Thinking questions

RNA polymerase II commonly terminates transcription of the HIV (the human AIDS virus) genome a few hundred nucleotides after it begins, unless helped along by a virus-encoded protein called Tat, which binds to a speciic hairpin structure in the nascent viral RNA. Tat then recruits a collection of proteins, including the protein kinase Cdk9, which phosphorylates RNA polymerase, enhancing its ability to continue transcription. Flavopiridol is the most potent inhibitor of Cdk9 yet discovered; it blocks Cdk9-mediated phosphorylation. Would you expect lavopiridol to interfere with HIV transcription? Why or why not?

Flavopiridol blocks the ability of Cdk9 to phosphorylate RNA polymerase; thus you would expect lavopiridol to interfere with the conversion of RNA polymerase to the form required for productive HIV transcription. Although this outcome seems likely from the brief summary of the effects of Tat on HIV transcription given in the problem, there are other protein kinases in the transcription complex that might mediate the critical phosphorylation of RNA polymerase. Direct tests, however, have shown that lavopiridol effectively blocks Tat-activated transcription and interferes with HIV replication.

The yeast Gal4 transcription activator comprises two domains: a DNA-binding domain and an activation domain. The DNA-binding domain allows Gal4 to bind to appropriate DNA sequences located near genes that are required for metabolism of the sugar galactose. The activation domain binds to components of the transcriptional machinery (including RNA polymerase), attracting them to the promoter, so the regulated genes can be turned on. In the absence of Gal4, the galactose genes cannot be turned on. When Gal4 is expressed normally, the genes can be maximally activated. When Gal4 is massively overexpressed, however, the galactose genes are turned off. Why do you suppose that too much Gal4 squelches expression of the galactose genes?

In order for Gal4 to work properly, the DNA-bound Gal4 must recruit many proteins, including RNA polymerase, to the promoter. When there is too much Gal4 in the cell, the free and DNA-bound Gal4 will compete for the limited quantities of these other components. In the presence of excess Gal4, those components are tied up in unproductive complexes with free Gal4, thereby preventing their recruitment to the promoter. As might be expected, cells that massively overexpress Gal4 grow poorly because of the reduced availability of critical components of the transcription machinery.

How are histone modification enzymes and chromatin remodeling complexes recruited to unmodified chromatin, and how are they thought to aid in the activation of transcription from previously silent genes?

Histone modiication enzymes and chromatin remodeling complexes are recruited to specific regions of chromatin by transcription activators that can bind to DNA in unmodified chromatin. Once bound, histone modification enzymes can add groups to histone tails, altering their packing properties and

providing binding sites for additional speciic proteins. Similarly, chromatin remodeling complexes, once recruited, alter the local chromatin structure. This facilitates the binding of additional transcription activators that cannot bind to unmodiied chromatin. Together, these changes in histones and chromatin allow the transcription machinery to be assembled at specific promoters and to initiate transcription.

How is it that protein–protein interactions that are too weak to cause proteins to assemble in solution can nevertheless allow the same proteins to assemble into complexes on DNA?

In a sense, the DNA acts as a tether, holding the proteins in close proximity so that inherently weak interactions between them can occur readily.

Multiple Choice Questions

1. Which of the following statements are INCORRECT for transcription regulators:
    a. Transcription regulators bind to very long DNA sequences called cis-regulatory sequences
    b. Transcription regulators control the time and place of transcription by switching genes on and off
    c. Dimer formation increases specificity of DNA recognition
    d. Transcription regulators can only form homodimers (complex of 2 identical proteins)

A is incorrect as cis-regulatory sequences are short. D is also incorrect as transcription regulators can work as monomer, homodimer, heterodimer, etc.

2. DNA binding proteins recognize their target sequence by
    a. a complementary DNA sequence
    b. protein surfaces matching the shape of DNA
    c. a complementary RNA sequence
    d. a protein surface establishing interactions with a specific portion of DNA

D is correct. Transcription regulators are proteins, which can recognize specific regions of DNA based on hydrogen bonds, ionic binds, and hydrophobic interactions. Their surface properties match features of a specific DNA region perfectly.

3. Which statements are CORRECT about eukaryotic transcription control?
    a. The transcription control is identical to the processes in prokaryotes
    b. Cis-regulatory sequences can be distributed over a large DNA stretch, but on the same chromosome
    c. The gene control region is just the promoter

   d. Eukaryotic transcription regulators recruit the same RNA polymerase as in prokaryotes

B is correct. The process is generally more complex than in prokaryotes and the control region contains the promoter and regulatory DNA sequences. Prokaryotes and eukaryotes have different RNA polymerases, so the regulators will recruit different polymerases in eukaryotes and prokaryotes.

4. Activator proteins…
   a. …are transcription regulators
   b. …block gene transcription
   c. …are required to make DNA more accessible for transcription
   d. …are the same as enhancers

A and C are correct. Activators activate gene transcription. The activator is the protein responsible for transcriptional regulation by binding to DNA and recruiting the RNA polymerase. DNA sequences recognized by activators are called enhancers.

5. How can repressors prevent transcription?
   a. Competitive binding to regulatory DNA sequences
   b. Interacting with general transcription factors
   c. Masking activation domains of activators
   d. Remodeling chromatin

All answers are correct.

6. Which statements are CORRECT for the Gal4-UAS system?
   a. Gal4 gene encodes a transcription activator
   b. It can be used to study gene expression only in Drosophila
   c. UAS is an enhancer sequence
   d. It can be used to study gene expression in many eukaryotes

A, C and D are correct. The Gal4-UAS system is widely used in Drosophila, but in principle it can be used in any eukaryote as they all share the same RNA polymerase II for mRNA synthesis.

7. How can the activity of transcription regulators be modulated?
   a. Transcription regulators are not modulated. They modulate transcription
   b. By binding of ligands or proteins
   c. By covalent modifications such as phosphorylation
   d. By binding to inhibitory proteins

B, C and D are correct. The activity of transcription regulators can be modulated in many ways including binding of other proteins (inhibitory or activating), ligands, and covalent modifications.

8. What are evolutionary advantages of transcriptional control?
    a. It allows organisms to adapt their set of expressed genes to environmental stimuli
    b. Researchers can use the systems for their experiments
    c. It has no evolutionary advantage
    d. It enables development of complex multicellular organisms based on the same genome

A and D are correct. B is also true but it is not an evolutionary advantage. Transcriptional control enables organisms to adopt to environmental situations (e.g. araBAD to regulate the metabolism of E. coli depending on the presence of arabinose) and it allows to create multi-cellular organisms based on the same genome where each cell has a specialized task to form a functional, highly complex organism.

True and False

1. Any cell in an organism expresses the exact same set of genes because they all have an identical genome.

TRUE or **FALSE**

Cells only express a subset of their genome and the exact genes expressed depends on the cell type and time.

2. Transcriptional control refers to the modulation of protein synthesis from mRNA to regulate the expression profile of a cell.

TRUE or **FALSE**

Transcriptional control is a mechanism controlling the transcription (not translation).

3. Activator proteins block the binding of additional regulators

TRUE or **FALSE**

Activators promote the binding of additional regulatory proteins

4. Activator proteins recruit RNA polymerase to the gene which is supposed to be transcribed and release it to begin transcription

TRUE or FALSE

5. Absence of insulator DNA sequences can lead to activation of inappropriate genes

TRUE or FALSE

Insulator DNA sequences prevent cis-regulatory elements to activate the expression of inappropriate genes. If insulator sequences are missing, cis-regulatory elements may activate the expression of the wrong gene.

6. The tryptophane operon uses transcriptional activators to activate gene expression if the tryptophane concentration is low.

TRUE or **FALSE**

The tryptophane operon uses a transcriptional repressor to control the expression

7. There is a single activator or repressor for each cell type in the organism

TRUE or **FALSE**

Cell types are the result of different combinatorial gene control. The gene expression pattern for each cell type is controlled by a combination of transcriptional regulators.

8. Transcription factors determine the cell type and can even be used to reprogram differentiated cells into pluripotent stem cells.

TRUE or FALSE