Biochemistry 401 Lecture 38
Today we're going to talk about transcriptional regulation and we're going to compare and contrast regulation in *E. coli* and in eukaryotes. We'll talk about the *cis* and *trans* transacting elements, and classes of transcription factors and to do this we're going to use to model systems. For prokaryotes we're going to look at *E. coli* and for eukaryotes we're going to look at the human model system. So let's get started.

We'll start with an overview of the regulation of transcription. Now the expression of genes refers to how a specific gene is transcribed to yield RNA. Those genes that are highly expressed are highly transcribed. In many cases this RNA is translated to protein. The expression of genes is temperospatially regulated, and what this means is, that genes are regulated differently at different times in different spaces, so the needs of the organism changes with developmental state. As an organism grows their needs change. Organisms must also be able to respond to different environmental cues, by up-regulating or down-regulating genes to more effectively utilize those things that are available in the environment. Additionally, genes are differentially regulated according to tissue type, and cell type, and it is these differences in gene regulation that allows cells to differentiate and to function properly.

Not the regulation of transcription happens differently in prokaryotic systems and eukaryotic systems. This is due to differences in genome organization, in RNA polymerase, and in differences in the organisms themselves. Eukaryotes have a very complex chromosomal structure, in which the DNA is tightly packed, first into nucleosomes and then into solenoids. One of the jobs of the transcriptional machinery is to get into the target DNA, but it has a hard time doing that if the DNA so highly packed, and so a lot of transcriptional regulation in eukaryotes is just providing accessibility to the transcriptional machinery. Now with gene organization, that's different in eukaryotes and prokaryotes as well. Prokaryotes try to maximize efficiency. It's one cell that has to respond to a changing environment rapidly and efficiently, and so in many cases genes that operate within the same system, for instance the utilization of a specific food source, are organized in a specific stretch of DNA that's turned on all at the same time. So that the genes necessary for that particular pathway are all turned on concurrently. There are also differences in RNA polymerase, in how the polymerase is targeted, and in the structure of the RNA polymerase itself. Now

when we looked at RNA polymerase in prokaryotes we saw that it is a heterotetramer it is made up of two alpha subunits a beta, a beta prime, and then a sigma factor. The sigma factor actually targets the RNA polymerase to the site of transcription, so it is the polymerase itself that helps to target the promoter, But with RNA polymerase in eukaryotes there are several proteins that are involved in targeting the RNA polymerase to the start site of transcription, and so the targeting does not come from the polymerase itself but rather comes from a whole host of accessory proteins that help localize the polymerase to the start site of transcription. There are also differences in the organism itself. Prokaryotes must respond efficiently and effectively to changes in the environment, and they must be able to utilize things from their environment efficiently so that food sources and energy sources are not wasted. They also might find themselves in a particularly inhospitable environment and have to regulate gene transcription based on that. Now with eukaryotes, and especially higher eukaryotes, there are specialized cells that carry out specialized functions, and so it's necessary to have specialized gene transcription in those particular cells. These cells must be able to respond to changes in the environment, as well. Another major effect is that prokaryotes to not have nuclear membrane. Transcription and translation can happen concurrently, and it's more difficult to separate transcription factors from the DNA itself, whereas in eukaryotes, because there's nuclear envelope, it's easy to be able to exclude transcription factors from the nucleus, and thus help to regulate transcription in that way. Also RNAs that are made in the nucleus must be transported out of the nucleus for translation.

These figures are from a paper by Gary Struhl on the left we see panels that look at the differences between chromatin organization in prokaryotes and eukaryotes Now with prokaryotes, the chromatin itself is generally not restrictive, and so by removing a repressor, the RNA polymerase can have access to the chromatin. And so how well that particular piece of chromatin is used depends on activators that can help to bind the polymerase to the site of transcription, or it also can depend on the activity of the promoter itself, on how close to the consensus sequence that promoter is, and again the polymerase is targeted by the sigma factor. Eukaryotes have a significantly more difficult time. Their repressed state or the silent state can include repression by accessory proteins, and also by chromatin modifying activity. The silencing of genes involves the repressors that recruit chromatin modifying proteins, that through modification of histone tails, change how histones interact with the DNA, and with each other. This can

effectively close down the gene, preventing the RNA polymerase from getting to the target. Once the repressor and the chromatin modifying protein are gone, the gene is still in the restrictive state, and this is because the chromatin is tightly wound around histones. What needs to happen is that activators, which recruit chromatin modifiers modify the histone tails in the nucleosomes surrounding the gene's promoter, and thus allow the polymerase (access) - and at this stage the gene is at what's called a poised state - the promoter itself is accessible, but the transcriptional factors have not bound to the site of transcription, yet. Those proteins that help to target the polymerase itself to the start site of transcription can further activate these genes. So with eukaryotes, the ground state is still restrictive. The DNA is wound around nucleosomes. we can further make this even more restrictive by modifying the histones to make them more compact and more difficult to unwind this is caused by modifying the histone tails and also by having proteins that bind to the chromosome in this state. In the panel to the right we see differing stages of chromatin accessibility. In the first stage we have an activator A that is going to recruit chromatin-modifying activity. These are enzymes that modify histone tails. Now in so doing, these modified regions of the DNA can now act to recruit more chromosome modifiers, and so in this way we can have a localized remodeling of the chromatin to allow greater accessibility to the gene itself and to the promoter region. Finally an activator B attracts TFIID and other proteins involved in targeting the polymerase to the start site of transcription, and so it each one of these steps, one through six, there is just an interplay of different proteins in different regions of the DNA, and so by modifying what proteins are available, and what reaches of the DNA are accessible, you can have a really tight control on which genes are expressed, and at what level those genes are expressed.

So let's talk a little more specifically about the steps. Now we're looking at scenarios like we saw in the right-hand panel, what we're looking at is the interplay between *cis* factors and *trans* factors. *Cis* factors are actual DNA sequences. These may be promoters, or enhancers and silencers. A silencer is a region of the DNA, to which the repressor will bind, to down regulate transcription from that gene. Silencer and enhancer elements are *cis* regulatory elements that are outside of the promoter region. These may be several base pairs, several hundred base pairs, or thousands of base pairs away from the start side of transcription. These regions of DNA are sites for transcription factor binding. Now these transcription factors themselves are *trans* factors. These are proteins that