Perspective

Pharmaceutical BIOPROCESSING

Control in avian retroviruses by RNA processing

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Retroviruses and RNA Processing

Retroviruses employ a singular replication scheme during which an extended, single-stranded RNA genome is converted into a double-stranded DNA molecule that's inserted into and becomes a permanent resident of the host genome. From the chromosomal position, the integrated viral DNA (the provirus) is transcribed by the host RNA polymerase II (pol II) to get genome-length viral RNA that has an equivalent modification as typical host mRNAs (a 5′ cap and a 3′ poly(A) tail). Some of this full-length viral RNA is packaged into progeny virions, and a further pool is translated into structural and enzymatic proteins that compose the virus particles. However, some viral proteins are synthesized from spliced transcripts, therefore the primary transcript also is a substrate for RNA splicing. The amount of spliced mRNA species is often quite large, as is that the case for complex retroviruses like human immunodeficiency virus (HIV). Clearly, the extent of splicing must necessarily be controlled to preserve the genome-length RNA, which usually represents ~50% or greater of the entire. Another issue raised by the recent appreciation that splicing and polyadenylation are coupled is how the full-length viral RNA is efficiently polyadenylated within the absence of splicing.

Control of RSV RNA Splicing: The Splice Sites

Splicing control in RSV involves the upkeep of suboptimal splice sites and therefore the regulation of splice site use by a positive element and a number of other negative-acting elements. These control regions are summarized in and discussed below.

One of the mechanisms by which splicing is controlled in RSV is that the maintenance of suboptimal splicing signals. Presumably, a population of viral RNAs is in a position to flee splicing by going unrecognized by the splicing apparatus. While it appears that the 5′ ss isn't involved in splicing control, evidence for a suboptimal env 3′ ss stemmed from the examination of an epidemic during which a 24 nt oligonucleotide was inserted 12 nt upstream of the env 3′ ss. This virus showed a marked delay in replication that correlated with substantial over splicing to the env 3′ ss. Upon longer-term passage, a category of phenotypic revertants was isolated that had mutations within the original insert that lowered splicing to wild-type levels. The results were according to the replication defect stemming from a paucity of unspliced RNA. A second class of revertants was identified that had deletions downstream of the env 3′ ss within the env exon. Within the context of a wild-type virus, an equivalent deletion caused replication defects and resulted in little or no env splicing, which suggested that the deleted sequences played a positive role in env splicing. It's now known that the deletion eliminated an RNA splicing enhancer.

Control of RSV RNA Splicing: Negative Elements Distinct From the Splice Sites

RSV RNA splicing in mammalian cells is sort of different than in chicken cells. In NIH3T3 or human fibroblast cells, little or no env splicing was detected whereas ~50-60% of the RNA represented spliced src transcripts. These results suggested that mammalian cells might lack a negative factor required for correct splicing control, which could partially

explain the no permissive nature of those cells for RSV replication. Subsequent work with wild-type and SSS mutant viruses showed an identical, high level of src splicing in human fibroblasts, according to a scarcity of SSS function in those cells. Furthermore, using an in vitro splicing system derived from cells, there was no difference in splicing of minigenes containing or lacking the SSS, but SSS-specific repression was observed upon addition of chicken cell extract. These data suggested that an inhibitory factor present in chicken extracts, but not mammalian extracts, is liable for splicing regulation by the SSS. However, the identity of the factor and therefore the mechanism of action remain to be elucidated.

Polyadenylation of RSV RNA

Most cellular mRNAs undergo 3′-end processing reactions involving cleavage of the RNA at its eventual 3′ terminus and addition of a poly(A) tail. The polyadenylation process is intimately coupled to other RNA processing reactions, including 5′-end capping and splicing, which ensures efficient and faithful processing of the mRNA. Retroviral mRNA is additionally polyadenylated by the host cell machinery but because the poly(A) signals are present within the LTRs at each end of the provirus, use of the 5′ poly(A) site must be suppressed. Additionally, polyadenylation in many retroviruses is inefficient and viral transcripts terminate at poly(A) sites within downstream cellular genes. This is often important for the incorporation of cellular sequences into retroviral genomes and for activation of downstream cellular genes, which within the case of oncogenes leads to tumor genesis.