Control of Prokaryotic and Eukaryotic Genome

	Regulation	Only Eukaryotes
Genomic Level	Gene Amplification	Gene amplification allows the **upregulation** of gene expression by **increasing the copy number** of a **specific gene** in order to meet the higher demand for the gene product which **cannot be met** by transcription and translation of a **single gene**. In the ***Xenopus* oocyte**, the **rRNA gene cluster** undergoes gene amplification. The chromosome gives rise to **extrachromosomal circular DNA** carrying the rRNA gene cluster. From this ring, many **more copies** of circular DNA are synthesized via the **rolling circle mechanism** where a **nick** occurs in **one strand** of the circular DNA, and **DNA polymerase** uses free **3' end** to synthesize a new strand of DNA, **displacing** the **5' end** is displaced. **Another nick** is made to **release** the displaced strand that **recircularizes** and acts as a **template** to synthesize a **complementary strand**. Gene amplification allows more **rRNA** to be **transcribed** and more **ribosomes** to be formed, allowing for much more **proteins** to be synthesized **after fertilization** for **rapid growth** of the embryo. In the **follicle cells** of ***Drosophilla*** ovaries, the **chorion gene cluster** undergoes gene amplification. **Multiple replications** of a small region of the chromosome containing the chorion gene cluster occurs as replication is **initiated and terminated randomly** within the chorion gene cluster, giving rise to **replication bubbles nested within larger replication bubbles**. Gene amplification meets the demand for high levels of **chorion mRNA** and hence **chorion protein** which **envelopes and protects** the zygote.
	Histone Acetylation and Deacetylation	The addition of an **acetyl group** on **lysine** residues of **histones** by **histone acetylase removes** the **positive charge** of histones, reducing the **electrostatic attraction** between the **positively charged histones** and the **negatively charged DNA**. Hence, DNA is **less tightly wound** around histones and the **promoter** is **more accessible** to **general transcription factors** and **RNA polymerase** to form the **transcription initiation complex**, hence increasing the **rate of transcription**. The removal of an **acetyl group** on **lysine** residues of **histones** by **histone deacetylase restores** the **positive charge** of histones, increasing the **electrostatic attraction** between the **positively charged histones** and the **negatively charged DNA**. Hence, DNA is **more tightly wound** around histones and the **promoter** is **less accessible** to **general transcription factors** and **RNA polymerase** to form the **transcription initiation complex**, hence decreasing the **rate of transcription**.

	Regulation	Only Eukaryotes
Genomic Level	Chromatin Remodeling Complex	Chromatin remodeling complexes alter the **structure of nucleosomes temporarily** and change the **accessibility** of the **promoter** to **RNA polymerase** and **general transcription factors** by causing DNA to be more or less **tightly coiled** around **histones**. This decreases or increases the **rate of transcription** respectively.
	DNA methylation	The addition of a **methyl group** to selected **cytosine** nucleotides in the sequence **CG blocks** the binding of **general transcription factors** to the **promoters** and hence preventing the formation of the **transcription initiation complex**. It also **recruits DNA-binding proteins** such as histone deacetylase to **condense chromatin**. In doing so, it **prevents transcription**.
	Organization of DNA	Heterochromatin is **highly compacted** DNA that winds more **tightly** around **histones**, limiting the access of **RNA polymerase** and **general transcription factors** to the **promoters** of genes, preventing the **transcription initiation complex** from forming and hence is **transcriptionally inactive**. Euchromatin is **less compacted** DNA that winds **less tightly** around **histones**, promoting the access of **RNA polymerase** and **general transcription factors** to the **promoters** of genes to form the **transcription initiation complex** and hence is **transcriptionally active**. <u>Explain why the genome of eukaryotes is condensed.</u> Organization of DNA allows the long molecules of DNA to **fit** into the **nucleus** and **prevents entanglement** which might result in **DNA breakage**. It also allows for **regulation of gene expression** as DNA wound around **histones** prevents **general transcription factors** and **RNA polymerase** from accessing **promoters** of genes that are not required in **differentiated cells**. A **large percentage** of the genome is **non-coding regions** which may be highly condensed.

	Regulation	Eukaryotes	Prokaryotes
Transcriptional Level	Promoters	Critical elements within the promoter such as the **TATA box** at the **-25** region determine the precise **location** of the **transcription start site**. **CAAT** and **GC boxes** help to **recruit general transcription factors** and **RNA polymerase** to the promoter to form the **transcription initiation complex**, making transcription more **efficient**. The **similarity** of critical elements to consensus sequences is **not that crucial** in controlling gene expression.	Critical elements in the promoter such as the **Pribnow box** at the **-10** region and the **-35** site determine the precise **location** of the **translation start site**. The greater the similarity of critical elements to the **consensus sequence**, the **stronger** the promoter and the higher the **frequency of transcription**.
	Promoter Recognition	**Activators** bind to enhancer sequences, increasing the **frequency of transcription** by **promoting** the **assembly** of the **transcription initiation complex** through the **bending** of **spacer DNA** that brings the **activators**, **RNA polymerase** and **general transcription factors** together at the **promoter**. **Repressors** bind to silencer sequences, decreasing the **frequency of transcription** by **preventing** the **assembly** of the **transcription initiation complex**.	The **sigma factor** binds to the core RNA polymerase to form the RNA polymerase holoenzyme which scans along the DNA and recognizes and binds to the promoter. **Different sigma factors** recognize **different promoters**. Hence, controlling the **availability of sigma factors** determines the genes that are transcribed.
	Operons	Operons are **not present** as it does not allow for **differentiation** of cells. In addition, a gene product may be involved in **several biochemical pathways** and hence it is more efficient to express each gene **independently** than to have **multiple copies** of the same gene in several **different operons**. There are also **homeostatic mechanisms** that keep the internal environment **stable** reducing the need for **fast responses**.	Genes coding for proteins involved in **same biochemical pathway** are usually **clustered together** on one **operon**. The expression of these genes is regulated by the **same operator** same **promoter** and **transcribed** into a **single polycistronic mRNA**. An operon allows the regulation of a group of genes that encode functionally related gene products together. **Repressor proteins** that bind to the operator prevent **RNA polymerase** from binding to **promoter**, preventing gene **transcription**.

	Regulation	Only Eukaryotes
Post-Transcriptional Level	5' Cap	A **7-methylguanosine cap** is added to the **5'** end of pre-mRNA shortly after transcription begins. This helps the cell to **recognize mRNA** amongst other RNA molecules so that **subsequent steps** such as splicing and polyadenylation **can occur**. The 5' cap acts as a **signal** to **export mRNA** out of the nucleus via **nuclear pores**, **protects** the growing pre-mRNA chain from **degradation** by **ribonucleases**, and is recognized by **translation initiation factors** bound to the small ribosomal subunit such that **initiation of translation** can occur.
	Splicing	**Spliceosomes**, a complex of **proteins** and **snRNA**, **excise introns** and **join together exons** by recognizing the points of excision determined by the **sequence of nucleotides** at **intron-exon boundaries**. Splicing allows for **alternative splicing** to occur where different exons of a single pre-mRNA can be spliced such that different mature mRNAs are produced, allowing one gene to code for many different polypeptides.
	Poly-A Tail	The **3'** end of pre-mRNA is cleaved enzymatically at a site downstream from the **polyadenylation signal**, AAUAAA. Immediately after the cleavage, **poly-A polymerase** adds a long sequence of **adenosine** monophosphate ribonucleotides, forming a **poly-A tail** during **polyadenylation**. The poly-A tail acts as a **signal** to **export** mature mRNA out of the nucleus via **nuclear pores**, **protects** mature mRNA from **degradation** by **ribonucleases**, and works with the 5' cap to **regulate translational efficiency** during initiation of translation.

	Regulation	Eukaryotes	Prokaryotes
Translational Level	mRNA Stability	The **stability** of mRNA is determined by the **length** of its poly-A tail. The longer the poly-A tail, the **longer** the mRNA can be used as a **template** to make proteins. The **poly-A tail** is removed by **ribonucleases** in the **3' to 5'** direction until a **critical length** is reached, triggering **removal of the 5' cap** and degradation of the mRNA from the **5' end** as well.	mRNA has a **relatively short half-life** as they are rapidly degraded by **RNases** soon after they are synthesized. This allows bacteria to **rapidly adjust** the synthesis of proteins in response to **environmental changes**. **Anti-sense RNA** which is **complementary** to part of the mRNA to be degraded can be synthesized, complementary base pairing with mRNA to form a **double stranded** RNA which then is **targeted for degradation** by **ribonucleases** and will **block translation** of mRNA.
	Binding of Small Ribosomal Subunit	During translation initiation, the small ribosomal subunit binds to the 5' cap of mRNA. This can be prevented by the binding of **translational repressors** to the **5' cap** or the **3' untranslated region**, which interferes with the interaction between the **3' poly-A tail**, the **5' cap** and the **small ribosomal subunit**. During translation initiation, **translation initiation factors** bind to the small ribosomal subunit and facilitate its binding to the 5' cap. The availability of **activated** initiation factors can be regulated through **phosphorylation**.	Initiation factors bind to the small ribosomal subunit and facilitate its binding to the Shine-Dalgarno sequence so that the start codon can be correctly position before the initiator tRNA and large ribosomal subunit can bind. Binding of **translational repressor proteins** at the **Shine-Dalgarno sequence** prevents the small ribosomal subunit from binding. Binding of **anti-sense RNA** complementary to the mRNA **near** the **Shine-Dalgarno sequence** prevents the small ribosomal subunit from binding. The **availability of translation initiation factors** can be regulated.

	Regulation	Both Prokaryotes and Eukaryotes
Post-Translational Level	Modification	**Polypeptides** can undergo **covalent modification**, such as attachment of prosthetic groups, glycosylation and disulfide bond formation, or **cleavage** to form **functional proteins**. In eukaryotes, this occurs in the **rough endoplasmic reticulum** and the **Golgi apparatus**.
	Phosphorylation	Proteins can be **phosphorylated** or **dephosphorylated** to **activate** or **deactivate** them.
	Degradation	Proteins that are no longer needed can be **degraded** by **proteasomes**, determining how long the protein remains in a cell. **Ubiquitin ligase** catalyzes the addition of **ubiquitin** to the protein, which is then **recognized** by a proteasome and degraded.
