mRNAs contains a 5′ *s.s.*, which in the smaller mRNA is utilized for splicing. This retained splice site on the larger T antigen mRNA does not appear to impair the transport.

One possible explanation is that not all splice sites are created equal; some can escape recognition while others cannot. The nature of the specific differences has been difficult to establish since little is known about the recognition of splice sites. Firm evidence now exists that a few positions in the 5′ *s.s.* consensus sequence GUAAGU and the branch site consensus sequences UACUAAC are recognized by complementarity to U1 and U2 snRNA, respectively (*7*). Beyond these, it is probable that recognition of sequences involved in splicing is mediated by protein-RNA interactions. Several proteins in extracts of mammalian cells have been shown to interact specifically with sequences at or near the 5′ *s.s.*, pyrimidine-rich sequences common to the 3′ *s.s.*, and the 3′ *s.s.* (*8*). How or whether the binding of these proteins regulates early commitment steps in splicing, that is, splice site recognition and nuclear retention, is unknown.

The early commitment steps in splicing have been studied in yeast with a reporter gene constructed so that only the intron containing RNA could be translated to produce β-galactosidase—splicing of the intron disrupted the reading frame (*9*). Such an approach allowed selection for transport of unspliced mRNA and allowed identification of cis or trans mutations defective for early steps of splicing. Retention of this unspliced RNA within the nucleus was shown to depend on wild-type sequences at both the 5′ *s.s.* and branch site (in yeast the branch site seems to be the major determinant for the 3′ *s.s.*). Similarly, at least two transacting gene products, previously isolated as temperature-sensitive mutants defective in splicing, were found to be essential to prevent the transport of unspliced RNA to the cytoplasm.

The connection between RNA splicing and mRNA transport has become more apparent from the studies of human immunodeficiency virus type–1 (HIV-1) gene regulation. The transport of HIV-1 mRNA from the nucleus of cells is regulated during the course of viral infection (*10*). Initially, only spliced viral RNA is transported to the cytoplasm for the translation of the regulatory polypeptides, Tat, Nef, and Rev. Of these, Rev exerts a novel regulation by binding to a particular RNA sequence, the Rev response element (RRE), to effect the transport of singly spliced or unspliced RNA to the cytoplasm (*11*). In the presence or absence of Rev, the level of unspliced or singly spliced viral RNA in the nucleus does not change. Thus, Rev does not appear to regulate the cytoplasmic level of unspliced viral RNA simply by inhibiting splicing reactions.

A model for Rev action was that it facilitated transport of unspliced viral RNA by a novel pathway. However, a more general mechanism was suggested from a study of the relationship between Rev regulation and splicing of a β-globin intron (*12*). An RNA containing a β-globin intron with a defined point mutation at both the 5′ and 3′ *s.s.* (double *s.s.* mutants) was readily transported to the cytoplasm as unspliced mRNA; the nucleus contained very little unspliced β-globin pre-mRNA. Insertion of the RRE into the intron and coexpression of Rev in the cell had no effect on the fraction transported. However, if either the 5′ or 3′ *s.s.* was reverted to wild type (single *s.s.* mutants) in these mutant β-globin–RRE intron constructs, unspliced RNA did not appear in the cytoplasm.

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Coexpression of Rev with the single *s.s.* mutants, resulted in the transport of unspliced globin mRNA. This suggests that recognition of one splice site, even in the absence of the other splice site, by cellular components in mammalian cell can retain the RNA in the nucleus. Furthermore, the pre-mRNA retention in the nucleus brought about by splice site recognition apparently can be regulated.

The regulation by Rev requires suboptimal splice sites. Mutations introduced into β-globin splice sites delay the progression of splicing to latter steps involving the intron excision and the exon ligation. The splice sites of HIV also are not efficiently utilized as unspliced viral precursor RNAs accumulate to impressively high levels in the nucleus. All classes of retroviruses must transport to the cytoplasm a balanced level of spliced and unspliced viral RNA for synthesis of new virion components and genomic RNA—mutations that enhance the rate of viral RNA splicing are defective for reproduction (*13*). HIV controls this balance by using a combination of ineffective splice sites and the viral protein Rev. Rev promotes the dissociation of the unspliced HIV RNA from splicing components that bind and retain the RNA in the nucleus, but execute splicing slowly. In view of the growing list of alternatively spliced transcripts where functional splice sites remain on mature mRNA, Rev-like activities may represent a common form of cellular regulation.

The vectorial aspect of mRNA transport across the nuclear membrane still remains an enigma. Messenger RNA transport, however, should not be viewed as an isolated event centered about this process. To understand how mRNA gets to the cytoplasm, the successive steps of mRNA maturation in the nucleus need to be emphasized. Studies on regulation of RNA transport by HIV-1 Rev protein have provided only a glimpse into the complex interplay between nuclear RNA splicing and RNA transport.

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