Why study genetics?
- Understanding/detection/treatment of inherited disorders
- DNA forensics
- Evolutionary studies: population movements
- GM crops
- Genealogical studies
- Gene regulation, developmental studies

DNA

- Chromosome - Discrete unit of genome carrying many genes.
- Each chromosome consists of long molecule of duplex DNA & equal mass of proteins, & is visible only during cell division.
- Structural Gene - Gene that encodes any RNA or polypeptide product other than a regular.

Genetic Material of Bacteria & Viruses
- Bacterial transformation provided first support
- During transformation, genetic properties can be transferred from 1 strain to another by extracting DNA & adding it to the 2nd.
- Transforming Principle - DNA is taken up by bacterium & whose expression changes properties of recipient cell.
- Phage infection showed DNA is genetic material of viruses. When DNA & protein components of bacteriophages are labeled with radioactive isotopes, only DNA is transmitted to progeny phages produced by infecting bacteria.

Genetic Material of Eukaryotic Cells
- DNA can be used to introduce new genetic traits into animal cells or whole animals.
- Transfection - In eukaryotic cells, the acquisition of new genetic markers by incorporation of added DNA.
- In some viruses the material is RNA.

Polynucleotide Chains
- Nucleoside consists of purine or pyrimidine base linked to 1' carbon of pentose sugar.
- DNA has deoxyribose sugar (2'-H); RNA has a ribose sugar (2'-OH).
- Nucleotide consists of nucleoside linked to phosphate group on either 5' or 3' carbon of (deoxy)ribose.
- DNA contains 4 bases: adenine, guanine, cytosine & thymine; RNA has uracil instead of thymine.
- Successive (deoxy)ribose residues of polynucleotide chain are joined by phosphate group between 3' carbon of 1 sugar & the 5' carbon of the next
- 1 end of the chain (written on left) has free 5' end & the other has a free 3' end

Supercoiling
- Coiling of closed duplex DNA in space so it crosses over its own axis
- Circular or linear DNA which ends are anchored & not free to rotate
- Linking number (L) = Twists (T) + Writhe (W)

Double Helix
- B-form of DNA is double helix consisting of 2 polynucleotide chains that run antiparallel
- Hydrogen bridges between A-T (2 bonds) & G-C (3 bonds) pairing
- Nitrogenous bases are flat purine or pyrimidine
- Diameter of double helix is 20A, & a complete turn every 4A, with 10 base pairs per turn
- Helix has a major & minor groove

Semiconservative Replication
- Separation of strands of parental duplex, each strand acts as template for synthesis of complementary strand.
- Sequences of daughter strands are determined by complementary bases.
- Meselson-Stahl Experiment - "Heavy" isotope labelling showed single polynucleotide strand is the unit.

Polymerases
- Replication is undertaken by a complex of enzymes that separate parental strands & synthesise daughter strands.
- Denaturation -Separation of 2 strands due to breaking of hydrogen bonds between bases.
- Renaturation - The resassociation of denatured complementary single strands of double helix
- Replication fork is point at which parental strands are separated.
- DNA Polymerase - Enzyme that synthesise DNA
- Nucleases - Enzymes that degrade nucleic acids - DNases & RNases categorised as endonucleases or exonucleases.

Genetic Information
- Cellular genes are DNA but viruses may have genomes of RNA.
- DNA is converted into RNA by transcription.
- Central Dogma - Information cannot be transferred from protein-protein or protein-nucleic acid, but can be transferred between nucleic acids & from nucleic acids-proteins.
- Translation of RNA into protein is undirectional

Nucleic Acids Hybrid by Base Pairing
- Heating causes 2 strands of DNA duplex to separate.
- Melting temperature (Tm) is midpoint of temperature range for denaturation.
- Complementary single strands can renature or anneal.
- Denaturation & renaturation/hybridization can occur with DNA-DNA, DNA-RNA or RNA-RNA
- Hybridization can be intermolecular or intramolecular.

Mutations
- Changes in sequence of DNA
- Occur spontaneously or induced by mutagens
- Point mutation changes single base pair.

Effects:
- Transition replaces G-C with A-T base pair
- Transversion replaces purines with pyrimidine, A-T to T-A

Other Factors:
- If glucose is plentiful, no need for *E. coli* to utilise lactose
- Another regulatory protein, catabolite activator protein (CAP) controls transcription of lac operon
- CAP binds to specific site upstream of lac promoter & stimulates binding of RNA polymerase to promoter & transcription
- CAP only binds in presence of cAMP
- Adenylate cyclase converts ATP to cAMP
- It is inhibited by glucose.
- Amount of cAMP is regulated by concentration of glucose
- CAP binding & transcription of lac operon is also regulated by concentration of glucose.
- High glucose = Low cAMP
- Low glucose = High cAMP
- Catabolite Repression - High glucose levels prevent CAP binding
- Glucose regulation means lac operon is only transcribed at a low rate, even when presence of lactose prevents repressor protein attaching to operator

Diauxic Growth - Sequential use of different substrates

trp Operon: A repressible operon
- 5 structural gene encoding enzymes involved in tryptophan synthesis
- When tryptophan absent, transcription of operon proceeds unhindered.
- When tryptophan present, binds & activates a repressor protein that binds to an operator region & prevent transcription of trp operon genes.
- When tryptophan is abundant, transcription of trp operon can be reduced 70-fold.

Fine-tuned by Attenuation:
- Attenuator sequence situated in leader region (between operator & first scriptural gene).
- Causes premature termination of transcription: proportion of full length to truncated transcripts is related to amount of tryptophan present.
- Attenuation further reduces transcription by another 8-10fold.
- trpL contains attenuator region with 4 GC-rich sequences with complementarity, allowing secondary structures to form.
- Translation immediately follows transcription 1st part of mRNA leader sequence is translated while rest is still being transcribed.

Tryptophan Absent
- Region 1 contains 2 trip codons (UGG), but not translated because no trp-tRNA available. Ribosome stalls, but transcription continues.
- Region 1 now covered by ribosome, 2 & 3 form alternative hairpin structure ('anti-terminator')
- Prevents formation of terminator (3 & 4), transcription continues.
- Transcription & translation of structural genes

Tryptophan Present
- Charged trp-tRNAs available, ribosome quickly asses two trp codons & partially covered region 2, allowing region 3 & 4 to for hairpin terminator structure.
- Leads to termination of transcription
- No transcription of structural genes
- In Bacillus subtilise, attenuation is the sole method of regualating the trp operon. (No repressor system).

Level of tryptophan	Ribosome stalls?	Position of ribosome when region 3 is	Secondary structure formed	Transcription of trp operon?
High	No	Covers region 2	3+4 hairpin	No
Low	Yes	Covers region 1	2+3 hairpin	Yes

REGULATION OF GENE EXPRESSION IN EUKARYOTES

- Can be regulated at transcriptional, processing or translational level.

Nucleus → Transcription → Processing: Capping 5' poled at 3', RNA splicing, Compartmentalisation & Regulation → Translation

Controlled Transcription of DNA
- Eukaryote:
  - Intracellular signalling & intercellular communication important for transcriptional regulation in eukaryotes
  - Positive & negative regulate proteins called transcription factors bind to specific regions of DNA & stimulate or inhibit transcription.
- Prokaryote:
  - Protein/DNA interaction: negative (lac repressor) & positive (CAP/cAMP)
  - RNA polymerase

Alternate Splicing of the Rat Troponin T Gene
Alternate Splicing of RNA
- Splicing: removing introns-spliceosomes
- Alternate splicing of transcripts makes it possible for asingle gene to encode several polypeptides
- Prominent mechanism to generate protein diversity

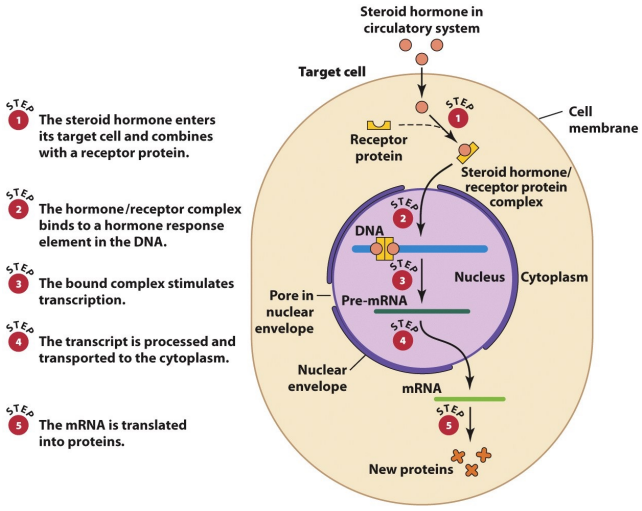
Cytoplasmic Control of mRNA Stability
- mRNA degradatiohn
- mRNA stability is influenced by several factors:
  - The poly(A) tail
  - The seuquence of the 3' UTR
  - Chemical factors (e.g.hormones)
  - Small interfering RNAs (siRNAs) or microRNAs (miRNAs)

Induction of Transcriptional Activity by Environmental & Biological Factors
- Eukaryotic gene expression can be induced by environmental factors such as heat & signalling molecules e.g. hormone & growth factors

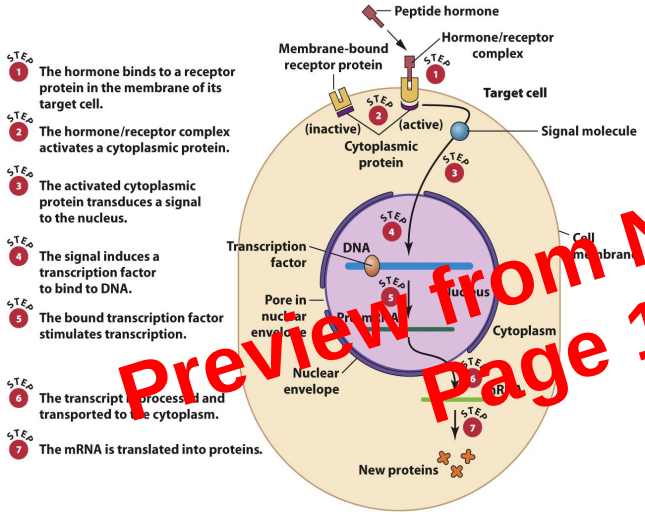
Heat-Shock Genes (Proteins)
- When organisms are subjected to stress of high temperature, they synthesise heat-shock proteins that help to stabilise internal cellular environment

- Expression is regulated at transcriptional level; transcriptional level; transcription of heat-shock genes is induced by heat.

Regulation of Gene Expression by Steroid Hormones



Regulation of Gene Expression by Peptide Hormones



Hormone Response Elements (HREs)
- Analogous to heat-shock response elements
- DNA specific sequences located near genes they regulate that bind specific protein that act as transcription factors.

Activation of Transcription by Hormones
- Steroid hormone/cytosolic receptor complex binds to HRE sequence to stimulate transcription
- For peptide hormones, receptor stays at cell membrane; signal is conveyed through cytoplasm by other protein, causing transcription factor to bind to regulatory sequence near gene

Molecular Control of Transcription in Eukaryotes
- Transcription of eukaryotic genes regulated by interactions between proteins & DNA sequences within or near genes.

DNA Sequences that Control Transcription
- Basal Transcription Factors - Proteins bind to specific DNA sequences within promoter to facilitate RNA polymerase alignment.
- Special Transcription Factors - Proteins bind to response elements or sequences called enhancers located near a gene & facilitate action of basal transcription factors & RNA polymerase

Properties of Enhancers
- Enhancers act over large distances
- Influence of an enhancer of gene expression is independent of orientation
- Effects of enhancers are independent of position. May be upstream, downstream, or within an intron.

Types of Transcription Factors
- Basal Factors (Fs) & RNA Polymerase - Bind to promoter & TATAA box.
- Activators - Proteins that recognise specific short DNA sequences inducing the efficiency of promoters. Bind at promoter.
- Co-activators - Protein required for a more efficient transcription. Don't bind DNA. Connect activators to basal factors.
- Regulators - Chromatin structure. Act on local structure of gene.

Regulation of Transcription:
- Proteins that bind to enhancers influence activity of proteins that bind to promoters, including basal transcription factors & RNA polymerase.
- Proteins are brought into contact with one another by mediator complex.

Proteins that Control Transcription
- Transcription factors usually have 2 domains (fragment) that may be in separate parts of molecules or overlapping:
- DNA binding domain that binds enhancer
- Transcriptional activation woman
- Transcription factor bound to an enhancer element may interact with other proteins bound at promoter to facilitate RNA polymerase alignment

Structural Motifs (Smaller fragment of Transcription Factors)
a) Zinc-finger motif
b) Helix-turn-helix motif
c) Leucine zipper motif
d) Helix-Loop-Helix motif

Anatomy of a Typical Eukaryotic Gene with its Core Promoter & Proximal Control Region