

**BMB400**  
**Part Four: Gene Regulation**  
**A Summary**

**Themes in mechanisms regulating the level of gene transcription**

**1.** Changes in chromatin structure can make the DNA template more accessible for assembly of the transcription preinitiation complex.

- a. Examples include the  $\beta$ -globin gene clusters in chickens and mammals. A locus control region (LCR) is a distal regulatory sequence that is needed for opening of the chromatin domain and for high level expression of the genes. Both LCRs and some enhancers can work by increasing the probability that a gene is in a permissive environment for transcription (putative accessible chromatin).
- b. Much evidence has implicated enzymes such as histone acetyl transferases (HATs, e.g. protein complexes containing Gcn5p + Ada2 in yeast, PCAF + P300/CBP in mammals) and nucleosome remodeling ATPases (e.g. yeast SWI/SNF and its homologs and analogs) in altering the chromatin so that the template is accessible. These are one type of *co-activators* of transcription, and can also be called adaptors and mediators.
- c. Particular *trans*-activator proteins can be used to recruit activities such as HATs and remodeling enzymes to particular loci.
- d. One might expect chromatin-based mechanisms to be seen not only in eukaryotic organisms but also in archaeobacteria.

**2.** Some negative regulatory proteins can block access of the transcriptional machinery to promoters.

- a. Binding of the yeast  $\alpha 2$  repressor to its operator (binding site in DNA) will position a nucleosome over the TATA box of the promoter, thus blocking access.
- b. An extensive complex of proteins is recruited to the silent mating-type loci in yeast and prevent expression of these genes by keeping them in an inaccessible chromatin. Models postulate that these SIR proteins build a cage around the nucleosomal chromatin, keeping it inaccessible.
- c. The bacteriophage  $\lambda$  has a repressor and another negative regulator, called Cro, that bind to specific sites (their operators) in the DNA. When bound to these sites, they block access of the RNA polymerase to the -10 and -35 boxes of the target promoters. The operator and promoter overlap in this case.

**3.** Other negative regulatory proteins can still allow RNA polymerase to bind to the promoter to form a closed complex, but they restrict the activity of the polymerase so that it does not initiate productive transcription.

An example is the *lac* repressor bound to its operator. The operator does not overlap the promoter, so both proteins can bind. However, the close proximity to the repressor prevents isomerization of the DNA-polymerase complex to the open complex.

4. Some positive regulatory proteins (*trans*-activators) can increase the rate of transcription initiation by directly contacting the RNA polymerase and recruiting it to bind more avidly to the promoter.

- a. An example from eubacteria (*E. coli*) is the CAP protein, which in the presence of cAMP will bind to a site centered at 62 bp upstream from the initiation site (-62) of the *lac* operon. When bound, the protein contacts the  $\alpha$  subunits of RNA polymerase and increases the rate of transcription from the promoter. In other operons, cAMP-CAP binds to a site centered at -42 and again increases the rate of transcription. Note that the same regulatory protein (cAMP-CAP) can make different contacts with RNA polymerase at different operons and activate transcription by different mechanisms, affecting the affinity of the polymerase for the promoter in one case and the rate of closed to open complex in the other.
- b. Another example is the repressor from bacteriophage  $\lambda$ , which infects *E. coli*. Its operator not only overlaps the promoter for the gene that it negatively regulates (see above), but it is adjacent to a promoter oriented in the opposite direction. It can stimulate transcription from this second promoter by direct contact with the RNA polymerase. The  $\lambda$  CII protein stimulates transcription from the otherwise weak promoters *PRE* and *Pint*.
- c. In eukaryotes, there are several studies showing that the activation domains of some *trans*-activators (e.g. GAL4-VP16) can bind to components of the basal transcriptional machinery, in particular some of the TAFs that are associated with TBP in the general transcription factor TFIID. This may be a mechanism analogous to the CAP-RNA polymerase interaction for recruitment of the transcriptional machinery to the promoter. It may act in concert with the effects of *trans*-activators in establishing an open chromatin domain.

5. Transcriptional regulators can change the conformation of the DNA, e.g. bending it, and thus allowing activation or repression. Examples include the action of cAMP-CAP at a MalT protein-DNA complex and HMG(I)Y in mammals.

6. Although regulation of transcription initiation is a primary level of control in many genetic systems, all subsequent steps in the pathway to gene expression are subject to regulation.

- a. Examples of regulation at transcription elongation include the *trp* operon in *E. coli*, in which the extent of translation of a leader peptide determines whether or not a  $\rho$ -independent terminator of transcription is used. An example from mammals is the HIV virus, in which a Tat protein acting at a TAR element close to the 5' end of the mRNA will determine the efficiency of elongation past this sequence.
- b. Anti-termination at  $\rho$ -dependent sites in  $\lambda$  regulates the expression of genes whose products allow progress through the lytic cycle. [pp. 498-508; problem 4.16.]
- c. Examples of regulation of splicing abound in any system with alternative splicing. Particular splice enhancers and the proteins that interact with these segments of exonic RNA are intensively studied in transcripts of sex-determination genes in *Drosophila*. [Part Three of the course.]
- d. Further regulation can be achieved during export of RNA from the nucleus, translation, post-translational modification, and degradation of both the mRNA and the polypeptide. These are important steps, but beyond the scope of this course.