Molecular Biology

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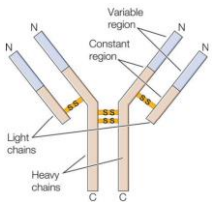
ARKAN ACADEMY

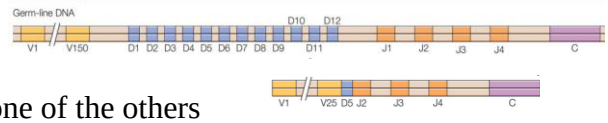
Gene Rearrangement and Recombination

- ***Immunoglobulins (antibodies)*** are proteins produced by B cells to protect the body against foreign bodies



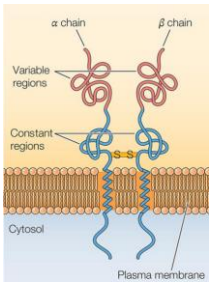
  - ➢ They consist of 4 subunits (***2 light*** and ***2 heavy***) linked together via disulfide bonds, and they contain many regions including constant and variable regions

- The human body can possess approximately 10^{12} different B lymphocytes
  - ➢ Each B cell can produce one type of an immunoglobulin which has a unique antigen-binding variable region (the site for binding antigens) that is encoded by unique genes formed ***by site-specific recombination*** during B-lymphocyte development (from naïve to mature)
  - ➢ Each heavy gene consists of 150 variable regions (V), 12 diversity exons (D), 4 joining (J) exons, and one constant exon (C), one of each is combined with one of the others



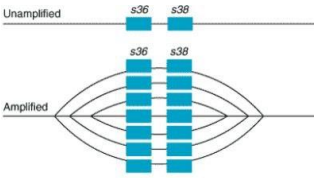
  - ➢ During lymphocyte development, one from each the total number of ***heavy*** chains that can be generated is about 7200 (150 × 12 × 4) and 600 ***light*** chains are produced by the same mechanism resulting in a possible 4×10^6 different combinations, the ***joining*** of the different segments often involves the <u>loss or gain</u> of one to several nucleotides resulting in 10^{11} different immunoglobulins and also ***somatic hypermutation*** introduces mutations during DNA replication enhances variety

- The T cell receptor on the surface of T lymphocytes is produced by site-specific recombination as well
- A new type of <u>cancer treatment</u> (***CAR-T cell therapy***) utilizes a patient's T cells that have been engineered to express an artificial T-cell receptor that recognizes antigens on the surface of tumor cells



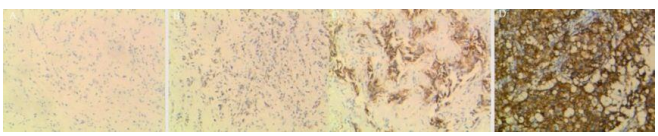
Gene Amplification

- It is an *increase in copy number* of a restricted chromosome region increasing the *quantity* of DNA in these regions and, hence, *increasing RNA and protein* production

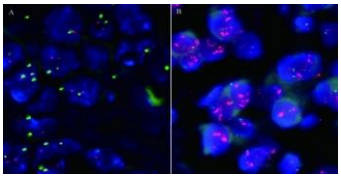


- ***Cancer cells*** use it to develop *resistance from methotrexate* by the amplifying <u>dihydrofolate reductase</u> gene (enzyme plays a key role in DNA synthesis)
- ***Breast tumor cells*** become amplify human epidermal growth factor receptor 2 (***HER2***) making them more aggressive in growth and progression
  - ➢ *Herceptin (trastuzumab)* is a treatment for HER2 enriched cancers, where it represents ***monoclonal antibodies*** that bind the HER2 on the cancer cells and <u>prevent proliferation</u>, and induce the activity of <u>immune cells</u> to get rid of them

- Gene amplification is detected by:
  - ➢ ***Immunohistochemistry*** (involves antibodies) to detect staining intensity
    - ✓ If the score was 0 or 1 (no amplification, 2 (unequivocal), 3 (amplification is ensured)
    - ✓ In the case of **<u>unequivocal</u>**, FISH is be done

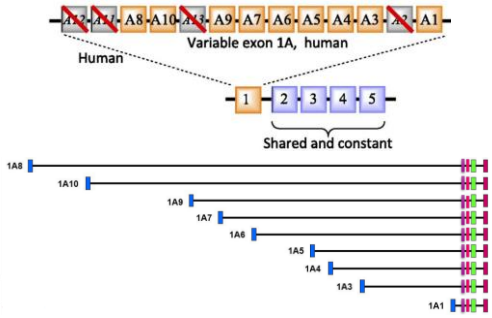


- *Fluorescence in situ hybridization (**FISH**)* uses probes specific for the gene amplified, as the number of fluorescent dots increases, that indicates amplification



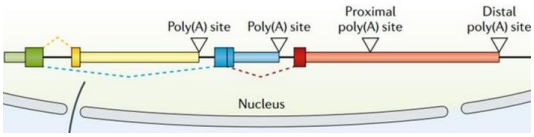
Applications for alternative splicing and polyadenylation

- *UDP-glucuronosyltransferase (UGT)* enzymes transfer glucuronic acid (glucuronidation) to xenobiotics and endogenous compounds making them water soluble allowing them for biliary or renal elimination
  - It acts on hundreds of compounds, including hormones, flavonoids, and environmental mutagens
- These enzymes have the ***same catalytic activity***, with ***different substrate-binding site***
- These enzymes are encoded in a gene containing 5 exons
  - Exons from (2 – 5) encodes the catalytic domain (unchanged)
  - ***Exon 1*** encodes the substrate-binding domain (*specificity*)
  - It contains 9 sub-exons each one has its own promoter and they are spliced generating 9 possible UGT1A transcripts



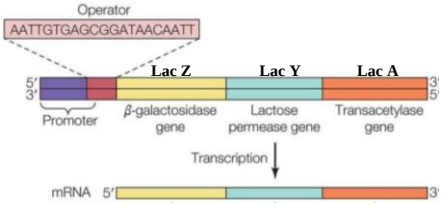
- ***Alternative splicing*** produces **different proteins**

- Some exons and genes can have ***many poly-A sites*** where transcription is terminated affecting the *length* of the transcript produced which affects the **regulation of expression** of this gene
  - Transcription can be terminated at different poly-A sites generating short and long mature mRNAs

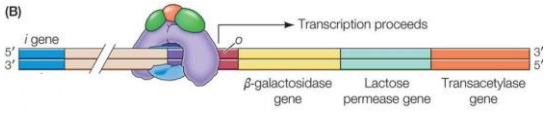


Regulation of Transcription in Prokaryotes

- **Lac operon** is a polycistronic gene that encodes for 3 different proteins with different structure and function but act together in the same metabolic pathway (lactose metabolism)
- The 3 proteins are:
  - ***Permease:*** *Transport* of lactose into the cell
  - ***β-Galactosidase:*** *Cleavage* of lactose into galactose and glucose
    - ✓ It can also convert lactose into *allolactose*
  - ***Transacetylase:*** *Acetylation of toxic* thiogalactosides to protect bacteria from toxicity



- *Operator* is a sequence *downstream the promoter*, represents the binding site of the ***lac repressor*** which prevents (blocks) the binding of the polymerase to the promoters, and so *inhibiting transcription*
  - Lac repressor is produced from "***lac I gene***"
  - ***Allolactose*** binds the repressor and prevents it from binding to the operator which *induces transcription* so it is considered as **positive regulation**



- Some promoters are ***leaky*** in some cells, and explains the relation in the expression of β-Galactosidase and synthesis of allolactose

- ***Catabolite activator protein (CAP)*** is a regulatory protein that binds to a sequence *upstream* of the promoter where CAP can then interact with the RNA polymerase to facilitate its binding to promoter (P)
  - CAP *activates the expression* of lac operon

- ***High glucose levels*** *inhibit expression* by inhibiting adenylyl cyclase causing decreased cAMP which affects the activity of CAP, even if lactose and allolactose are present because glucose is utilized by the cells preferentially (**negative regulation**)

Lactose ✗ Glucose ✗ No expression	Lactose ✓ Glucose ✗ Expression	Lactose ✗ Glucose ✓ No expression	Lactose ✓ Glucose ✓ No expression

- ***Cis regulatory elements:*** DNA regulatory sequences affect the expression for only genes linked on the ***same*** DNA molecule or same domain (close-by)
  - Examples: Promoter, Enhancer, Silencer

- ***Trans regulatory elements:*** Proteins or RNA molecules affect the expression of genes located on chromosomes or domains ***different*** than that where they are encoded
  - Examples: Repressor, transcription factors

- Some mutations can cause:
  - *Constitutive expression* (*always on*) such as defecting operator or Lac I mutations
  - *Non-inducible or repressed expression* (*always off*) such as defective promoter and RNA polymerase mutations, in addition to hyperactive repressor or gene I mutations

Regulation of Transcription in Eukaryotes

- It is similar to that in the prokaryotes, but more complex
- Transcription in eukaryotic cells is controlled by:
  - *Cis-acting elements* (location sensitive) such as Promoters, PPE, enhancers, and silencers
  - *Trans-acting factors* such as transcriptional regulatory proteins (activators, repressors)
    - ✓ It involves DNA and chromatin structural and chemical modification, and noncoding RNA
    - ✓ TFs can regulate transcription by ***epigenetics*** which alters gene expression *without affecting the DNA sequence* via structural and chemical modifications

- DNA exists as chromatin (mixture of DNA and Proteins)
  - ***Nucleosome:*** The *first level* of chromatin packing, in which ***DNA is wrapped*** around an ***octamer*** of histone proteins (***H2A, H2B, H3, and H4***) and a ***Histone 1*** molecule that stabilizes the interaction
  - The octamer with the wrapped are called ***nucleosome core particle***

- ***Free linker DNA*** between every two nucleosome core particles
- DNA can either be *loosely* (*euchromatin*) or *tightly* (*heterochromatin*) condensed

- Histones has 2 main domains:
  - A *histone-fold*, which is involved in interactions with other histones and in wrapping DNA around the nucleosome core particle
  - An *amino-terminal tail*, which extends outside of the nucleosome, and is ***rich in lysine (K)***

- The packaging of eukaryotic DNA in chromatin can regulate transcription
  - ***Active*** genes exist in ***euchromatin*** and ***inactive*** genes (inaccessible) exist in ***heterochromatin***
    - ✓ Insulin gene in the pancreas is euchromatic and in neurons is heterochromatic
  - Regulatory proteins switch between the two structures of chromatin

- About 2000 transcription factors are encoded in the human genome (10% of protein-coding genes)
- ***Positive TFs (activators)*** have **at least two** independent domains:
  - *DNA-binding domain*
  - *Activation domain* or functional domain which stimulates the transcription by:
    - ✓ *Interact* Mediator proteins and general TFs, such as TFIID, to recruit the RNA polymerase and facilitate the assembly of a transcription complex on the promoter
    - ✓ *Modifying chromatin* with the aid of coactivator

Domain: A 3D structure that is part of a protein's structure, has **independent** *structure* and *function* from the rest of the protein (can be separated from the protein and still be functional)

- ***Eukaryotic repressors*** can consist from a binding domain only or can have a repressor domain
  - *Block* the binding of activators to regulatory sequence
  - It can have *active repression* domains that inhibit transcription by *interactions* with Mediator proteins or general transcription factors
  - *Modifying chromatin* with the aid of corepressors

- How are chromosomal structures altered?

Change the structure and position of nucleosomes

- Chromatin remodeling factors (eukaryotic only) facilitate the binding of transcription factors by:
  - *Removing histones* from DNA and altering nucleosome structure allowing protein binding to DNA
  - *Repositioning nucleosomes* making DNA sequences accessible

Chemically modifying histones

- ***Histone acetylation:*** Is the addition of *acetyl group* that hides the positive charge on lysine which is responsible for the interaction between the histone and DNA which generally *loosens* the chromatin and *promotes the initiation* of transcription (**Actively transcribed chromatin**)

- Histone can also be *methylated* or *phosphorylated*
- The effect, whether transcriptional activation or repression, depends on the modification sites
- Histone modifications can: alter chromatin structure and provide binding sites for other proteins that can either activate or repress transcription

- Transcriptional activators and repressors are associated with coactivators and corepressors
  - ***Coactivators*** have *histone acetyltransferase (HAT)* which activates transcription
    - ✓ ***TFIID*** associates with histone acetyltransferases
  - ***Corepressors*** have *histone deacetylase (HDAC)* which inhibits transcription

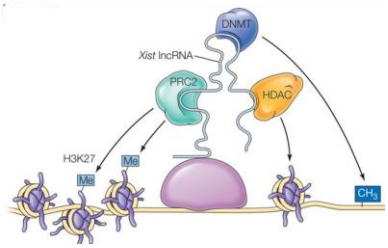
Chemical modification of cytosine

- Cytosine residues can be ***methylated*** at the *5'*- carbon position specifically at *CG sequences* called ***CpG islands*** near promoters
- DNA methylation reduces (repress, inhibit) gene transcription by:
  - *Blocking* activator binding to DNA
  - Inducing *heterochromatin* formation by recruiting chromatin compacting proteins

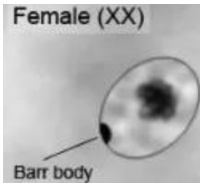
- Methylation is a mechanism of ***genomic imprinting*** (either the paternal or maternal genes is active)
  - This is the case for *75 genes*
  - Methylation is *inherited* following DNA replication

Binding of noncoding RNAs to DNA

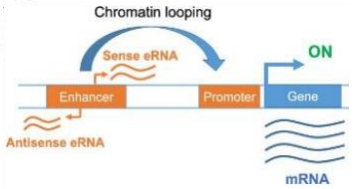
- More than 50,000 long noncoding RNAs (lncRNA, each >200 nucleotides long), in human genome
  - ***LncRNAs*** can be *homologous* to certain DNA sequences (more sensitive and specific)
  - They can *form complexes with chromatin and DNA* modifiers to activate or repress gene expression via chromatin modification and histone methylation
  - They can complex *with transcription factors* (e.g. TFIIB), Mediator, or RNA processing proteins
  - LncRNA can act in **cis or trans**



- ***X chromosome inactivation:*** lncRNA is transcribed from *Xist gene* located on one of the two X chromosomes in *females* where Xist RNA *coats* the X chromosome and promotes the recruitment of a protein complex that *methylates* histone 3 leading to ***chromosomal condensation***
  - ***Dosage compensation:*** X-chromosome inactivation in females to *equate* the number of X chromosomes between **males and females**



- ***Enhancer RNA (eRNA):*** RNA <u>transcribed from enhancer</u> sequences and complementary to it where it can regulate transcription of adjacent genes
- Also, promoters and telomeres can be transcribed (telomeres can produce lncRNA called TERRA)



- **Identical twins** have the exact *same genetic* material but they can have some *differences* due to *epigenetics* which changes over time, which is called <u>non-sequence dependent inheritance</u>
  - Epigenetics can be <u>inherited</u>
  - <u>Life style</u> can affect epigenetics which can affect the risk for getting a disease

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