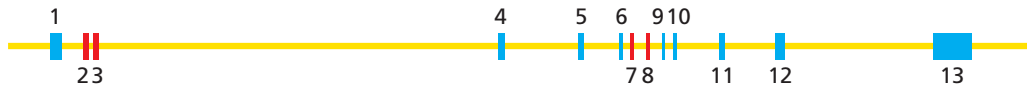
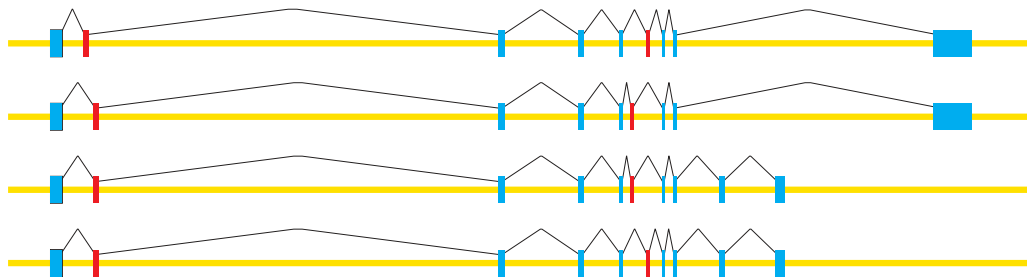


Discuss the following exercises in pairs

The human  $\alpha$ -tropomyosin gene is alternatively spliced to produce different forms of  $\alpha$ -tropomyosin mRNA in different cell types. For all forms of the mRNA, the protein sequences encoded by exon 1 are the same, as are the protein sequences encoded by exon 10. Exons 2 and 3 are alternative exons used in different mRNAs, as are exons 7 and 8.

(A) HUMAN  $\alpha$ -TROPOMYOSIN GENE

(B) FOUR DIFFERENT SPLICE VARIANTS



Which of the following statements about exons 2 and 3 is the most accurate? Is that statement also the most accurate one for exons 7 and 8? Explain your answers.

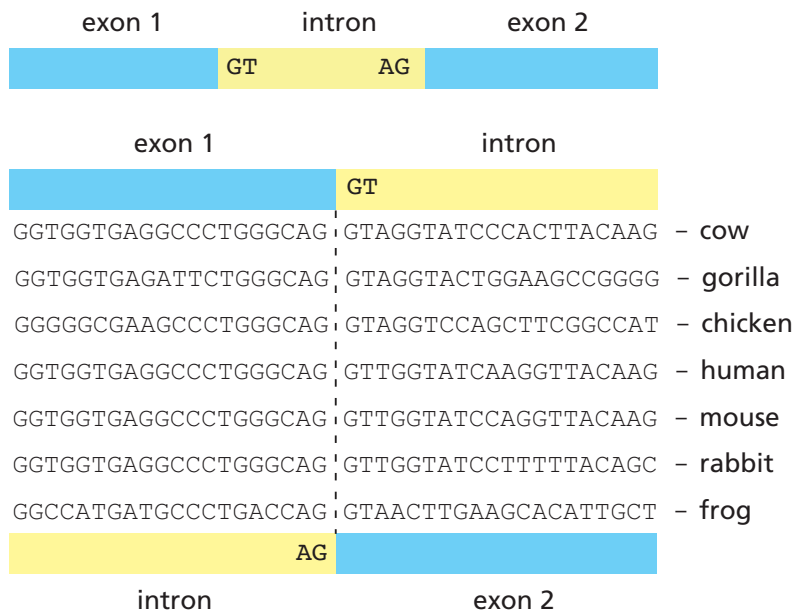
- Exons 2 and 3 must have the same number of nucleotides.
- Exons 2 and 3 must each contain an integral number of codons (that is, the number of nucleotides divided by 3 must be an integer).
- Exons 2 and 3 must each contain a number of nucleotides that when divided by 3 leaves the same remainder (that is, 0, 1, or 2).

**Statement C is the only one that is necessarily true for exons 2 and 3. It is also the only one true for exons 7 and 8. While the conditions given in A and B could be the case, they need not be. However, because the encoded protein sequence is the same in segments of the mRNA that correspond to exons 1 and 10, neither choice of alternative exons (2 versus 3, or 7 versus 8) can be allowed to alter the reading frame. To maintain the normal reading frame—whatever that is—the alternative exons must have a number of nucleotides that when divided by 3 (the number of nucleotides in a codon) gives the same remainder.**

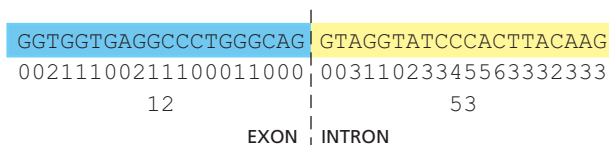
You have printed out a set of DNA sequences around the intron/exon boundaries for genes in the  $\beta$ -globin family, and have taken the trip to the country to study for the weekend. When you look at the printout, you discover to your annoyance that there's no indication of where in the gene you are. You know that the sequences in **the figure below** come from one of the exon/intron or intron/exon boundaries and that the boundaries lie on the dotted line, but you don't know the order of the intron and exon. You know that introns begin with the dinucleotide sequence GT and end with AG, but you realize that these particular sequences would fit *either* as the start *or* the end of an intron.

If you cannot decide which side is the intron, you will have to cut your weekend short and return to the city (or find a neighbor with Internet access). In desperation, you consider the problem from an evolutionary perspective. You know that introns evolve faster (suffering more nucleotide

changes) than exons because they are not constrained by function. Does this perspective allow you to identify the intron, or will you have to pack your bags?



Since introns evolve faster than exons, the introns of the different species will be more variable than the exons. It is difficult to scan these sequences by eye and decide, with confidence, which side is the more conserved. One way to quantify the differences is to pick one sequence, for example, the cow, and count up how often the other sequences differ at each position, as shown below. Summing the differences on each side of the junction makes it clear that sequences on the left are much more similar to one another than are the sequences on the right. (Similar differences exist no matter which sequence is chosen for comparison.) Thus, the more conserved sequences, which are on the left, correspond to exons, and the less conserved sequences, which are on the right, correspond to introns.



### Multiple Choice Questions

- What can be the impact of a mutation in the -10 or -35 consensus sequence of the bacterial promoter?
  - Increase in transcription speed.
  - Decrease in RNA polymerase binding to the promoter.
  - Transcription starts but with systematic errors.
  - It does not affect the transcription process.
  - RNA polymerase will not be able to finish transcription.

**A mutation in the -10 or -35 consensus sequence typically affects the binding of RNA polymerase to the promoter (B), leading to decreased transcription efficiency.**

- RNA polymerases do not require an RNA primer
  - Because ribonucleotides are more stable than deoxyribonucleotides.

- b. Because RNA polymerases can directly bind to the template strand promoter and initiate transcription.
- c. Because RNA is single-stranded.
- d. Because RNA polymerases use different nucleotides.
- e. Because RNA polymerase is more accurate than DNA polymerase.

**The key reason is that RNA polymerases can initiate transcription directly from the DNA template without needing a primer (B).**

3. What is the main difference between transcription initiation in prokaryotes and eukaryotes?
- a. Prokaryotes do not require transcription factors.
  - b. Prokaryotic RNA polymerase requires a double-stranded promoter to function.
  - c. Eukaryotes have multiple RNA polymerases, whereas prokaryotes have only one.
  - d. Prokaryotes have many transcription factors, but Eukaryotes do not.
  - e. No significant difference.

**Eukaryotes utilize multiple RNA polymerases for different types of RNA synthesis, while prokaryotes have a single RNA polymerase (C).**

4. What is the direct consequence of a mutation in the TATA box in Eukaryotes?
- a. Failure of RNA polymerase to bind to the promoter.
  - b. Halting of transcription elongation.
  - c. An error in RNA cleavage.
  - d. Alteration of the mRNA translation mechanism.
  - e. No observable change.

**A mutation in the TATA box would prevent RNA polymerase from effectively binding to the promoter (A), which is crucial for transcription initiation.**

5. What is the main effect of elongation factors during transcription in eukaryotes?
- a. Helping to open the DNA double helix.
  - b. Stabilizing RNA polymerase to prevent premature dissociation from the DNA.
  - c. Speeding up transcription by binding to mRNA.
  - d. Modifying the promoter sequence for better transcription.
  - e. Inhibiting transcription once the mRNA is completed.

**Elongation factors primarily stabilize RNA polymerase, preventing its premature dissociation from DNA, which is crucial for efficient transcription (B true).**

6. What is the function of topoisomerases during transcription?
- a. Prevent RNA polymerase from dissociating from DNA.
  - b. Relieve supercoiling tension created during DNA unwinding.
  - c. Add ribonucleotides to the RNA strand.
  - d. Facilitate transcription initiation by stabilizing DNA.
  - e. Help transcription factors associate.

**Topoisomerases are responsible for relieving the tension caused by supercoiling during DNA unwinding, which is essential for the transcription process (B).**

7. What is the main effect of supercoiling tension during transcription?
  - a. Prevent RNA polymerase from binding to DNA.
  - b. Facilitate wrapping of RNA around histones.
  - c. Help open and unwind DNA for transcription.
  - d. Promote RNA elongation by aiding ribonucleotide binding.
  - e. Slow down topoisomerase activity.

**Supercoiling tension primarily aids in the opening and unwinding of DNA to allow transcription to occur (C).**

8. Which of the following RNAs is mainly involved in protein translation?
  - a. tRNA
  - b. miRNA
  - c. piRNA
  - d. siRNA
  - e. snoRNA

**Transfer RNA (tRNA) is the main type of RNA involved in translating mRNA into proteins (A)**

TRUE or FALSE

1. Prokaryotes and eukaryotes only use one RNA polymerase for transcription.

**False, prokaryotes use one RNA polymerase, but eukaryotes use multiple RNA polymerases.**

2. Multiple RNA polymerases can simultaneously transcribe the same gene.

**True**

3. The sigma factor is essential for transcription initiation in eukaryotes.

**False, the sigma factor is only relevant in prokaryotic transcription**

4. Elongation factors reduce the likelihood that RNA polymerase dissociates prematurely from the DNA strand in both prokaryotes and eukaryotes.

**True**

5. The consequences of errors in transcription are less severe than those of errors in DNA replication.

**True. The consequences of errors in transcription are generally less severe than those in DNA replication because transcription produces temporary RNA copies of genes that can be corrected or degraded, while replication leads to permanent alterations in the DNA sequence that can affect the entire organism.**

6. The splicing process allows prokaryotes to produce multiple RNAs from a single gene.

**False, splicing is a process specific to eukaryotic RNA processing, allowing for alternative splicing and the production of multiple RNA variants from a single gene.**

7. The transcription of non-coding RNAs (such as siRNA and miRNA) does not require an RNA polymerase.

**False, non-coding RNAs are transcribed by RNA polymerase too.**

8. Capping and polyadenylation enzymes function exclusively after RNA polymerase has completed mRNA transcription in eukaryotes.

**False, the 5' cap occurs co-transcriptionally after the synthesis of the first 20-30 nucleotides of the nascent pre-mRNA, while polyadenylation involves the addition of a poly(A) tail to the 3' end of mRNA at the end of transcription. Both processes are crucial for mRNA stability, nuclear export, and translation.**

**Note: During transcription elongation, the pre-mRNA being synthesized is accessible to the splicing machinery, allowing splicing to occur before the termination of transcription, thus enabling co-transcriptional splicing.**