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# Cell and tissue engineering

## Quantum Information Processing and Genetic Engineering

DNA is condensed into chromatin and chromatin loops and then finally chromosomes.

Human to human we differ of one to two nucleotide pairs per 1000.

We have roughly 3,000 nucleotide pairs.

Mice quite genetically similar to a human. Mouse contains the same genetic material, just in a rearranged state.

Naturally genetic mutations occurring in human also occur in mice, and can result in same effects.

* **KIT gene**: gene required for maintenance of pigment. KIT genes in human and mice are Orthologs: same gene in different species.
* Paralog: duplicate gene within the same species.
* Paralog and orthologs are formed form homologs.

Genome size does not correlate with organism complexity.

Diploid: two copies of the genome one from the mother and one from of the father.

Although genome size varies, they contain the same number of functionally distinguished genes.

Gene is comprised of **exon**: code for segments of proteins, separated by introns. When gene is active, it is transcribed into RNA. Then the intronic RNA is spliced out, and **the exonic RNA** is assembled into mRNA. Non coding intronic RNA may serve regulatory role, interacting with DNA and RNA to help perform specific functions.

Most genes have under 20 exons.

Number of exons varies with the size of the protein and the size of the gene.

* **Genotype**: genetic make-up of the cell or the organism.
* **Phenotype**: function characteristics traits or behavior of the cell or organism. Determined by the genotype but also the environment.

Genes are only transcribed from one strand of DNA.

They are read from 3’ to 5’.

**Transcription**: DNA is transcribed to RNA via RNA polymerase (5’ to 3’). It happens on the anti-sense or non-coding strand. Process of transcription is facilitated by RNA polymerase which marches down the DNA strand, unwinding it to expose an active site, adding in a nucleotide.

RNA has directionality similar to DNA, denoted by 5’ and 3’ thus it is only read and translated into a protein in one direction. Unlike DNA which needs to be open for transcription, RNA is already opened for translation.

A direct RNA transcript is spliced to become mRNA used in **translation**.

Process of transcription from DNA to RNA occurs in the nucleus. Then the transcript moves to the cytoplasm where the nucleotide code is converted into amino acid.

In eukaryotes, translation occurs in across the membrane of the ER (endoplasmic reticulum). The process of decoding RNA is completed by a ribosome and tRNA. Step 2: the amino acid forms a peptide bond with the prior amino acid. Step 3: the mRNA moves a distance of 3 nucleotides through the small subunit, ejecting the spent tRNA molecule, the tRNA no longer carrying an amino acid.

**Codon**: 3 nucleotides in the mRNA transcript; anti-codon, its complement.

4 nucleotides: 4^3 = 64 possible combinations (amino acid) but only 20:the code is redundant.

**Restriction endonuclease:** to cut DNA; they are found in bacteria.

**DNA ligase**: to undo these cuts.

* **Hybridization**: used for related but not identical DNA sequences. Use DNA probe to look for related RNA sequence is one way to find out if a gene is being expressed. Hybridization technics can also be used to eliminate patterns of gene expression during the development of a disease.
* **DNA sequencing**: to determine the exact nucleotide sequence of the DNA to work with. **Sanger method** the most popular. DNA is made up of dNTPs. Each nucleotide is added together using an OH group. Sanger method uses synthetic dNTPs which only have an H group. This blocks the addition of nucleotides and therefore terminates the chain. After sufficient incubation, the samples run through gel electrophoresis separating the strands from largest to smallest. The colors are then read by computers for analysis.
* **DNA cloning**: to produce large quantities of a DNA sequence. PCR or vector chemistry.
* Also used to amplify trace amount of RNA. Scientists can investigate gene expression patterns and levels.
* **Vector chemistry or technique**: insertion of DNA of interest to the genome of a self-replicating genetic element, a virus or plasmid. Plasmid vector usually is a circular double-stranded DNA molecules derived from larger plasmids, which occur in bacteria and separate from the bacterial chromosome. Next step is to introduce the recombinant DNA to bacterial cells.
* As these cells grow and divide, they replicate the plasma in the sequence.

**DNA engineering**: mutation can mean overexpression of a gene, change of location of a gene, change of activity from one tissue to another, or changing the timing of that activity.

## Making a transgenic animal

* **Embryonic stem cell method**: cells can grow in culture, and desire genetic modification can be verified before cells are transformed and put into the blastocyst. Another advantage is targeting by homologous recombination.
* **Pronucleus method**: DNA construct directly injected into the pronucleus of a fertilized egg, prior to the fusion of the pronuclei which forms a diploid zygote nucleus. When the zygote is a two cells embryo it is implanted in the pseudo-pregnant mother just as method 1.

## Type of mutations

### Random vs. Targeted Mutations

In order to knock-out or knock-in a gene you must be able to target the location of that gene in the genome.

Targeting strategy is easier in haploid systems and lower eukaryotes.

### Antisense RNA to knock out gene

* Only genetic material in (no need to get material out): integration site is much less important.
* Create a gene when is transcribed is complement of the RNA for the targeted gene.
* When the target RNA to the compliment antisense RNA, this hybridization blocks translation of the protein.
* Short anti sense RNA peptides can also be directly injected (a synthetic version is morpholino).
* Introducing double-stranded RNA containing both the sense and anti-sense strands, is more effective at knocking out gene expression: **RNA interference**:

Long double-stranded RNA administered, is cleaved by a protein called **dicer**, into small interfering or SI RNA. These are then assembled into an RNA-induced silencing complex, or RISC complex. In this RISC complex, RNA is unwound and directed to its mRNA complement. Binding to the complement results in splicing of the mRNA, degradation and ultimately gene silencing.

## Cellular Dynamics And High Throughput Biological Datas

### Measuring Protein Dynamics

* After translation, proteins are folded up into their final conformation then post-transtionally modified. Sometime they are forming protein-protein complexes: integrin made of 2 protein chains. Sometimes proteins are changing conformation. Movement coupled with protein-protein interaction allows communication within a cell: protein signaling network.
* The ligand is a protein that binds to a specific site (binding site) on another protein.
* Noncovalent bonds: hydrogen binding, ionic binding, or Van Der Waal forces.
* Enzyme and substrate binding: very optimized surface area of binding.
* Antibodies or immunoglobulin are produced by the immune system in response of foreign molecules. Role of antibodies is to recognized and antigen either inactivate or mark it for destruction. They are good as selectively binding their target: have multiple loops that fold back repeatedly extending finger like structures extending into the binding pocket increasing the surface area; the strength and specificity of their match.

### Immunofluorescence Imaging

* Used to look at both protein localization and protein-protein interactions.
* Start with an antibody that is specific to protein X: primary antibody where the light chains are the primary antibody interact with the antigen. The primary antibody can be conjugate with a fluorescent marker or you can use a secondary antibody conjugated to a marker specific to the primary antibody.
* To visualize the fluorophore, you must use special imaging tools of fluorescent confocal, or multiphoton microscope.
* The **fluorophore** is a molecule that can absorb light at a particular wavelength. This light excites the fluorophore causing to emit or give off a photon. The specialized microscopes can capture the emission signal. A tunable laser is used for excitation. The emission is at a lower energy, a larger wavelength, than the excitation.
* **Quantum dots**: long-tern photostability and narrow emission spectra
* **Fusion protein**: a protein that maintains its natural function but also has GFP attached to it.

DNA technologies have increased brightness, resistance to pH changes and photostability: how quickly a photo-induced alteration in a molecule extinguish its fluorescence.

Spectral diversity allows to see more than one protein at a time. Narrow spectrum overlap allows you to differentiate proteins are colocalizing or near each other.

* **FRAP**: a small region of the cell, containing fluorescence proteins, is photobleached, with a high-power laser beam. A low power laser is used to record the movement of fluorescently labeled proteins in the surrounding area backed into the bleached area. Can measure a diffusive flow but also active transport mechanisms like molecular motors.
* **Mobile fraction**: fraction of fluorescent proteins that can diffuse in the bleach area. Changes in mobile fraction can indicate anchoring to fix molecules or confinement of a protein to a specific compartment.
* **Diffusion constant**: measures the rate of protein movement in the absence of flow or other active transport mechanisms. Membranes have higher viscosity than the cytoplasm: diffusion through is slower resulting in smaller diffusion constant. Membrane-spanning proteins the radius of the membrane portion is what dominates the diffusion constant equation. Diffusion can also be limited by protein-protein interactions, binding or simply colliding with other proteins. Diffusion is slower could indicate protein complex; higher there maybe flow-directed or motion directed by a motor protein.
* **FLIP:** bleaching occurs repeatedly (in FRAP only a single event of bleaching). Measurements are taken non in bleached zone but in the bleached region. Used to determine if there is a connection between two compartments.
* **FRET:** used to measure protein-protein interaction and a protein is regulated, detect 2 proteins that are in a very close proximity in the same intracellular compartment. Label two proteins of interest with different fluorophores. then Emission-Absorption mechanism: emission of the first overlaps with excitation of the other. **Rate of energy transfer**. Used to investigate protease activity in gene expression, measures the rate and duration of receptor activation via phosphorylation events.
* **FCS and FCCS (dual color FCS, 2 proteins are labeled):** very small in fluorescence intensity are measured in fluorescent labeled protein move in and out of a small volume. **FCSS** uses auto-correlation function G. The auto-correlation function: compares the intensity at one step to the intensity at a time lag step: values are the same then high-correlation. Amplitude of G is a function of concentration. One molecule effect on small vs large group: decreases ass the concentration or group size increases. Length of time that a molecule spent in a defined volume indicated by the time before the curve drops off. When molecules are bound, they move more slowly, this time increases and the curve will shift to the right.

### Kinetics Equations

Models are useful in modeling the behavior of complex signaling pathways, and identification of critical nodes or steps in the pathway that may be potential therapeutic targets.

dC/dt = Sum of production rates – Sum of consumption rates

Law of Mass action: assumes the.

1. Reaction rate is proportional to probability of collision (concentration).
2. Number of receptors is constant allowing: total numbers of receptors = free receptors + bound receptors.
3. The receptor is the limiting reagent when there is plentiful ligand available: amount of ligand at T0 is constant through the duration of the experiment.

The ratio koff/kon proportional to the binding species = dissociation equilibrium constant.

If we increase the initial amount of ligand, the complex formation [LCR] saturates. The saturation is due to that limited number of receptors. Ligand thought as an agonist that it binds to the receptor and this leads to signaling events. Maybe it’s phosphorylation, contraction, secretion, eventually migration of the cell. But a ligand is not only an agonist, sometimes a neutral agonist. When a ligand is sitting in a receptor, it may not lead to a downstream signaling, but instead it may be blocking non-neutral ligands from binding another ligand. Now we cannot bind to that receptor (L2) and cannot lead to phosphorylation. A ligand can also work to reduce or downregulate activity this way. It’s an inverse agonist. Downstream negative feedback loops which reduce activity after a certain threshold (signaling achieved) and would turn an agonist into an inverse agonist.

### High Throughput Data

10Millions of SNPs in a human genome, most of which have unknown function. However, many have been linked to specific traits and diseases. Some of the 2K diseases associated with SNPs include: diabetes, cancers and Alzheimer’s disease.

* Nucleotide (small DNA fragments) clones are robotically printed or spotted on expression arrays.
* **Photolithography**: also used for regeneration.
* Next step: RNA is reversed transcribed from RNA into cDNA. During this process, fluorescently labeled dNTPs are incorporated. Both samples are then hybridized to the array.
* **Each array** is fluorescently imaged using an excitation specific to each fluorophore. By superimposing the images, taking up both excitations, we get a map of the changes. Ratio of one color to the other on each spot: the relative upregulation, or down regulation of a gene, relative gene expression changes between each sample.
* **Two-fold difference** as the threshold for differential expression.
* **Cluster analysis**: genes are grouped by the expression: allows to see genes that are behaving similarly, and maybe identify links or pathway connections that weren’t already known.
* **Gene expression profiling**: disease classification, cell differentiation: waves of gene expression, patterns during development of specific tissues or cell specification.
* **Proteomics**: study high-throughput biological data on protein structure, expression and function. More proteins in a cell than transcripts due to alternative splicing. Proteins form complexes and have functional states which change rapidly over time. **Expression profiling**: look at all the proteins present in a cell. **Interaction map**: first done using large, clumsy 2D gels, new technologies include antibody array, instead of an array of oligomers, antibodies specific to different proteins are arrayed and then incubated with solution from one sample, show differential protein expressions. Interaction maps aim to describe all the interactions between proteins. Most popular method is the yeast to hybrid system ([Yeast Two-hybrid Description)](https://www.creative-biolabs.com/custom-yeast-two-hybrid-screening-service.html) The two required parts of the transcriptional activator are split and bound to the two proteins of interest (bait and prey proteins). Only in the case of the two proteins bind together, both require the activator part be present and able to transcribe the reporter. This system is capable to identify weak and transient interactions.
* **Metabolomics**: the high-throughput stays simultaneously measures all of the metabolites in the cell: intermediate small molecules and metabolism: sugar, nucleotides, amino acids, steroids, fatty acids, lipids, phospholipids and organic acids. These measurements typically done via NMR and mass spec technology.
* **Phenomics**: assay use whole cells rather part of cells. Liver cells are in culture model that maintains them in a physiological state (state similar to what they experience when in the human body). **Human Variome Project**: catalog matching mutation to characteristics.