

Ch 17 control of gene regulation in eukaryotes



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What are the differences between prokaryotic and eukaryotic gene regulation? Prokaryotes: 1. bacteria grows in competitive rapid conditions 2. simple needs, and single celled 3. don't have cell specific gene expression 4. have to respond quickly to changes in their environment 5. organized in operons and transcribed by a single mRNA 6. polycistronic mRNA 7. transcription and translation can occur at the same time

Eukaryotes: 1. have other regulatory needs so they have a different set of regulatory controls 2. are NOT regulated by operons 3. each structural gene has its own promoter and is transcribed independently 4. monocistronic 5. chromatin structure affects gene expression 6. transcription requires unwinding of the DNA from the histone proteins 7. eukaryotic cells have many more transcriptional activators as well as gene repressors (DNA binding proteins) 8. transcription and translation are physically separated so the regulatory mechanisms must differ from those in bacteria

What are 2 major differences between prokaryotic and eukaryotic gene regulation? Eukaryotic genes are NOT regulated by operons.

In eukaryotes transcription and translation are separated by the nuclear membrane

What do prokaryotes and eukaryotes have in common in terms of gene regulation? Both have DNA binding proteins What is another term for DNA binding proteins? transcriptional activators and gene repressors monocistronic mRNA each mRNA codes for a single polypeptide; therefore each gene codes for a single polypeptide polycistronic mRNA the mRNA codes for two or more polypeptides; only found in prokaryotes What is

a cistron? a gene How does chromatin structure affect gene regulation? DNA tightly wound around the nucleosomes tends to repress transcription.

DNA is associated with the histones and its hold must be released "loosened" in order to have transcription

Efficient transcription requires changes in chromatin structure to make the DNA more accessible to transcription factors, activator proteins, and RNA polymerase

What is DNase I? it is used as a tool to "probe" chromatin structure it is not a sequence specific enzyme DNA that is tightly packed or would be resistant to it. it is an enzyme that cleaves the DNA if it has access to it How does DNase I affect gene regulation? DNA tightly wound around nucleosomes will be resistant to the nuclease since the DNA is protected by the histone proteins

changes in chromatin that make the DNA less tightly associated with the nucleosomes will be seen as an increased sensitivity to degradation by a nuclease

hypersensitive DNA that is most accessible to the enzyme What DNA is hypersensitive to cleavage by DNase I? DNA located ~1000 bp upstream of the promoter of transcriptionally active What are nucleosomes composed of? 8 histone proteins What are histones? basic proteins that are positively charged at the physiological pH and bind with the negative charge of the DNA. Thus, holding the DNA tightly together on the nucleosomes. What is acetylation and what does it do in the case of histones? It is the process of

adding an acetyl group. It neutralizes the positive charge on the tails of the histones causing the DNA to become more loosely associated with it. (DNA becomes less tightly packed) How do histones attach to the DNA? Histones have positively charged tails that interact with the negatively charged phosphates of the DNA. Describe the process of histone acetylation? Acetyl group can be transferred to the lysine and arginine amino acids that have the positive charge by acetyl transferases

neutralizes the positive charge so the DNA is more loosely associated with the histone

allowing some transcription factors to bind to the DNA

chromatin remodeling proteins* a range of proteins whose function is to change the conformation or organization of the chromatin* some transcription factors or other regulatory proteins alter chromatin structures without affecting histones directly* in some cases the actual positions of nucleosomes get moved relative to where they were on the DNA. What is the goal of chromatin remodeling? GOAL: if the genes are going to be transcribed they must be in a more euchromatic form / configuration. What is chromatin remodeling? Nucleosomes that are repositioned to allow binding and initiation of transcription. methyl group CH_3 group. Where will DNA methylation occur as a mechanism for affecting transcription? It will almost always occur on a cytosine residue at position 5. Heavily methylated DNA is associated with _____? repression of transcription; the genes are not expressed. Unmethylated DNA is associated with _____? DNA that is available for transcription; genes that are expressed. DNA Methylation usually

occurs in _____? CpG islands
 What are CpG Islands?* this means that a methylated C is usually followed by a G* methylated C= CG sequences* generally found in heterochromatic areas* p represents the phosphodiester bond* inactive genes have heavily methylated CpG

(there are methylated cytosine on opposite strands)

What is the purpose of CpG islands? to block transcription
 Where do you find heavy methylation of CpG? With fully repressed genes such as those on the inactive X-chromosome of mammalian females. (ex: insulin promoter in brain tissue)
 What is epigenetics? changes in chromatin by NOT changes in the DNA sequence that are passed on to future generations (affect the germ line)
 What is the molecular mechanism for DNA methylation and epigenetics?
 * changes in the chromatin must be stable through chromosome replication if the epigenetic effect is inheritable* key mechanism appears to be DNA methylation (CpG islands)
 How is CpG methylation used in epigenetics?* CpG methylation means that there are methylated cytosine on opposite strands* after replication the old strand is methylated resulting in hemimethylation* methyltransferase methylates adds -CH₃ to unmethylated "new" strand* siRNA, miRNA, and piRNA also play a role in some cases
 When does DNA methylation occur? after replication
 Describe the overall process of DNA methylation.
 1. before replication DNA is fully methylated at CpG dinucleotides
 2. during replication the new strands are synthesized without methyl groups
 3. after replication each new DNA molecule will have methylation on one strand but not the other; hemimethylated
 4. methyl groups attract methyltransferase enzymes, which add methyl groups to the unmethylated strand
 5. resulting in fully methylated DNA
 What is

hemimethylation? When only one strand of DNA is methylated. Occurs in a very short window of time. What is the epigenome? An individual's unique chromatin modifications.

* it can be passed on to daughter cells during mitosis* it can be maintained through DNA replication* it can affect which genes are active in an individual

What is the basal transcription apparatus? General transcription factors assemble and bind to the core promoter that is located immediately upstream of the gene. Transcriptional activator proteins enhance the rate of transcription by stimulating (or stabilizing) the basal transcription apparatus. What are the 2 distinct functions of the transcriptional activator proteins? 1. One domain of the proteins binds to specific DNA sequence. 2. Another domain can interact with a different component of the transcriptional apparatus to enhance the rate of transcription.

* Requires a coactivator.!! Transcription will not happen until the transcriptional activator proteins are in place. !!

What is a coactivator? A protein that can serve to link the transcription factor to another component of the transcription machinery. Transcriptional activators usually bind to either: * a regulatory promoter that is upstream of the core promoter * an enhancer which may be located several thousand bases from the promoter * (sometimes a sequence can fall in between the 2 categories) What is a regulatory promoter? * located upstream of the core promoter * must be close to the core promoter * strict requirements on positioning What is an enhancer? * function the same as a regulatory

promoter* may be located 1000's of base pairs away
What is Gal4? an example of a transcriptional activator protein

regulates transcription of several yeast genes involved in galactose metabolism

(widely used in molecular biology as a research tool since most other eukaryotes do not have a homologous transcription factor)

UASg for Upstream Activator Sequence
The DNA binding site for the GAL₄ protein
What does the GAL₄ protein do? Binding activates transcription
What is GAL₈₀? It regulates GAL₄ activity. In the absence of galactose, GAL₈₀.... binds to GAL₄, which prevents binding to UASg so transcription cannot occur. In the presence of galactose, GAL₈₀.... binds galactose

allosteric change prevents binding of the GAL₈₀-galactose complex to GAL₄, GAL₄ is free to bind to the UASg and transcription initiates

Summarize GAL₄ regulation:
* Galactose is added to the system. (affecter molecule)
* UASg is the upstream activator sequence.
* GAL₄ binds to it and activates transcription.
* GAL₈₀ binds when galactose is not present to repress transcription

Describe the characteristics and functions of an enhancer.*

Enhancers can affect transcription even when they are located at a

considerable distance from the target gene* In general, activator protein

binds to the enhancer* The enhancer " loops out" to bring the activator

protein closer to the basal transcription apparatus.* most enhancers could

affect any gene within their proximity. What is the goal of enhancers and

insulators? To avoid cross talk across genes. What is an insulator? (also

called boundary elements) DNA sequences that function to limit (or isolate) the activity of an enhancer.

Provide a physical barrier to keep the enhancers from enhancing the wrong gene.

What is coordinated gene regulation? some genes need to be expressed in a coordinated manner, but eukaryotic genes are not organized in operons (ex: heat shock response requires a coordinated response of ~20 genes) What is a consensus sequence? a DNA binding sequence that is the same response element A common regulatory DNA sequence shared by genes that respond to a specific condition in the cell (ex: heat shock)

Usually have a short consensus sequence.

(One gene can be activated by a variety of stimuli and have several different response elements)

What are the common features of eukaryotic transcriptional control? * a single gene can be activated by several different response elements * multiple response elements allow a single gene to be activated by different stimuli * presence of the same response element on different genes allows coordinated expression of a set of genes in response to a common stimulus What are 3 methods of eukaryotic transcriptional control? mRNA Processing RNA Stability RNA Silencing What is mRNA processing? Alternative splicing of the pre-mRNA to give different variants of the mRNA (causes the exons to splice together in different ways.) What is Sxl (sex-lethal) ? An RNA binding protein that is synthesized in female Drosophila cells.

It binds to the tra (transformer) pre-mRNA and affects splicing.

What is tra (transformer) mRNA? a protein that is coded for in both sexes of *Drosophila* but there is a difference in the splicing of the pre-mRNA

it is required for female development; males have a nonfunctional form of this protein

(males don't have a functional tra protein not because the gene wasn't transcribed but because it was spiced in a way that lead to a non-functional protein.

How is Sxl used as a transcriptional control in eukaryotes?* In the absence of sxl protein (males), splicing of the tra mRNA occurs at a different place.* In females, the sxl protein binds to the pre-mRNA to prevent splicing. The result is splicing at an alternative site.* The alternative splicing in females leads to a tra mRNA that codes for a functional protein. How does eukaryotic RNA stability influence eukaryotic transcription?* eukaryotic mRNA is, in general, more long lived than prokaryotic mRNA* some eukaryotic mRNAs are stable for hours, days or even months* can result in very large differences in the amount of protein made* (ex: red blood cells)Where does degradation begin on RNA? a mechanism for silencing gene expression by targeting the RNA for early degradation rather than allowing it to degrade at its own rate

(possibly as many as 30% of genes utilize RNA silencing)

What is translational control?* processes that occur during protein synthesis or after the protein has been synthesized* there are factors that can bind the mRNA in the 5' end UTR (untranslated region), that can affect the rate of

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translation* rare but, some factors bind to the 3' UTR to affect

translationWhat is post-translational control?* many proteins are initially made in an inactive state

* activation requires addition processes such as removal of some amino acids, as well as chemical modification (methylation, acetylation, phosphorylation etc)