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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.

## Morphogenesis

### Early Transformations

A zygote in pro nuclear phase, before the nuclei is joined, the **zygote** splits evenly in two cells called **blastomeres**. This type of cell division is called a **holoblastic cleavage**. Cleavage cell cycles are about 12 hours in mammalian embryos. At this point, the cells at the center are connected by **gap junctions**. They’ll readily allow the passage of ions and small molecules between cells; they are used mainly for cell-cell communication. The cells at the outside are connected by **tight junctions** (nothing comes out). These cells are polarized.

* **“Inside-out” hypothesis**: when the embryo is around the 16th cell stage, cells in the middle, already have an interior identity. Cells on the exterior have an exterior identity.
* **“Polarization” hypothesis**: all the cells in the embryo become polarized and when cleavage happens, the cells will divide into two very different daughter cells. A daughter cell: interior identity, another: exterior identity.
* **“Cleavage-driven” hypothesis**: early cleavage events specify inner and outer cells. Before the embryo reaches the 16th stage, identity of these cells already decided.
* The embryo continues to develop into the **morula**. When morula is between 16 and 32 cells, it will collect fluid at its center, creating an internal cavity: the **blastocoel**. Two distinct cell populations the embryo is now a **blastocyst**. The outer cell population: the **trophoblast**. The inner cell population: the **inner cell mass**.
* **Variable cleavage**: (from embryo-to-embryo same number of cells in the inner cell mass) in the trophoblast, it varies.
* **Regulated development or stepwise approximation**: cells from trophoblast, can convert to cells in the inner mass. Important because the inner cell mass becomes the **fetus**, the trophoblast becomes the **placenta**.
* The inner cell mass will continue to develop through the **gastrulation**. This begins when a layer of inner cell mass moves to cover the interior: the **blastocoel cavity**. This process is called **delamination** because a layer peeling off that inner cell mass to coat the interior cavity. What result, two structures: the **hypoblast**, sitting on top of what used to be the inner cell and the **yok sac**. At this point the inner cell mass is the **epiblast**. A second delamination creates a new fluid-filled space: the **amniotic cavity**. This cavity will surround the fetus and will protect it up until birth. The epiblast will develop into the fetus. Fluid-filled structure is being prompted by diffusion limitations, **diffusion of nutrients**. As the embryo develops, and grows thicker in cell layers, nutrient demand will no longer be met by just cavity formation, that is creating thinner layers. Instead, it will be met by the circulatory system, the development of blood vessels, that will be able to get nutrients and oxygen deep into tissues.
* In the primitive streak, significant movement takes place. What result is the formation of 3 germ layers: the **endoderm, mesoderm and ectoderm**.
* A group of cells in the ectoderm will travel down along the primitive node (Hensen’s node) to form the **notochord**, which supplies signals to all 3 germ layers, will help in the formation of the CNS from the ectoderm, connective tissue from the mesoderm, and the respiratory tract from the endoderm.
* Folding of the ectoderm continues and a tube is formed, eventually dissociated from the ectoderm, siting on top of notochord and beneath the **neural crest**: this tube will from the brain, brain stem and the spinal cord.
* As the embryo develops more, the amnion will pull down, and the cavity will pull down and close around the neural tube. To enclose the 3 germ layers, it swill begin to pinch off a portion of yolk sac. One cavity is being pinched off by this folding is the **gut cavity**, and progressing a fully tubular structure for the gut. At the end of these folds and pinches, embryo fully encased within the amniotic sac.
* **Fate mapping**: depictions of an embryo with the fate of each cell identified, label cells, and see where cells end up over time. Originally **Nile blue dye**, carried though cell division so lineage can be tracked. **Genetic inducible fate mapping**: pattern formation of gene expression. Expression of gene of interest is linked to a reporter gene. System used: **Cre-lox** system: in front of reporter gene a stop sequence. Only when animal is given **tamoxifen**, when Cre molecules become freed from the cytoplasm, go and induce recombination between the two lox P sites that results in the removal of the stop sequence, and allows the reporter gene to be made (reporter gene was linked to a gene expressed in the hindbrain). Critical to the success of this technique is that the recombined allele is constitutive and inheritable.
* **Mitotic wave**: a wave of proliferation that runs down the length of the embryo.
* Morula: cells totipotent, blastocyst: pluripotent, after step wise approximation, the trophoblast and inner mass cells have discrete fate: unipotent.
* Two mode of cell specification:
  + **Autonomous specification**: all signal cells need to determine fate, are contained within cells cytoplasm. Asymmetric cleavage can result in daughter cells of different fates.
  + **Conditional specification**: cells depend on the condition of their neighboring cells and environment for fate determination.
* **Positional control or conditional environment control**. **Blastema**: group of dedifferentiated cells from which all the new cells and regenerated limb come from. Blastema has environmental memory or conditional memory of the amputation.
* Every cell has the same genes. Genes are not lost as cell specialized they are silenced.

### Mechanisms of Development

* **Chemical regulation**: regulation by soluble cues, gradient of morphogens that can simulate or block cells movements or specification. Gradient expression of a gene, result of diffusible BMP, retinoic acid, and actin. Many cues and overlapping ingredients responsible for complex pattern formation. Sink: morphogen is degraded. Slide 6: two-tail gradient. Gradient depends on the shape of the tissue, the source and sink. Tissue constructs are artificially engineered sources like beads or polymers loaded with a particular molecule.
  + When soluble morphogen gradients influence cell migration, they are called **chemokines**: **chemoattractant**, causing cells to walk towards them or **chemorepellent**. Act of moving up or down a chemokine gradient is **saxis**. In development chemokines are used to direct the cells to their final destination. The neuron descends down the spinal cord, as it comes down, it is attracted to the midline by a gradient of molecules called **netrin**. Once it crosses.
  + The midline, the neuron upregulates a family of receptors: **robos** which bind too slits. Midline produces a gradient of slit. Neuron can respond to **slit** until it upregulates the receptors, after crossing the midline, it upregulates the receptors and suddenly it senses the repulsive cue: the **slit** molecule. This causes the neuron to go away from the midline and not come back across.
  + Blood vessel patterning: zebrafish expression among the somites of the semaphorin molecule. The blood vessels are expressing the molecule plexinD1, which is a receptor for semaphorin. When semaphorin bind, this is a repulsive cue to help the blood vessel to stay out of the somatic space. So as the blood vessels are growing along it, they will come up and in-between the somatic instead of traversing through them. PlexinD1 morpholino: endothelial cells starting to branch into the somatic space and blood vessels invade it.
* **Mechanical regulation**
  + Cells have 3 main types of filaments: **microtubules, intermediate filaments, actin microfilaments**. Fibers used to set up shop but also to adhere. They connect to focal adhesions which help the cell to hold onto the matrix and migrate. Actin fibers will polymerize at the leading edge and disassemble at the back: this how cell move.
  + **Microcontact printing**: start with an elastomeric stamp, ink that stamp with a protein of choice, then dry it, flip it, and stamp the substrate. Then you wash in a substance which blocks the cell adhesion: precise control on both the shape and the area of the features.
  + Cell shape has been linked to cell specialization in adult mesenchymal stem cells.
  + In presence of mixed media, cell specification depends on their spread area or shape. Small island cell: adipogenesis, large island cell results: osteogenesis.
  + Signaling pathway: **RhoA** required for polymerization of actin microfilaments therefore necessary for generating internal stress in the cytoskeleton.
  + Patterning can be used not only for cell differentiation, and specification, but also for creation of engineered tissues.
  + **Cell adhesion**: integrally linked to cell shape, between the cell and ECM. ECM receptor integrin binding to membrane protein. Cell adhesion can pass both chemical and mechanical signals. **Ligation** at one cell adhesion receptor with a neighboring cell will trigger a host of downstream signaling. Force can also be transmitted through these adhesions. **Cadherins** can link intermediate filaments together and the desmosome or actin filaments together in the hegemon bands or adhesion belts. Since 2 cells are needed for the cadherins to engage cells of like stickiness or like adherent expression, tend to group together. Cells with lot of adherences will group together.
  + Lower cohesion causing external positioning and higher cohesion causing internal positioning.
  + Neural plate cells sorting into the middle while epidermal cell sort to the outside.
* **Migration**
  + In order for cells to sort by adhesion, they must migrate. Migration invokes a particular type of force or attraction: force that a cell applies to the substrate of pushing and pulling that is to migrate. To migrate: cells have to generate force and subtraction with the substrate to push off of it. Adhesion between them, and the substrate, allow for the cell to pull itself forward in the leading edge: **propulsive traction**. Release of adhesion at the back of the cell, let go a **frictional traction**, and allow the cell to move forward.
  + **Wrinkle assay:** a cell is plated in an extremely thin flexible substrate: a cell starts to generate traction on that substrate, the substrate will wrinkle and it will deflect. The arrangement and length of wrinkles can be used to calculate the traction force.
  + **Microneedles:** cells are plated on a thin elastic post with known elastic modulus. Deflection of the posts as the cell tries to move or contract, can be used to back out traction forces.
  + **Traction Force Microscopy:** cells are plated on a solid elastic substrate. The substrate is populated with tiny fluorescent microspheres. As the cell contracts and generate tension on the substrate, it will display some of these beads. The substrate displacement field is used to calculate the traction force field.
* **Haptotaxis**
  + Directed migration along a gradient. Here the gradient is bound to the substrate. These can be gradient of ECM proteins, molecules bound to the ECM.
  + Bridge between chemical and mechanical regulation. Cues: adhesive or non-adhesive.
* **Mechanotaxis**
  + Cell directed purely by mechanical cues.
  + **Durotaxis**: cells directed to move by the stiffness of the substrate. Tissue stiffness predictor of cancer metastasis: stiffer tissue has a greater likelihood of spreading because cells are more eager to migrate.
* **Fluid**-**mediated motility**: fluid flow over cells can impart a shear on the apical surface of the cell. It can cause convection of molecular products creating a soluble gradient. Many cell types preferably migrate in the direction of the flow. Kartagner’s syndrome caused by autosomal recessive genetic defect. Hallmarked by two abnormalities, both results of defects in fluid motion. The defect is in the motion of cilium, small hair-like structures which exists in many columnar epithelial tissues. Cilia will direct the flow from right to left across the ventral node.
  + - Situs inverses: organs on opposite side.
    - Primary cilia dyskinesia, all. The cilia in respiratory and other locations, become immobile: effect on the function of the airway and other ciliated tracts like GI tract.

## Cell Adhesion and Migration

### Cell Numbers

* Basic function unit:
  + Kidney: **nephron**
  + Heart: a set of **myocytes** in between the interpolated disc.
* To build a functional unit of tissue: we need 500 and 1,000 cells. That unit is made of many different types of cells and a specialized connective matrix.
* Chondrocyte transplants are used primarily in knee replacement.
* Lymphocyte therapies and bone marrow transplant often used as an arm of cancer treatment.
* Cells proliferate from basic tissue maintenance.
* In somatic phase M phase is less than an hour and starts nuclear division or mitosis followed by separation of cytoplasm or cytokinesis.
  + G1-S-G2 interphases: proteins, organelles are doubled, DNA is replicated.
  + G1-G2:
  + Spacers to allow for growth between S and M phase.
  + Take 24 hours.
  + Checkpoints where the cell evaluates both the internal and external environments.
  + If external environment deemed unfavorable, when in G1, delays the start of DNA replication, and go into cell cycle arrest of G0. In G0 cells are called quiescent (terminally differentiated cells like neurons, fat cells, hair cells).
* Once entering in S phase, a cell will continue regardless of internal or external cues.
* **FACS**: Fluorescent Activated Cell sorting. This procedure uses a fluorescent dye which binds to DNA when you measure that fluorescence.
* Majority of cells are in G1 and minority in G2.
* G1: longest part of the interphase.
* How is cell regulated:
  + Regulation in term oof time is done by cyclins.
  + Production and destruction of both the CDKs and cyclin proteins which regulate CDK activity are the major regulators of the cell cycle.
  + CDK activated through the binding of a cyclin, then acts on downstream substrate. These phosphorylations regulate the necessary intracellular behavior required for cell division. Mitogens jump-start the cycle, by initiating activation and up-regulations of cyclins.
* Using apoptosis for pattern formation: hand, proteins responsible for apoptosis and destruction of the webbing: **BMP** (bone Morphogenic Protein) proteins, **syndactyly**: regulation is not correct of BMP.
* Up to age 2: more and more synaptic connections, many don’t work well and not very strong, prune back of the poor connections and strengthen connections with the highest level of conductivity.

### Cell Culture Expansion

* Bone marrow is the source of all blood cells in the body.
* **Hematopoietic** stem cells reside in the niche.
* **Autologous** bone marrow transplants: hematopoietic stem cell population is saved.
* Issue with allogenic transplant is matching: human leukocyte antigens or **HLA** must match. HLA: proteins expressed on the surface of bone marrow cells. Without a perfect match, recipient’s immune system will reject donor’s bone marrow.
* **Thrombocytopenia**: low platelet count, could be the result of auto-immune issue like idiopathic thrombocytopenia purpura, or with children with leukemia as a side effect of treatment. Another source of transplant material: umbilical cord blood (**UBC**) does not require same stringencies and HLA matching but limited number compared to the ones harvested from the iliac crest of the adult hipbone. These cells have great proliferative potential when in body, does translate to proliferation in culture.
* **Telomeres:** non-coding DNA, protect the chromosome from fusion with other chromosomes and deterioration. During chromosomes replication, the end of the chromosome shortens by 50 and 200 base pairs per division. When telomere loss reaches critical level around 5-7 kilobase pairs, cell division stops. When it stops, it reaches the Hayflick limit.
* **Fibroblast and skeletal myocytes** grow extensively in culture and give 1010 - 1015 cells.
* **Cultures condition** can also severely limit growth.
* **Liver cells** can proliferate and regenerate tissue in the body, but difficult to grow in culture.
* **Cell dissociation** is the most common and works if we don’t care about the matrix being present in the explant or if your cell type does require macrostructure for growth.
* Cells are isolated through the use of enzymes or mechanical forces; both will break cell matrix and cell-cell bonds.
* Other route tissue is put on culture, cells and explants are typically cultured on substrates conducive for cell attachment, like polystyrene.
* **Contact or density-dependent inhibition of cell growth**: in mammalian cell culture, many cell types display inhibited cell movement and growth with increased cell contact or cell density. Once cells reach **confluence** (completely surrounded by neighbors or critical density has been reached), proliferation in culture will stop and cells will remain in a single monolayer. In body necessary for organogenesis and proper wound healing: for ex. enclosure of the epidermis after skin laceration, cells just close the open area, they don’t continue to grow past the wound site.
* To continue growth, you must subculture cells, reducing their densities by splitting them and giving them more area to grow: utilize enzymes to break the cell-matrix and cell-cell contacts, and then replacing the cells in new dishes. Depending the rate of growth and dependencies, the cells have one paracrine signaling, signals produced by their neighbors, you may split the cells at different densities. If the cells only rely on the factors from the media, or autocrine signals then the splitting density is less an issue.
* Most cells want to live around a physiological pH around ,7.4 super pink media: pH > 8.2.
* Some cells like cancer cells are not limited by cell density, and will continue to grow, pilling up deeper and deeper.
* You can force **transformation** through expression of oncogenes or reduction of tumor suppressor genes. Like **p53**, a molecule which acts a guardian of the genome, causing apoptosis of DNA damage and stopping cell cycle.
* **HeLa** cells are immortal and continue to proliferate through **passaging**.
* **Serum:**  acellular, non-clotting component of blood, contains hormones and growth factors.
* **Antibiotics:** decrease the growth of bacterial contaminants
* Media needs to be replaced routinely to remove waste products and replenish the cells with necessary nutrients. Important to measure relative growth rate, sensitivity to enzymes, and hormone concentrations, and which cells may be contact inhibited and which aren’t.
* Cell growth kinetics: kp rate constant related to doubling time.

## Cell Adhesion And Migration

### Cell Adhesion

The growth of most cell types is adhesion-dependent.

Strength of adhesion determines:

* Migration speeds
* Pattern of cell-cell aggregation

Changes in ECM composition influences the adhesion and migration of the cells, leading to a host of pathological conditions.

**Cell-cell adhesion**:

* transmit mechanical signals between cells
* defines cell polarity and set up barrier between tissues

**Cell-matrix adhesion: focal adhesion** from extra-cellular matrix to cytoskeleton of the cell.

**Cell shape** can regulate cell function and cell phenotype (slide1: adipose and osteogenic phenotypes).

**Bi-layer membrane** coated in carbohydrate molecules, contains **membrane-associated** and **membrane-spanning** proteins. The phospholipids give the membrane a net negative charge.

Some proteins are for binding other cells, the matrix or other soluble molecules.

**Mechanics of cell adhesion**: attractive and repulsive forces.

Electrostatic, steric and Van Der Waals interaction do not require specific binding of service molecules.

**Poly(L-lysine) surface**: substrate coding has a net positive charge.

* As two surfaces get closer together, water is pushed out of the gap space, creating an osmotic imbalance => higher concentration of proteins in resulting gap between cells compared to. jest adjacent. This imbalance pulls water into the gap, generating a repulsive force which becomes greater as the gap between cells decreases.
* Compression of cell surface molecules also acts as a repulsive force.

**Cell attracted to each other due to Van Der Waals interactio**ns **between polarizable but uncharged molecules** dominates at distance > 200 A0.

**Extra visitation sites for immune cells**: for an immune cell to leave the bloodstream, and get to an injury site in the tissue, it must first adhere to the walls of the blood vessel, then crawl through a gap between adjacent endothelial cells. In order to adhere to the vessel walls, the attraction forces must dominate (happens in post-capillary venules).

**Kd**: dissociation constant, indicator of the affinity of receptor-ligand pair.

Directly related to bond strength or tensile strength

Low-affinity bonds have lower adhesion or tensile strength, and higher bond have higher strength.

Methods for measuring cell adhesion

**Sedimentation-detachment**: counts determined by **microscopy measurement** of cell spreading or counts of adhesion complex staining. To keep a constant attachment force when washing cells is through usage of **centrifuge** (radio-labeled cells).   
**Fluid flow chambers:** flow between two parallel plates: results in non-constant shear stress => cone rotates and produce a constant shear stress across the entire monolayer cells: can **measure adhesion, kinetics of cell attachment and of cell rolling**.

**Micropipette or Laser Trap**: hold cells or beads coated with adhesion proteins => readout. On bond strength. Measurement of the force attachment of a myosin motor pulling on this actin filament.

**3 types of functionally different cell adhesions**

* **Tight junctions**:
  + Mechanically active
  + Form barriers
  + Permeability depends on density of the proteins
  + Restricts the movement of bound proteins from one side of the junction to the other
* **Anchoring junctions**:
  + Adhesion belts, desmosome, integrin
  + Mechanically active links that incorporate the cytoskeleton and allow transmission of force transmission.
  + Cell-cell and cell-matrix adhesion
  + **Adherens junction** 
    - Incorporate actin filaments of the cytoskeleton
    - Skin epithelium cells or keratinocytes
  + **Desmosome**
    - Links to the intermediate filaments instead of actin filaments
    - Dense plaque of clusters
    - Anchoring muscle cells to one another (many. Linked together to form a continuous piece of muscle) and epithelium tissue of the body
  + **Hemidesmosome and Focal contact** (focal adhesion)
    - Cell matrix contact
    - Hemidesmosomes link to the intermediate filaments
    - Hemidesmosomes connect epidermal cells to underlying basal lamina
    - Focal contacts link to actin cytoskeleton
    - Focal adhesions used in migration
* **Communicating junctions**:
  + signal transmission, via exchange from molecules from the cytoplasm of one cell to the cytoplasm of another cell.
  + Gap junctions
  + All cells except blood and skeletal muscle communicate with gap junctions.
  + 12 connecting proteins form a junction channel with 6 proteins in the neighboring cells forming the channel. These channels allow small ions and water (<= 1,000 Da) to pass. Unique in connect and composition determines what can pass through therefore what function the junction regulate. Conjunction of electrically excitable cells, but abundant as well in non-excitable cells where they smooth out coordinated cell behavior by evenly distributing signaling molecules.

Cell-Adhesion receptors

* **Homophilic**: bind each other
* **Heterophilic**: bind to a different molecule
* **Integrins**
  + Focal adhesion and binding to the ECM
  + Each integrand has an alpha and beta subunit which determines their affinity to ECM molecules
* **Cadherins**
  + Adherence junction
  + Nerve and cardiac cells
* **Selectins**
  + Used in a **leukocyte adhesion cascade**
  + Expressed by endothelial cells in inflamed tissue. Facilitates the captures d of leukocytes as they pass by.
* **Immunoglobulins** or **Ig-like CAM**
  + Used in leukocyte cascade but not for capture
  + **PECAM** or platelet endothelial cell adhesion molecule used during the transmigration of the leukocyte through the endothelial cell-cell junctions
  + Bonds weaker than cadherin bonds

**Extracellular matrix components**

* Scaffold that defines the structure of the tissues
* Secreted and modified by cells
* Interacts with adhesion receptors, influencing the adhesion, migration and other functions of cells (growth and phenotype)

### Cell Migration

* **Sensing** the gradient topography or an electrical current (filopodia).
* **Extension** (lamellipodium: actin filaments)
* **Anchoring**: actin filaments cluster together into focal adhesions.
* **Contraction**: myosin motor pull on the actin cytoskeleton
* **Rear release**: fewer contact at the rear or differences in Ca2+ availability at the front and the rear of the cell and Ca2+ being required for integrin attachments.
* **Recycling**: integrins receptor at the rear of the cell are recycled to the front of the cell.

**How to measure cell migration**

* **Scratch assay**
* **Cell exclusion zone assay**
* **Electrical cell-substrate impedance sensing** (ECI)
* **Microfluidics**: generate a soluble bound gradient (chemotactic or half detactic gradient). Xmas chip tree supports only laminar flow therefore mixing happens only through diffusion and in perpendicular direction to flow. Length of channel is maximized and that generates serpentine appearance. Feature allows enough time for mixing to happen to generate this gradient. Technology allows for precise control of concentration gradients and steady gradients.
* **Boyden chambers (transwell assay)**: cells migrate through a porous membrane. Cells are seeded in upper compartment, a chemo attracting or some stimulus such as coating ECM proteins or other materials. Cells at the top will migrate through the porous membrane as they react to the stimulus, end up at the bottom side of the membrane.
* **Gel invasion assay**: measure individual cells. In vivo the gel is placed or construct into an animal and cell migration is monitored in real-time with MRI or fluorescence trackers. (Slide 8: luciferase expression in neural stem cells).

**Migration speed**

Migration speed is **biphasic** wMimic trt to ligand concentration. At low concentration, few adhesions are made and cells cannot develop the necessary traction force to propel themselves forward. If concentration too high, steric hindrance will inhibit movement. Motility faster on collagen than quartz, even though quartz is a highly adhesive substrate. Bond strength displays same behavior than concentration vs speed: at a low detachment force, cells break bonds before they can establish necessary propulsion or traction force. At a high detachment force, cells can’t release the rear adhesions, and are left in place. Ligand concentration, affinity and bond strength matter (single bond matters) but also total number of bonds in adhesion determining the detachment force, force of the overall adhesion. Slide 13: we may want rapid infiltration at the edges of the material for investment of cells into the construct, and limited migration at the center to maintain population within the construct.

* Related to function: neutrophils need to get where they’re going quickly and solve the problem there, they use an **integrant independent migration**.
* **Persistence time**: how long a cell continues in a given direction. The shorter: more random migration is. Directed migration: long persistence time. Cells following directed migration display a unique morphology with a single **lamellipodia**.
* **Directed migration not always desired**: highly metastatic cells preferentially migrate along collagen fibers.

**Contact guidance for the design of in vitro models and scaffold**

* Mimic the natural tissue
* Grooved topography promoting extension and growth of cardiac cells in highly aligned fashion.
* Deeper and more narrow grooves for long-range guidance of an axon
* Half-sphere or cups for embryonic cells