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# POST-TRANSCRIPTIONAL GENE CONTROL

## REVIEW THE CONCEPTS

1. 1) controlling the stability of the corresponding mRNA in the cytoplasm; 2) controlling the rate of translation; and
   3) controlling the cellular location so that newly synthesized protein is concen-trated where it is needed.

2. True. Enzymes involved in mRNA capping, splicing, and polyadenylation are recruited to the phosphorylated CTD, which activates them. As only RNA polymerase II possesses a CTD, and RNA polymerase II is responsible for mRNA transcription, this ensures that these forms of processing only occur with mRNA.

3. These sequences are found near the intron/exon junctions, not the middle of the intron. Because of these sequences, the snRNPs of the spliceosome are recruited to the proper location on the mRNA. The role of the branch point A is to perform the first transesterification reaction, which eliminates the phosphodiester bond connecting the intron and the upstream exon. While RNA nucleotides have an OH group at both the 2′ and 3′ carbons, the 3′ carbon of the branch point A is connected to an adjacent nucleotide. Thus, the OH group involved in this reaction must be at the 2′ carbon.

4. 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 rRNA processing and can help to position

methyltransferases near methylation sites or convert uridines to pseudouridines. 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.

5. 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 form 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.

6. 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.

7. 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 mitochondria 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.

8. The nucleus is composed of a double membrane bilayer. These bilayers are amphipathic in nature, so it is extremely unfavorable for hydrophilic molecules to diffuse through. Instead, transport is restricted to the nuclear pores.

9. The mRNP exporter is a heterodimeric protein composed of the nuclear export factor 1 (NXF1) and the nuclear export transporter 1 (NXT1). NXF1 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.

10. Short interfering 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 intro-duced 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 mes-sage.

11. 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.

12. The deadenylation-dependent decapping pathway involves the shortening of the poly(A) tail, which destabilizes the interaction between the 5′ cap and translation initiation factors. This results in removal of the 5′ cap, at which point the mRNA is degraded by nucleases from both the 5′ and 3′ ends. The adenylation-independent decapping path-way only involves 5′ degradation due to a 5′ cap that is more sensitive to decapping. Finally, in the endonucleolytic pathway, it is possible for nucleases to degrade from within the mRNA and not at the 5′ or 3′ ends. P bodies are the sites of translation repression and/or de-gradation of mRNA and have a high concentration of mRNA processing enzymes. If a decapping enzyme such as DCP1 had lower activity in a mutant cell, it likely would result in a build-up of mRNA molecules, increasing the size of the P bodies in the cell.

13. Proteins work at specific locations within the cell. Traditionally, it was thought that translation happens either in the cytoplasm or the ER, at which point the protein would be directed to its area of function. By localizing an mRNA, it is possible to synthesize proteins at specifi c places in the cell, thus eliminating the need to traffi c this protein after it is translated. Ash1, a protein that inhibits mating type switching in yeast, has been shown to localize to the bud tip so that it is only present in the smaller daughter cells after budding. In the sea slug, neuro-nal mRNA were shown to localize to the synapse.