8 Post-transcriptional Gene Control

Review the Concepts

1. In the case of a protein-coding gene, gene control beyond regulation of transcriptional initiation can be regulated in several ways: 1) by controlling the stability of the corresponding mRNA in the cytoplasm; and 2) by controlling the rate of translation; and 3) by controlling the cellular location so that newly synthesized protein is concentrated where it is needed.

2. You should be surprised! A 5′ cap is a well-conserved structure among all eukaryotes and it contributes to several processes. For example, during transcription, the CTD of RNA polymerase II interacts with capping components. During translation, the cap promotes engagement of ribosomes and mRNA by binding to translation initiation factors (eIF-4E). During splicing, the cap promotes splicing via the cap binding complex which enhances U1 snRNP and U6 snRNA 5′ site interactions. As well, polyadenylation requires the cap binding complex.

3. Biochemical and chromatin IP experiments suggest that the polyadenylation complex is associated with CTD of RNA polymerase II following initiation.

4. A mutation in the TAR sequence that abolishes Tat binding would result in shorter HIV transcripts (i.e., no antitermination). Cdk9 would not be positioned correctly to phosphorylate the CTD of RNA polymerase II, which would terminate transcription at the transcriptional block. Loss of Cdk9 activity would also result in no antitermination and shorter HIV transcripts.

5. Electron microscopy studies with adenoviral DNA complexed with the mRNA encoding a viral capsid protein revealed that such RNA-DNA hybrids contained "loops" of DNA that had no counterparts in the RNA (see Figure 8-6). The locations of exon-intron junctions are predicted by comparing the genomic and cDNA sequences of a gene.

6. The term *hnRNA* describes heterogeneous nuclear RNAs that consist of several different types of RNA molecules that are found in the nucleus. Small nuclear RNAs (snRNAs) bind to splice sites and participate in splicing reactions. Small nucleolar RNAs play a similar role in tRNA processing and can help to position methyltransferases near methylation sites. Micro RNAs (miRNAs) and short, interfering RNAs (siRNAs) are involved in gene silencing. Both are derived from longer precursor molecules and become part of the RISC complex.

7. Both types of splicing involve two transesterification reactions and similar intermediates and products. In group II intron self-splicing, the introns alone form a complex secondary structure involving numerous stem loops, whereas spliceosomal splicing utilizes snRNAs interacting with the 5′ and 3′ splice sites of pre-mRNAs, which forms a three-dimensional RNA structure functionally analogous to the group II intron. Evidence that supports the idea that introns in pre-mRNAs evolved from group II self-splicing introns comes from experiments with mutated group II introns. In these experiments, domains I and V are deleted, and this yields a group II intron incapable of self-splicing. When RNA molecules equivalent to the deleted portions are added back "in trans" in the in vitro reaction, self-splicing is restored. This shows that portions of group II introns can be trans-acting like snRNAs.

8. Researchers believe these events occur mostly within discrete foci in the nucleus. Supporting evidence for discrete nuclear regions comes from imaging studies utilizing rhodamine-labeled poly(dT) to detect polyadenylated RNA. In the

nucleus this probe binds to about 100 discrete foci.

9. In muscle cells, the internal polyadenylation site could be spliced out of the mature RNA when the fifth intron is removed. This would leave the site in the 3′ UTR as the sole polyadenylation site. In other cells, the fifth intron may not be spliced out. This would result in earlier polyadenylation and a shorter mRNA transcript. In this scenario, a muscle-specific splicing factor could facilitate removal of the fifth intron.

10. RNA editing is a type of pre-mRNA processing, altering the sequence of the pre-mRNA that results in a mature mRNA differing from the exons encoding it in genomic DNA. Although half of the sequence of some mRNAs may be altered in *Trypanosoma* and plant mitchondria and chloroplasts, only single-base changes have been observed in higher eukaryotes. A case for RNA editing in humans involves the serum protein apoB, which forms large lipoprotein complexes that carry lipids in serum. The apoB gene encodes two alternative forms of the protein, the ~240-kDa form (apoB-48) in intestinal epithelial cells and the ~500-kDa form (apoB-100), which is expressed in liver. RNA editing occurs in intestinal cells, where a single base alteration converts a codon for glutamine into a stop codon. The truncated protein is smaller and has a function distinct from the larger apoB-100 form, which as part of the low density lipoprotein (LDL) particle, is responsible for transporting cholesterol to body tissues.

11. The mRNP exporter is a heterodimeric protein composed of the nuclear export factor 1 (NXF1), also known as TAP, and the nuclear export transporter 1 (Nxt1). TAP binds in multiple places along mRNPs, together with other mRNP adapter proteins, including REF (RNA export factor) and SR proteins. Both exporter subunits interact with FG-nucleoporins, allowing them to move through the nuclear pore complex and into the cytosol. Protein kinases and phosphatases are thought to play a key role in the directional movement. In the nucleus, REF and SR proteins must be dephosphorylated in order to bind the mRNP exporter. In the cytoplasm, however, a kinase phosphorylates the adapter proteins, promoting the dissociation of the exporter from the mRNP. This dissociation results in a lower concentration of mRNP exporter-mRNP complexes in the cytoplasm than in the nucleus, allowing the complex to diffuse down its concentration gradient into the cytoplasm.

12. Short interefering RNAs (siRNA) can be synthesized to inhibit the function of any desired gene. siRNAs contain 21–23 nucleotides hybridized to each other so that two bases at each of the 3′ ends are single-stranded. The siRNA is introduced into cells, where it forms a complex with RISC. It then base-pairs with its target RNA and induces its cleavage, thereby eliminating the endogenous message. Cells containing siRNAs to TSC1 are likely to undergo uncontrolled cell growth because the loss of the TSC1 protein eliminates Rheb-GAP activity. This loss of activity causes abnormally high and unregulated levels of Rheb-GTP, which when bound to the mTOR complex would result in a high, unregulated activity of mTOR serving as an active kinase to phosphorylate a variety of substrates required to promote cell growth.

13. A plant deficient in Dicer activity shows increased susceptibility to RNA viruses because Dicer is not present to degrade a portion of the viral double-stranded intermediates that viruses synthesize during replication. Without Dicer, all of these viral mRNAs are available for further viral infection.

14. b-actin mRNAs can be seen to accumulate on the leading edges of myoblasts during in situ hybridization experiments. Further, specific portions of the actin genes (the 3′ UTRs) can be linked to a reporter gene and transfected into cultured cells. When the cells are assayed for activity of the reporter gene product, the results show that the 3′ UTR directs localization of the reporter gene.

Analyze the Data

a. Dicer is needed to cleave microRNAs from a larger transcript. The scientists tested the

hypothesis that *LAT* encodes a miRNA by interfering with Dicer expression. Without Dicer, there should be no miRNA. If the *LAT* gene encodes an miRNA that protects cells from apoptosis, then cells that lack Dicer should not survive in the presence of the apoptosis inducing drug. Indeed, in the presence of the *LAT* plasmid but in the absence of Dicer, the *LAT* gene did not protect cells from apoptosis. The observation that the Pst-Msu fragment of the *LAT* gene provides protection from apoptosis suggests that an miRNA, if produced, would be derived from this region of the gene.

b. The region of the *LAT* gene between the Sty-Sty sites is both necessary and sufficient to protect cells from apoptosis.

c. Cells infected with either wild-type virus or rescued virus make RNAs of ~55 or 20 nucleotides containing sequence from the Sty-Sty region of the *LAT* gene. These RNAs are not observed in mock infected cells or in cells infected with the DSty virus. The probe corresponding to the 3′ end of the predicted stem can hybridize to RNAs containing the sequence of the 5′ stem to which it is mostly complementary. It detects both the ~55- and 20-nucleotide RNAs. The probe corresponding to the 5′ end of the predicted stem can hybridize to molecules containing the 3′-stem sequence to which it is complementary. This probe detects the ~55 nucleotide RNA. Thus the ~55-nucleotide RNA contains both the 5′-stem sequence and the 3′-stem sequence, whereas the 20 nucleotide RNA contains only the 5′-stem sequence. These results strongly suggest that a 20-nucleotide miRNA is derived from the 5′ end of a ~55-nucleotide pre-miRNA. The ~55-nucleotide RNA was probably processed from *LAT* RNA by Drosha in the nucleus. The 20-nucleotide RNA was probably processed by Dicer in the cytoplasm. The findings that a fragment of the *LAT* gene that includes the Sty-Sty region protects cells from apoptosis, that Dicer is needed to confer this protection, that part of the Sty-Sty region is predicted to form a stem-loop, a structure found in pre-miRNAs, and finally that this region gives rise to an ~55- and a 20-nucleotide RNA, the latter being the size of miRNAs, all strongly suggest that the *LAT* gene encodes an miRNA that protects cells from apoptosis.

d. By forming an imperfect duplex with TGF- mRNA, miR-LAT would inhibit translation of TGF-. Accordingly, TGF expression would be reduced in cells infected with wild type HSV-1. Because TGF expression potentiates cells to undergo apoptosis, a reduction in TGF would likely mean fewer apoptotic cells, as is the case when cells are infected with wild type HSV-1. Thus HSV-1, which encodes miR-LAT, can inhibit the cells it infects from undergoing apoptosis. Because no other HSV-1 genes are expressed in latently infected cells, the latently infected cells are not recognized by the immune system. Furthermore, because of the expression of LAT, they are more resistant to the induction of apoptosis than uninfected cells. Eventually, latency in some latently infected cells is overcome and those cells produce new progeny virus particles. These virus particles are transported down the axons of the virus-producing cells where they escape from the neuron and infect surrounding cells in the region of skin enervated by that neuron, causing a recurrent cold sore.