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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.

## Tissue Organization And Dynamics

### Tissue Organization

* Understand how tissue structure is regulating tissue function.
* The fabric of human tissue is made up of specialized cells and extracellular matrix components.
* Every tissue has a defined set of functions.
* **Liver biopsy using hematoxylin**: non-nucleic acids thus the nucleus is stained blue by hematoxylin.
* **Eosin** is an acidic dye stains proteins non-specifically, including those found in ECM and cytoplasm.
* Prior to staining analysis, most tissues must be sectioned.
* **Biopsy punch**: the XI sample contains multiple layers of skin and possibly muscles.
* With samples oriented appropriately, it is used by **microtome**: slices the blocks very thinly.
* Each tissue type is composed of two main pieces: cells and cell products: Extracellular matrix.
* **Epithelial**: derived from endoderm and ectoderm during morphogenesis.
  + Organs primarily epithelial: lung, kidney, and liver.
  + Epithelial cells are constantly interacting with an open environment or ducted.
  + They are polarized: they have an apical surface that faces the environment and is different from the basal surface, typically bound by the basement membrane.
  + Dark blue stained by PAS stain. Basement membrane: sheet of collagen and other glycoproteins, made by the epithelium and underlying connective tissue.
  + **Stratified squamous**: many cell layers verry thick, good barrier.
  + **Simple squamous**: very thin and flat: good for filtration and diffusion.
  + Circulatory system: lined with a specific squamous epithelium: **endothelium**.
  + A polarized columnar epithelial cell in the trachea: **cilia** is modal and used to move material in a direction parallel to the epithelial surface.
  + In small intestine, absorptive cells are arranged in a **microvilli** structure, served to maximize surface area for nutrient absorption.
  + Liver: **hepatocytes** (secretory cells) arranged in columns or strings: max. surface with the sinusoids and bile duct.
* **Connective**: cells are derived from mesoderm. Connective tissue is a thick matrix interspersed with cell. ECM components: collagen and elastin. Easy to tell them apart because of their diameters: collagen much larger diameter because it’s bundled; elastin fibers are thin. Elastin helps restore tissue, to its shape after deformation. Ground substance between fiber and cells: water stabilized by glycosaminoglycans, proteoglycans and glycoproteins. In bone ground substance is mineral and blood it is plasma.
  + **Areolar**: loose structure that holds organs in place, surrounds blood vessels and nerves. Purple is the nuclei of the interstitial fibroblasts. **Fibroblasts**: tissue repair and aid in the development.
  + **Dense connective tissue** found where tensile strength needed and fibrillar align collagen necessary. Found in tendons, ligaments and dermis.
  + **Bone matrix: osteoblasts** responsible for producing mineralized ground space. **Osteoclasts** which break it down and allow hollow bones, and **osteocytes** which are bone cells which reside within the matrix.
  + **Chondrocytes:** joining bones and joints **cartilage** whose primary cells are chondrocytes. **Image**: hyaline cartilage form trachea. Chondrocytes found in small cavities of a lacuna.
  + **Adipose:** fat tissue,main function to store energy in form of lipids. Tissue produces its hormones including estrogen.
  + **Blood:** very specialized form of connective tissue. Has no fibers, in a very modal ground substance. RBC have no nucleus; white blood cells have a nucleus that stains dark purple.
* **Muscular:** 
  + **Skeletal muscle**: connected to bones via tendons. Contraction of these muscles move skeleton and movement is voluntary. Cells in this tissue are multinucleated muscle fibers (sarcomeres, A band – dark stripe: myosine, I band light stripe: actin). Others cells include satellite cells adult stem cells, are implicated in repair. Coordinated long contraction.
  + **Cardiac muscle:** helps move blood through the blood vessels. Striated like skeletal muscle., but branches and rejoins at meeting points: interpolated discs. Contraction is involuntary. Coordinated rapid depolarization of the heart.
  + **Smooth muscle:** not striated. Used to squeeze material through hollow organ like the bladder. Alternate between contraction and relaxation. Smooth muscle is involuntary. Cells in this tissue have single nucleus. Contraction not as strong, slow and low amplitude contraction, as the striated ones but longer.
* **Nervous**
  + Support cells: glial, Schwann cells and oligodendrocytes: to produce myeline, wrapped around the neural axon. Cross section of neurons: axon is dark pink.
* Artery have a thick elastic lamina, makes them more rigid
* Systemic veins carry low pressure deoxygenated blood, thin wall, collapse easily, contains a much larger blood volume (2/3 blood volume in whole body).

### Tissue Dynamics

* **Epimorphosis**: in this process, cells dedifferentiate and rapidly proliferate.   
  Mass of dedifferentiated cells: **blastema**.
* In human regeneration s limited to: musculoskeletal system, skin and liver.
* In human development of specialized cells is completed in the first semester.
* But during life time, our bodies constantly replacing cells. This apoptosis is due to combination of internal stresses, external stresses and injuries.

Dynamic states of tissues:

1. **Homeostasis**: includes physiological adaption, constant turnover of cells. Highly regulated state.

**Small intestine**: cryptos: small invaginations, where epithelial cells are replenished. At the base slowly diving tissue-specific stem cells. After diving the daughter cells move up the crypt, and become transient amplifying cells. When pushed out of the crypt, they are mature. By 5 days, they reach the top of microvilli, and die and slough off. Another replenishing tissue is hair. The epidermis is also proliferative, and maintained by tissue resident stem cells located in the basal lamina, dark color: stem cell niche.

1. **Repair**: deals with wound healing.
2. **Formation**: new tissue, concerns morphogenesis and embryonic stem cell biology.

When cell proliferation goes haywire leading to rapid cell growth, or cancer, is attributed to DNA mutation.

One way homeostasis could be interrupted is through changes of matrix composition and structure.

**Bladder**: bottom type 1 and type 3 collagen fibers. Connective tissue supported by bladder smooth muscle cells. When tension, stress produces metalloproteinases, that degrade in stages the extracellular matrix. This structure changes alter the mechanical properties of the bladder, and alters the amount of free or diffusible growth factors. This burst of growth factors leads to **hypertrophy** and **hyperplasia** of the smooth muscle cells. The change is ECM structure is the tipping point of progression of bladder disease.

**Tissue repair**: healing is highly coordinated. Fibrotic tissue diseases including cirrhosis of the liver, and scleroderma contained similar processes to scar formation. Fibrosis is considered irreversible clinically and is treated with anti-inflammatory and immunosuppressive drugs:

1. **Hemostasis**

In flux of platelets from the bloodstream, adhere to the wound site and dump their granules. The signal from these granules recruit other platelets and thrombus starts to form. Hemorrhaging blood vessels will respond via constriction and combined with the thrombus; this will shut down bleeding.

1. **Inflammation**:

White blood cells infiltrate, and help clean up bacteria and dirt. Neutrophils migrate to the wound, from dilated and permeable surrounding blood vessels. Monocytes mature into macrophages and clean up or further clean up the injury site. Macrophages please another role in wound healing by releasing a host of stimulatory factors which signal to surrounding cells.

1. **Migration and Proliferation:**

Eventually, granulation tissues replace the clot. This tissue contains fibroblasts which produce collagen and this works to hold the wound together. It also helps support the growth new capillaries into the injury zone, necessary for providing vital nutrients and waste removal. proliferation and migration of the epidermis closes the top of the wound. While contraction of myofibroblasts which are a differentiated form of fibroblasts will close the internal edges of the wounds

1. **Maturation**:

Changes made during the proliferation phase are reinforced. Collagen is rearranged and only the necessary capillaries are maintained.

**Actin “purse-string” closure**: instead of epithelial migration epithelial purse-string closure where the epithelial cells around the periphery of the wound link their actin cytoskeleton together and pull inwards. The underlying collagen structure is different as well. Fetal case: basket weaves or randomly oriented fibers, in adult typical bundle of collagen.

Tissue engineered solutions to wound healing

Cells-based, biomaterials-based, and combined cell-scaffold approaches. Some with growth factors, and some without.

* **Xenograft**: from pig
* **Allograft**: from a cadaver, same species but not the same patient.
* **Autograft**: cells used to create the skin where derived from the patient, stem cells, collagen derived from cows. Skin equivalent being developed for both skin replacement therapy and platforms for testing drug permeability, toxicity and efficacy.

**Artificial matrix of proteins or peptides**: advantages of using matrix without cells is readiness.

Biomaterials

* Properties customized for the goal.
* Porous scaffold made of collagen used as ECM substitute for wound healing in the skin:
  + Very small pore diameter fibroblasts cannot penetrate the scaffold.
  + Very large diameter not enough surface area for the fibroblast to adhere.
  + Scaffold rate has also an optimum: when template degradation rate proportional.to normal wound healing rare best healing occurs.
  + For skin normal wound healing rate: 3 weeks, 6 weeks for peripheral nerve.