**Differences between prokaryotes and eukaryotes:**
In eukaryotes, as in prokaryotes, the initiation of transcription is a major regulation point for gene expression. However, at least three general themes encountered in eukaryotic gene regulation distinguish it from the prokaryotic regulation we have already discussed.

1). The activation of transcription is associated with multiple changes in the structure of the chromatin in the region being transcribed.

2). Although both positive and negative regulatory elements exist, positive regulatory mechanisms predominate in the systems characterized to date.

3). Finally, the physical separation of transcription, which occurs in the nucleus, and translation, which occurs in the cytoplasm, means that certain forms of regulation found in prokaryotes are not possible in eukaryotes (mechanisms such as attenuation).
Eukaryotic Promoters are Positively regulated:

In general, eukaryotic RNA polymerases have little or no intrinsic affinity for their promoters. Initiation of transcription is almost always dependent on the action of one or, more often, several regulatory (activator) proteins. The extensive use of positive regulatory mechanisms is probably a consequence of the larger size of the eukaryotic genome.

There are at least two possible reasons for the many examples of positive regulation:

1). **Nonspecific DNA binding of regulatory proteins becomes a greater problem with a large genome.** The chance that a specific binding site will occur randomly at an inappropriate site also increases. One way to improve specificity is to use multiple regulatory proteins. Then, the probability of randomly binding the correct set of regulatory proteins is very small. Using several negative regulators will generally not improve specificity, because binding of one is sufficient to adequately block RNA polymerase action. Specificity can be improved, however, by requiring the binding of several positive regulatory proteins to a variety of specific DNA sequences and then forming a complex that activates transcription. The average number of regulatory sites for a gene in a multicellular eukaryote is probably at least 5.
2). **Positive regulatory mechanisms are also more efficient in a large genome.** If the 100,000 genes in humans were negatively regulated, each cell would have to synthesize 100,000 different suppressors in sufficient concentration to permit specific binding of each. In positive selection, most of the genes are normally inactive (RNA polymerases do not bind the promoters) and the cell only has to synthesize a select group of activator proteins needed to activate transcription of the small subset of genes required in that cell.
Eukaryotic cells have three different RNA polymerases that are specific (to a first approximation) for different classes of RNA. **RNA polymerase II** is used to transcribe mRNA. Two types of generalized RNA polymerase II promoters and regulatory regions are diagrammed above.

**TATA Box:** (consensus sequence TATAAAA) TATA boxes are located 25-30 base pairs from the mRNA initiation site. These sequences act as binding sites for a transcription factor called TFIID that is required for RNA polymerase binding.

**GC boxes** (consensus sequence GGGCGG) and **CCAAT boxes** (consensus GCCAAT) are short sequence elements that are found within several hundred base pairs of the transcription start site. Additional regulatory sequence elements are more complex and are termed **enhancers**. Enhancers can act at a distance of several thousand base pairs and their orientation and location to the transcription start site is relatively unimportant. Each of these sequence elements is recognized and bound by one or more regulatory proteins (transcription factors). Regulation involves protein-protein interactions between different regulatory proteins and the RNA polymerase. Because these DNA binding sites are often at great distances from each other and the transcription start site, the protein-protein contacts often require extensive DNA looping.
Examples of Translational Regulation in Eukaryotic Cells:

Example 1. Regulation of Translation Initiation Rate by a 5' Hairpin structure in the Untranslated Region of a mRNA molecule.

A Key difference between translation initiation in Eukaryotes vs. Prokaryotes:

Prokaryotes: Identify start (AUG) codons using the Shine-Dalgarno sequence: 16S rRNA interaction. Bind directly to the internal start codon region of mRNA.

Eukaryotes: 40S Ribosome subunit binds to the 5' Cap of mRNA and then scans down mRNA until it finds the first AUG codon. Therefore, the Translation initiation Rate for eukaryotic mRNAs can be affected by the sequence of the 5' untranslated region of the mRNA.

How does this work?

If the sequence of the 5' untranslated region of an mRNA forms a hairpin structure, the 40S ribosome will have a difficult time scanning through this sequence and tends to fall off. This would reduce the translation initiation rate for this particular mRNA relative to an mRNA that did not have a hairpin structure in its 5' untranslated region.

Example 2. Synthesis of Hemoglobin in Red Blood Cells

Hemoglobin is a protein that carries oxygen from lungs to the cells of other tissues. Red blood cells are quite specialized; they have no nuclei but have a large reserve of mRNA molecules. Their major function is to synthesize globin.

Heme Group at the center of hemoglobin has an iron atom that serves to coordinate molecular oxygen. Hemoglobin needs the heme group to be functional, without heme there is no point in making hemoglobin (it would not be functional).
Cells have a way of making hemoglobin protein only when sufficient heme groups are present.

A protein called the **Heme-Controlled Inhibitor, or "HCl"** a protein kinase (adds phosphate group from ATP to specific amino acid side chains) acts to regulate hemoglobin synthesis.

HCl phosphorylates and inactivates **eIF-2 (Eukaryotic Initiation factor-2)** same function as bacterial eIF-2).

When eIF-2 is inactivated, translation can't initiate and **No globin protein is made**.

**How is this controlled by levels of heme present in cells?**

Heme causes the HCl to be converted to an inactive state. In its inactive state, HCl can't phosphorylate eIF-2.

Translation can initiate, globin protein is made.

![Diagram of eIF-2 and GTP/GDP interactions]
Summary:

When Heme groups are abundant, HCl is inactive, eIF-2 is active, translation initiates and the globin protein is produced and associates with heme groups to form functional hemoglobin.

When Heme groups are scarce, HCl is active, eIF-2 inactive, translation can't initiate so no globin protein is produced. This makes sense because when there are no heme groups to associate with, globin is not able to form functional hemoglobin.