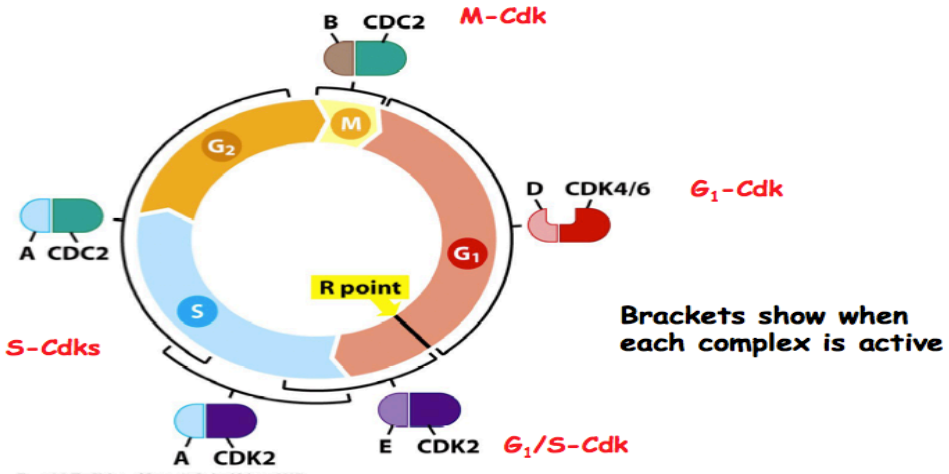
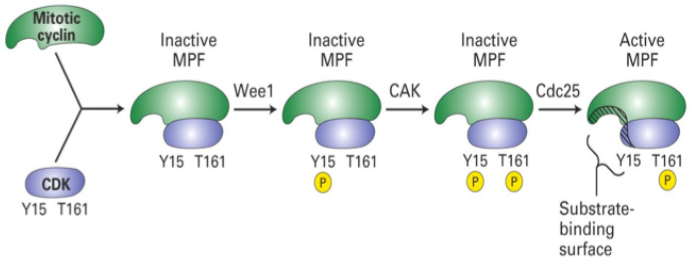
SESSION 91: CELL CYCLE BASICS

- Normal cell replication
  - Replication is **mitogen- (growth factor) dependent** (only divides when told to)
  - Replication is **anchorage-dependent** (must be anchored to extracellular matrcix)
  - Replication is **contact-inhibited** – cells normally stop growing when available space is filled
  - Cells are **mortal** – normally have a limited number of divisions before they die (telomeres shorten)
  - Cell division
    - Complex network known an **cell-cycle control system** or **cell cycle clock** governs progression through cell cycle
- The cell cycle
  - Two most basic functions
    - DNA replication (accuracy)
    - Chromosomal segregation (each daughter receives copy of entire genome)
  - How do cells duplicate their contents?
    - Mitochondria are very plentiful – doubling number with each cycle is sufficient to ensure nearly perfect segregation
    - Other organelles (ER, Golgi) break into small fragments which increases their chances of equal distribution – grow in size in daughter cells
  - Cell-cycle times
    - Vary between species
    - Examples: intestinal epithelial cells – 12 hours; fibroblasts – 20 hours; human liver cells – 1 year
  - Stages
    - M phase: mitosis, about 1 hour
    - Interphase: about 23 hours (S-phase, chromosome duplication about 11 hours)
- Regulation
  - Checkpoints:
    - G1: **Restriction Point** – start checkpoint in yeast, are there growth factor signals present? Has the cell grown sufficiently?
    - **S Checkpoint** – DNA damage checkpoint, DNA replication halted if genome is damaged
    - **G2/M Checkpoint** – entrance into M blocked if DNA replication is not completed
    - **Spindle Assembly Checkpoint** (metaphase-to-anaphase transition checkpoint) – anaphase blocked if chromatids are not properly assembled on mitotic spindle
  - Cell cycle is controlled by protein kinase complexes
    - **Cyclin-dependent kinases (Ckds)** – ser/thr kinases, inactive unless bound to cyclins, phosphorylates proteins involved in cell cycle when active
    - **Cyclins** – have no enzymatic activity themselves, are a regulatory (activating) subunit of Ckds that direct to target proteins
      - **G1-cyclins (D)** – induced by mitogens, regulates the activities of G1-Cdks (needed to go through restriction point)
      - **G1/S-cyclins (E)** – help trigger progession through restriction point
      - **S-cyclins (A)** – bind Cdks right after restriction point, stimulate chromosome duplication and control many early mitotic events
      - **M-cyclins (B)** – activates Cdks that stimulate entry into mitosis

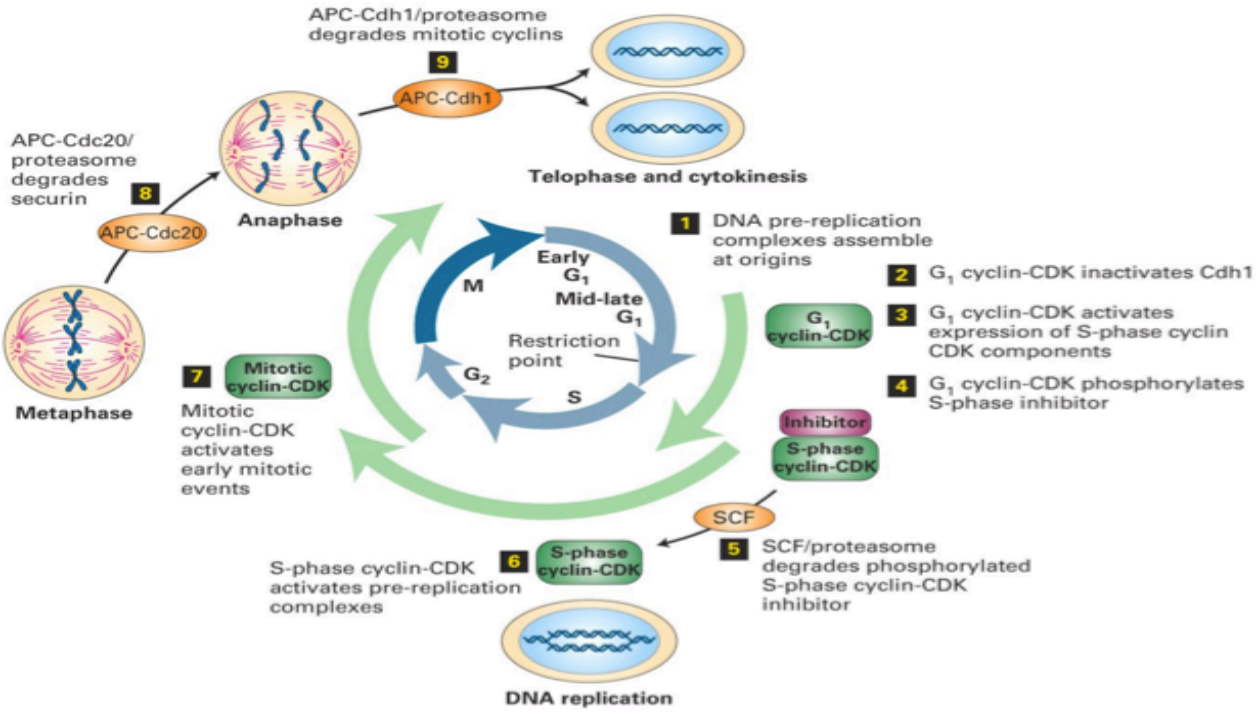


CYCLIN–CDK COMPLEX	VERTEBRATES		BUDDING YEAST	
	CYCLIN	CDK PARTNER	CYCLIN	CDK PARTNER
G_1-Cdk	cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	cyclin A	Cdk2	Clb5, 6	Cdk1
M-Cdk	cyclin B	Cdk1**	Clb1, 2, 3, 4	Cdk1

        - Regulation of Cdk Activity
            - Controlled degradation of cyclin subunits, some Cdks degraded during cell cycle (each cyclin only present during specific time during cell cycle)
            - Phosphorylation and dephosphorylation regulates activity
            - Cdk-inhibiting proteins (CKIs, CIPs, INKs) interfere with kinase activity
            - Transcription of the cyclins and CKIs
    - Controlled degradation of cyclins during the cell cycle
        - Cyclins "rule the cycle" – undergo a cycle of synthesis and degradation
        - Cycle ensures that
            - Each checkpoint is 'checked' at each cycle
            - Only one round of cell division occurs unless mitogens still present
        - During G1 and S phases, the **SCF E3 ubiquitin ligase** complex polyubiquitylate and destroys G1/S cyclins (D, E, A)
        - In M phase, **anaphase-promoting E3 ligase complex (APC/C)** polyubiquitylates and destroys M-cyclins (B)
    - Phosphorylation/dephosphorylation of the Cdks (ex M-Cdk)
        - Mostly used to regulate M-Cdk activity
        - **MPF**: M-Cdk in vertebrates
        - **Wee1**: tyrosine kinase
        - **CAK**: Cdk-activating kinase
        - **Cdc25**: protein phosphotase



    - CKIs Inhibit Cyclin-Cdk Activity
        - CKIs wrap themselves around cyclin-Cdk complex to inactivate it
        - Ex: $p16^{Ink}$, $p15^{Ink}$, $p18^{Ink}$, $p19^{Ink}$, $p21^{Cip}$, $p27^{Kip}$
    - Transcription of key genes regulates the cell cycle
        - Transcription of cyclins, CDKs, etc



SESSION 92: REGULATING THE CELL CYCLE

- Cyclin-Cdks required by ALL cells
  - **G_1/S Cyclin-Cdks (cyclin E-Cdk)**: control entry into S phase (progression through restriction point)
    - Phosphorylates transcription factors controlling genes whose proteins are needed for DNA replication
  - **S-Phase cyclin-Cdks (cyclin A-Cdk)**: controls DNA synthesis
    - Phosphorylates protein components of the prereplication complexes at origins or replication
  - **Mitotic Cyclin-Cdks (cyclin B-Cdk)**: control mitosis
    - Phosphorylates hundreds of proteins
- Fourth class required by MOST cells
  - **G_1 cyclins (cyclin D-Cdk)**: control the activities of G_1/S cyclins
    - Induced by mitogens
    - Note: mitogens not required by embryonic stem (ES) cells
      - ES cells respond to an intrinsic timer or oscillator instead of mitogens
      - Don't really have a restriction point
      - Only WT cells that are *tumorigenic*
- Cell Cycle 'Clock'
  - Integrates signals from outside and within cell to control cell cycle initiation and progression
    - Signals include tyrosine kinase, GPCRs, TGFB, nuclear receptors, nutrient status
- G_1 Checkpoint:
  - M-cyclin (cyclin B) degradation and Cdk Inactivity ends M-Phase and begins G_1
  - Key point where cells decide whether or not to divide
  - Based on whether *mitogenic signals present, DNA damage, cell has grown sufficiently*
  - → Cells enter G_0 between divisions
    - May be terminal (neurons, muscle cells)
    - Can be quiescent state but one where cell cycle machinery is intact (liver)
    - Can by transient, cells enter and leave rapidly (fibroblasts, intestinal cells)
    - Imposed on all cells, at least temporarily, by degradation of the M-phase cyclin after mitosis
  - Mitogens stimulate synthesis of the D-cyclins (MAPK, JAK/STAT, Wnt pathways, etc), induction of Myc, AP-1 (Fos and Jun), B-catenin, STATs, SP1…

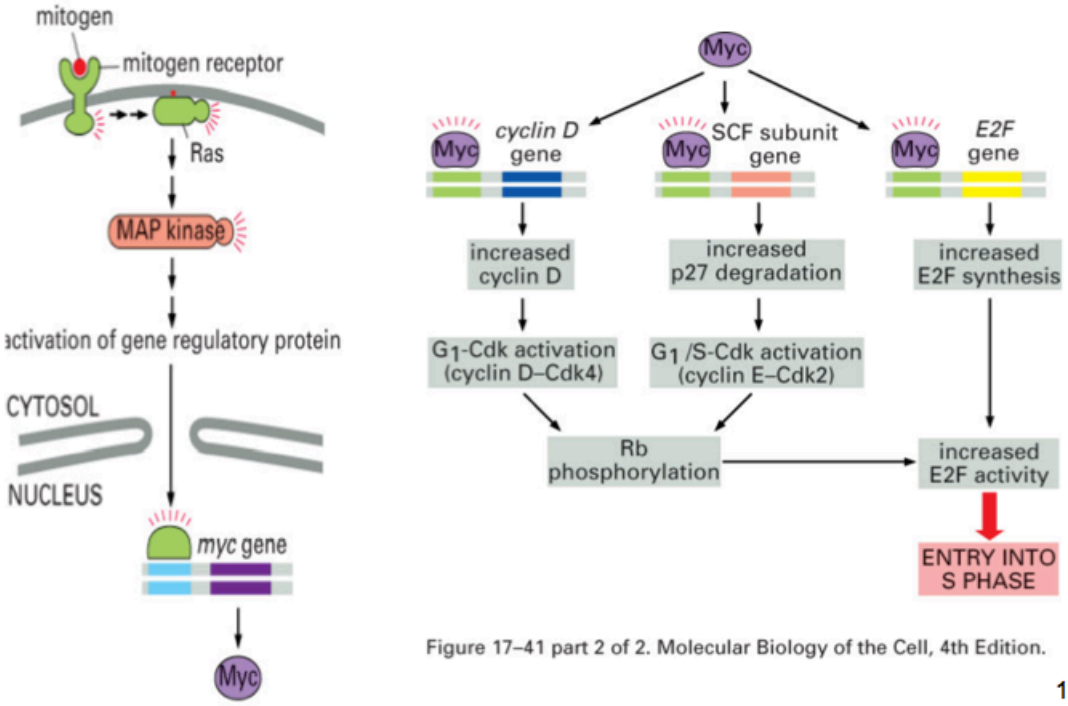


Figure 17–41 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

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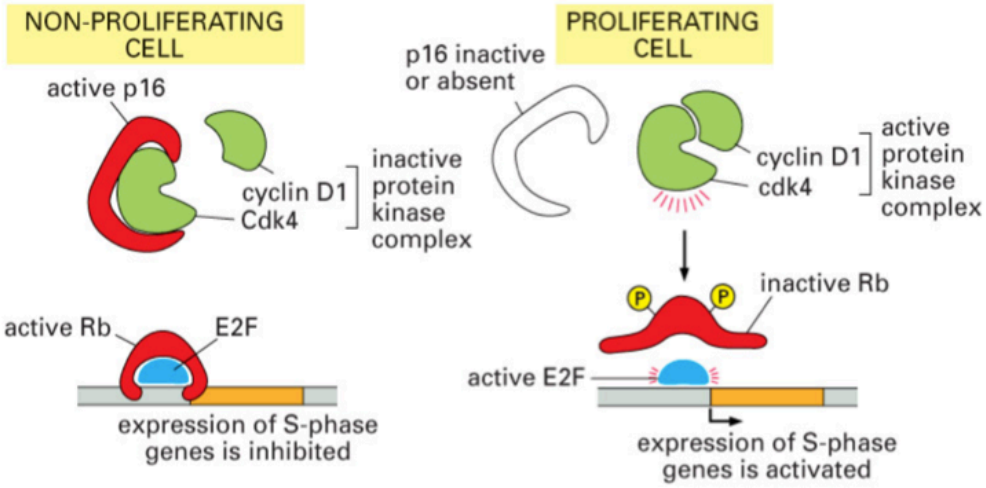
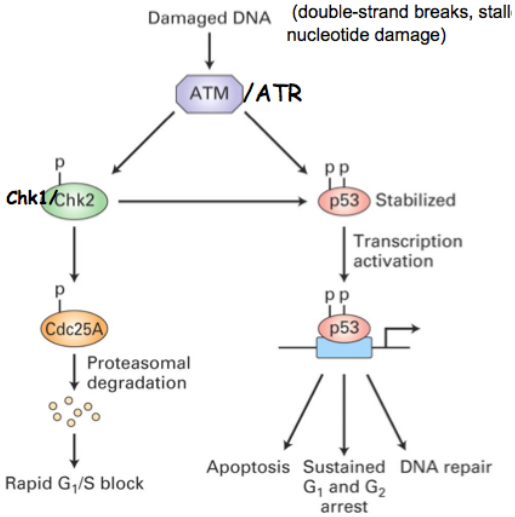
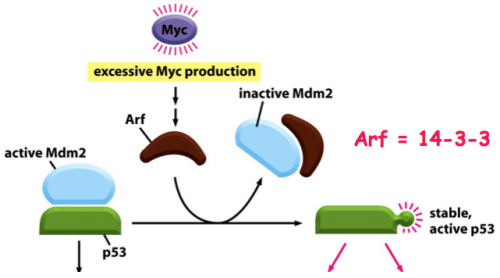
  - Inactivation of Rb and activation of E2F
  - Induction of SCF and degradation of CKIs
  - Synthesis of G1/S cyclins and other proteins needed for DNA synthesis



        - When TGFB is acting as a tumor suppressor, it opposes cell proliferation by preempting the mitogenic pathway
            - Increases expression of CKIs
            - Blocks phosphorylation of Rb
            - Prevents expression of Myc
    - Embryonic Stem ells are not subject to the G1 checkpoint
        - Rb is usually hyperphosphorylated all the time and thus inactive
        - Mitogens and MEK signaling not needed for progressiong G1
        - No DNA damage checkpoint in G1
        - Cyclin E expression is constant, not cyclical
        - Net result: ESC pass through G1 rapidly, allowing rapid cell proliferation
- DNA damage checkpoints (G1/S-Cdk, S-Cdk, M-Cdk phases)
    - DNA damage (esp single- double-stranded breaks) is detected by Kinases
        - Activate the **ATM** and **ATR** kinases
        - ATM and ATR activate the **Chk1** and **Chk2 kinases**
            - These phosphorylate **Cdc25 phosphatase** → leads to degradation
            - Cdc25 normally removes inactivating phosphoryl from Cdks, without this signal Cdks cannot be activated → cell cycle is blocked
        - Both ATM and Chk kinases increase **p53** levels by phosphorylating p53, which kicks off inhibitor Mdm2 (remember???)
            - Binds to many target genes
            - Signals DNA repair
            - If this fails → apoptosis
            - Inhibits cell cycle (CKI activation)



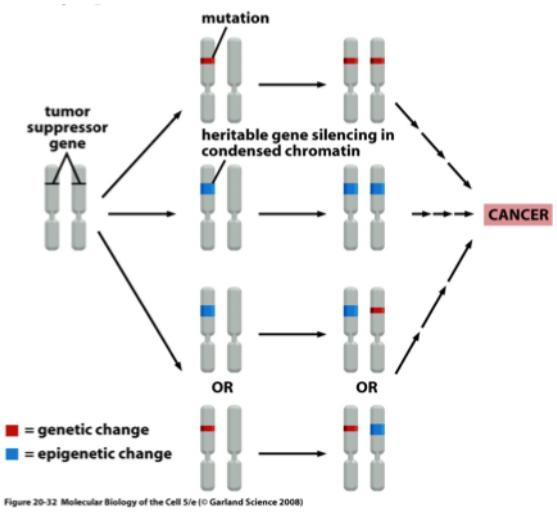
- Unreplicated DNA Checkpoint (M-Cdk phase)
    - Also activates Chk1, inactivates Cdc25, etc
- Spindle-Assembly Checkpoint (APC/C)
    - **APC/C** activated → degrades **Securin** (from Securin-separase complex) → active **separase** cleaves cohesins that hold chromatids together
    - Prolonged activation of the checkpoint → cell death
    - Many anti-cancer drugs target this step

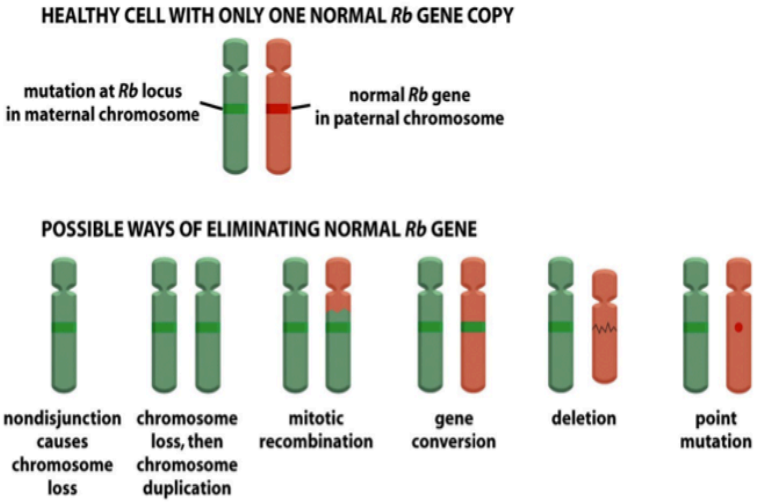


SESSION 93: THE CELL CYCLE AND CANCER

- One check against mitogen overstimulation: increased p53
    - Excessive Myc production → activates Arf (14-3-3) → complexes with Mdm2 (inhibits) → increased p53

- Cells can overcome their control systems
  - Cancer from deregulated cell proliferation
    - Mutations that short-circuit the need for mitogens – activating proto-oncogenes (RTKs, Cyclin D, Ras, PI3K, etc); or inactivating tumor suppressors (PTEN, p53, TGFB)
    - Mutations that target the G1 checkpoints – inactivation tumor suppressors (Rb, CKIs, p53); mutations that increase Myc or AP-1
  - Cancer from deregulated cell survival
    - Mutations that suppress apoptosis – activating mutations in PI3K cascade, inactivating mutations in PTEN, p53
- Mutations of critical genes
  - *Gain of function* of proto-oncogene creates **ocogene** – dominant → only in somatic cells
  - *Loss of function* of **tumor suppressor** genes – (typically, not p53) recessive → in somatic and germ cells (can be heritable), can have *strong tissue preference*
- Common conversion of proto-oncoproteins → oncoproteins
  - Missence **mutation in transmembrane regions** of Her2/neu receptor → leads to *dimerization* even in absence of ligand
  - **Deletion of external domain** of EGF receptor → *dimerization* without ligand
  - **Chromosome translocations** can create fusion proteins with oncogenic proteins
    - **Philadelphia chromosome** created by translocation of tips of 9 and 22
    - Creates **BCR-ABL fusion protein**, which is a constitutively active tyrosine kinase
    - Phosphorylates many signaling molecules, such as *JAKs*
    - Leads to chronic myelogenous leukemia (CML) if occurs in bone marrow
    - **Imatinib (Gleevac)** targets Abl kinase → first cancer drug targeting to a signaling protein unique to cancer cells
- Mechanisms for inactivating a tumor suppressor gene
  - Rb gene example (major tumor suppressor for cell cycle progression)
  - **All except point mutations lead to LOH**
  - Tumor suppressor genes can be inactivated by both *epigenetic* and *genetic* mechanism
    - Epigenetic changes are much more common → could inactivate only good copy of gene

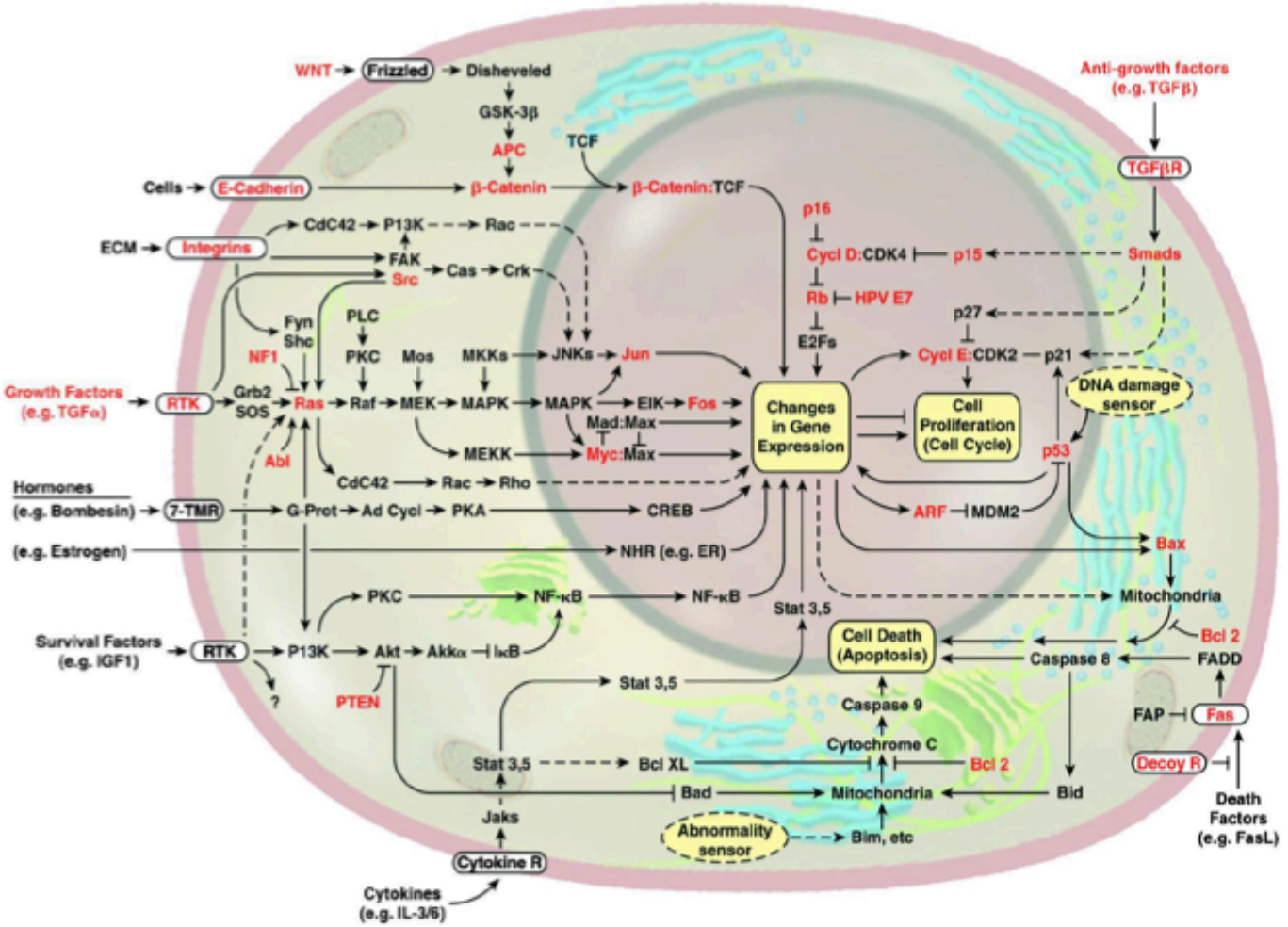




- Why isn't cancer more frequent?
  - 10^{16} cell divisions occur in humans in a lifetime
  - Spontaneous mutations in a carcinogen-free environment occur about 10^{-6}bp/gene/cell div
  - In a lifespan, each gene is likely to have been mutation 10^{10} times
  - **Multiple genetic events are needed**
  - Cancers are though to arise from a **single cell with more than one mutation**
- Evidence supporting **Multi-Hit Model** of cancer induction
  - All of the cells in a tumor should have at least some genetic alterations in common
    - Supported by microarray analysis; in female tumors all cells have same X-inactivation
  - Cancer *incidence increases with age* → more chance for multiple mutations to occur
  - In mouse models, even with overexpression of potent oncoproteins, cancer initiation is extremely slow unless more than one is introduced

- Successive mutations have been traced in colorectal cancers

- Mutations in **p53** are particularly devastating – *'guardian of the genome'*
  - Reponds to many different signals (lack of nucleotides, UV damage, hypoxia, etc)
  - Promotes cell cycle arrest when DNA is damaged
  - Triggers DNA repair mechanisms
  - Initiates apoptosis when damage is too severe
  - Blocks angiogenesis, excessive mitogen signaling
  - p53 functions as a **tetramer**, mutations in **only one allele** of TP53 can cause cancer
    - Mutations that inactivate p53 usually occur in DBD → typically recessive, usually require both alleles to be mutated, but in some cases can bind to WT p53 in dominant fashion
    - Other mutations are in the oligomerization domain → **dominant negative** mutations, only one allele needs to be affected
    - Ex: *Li-Fraumeni syndrome* – individuals with this disease develop tumors early in life (AD)
    - 50% of cancers have mutations in p53, other 50% have mutations in p53 regulators (Chk1)
- Characteristics of Cancer Cells
  - Abnormally *high mitotic rate* - used to estimate malignant potential
  - Signs of *de-differentiation* and assume features of immature stem cells
  - *Disordered growth* – not subject to contact inhibition and grown in a sprawling mess
    - **Anaplasia** – de-differentiation and disordered growth, degree of anaplasia is predictive of malignant behavior, patient's survival
  - Can *metastasize* – ability to migrate, cross basement membrane, grow in a strange environment
  - Are *genetically unstable* – keep mutating because they cannot prevent or repair DNA damage
  - Are *immortal*
  - Become *self-sufficient for growth* and proliferation
  - Induce help from local stromal cells
  - Induce *angiogenesis*



SESSION 95: CANCER GENETICS

- Cancer Basics
  - Common – 1/3 of population will have cancer in lifetime, 20% of deaths in developed nations
  - Early diagnosis improves outcome
  - Cancer causes (sporadic most common)
    - **Sporadic** – frequently only one person in family with cancer, no germline mutation
      - Typically occur later in life
    - **Familial** – number of primary/secondary relatives with cancer, no evidence of a cancer syndrome or germline mutation
    - **Hereditary** – heritable germline mutation, inherited in autosomal dominant manner
  - Multistep process, requires accumulated sequential mutations
- **Oncogene**
  - Mutations in genes that normally function to promote cell survival or limit cell death
  - Examples: telomerase, Bcl2, Myc
  - Typically cause cancer by a *gain of function* mutation
- **Tumor Supressor Genes**
  - **Gatekeepers** – control cell growth by regulating cell cycle checkpoints or promoting apoptosis
  - **Caretakers** – guardians of the cell's genome, correct normal day to day errors that occur in genome
  - Typically cause cancer by *loss of function* mutations
- Hereditary Cancers
  - Autosomal Dominant
  - Penetrance is not 100% but quite high
  - If the mutation is in a tumor suppressor gene, requires a second hit for the cancer to start
  - Frequently cell type (cancer type) specific but may have patterns of tumors that would be indicative of the cancer syndrome
  - Tend to be bilateral in paired organs (breast cancer, retinoblastoma)
  - Tend to be early onset
  - Hereditary 54 described hereditary cancer syndromes
- **Retinoblastoma**
  - Caused by germline mutations in the *Rb gene*, requires second Rb mutation to develop tumor
    - Rb encodes a *gatekeeper*
    - Inherited in *dominant manner*, but in the cell it is *recessive* (as second mutation must happen)
  - Clinical presentation
    - Very early onset: sometimes present at birth
    - Tumors can be bilateral and at multiple sites in retina
    - Later in life, individuals are at risk for osteosarcoma
- **Colon Cancer Syndromes**
  - **Familial Adenomatous Polyposis (FAP)**
    - Autosomal dominant mutations in *APC gene* → controls action of beta-catenin in Wnt pathway
    - Diagnostic charactertics
      - >100 colorectal adenomatous polyps (typically in 1000s)
      - <100 and a relative with FAP
      - Attenuated (milder) forms may have fewer polyps
      - Onset of polyps early in life; median age is 16 years (range 7-36 years)
    - Polyps are first benign → progress to cancer
    - >95% with APC will develop cancer
    - 12% will develop duodenal carcinoma
    - Screening (sigmoidoscopy), many individuals eventually have colon removed
  - **Lynch Sydrome (Hereditary Non-polyposis Colon Cancer, HNPCC)**
    - Early appearance of colon cancer (average is 43, sporadic is typically early 60s)
    - Family history of colon/endometrial cancer
    - Cancers grow quickly and are aggressive
    - Mutations in four genes: MLH1, MSH2, PMS2, MSH6 (possibly MSH3, PMS1)
    - Genes involved in *mismatch repair*
      - Hallmark '*microsatellite instability*' – DNA slippage events in short repeat units are not repaired, show slightly different microsatellites sizes when run on gel
    - Cancers in lynch syndrome
      - Colon cancer: tends to be 'right-sided', risk of 70% by age 70

        - Endometrial cancer: 30-60% by age 40
        - Other tumors with a high percentage: gastric, biliary, ovarian, urinary tract, etc…
        - Requires screening with consideration of removal of colon, uterus, ovaries
- **Li-Fraumeni Syndrome**
    - Caused by mutations in *p53* (TP53)
        - TP53 causes cell cycle arrest that is required to permit repair of damaged DNA
    - Clinical presentation
        - Early onset cancer syndrome with multiple primary cancers throughout lifetime
        - 90% incidence of cancer by age 70
    - Cancers
        - Breast cancer 25%
        - Rhabdomyosarcoma 20%
        - Brain cancer 13%
        - Adrenocortical carcinoma 10%
        - Osteogenic, chondrosarcoma 15%
- **Hereditary Breast Cancer Syndromes**
    - BRCA1 and BRCA2 mutations found in 2/3 of families with 3+ individuals with breast/ovarian cancer, found in 10% of women regardless of family history
    - High frequency of BRCA1/2 founder mutations in Ashkenazi Jewish women
    - Lifetime risk of breast cancer (men are also at risk for breast cancer)
        - BRCA1 by age 70
            - 65% will have breast cancer, 39% will have ovarian cancer
        - BRCA2 by age 70
            - 49% will have breast cancer, 18% will have ovarian cancer
    - Role of BRCA1/2
        - BRCA1 – multifunction E3 ubiquitin ligase involved in DNA damage, signaling, DNA repair by homologous recombination
        - BRCA2 – repair of double stranded breaks through homologous recombination
    - Cancer screening
        - Monthly self examinations starting at age 18
        - Clinical examination twice yearly starting at age 25
        - MRI and mammogram yearly starting at age 25
        - Twice yearly transvaginal ultrasound/CA125 blood test (ovarian cancer marker)
        - Consideration of salpingo-oophorectomy after age 35-40 or childbearing
        - Consideration of prophylactic bilateral mastectomy

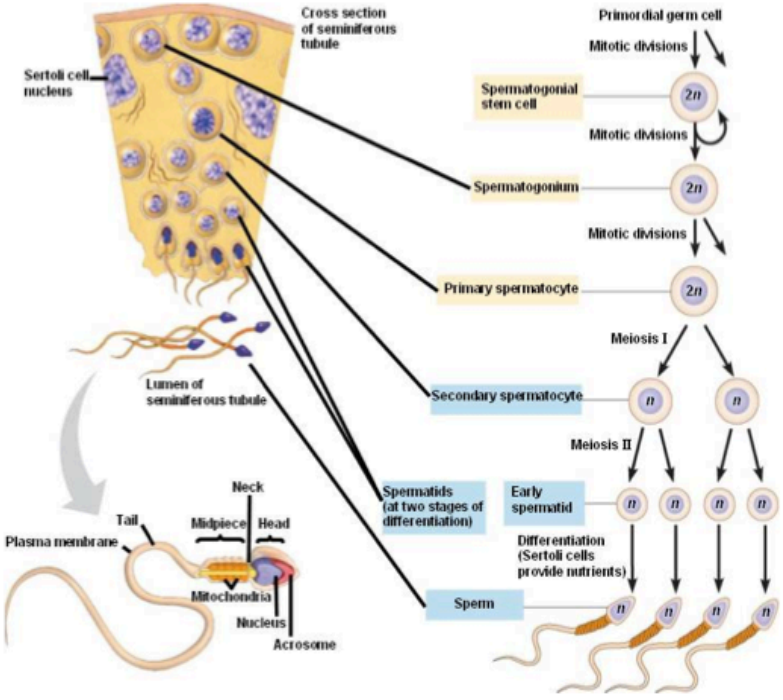
SESSION 97: ETHICS AND GENETIC COUNSELING

- Ethics
    - Establishment of a set of *guidelines* for morally acceptable *conduct* within a theoretical *framework*
    - Principles
        - **Beneficence** – benefit
        - **Nonmaleficence** – no harm, for example doing research/clinical trials
        - **Autonomy** – rule of self, patient makes independent decisions
        - **Justice** – equal care
- Ethical/professional issues in genetics
    - **Informed consent** – to make a decision, patient needs to understand treatment, other options, privacy issues, etc…
        - *Examples*: predictive testing for adult onset diseases (ALS, BRCA), testing minors, research
    - **Confidentiality** – situations in which a professional must decide whether or not to share privileged information with family members, insurance companies, fellow professionals, third parties
        - *Example*: duty to warm family members of risk, must counsel patient about this
    - **Withholding information** – situations in which a professional must decide whether or not to share information with patient
        - *Examples*: unanticipated information (non-paternity, whole genome sequencing), withholding is in patient's best interest?
    - **Uncertainty** – situations where there is ambiguity about how genetic information
        - *Examples*: test results from cancer susceptibility test (lack of data), variants of uncertain significance

- **Value Conflicts** - situations where there is a difference in a personal/professional values
  - *Examples*: sex selection, patients selecting <u>for</u> such traits as deafness of dwarfism
- **Directiveness** – to what extent should a professional influence a patient's decision making
- **Discrimination** – situations in which a professional or patient is concerned about unfair treatment by an insurance company or employer based on results of a genetic test
  - MN statute and GINA protect in some circumstances
- **Diversity** – situations in which ethnicity, religion, socioeconomic status, language etc present an obstacle in the genetic counseling process
- **Who is the patient**? **–** situations in which parents disagree about what to do with respect to child or families disagree about procedures/testing
- **Documentation** – illegal not to record, even if patients doesn't want it to be

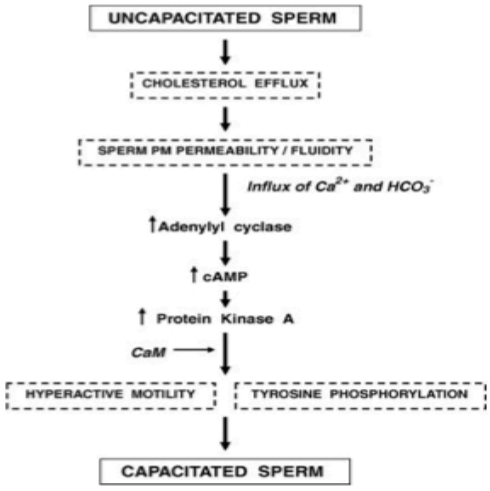
<u>SESSION 99: GAMETOGENESIS AND FERTILIZATION</u>

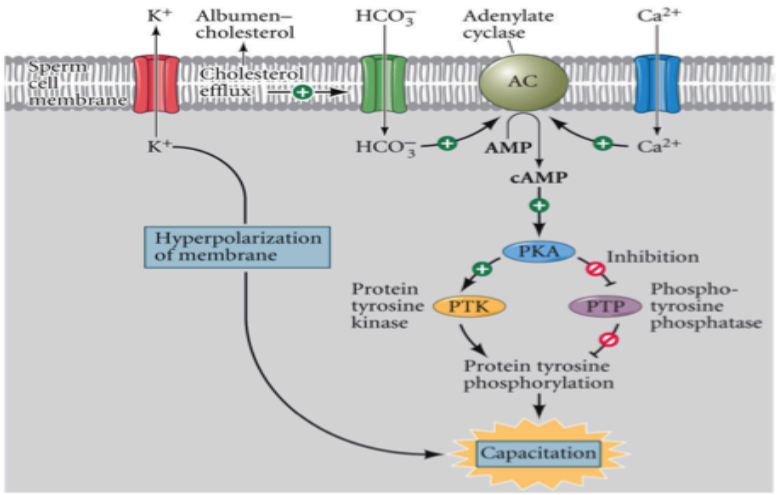
- Sexual Reproduction
  - Advantages: *Genetic variation* and *elimination of deleterious genes* (through sexual selection)
  - Timing, onset, duration of meiosis are **sexually dimorphic**
- **Retinoic Acid** (vitamin A derivative) regulates the timing of entry into meiosis
  - RA binds to RAR, a nuclear receptor
  - <u>Females</u>: in embryonic ovary, high levels or RA induce proliferating germ-line cells to enter meiosis at about 11-12 weeks of gestation
  - <u>Males</u>: in embryonic testis, sertoli cells produce an enzyme (Cyp26b1) that metabolizes RA, only after puberty do enzyme levels decrease, allowing RA levels to rise and meiosis to resume
- Gametes specialization
  - **Sperm**: very small, highly motile, no organelles except for mitochondria, highly competitive
  - **Ovum**: very large, nonmotile, contains many materials/organelles to support embryo growth
- **Spermatogenesis**
  - Sperm develop as a syncytium – connected together to synchronize development and so X containing sperm get same proteins as Y containing sperm
  - **Acrosomal vesicle** contains hydrolyic enzymes to penetrate ovum's outer coat
  - DNA is tightly condensed by **protamines** and sperm-specific histones
  - **Sertoli cells** (nurse cells)
    - Secrete **anti-Mullerian hormone (AMH)**
    - Convert testosterone to estradiol
    - Form blood-testes barrier
    - Secrete inhibin and activins after puberty to regulate FSH secretion
  - **Leydig Cells** (interstitial cells)
    - Produce testosterone in response to LH



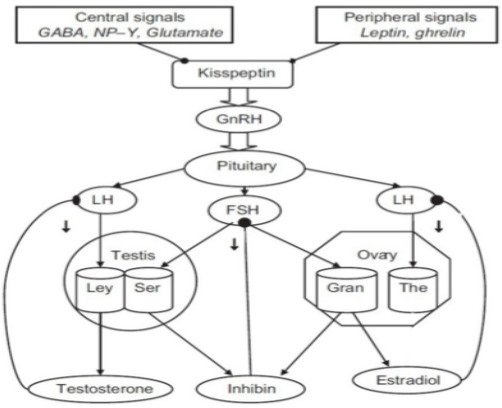
  - Sperm are not capable of fertilization until they undergo **capacitation** in the female genital tract
    - Occurs in fallopian tubes, takes 5-6 hours
    - Involves extensive biochemical and functional changes in the sperm
      - Cholesterol efflux, membrane becomes hyperpolarized, increased cytosolic pH, unmasking of cell surface receptors that bind the sperm to the ovum
    - Stimulus for capacitation is unclear, *in vitro* fertilization requires albumin, Ca2+, HCO3-
      - Albumin helps extract cholesterol, increasing the ability of plasma membrane to fuse with acrosome during **acrosome reaction**

- Ca2+, HCO3-Eenter the sperm and activate a soluble adenylyl cyclase enzyme to increase cAMP → leads to tyrosine phosphorylation of many proteins

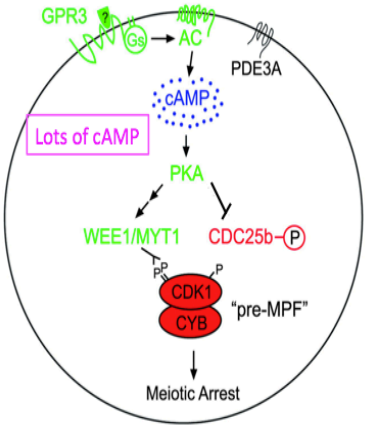


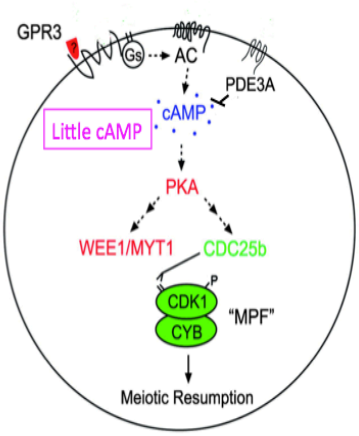


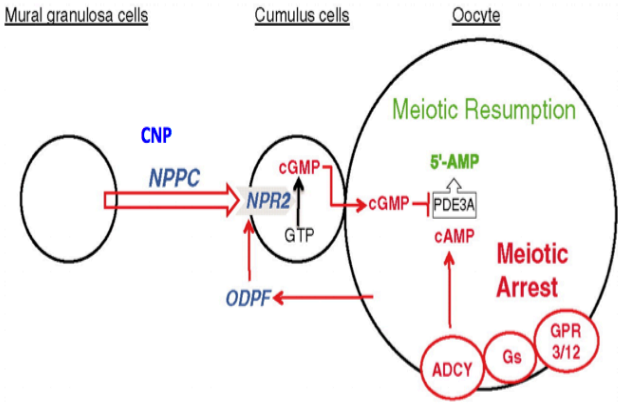
- Ovum maturation
  - **Oocyte**: developing egg/ovum that cannot bind sperm of be fertilized
  - **Egg = mature ovum = ovum**: capable of being fertilized, arrested in metaphase II
  - The primary oocyte uses special strategies to grow:
    - Most of the growth occurs when the cells are bivalent (4N), so there is twice as much DNA to use for RNA synthesis
    - Some genes such as ribosomal RNA genes become amplified
    - Follicle cells link to oocyte via gap junctions to provide precursors for protein synthesis
  - Ruptured follicle → corpus luteum → secretes progesterone to maintain pregnancy
  - Gonadotropins **FSH** and **LH** regulate Ovarian and Testicular Development
    - During reproductive years, LH and FSH stimulate ovulation
    - During puberty, **Kisspeptin** is the key regulatory of the onset of puberty
      - It binds to a GPCR in the pituitary (GPR54)
      - This signals the pituitary to release LH and FSH
      - FSH → drives maturation of Sertoli, Granulosa cells
      - LH → stimulates Leydig, thecal cells



  - Regulation of Meiosis in Mammilian Oocytes
    - Meiotic arrest depends on high cAMP levels in oocyte
    - The FSH/LH surge acts on granulosa cells to trigger the oocyte to resume meiosis
    - CNP and NPR-B (natrieretic peptide receptor) contribute to maintaining meiotic arrest
      - NPR2 → GTP to cGMP → inhibits PDE3A → stops degradation of 5'-AMP



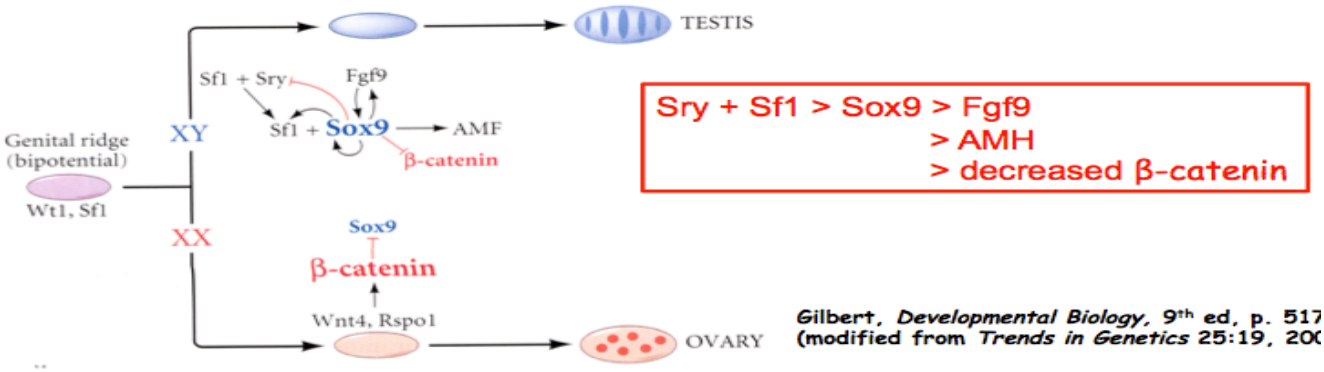




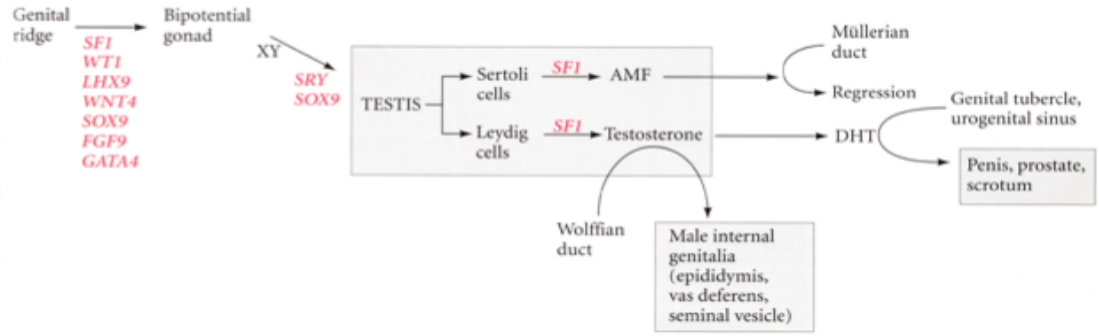
- Fertilization
  - Around 300 million sperm are deposited at the opening to the cervix
  - Only about 200 reach the ovum
  - Ovum surrounded by glycoprotein matrix called **zona pellucida** and layer of follicular cells called corona radiata
  - Meeting of sperm and ovum
    - Ovum secretes chemoattractant peptides
    - Sperm binds to the **zona pellucida**
    - Sperm passes through ZP, contains glycoproteins ZP1, ZP2, ZP3 (may be sperm receptor)
    - ZP induces the **acrosomal reaction**, contents of the acrosome are exocytosed in a Ca2+ mediated reaction
  - Blocking Polyspermy
    - *Fast reaction*: electrical potential of the ovum membrane is changed within 1-3 seconds as Na2+ enters ovum → lasts 1 min (not detected in mammals)
    - *Slow reaction*: the **cortical granules** fuse with the ovum membrane, modify proteins in the ZP including 'sperm receptor' ZP3 → **cortical reaction**
  - Ovum activation after sperm binding begins development program (involves PLC pathway → Ca2+ ^^)
    - Exocytosis of cortical granules
    - Resumption of meiosis and extrusion of 2nd polar body
    - Release of inhibition on maternal mRNAs
    - Stimulation of protein synthesis, DNA replication, etc
  - Sperm contributes centrioles needed for first mitotic division

SESSION 100: SEX DETERMINATION AND DIFFERENTIATION

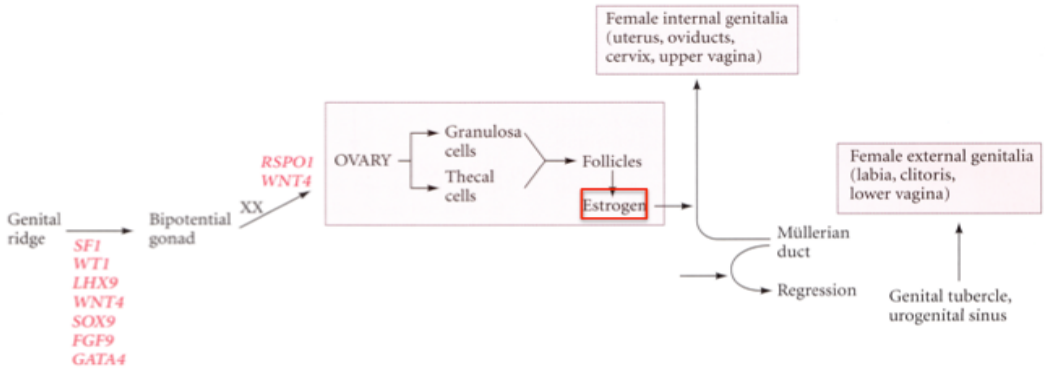
- Primordial Germ Cells (PGCs)
  - **PGCs** are the cells that give rise to the gametes
  - PGC **specification** begins at *gastrulation*, initiated by high levels of BMP family members (in mouse)
    - Part of TGFB family, bind to Ser/Thr kinase receptors
  - **Specification**: the first stage of commitment of a cell or tissue to its fate
- Mammalian Gonad
  - **Bipotential (indifferent)** – can become male or female from weeks 4-7
  - Sex chromosomal composition of the somatic cells surrounding the PGCs determines differentiation
  - Male: *wolffian duct*, XY or XXXXXXXXXXXY
  - Female: *Mullerian duct*, XX or X0
- Development of Male Phenotype
  - Testis determination controlled by **SRY** (sex determining region of the Y chromosome) on short arm of Y chromosome
    - Encodes 223 aa tranction factor, expressed in a number of tissues in fetal development
    - Activates the male-specific transcription factor **Sox9**
    - Somatic cells expressing Sry gene become *sertoli cells*
  - Sertoli Cells direct development
    - Stimulate PGCs to develop along pathway that produces sperm and inhibit from entering meiosis, which oocytes must do
    - Secrete **AMH (anti-Mullerian hormone)** → causes regression of Mullerian duct
    - Stimulate development of other somatic cells to become Leydig cells
    - Leydig cells secrete **testosterone**, induces male reproduction structures and secondary sexual characterstics and masculinizes the brain
  - Initiation of sex determination



        - In the default female pathway, paracrine signals and transcription factors activate **Wnt4** and **R-spondin1**
            - Wnt4 increases beta-catenin, which inhibits Sox9 → male pathway cannot proceed
        - If **Sry** is present, it acts with **Sf1** to induce **Sox9**
            - Sox9 induces **Fgf9**, stimulates testis development and feeds back to increase Sox9
                - Fgf9 – growth factor, binds to tyrosine kinase receptor
            - Sox9 inhibits beta-catenin's activation of ovary
    - Mechanism of action of **Sry**
        - Contains two nuclear locatization domains, must be bound by **CaM** and **Impbeta** to move into nucleus
        - Sry and Sf1 bind directly to enhancer region (TESCO) of Sox9 gene → induce expression of Sox9
        - Sox9 also binds directly to TESCO site with Sf1 to amplify Sox9 expression
    - **Sox9**: the autosomal testis-determining gene
        - Transcription factor that induces testis formation in all vertebrates
        - Functions
            - Upregulates its own promoter, prolonging its own expression
            - Blocks ability of beta-catenin to induce ovary formation
            - Regulates numerous genes requires for testis formation – AMH, FGF9
        - NOTE: although Sry is the trigger for male sex determination, Sox9 orchestrates and stabilizes Sertoli cell differentation to lock in the testis-determining program
    - **FGF9** is also essential – induces proliferation of Sertoli cell precursors and stimulates their differentiation, without sertoli cells, the male structures do not form
    - **Sf1** (steroidogenic Factor 1) – link between Sry and male development pathways

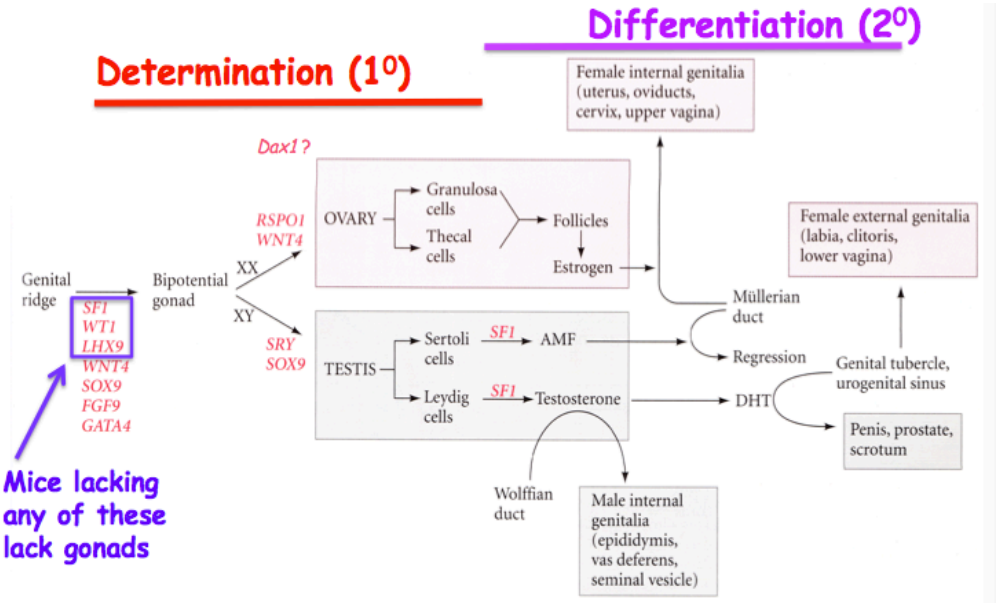


        - Nuclear orphan receptor (ligand not known)
        - Binds to DNA in monomeric form
        - Activates AMH in sertoli cells and the enzymes that make testosterone in Leydig cells
        - Individuals heterozygous for Sf1 have malformed gonads, retain Mullerian duct
- Development of Female Phenotype
    - Initiation of sex determination (see figure above)
        - Female pathway is default, Wnt4, Rspo1 (inhibits Wnt inhibitor)→beta-catenin→ *inhibits Sox9*
            - Beta-catenin prevents Sf1 from binding to TESCO enhancer



        - **Wnt4**, **R-spondin1** required for normal ovarian development

- Sex determination vs. differentiation: development of female and male phenotypes in response to hormones secreted by the gonads



SESSION 101: DISORDERS OF SEX DEVELOPMENT

- Disorders of sex development
  - Genotypic sex based on presence of the type of sex chromosomes and the phenotypic sex are disconcordant or external genitalia are sufficiently ambiguous to preclude sex assignment at birth
  - Umbrella term that supplants terms like intersex, hermaphrodite, etc…
  - Many are characterized by ambiguous genitalia: hypospadias, chordee, absent testes, enlarged clitoris, masculinized labia majora, extrophic bladder
- Terms
  - **Wolffian structures**: male structures – epididymis, vas deferens, seminal vesicles
  - **Mullerian structures**: female structures – fallopian tubes, uterus, upper 2/3 vagina
- Sex Chromosome Disorders

Sex Chromosome DSD	46, XY DSD	46, XX DSD
46, X Turner Syndrome	Gonadal DSD: Complete/Partial/Mixed Gonadal Dysgenesis	Gonadal DSD: XX Testicular DSD XX Ovotesticular DSD
47, XXY Klinefelter Syndrome		
45, X/46,XY Mixed Gonadal Dysgenesis, Ovotesticular DSD	Disorders of Androgen Synthesis or Action	Androgen Excess
46, XX/46, XY Chimeric, Ovotesticular DSD	Severe gonadal malformations: hypospadias or bladder extrophy	Severe gonadal malformations: MURCS, cloacal malformations, vaginal atresia

  - **Turner Syndrome**
    - 45, X (most common); 46, X, iso Xp (has two short arms); 46, x, iso Xq; X translocation to an autosome or Y chromosome, 45, X/46, XY mosaicism (ratio determines sex characteristics)
    - 1/5000 live births
    - Clinical characteristics:
      - Short stature, infertility, congenital heart disease, streak gonads (no follicle cells in ovaries), kidney malformations, hand/food edema, neck webbing, wide carrying angle, low hair line, wide spaced nipples, late onset hearing loss
  - **Klinefelter Syndrome**
    - 1/500 males
    - 47, XXY
    - Clinical characteristics

            - Tall stature with long limbs, small testes, low testosterone – leads to under-virilization and decreased bone density, azoospermia, learning disability or behavioral changes
    - **46, XX/46, XY Chimerism**
        - Two cell lines that occurred from different zygotes
        - Phenotype depends on percent and location of one cell type vs another
- 46, XY Disorders
    - **Partial and Mixed Gonadal Dysgenesis (PGD)**
        - Characteristics
            - Ambiguous genitalia, mild to severe penile scrotal hypospadias, dysgenetic testes may become tumors later in life, reduced to absent sperm production, various degrees of both Mullerian and Wolffian structures
    - **Complete Gonadal Dystenesis (CGD)**
        - Individuals appear as normal woman and may not present until puberty when they fail to develop menses
        - Completely underdeveloped gonads, need to be removed by adulthood to prevent tumors
        - Normal Mullerian structures
        - Woman are infertile, but may carry a donated pregnancy
    - Genetic basis
        - Deletions of small regions on 9p, 2q, 10q
        - Duplications of 1p (Wnt4) or Xp (Dax1 aka NROB1)
        - Single gene mutations
            - Sry – 1% of 46, XY PGD; 15% of 46, XY CGD
            - Sf1 – 13% of 46, XY DSD
            - DHH – 20% DSD, 50% CGD
            - WT1
- 46, XX Disorders
    - **Testicular Disorder of Sex Development**
        - 46, XX Karyotype
        - clinical findings
            - male appearance (80%) or ambigiuous male (20%), two testicles, no Mullerian structures, low testosterone, gynecomastia
        - Genetic basis
            - Translocation of Sry from Y to X chromosome found in majority
            - De novo event
    - **Ovotesticular Disorder**
        - Formerly known as True Hermaphroditism
        - Causes are variable: chimerism – 46, XX/46, XY; mosaicism, etc
        - Characteristics
            - Mullerian and Wolffian structures may both be present
- Hormonal basis of DSD
    - Abnormalities of sex differentiation
        - In production or response to hormones that are produced by the testes, adrenal glands, or other sources that change the external appearance of genitalia
    - **Congenital adrenal hyperplasia (CAH)**
        - Mutation in genes that encode enzymes needed for the biosynthesis of cortisol
        - Most common is 21-hydroxylase deficiency
        - Have increased 17-hydroxyprogesterone (used to screen)
        - Characterstics: virilized female genitalia – ambiguous genitalia appears male
        - Infants can have salt wasting – leads to death in first days of life if not treated
        - Common – 1/10000 in Europe, 1/300 in Yupik Eskimos
    - **5 alpha-reductase deficiency**
        - 46, XY
        - Ambiguous genitalia, testes present in abdomen or inguinal canal or labia, virilization at puberty due to effect of testosterone
        - Most individuals are raised as girls but adopt male identity after puberty
        - Testosterone → DHT by 5-alpha reductase, induces transcription of proteins needed for male differentiation

- **Complete Androgen Resistance**
  - 46, XY but unambiguous female
  - Mutation in gene encoding the Androgen Receptor in Xq11-12
  - Testosterone is produced but cells cannot respond to its signal
  - 2-5/100000 individuals (fairly rare)
  - Individuals have normal breast development at puberty, testes present in abdomen or inguinal canal or labia
  - Most individuals raised as women

- Sex assignment
  - 2/10000 babies are born with ambiguous genitalia
  - Both a *medical* and a *social* crisis
  - Do not risk assignment until you have all the data you need to help the child
  - Testing needed:
    - Karyotype, steroid measurements, electrolytes to rule out salt wasting, blood pressure, ultrasound or MRI of pelvis
  - Final assignment may take days, and genotypic sex may not be the same as phenotypic sex
- Surgical Management
  - Controversial intervention
  - Arguments against early surgery
    - Young girls have no use for 'functional' vagina until menses, intercourse
    - We could reduce total number of operations needed to achieve vaginal length, while reducing risk of stenosis and give patients greater control over their lives
    - No evidence that early surgery improves gender or psychosocial development
  - Arguments for early surgery
    - There is more mobility of infant tissue
    - There is a shorter pelvis length that allows easier mobilization of urogenital sinus
    - Many still argue that psychosocial rearing/bonding with parents is easier (no evidence)
  - Consensus
    - Surgery recommended for patients with high confluence by 2-6 months of age
    - Never remove clitoris, preserve neurovascular bundles
    - Females with CAH have low risk for gender identity problems
  - Evaluation before surgery
    - Defining vaginal confluence with UG sinus is most important step
    - Genitography is recommended for pre-operative planning
  - Procedure
    - Goals are to recreate normal appearance and function external genitalia
    - Preserving bladder function

SESSION 102: GENETIC ASSOCIATION STUDIES

- High sensitivity = genotype catches all cases, Low Specificity = many unaffected have same genotype
- Low sensitivity = miss the correct genotype for cases, High specificity = avoid misdiagnosing unaffected
- Clinical utility
  - Is it sensitive and specific?
  - Does it provide predictions of disease occurrence and outcomes?
  - Does it replace or contribute to other clinical diagnostic tests?
  - Is it cost effective?
- What tools do we have?
  - Genome variations associated with disease (DNA)
    - Genome Wide Association Studies – GWAS
      - Manhattan plot shows SNP association with disease vs control
      - Pros: high throughput screening with no needed hypothesis, use of haplotype blocks and LD can simplify search, can show significant association even if low penetrance
      - Cons: multiple testing error = 1/20 association may occur by chance, need to overcome false positives by large case-control numbers, may identify a haplotype block not a gene or specific cause, OR may be low association, may have significant association and NO UTILITY
    - Make use of HapMap information
  - Gene expression profiling (mRNA)

- Whole genome sequencing

SESSION 103: CANCER CYTOGENETICS

- **Constitutional** Abnormalities – germline; in most cases, arise during meiosis
- **Acquired** Abnormalities – acquired in association with the development of a malignant process (sometimes in utero, usually after birth)
- Most cancer cells have associated chromosomal abnormalities
  - Abnormalities are acquired, clonal (two or more cells have the same abnormality), limited to the tissues involved in the malignancy
  - Identification of abnormality is important for diagnosis, (gene-product targeted) therapy, prognosis
- **t(9;22) translocation**
  - Example karyotype: t(9;22)(q34;q11.2)
  - Diagnosis: *Chronic myelogenous (myeloid) leukemia (CML)*
  - Derivative chromosome 22 is called **Philadelphia chromosome Ph+**
  - Translocation results in fusion of the breakpoint cluster region (**BCR**) in 22q11.2 to the Abelson murine leukemia virus oncogene (**ABL1**) in 9q34
    - BCR-ABL1 fusion on 22 is Philadelphia chromosome
    - ABL1-BCR on 9 is not involved in leukemia
    - Can be detected by FISH
    - Translocation results in the formation of a **novel (chimeric) BCR-ABL gene** that encodes a protein with altered tyrosine kinase activity
  - Cytogenetics used in CML
    - Confrism diagnosis, determine stage, monitor response to therapy
  - Natural history of CML
    - **Chronic phase**: t(9;22) is typically the sole abnormality, symptoms very mild
    - **Accelerated phase**: 75% of cases gain additional abnormalities (e.g. +8, i(17)(q10), +der(22)t(9;22)), secondary abnormalities are no consistent but some are commonly seen
    - **Blast crisis**: additional abnormalities typically present as in accelerated phase, lethal if not treated
  - Current therapies
    - Hematopoetic stem cell transplant
    - Gleevec - Therapy targeted at the specific abnormal tyrosine kinase generated by the t(9;22)
      - Don't know if individuals need to take therapy their whole lives
- **t(15;17) translocation**
  - Example karyotype: t(15;17)(q24.1q21)
    - **PML** (promyelocytic leukemia) gene on 15q24.1 fuses with **RARA** (retinoic acid receptor alpha) on 17q21
    - RARA transcription factor regulating transcription of genes important in the maturation of white blood cells beyond the promyelocyte stage
    - PML acts as a tumor supressor
  - Diagnosis: *Acute Promyelocytic Leukemia*
    - Arrest in development of promyelocytes, have fibrous-looking rods in cytoplasm
  - Recognition of the PML-RARA fusion is critical for care
    - APL associated with coagulopathy
    - Targeted therapy: **ATRA + Arsenic + chemo**
    - Recent study in blood: 98% sustain complete remission
    - ATRA should be given within 24 hours of diagnosis – given to patients when PML is suspected
- **t(4;11)(q21;q23) translocation**
  - Presentation: somewhat elevated WBCs, very high circulating blasts, anemia
  - A specific recurring abnormality
    - Accounts for 60% of cases of **acute lymphoblastic leukemia of infancy**
    - Very poor prognosis – immediate planning for bone marrow transplant
  - **MLL** gene is a human homolog of Drosophila trithorax gene that regulates HOX genes
  - **AF4** is thought to be involved in lymphocyte development
- **High hyperdiploidy** (>52 chromosomes)
  - Associated with **B-cell lineage acute lymphoblastic leukemia** of childhood
  - Very favorable prognosis
  - Presence of trisomies for 4,10 now used to stratify to a 'low risk' leukemia therapy group

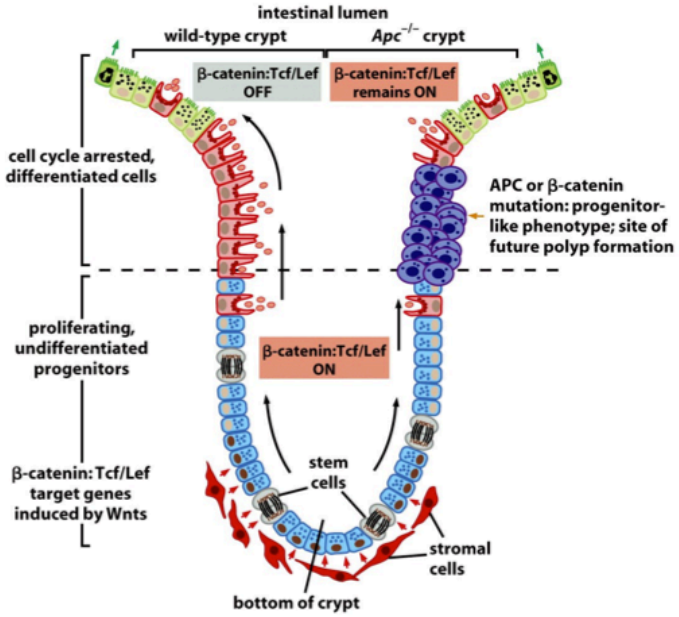
- Case 5: 22 yr old female, B-acute lymphoblastic leukemia
    - Presentation: hypercellular marrow, very high blasts, B markers present, normal G-banding cytogenetics, normal FISH for the recurring abnormalities
    - Array showed 21 abnormalities not shown through G-banding
    - Found 7 important abnormalities
        - Small deletion in 7p12.2: **IKZF1** gene
            - Associated with **increased risk of relapse and adverse events**
            - 74% will relapse
        - Small deletion in 9p13.2: **PAX5** gene
- Recent studies show some patients with high-risk, active lymphoblastic leukemia have mutations affecting tyrosine kinase and cytokine signaling → can be targeted
- **HER-2/neu**
    - c-*erb* B2
    - A proto-oncogene
    - Encodes a tyrosine kinase receptor
    - The ligands of HER-2/neu and related growth factor receptors are known as *heregulins*
    - HER-2/neu amplification
        - Pts with multiple copies of the gene in tumor tissue had a shorter time to relapse and a shorter overall survival
        - Amplification occurs in 25-30% of human breast cancers, also in some ovarian malignancies
    - Therapy
        - Use of recombinant anti-HER-2 monoclonal Ab (**Herceptin**/trastuzumab) together with cisplatin → clinical response in patients with HER-2 overexpressing metastatic breast cancer refractory to other chemotheraputic regiments
        - mAb therapy may also increase efficacy of radiotherapy and other chemotherapeutic agents

SESSION 104: STEM CELLS

- Two major categories of cells in adults
    - Cells that are formed to last a lifetime (ex. Auditory hair cells)
    - Cells that are replaced
        - By simple duplication: differentiated cells divide (ex. Differentiated hepatocytes in liver, beta cells in pancreas)
        - From stem cells: to replace cells that undergo rapid turnover (ex. Blood, skin, intestine)
- **Stem cells**
    - Cells that can reproduce themselves as well as generate specific types of more specialized cells
    - Properties
        - Can undergo endless asymmetric cell division
        - No replicative senescence (telomerase continually expressed)
        - Each daughter cell can remain a stem cell or commit to a pathway that leads to terminal differentiation
        - **Self-renewal** is the ability for a cell to proliferate in the same state
        - Note: Rb is always phorphorylated (remember??)
    - Asymmetric division
        - Not fully understood
        - Can be *environmental* (due to different environment of daughter cells) or *divisional* (division of RNA, proteins is asymmetric between daughter cells)
- **Progenitor cells** (transit amplifying cells)
    - Staged between stem cels and differentiated cells
    - Also called **transit amplifying cells** since they usually divide while 'transiting away' from the stem cell niche
    - Related to stem cells but do not have the unlimited capacity for self renewal
    - Usually more differentiated than stem cells and have become committed to a particular cell type
    - Use of progenitor cells keeps the number of stem cells low and slowly dividing
        - Reduces the potential for genetic damage and cancer
    - Example: Hematopoietic Stem Cells
- Stem cell '**Potency**'
    - **Totipotent**: can form every cell type including the trophoblast cells of the placenta (zygote)
    - **Pluripotent**: can form every cell type except trophoblasts (ESCs)

- **Multipotent**: can form a limited number of adult cell types
- **Unipotent**: can form only one cell type

- Normal stem cells grouped into:
  - **Embryonic (pluripotent) stem cells**: capable of developing into all cell types of the body, isolated from **inner cell mass** of blastocysts
  - **'adult' stem cells**: involved in replacing and repairing tissues of a particular organ, can typically forms only a limited subset of cell types, multipotent or unipotent
- Adult Stem cells
  - Many/most? Adult organs contains committed stem cells
  - Difficult to identify, isolate, purify as they are rare (estimated .1-3% of cells)
  - Low rate of cell division, do not proliferate readily
  - In use medically → ex: bone marrow transplants are essentially stem cell transplants
  - Tissues and organs that undergo continual renewal (skin, intestine, breast, blood, etc) contain stem cells in areas known as adult **stem cell niches**
    - Allows for controlled stem cell proliferation, differentiation of progeny that leave niche
    - Produce **paracrine factors** that regulate proliferation, prevent differentiation, when cells leave niche they begin differentiation
    - Intestinal niche: Wnt signaling maintains stem cell niche

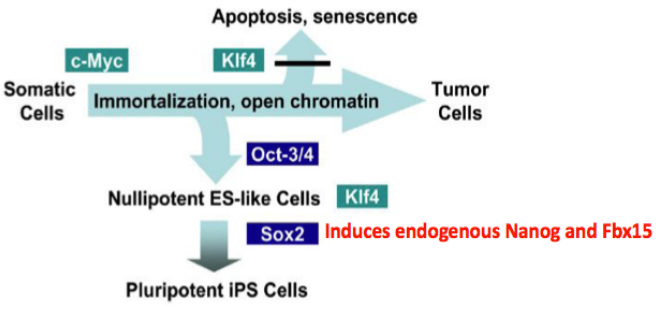


- Other sources of multipotent stem cells:
  - **Mesenchymal Stem cells (MSCs)**
    - Multipotent
    - Found in several adult tissues: bone marrow, adipose, dental pulp, breast milk, intestine
    - Able to give rise to numerous mesenchymal (stromal) cell types: bone, cartilage, muscle, fat
  - **Amniotic epithelial cells**: from the amniotic membrane in human term placenta
    - Do not express telomerase and therefore do not make teratomas after transplantation
    - Express markers that are present on ESCs, can differentiate into 3 germ layer *in vitro*
    - Not used extensively now but might prove to be a source of SCs for tissue regeneration
  - **Fetal stem cells**: found in the organ of fetuses
    - Are somewhat more differentiated than ESCs, generate a limited number of cell types
    - Do not form teratomas *in vitro*
    - Up to 12 weeks, cells have less chance of rejection than cells derived from umbilical cord, marrow
    - Even more controversial than ESCs
  - **Umbilical cord stem cells:** Derived from the umbilical cord epithelium and cord blood
    - More primivitive subpopulation of mesenchymal stem cells than bone marrow, less likely to generate host response
- **Embryonic stem cells**
  - All cell types can be generated (in theory) from ES cells
  - Most differentiated cells express only 10-20% of genes, ESCs express 30-60%
  - Thought to be due to accessible chromatin structure
  - Low-level expression of many cell surface receptors, enabling them to respond to many signals
  - **DNA methylation** pattern is critical
    - DMRT1 remethylates DNA after it becomes demethylated in the zygote
    - Remethylation is required for pluripotency
  - ES cells rely on 'master' transcription factors
    - **Oct4, Sox2, Nanog** activate genes encoding proteins and miRNAs for self-renewal and pluripotency and repress genes that induce specific differentiation pathways
- Possible use of ES cells therapeutically to restore or replace damage tissue → research and controversy

- Undifferentiated ES cells can form *teratomas* (can contain hair, teeth, bone, eyes, limbs, etc), so ES cells must ALL be differentiation before implantation
- Primary experimental sources
  - **Therapeutic cloning** (somatic cell nuclear transfer = SCNT)
    - Involves replacing the genome of an oocyte with that of an adult cell
    - Need an oocyte donor and a nuclear donor
    - Remove egg → remove spindle apparatus → transfer nucleus into enucleated egg → egg and cell fused with electric current → culture embryo
    - Ex: Dolly the sheep, BUT she died, thought that she was actually the chronologic age of the mother because of telomere shortening
    - Advantages: reduces ethical concerns as doesn't involve ESCs, no need to identify and clone genes to screen for traits of interest
    - Disadvantages: very inefficient, clones have medical problems (bad epigenetic programming), human oocytes manipulated to SCNT don't develop to blastocyst stage, some concerns about obtaining human oocytes
      - One study was able to get to blastocyst stage by injecting fibroblast nucleus into haploid cell → triploid cell
  - **Excess eggs** from *in vitro* fertilization (IVF)
    - Ethical debate regarding the use of ESCs from IVF embryos
    - Pro: ESC research fulfills ethical obligation to alleviate human suffering, IVF embryos will be discarded anyway
    - Against: ESCs are taken from a blastocyst that is then discarded (murder?), risk of commercial exploitation of participants, ESC research will lead to human cloning

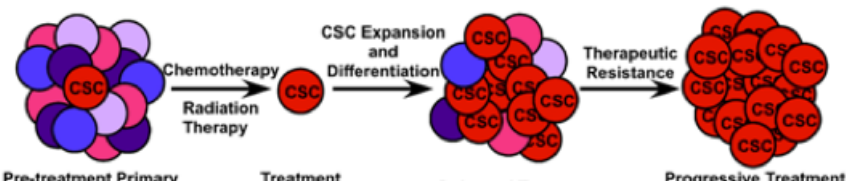
SESSION 105: STEM CELLS AND DISEASE

- Current role of stem cell therapy in regenerative medicine
  - Bone marrow, umbilical cord, and peripheral blood stem cells are the only SC therapies routinely available
    - Bone marrow transplant has been used to treat leukemias, other blood disease for 30 years
      - Recently being used experimentally for other diseases: epidermolysis bullosa
    - Umbilical cord has a relatively high number of MSCs, less prone to rejection
    - Peripheral blood SCs can be used instead of bone marrow, less invasive to obtain
- Potential role of stem cell therapy
  - Repair of degenerating or lost tissues
    - Repair of neurons for spinal injuries, cardiac muscles after MI, neurodegenerative diseases
  - Gene therapy for diseases
    - Current treatment for muscular dystrophy, Type 1 diabetes, leukemias and other hematopoietic disorders, but potential for treatment of many other diseases
- The 'Selling' of Stem Cells
  - Illegal since 1984 to sell any body parts (National Organ Transplant Act)
    - BUT does not apply to blood stem cells obtained by apheresis – used in 2/3 BMTs
    - Problem for poor because of pressure to sell body parts (blood, plasma not covered)
  - Why allow the sale of stem cells (or body parts)?
    - Very difficult to obtain matches, especially for mixed race individuals
    - Children with with leukemia and aplastic anemia are in desperate need of stem cells
- **Induced pluropotent stem cells (iPCSs)** – most prominent type of stem cell therapy
  - Can be 'made' from multipotent cells by forcing the expression of certain transcription factors
  - First done in 2006 by Shinya Yamanaka (nobel prize in 2012) with cocktail of *4 transcription factors*
    - Transformed a mouse fibroblast into a cell that appeared identical to an ES cell
    - Transcription factors **c-Myc, Klf4, Oct-3/4, Sox2**
      - Klf4 binds to beta-catenin, activates telomerase gene
      - Oct-3/4 induce Sox2, which induces Nanog and Fbx15



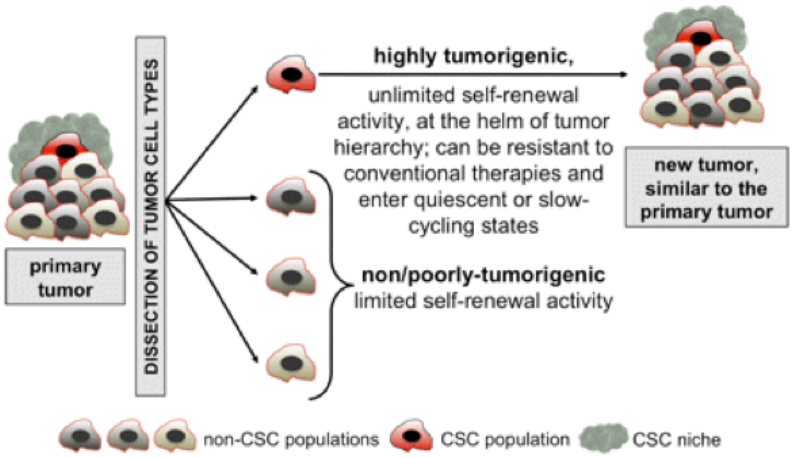
- Note: dedifferentiation and regeneration has been known for some time in lower organisms
  - Ex: 'immortal jellyfish' dedifferentiates back into an amoeba-like blob and then regenerates back into a jellyfish
- *Testing for pluripotency*
  - When aggregated together, cells form a teratoma: tumor-like structure with all 3 germ layers
  - Transcription and DNA methylation pattern were found to be almost identical to that of normal mouse ESCs (the concordance between the two varies with the cells and the labs)
- Curing a human disease in mouse using ePSCs
  - Humanized sickle cell anemia mouse model
    - Harvest tail tip fibroblasts → infect with Oct4, Sox2, Klf4, c-Myc viruses → correct sickle-cell mutation in iPS cells by specific gene targeting → differentiate into embryoid bodies → transplant corrected hematopoetic precursors back into irradiated mice
  - *In vivo* reprogramming of adult pancreatic exocrine cells to beta-cells
    - NGN3, Pdx1, Mafa transcription factors put into viral vector
    - Injected vector into exocrine pancreas cells → 'exocrine' pancrease cells produce insulin like beta-cells and *corrected diabetic phenotype!*
- Technical considerations for making iPSCs
  - Choice of factors, methods of factor delivery, choice of cell type, parameters of factor expression, derivation of conditions, identifications of iPSC colonies, expansion and characterization
  - Only about 1% of the cells de-differentiate completely, making it rather inefficient
  - Regulation of expression is key – difficult to regulate post-translationally regulated events
- Uses of iPSCs – huge potential in medicine!
  - Cell/organ therapies
  - Disease modeling – *neurological diseases*, cardiovascular modeling, hepatic
    - Can use diseased iPSCs to study disease (better than mouse models)
  - Drug development – especially for mixed races and races not commonly tested
  - Regenerative medicine
    - Can generate early stem cells that have the exact genotype of the patient
    - Can theoretically correct genetic diseases
    - Minimal ethical issues
    - Propensity to be tumorigenic (readily forms teratomas as with ESCs)
    - Likely to be *more* tumorigenic because of use of viral techniques for DNA insertion
    - Throughput is low; only a few cells are 'induced'

- Cancer stem cells (CSCs)
  - Some cancer may be considered a disease of stem cell regulation
    - Evidence is accumulating that indicates that tumors *can* arise from adult stem cells
    - Normal stem cells have a hierarchy of slowly dividing cells producing more differentiated cells
    - Cancer cells seem to be organized in the same way
      - Cancers of skin, intestine, blood are very frequent, yet the only cells that are around long enough to accumulate enough mutations are the adult stem cells
  - Thought that a stem cell may undergo oncogenic transformation, lose important homeostatic control mechanisms
  - Cancer stem cells may be responsible for cancer *recurrence* in some cases
    - CSCs appear to repopulate cancers through self-renewal and differentiation of all the tumor cell types
    - Origin of these CSCs is unclear
  - Few cells in tumors are tumorigenic
    - <1% chance that random tumor cell will generate a new tumor when transferred
    - Thought that few tumorigenic cells are cancer stem cells
  - Cancer Therapies
    - **Current therapies may promote CSC survival and propagation**



- Radiation and many chemotherapies target rapidly dividing cells, BUT CSCs would replicate infrequently → Thought therapies kill off the bulk of the tumor but NOT the cancer stem cells
- SCs and CSCs are naturally resistant to chemo agents as they have ABC transporters that pump out the drugs (so that the stem cells won't accumulate mutations) → only the susceptible cells die and the resistant cells live
    - Ex: paclitaxen in ovarian cancer

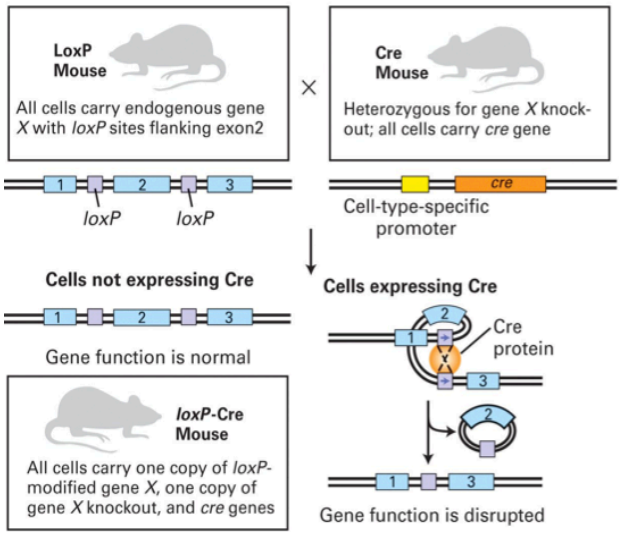
- Prevailing hypothesis is that **recurrent** cancers are largely the consequence of CSCs slowly repopulating the area



SESSION 106: GENETIC MODIFICATIONS IN MEDICINE

- Genetically Modified Organisms (GMOs)
    - Genome of an organism is modified by genetic engineering techniques
    - Routinely used:
        - Pharmaceutically important drugs (insulin, growth hormone)
        - Agriculture to enhance the resistance, storage, taste, amount of food products
        - Environment (clean up spills, 'sterile' mosquitoes)
        - Research purposes
        - Fun – GloFish, GFP Axolotls!
- **Transgenic Mice**
    - Have 'foreign' gene introduced into their genomes – **knockin**
    - Used to study 'normal' gene function in mammals and to model human diseases
    - The inserted DNA (**transgene**) usually confers gain of function of that gene
        - Random insertion of gene, doesn't typically disrupt genes
    - Typically done by pronuclear injection
    - Gene is inserted into an **Expression vector**, typically a bacterial plasmid
        - Requires promoter, multiple cloning site (MSC) with restriction sites where gene can be inserted, region that encodes a peptide that can be recognized by an antibody, resistance genes for screening purposes (such as ampicillin resistance marker for bacteria)
    - **Pronuclear injection**
        - Expression vector injected into male pronucleus via non-homologous recombination, then egg is transferred into foster mother
        - About 10-30% of offspring will contain foreign DNA in chromosomes of all their tissues and germ line, then can breed mice
    - Advantages:
        - Quick and easy, transgene usually not lethal so some phenotype will be observed
        - Used extensively for GMOs
    - Disadvantages:
        - Tegulation of the transgene is usually not normal, there can be 'dosage' effects because of the multiple gene copies
        - Endogenous gene is still active so looking for an effect 'on top' of the endogenous gene
        - Transgene protein must be identified separately from the endogenous protein
- **Null Mice**
    - A **knockout** is the germ line deletion of a specific gene
    - Advantages:

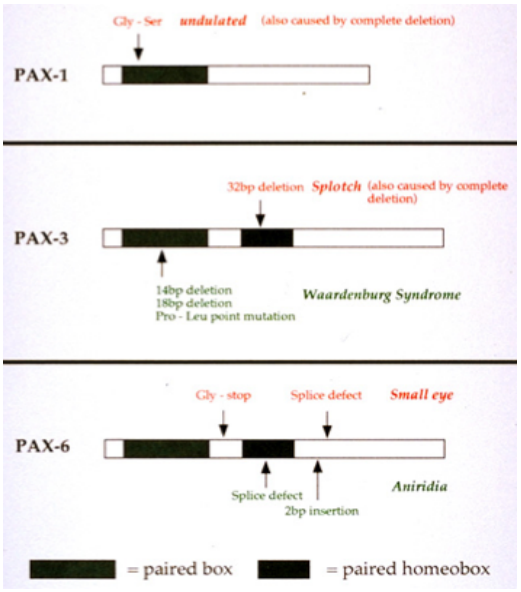
        - Very useful for exploring functions of genes as it assesses what happens in an organism when a gene is missing
        - Very useful to study development
    - Disadvantages:
        - Interpretation can be complicated by 'compensatory' increases in other genes
        - Deletion may be lethal, slow and a lot of work (and very expensive!)
    - **Creating gene knockouts** in mice
        - Gene introduced by homologous recombination such that the endogenous gene is removed and a new one is inserted – done is ES cells growing in culture
        - ES colony with knock out injected into early embryo, which is then implanted into foster mother
        - Breed heterozygous offspring to get the null animals and hope its not lethal
    - Creation of '**conditonal' knockouts**
        - Gene can be disrupted only in a specific tissue or at a specific time in development
        - Most commonly uses the **Cre-Lox system**
            - Target gene replaced by the same gene that is flanked by the LoxP sites, which are recognized by the Cre recombinase → recombinase enzyme then does site-specific recombination using the LoxP sites
            - Lox mouse in then mated with a Cre mouse that has the recombinase under the control of a tissue-specific promoter or one that can be induced
            - Ex: Estrogen receptor that is sensitive to tamoxifen, the Cre-Lox mouse can be injected with tamoxifen at any time, recombination is induced to remove the targeted gene, effects of the loss can be determined



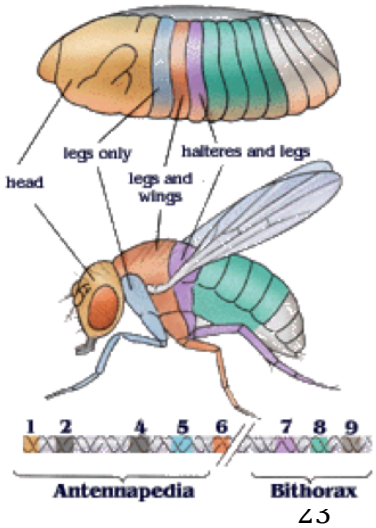
- Human gene therapy
    - Requires the same site-specific knockin and knockout technologies or analogous methods
    - Germline human gene therapy – illegal at this time
    - **Somatic human gene therapy**: therapeutic genes are transferred into the somatic cells of a patient
        - Becoming increasing useful, but not fulfilled expectations
        - Problems limiting somatic gene therapy
            - Vectors often have viral particles, leading to potential problems with toxicity, immune and inflammatory responses
            - Corrections are often transient (gene methylation?)
            - Chances of inducing tumors
            - Regulation and **delivery** are difficult
- **Cloning**
    - A 'clone' is a set of individuals that are genetically identical because they descended from a common ancestor
    - Human cloning refers to making an identical copy of a human individual
- Human reproductive vs. therapeutic cloning
    - **Reproductive cloning (SCNT)**: embryo generated is implanted into the uterus of a foster mother to create a new individual (could also use iPSCs to get embryo) → **human cloning**
        - Only require the genome of one individual, a form of *asexual reproduction*
        - Currently unsafe with about 95% of cloning attempts ending in miscarriages, stillbirths, etc
        - Cloned individuals are often biologically damaged
        - In the future, could replace of cherished loved one OR provide children for those who are sterile or homosexual
    - **Therapeutic cloning (SCNT)**: embryo generated is used as a source of ESCs → **cell or tissue regeneration**

SESSION 107: INTRO TO DEVELOPMENT AND DISEASE

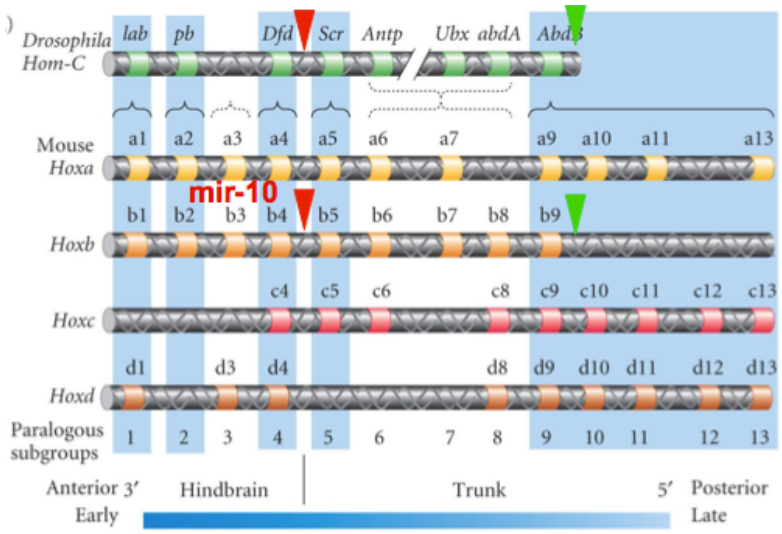
- Basic components of development
  - Increase is number of cells and size of organism
  - Increase in complexity with diverse cell types
  - **Patterning** (blueprint) and **morphogenesis** (construction)
  - Still occurs after birth
  - Influenced by both genes and the environment
- Animal models used to study development (for ethical reasons)
  - Experimental models (develop outside mother) – xenopus, chick
  - Gentic models: mouse and zebra fish (vertebrates), *c elegans* and *drosophila* (invertebrates)
  - Much of what we know about development is universal
    - 3 common germ layers: **endoderm**, **mesoderm**, **ectoderm**
    - 50% of human genes are conserved in *c elegans* and *drosophila*
- Essential genes in embryonic development (first found in *drosophila*)
- **Pair rule genes** are transcription factors
  - Vertebrate **Pax genes** – expressed in segments
    - Mutant Pax1: undulated mouse with shortened vertebral column
    - Pax3 homozygous mutants (splotch mouse) show spina bifida, brain/neural crest defects
    - Pax3 heterozygous similar to splotch mouse, pigment defects
    - *Waardenburg's syndrome* patients have mutations in the human homologue of the Pax3 paired box gene → allowed sequencing of human Pax 3 analog
    - **Synteny**: conserved organization of human and mouse chromosomes



- Regulating expression of different genes in **time and space** gives rise to diverse cells types
  - Regulated expression at *transcriptional, post-transcriptional* level, or *post-translational* level
    - Transcriptional – transcription factors (enhancers, etc), chromatin remodeling
    - Post-transcriptional level – RNA processing, inhibitory proteins, miRNA
    - Post-translational level – phoshorylation state, nuclear vs. cytoplasmic
  - Regulation of **master transcription factors** and **hox transcription factors**
- **Master transcription factors** – Cell specification/fate
  - Cell face specification: Ex. Fat cell vs. muscle cell
  - **Pax-6**
    - In *drosophila*, artificial expression in leg gives ectopic eye on leg
    - Can *induce undifferentiated cells* into an eye, required for eye formation
    - In humans, *aniridia* is caused by mutation in the pax-6 gene (black iris, poor vision)
    - Transcription factor, contains conserved motif for DNA binding (pax-1, pax-3, pax-6)
      - Paired box and paired homeobox
      - Mutation in these causes impaired function – required for DNA binding
  - **myoD** – master transcription factor for muscle
    - Fibroblast → myoD → muscle cell
- **Hox genes** – specification of cell identity (brain vs. spinal cord, what KIND of neuron) along anterior-posterior axis
  - Transcription factors that have a homeobox DNA binding region
  - Found in a cluster along the same region of the chromosome
  - Position of the genes in the homeotic complex corresponds to body segment
  - Mutations in Hox genes cause *homeotic transformation*

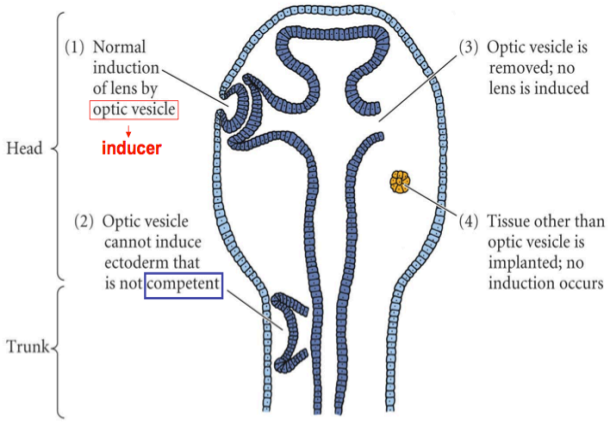


- Humans have multiple redundant copies of Hox genes, but can still have homeotic transformations
  - Ex: Hoxb-2 expressed from shoulder to legs, Hoxb-4 expressed from arms down
  - Hoxb-4 knockout mouse: C2 vertebra transformed into more anterior C1
- Hox genes and human disease
  - HoxD13: synpolydactyly – short, fused fingers
- **Retinoic Acid** is a teratogen – caused misexpression of hox genes
  - Causes homeotic transformation in the hind brain
  - Causes more anterior expression of Hoxb-1 in hind brain → rhomboid 2/3 intro rhomboid 4/5
- How is A/P pattern of Hox gene expression set up?
  - miRNAs play an important role in where Hox genes are expressed
    - miRNA-10 targets Hoxb4, miRNA-196 targets Hoxb8
    - Wherever these miRNAs are expressed, you get targeted destruction, silencing

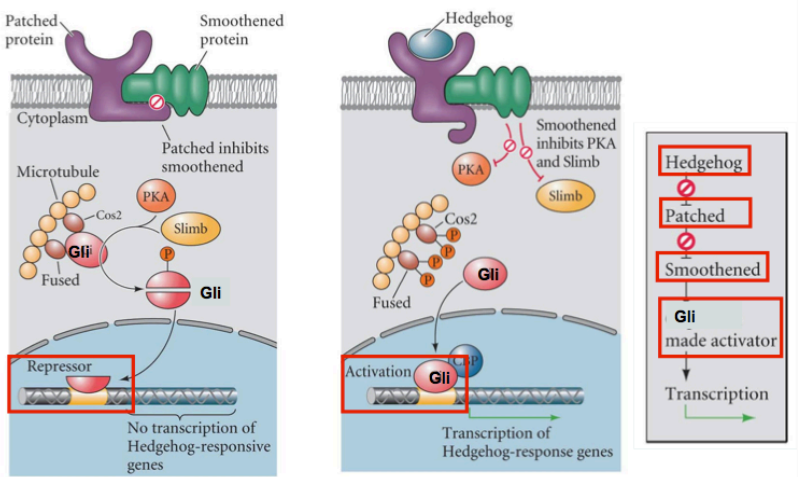


SESSION 108: SPATIAL AND TEMPORAL SIGNALING IN DEVELOPMENT

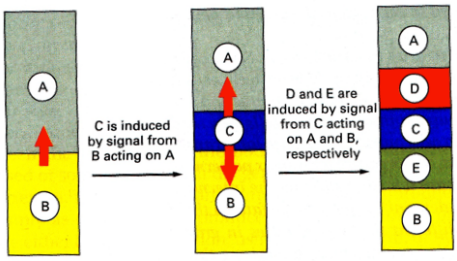
- **Induction** – inducer, responder, competence
  - A process by which one population of cells (**inducer**) affects the development of another (**responder**) through signaling
  - Two types
    - **Paracrine** – involve diffusible molecules
    - **Juxtacrine** – involve cell contact
  - Example: Eye development
    - Normal induction of lens by *optic vesicle* (inducer)
    - Only ectoderm in head region is **competent** to receive inducer signal and respond
    - Optic vesicle has high expression levels of **FGF8** → *inducing factor*
    - Head ectoderm expresses **pax-6**, required for ectoderm to be *competent* for lens induction



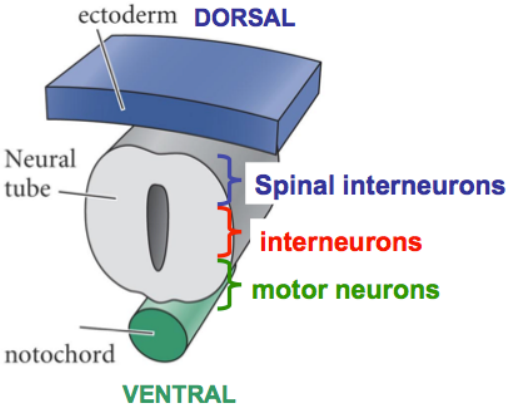
- Most embryonic inductions are mediated by secreted signaling factors
  - Can act on remote cells in paracrine fashion
  - Can form **gradient**, affecting cells differently depending on the concentration of the signaling factors
  - Always activate intracellular signaling pathways
- Four **key signaling pathways in development** - involved in INDUCTION
  - **FGF** pathway – binds to tyrosine kinase receptor, activates MAP kinase pathway; mutations typically affect bone development
    - **BMP7**: kidney and eye development, skeletal patterning
    - **BMP2**: heart development
    - **BMP8**: spermatogenesis
  - **Hedgehog** (Hh) – membrane receptor Patched → stops inhibition of Smoothened → Gii

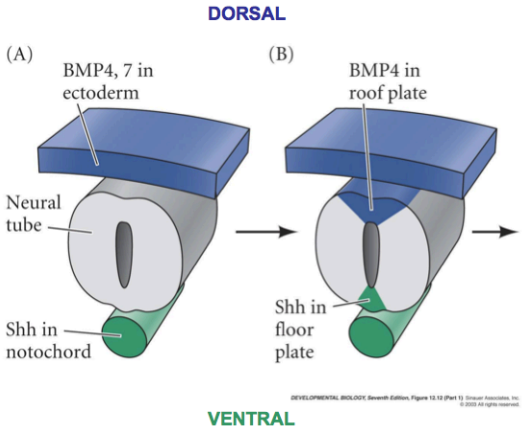


enters nucleus → transcription, Gli is either an *activator* or a *repressor*
    - **Wnt** pathway – Frizzled → Disheveled → inhibits GSK-3 → b-catenin not degraded
    - **TGFb** – Smads (see previous)
        - Associated with diseases of limb formation



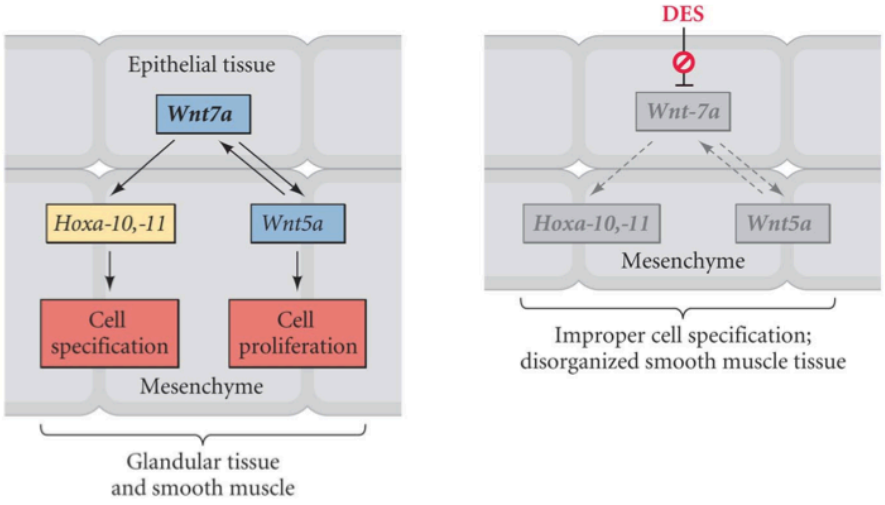
- Sequential inductive interactions lead to pattern formation
    - Specification of embryonic axis
        - A/P axis
        - D/V axis
        - L/R axis
    - *Eye development*
        - Optic vesicle induces formation of optic placode → lens placode induces formation of optic cup → lens capsule induces formation of cornea
    - Neural Tube Patterning – dorsal/ventral patterning





        - Two signaling pathways – **sonic hedgehog** (notocord) and **BMPs** (ectoderm)
        - Ventral patterning of neural tube induced by Shh secreted by notocord (HEDGEHOG signaling pathway) – D/V *concentration gradient*
            - High concentration in floor plate → motor neurons
            - Lower concentration towards roof plate → different types of interneurons
        - Dorsal patterning of neural tube induced by BMP4 and 7 secreted by epidermis and roof plate turns on TGFb signaling pathway
            - High concetration in roof
        - Gradients of the two paracrine factors on opposite ends of D/V axis results in production of different transcription factors, which specify different neuronal cell fates
- Hedgehog signal transduction pathway mutations
    - Gli truncation leads to **Pallister-Hall syndrome**
        - Gli is continually active as a repressor
        - Lots of digits – no proper patterning
    - **Greig cephalopolysyndactylyl** is due to loss of function mutation of Gli
        - Gli cannot act as an activator or a repressor
        - Megalocephaly, broad thumb, duplicated big toe – duplicated fused digits
    - Mutations in patched receptor lead to **Gorlin's syndrome/basal cell syndrome**
        - Overactivation of Hh signaling due to constitually active Smoothened signaling even without hedgehog ligand (Patched normally inhibits Smoothened in absense of Shh ligand)
        - Cancer due to overproliferation of cells in skin, eye
    - Loss of hedgehog molecule leads to **holoprosencephaly cyclopia**
        - Major developmental disorder
        - Affects *midline patterning*
        - Can be as mild as only having a single front tooth to having a single eye (cyclopia)
        - Can also be caused by *defects in the synthesis of cholesterol*
            - Hh requires cholesterol modification to create smooth Hh gradient through signal diffusion

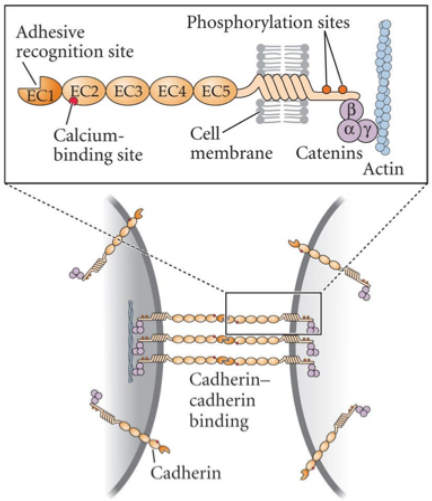
- Jervine alkaloid inhibits cholesterol synthesis → causes similar symptoms
- FGF signaling pathway mutations – generally affect bone formation and limb development
  - Crouson's, Pfeiffer, and Apert Syndromes
  - Achondroplasia – congenital dwarfism
- Wnt Signaling and Disease – generally gives rise to cancer
  - DES (diethylstilbestrol) – teratogen causing abnormal reproductive tract in fetus



    - DES inhibits production of Wnt7a in epithelial tissue
    - Loss of Wnt7a signaling means Hox and Wnt5a are not induced in mesenchyme
  - APC mutations lead to FAP hereditary colon cancer

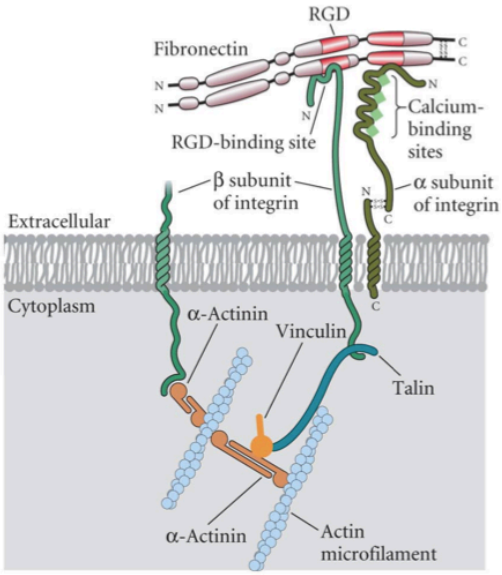
SESSION 109: MECHANICS OF MORPHOGENESIS AND CELL ADHESIONS

- **Morphogenesis**
  - How are tissues formed from populations, how do they migrate to the correct layer, etc…
- Three germs layers
  - Holfreter's experiment to show cells sort themselves
  - Reconstruction of dissociated skinc ells from 15 day mouse embryos
- Cell adhesion as a mechanism of morphogenesis
  - Generates boundaries between different cell types
  - Formation of tissues from individual cells, and maintenance of tissue integrity
  - Formation of organs and maintenance of organ integrity
  - Establish connections between different cell types that need to communicate (neurons and mucle)
- Cell adhesion molecules
  - **Cadherins** – large family (desmosomes, adherent junctions)
    - Classic cadherins: E-cadherin, P-carherin, N-cadherin
    - *Homophilic adhesion*
    - Injection of cadherin mRNA in xenopus embryo results in loss of cell adhesion
    - N-cardherin *establishes boundary* between neural and epidermal ectoderm
    - To form adhesion, *Ca2+* must be in solution and must be associate with <u>*actin cytoskeleton*</u>
      - Associated with cytoskeleton by *catenins* (remember Wnt pathway???)
    - Signaling pathways and disease (crosstalk)
      - Wnt signaling pathway → b-catenin: colon carcinomas, melanomas probably due to loss of adhesion, contact inhibition through crosstalk with Wnt signaling pathway



    - Diseases associated with cadherins – normally loss of classical cadherins leads to death of embryo due to importance in embryogenesis
      - E-cadherin: **<u>tumor malignancy</u>** (loss of contact inhibition → cell proliferation)

        - Demosomal cadherin: **<u>pemphigus vulgaris</u>** (autoimmune disease, Ab made to desmonsomal cadherin)
            - Skin, mucous membrane blistering due to loss of desmosomes in stratum spinosum layer of skin
        - Cadherin 23, protocadherin 15: **<u>usher syndrome</u>**
            - Common form of hereditary deafness – disorganization of hair cells due to loss of stereocilia connection by cadherin molecules
            - Retinitis pigmentosa
    - **IgCAMs** – very large family
        - Calcium independent
        - Extracellular globular domains held together by disulfide bonds
        - Mediate *homophilic* and *heterophilic* binding with other molecules, including extracellular matrix (collagen, proteoglycans, fibronectin, laminin)
        - Weaker adhesions than cadherins
        - Generally thought to be of importance for transient cell adhesion
        - Generally found in neuronal cells (NCAM) → important in axon guidance and neurite growth
        - Associated Diseases
            - <u>CRASH and L1</u> (IgCAM linked to actin cytoskeleton by ankyrin)
                - Corpus callosum hypoplasia
                - Retardation, mental
                - Adducted thumbs
                - Spastic paraplegia
                - Hydrocephalus
            - Autism, Schizophrenia, Cancers
    - **Integrins** – large family (hemidesmosomes)
        - *Heterodimeric proteins* composed of one alpha and one beta subunit
        - Cell-cell (typically *heterodimeric*) and cell-extracellular matrix (ECM) adhesion
            - Links EXTRACELLULAR matrix (via **talin, viculin, alpha-actinin**) and INTRACELLULAR actin cytokeleton
        - Dependent of extracellular divalent cations (*Ca, Mg*) for ECM binding
        - Much weaker than cadherins, but typically present in high concentrations (like Velcro)
        - Important in **cell migration**
        - Associated Diseases
            - Angiogenesis, Inflammatory diseases, cancer, myopathy
            - **<u>Epidermolysis Bullosa</u>**
                - Skin blister disease, may be fatal
                - Defect in cell adhesion, but defect in hemidesmosomes in basal layer of skin that connect cells to basement membrane
        - Play important role in cell migration – migration of neural crest cels, migration of blood cell precursors to liver, bone marrow, etc…



- Cell Adhesion are important
    - **Tissue boundaries**, cell sorting
    - **Epithelial adherens junctions** maintain tight protective layer
    - **Cell migration**, neural crest cells must migrate, requires cell adhesion molecules
    - **Axon guidance**, synapse targeting, and adherens (connection between neurons, muscle cells)
- Types of cell adhesion
    - Homophilic binding – one adhesion molecule binds to same molecule on other cell
    - Heterophilic binding – adhesion molecule binds to different molecule on other cell
- **Cell migration** as a mechanism of morphogenesis – *extension, attachment, translocation, de-adhesion*
    - Migration of cells during **gastrulation**
        - Migrating ectoderm forms endoderm and mesoderm
    - **Neural crest cells** undergo extensive migration

        - Neuroectoderm cells that for neurons, Schwann cells, pigment cells
        - Defects can give *piebaldism*, where pigment is missing from forehead, stomach
        - **Hirschspring's Disease** – congenital constipation of lower bowels caused by absence of ganglia that regulate preristalsis
    - How cells migrate
        - Cells extent filopodia, lamellipodia and form *contacts* with substratum
        - This connected in mediated by **integrins**
            - Helps create force/traction so cells can move against substratum
    - How do cells decide where to migrate?
        - **Haplotaxis**: migration based on changes in adhesiveness of the substratum
        - **Specific substrate guidance**: migration along a pathway made of a specific substance (eg laminin, collagen) – sensory neuron grows processes onto laminin but not collagen
        - **Chemotaxis**: migration regulated by a gradient of diffusible substances sensed by the cell via cell surface receptors (like bread crumbs) – neutrophil migration
    - Example: directing axon migration in neural tube
        - Floorplate neurons must migrate ventrally, then anteriorally
    - Chemotactic cues can be attractive and/or repulsive
        - Attractive: N-formylated peptides produced by bacteria attract neutrophils which sense cue via a cell surface receptor
        - Repulsive: **Slit** repels axons expressing the Slit receptor, **Roundabout** (**Robo**)
            - Slit found at midline, so axons/neurons expressing Robo won't be seen at midline
            - Slit knockout → axons with Robo found at midline
        - Both: **Netrins** are secreted by floorplate on the central part of neural tube, direction the ventral migration of some axons and repelling migration of others
            - Some neurons are attracted to Netrins, some are repelled
            - Why? Different neurons express *different receptors* → type of guidance molecule and receptor

SESSION 110: HUMAN MALFORMATIONS AND TERATOGENESIS

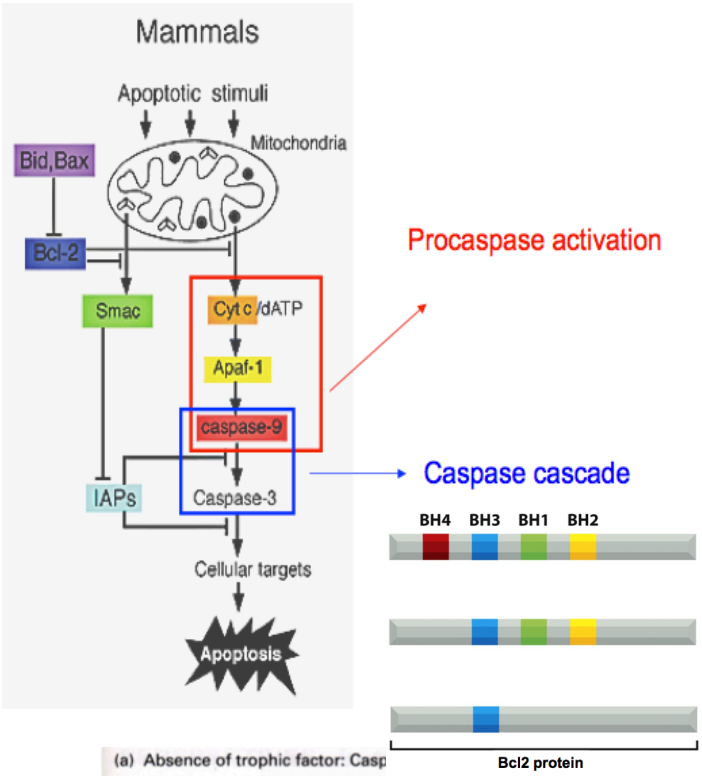
- General
    - Typically occur in the first 12 weeks of gestation – period of organogenesis
    - Birth defects are common: 1/30 babies are born with a 'birth defect'
    - 5th leading cause of death in children 5-14 years
    - 4th leading cause of infant mortality worldwide
    - Causes
        - 95% genetic – complex of multifactorial 50%
        - 5% environment – infections, prescription drugs, recreational drugs/ethanol, method of conception
- **Genetic** Malformations
    - Chromosomal basis of birth defects
        - Standard chromosome studies will be abnormal in about 4% of infants with birth defects
        - With array CGH up to 20% will be identified to have a significant chromosome abnormality
    - Single Gene Disorders
        - Although there are many single gene disorders that cause birth defects, there are no current means for screening multiple genes at once
        - Testing relies of ability of clinician to recognize pattern of malformations – syndrome
        - Syndrome Recognition
            - Clinical recognition of a constellation of findings that when identified together in a patient constitute a known condition
- We do not known the exact cause for the majority of birth defects - **Multifactorial**
    - The 'complex' or 'multifactorial' model is used to explain the majority of birth defects
    - Most common defects – congenital heart defects, cleft lip and palate, neural tube defects
    - **Neural tube defects**
        - 1930's – noted that women with good nutrition had lower risk
        - 1980's – found folic acid was protective, confirmed in early 90's through case control studies
        - Folic acid is now given prenatally to women to prevent NTD
        - Cereals and grains are now fortified with folic acid
        - Rates have decreased since fortification with folic acid

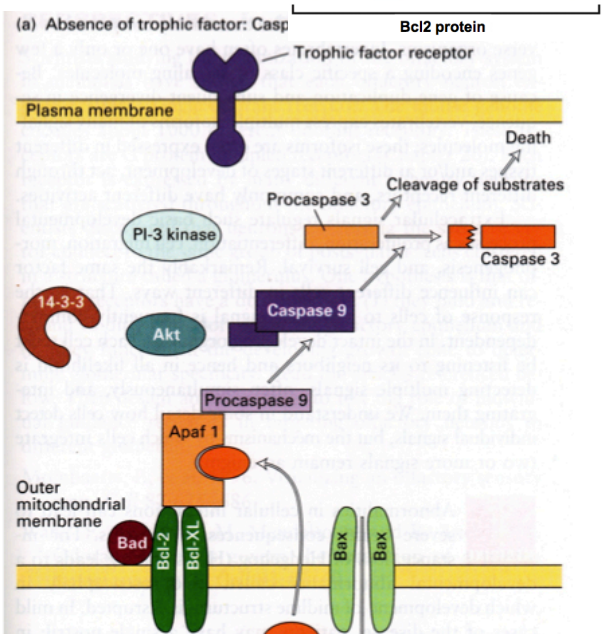
        - Hypothesis: since folic acid lowers the risk of NTD, then the genetic cause may lie in genes that encode proteins or enzymes that effect folate metabolism
            - Found that MTHFR polymorphism T/T homozygotes (10% of population) increase risk – homozygous mothers have relative risk of 1.6
        - May be *gene/ environment interactions*
    - **Cleft lip and palate**
        - Like NTD, there is an increased risk of cleft lip in palate in subsequent pregnancies (5-7% risk of recurrence)
        - Complex inheritance model
        - Multiple studies have shown an increased risk of cleft lip/palate to mothers who smoke – odds ratio of 1.3
- **Environmental** causes of birth defects - **teratogens**
    - Timing
        - There are critical periods in embryonic periods where embryo is most susceptible
    - **Alcohol** – 30-60% fetuses affected
        - Recognizable pattern of facial findings (short nose, flattened midface, thin upper lip, etc), growth delay, heart defects, cognitive disability and behavioral difficulties
        - Later exposure results in cognitive disability and behavioral difficulties without facial findings
        - There is no known safe amount of alcohol during pregnancy
        - Annual costs related to caring for ethanol exposed infannts is in the BILLIONS
    - **Prescription medications**
        - Isotretinoin – 30%
        - Thalidomide – 10%
            - Associated with severe malformations of their arms and legs
            - Thalidomide was used as a sedative to help pregnant women remain 'calm'
        - Warfarin – 8%
        - Diazepam – 1%
    - **Maternal Infections**
        - **Cytomegalovirus** (CMV)
            - 1% of infants are infected with CMV at birth
            - 10% of infected infants can have sequelae
                - Hearing loss, microcephaly, brain malformations, rash
        - **Rubella**
            - Common prior to universal immunization in the 1960s
            - Concerns with re-emergence in area with low immunization compliance
            - 90% of women infected during the first trimester will have infected offspring
            - Clinical Findings
                - Cataracts, deafness, brain malformations, microcephaly, rash (blueberry muffin baby)
    - **Assisted Reproduction**
        - 10% of couples experience infertility
        - Now ask mode of conception as part of history taking in genetics clinic
        - In vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSE)
            - Higher incidence of imprinting disorders (especially maternal imprinting): Angelman, Beckwith-Wiedmann
            - Higher incidence of cleft lip/palate, hypospadias, esophageal atresia, imperforate anus

SESSION 111: APOPTOSIS IN DEVELOPMENT

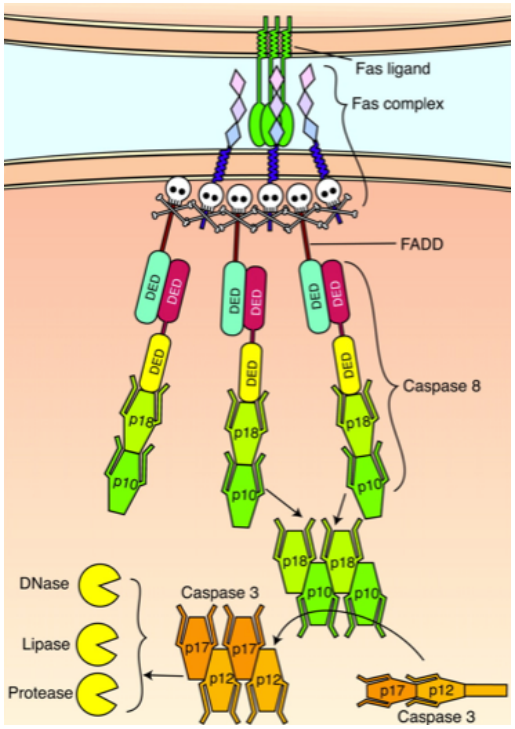
- Apoptosis is genetically programmed
    - **Apoptosis** is **programmed cell death** – genetically controlled
        - Cell membrane is intact, no cytoplasm leaks out
        - Genes are turned on to induce morphological changes – degradation of cell contents
        - **Chromatin compaction** gene causes DNA condensation and cleavage
    - **Necrosis** is cells that are damaged by injury or exposure to toxic chemical, cells swell, lyse, cause inflammation of surrounding tissues
- Apoptosis in tissue morphogenesis
    - **Sculpting tissue/organ**

        - Fingers, limb development
        - Tube formation (hallowing out)
        - Bone formation – hypertrophy and apoptosis of chondrocytes to for bone
    - **Deletion of unwanted structures**
        - Vestigial structures removed – like tadpole's tail
        - Tissue homeostasis – mammary secretory epithelial cells that increase during lactation die after weaning
        - Development of male and female reproductive organs (Mullerian and Wolffian duct)
    - **Regulation of number of cells**
        - Number of motor neurons are determined by size of target tissue to be innervated, neurons that don't 'make it' undergo apoptosis
    - **Elimination of damaged or harmful death**
        - 95% of thymocytes generated in thymus (self-reactive) die
        - Cells that have incurred DNA damage
    - **Production of specialized cells like lens epithelial cells, keratinocytes, RBCs**
        - Lens cells are actually dead – apoptosis of nucleus occurs but cell is not phagocytosed
- Caspase cascade
- Apoptosome
- **Suicide (intrinsic) pathway** via mitochondria
    - First discovered in *c elegans*
    - Induction of **procaspase** activation
    - Caspase-9 – protease, induces caspase-3 (**caspase** cascade)
    - **Caspase Cascade:**
        - Release of **cyt c** → binds to **Apaf1** → causes conformational change → → forms **apoptosome** → recruitment, cleavage of procaspase-9 → active caspase → activates other caspases (caspase-3) → cleavage of cytosolic proteins, nuclear lamins, etc…
    - **Bcl2 proteins**
        - Anti-apoptotic proteins (Bcl2, Bcl-Xl) – 4 BH domains
            - Binds to pro-apoptotic BH123 proteins to block pore formation → cyt c cannot escape for mitochondria
        - Pro-apoptotic BH123 proteins – 3 BH domains (Bax)
            - Found in mitochondrial membrane
            - Aggregation causes formation of pores to release cyt c → caspase cascade
        - Pro-apoptotic BH3-only protein – 1 BH domain (Bid, Bad?) – sequester Bcl2 away from Bax channel
    - Expression of Bcl-2 is dependent on the Mift transcription factor
        - Mift is activated by MAP kinase cascade → transcribes genes that ensures cell survival of melanocytes
        - Piebaldism results in loss of melanocytes regulated by the Mift transctiption factor
    - Bcl-Xl expression controlled by erythropoietin
        - Bcl-X regulates how many red blood cells go into circulation



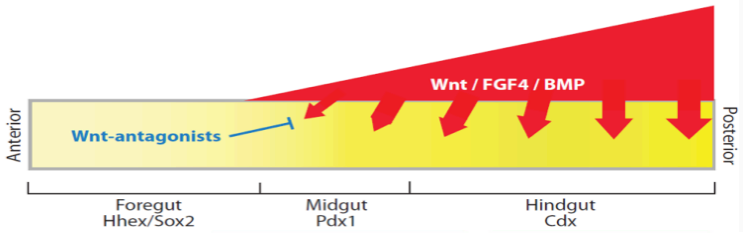


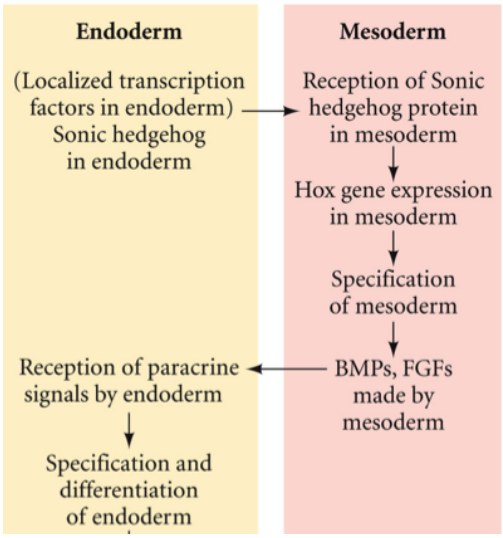
        - Binding of erythropoietin to receptor causes downstream transcription of Bcl-Xl → anti-apoptotic protein → more RBCs survive
- Signals inducing apoptosis
    - *Loss of trophic factors*
        - In presence of survival factors, Bcl2 production is increase → apoptosis blocked; inactivation of pro-apoptotic BH3-only Bcls protein
        - Absense of survival/trophic factors promote apoptosis
            - Activation of caspase cascade
    - *Damaged cells via **p53***
        - **PUMA** (p53 Upregulated Modulator of Apoptosis)
            - Pro-apoptotic Bcl2 protein – contains BH3 domain, binds to anti-apoptotic Bcl2 proteins
- **Murder (extrinsic) pathway** receptor mediated
    - Generally *induced by cytotoxic T cells*
    - T-cell receptor binds to histocompatability molecule → produced **FasL** ligand → binds to **Fas receptor** on target cell (ALL cells express Fas receptor)
    - Causes trimerization of receptors → **death domains** aggregate (allows formation of **DISC**) → recruits and actvates **caspase 8** → more caspases recruited (caspase 3)
    - Mutations in FasL or FasR or caspases in pathway, have defective apoptosis
    - FasL and immune privileged sites
        - Very little immune response in brain, eye, etc
        - Have physical structures AND immune blockers
        - Eye cells have FasL that is constitually active → induces apoptosis in immune cells that enter the eye
        - In these tissues, immune response (inflammation) would cause a lot of damage
- Homeostasis – balance between cell division and cell death
    - Too much cell proliferation causes cancer, SLE, rheumatoid arthritis, polycythemie
    - Too much cell death causes neurodegenerative diseases (huntington's, ALS), autoimmune diseases (AIDS), stroke, MI



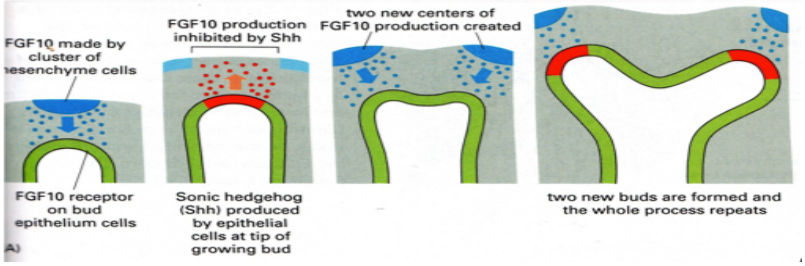
SESSION 112: ORGANOGENESIS

- How the endoderm gives rise to the **digestive** and **respiratory tracts**
    - Tissue becomes specified to a certain organ, then cell differentiation occurs
- **Gut development**
    - Differentiation into foregut, midgut, hindgut is caused by relative levels of Wnt, Fgf4, BMP signaling molecules
    - Different parts of gut express different transcription factors (see figure)
    - Know **Pdx1** → required for pancreas formation
    - As gut invaginates, Sonic Hedgehog (**Shh**) is expressed in hindgut first → turns on expression of specific **Hox genes**
    - Paracrine signals from cardiogenic mesoderm (**FGF**)
        - Highest levels received by *lungs*, then *liver*
    - **Liver formation**
        - Receives FGF signal from cariogenic mesoderm
        - Liver endoderm made competent to receive signal by BMPs
        - Induces formation of hepatocytes, begins expressing **Prox1** → allows cell proliferation

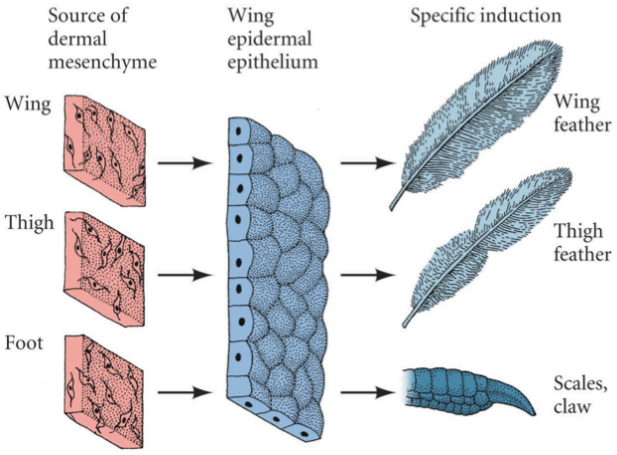




        - Reduces levels of **E-cadherin**
        - Proliferating cells need space, start proliferating into mesenchyme (which produces hepatocyte growth factors)
        - Prox1 mouse mutant: no cell proliferation, no cell migration, high levels of E-cad
    - **Pancreas formation**
        - 2 buds formed → one next to liver (ventral bud), one dorsal bud
        - Eventually migrate and fuse
        - Ventral endoderm
            - Low FGF, BMP, but does express **PDX1** (master transcription factor for pancreas)
            - Migrates to dorsal bud, where they fuse
        - Dorsal endoderm
            - Also expresses PDX1
            - Signaling from notocord, expresses **FGF2** and **activin**
            - Induces endoderm to *turn down Shh* → induces pancreas formation
                - Forcing Shh expression means dorsal pancreas doesn't form
        - Pdx1 transcription factor
            - Master transcription factor
            - Induces budding from the gut epithelium
            - Represses gene expression characteristic of gut tube other than pancreas region
            - Maintains repression of Shh in pancreas region of gut
        - Pdx1 and disease
            - Loss of Pdx1 results in loss of pancreas formation
            - Humans homozygous for Pdx1 mutation do NOT develop a pancreas → die
            - Patients heterozygous for Pdx1 mutation develop **MODY4 diabetes** (Pdx1 is also important in beta cell formation)
- **Lung formation**
    - **Tbx4** helps tracheoesophogeal folds to fuse
        - Loss of tbx4 leads to tracheoesophogeal fistula
    - *Mesenchymal interactions required for bronchial branching*
        - **Fgf10** found in mesenchymal cells → acts as a chemoattractant for lung epitherium
        - Tips of lung buds express Fgf receptor that responds to signal → migrate and grow towards cells expressing Fgf10
        - Lung bug epithelium exposed to Fgf10 expresses sonic hedgehog (**Shh**) → this signal diffuses to mesenchymal cells, which stop Fgf10 production
        - This leads to BRANCHING



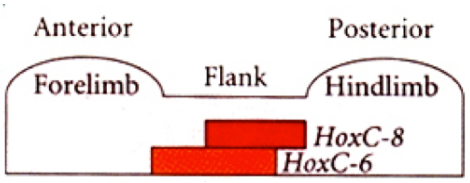
- **Epithelium-mesenchymal interaction** for organ fine tuning (found in many different tissues)
    - All organs have an epithelium (one of three germ layers) and mesenchyme (mesoderm or neural crest)
    - Epithelial-mesenchymal inductions display regional specificity; mesenchyme specifies the structure to be formed
        - In chick embryo, mesenchyme from wing, thigh, and foot transplanted with wing epidermal epithelium induces formation of wing feather, thigh feather, or scales, respectively
        - Epithelium is a 'blank slate' → induced to form different structures by mesenchyme



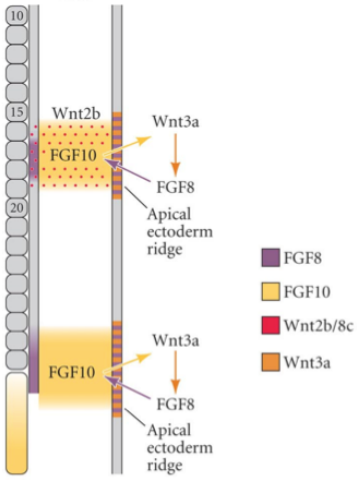
SESSION 113, 115: LIMB DEVELOPMENT

- Limb development is the most well understood organ development

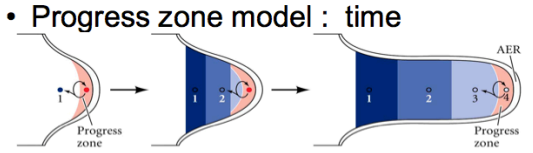
- Malformed limbs are not lethal, unlike malformation of many other organs
- Chick wing development is very similar to human limb development

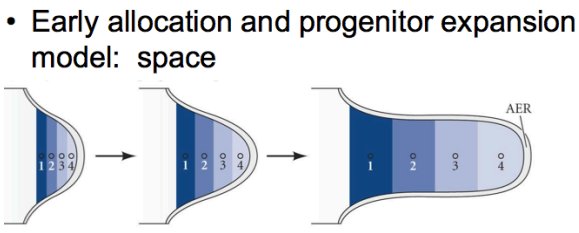


- **Initiation** (where limb growth starts)
  - What determines **where** limbs are formed? → Hox genes (important in patterning along A/P axis)
    - Hox genes specify *region* of the embryo, then master transcription factors are turned on
    - Most anterior region where **HoxC-6** is *not expressed* is where forelimb develops
    - Most posterior region where there is no **HoxC-8** expressed is where hindlimb develops
  - What induces limb bud formation?
    - High expression of **FGF10** in mesoderm where limb bud develops
    - First expressed uniformly, then localized to where limbs develop (probably based on Hox genes)
    - Induces epithelium (**apical ectodermal ridge**) to express **Wnt3a → FGF8** → signals back to mesoderm to keep expressing **FGF10**
    - Ectopic FGF10 can induce additional limb formation
  - What specifies fore vs. hindlimbs?
    - **Tbx5 (forelimb)** and **Tbx4 (hindlimb)** are important transcription factors
    - Ectopic FGF10 between fore and hindlimb induces formation of *chimera limb* that expresses both Tbx5 and Tbx4
    - <u>Clinical: Holt-Oram syndrome</u>
      - Patients are heterozygous for mutations in Tbx5
      - Also known as heart-hand syndrome
      - Heart and upper limb malformations: absent thumbs, distally placed, duplicated, or triphalangeal thumbs
      - Partial or total absence of forearm

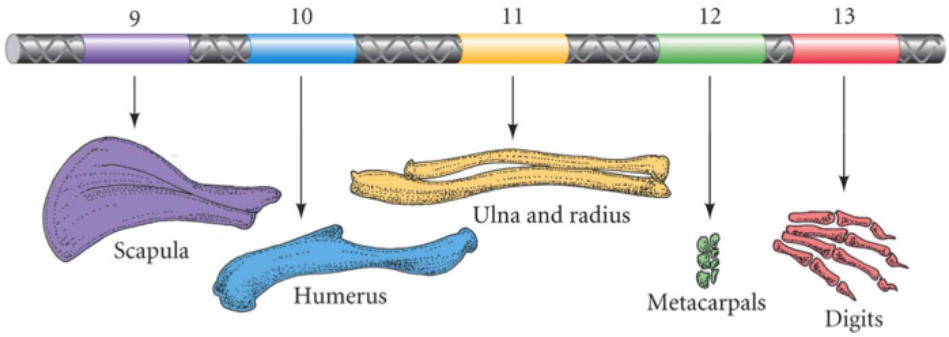


- **Proximodistal** axis: FGF pathway
  - Mesenchymal cells hold a lot of information, epithelium is a 'blank canvas'
    - '**Progress zone**' (regional of mesenchymal cells) that undergoes proliferation
    - Forms all cells of limb
    - **Apical ectodermal ridge** responds to FGF signaling from 'progress zone' (see above)
      - Produces Wnt3a and FGF8
      - *FGF8 is required to maintain cell proliferation in progress zone* (also maintains FGF10 expression) *but contains no positional information*
    - <u>Mesenchyme induces and sustains AER and specifies type of limb; AER sustains outgrowth and development of limb and directs P/D growth</u>
    - AER *only* maintains proliferation of progress zone – implantation of old AER into young limb bud results in a normal limb (vice versa with young AER)
    - Positional information lies with mesenchyme (progress zone) – implantation of old limb bud onto young limb bud results in only distal structures
  - How does mesenchyme specify the proximal-distal axis?
    - **Progress zone model: time** - how much time is spent going through cell division determines what kind of structure develops (proximal vs distal)
    - **Early allocation and progenitor expansion model: space** – there are four regions of limb bud, each region just expands in size as limb grows (cell fate is specified from the beginning to become proximal vs distal stuctures)
  - **Hox** (A and D) **genes** specify the P/D regions of the limb (cell fate)

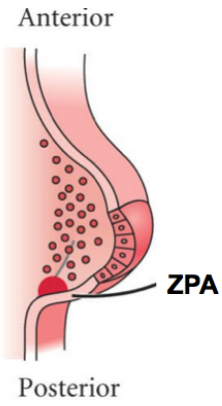




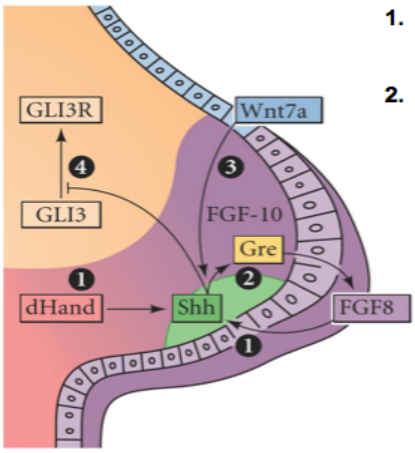
- Mutations in HoxD13 give mutations in most distal part of arm (digit formation)
- Deletion removing HoxD cluster leads to severe developmental defects in distal regions (11, 12, 13 → ulna, metacarpals, digits)



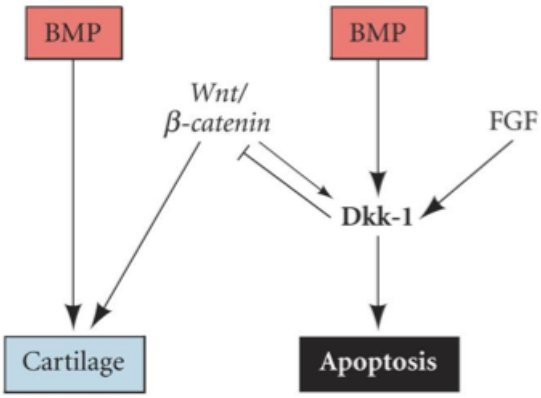
- **Anteroposterior** axis: SHH pathway
  - Anterior = thumb; posterior = pinky
  - **Zone of polarizing activity (ZPA)** – region of the limb mesenchyme in the posterior limb bud
    - Required for A/P patterning of the limb
    - Secretes **sonic hedgehog (SHH)** (induced by FGF8 secreted by AER)
      - Increases **activator Gli3:repressor Gli3 ratio** with the highest levels of activator in the posterior
      - Initiates and sustains a gradient of **BMP** (2, 7 - TGFb family) signals in interdigital mesoderm → specifies digit identity
      - *BMPs induced in webbing between digits specify digit identity*
  - Clinical: SHH misexpression and congenital limb defect
    - SHH contains enhancer far away (in different gene); important for direction expression of SHH
    - Mutations in enhancer lead to limb malformation → extra digits, mirror imaged limbs
    - Gli-3: Grieg cephalopolysyndactyly and Pallister-Hall syndrome with polydactyl, abnormal facial, cranial formation



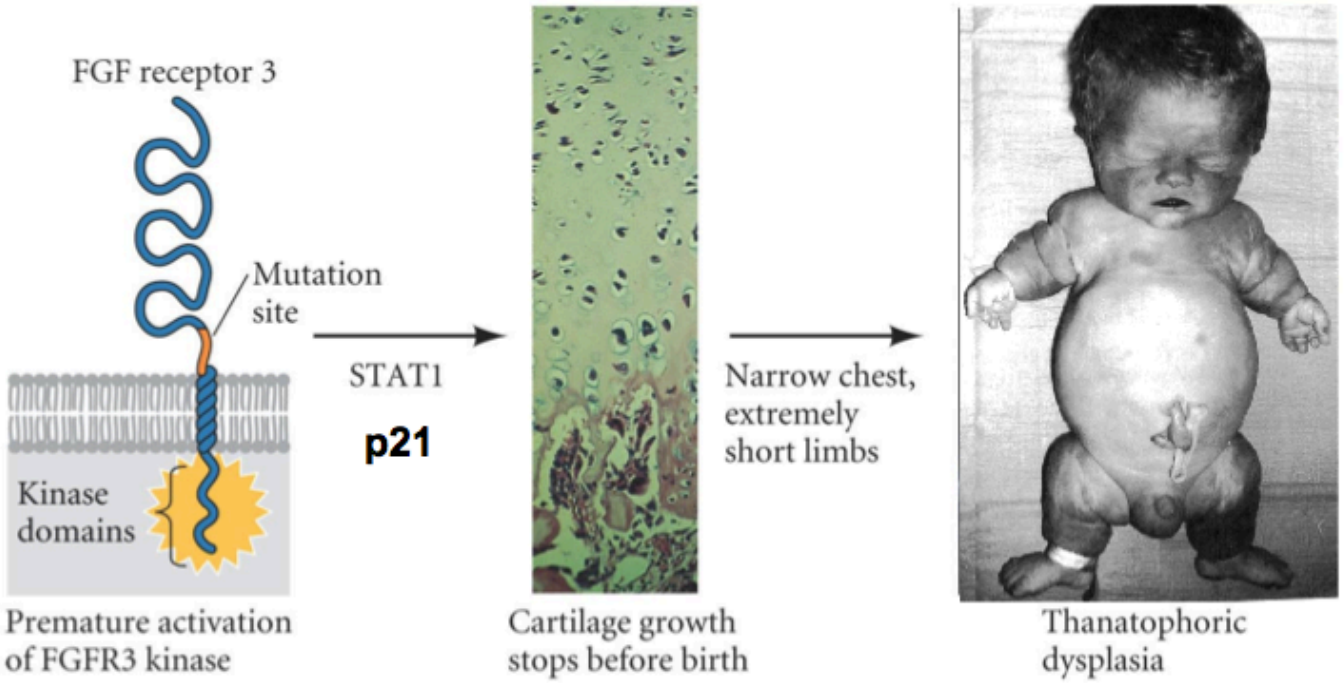
- **Dorsoventral** axis: Wnt pathway
  - Dependent on surface ectoderm
  - Wnt7a is expressed in the dorsal but not ventral limb ectoderm
  - 180 degree rotation of ectoderm partially reverses polarity on distal structures
  - **Lmx1** transcription factor, activated by Wnt7a
    - Specifies dorsal identity in limbs
    - Expressed only in the dorsal limb mesenchyme
    - Ectopic expression of Lmx1 in ventral limb mesenchyme induces dorsal phenotype
    - Lmx1 mutant mouse knockout results in mice with no dorsal limb
    - Clinical: Nail-patella syndrome – dominant disorder resulting from mutations in Lmx1 gene
      - Dorsal tissues are partially ventralized
- **Coordinating three axis**
  - Fgf8 is required to induce Shh expression, thus coupling A/P patterning to P/D growth
  - Wnt7a is required to maintain Shh expression, coupling A/P patterning to D/V
    - Knocking out Wnt results in abnormalities of D/V patterning and results in loss of 5th digit as well
  - AER forms in response to FGF10 only where dorsal and ventral ectoderm are juxtaposed, coupling P/D growth to D/V patterning
- **Morphogenesis** – adhesion, apoptosis, migration; occurs after patterning, limb bud formation
  - Sculpting the autopod (hand)
    - Cell death is required
    - Apoptosis occurs in intidigital regions, as well as space between radius and ulna



        - **BMPs** are important for inducing cell death
            - Inhibition of BMPs (by noggin) maintains interdigital tissue
            - Also involved in inducing chondrocyte formation
            - Note: *BMP turns on FGF pathway*
        - TGFb/BMP signaling and disease (loss of GDNF?)
            - Symphalangism – loss of second phalanges
            - Brachydacyly – fusion of joints (no cartilage formation)
    - Bone formation
        - Bones must undergo apoptosis and chondrocyte formation
        - Apoptosis shapes bone



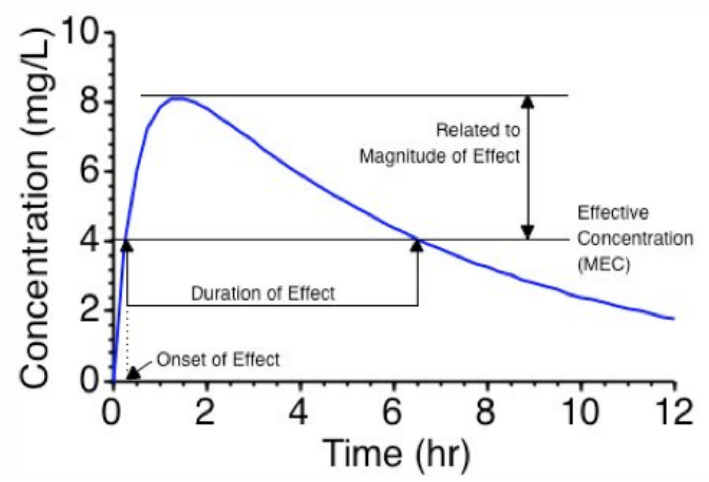
- FGF and Disease
    - Directs chondrocyte formation, and thus is important in bone formation
    - Mutations give Pfeiffer syndrome (early closure of sutures, very severe), Crouzon syndrome, Apert Syndrome (includes syndactyly), achondroplasia (short limbs due to disturbed growth)
    - Activation of FGF receptor is constitutive after mutation → leads to **p21** activation → brings cells out of cell cycle (Cdk inhibitor) → leads to cartilage growth stopping early → stunting of growth



SESSION 116: PHARMACOGENOMICS

- What happens to a drug when it enters the body?
    - **A**bsorption
    - **D**istribution
    - **M**etabolism
        - Biochemical modification, *xenobiotic metabolism* often converts lipophilic chemicals to more readily excreted polar products (p450s)
        - Metabolism can result in *activation* or *deactivation* of drug
    - **E**xcretion
    - **ADME** process – each step is affected by genetic differences in people

        - Receptors, ion channels, transport molecules, signaling pathways, metabolic pathways – all part of GENETIC PROGRAM of an individual
- **Pharmacokinetics**
    - Time course of drug and metabolite levels in different fluids, tissues, extreta of body
    - Different for different individuals
    - Drug dose can be represented as the *area under curve (AUC)*
    - *Therapeutic window* – amount of medication between the amount that gives the effective dose and the amount that gives more adverse effects (toxicities) than desired effeects
- **Pharmacodynamics**
    - Biochemical and physiological disposition of the drug within the body (often related to receptor interactions and transport)



- Example: Cetuximab
    - An EGFR inhibitor, given by IV for treatment of metastatic colorectal cancer, head/neck cancer
    - About 75% of metastatic colorectal cancers have EGFR+
    - BUT…40% do not respond to Cetuximab – have an activating mutation in the RAS gene
        - EGFR signaling is not required to activate RAS pathway
    - *Genetic testing recommended to look for mutations in RAS before prescribing Cetuximab*
- **Pharmacogenomics**
    - Using what we know about genetic variations in individuals to predict drug response in an individual
    - FDA currently has about 80 recommended companion genetic diagnostics with drugs
- Drug metabolism
    - *Phase I Enzymes*: add or expose polar groups to inactivate enzymes or activate proenzymes by oxidation, reduction, hydrolysis
    - *Phase II Enzymes*: conjugate other molecules to drug (methylation, sulphation, acetylation, glucuronidation) to increase mass of drug → most often inactivate drug
- Drug dose and response is related to genetic variations
    - Ex: Prozac overdose in 9 year old child
        - There are at least four genetic variations that effect **Cyp 2D6**
        - Variations cause differences in metabolism (poor → intermediate → extensive → utra)
        - Can either cause *reduced ability to clear or activate drugs* or *increased activity accelerating clearance or activation*
        - 7% of Caucasians are poor metabilizers vs <1% of Asians
            - Frequency of variations is different in people of different ethnic backgrounds
            - Also is more common in redheads → *linkage disequilibrium* with Cyp 2D6
    - Cyp 2D6 affects metabolism of many psychiatric drugs → important to test for Cyp 2D6 genetics to find the ideal dose for each individual
    - *NOW, dose adjustment is typically done with trial and error, in the FUTURE, genetics will probably be used to find a more ideal dose from the outset* → ***personalized medicine*** (family history, clinical data, genomic profile all used together)
    - Ex: Tamoxifen used to treat ER+ breast cancer
        - Cyp 2D6 metabolizes prodrug tamoxifen to MUCH more effective endoxifen
        - Those with ineffective Cyp 2D6 activity do not effectively convert prodrug to active drug
        - IDEA: if cancer patient isn't experiencing any side effects, must think about genetic testing to see if patient has appropriate metabolic activity to activate drug
- **Health care impact of genomics**
    - Pre-genomic era: disease description, uniform disease classification, patient homogeneity, universal therapy
    - Post-genomic era: disease mechanisms, disease heterogeneity, individual variability, targeted therapies
- **Genome testing issues**
    - Privacy and confidentiality, stigmatization as 'untreatable', need for new guidelines, incidental findings
    - Personal information is not unique to the patient, it also has implications for family members

    - GINA: employers, health insurance cannot discriminate based on genetic status, but life insurance, military can discriminate
    - Race/ethnicity
        - Does the emerging data of race/ethnic difference in genetic variations lead to racial profiling in health care delivery?
        - Many issues with drugs targeted towards certain ethnic groups
- Genetic variations leading to variability in Warfarin response
    - Two genes – CYP2C6 and VKORC1 – affect metabolism of Warfarin
    - Mayo study found that using genetic information to determine dose led to a 30% decrease in hospitalization costs