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TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION

REVIEW THE CONCEPTS

1. RNA polymerase I is responsible for transcribing 18S and 28S rRNA genes. RNA polymerase II is responsible for mRNA transcription. RNA polymerase III transcribes tRNAs, 5S rRNAs, and several other small RNAs. Since RNA polymerase II is uniquely inhibited by a low concentration of α-amanitin, one can determine if any gene requires this polymerase by measuring gene transcription in the presence and absence of this compound. If RNA polymerase II is responsible for transcribing a gene, then transcription should only occur in the absence of α-amanitin.

2. The CTD becomes phosphorylated by a subunit of TFIIH during transcriptional initiation. The CTD is then further phosphorylated by cyclin T-CDK9. CTD phosphates are removed when RNA polymerase II terminates transcription.

3. TATA boxes, initiators, and CpG islands are all promoter elements. The TATA box was the first to be identified because it is found in the promoters of most genes expressed at high level. These were the first genes to be subjected to in vitro transcription. Since the TATA box occurs at a fixed location relative to the transcription start site (~30 bases upstream), it was relatively easy to recognize in the DNA sequence.

4. To identify DNA-control elements within promoter-proximal regions, investigators utilize linker scanning mutagenesis. A set of constructs with contiguous overlapping mutations are assayed for their effect on the expression of a reporter gene or production of a specific mRNA in cultured cells or a transgenic animal. To further pinpoint the DNA-control element, point mutations are then introduced.

5. Promoter-proximal elements are located within ~200 base pairs of the transcription start site. Enhancers are located at greater distances, either upstream or downstream of the transcription start site. Both continue to activate transcription when they are inverted and both can be cell-type specific. Both are composed of several functional sequences.

6. Once a putative control region is identified, DNA footprinting with a nuclear extract can identify the precise DNA sequence that is bound by a protein in the extract. This assay depends on the ability of protein factors to "protect" DNA from DNaseI digestion. The electrophoretic mobility shift assay can also be used to determine whether proteins in a cell extract bind specifically to a DNA sequence within the probe used. In this assay, an extract is incubated with a labeled fragment of DNA. If protein binds to the DNA, then it "shifts" in terms of its migration on a polyacrylamide gel. This technique is often used as an assay to purify DNA-binding proteins.

7. Transcriptional activators and repressors contain a modular structure in which one or more transcriptional activation or repression domains are connected to a sequence-specific DNA-binding domain, usually through a flexible domain.

8. The location of the sequence on the chromosome may influence whether it is expressed; for example, if the sequence is adjacent to a telomere, it will be in a silent (heterochromatin) locus and will therefore not be transcribed due to condensation of the chromatin at that site. Second, DNA/histone methylation or deacetylation can lead to chromatin condensation and prevent transcription from that site.

9. CREB binding to its co-activator (CBP) is regulated by cAMP, which stimulates phosphorylation of CREB. The phosphorylated acidic activation domain within CREB is a random coil in the absence of CBP. However, in the presence of CBP the phosphorylated activation domain undergoes a conformational change to form two a helices that wrap around a larger globular domain of the co-activator. In contrast, nuclear receptors contain a larger activation domain that is regulated by the binding of a hydrophobic ligand. When ligand binds to these domains, they undergo a conformational change that generates a groove in the globular activation domain that binds a short a helix in a coactivator. Thus, while the phospho-CREB activation is a relatively short random coil until it interacts with a larger globular domain in a coactivator, nuclear receptors have a large, folded, inactive activation domain that undergoes a conformational change allowing it to bind a short a helix in a co-activator.

10. The first protein to bind to a RNA-polymerase II promoter is the TATA box–binding protein (TBP), a subunit of TFIID. This protein folds into a saddle-like structure that binds to the minor groove of DNA near the TATA box and bends the DNA. TFIIB then binds and makes contact with both TBP and DNA on either side of the TATA box. TFIIF and Pol II bind and Pol II is positioned over the start site. TFIIE and TFIIH bind and a helicase activity unwinds the DNA generating an "open" complex with the template strand in the active site of the polymerase.

11. Integration of gene *X* near the telomere is not ideal for good expression of gene *X*. Telomeres are usually contained in heterochromatin, which is tightly packed and less accessible for the transcriptional machinery. If the yeast line used for expression contained mutations in the *H3* and *H4* histone genes, the outcome could be different, depending on the specifi c mutations. For example, if the DNA sequence encoding lysines in the histone N-termini were mutated so that glycine residues were substituted in their place, then repression of gene *X* would not take place. Repression would not take place in such a mutant because the gly-cine residues are not positively charged, similar to acetylated lysine residues. This prevents binding by SIR3 and SIR4, preventing the formation of heterochro-matin. The resulting "open" chromatin structure at the telomere would facilitate RNA polymerase II and general transcription-factor binding, allowing gene expression.

12. A good prediction is that STICKY functions as a transcriptional repressor. Repressors contain two domains, one that binds DNA and a second that represses transcription. The bHLH domain is a DNA-binding domain that has been found in many different transcription factors. The Sin3-interacting domain is likely to associate with a Sin3 containing histone deacetylase complex. This complex can repress transcription because deacetylation of histones promotes a more closed chromatin conformation.

13. Enhancers and promoter-proximal elements.

14. Use recombinant DNA technology to make constructs with short fragments of gene X (including upstream and downstream sequences) adjacent to a reporter gene such as green fluorescent protein (GFP). These constructs can be used in cell lines or to make transgenic organisms that can then be analyzed for GFP expression to identify the sequences capable of activating reporter gene expression.

15. The RNA-binding protein Tat binds to the RNA copy of a sequence called TAR, which then binds cyclin T. Consequently, the CDK9-cyclin T complex becomes activated, phosphorylates its substrates, and facilitates polymerase-mediated transcription elongation. In the absence of Tat, the transcripts are terminated prematurely. Antibodies against Tat would therefore prevent the infected cells from transcribing the viral genome because Tat would be unable to bind the viral RNA.

16. No—low/medium level transcripts such as housekeeping genes often have a high frequency of CG sequences.

17. **Leucine zipper proteins:** contain the hydrophobic amino acid leucine at every seventh position in the sequence; bind to DNA as dimers.
bHLH proteins: contain an N-terminal α helix with basic residues that interact with DNA; bind to DNA as dimers.
Homeodomain proteins: conserved 60-residue DNA-binding motif similar to the helix-turn-helix motif of bacterial repressors; bind to DNA at positively charged (basic) residues that interact with phosphates in the DNA backbone as well as residues that interact with specifi c bases in the major groove of DNA.
Zinc-finger proteins: 23- to 26-residue consensus sequence providing proteins with a region that folds around a central Zn^{2+} ion; found in DNA and non-DNA–binding proteins.