Cell cycle G1→S→G2→M

Cyclin present during TRANSITIONS, specificity → regulation thru 1) phosphorylation → CDKs
2) protein ones and stability and ubiquitin
& temperature sensitive sometimes

CDKs always there

- temperature sensitive mutations
  - switches b/w active 3D conformation and inactive conformation

*checkpoints help regulate and fix mistakes

Science things
transgenic organisms

CANCER

Concepts
- Contact inhibition prevents cancer (can't form colonies)
- Ames

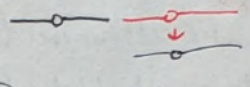
- oncogenes turn on growth, inhibit cell death → normal = proto-oncogene → only one homologue needed for a gain of function to increase function for cancer.
- Tumor suppressors - protects cell from cancer → recessive mutation needed for cancerous type

Ames Test - can this chemical cause mutations?
- Direct
  1. His- bacterial cells → plate / plate + mutagen
  2. if mutagen+plate has colonies bc His- → His+, then it has higher mutagenic potential
- Indirect Acting Mutagen: adding liver extract to make things reactive

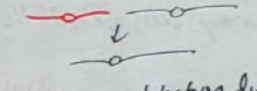
Things
- RAS
- MYC
- oncogenic

Ex - Retinoblastoma and loss of heterozygosity

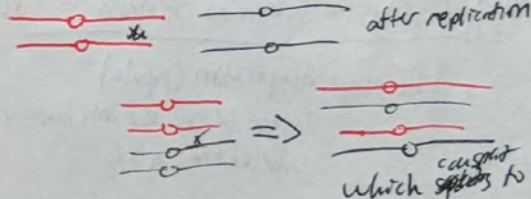
$Rb^{+/-} \rightarrow Rb^{-/-}$ ① De novo
↳ $Rb^{+/+}$



② Chromosome Loss



③ Mitotic recombination during repair



and

Mutations needed for cancer
- Multiple hit hypothesis (1+ gene mutation)

Normal → hyperplasia → dysplasia → tumor → metastasis

- old age
- radiation

DEVELOPMENT

- Model organisms
- genes can be ubiquitous, restricted, or cell-type specific
- FACTORS impact cell type
  - Effector proteins differentiate cells
  - Regulatory genes help cells commit
    - → cell-cell signalling
    - → determinants
    - → inherited transcription factors

haploid → diploid zygote → embryo (multicellular) → fetus
→ ectoderm, epidermal, mesodermal

Lengthening an Epithelial sheet
- Epiboly (lengthening)
- intercalation

Organogenesis - organ - many cell types in 3D org
- MIGRATION
- SEQUENTIAL INDUCTION
- COINDUCTION

Tissue engineering

- platform
- cell types
- signaling molecules
- 3D conformation

Morphogenesis – acquire shape

- stick together through junctions
  - tight (closed)
  - gap
  - adhesion junctions
- sort
  - cadherins (same cells attach to each other)
- migrate
  - F-actin poly

Mesenchyme – single units/cells that are loosely connected, no junctions

Epithelium
- cell sheets, yes junctions

E → M (metastasis)
MET

Stem Cells

Pulse/chase assay – measures cell turnover ($t_{1/2}$) (implies stem cells by cell replacement) → measure lifetime, migration, differentiation by tracking lineage of a certain cell

1. pulse w/ BrdU or other label, chase w/ regular thymidine/base
2. Measure how many cells are still there.

Assays to determine "stemness" → Measure SC potency

They all solve "Is this a stem cell"

1) Embryo incorporation (popular)
   - inject labeled SCs into blastocyst + pseudopregnant mouse
   - labeled forms of SC
2) repopulation
   1) remove endogenous cells (like w/ radiation)
   2) replace w/ possible SC, see survival
3) In vitro induction ~~this~~
   1) dump in diff inducers to see differentiated cell types
   2) see the diff cell types

FACS → Isolate SCs / sort cells

- use labels and antibodies to ISOLATE stem cells
- use labeled antibodies

Making Stem Cells

- Embryonic SC
- iPSC

Potency

Totipotent – everything
Multipotent/Pluripotent
Bipotent
↓ 3 germ layer potential
Medium Potential

Stem Cells

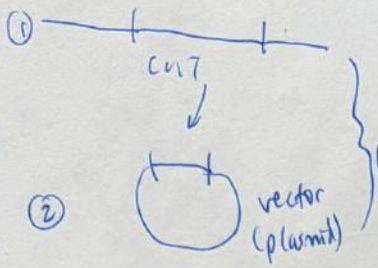
- divide asym
- low acetylation ↓ stem (over diff, increases acetylation)
- low methylation ↑ stem

Stem cell niche – micro-environment "non stem cells + SCs

Cloning – SCNT

Organoids – scaffolds,
- hydrogels 3D!
- matrices

Recombinant DNA – Cloning



Restriction enzymes
- recognize
- bind
- cut

Ligase puts back together

Ex 5' G/AATT C 3'
3' CTTAA/G 5'

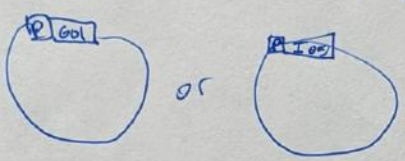
↓

5' overhang, Staggered cut

(3) Vector can replicate in host
* Selection – some vector confers bacteria
* Screening

SNPs → substitution of one base pair
- noncoding SNPs change gene exp
- certain SNPs are related to certain alleles

Testing how well you pasted: restriction mapping



– use REs and gel electrophoresis

CRISPR
1) Target gene → delete/edit
2) guide RNA complementary
3) Cas-9 makes cuts

guide RNA design
- Find AUG
- Find NGG → on target
- Box x 20 bp before NGG
- gRNA is complementary to template (so identical to target)

Fusion proteins
* Don't mess up protein 2's reading frame
* include protein 1's AUG
* include stop codon
* to connect/cut → RE + ligase

•usu protein 2 is a GFP or label for localization or something

DNA Sequencing
(1) put some ddNTPs in solution with replicated DNA strand
↳ probability of terminating
↳ separate DNA pieces synthesized by GE
↳ read 5'→3'

PCR – amplifies sample of DNA – ds DNA + primers
(1) Denature DNA @ High temp
(2) Anneal DNA primers @ Low temp
(3) Taq DNA pol @ med temp

Libraries – Genomic → all DNA

cDNA
reverse transcribed mRNA
ie all coding sequences (identify protein exp)

ENZYME activity impacted by:

- active site regulation

activators increase enzyme activity
- reversible

inhibitors (dec enzyme activity)

competitive
- mostly rev.
- binds to active site

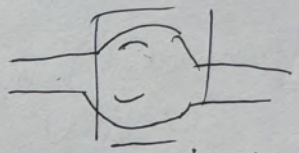
allosteric / noncompetitive
- binds elsewhere

sometimes (irreversible) covalently bonds to enzyme

Uncompetitive inhibitors bind to ES, prevent → P

Molecular Bio: DNA → RNA → protein
- DNA → DNA: replication
- RNA → protein: translation

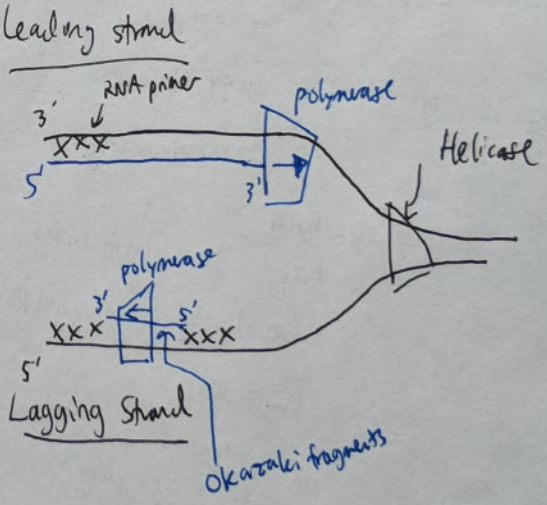
Replication:



oriC tends to T-A rich

4 Rules of DNA:

1) Complementary base pairing
2) Antiparallel strands
3) Nucleotides added to OH 3'
4) Double stranded DNA can denature & renature



DNA repair

- proofreading 3' → 5' exonuclease DNA pol (during rxn)
- mismatch repair - single nucleotide rmvl - identifies by methylation 5' C cytosine (diff in methylations)
- Nuclear excision repair - thymine covalent bond errors

telomerase repairs shortening of chromosomal ends

INGREDIENTS FOR REPLICATION:

- nucleotides
- polymerase - elongate from primers, seals gaps from primers
- RNA primase - attaches primers
- RNAase - removes primers
- clamp - keeps polymerase on template
- SSDBP - keep DNA single stranded
- Helicase - breaks H bonds, unwinds DNA
- ligase - binds on lagging strand

BIOCHEM

1. Chemical bonds/structures

CHNOPS

Bonds: (by strength)

1) covalent (within molecule
2) ionic (diff charges)
3) H bonds (IMF) (partial δ^- and partial δ^+ on H)
4) hydrophobic - avoids water (nonpolar)
5) VDW - doesn't happen as much

} functional groups regulate 3D struct of molecule, interactions with other molecules

2. Macromolecules

Carbohydrates (monosaccharides) - hydrophilic
$C:2H:O$
- energy, recognition markers

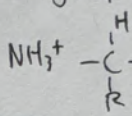
LIPIDS
- hydrophobic
- energy storage
- cell signaling

steroids - rings

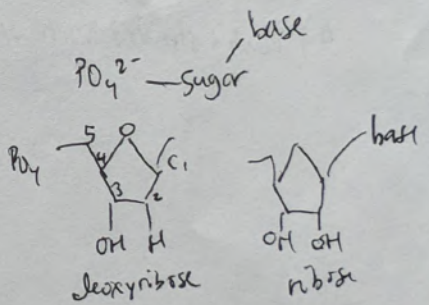
phospholipids
- POy
- Glycerol
- Fatty acids

Triglycerides
- glycerol + 3 fatty acids

PROTEINS - R groups determine polarity



NUCLEIC ACIDS
- nucleotides
- hereditary info



3. Energy & Enzymes

Exergonic: $\Delta G < 0$
Endergonic: $\Delta G > 0$
$\Delta G = \Delta H - T\Delta S$

Anabolic: build new molecules → generally endergonic
Catabolic: breakdown of more complex organic molecules → sometimes gives off energy → generally exergonic

ENZYMES - rxn specific! $S + E \rightleftarrows S\text{-}E \rightleftarrows P + E$

- reversible
- lowers E_a
- makes rxns faster
- behavior impacted by
  - pH, temp
  - coenzymes

Impacts substrate by:
- promoting transition state
- changing structure
- changing orientation
- creating conformational change