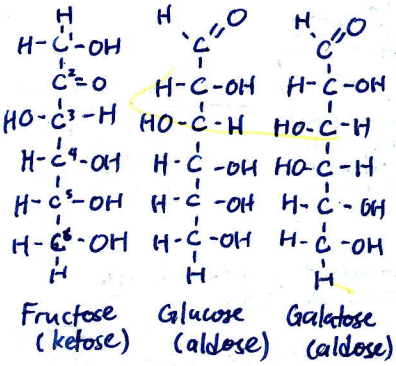
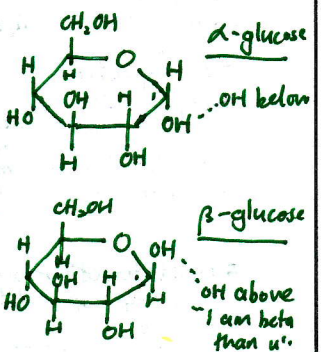
BIO H2: ① BIOLOGICAL MOLECULES

NIGEL FONG

① CARBOHYDRATES

(a) Monosaccharide Isomerism $(CH_2O)_n$

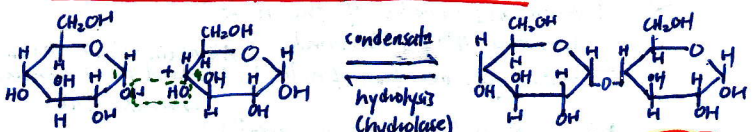




(b) Sig of molecular str. of monosacc.

1. Soluble – Small size, – H-bond (OH) ⇒ Easy tpt in H_2O
2. Ring forms (pentose, hexose) ⇒ stable building blocks
3. α/β-isomerism ⇒ ↑diversity, sp. func
4. C=O carbonyl gp ⇒ reducing

(c). Disaccharide synthesis & catabolism



— α(1-4) glycosidic linkage — $+H_2O$ DON'T FORGET

* Sucrose non-reducing as 1→2 glycosidic bond uses the carbonyl gps of both glu & fruc.

Maltose – glu + glu
Lactose – glu + galac
Sucrose – glu + fruc

(d). Benedict's test (reducing sugars) & Acid Hydrolysis (non-red)

$R\text{-}COH + 2CuSO_4 + 4NaOH \xrightarrow{\Delta} RCOOH + Cu_2O + 2H_2O + 2Na_2SO_4$

blue soln → red ppt

B. test.: 2 cm³ test soln (RED SUGAR) + 2 cm³ Benedict's reagent (blue) → mix → boiling H_2O bath, 3 min → IF red sugar: ppt. Green → Yellow → Brown → Brick Red.

A. hydroly.: Boil test soln w/ dil HCl, 1 min → Cool → Neutralise w/ $NaHCO_3$ (alkaline cond for B. test) → hydrolyse disacc → monos

* If both present, do 2 times to quantify:
- w/o acid hydrolysis → amt red
- w/ acid hydrolysis → red + non-red

find amt non-red

(e) Starch & Glycogen (storage polysacc)

amylose 20% + amylopectin 80% | glycogen] α-glu.

Basic str. - α-glucose. α(1-4) glycosidic bonds
- coils into helix.

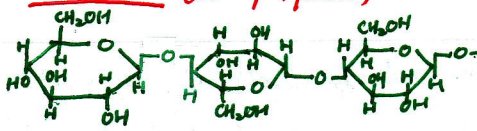
Branching
- amylose × branching
- amylopectin √ branching (every ~20-30 glu)
- glycogen ↑↑ branching (~12 glu)
- α(1-6) glycosidic bonds @ branch pts
- str. sim to amylose, helical side chain attach @ branch pts

Str → Func
- ↑α-glu residues → large energy store
- large molecule → insol, × affect H_2O pot
- helical str → compact, ↑energy/vol
- branching → multiple hydrolytic enz can work on diff ends, ↑rate of release glu. ↑energy gen/unit time

(f) Iodine test for starch

- Triiodide ion fit in each turn of amylose helix. ⇝ cplx: blue black. √ starch
- Boil: temp unwinding of helix, × col.
- Procedure ~ add drops of Iodine soln to 1 cm³ test soln

(g) Cellulose (str. polysacc)



- β-glu
- β(1→4) gly linkg
- Str, unbranched.
- OH gps in both dir

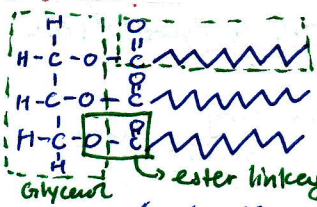
Suitability as cell wall
1. H-bonds between chains (Parallel chains → Micro fibril → Macro fibril) ⇒ ↑tensile str, rigid; ⇒ support plant; ⇒ × cell wall burst (pressure pot)
2. Insolub. (size)
3. Cellulose × commonly avail in organisms. ⇒ × digest
4. Porous ⇒ allow free mvmt of subst in/out cells

(h) Uses of carbs

1. Energy storage – starch, glycogen
2. Energy Tpt – ATP; – sucrose (suitab – × reactv); – glucose (↑osmotic conc, ↑flow phloem)
3. Cellular recog/sig – glycoproteins.
4. Structural – cellulose, chitin, hemicellulose
5. Raw mat – starting mat for synthesis of other cpds

② LIPIDS

(a) Str. of fats



Saturated	Unsat
- all C-C	- w/ C=C
- no kink	- kink in tail
- pack closely	- pack ↓closely
- ↑hydrophob intxn	- ↓hydrophob intxn
- ↑mp, ↓fluid (fats)	- ↓mp, ↑fluid (oils)

(b) Str ~ Func of fats

1. **Energy Storage**
   - ↑reduced C-H → ↑energy released per unit mass (β-oxidation)
     ↳ compact, ✗ bulky
   - insoluble → ✗ affect H_2O pot
     → ✗ easily tpt out of cell
   - prod metabolic H_2O ~ esp desert animals
   - light + ✗ bind H_2O of hydratn
2. **Cushioning** - internal body fat
   - Bulky, ✗ compressible → cushion vital organs eg kidney
3. **Insulation** - subcutaneous fat (eg seals, mammals)
4. **Buoyancy** - eg. whales.
   - to sink/float ~ modify membrane sat: ρ↑ → sink.
5. **Membranes**

(c) Phospholipids Str (choline, sphingolipids)

- 3rd hydroxyl gp of glycerol linked to phosphate gp / other small gp
- Amphipatic ~ hydrophillic head [-ve charge of phosphate]
  ~ hydrophobic tail (lipid)

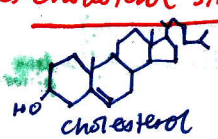
↓

Self-assembly into bilayer / micelles

(d) Phospholipids in cell memb

1. Cell mem - hydrophillic head face aq. env, hydrophob. tail face inwards ⇒ lipid bilayer
   ⇒ compartmentalize in cell.
   ~ hydrophob reg ✗ permeable to polar, charged solutes.
2. Liposomes → artificial lipid spheres w/ aq. core
   - carry therapeutic DNA into cell.

(e) Cholesterol Str & Func



cholesterol

- largely hydrophobic → tuck into hydrophob core of mem
- -OH gp: slightly amphipatic → align w/ phosphate heads of phospholipids
- rigid 4-ring str

↓

Regulates memb. fluidity
- Warm: ✗ overly fluid ~ restr. phospholipid mvmt
- Cold: ✗ overly firm ~ prev. close packing, ✗ crystallize.

(f) Ethanol Emulsion Test

2 cm^3 ethanol + 2 drop sample/ grounded sample (mix well) → Decant into 2 cm^3 H_2O. Shake. vig

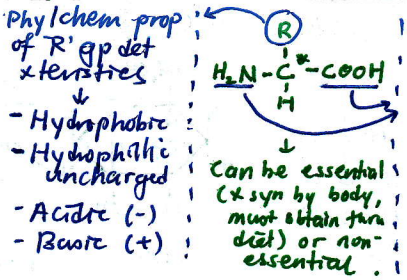
- 1. Homogenous (clear) soln of ethanol, 2. remain homog. when H_2O added → Lipids absent
- 1. Homog of ethanol 2. Emulsion of H_2O → Lipids present

③ PROTEINS

(a) Amino acids & Pri. str

= Seq & No of aa in polypeptide chain

Phy/chem prop of R' gp det x teristics
- Hydrophobic
- Hydrophilic uncharged
- Acidic (-)
- Basic (+)



Can be essential (✗ syn by body, must obtain from diet) or non-essential.

Zwitterion - both +/- charge
$NH_2 \rightarrow NH_3^+$; $COOH \rightarrow COO^-$

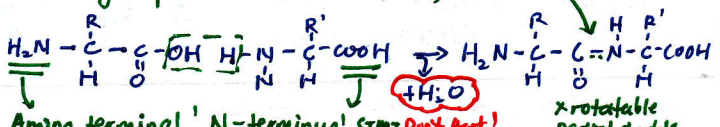
↓

Amphoteric - both acid/basic groups

↓

Acts as buffers
↳ resist ΔpH

= Held together by covalent peptide bonds (R-group not involved in p. str)



Amino terminal; Carboxyl; N-terminus; C-terminus; START; END; Don't forget!

x rotatable partial double bond x teristic

(b) Secondary Str

- Local spatial arr formed by reg coiling/pleating of chain
- maintained by H-bond at reg int between -CO & -NH of polypep. backbone (R-gp ✗ involved)

1. α-helix (eg. hair keratin)

- Coiled, helical / spring-like
- Turns linked together by H-bonding between NH of 1 turn and COOH of the next
  - every 4th peptide bond
  - 3.6 aa. per turn of helix
  - } All main-chain CO/NH involved in H-bonding ↳ max. stab
- * Bulky R gp (tryptophan), when↑, proline/hydroxyproline insert kink & distrupt helix. } → String of prolne ✗ 2° seq.

2. β-pleated sheet (eg. silk fibroin)

- 2 or more reg of single pp chain linked by H-bond
- H-bonds formed between NH & CO gps of 1 chain and NH & CO gps of adjacent chain.
  ↳ all CO & NH gps involved — max stab
- Chains: parallel / anti-parallel)

↓

- Flexible but inelastic
- ↑tensile strength.

(c) Tertiary Str

= Str formed by extensive folding & bending of pp chain
↳ forming compact shape to give overall 3D conformatn
→ Intxn between R-gps on adj/distt pp chains
↳ lost on denaturation

1. **Disulfide bridge** - -SH + HS- → -S-S- (ox. of SH on cysteine aas)
   - strong covalent bonds → ↑heat stab.
2. **Ionic bond** - between opp. charged gps of AA (COO^-, NH_3^+)
   - ΔpH env, Δcharge, Δ ionic B
   - weak under aq. cond ~ charged gps surr by H_2O
3. **Hydrogen bonds** - between electroneg O of CO / N of NH and electropos H of OH / NH
   - collectively strong.
4. **Hydrophobic intxn** - non-polar R gp cluster @ ctr, hydrophob
   - hydrophillic gps face ext aq env

(d) Quaternary Str

= Associate of 2 or more pp chain into 1 complex. protein (not all proteins quaternary str ↳ some monomeric)

- Each polypeptide = subunit
  - ↳ held together by disulfide bridge, ionic B, H-B, hydrophobI
  - ↳ constituent chains of multimeric protein — same/diff.

Advantages of assoc:

1. Cooperativity (see enz)
2. Bring catalytic site together
3. Less DNA req to code for monomer
4. ↓ SA/Vol ratio (possibly)
5. Defects can be fixed by repairing flawed subunit.

(e) Fibrous & Globular Prot.

	FIBROUS	GLOBULAR
STR	Long fibres/sheets	Spherical shape
SPEC	length/aa seq may vary	Length/aa seq always same
SEQ	Repetc, reg aa seq	Irreg aa seq
SOL	Insolub — large, ↓H-bond	Solub
FUNC	Structural, Contractile	Metabolic & other func

(f) Collagen (most abundant fibrous protein in body)

↳ Connects tissue — bones, tendon, skin, teeth.

INDV CHAIN — Tripeptide seq, gly – proline – hydroxyproline or hydroxylysine
- ↓ helical (not α-helix)
- ↳ small size, allow coiling, fits into places where 2 str meet tight triple-helical str

TROPOCOLLAGEN
- 3 indv chains wound round each other
  - ↳ H-bonds (NH, OH, CO) → rigidity, tensile strength
  - ↳ bulky & inflexible proline → rigidity

FIBRILS
- Covalent cross-links form between N & C-term lysine of adj tropocollagen → fibrils
- Staggered arrangement — minimize pts of weakness.

↓

FIBRES
- Tensile strength ↑

(g) Haemoglobin (globular, tpt O_2 in RBC)

STR
- 2 α-globin & 2 β-globin subunits
- hydrophobic gps face interior, hydrophilic gps face aq ext ⇒ soluble
- 1 prosthetic heem gp per subunit — Fe^{2+} bind O_2
- Fe^{2+} held by 4 N in polyphyrin ring
- 4 subunits held by weak hydrophob intx & H-bond
  - ↳ allow subunits to move wrt each other — allosteric mech

ALLOSTERIC MECH (COOP O_2 BINDING)
- Binding of 1 O_2 to Hb subunit
- Structural Δ in remaining 3 subunit — ↑ O_2 affinity
  - ↳ hesitant loading of 1st O_2 → rapid loading of other 3
  - ↳ 1 O_2 unloaded → other 3 quickly unload

IMPTCE OF PRI STR — Sickle Cell Anaemia
- 1 bp mutation → Glu → Val (6th AA)
- Δ str β-globin
- Hb aggregate @ low [O_2], ppt
- RBC Δ shape (sickle)

(h) Protein func

Specific 3D conformatn ⇝ Specific intxn wt a wide range of molecules | Func dpd on conformatn

1. Enzymatic/catalytic · Lysozyme, Sucrase, Maltose
2. Regulatory · Insulin, Glucagon (pep. hormones)
3. Receptors · Insulin receptor, neurotransmitters
4. Movement · Actin & Myosin
5. Transport – circ · Haemoglobin
6. – memb · Na^+/K^+ pump
7. Structural · Collagen.

④ ENZYMES

→ globular protein
→ RNA (ribozyme)

Enz = bio catalyst, ↑rate of rxn w/o being consumed.

(a) Catalytic Action - Models

GEN PROP
- → ↑rate of rxn
- → Mild conditions (temp, pH, p.)
- → Specific – abs · sp · sp. to 1 rxn
  - group sp · act on 1 type of chem. bond found in a variety of substances

LOCK & KEY
- → Enz: specific surf conf due to 3D folding.
- → Substrate = key, complementary to Enz = Lock
- → When enz & sub collide in correct orientatn, enzyme-substrate cplx (ESC) formed
- → Once formed, pdt ✗ fit into actv site & leave.
- → Actv site free to receive further subs.
- ~ Prob: some enz can receive more than 1 sub. more than 1 key? specific?

INDUCED FIT
- → Both enz & substrate Δconf during rxn
- → Actv site of enz complementary but ✗ perfect fit for substrate
- → When substrate bind, induce Δ in enz.
  - ⇒ allows actv site to be moulded into precise conf. ⇝ ↑effectv cat-func

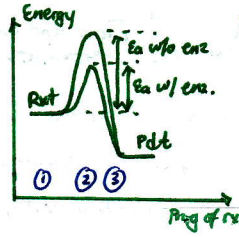
(b) Catalytic action - Molecular Basis for ↓Ea

1. Strain Effects — Rxt distorted slightly – bonds to be broken strained, ∴ ↓energy to break
2. Proximity Effects — Temp. binding of rxts next to each other ↑chance of rxn (not just dpd. random collision)
3. Acid-Base Cat — Transfer gps between rxts
4. Microenv Effects — Hydrophobic gps create water-free enz ↳ allow non-polar rxts to rx. more easily
5. Orientatn Effects — Enz hold rxt in perfect orientn ↳ bonds exposed to attack.

→ Roles of diff aa. residues

1. Contact/binding aa — Temp. bind to subs (weak intxn); det enz specificity
   - ↳ posits sub in correct orientn
2. Catalytic aa — Act on bonds in substrate molecule. (R gps involved)
3. Structural aa — Maintain 3D conf of protein
4. Non-essential aa — No func, usually on protein surface

(c) Enzyme energetics ∫ E_a

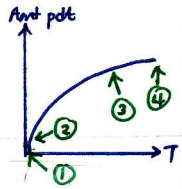


① Rxts absorb energy fr surroundings
↳ ↑KE
↳ ↑effects collisions
↳ thermal agitatn → bonds ↑likely to break

② If absorb enough energy (= E_a) ⇒ ESC.

③ Energy released.

(d) Measuring enz kinetics



① At 0... rate = 0...

② Initially, substrate in excess, enz limiting
↳ all acte site occupied

③ After T, substrate limiting, enz in excess...

④ All rxt → pdt - Horiz line.

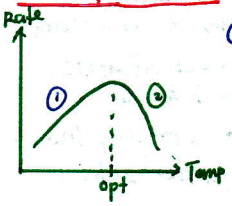
MEASUREMT.

1. Rate of pdt formatn - eg. amt gas
2. Rate of disapp. of sub.

- -ve control → boil rxts ⇝ denature enz.
* All other factors constant

(e) Factors affecting rate of enz-cat rxn

→ Temperature

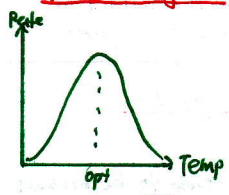


① Below opt : ↑KE → ↑effects collisions
↑KE → ↑molecules have enough energy to pass over E_a barrier ∫ form pdt
∴ ↑temp, ↑rate.

Q_{10} (Temp coeff) = Rate at (x+10)°C / Rate at x°C } usually, $Q_{10} = 2$

② Above opt : Break H-b, I-b, hydrophb. interm that stabilise acte conformatn.
⇝ enz denatures
⇝ acte site ✗ complementary to substrate, ∴ ↓ rxn

→ pH changes

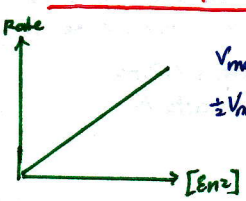


Excess H^+ or OH^-

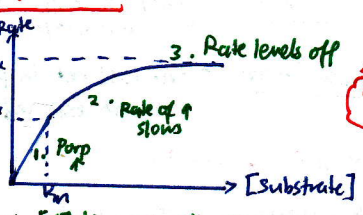
1. Δcharges of catalytic ∫ contact gps on acte site ⇝ ✗ catalysis / binding
2. Δioniz of structural acc
↳ disrupt ionic ∫ H-bond
↳ Δ3D conf. ⇝ denature.
3. Δ charges on substrate
↳ ✗ fit into acte site.
⇩
∴ no E-S cplx. No catalysis.

Ans must inc ES-complex

→ Concentratn of enz ∫ substrate



Rate ↑ w/ [enz] when enz-limiting



Name which is lim

1. [S] lim → acte sites on enz ✗ fully occup
2. [S] still lim but more ∫ more acte sites used
3. ↑ [S], [enz] limiting, indep of [S]

K_m Michaelis cnst = [S] req to make rxn go at ½ max rate
↳ measure of affinity of enz for sub.

Low K_m : ↓ [S] needed for ½V_{max} - ↑affin.
High K_m : ↑ [S] needed for ½V_{max} - ↓affin.

(f) Enzyme inhibition

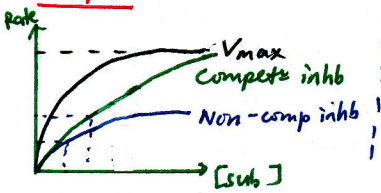
→ Competitive

Inhibitor : str. resemblance to sub
Bind rev. to acte site (compete w/ sub)
↓
↓available acte sites for substrate binding
↓
Effect can be overcome by ↑ [S], ↑chance sub binding to acte site. compared to inhb.
↓
High [S], rxn V reaches V_{max} w/o inhb.

→ Non-competitive

Inhibitor : ✗ str res to sub
Forms enz-inhb cplx on pt other than acte site.
↓
Δ globular str of enz
↳ acte site ✗ recepte to sub
↓
Effect ✗ be overcome by ↑ [S].
↓
Rate of rxn ↓s with ↑ inhibitor conc.
⇝ inhb sat, rate = 0

→ Graphs.



	Comp.	Non-comp.
K_m	↑	same
V_{max}	same	↓

(g) Enzyme regulatn ∫ acte

→ Control enz availability

1. Rate of enz prod vs rate of enz degradatn
2. Cellular compartmentalizatn - multiple simult rxn.
- isolate sub/pdt ∫ competing rxn
- provide for enz ∫ rxn
- organize enz into purposeful pathways - eg. Krebs.

→ Control enz acte

1. Post-translational modificatn
- phosphorylatn, glycosylation - phosp. of glycogen synthase
- inacte precursor (zymogen) - pepsinogen → pepsin.
2. Acte enz when localz to diff env.
- Δ oxidising/reducing env
- low pH → high pH → hemagglutinin of flu virus conf Δ on encountering acidic env of host cell vesicle → activatn
3. Inhibitors ∫ Activators - feedback (end pdt) inhibits (end pdt inhibit enz early in pathway)

→ Allosteric regulatn

- Protein's func @ 1 site affected by binding of regulatory molecule to another site.
↳ coenzymes - organic cofactors (eg. Vitamins)
↳ cofactors - non-protein sub nec for cat actv (eg Ca^{2+}, Zn^{2+}...)
- Allosteric enz → 2 or more subunits (4° str)
↳ each subunit has its own acte site
2 conf states → - Cat acte state (relaxed) ... ↑affinity
- Cat inacte state (tense) ... ↓affinity
- Binding of allosteric acte stab cat-acte site
- inhb stab cat-inacte site.
- Conformational Δ in 1 subunit Δ all other subunits
↳ i.e. binding of 1 acte affects all subunits
- Sigmoid curve

BIO H2 : ② CELLULAR STRUCTURE & FUNCTN

NIGEL FONG

① MICROSCOPY

(a) SEM, TEM

Stain cell w/ gold - pref bind to certain organelles
→ less light/e⁻ passes through this

SEM Scanning Electron Microscopy
→ show surface of specimen (3D look)

TEM Transmission Electron Microscopy
→ show thin sects (int str) of specimen (2D look)
→ some longitudinal sects, some x-sections

x-sec movement

(b) Res & Mag

Resolutn: Min dist whereby 2 pts can be distinguished fr each other (2nm for e⁻ mic, 200nm for light)

Magnificatn: How much viewed image larger than actual

Calc mag fr. eyepiece graticule . . .

Stage micrometer 1 unit = 100 μm
↳ no of units on eyepiece graticule this coincide w/
↳ length each div of eyepiece graticule rep (μm)
×40 = 25 μm | ×100 = 10 μm | ×400 = 2.5 μm | ×600 = 1.5 μm

$$\text{Magnificatn} = \frac{\text{Image size}}{\text{Object size}} = \times \_\_\_\_ .$$

(c) Cell fractionatn

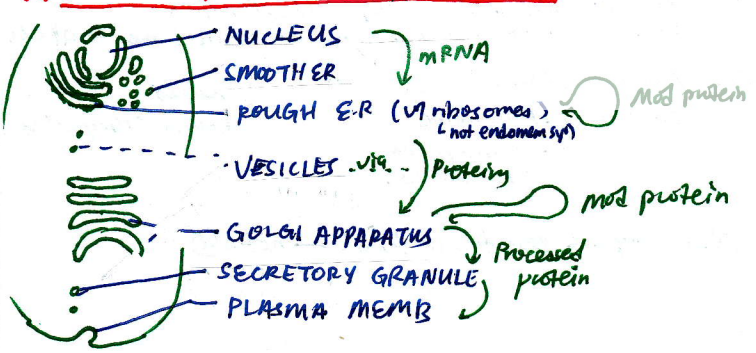
- Homogenize cell → distrupt cell walls, mem. (eg sonicate)
- Centrifuge : separate organelles by size/density.
  → most dense (usually largest) organelles ppt first
  → supernatant removed & centrifuged @ even ↑spds
  - smaller/less dense organelles ppt out

(d) Size of cellular components

Nucleus	5–10 μm	Lysosome	0.2–0.5 μm
Chloroplast	1–10 μm	Centrioles	0.3–0.5 μm
Mitochon	1–5 μm	Ribosome	0.02 μm (20nm)
		Plasma mem	7.5nm .

② CELL STRUCTURE

(a) Overview of endomembrane sys & prot

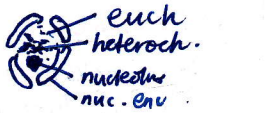


* Flow of proteins prod
* Flow of membrane; dynamic org.

→ Varying prop: eg cells acte secreting ↑prot.
→ ↑R.ER. etc.

→ Singular vs plural

(b) Nucleus



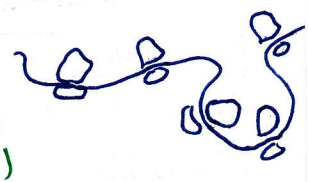
NUC ENVELOPE
- Double membrane.
- w/ numerous nuclear pores
  IN: free nucleotides, proteins (histone, promoter)
  OUT: mRNA, ribosomal RNA.

DNA
- In interphase cell, appear as thin elongated threads ⇝ chromatin (DNA + histones)
  - EUCHROMATIN — loosely coiled, ext, diffuse ⇝ involved in mRNA prod.
  - HETEROCHR. — tightly coiled, ↑stain (↑dark) ⇝ x active in mRNA syn.

NUCLEOLUS
- one or more nucleoli ⇝ look like dark spheres ⇝ w/ DNA, RNA, prot
- Where ribosomal subunits assembled
- ↑nucleoli ⇒ cell ↑ acte in prot syn

SIG OF NUC
- Contain hereditary mat of cell (DNA also in mitochon & chlorop)
- Regulate prot-syn.

(c) Ribosomes



- Ribosomal RNA + protein
- large subunit + small subunit
  ↳ prokaryote: 70S (30S + 50S)
  eukaryote: 80S (40S + 60S)
- polysome: "string on bead" ⇝ multp ribosome syn 1 protein chain.

LOCN: ↑Cytosol — prod proteins that func within cytosol
: R. ER — proteins to 1. insert into memb.
2. package within organelle (eg lysosome)
3. secretn out of cell

[ribosome can go to either. signal pep on prot syn makes rib attach to RER] [fall into lumen of rough ER]

: Mitochon & Chlorop.

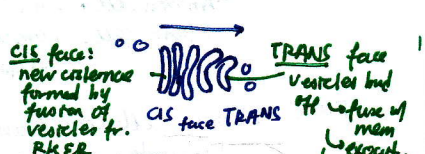
(d) Endoplasmic Reticulum

- Extensive network of cisternae (membraneous tubules & sacs)
- Outer mem of nuc env. continuous w/ ER.

SMOOTH ER	ROUGH ER
- Mem appears smooth	- ribosomes stud outer surf ⇝ appear rough
- cisternae appear tubular	- cisternae appear flattened
- may connect w/ golgi / cell mem	
FUNC	
1. Lipid synthesis - steroid horm - phospholip.	1. Tpt of prot syn by rib on surf ⇝ enter cisternal sp, fold into nate conf → vesicles bud off.
2. Carb synthesis	2. Modificatn of prot (eg glycosylation)
3. Detox drugs & proteins (eg liver cells ↑SER.	3. Membrane / phospholipid syn.
4. Sarcoplasmic ret (muscles) store Ca^{2+} — contracte	

(e) Golgi Apparatus

- Stack of flattened, mem-bound sacs (cisternae) + golgi vesicles



FUNC
1. Chem mod. proteins / lipids fr RER / SER . . . eg. carb chain on glycoprot
2. Sort & target completed mat to diff parts of cell
   ↳ out of cell — secrete
   ↳ formate of lysosomes
3. Vesicles budded off fuse w/ cell mem — replace mem lost to endocyt.

(f) Lysosome

STR - Single mem
- Hydrolytic enz inside - - - proteases, lipase, nuclease, lysozyme (lyse cell w), hydrolase ...
- Acidic contents (nec for enz func)
  → ∴ contents of cell ✕ dtg. if hydrolytic enz accid released

FORM. ATN - Syn on RER → Tpt to Golgi → bud off.

FUNC 1. Digests of mat taken in by endocytosis (food, foreign particles)
  └ lysosome fuses w/ endosomes to digest ē contents
  └ useful pdts absorbed, unwanted pdt expelled (exocytosis)

2. Autophagy
  └ breakdown unwanted str within cell eg old organelles
    → return broken down pdts to cytop. → reuse

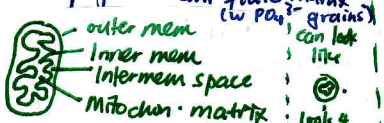
3. Release enz outside cell by exocytosis
  └ breakdown of extracellular content
    └ eg. sperm release hydrolytic enz to reach ovum

4. Autolysis
  └ cell - apoptosis (eg. tail of tadpole)

(g) Mitochondrion

- Spherical / rod-shape
- Double mem
  └ outer mem
  └ intermem space
  └ inner mem: ↑infolding to form cristae

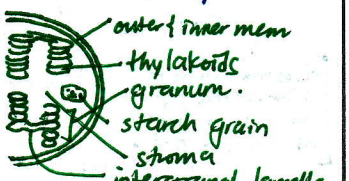
proj into semi-fluid matrix (w PO4 3- grains) can look like ◎. look ∮ cristae



↓
Site of aerobic resp.
  └ gen ATP through ox. sugars, fats, fuels w O2 use

Chloroplast

- Lens-shaped / Disc-shape
- Double mem
- Stroma (w/ starch grains)
- Granum (grana: plu)
  → stacks of thylakoids



↓
Site of photosyn.
  └ conv solar Ē → chem Ē
  └ use sunlight to syn org cpd fr. CO_2 ∮ H_2O.

→ ATP synthase (stalked particles)

A	Intermem space	Thylakoid sp	↓pH
B	Inner membrane	Thylakoid mem	⟩grad
C	Mitochon matrix	Stroma	↑pH

→ Endosymbiotic theory

- Mitochon ∮ chlorop. derived fr. prokaryotic organ.
  → ancestral prokaryote engulfed by eukaryote.

→ Mitochon matrix, Chlorop. stroma } contain 70S ribosome (prok) cDNA. enz to syn DNA ∮ prot..

(h) Vacuole

STR - Fluid-filled sac bound by single mem
  └ Animal cell: small, more num vac
  └ Plant cell: Large central vac surr by tonoplast (mem)
    └ min. salts, sugars, enz, pigments, waste pdt

FUNC 1. Conc. cell sap draws H_2O into vacuole
  └ maintain turgor pressure for support in herbaceous p

2. Plant cell growth - vacuole enlarge (↑H_2O) w/ min ↑cytoplasm → easy growth

3. Pigments → colours in flower / fruits ~ attract animals

4. Storage - food, waste pdt (ca. oxalate crystals, latex)

(i) Microtubules

MICROTB - Tubulin monomers ~ helical arrangement
  form hollow, tubular, slender str. - stiff, str

- Growth +/-: add/rmv of tubulin subunit
  inhb: chemicals eg colchicine

FUNC 1. Maintain shape of cells ~ rgid cytoskeleton

2. Chromosome movement
  → spindle fibre pull chromosomes apart during cell div

3. Intracellular tpt
  └ channels for oriented flow of cytoplasm (eg. cytoplasmic streaming)
  └ str on which vesicles move (dynein arms)

4. Str. component of centrioles, cilia, flagella.

(j) Centrioles

- Pair of cylindrical, rod-like str. pos next to each other
- 9 triples of microtubules in a ring
- found @ centrosome (microtub org ctr) close to nuc

centrosome: all cell
centriole: ✕ in higher plants (ie. animals, ferns, algae)

- During cell div, centriole replicate ∮ move to opp end

FUNC: Role in nuclear div. - org. formats of spindle fibres

(k) Cell Wall (plant / fungi / bacteria)

└ Carbs (cellulose, hemicellulose, pectin)
└ Ext. surf of cell.

FUNC 1. Confer pressure pot
  - H_2O enter cell via osmosis } structural supp. in herbaceous plants
  └ cell become turgid, xlyse

2. Porous (space between microfibrils)
  └ allow mineral salts to move along CW
    eg. apoplastic route - root cortex).

(l) Prokaryote v Eukaryote

	euk	prok
cell size	Larger: 10-100 μm ⌀	Smaller: 0.5-5 μm ⌀
Nuc.	Nucleus w/ nuc. envelope	No true nucleus.
RIB.	80S (40S+60S)-ribosomes Attached to ER or free.	70S (30S+50S) No ER for ribosomes to attach
org.	Many mem-bound organelle Double mem: Nuc, mitoC Single mem: Golgi, lysosome, ER, vesicles.	Few organelles No mem-bound organelles.

(also compare genetic material - notes ③)

③ MEMBRANES

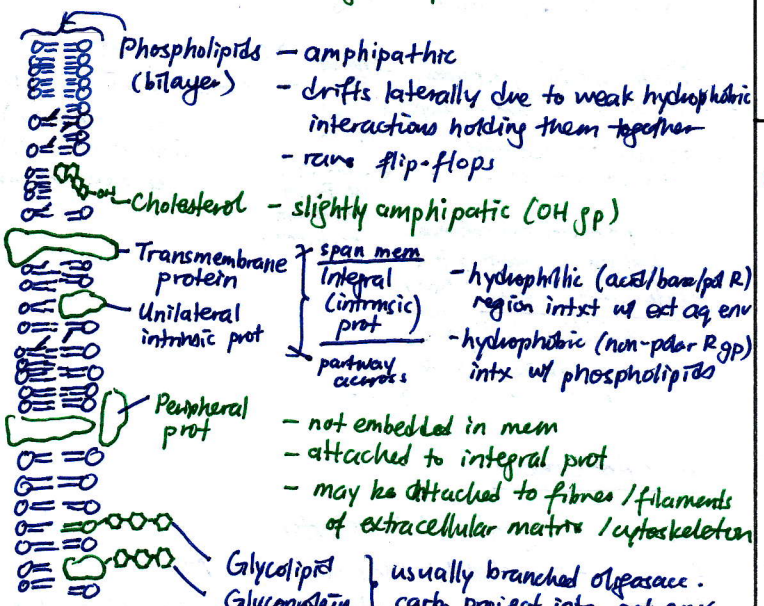
(a) Membrane structure

7-5nm thick

prot: must state → intrinsic/extrin. → trans/uni..

"Fluid" - dynamic str, prot & phospholp. in cont mvmt

"Mosaic" - random arrangement of embedded/attached prot



- Phospholipids (bilayer) – amphipathic
  - drifts laterally due to weak hydrophobic interactions holding them together
  - rare flip-flops
- Cholesterol – slightly amphipatic (OH gp)
- Transmembrane protein / Unilateral intrinsic prot – span mem / Integral (intrinsic) prot / partway across
  - hydrophilic (acid/base/pol R) region intxt w/ ext aq env
  - hydrophobic (non-polar R gp) intx w/ phospholipids
- Peripheral prot
  - not embedded in mem
  - attached to integral prot
  - may be attached to fibres/filaments of extracellular matrix/cytoskeleton
- Glycolipid / Glycoprotein – usually branched oligosacc. carbs project into ext env.

(b) Regulation of mem. fluidity (w/o reg: var w/ T)

1. % unsat: sat phospholipids
   - unsat → kinks → pack less tightly → ↑fluid.
   - freeze @ ↓T if ↑unsat phospholip.
     - ↳ cold-tolerant plants Δsat → unsat in winter.
2. Cholesterol
   - Warm: ✗ too fluid – choles. restrict phospholipid mvmt
     - choles. is rigid. stab lipids
   - Cold: ✗ too firm – ✗ close packing.

(c) Role in cellular func/reg

1. Enz activ – mem: provide surf for chem rxn
   - prots built into mem / form cplxes on mem (immob) w/ activ site exposed to surr. aq env
   - eg. E. tpt chain, Alkaline phosphatase, ER.
2. Signal transductn
   - mem. prot binding site w/ shape complementary to chemical messenger (binding site – prot/glycoprot/lip)
   - On ligand binding → Δshape → bind to cytoplasmic prot
   - convey msg
   - eg: G-prot coupled receptors, liver cells activ by adrenaline/glucagon to convert glycogen → glucose.
3. Cell-cell recog
   - glycoprot/glycolipids → diversity of carb chain → loc on ext of cell
   - Recog by mem prot/gly. of other cells
   - eg. mucin (glycoprot)
     - sort out embryo cells into tissues & organs
     - self-recog of sponges
     - rejection of foreign cells by immune sys

(d) Role in inter-cellular structure

1. Cell-cell adhesion
   - mem prot/carbs adhere cells to each other
   - eg: cadherins
     - tight junctions – skin = waterproof
     - casparian strip in roots
2. Attachmt to cytoskeletal & ECM
   - stabilize cell shape
   - fix loc of some mem. prot
   - e.g. integrins.

(e) Partial permeability of mem

Partially permeable = some substance may cross more easily than others

↳ lipid ↑%: √small non-polar mol. ✗ polar/large mol

↳ integral tpt prot.

Func
- allow mvmt of cytoplasm (separate cell vs ext env)
- compartmentalizatn (within cell, within organelles)
  - ↳ unique env for specialized activ
  - ↳ spatial separatn/sequential op. of processes
  - ↳ allow enz to accumulate to ↑ conc (eg lysozyme)

Mvmt nec
1. To obtain nutrients/raw mat (eg glu)
2. To excrete waste substances (eg urea)
3. Secrete useful substances (eg hormones)
4. Generate ionic grad. (eg mitochon, neurons)
5. Maintain pH, ionic conc.

(f) Simple Diffusion

Def
- → mvmt of ions/molecules (through plasma mem)
- → down a conc grad (high conc → low conc)
- → w/o use of energy.

Note
- until $conc_{in} = conc_{out}$ (eqm)
- each molecule moves indep of other conc grad.

EG - diffusion of gases in/out of cells.

Mech - formation of transient pores in membrane (↑ fluidity of mem = more transient pores)
- solvatn in hydrocarbon layer

Factors
1. Molecule size – ↓size, ↑rate of diff.
2. Molecule charge
   - Ions ✗ pass thru hydrophb core
   - hydratn shell ↑effective size of ion
   - ↑charge/mass, ↓rate
3. Molecule polarity – non-polar √intxt w/ core – ↑rate
4. Temp (mol. KE)
   - ↑T, ↑KE, ↑diffusion
   - ↑↑T – mem loses integrity & selectivity...
5. Surf. area. – ↑surf area, ↑rate
6. Conc grad – ↑conc grad, ↑rate; countercurrent mech!
7. Distance – ↑dist, ↓rate.

(g) Facilitated diffusion

Def → movemt of substance down conc grad
→ by tpt prot
→ w/o direct use of energy

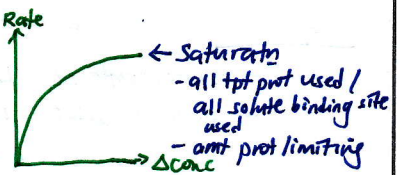
movement in both dir occurs by same mech to eqm.

Eg → aquaporins, V-gated Na^+/K^+ channels.

Mech Channel prot – transmem prot w/ fixed 3D conf
– provide hydrophilic pore across mem ~ selective for a certain solute.

Carrier prot – prot existing in 2 alt conf
– undergo Δ conf when sp. molecule binds.
– move solute across as shape Δ.

Factors 1. Conc grad.
2. No of carrier/channel
- Chance of collision
- ↑saturatn pt



(h) Osmosis & H_2O pot

Osmosis = Movemt of H_2O fr ↑ Ψ_w to ↓ Ψ_w through a partially permeable membrane

Ψ_w Water pot = tendency for H_2O to leave (PE_{H_2O}) } 0 = pure H_2O
Ψ_s Solute pot = Extent by which solute ↓ Ψ_p }

Ψ_p Pressure pot = Pressure by cell wall on plasma mem
= turgor p (by mem on cell wall)

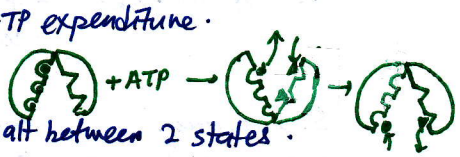
ANIMAL					PLANT				
$\Psi_{w,ext}$	Ψ_w	=	Ψ_s			Ψ_{wext}	Ψ_w	=	$\Psi_s + \Psi_p$
-2x (H₂O in)	-x		-x	lysed (H₂O)	Hypotonic soln	-2x (turgid)	-2x		-x -x
-x	-x		-x	O norm	Isotonic soln	-x (flaccid)	-x		-x O
-x/2 (H₂O out)	-x		-x	shrivelled/ crenated (H₂O)	Hypertonic soln	-x/2 (H₂O out) (plasma lysed) (H₂O)	-x		-x

* Pt of incipient plasmolysis → 50% cells plasmolysed
$\Psi_{ext} = \Psi_w$ cell $= \Psi_s$ cell → can tell Ψ_s cell.

(i) Act= tpt

Def → movmt of sub. against conc grad Mvmt in 1 dir!
→ via carrier prot
→ w/ Energy /ATP expenditure.

Eg → Na^+/K^+ pump.



Mech → Carrier prot alt between 2 states.
→ Na^+ bind from inside
→ ATP phosphorylate ... prot Δ conf ... expel Na^+ to ext
→ K^+ bind from ext
→ Loss of phosphate ... prot return to orig conf ... expel K^+

(j) Bulk tpt

Endocytosis ↑E expend! **Exocytosis**

Infolding/ext of cell surf mem to form vesicle, allowing cell to acquire macromolecule/particles → why not carrier prot? ↓ too big

← Secretion of macromolecules by fusion of vesicles w/ plasma mem.

1 → Phagocytosis (take up s) - eg. immune sys
2 → Pinocytosis (take up l) - eg egg cell take nut
3 → Receptor-mediated endocytosis ... - eg. cells take up Fe in transferrin fr. blood.

→ secrete of wastes
→ secrete of manuf pdt
(eg hormones)
(eg mucus cell)
(eg neurotransm)

- receptor prot on cell mem, clustered in coated pits.
- coated pits lined w/ fuzzy prot layer on cytoplasmic side to deepen pit.
- helps acquire bulk Q even if extracellular conc ↓.

Endocytotic mech

Recep-med – Ligand bind to receptor

ALL – Cytoskeletal filaments rx w/ ATP

Phago. Rest
– to form pseudopodia which engulf particle
– to form invaginatn of plasma mem.

ALL – Ends of extensions of plasma mem fuse
↳ vacuole containing solid matter pinched off & into cytoplasm (coated ves for recep-m)

– Process vacuole

Phago – Lysosome containing hydrolytic enz fuses
enz hydrolyse particles into soluble pdt

– Useful pdt absorb into cytoplasm.

Exocytotic mech

- Tpt vesicle (eg budded fr Golgi) moves to plasma mem.
- 2 mem fuse when in contact
- Contents of cell released to extracellular env.

③ MITOSIS & MEIOSIS

(a) Chromosomes

DNA → 'String of beads' (8-histone cplx) → Chromatin thread 30nm: DNA+histone euchromatin (loose coil) heterech (tightly coil) → Chromosome chromatin coiled many times, condensed.

homologous chromosomes

1. Sim size, shape, centrom pos & staining pattern (except X & Y homolog)
2. 1 fr. each parent → 2n diploid, n haploid. Humans: 2n=46, n=23
3. Sim genes (diff allele) @ corresponding loci

* 1 centromere = 1 chromosome

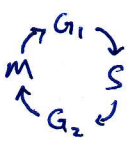
identical DNA. replicated fr same DNA molecule

tetrad

2 bivalents (paired up homologous chromosomes) that have X-over.

chiasma = sites where non-sister chromatids of homologous chroms. break & rejoin, xchange of genetic mat

(b) Cell cycle



G1, S, G2 = interphase

G1 (gap 1): Intensive cellular synthesis - Organelle, - Protein, - RNA, - ATP

↓ chem signals

S (synthesis): DNA replication

- DNA replicate, wrapped around histones
- each chromosome now has 2 identical, uncondensed DNA mol.
- centrioles & centrosome replicate

G2 (gap 2): Intensive cellular synthesis - Organelle, - Spindle prot, - ATP

↳ mitotic spindle begins to form...

↓ chem signals

M

(c) Mit & Mei Process

n = amt chromosomes (no.)
Y = amt of DNA molecules (no.)

	MITOSIS & MEIOSIS SPINDLE	NUC	MITOSIS & MEIOSIS II GENETIC MAT	MEIOSIS I ONLY. GENETIC MAT	MITOSIS Per cell	MITOSIS per nuclear region	MEI I Per cell	MEI 2 Per cell
PROPH.	- Centrioles move to opp. poles (higher plants no centrioles) - Asters dup - Spindle fibres form, attach to kinetochore	- Nucleolus disappears - Nuclear envelope disintegrates	 DNA alr replicated Chromatin condenses to form chromosomes. appear as 2 sister chromatids joined @ centromere	 bivalents pair up = tetrad. X-over. form 2 chiasma Chromatin condenses to form chromosomes Homologues pair up via synapsis to form bivalents. Non-sister chromatids of homologous chroms break & rejoin, allowing xchange of genetic 3 material (Crossing-over)	G1 2n Y ↓S↓ 2n 2Y @proph.	G1 2n Y ↓S↓ 2n 2Y @proph	G1 2n Y ↓S↓ 2n 2Y @proph	n Y
METAPH	- Spindle completely formed.		 - Chromosomes moved to metaphase plate by kinetochore microtubules - aligned @ met. plate @ right ∠ to spindle axes (align in single file) 4 (random order) - Each centromere attached to spindle fib fr 2 poles. 5	 - Chromosomes moved to metaphase plate by kinetochore microtubules - Homologues align along metaphase plate in random order (indep. assortment) (in pairs) 4 - Each homologue attached to a kinetochore microtubule fr. the pole it faces 5	2n 2Y	2n 2Y	2n 2Y	n Y
ANAPH	- Non-kinetochore microtubules lengthen ↳ motor prot between overlapping reg push microtub apart → Elongate cell.		 MEI II non-identical sis chromatids - centromere div into 2 6 - Daughter chromosomes 7 pulled to opp. poles by shortening kinetochore microtubules	 sister chromatids still attached - Homologues separate 7 to opp. poles, pulled by shortening kinetochore microtub.	4n cent rom. div	2Y still 1 cell	2n Y	2n 2Y centromere x div!	2n Y centrom div!
TELOPH	Spindle fibres disintegrate.	- Nucleolus reforms - Nuclear envelope reforms around each gp of chromosomes	 - Daughter chromosomes reach pole, uncoil & lengthen into chromatin (appear diffuse) - cleavage furrow / cell plate starts to form	 - Chromosomes, each consisting of 2 sister chromatids, reach opposite poles. - Chromatin sometimes decondenses but XREP.	4n	2Y still 1 cell	2n Y	2n 2Y	2n Y
CYTO KINESIS	ANIMAL CELL - cell membrane invaginates to region prev. occupied by spindle equator - Cell mem. in region of furrow join up & separate → 2 cells - Cytoplasm & organelles evenly dist between 2 cells (NOT organelles!)		PLANT CELL - A series of fluid-filled vesicles surr by mem (made by Golgi) appear @ former spindle equator - Vesicles fuse → form cell plate (across form. eq) - Contents of vesicles conv to pectin & cellulose ↳ middle lamella & cell wall of daughter cells - Mem of vesicles form cell surf mem of daug. - cell plate fuses w/ parent cell wall & cell mem.		2n Y	2n Y	n Y	n y/2

(d) Comparison : Mit vs Mei

	MIT	MEI
1. LOC	- Somatic cells	- Precursor sex cell @ reprod organ
2. TYPE OF CELL	- Haploid / diploid cells	- Only diploid cells
3. NO. NUC DIV	- 1	- 2
4. POT	- 2 genetically identical cells Same no. of chromosome as parent	- 4 genetically diff cells Half. no of chromosome as parent.

5. Compare phase by phase (no 1-7 on prev pg)

(e) Significance of mitosis

1. Genetic Stab.
   - 2 daughter cells : same no & types of chromosome as parent
   - Genetically identical — X var → genetic stab
   - Exact replicate & even dist of parental DNA
2. Growth
   - No. cells ↑, new cells identical to existg cells
   - X same no of cell, each cell just grow bigger! (↓surf area ↑vol, Xeff diffusion of substances)
3. Cell replacement
   - Repair damaged / worn out tissues
   - Damaged cells replaced by cells identical to orig
     ↳ eg. regen of tails in lizards, arms in starfish.
4. Asexual reprod
   - Prod genetically-identical offspring
   - Gd in stable env → propagate genes well suited to the env
   - Rapid reprod — colonize area
   - Eg vegetative reprod in plants. (Strawberry)
     parthenogenesis : unfert egg → adult (water flea, stick insect)

(f) Significance of meiosis

A. Form haploid gametes in sexual reprod
- Prod haploid gametes
- Fert : 2 haploid gametes fus → diploid zygote restore diploid cond
- w/o mei : fusion of gametes will result in n×2.
- ∴ mei keeps chromosome no. const.

B. Genetic var
- Diversity of genes allow adaptate of pop to Δenv
- Favoured genes for favoured traits ↑survival
  ↳ some ind have these, pass on to F_2.
  ↳ accumulate of fav. heritable traits in pop
  ↳ ↑ fitness of pop

1. Crossing-over (show diag)
- Crossing-over of segmts of non-sis chromatids at prophase I of meiosis I
- New combi of alleles on chromosome of gamete

2. Independent assortment

AA aa BB bb
↓ mei I
AABB or AAbb or aaBB or aabb
↓ mei II
AB Ab aB ab

- During metaphase I, arrangement of chromoso. in each pair of homologous indep of other ch.
- During anaphase I, chromosomes of 1 homologous pair separate indep of other pair (50% chance of daughter cell getting either homo)
- Also, during metaphase II, non-identical sister chromatids sep indep...
- Diff combi of maternal/paternal chromos. in daughter cells

2^n diff gametes.

(g) Cancer cells

Cancer : Uncontrolled cell division when cells escape ctrl mech

Mutate in genes controlling cell div

1. Proto-oncogene —mut→ Oncogene (gain in func mutate)
   - Δstr prot prod — ↑env acts
     — ↓ regulate (eg. X breakdown growth factor)
   - ↑prot prod — ↑exp
     — ↑prot stab
2. Tumour-suppressor genes —mut→ X (loss in func mutt)
   orig func : suppress cell div, promote apoptosis. —mut→ cell X stop dividing

Characteristics of	CANCER CELLS	NORM CELLS
1. Genes	- Oncogenes	- Proto-oncogenes
2.	- Mut. tumour-suppressor genes (↓ func)	- √ tumour-suppressor genes
3.	- Abnormal nuclei ch mut — some duplicated — some deleted	- Normal nuclei
4. Growth	- Uncontrolled growth	- Controlled cell growth
5.	- No need growth factor	- Need growth factors
6.	- No contact inhibition	- Contact inhibition.
7.	- Do not differentiate properly	- Differentiate properly to form specialised cells
8.	- Angiogenesis (growth of new blood vessels)	- No angiogenesis
9.	- No apoptosis (telomerase)	- Apoptosis : lim to no of div

Forms tumours — genetically identical
— derived fr 1 mutant cell
↳ Benign : slow growing, X spread
↳ Malignant : fast growing, metastasis can occur

Metastasis : - cells of malignant tumours break away fr pri site and travel to other parts of body (via blood / lymph)
- At new site, multiply further, form new tumour.

(h) Factors ↑ chance of cancer

1. Env. factors carcinogens — carcinogens (radiation, mustard gas, arsenic, ↑DNA damage UV, smoking, soot)
2. Certain infects — Certain viruses & bacteria & parasites
   ↳ ΔDNA — virus insert gene into host cell
   ↳ suppress immune sys.
   — ↑ mut → cancer
   - Eg : Human Papilloma Virus — cervical cancer
     Hepatitis B & C — liver cancer chance
     H. pylori bacteria — cancer of stomach (damage lining)

BIO H2 ③ GENETICS

— NIGEL FONG —

① THE CENTRAL DOGMA

(a) Defs

Gene = spec. seq of DNA nuc → coding for spec. seq aa @polypep
Central dogma = Flow of g info DNA → RNA → protein
* Gene expression = translate + transcription

(b) Features of g. code

TRIPLET CODE	Every aa coded for by 3 consec DNA bases Nec 3 bp: 1 nuc → 1 aa = 4 aa max; 2 nuc → 1 aa = 4^2 aa max; 3 nuc → 1 aa = 4^3 aa max — enough (insuff 20 aa)
DEGENERATE	Some aa coded for by multiple codon
NON-OVERLAP	Read as successive grp of 3
CONTINUOUS	Not punctuated, no nuc skipped
START/STOP	Start = AUG Stop = UAG/UAA/UGA Read in correct reading frame: GUG|ACU (not stop)
UNIVERSAL	Same triplet codes for same aa in any sp Basis of g engineering

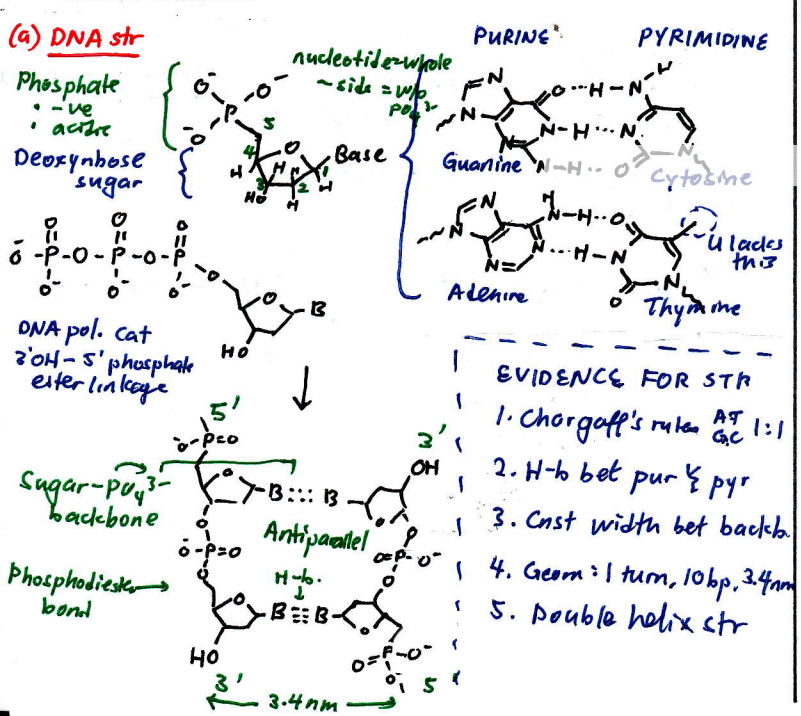
(c) Tracking sequences

DNA	5'	ATG	GCA	GTG	ACT	TAG	3'	Sense
	3'	TAC	CGT	CAC	TGA	ATC	5'	Template, read 3'→5'
mRNA	5'	AUG (start)	GCA	GUG (rmb U)	ACU	UAG (stop)	3'	Syn 5'→3'
tRNA		UAC	CGU	CAC	UGA	AUC		Trans 5'→3'
Prot		Met	Arg	His	Thr			→ based on codon not anticodon

codon = 3 consec nuc bp on mRNA codng for sp. aa

② STRUCTURES

(a) DNA str



EVIDENCE FOR STR
1. Chargaff's rules A:T, G:C 1:1
2. H-b bet pur & pyr
3. Cnst width bet backb
4. Geom: 1 turn, 10 bp, 3.4 nm
5. Double helix str

(b) Role of DNA/Why suitable store of info

STABLE MOLEC	H-b bet anti-ll → stable double helix strong cov. phosphodiester B – seq integrity	Pass on w/o loss of info
ACCURATE REPLN	Weak H-b allow sep of strand for rep, reform Each strand = template to form new str ↳ new str form thru ↑sp. b. pairing Proofreading by DNA pol	Acc rep of mitotic F, identical DNA copies
DOUBLE STRAND	Backup – can repair / damage/mut str using 2nd as template	Preserve integrity
INFO READILY UTILIZ.	Weak H-b: sep str for transcrip Comp bp: faithful DNA → RNA → Prot info transfer	Info accessib & useable by cell

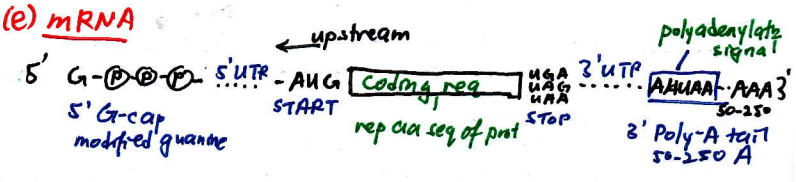
(c) Prokaryote v Eukaryote DNA str

	EUK	PROK
Size	10^8–10^{11} bp	10^5–10^7 bp
Str	Multiple linear mol	Single circ mol
Prot	Assoc w/ ↑prot (histone)	Assoc w/ ↓ prot
Packing	↑packs histone(+) w/ DNA(−) octamer 8H + DNA nucleosome 10nm (linker DNA) chromatin 30nm loop domain 300nm Chromosome	↓packing – loop or nucleoid-assoc prot – unfolded DNA 430nm (E.Coli) chrom. domain (prot-DNA int) supercoiling (gyrase) 1/μm
Locate	Nucleus	Nucleoid reg
Extrachromosomal DNA	None (except mitochon)	Yes – plasmids

(d) RNA v DNA

		DNA	RNA
MOL STR	SUGARS	Deoxyribose	Ribose
	BASES	A, T, C, G	A, U, C, G
	RATIO	A:T = C:G = 1:1	Varies – ss, no comp bp
MAC STR	STRAND	Double-str	Single str
	STR	Double helix	Complex twist/fold 2°, 3°
	STAB	Chem stable	↑unstable: +2'OH = ↑rxty; no comp bp
FUNC	FORMS	One basic form	Diff form w/ diff func – m, r, t
	LOCN	Nuc, mit, chlorop	Found thruout cell (mainly in nuc)
	AMT	Constant for all somatic c in a species	Varies

(e) mRNA



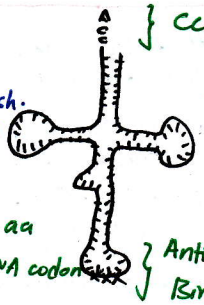
UNTRANSLATED REGIONS
1. Protect mRNA fr ribonuclease degradatn
2. Direct mRNA export thru nuclear pores
3. Promote transl – distng mRNA fr other mol
   5' recog sig for small rib subunit to bind
   3' intx w/ 5' to translate
4. 5' Target mRNA for recog
   "exon def" for splicing, 3' end formatn

mRNA FUNC Intermediate that carry info fr DNA → prot syn
↳ template for translatn
↳ 1 codon on mRNA codng reg = 1 polypep aa.

(f) tRNA

- Small (80 nuc)
- 3 loops, fold to form
  - 2D cloverleaf, 3D L-sh.
  - maintain by H-bond

Specificity
1. Bind to sp single aa
2. Anticodon bp mRNA codon

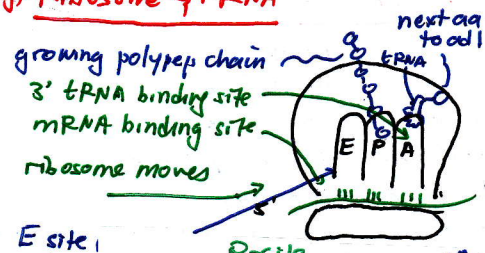


} CCA stem → specific aa attachment
Aminoacyl-tRNA synthetase
- recog 3D str of tRNA
- cat ester linkage btw CCA stem & sp aa corres to anticodon

} Anticodon → 3 sp unpaired base
Bind to sp mRNA via comp bp (A-T, C-G)

FUNC: Adapter molec in trans
↳ bring in specific aa in seq corres to seq. of codon in growing polypeptide.

(g) Ribosome & rRNA



Synthesize in nucleolus

	Euk	Pro
Large subunit	60S	50S
	+	+
small subunit	40S	30S
	=	=
	80S	70S

20-30 nm

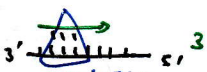
FUNC: Organelle, syn polypep fr mRNA codon (5'→3')
↳ provide env for sp. recog bet mRNA codon, tRNA anti
- close prox
- pos near aa for addtn to growing polypep
- PEPTIDYL TRANSFERASE (large sub) cat peptide B formatn bet 2 aa.

(3) PROCESSES

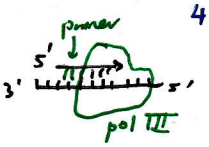
(a) Replicatn

1. Free dNTP manuf in cytop, tpt thru nuc pore into nuc

2. Initiatn
- helicase recog & bind to ori
- helicase unzip DNA str - disrupt H-b
- rep forks form, spread in 2 dir → rep bubble
- ss binding prot stab & keep apart ssDNA
- topoisomerase relieve overwinding ahead of rep fork
  ↳ break, swivel, rejoin str



3. Syn: RNA primer add to parental str by primase
prov free 3'OH for DNA pol to initiate syn



4. Elongatn (leading str)
- DNA pol III add nuc 5'→3'
- Parental str template → align free dNTP in comp bp
- Pol III cat phosphodiester B bet adj nuc of new str
- Proofread prev sectn, replace mistakes

5. Elongatn (lagging str)
- Syn opp dir to since antiptl & must syn 5'→3'
- more primers added as rep fork spreads
- Okazaki frag form thru discont syn of DNA
- Each okazaki frag lengthens 5'→3', join up w/ other frag to form cont str

6. Replace primer @ start of rep & make cont str fr Oka.
Pol I remove primer, rep w/ DNA
Ligase join 3' of new DNA to 5' of strand - phosph bond

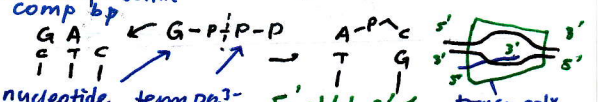
7. Multiple rep bubbles /ori ~ speed up rep / ↑pol sin
Rep bubble extend in both dir until meet & 2 helices form

(b) Rep: pro vs euk

	Euk	Pro
When	S-phase interphase	Prior to cell div (binary fission)
Where	Nucleus	Cytoplasm
No. ori	Multiple / DNA str	Only 1 (less g mat)
End-rep prob	Occurs, DNA linear	No, DNA circ
Rep ends	@ telomere	@ terminus
Rate	50 nuc/sec (hum)	500 nuc/sec (bact)

(c) Transcription

	Pro (cytosol)	Euk (nucleus)
INITIATN	1. G-factor + core pol = RNA pol holoenz	1. Gen TF assemble along promoter
	2. G fact of holoenz recog & pos pol on correct promoter	2. Gen TF recruit RNA pol, pos corr on promoter ⇓ Form transcrip-init cplx
	3. RNA pol unzip & sep 2str of DNA @ promoter - break H-b 1 strand used as template & transcribed	
	4. Free nuc fr nucleoplasm pair w/ template - comp bp ↳ RNA pol. cat phosphodiester B form bet adj nuc ↳ form Sug-phosp backbone	
ELONGATN	5. RNA pol move along template str 3'→5' cat assb of ribonuc comp bp	



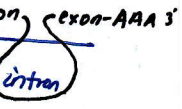
TERM	6. Af transcribing thru term seq terminator folds back to form hairpin loop mRNA released	6. RNA transcribes polyadenylatn sig (AAUAAA) Prot bind downstream of sig Cut & free pre-mRNA fr polymerase

(d) Post-transcriptional mod (Euk)

1. 5' mG cap & 3' poly-A tail added (quote func in prev sectn)

5'-PPP-exon—exon-3'
↓
5'-G-PPP-exon—exon-AAA 3' (200)

2. RNA splicing
introns excised, exons spliced tog
by spliceosome (snRNA-prot cplx)
- precise points of excision (seq @ boundary)
- w/ ATP use



↓
forms mature mRNA
5'-G-PPP—exons—AAA 3' (200)

Intron = noncoding seq between exons of gene
Exon = reg of DNA in interrupted gene found also in mature RNA pdt

(c) Translation

	PRO	EU
AA ACTV	1. Specific aa cov att to 3'CCA of sp tRNA w/ corres anticodon ↳ aminoacyl-tRNA ↳ cat. aminoacyl-tRNA synthetase w/ ATP use (1ene per aa) * L_1 specificity - sp aa ≡ sp anticodon → fidelity of code	
INIT	2. Translatn init factors facil b of small rib sub. w/ Shine-Dalgarno seq ↳ pos AUG start codon on ribosome	2. Euk init factors bind to small rib sub, pos init tRNA on rib 3 Small rib sub recog 5' G-cap move 5'→3', find start codon 4. Init tRNA assoc w/ start cod on mRNA — comp bp
	5. Large rib subunit bind — form transl init cplx P site: initiator aminoacyl-tRNA A site: vacant for addn next aminoacyl-tRNA } GTP use * L_2 specificity — sp bp codon/anticodon → code fidelity	
ELONG	6. 2nd tRNA w/ next aa bind to A site — H-b w/ 2nd codon (comp bp) 7. Peptidyl transferase cat. peptide B f. bet 1st & 2nd aa Initiator tRNA dissoc fr 1st aa 8. Ribosome shift 1 codon down in 5'→3'  E: 1st tRNA shift to E → release & recycle P: 2nd aminoacyl-tRNA shift fr A→P site A: Empty A ready to recv 3rd am-a tRNA ↳ anticodon comp to 3rd mRNA codon	
TERM	9. Stop codon reach A site release factors enter — hydrolyse polypep-P site tRNA bond polypep release fr ribosome ribosome disassem into subunits	

More than 1 ribosome can transcribe same mRNA simult — ↑ rate.

(d) Comparison

BIG IDEAS
- Comp bp (eg: A-T, C-G ... spell out)
- Unwinding & rewinding DNA helix
- Sep parental str progressively in short seg
- Proofreadng
- ATP use

	REPLICATN	TRANSCRIPTN	TRANSLATN
Locatn	Nucleus	Nucleus	Cytoplasm
Template	DNA	DNA	mRNA
Reading	3'→5'	3'→5'	5'→3'
Raw mat	dNTP	NTP	Aminoacyl-tRNA
Base pair	A+T, G+C	A+U, G+C	A+U, G+C
Enz Involvemt	Helicase, Primase, pol, lig	RNA pol	Ribosome, aminoacyl-t syn. ribosomes, tRNA
Products	DNA double helix	mRNA strand	Polypeptide
Type of B	Phosphodiester	Phosphodiester	Peptide

(4) ORGANIZ OF GENOME

(a) Intro

Noncoding = part of genome, X code for prot/RNA pdt
mostly rep seq e.g. tandem repeat — short seq, rep many X

	No. genes	Non-coding	Introns	Promoter	Enhancer Silencer	Repeat seq	Operon
EUK	25k	98%	Many	✓	✓	Many	Few
PRO	4.5k	15%	Rare	✓	rare	Few	Many

(b) Telomeres

END-REP PROB.
- 5' of newly syn str b/c primer removed w/o replacemt
- NO 3' to add nuc to growing DNA str
- Shortening of chromosome — could erode gene

TELOMERE
- Tandem-rep seq @ end of euk chromos. (non-coding)
- 3' overhang w/o complem. region

FUNC 1
- Ensure g X erode w/ rep
  - End-rep prob as DNA pol need 3'OH to ext 5'→3'
  - 3' overhang, 5' of new str: primer remove w/o replac.
  - Genes close to ch ends can erode w/o telom
    - ↳ loss of g. mat
  - But shortening of ch ends - first shorten telom, X deletrious effect
  - Telom in cell which b/c div many X shorter

FUNC 2
- PROTECT & STAB chromosomal ends
  - Form t-loop w/ prots - protects cap str
  - Else, ss end of ch may comp bp to anr term end
    - ↳ chrom. joining → recog as damage → apoptosis

GERM C
- Need to b/c chrom persist X Δ over 1gen
  - Else ch get shorter, g. eroded
- Telomerase lengthen telomeres
  - also pres in rapidly div somatic cell
  - func ↓ w/ age ~ telom shortening.

(c) Centromeres

DEF
- Constricted reg on chromosome where spindle fib attach
- Repetv non-coding tandem repeat

FUNC 1
- Allow sis chromatids to adhere

2
- Allow kinetochore prot & spindle fib attachmt
- so sis chromatids/homolog ch sep to opp poles
  - ↳ unique seq for kinetochore recog

ANAPHASE
- Centromere divide & move apart

FAILURE 1
- 0 centromere — improper alignmt/segregatn → aneuploid
- 2 centromere — came sis ch pull to opp pole → breakage

(5) CONTROL OF EXP

(a) Importance in euk

CPLX
- Euk DNA cplx — genes for 1 pathway scattered
  - transcrp & transl spatially & temporally sep

CTRL
- Specific ctrl for each gene — hormone → cause binding of ctrl elem
- Combi actv/rep ⇒ finer ctrl — no. mRNA, time to start transcrp.
- Nec as 1 gene may exp for diff resp / need diff str. of resp

COOP
- Usually >1 gene exp for 1 pathway
- Ctrl & coord: TF may bind to several reg genes

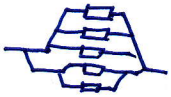
CTRL @ GENOMIC LVL

(b) Gene amplificatn

DEF Sp gene rep. multiple x, create more copies

FUNC Allow g. of int. to exist in ↑copy no.
↑copies transcribed → ↑m RNA → ↑prot
During dup, some g pdt req ↑Q: 1 copy ✗exp fast enough

CSI Chorion prot req ↑Q to protect Drosophila zygote - amplif meet DD
chorion gene rep x times in ovarian cell, other cell: single copy

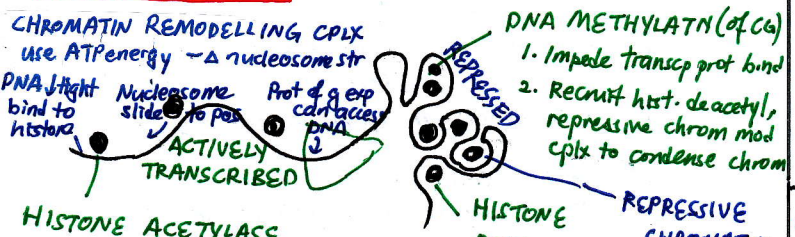


Rep init @ ori within gene of int
Randomly term → fork str

CSII Oocyte of S.Af clawed toad need 10^{12} rib for prot syn alf fert
Precursor cells 1k copies of rRNA gene... take 50yr
Need amp no rRNA genes 0.2% → 68%

Circ. extrachromosomal DNA form
Syn many more copies of circ DNA (rolling circ)
Circ extrachromosomal DNA @ nucleoli

(c) DNA & histone mod



* How condensed affect accessb fr transcrip

CTRL @ TRANSCRIPTN LVL

(d) Promoter (P,E)

= noncoding seq upstream of transcrip start
↳ RNA pol & gen TF recog, bind for transcrip init
→ Seq det promoter strength & freq. transcript.

PROK	EUK
-10 5'-TATAAT-3' Pribnow box	-25: 5'-TATAA-3'
-35 5'-TTGACA-3'	• Fixed pos, det transcrip start
Consensus seq: most commonly occurng bp in seq elem	-75: 5'-GGCCAATCT-3' CAAT box
More sim to consensus seq = stronger promoter - ↑transcrip	-90: 5'-GGGCGG-3' GC box
	• Loc variable, not always ✓
	• Gc can inv multiple copies
	• Recruits RNA pol, TF

(e) σ factor (P)

= subunit of RNA pol, recog & bind to promoter to init transcrip

- Diff σ factor recog diff promoter
- ctrl availability of σ factor det operon inv & g transcribed
- ↑efficient: Δσ F allow diff g transcrib. by same RNA pol core enz

(f) Operon (P) — see operon

- Repressor bound to operator block RNA pol, ✗transcrip.
- Small effector mol Δ rate
  - co-repressor tryptophan } bind to repressor
  - inducer allolactose }
  - co-activator cAMP } bind to activator

(g) ENHANCER SILENCER (E)

Non-coding short DNA seq
Can loc far away / near / within intron of gene

Bind to specific TF ACTIVATOR to upreg transcription	Bind to specific TF silencer to upreg transcripta
1. Promote transcp init cplx assb - intx w/ RNA pol/gen TF or hist	1. Dir intx TF, prevent transcrip init cplx assb
- looping mech, bend space DNA	2. Bind repressor - overlap act^n binding region → block
	3. Bind activator - act^n ✗intx w/ gen TF
2. Recruit histone acetylase / chromatin remod cplx Decondense chromatin ↑accessb for gen TF / RNA pol	4. Recruit repressive chromatin remod cplx / histone deacetylase Condense chromatin ↓accessb to TF / RNA pol

(h) Post-transcrip. mod (E) — see bf

(i) Alternative splicing

E1-E2-E3-E4
↓
E1 E2 E3 —
E1 E2 E4 —

Single pre-mRNA can form diff mature mRNA depending on where exons spliced

- 1 gene can code for ↑ polypep
- ↑no prot rel to no. gene
  - e.g. prot may be free / mem-bound } alt splice det free/b
    extra exon for transmem reg

(j) CTRL @ TRANSLATIONAL LVL

	PRO	EU
mRNA stab	Short ½ life - ribonuclease degrade in mins Allow induct^n exp to rev quick (responsive)	Stab ∝ poly A length Steadily remov by ribonuc 3'→5' Crit. ? → 5' cap remov → both end deg.
Small rib subu. binding	Tr init: binding of small rib subu to shine-dalgarno block - trans^l rep prot - antisense RNA block mRNA & Δ2°SS, SD seq ✗acc	Tr init: binding of small rib subu to mRNA 5' block by trans^l rep prot binding to - 5' cap region - 3' UTR: 3' poly A & 5' cap ✗intx
Availab of init factors	• IF req for proper pos. of small rib + init tRNA + mRNA • IF req - recruit large rib Availb IF affect init of tran	Sim, but diff & diff Q IF Availability det by phosphorylatn

(k) CTRL @ POST-TRANSL LVL

COVALENT MODIF	Eg glycosylatn, disulfide B formatn, prosthetic gp nec for prot func EU: mod @ RER/golgi PRO: mod @ cytosol
PHOSPHORYL	Δ act^n of prot
DEGRADATN	Ubiquitin ligase attach ubiquitin to prot ↳ tag for degradatn recog by proteosome ↳ cleave into smaller peptide & degrade by cytop enz ⇒ Det how long prot rem in cell

⑤ GENETIC PROBLEMS

(a) Mutatn

Mut = rare, random, permanent Δ in DNA sq of gene
↳ Δaa → Δ3D prot → Δ phenotype
- can be caused by carcinogen
- inheritable if germline cells mutate

SUBSTITN | 1 nuc rep by anr
- 3rd nuc Δ may still ⇒ same aa
- Reading frame ✗ affected
- May be non-coding non-reg seq

} May not alter aa seq / conseq

INSERTN DELETN | 1/sev nuc inserted in seq
1/sev nuc removed fr seq
} less common
- Frameshift mutn — ribosomes read incorrect triplets fr mut onwards — non-func prot

INVERSION | segmt of nuc seq rejoin @ orig pos but inverted

(b) Sickle-cell anaemia (autosomal recessive)

	β-globin	mRNA	AA @ pos 6	Δ prop RBC
Normal	CTT ↓subst	GAA	Glu (charged hydrophil)	Biconcave even ↓O_2
Sickle	CAT	GUA	Val (non-pol. hydrophob)	↓O_2: Hb crystallise into rod-like fibre RBC sickle shape

EFF
- RBC fragile — break — shortage
  - Poor O_2 tpt — breathless, weakness
  - Heart failure
  - ATP shortage
- Sickle cell crisis : sickle RBC elongated — lodge in capillar
  - Deprive organ of O_2
  - Damage to organ esp. ↑capillaries (spleen, lung)
  - Severe pain : tissue death

(c) Cystic Fibrosis (autosomal recessive)

CFTR | CF gene code for CFTR : cystic fib transmem conductance regulator — ctrl Cl^- in/out of cell ⇝ indir inf Na^+

MUT | Deletn of 3 nuc on ch7 exon 10 — loss of phenylalanine
CFTR missing / defective

E: LUNG
- Cl^- not tpt out of epithelial cell : Na^+ retain (charge)
- Water retained in cell (Ψ more -ve)
- Mucus in lumen undiluted — thick — ✗ flow
- Congestion: mucus remains too long in resp tract
- Bacteria growth / infectn
- ⇒ Severe breathing difficulty

E: DIGESTN
- Thick mucus in intestine : ↓abs digested food
- Pancreatic duct choked by thick mucus — ✗ rel enz — ↳ indigestion

E: SWEAT
- Usually, upper duct of sweat gland reabs Na Cl
- Defects CFTR — no reabs : salty sweat (diagnostic)

E
- Death by age 30

(d) Δ chromosomal aberratn

Deletion, Duplicatn } Non-sis chromatids break & rejoin incorrectly
Uneq crossover — 1 chromatid give up more gene

Inversion — new loc, inf by e.g. new reg elements ...

Translocatn : move segmt fr 1 ch to non-homologous ch

Aneuploidy
- Chromosome pres in extra/fewer copy
- Chr no not multiple of wild type
- Result of non-disjunc : homolg ch / sis chromatid ✗sep at mei I/II
  - ↓ mitosis transmit anomaly to all cell

Polyploidy
- 3x or more x haploid no
  - ↳ non-disjunc of entire chromosome mit/mei
  - ↳ common in plants
- Need even no. to pair up @ mei & form gamete

⑥ CANCER

(a) Cancer

DEF Uncontrolled growth & sp read of abnorm cell

BENIGN Non-cancerous : ↑div & growing

MALIGNANT
- Invasive (erode surr norm tissue)
- Can metastasize (spread to other part of body)

	NORM	CANCER
Differen	Differentiate : specialize cell	Undifferentiated
Contact	✓contact inhibitn : monolayer	✗contact inhibitn : multp layer
Growth	Controlled growth & prolif	Excessv growth & div
Apoptosis	✓undergo apoptosis	✗undergo apoptosis
DIV	Lim cell division	Indefinite div (upreg telomerase)
Adhesion	Cells adhere to form tissues	Able to detach fr surr cells
Angiogen	No angiogenesis	✓angiogenesis

(b) Protooncogene → oncogene (gain in func)

Enhanced cell growth & prolif — 1 allele mutate enough

CHROM TRANSLOCATN | Protooncogene next to enhancer : ↑transcrip
↳ ↑g products — oncogene

RETROVIR INTEGRATN | Provir enhancer Δ protooncogene promoter disrupt silencer carry viral homologue
- Hum Papilloma Vir ↑cervical cancer
- Hepatitis B, C ↑liver cancer

MUTATN | Δ protooncogene aa → prot : ↑resistant to degrad
Δ regulatory elem eg. enhancer

AMPLIFICATN | ↑copies of protooncogene — esp w/ carcinogen (UV, smoking, arsenic)

RAS | G-prot : transduce sig fr growth factor to downstream processes
Mutn — constitutively actv, ✗recep stim

(c) ↓ Tumour-suppressor gene (loss of func)

NORM FUNC & p53 FUNC / both copies mut for cancer

- Mod prolif sig ... When DNA damage arrest cell cyc, ✗prod mutant cell
- Actv DNA repair ... pres. gen integrity, ✗mut (onco)
- Actv apoptosis ... remove damage cell if beyond rep

(d) Multistep proc.

- Multp reg checkpt, genes @ each lv to mut for cancer
- Nec : gain in func @ protooncog, loss of func @ t supp, telomerase, ...
- Take many mut, not all mut Δ func ... t for mut to accum.

BIO H2 ④ VIRUSES & BACTERIA

NIGEL FONG

① VIRUS

(a) Xteristics

- Vir: obligate parasites, X live indep of host
  - : contain DNA/RNA but not both
  - : components must assb into complete viron to mov btw cells

Living? — G mat — Can evolve

X living? — Can only surv in host cell, X indep metabolic actv

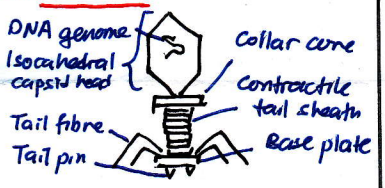
(b) Evolutionary origin

1. RNA vir: fr RNA strand which have acquired rep enz
   e.g. RNA-dependent RNA pol
2. DNA vir: fr transposons - able to escape host genome, exocytose w/ g & prot to inf & rep
3. Bacteriophage: fr plasmids that escape, infect other bact & self-replicate
4. Fr parasitic cellular org that reduced fr cellular → viral

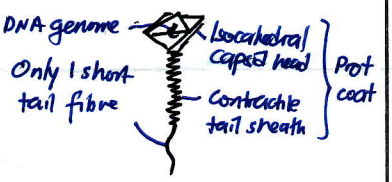
FURTHER EV

5. Antigenic drift: Random mut in viral genome
6. Antigenic shift: 2 diff vir infect single host, recombine

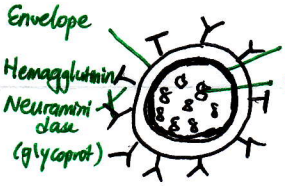
(c) Structure



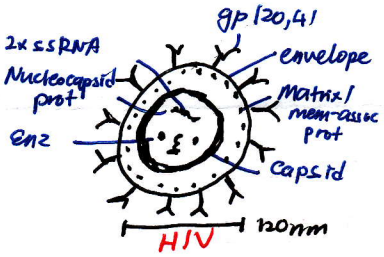
BACTERIOPHAGE



λ (TEMPERATE) PHAGE



INFLUENZA



HIV

GENERAL

GENOME
- Code for vir component & enz
- ↓ no. genes (3-100) ~det v complexity
- RNA/DNA, ss/ds, single/segmented, lin/circ

CAPSID
- Prot coat surr genome ~made up of capsomeres
- Protect & intro genome to host cell

4 MAIN VIR: helical, isocahedral, enveloped, cplx (env vs naked)

INFLUENZA

8 seg ss RNA pkg w/ prot → helical nucleoprot

3 pol pep per RNA: RNA dpd RNA pol
- replicate & transcript

Glycoprot: hemagglutinin (attachmt)
neuraminidase (release new vir)
↓
diff H & NA det strain

(-) strand RNA vir: gen comp mRNA

HIV

- 2 copies ss RNA
- Enz: rev transcriptase, integrase, protease
- Envelope fr host cell mem nuc/vacuolar/outer
- gp 41 & 120 bind to T_4 recep
- core = capsid and inside

(+) strand RNA vir: genome = mRNA

(d) Replicate

	BACTERIOPHAGE	λ PHAGE	INFLUENZA	HIV RETROVIR
Attachment	Attm site on tail fibre recog & att to comp recep on bact surf Weak bonds formed / cell wall, flagella, pili / specificity		Protruding glycoprot on outer mem bind to sp receptor Hemagglutinin: sialic acid	gp120: CD4 — ∆conf co-recep
Penetrate	• Tail release lysozyme: digest cell w, molec rel thru hole • Molec reach vir, ∆shape of base plate • Spring like contracts of tail sheath — thrust hollow core tube thru cell wall • Tip of core reach p. mem — DNA enter thru tail core • Capsid rem outside cell (empty) ALT: enter thru flagella/pili		ENDOCYTOSIS • Host mem invaginate & pinch off • Vir in endocytotic vesicle • Fuse w/ lysozyme, ↓pH • Vir env fuse w/ vesicle mem, nucleocapsid rel into cytop • Capsid degraded by cell enz • Nucleoprot dispatch to nuc	• Viral env fuse w/ host mem ↓ leave behind; ↓ capsid rel into cell • Capsid degraded • Enz & RNA rel into cytoplasm
Replicate	LYTIC CYCLE • Vir nuc acid —host RNA pol→ mRNA • Degrade host DNA └ reuse nucleotide └ vir DNA methylated, X deg • Vir enz take over bact syn mech (using host enz) ·syn phage DNA ·syn viral prot • Eclipse period — X find complete vir — only sep components	LYSOGENIC CYCLE • Phage DNA form circ — lytic → lysogenic • Recombine & bec part of bact circ DNA (prophage) • Prophage g. repressed by rep prot (1 of phage g.) • When bact div rep, prophag rep └ rem latent INDUCTN • Rep prot destroy by acts bact enz • Spontaneous, ↑freq w/ DNA damage • Prophage excises out, replicate	Viral ss RNA use as template ↓ Comp mRNA ↓ New viral RNA genome mRNA exit nuc - cytosol → transl capsid prot - ER → transl glycoprot for virion env	Rev transcriptase vRNA → DNA -syn DNA comp. RNA — RNA-DNA hybrid - RNA strand degrade -syn 2nd DNA comp 1st → provir Vir DNA enter host nuc └ Integrase: integ. into host DNA └ May persist in latent state PROVIR ACTV • Transcrip vDNA → vmRNA ↓ v. polyprot • Env glycoprot made @ ER vesicle tpt to mem • Viral mRNA = new vir g mat
Maturate	Spontaneous assembly - tail fib join w/ tail - DNA-filled head attach	Phage component prod using host enz Then assemble into complete vir	• Vesic fr ER tpt v glycoprot to p. mem • Capsid prot assoc w/ glycoprot @ p. mem • Viral genome assoc w/ prot nucleoprot assoc w/ capsid @ p. mem	• Capsid form around nucleocapsid proteins, enz, vRNA • Assemble @ cell mem into new vir
Release	Lysozyme (phage gene) break down bact cell wall P. mem of host lyse — release vir to infect new cells		BUDDING (evagmatn) • New vir bud fr cell, acquire host mem w/ embedded glycoprot - Host cell may/may not be lysed	

(e) Effects of bact vir

Bact w/ integrated prophage may exhibit phage conversion
- └ prophage: gene for diphtheriae toxin → bact prod toxin only w/ this prophage

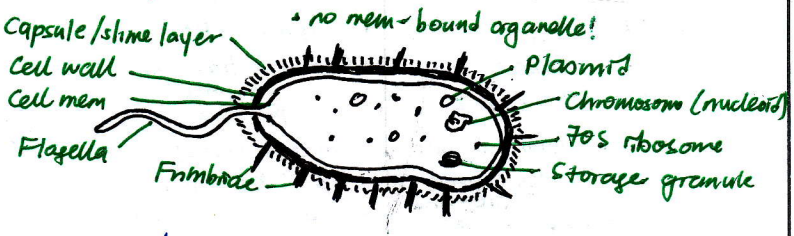
Def mech against phage
- Dup receptor site X recog by phage
- Dup restrict enz that recog vDNA
- Mod own DNA to prev rest enz attack
- Dup lysogenic rts w/ phage

(f) Pathogenecity of animal vir

	INFLUENZA	HIV
TARGET	Mucus lining of resp tract	Helper T cell
MECH	- Virus settle on muc lining - Neuraminidase: penetrate muc mucoprot - Hemagglutinin: bind to epithelial recep - Infected epit. cell destroyed (24hr) - Inflammate, build up dead cells - Weaken epit layer → 2° inf eg pneumon	- gp41, 120 bind CD4 - Destroy CD4 cells - Depress immune sys: ↑infectns - Pass cell→cell undetected - Mutate @ ↑rate: Δsurf prot └ X recog & elim by immune - Full blown: unmgt 2° inf
TRANSM	Moisture drop fr lung Infected bird dropping	Blood, sexual Mother→child: placenta, milk
TREATMT	Symptomatic Antivirals (tamiflu) Antibiotic (X 2° inf	AZT nucleoside analogue → X rep Rev transcrip inhb Protease inhb — X prod vir Fusion inhb — X ente cell

(2) BACTERIA

(a) Structure



NUCLEOID	Region w/ DNA
RIBOSOME	Translate site — give granular appearance
STORAG GRA.	Nutrient reserves (glycogen, lipid, ion) = inclusions
PLASMID	Small, circ, autonomously rep mol, sep fr chrom Genes may confer adv but X nec
CAPSULE SLIME LAYER	Polysacc / prot • Protect fr env danger — antibiotics, phagocyt • Adherence to surf • Biofilm = matrix of bact living in slime (eg dental plaque)
CELL WALL	= Peptidoglycan / murein (sugar + prot X-link) • Protect cell fr osmotic lysis • Gram − : Outer mem w/ lipopolysacc, endotoxin + : No outer mem; thicker CW
P. MEM	• Permeability barrier — tpt solutes • ETC for photosyn & resp • Syn mem lipid / peptidoglycan
PILI / FRIMBRIAE	For attachmt to other cell / sex pilus (bristle-like fib)
FLAGELLA	1 Long, hollow, cylindrical prot → Motility motor below cmem

(b) Binary fission (Replicate)

- DNA rep start fr ori
- Double helix sep — rep bubble
- Rep proceed outward fr ori - bothdir
- Enz cut, twirl, reseal helix
- New & orig ori move to opp end (assoc w/ p mem)
- Cell elongate
- P mem invaginate, deposit new CW

DIFF MITOSIS
- No spindle
- No pos chromosom
- Div immed aft rep

ASEXUAL
- Adv in stable, favenv
- Successful genotyp quickly colonize habitat

(c) Transformation

= uptake of foreign naked DNA fr env — Δgenotype, phenotype
- └ may be dead, lysed cells

UPTAKE	• Using cell surf prot — bind to & tpt DNA into cell • Make artificially competent: Ca^{2+}, heat shock
COMBI	• Foreign DNA incorp into chrom └ X over w/ homolg reg on bact chrom • Orig DNA degraded • If diff allele xchg — Δphenotype ⇒ recombinant

(d) Transducta

DEF	Phage carry bact gene fr 1 host to next due to abberatn in phage reprod cycle
GENERALIZ / random pan transfer	Phage enz hydrolyse bact ch Assb of phage genome — host DNA pkg instead of phage DNA Defect phage released: lysis ↓ bact DNA into anr cell — defect phage, X syn new
SPECIALIZ / gene adj to prophage transfer	Phage integ into bact chr as prophage (lysogenic) When prophage exit chr, vDNA improperly excised Bact DNA adj to prophage pick up Phage-host hybrid DNA in v progeny infect new host
RECOM	Foreign bact DNA rep homolog reg of recipient cell └ X over bet homologous reg OR specialize: integ of phage-bact hybrid If new allele — recomb cell

(e) Conjugation

DEF	Dir transf of g mat fr 1 bact cell to anr via sex pilus One way transfer F^+ (w/ F factor on F plasmid) → F^-
MECH	Due to F factor, F^+ prod sex pili Sex pili on F^+ make contact w/ F^- receptor Sex pilus retract — pull 2 cell into contact Form conjugate tube (mating bridge) 1 strand plasmid DNA transf across tube & recirc Each parental str = template for syn comp daughter str ⇒ 2 bact cell w/ F^+

③ OPERONS

Gene reg in prok / bact
& some euk e.g. C elegans, nematodes

(a) Purpose

GEN
- Δ rate prot syn w/ ΔDD
- Transcripta-level ctrl – efficient
- → Responsiveness to env
- → Economical use of energy/resources ✓ X prod w/ X need ✓ X prod w enough

LAC
- E coli in adult cow colon X exp to lactose but √ in calf
- – X prod lactose-metaboliz enz just in case → wasteful
- – but may need these enz

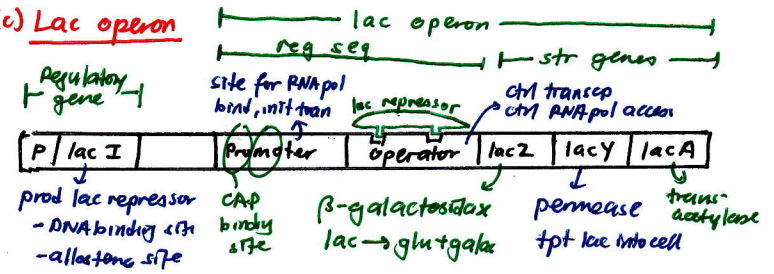
TRP
- Tryptophan essential for prot syn
- But when [trp] ↑, no need to prod

(b) Type of operon

	REPRESSIBLE	INDUCIBLE	CONSTITUTIVE
DEFAULT	On	Off	On
W/ EFFECTOR	Off	On	–
TYPE- PDT	Anabolic use/ nec	Catabolic nec only if substr	Housekeeping
REPRES. SYN IN	Inactive form	Active form	–
REP BIND OP	When corep bind to allosteric site	On its own	–
REP X BIND	On its own	When inducer bind to allosteric	–
EFFECTOR	Tryptophan (corepressor)	Allolactose (inducer)	
EG	Trp	Lac	

-ve reg: repressor turn off gene
+ve reg: activator turn on gene

(c) Lac operon

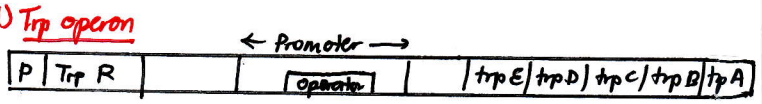


X LAC
- lac I constitute transcribed
- lac rep prod in active form → bind operator
- RNA pol X access operator → repressed

√LAC √GLU (+ve reg)
- Basal lvl lacZYA transcribed (leaky repressor)
  - permease: tpt lac in
  - β-galactosidase: lac → allolactose (inducer)
- Allolac bind to allosteric site of lac rep
  - Δ3D conf – DNA-b site X recog operator
- When all rep bound w/ allolactose – RNA pol bind to pro
- Transcribe lacZYA as polycistronic mRNA
- But lac prom ↓ affinity for RNA pol – lac only x each
  - Use glu first, energy req to conv lac → glu+galac

√LAC X GLU
- ↓ glu, ↑ cAMP – bind to allosteric s of CAP (catabolite activator prot)
- cAMP-CAP bind to CAP binding site @ promoter
- ↑ affinity of promoter for RNA pol → ↑ transcripts

(d) Trp operon



- Trp rep syn in inactive form, ↓ affinity for op
- Trp ↑, bind to trp rep @ allosteric site
- Δ3D conf of rep → active form
- Rep prot attach to operator → switch off

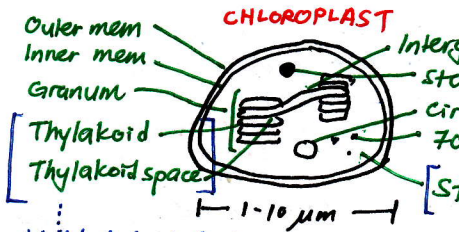
Single polycistronic mRNA
↓
ene to syn trp

Bio H2 ⑤ Physiology & Biochemistry

NIGEL FONG

① Photosyn & Resp

(a) Purpose & Overview



Light-dpd photophosrylatn & chemiosmosis
- non cyclic: 1 ATP : 1 NADPH, PSII→I
- cyclic: ↓ ATP : X NADPH, PSI
- 9 ATP : 6 NADPH

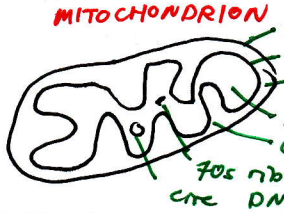
Calvin cyc → 1 G3P (3 CO_2, 3 O_2)

ENDOSYMBIOTIC THEORY

Forerunners of mit/chlorop were symbiotic prok living in larger euk

Eu-circ DNA, 70S rib
- replicate indp of nuc
- same prok antibiotic hinder prot syn @ mit/chlp
- size sim to bact (mit)

MITOCHONDRION



		CO_2	ATP	NADH	$FADH_2$
1 Glycolysis	Cytosol		2	2	
2 Link rx	Matrix			2	
3 Krebs cy	Matrix	4	2	6	2
4 Ox phosphr	Cristae			↓ ATP	↓ ATP
1 Glu 36-38 ATP		6	4	10	2

(10 ×3, 2 ×2)

→ some use to tpt pyruvate into mitochon

→ H^+ & e^- of gly NADH pass to mit NAD+ via shuttle sys (mem impermto NAD)
If pass to FAD → 2 not 3 ATP prod

TYPES

C source
- CO_2: autotrophic
- Org: heterotrophic

E source
- nitrobact
- Chem: Chemotroph
- Light: Phototrophic
  - non-S purple bact

- anabolic = build up
- catabolic = break down

PURPOSE

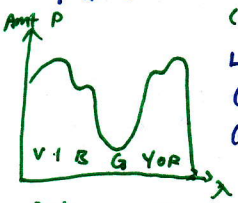
1. Syn sub - growth & repair
2. Mvmt - muscle, flagella
3. Actv tpt, nerve transm
4. Homeostasis
5. Reprod

WHY ATP

1. Mobile
2. Easy & rel.
3. Easy regen fr ADP
4. Inv in many rx.

(b) Photosyn : Photosys

Photosys = light-harvesting units on thylakoid mem

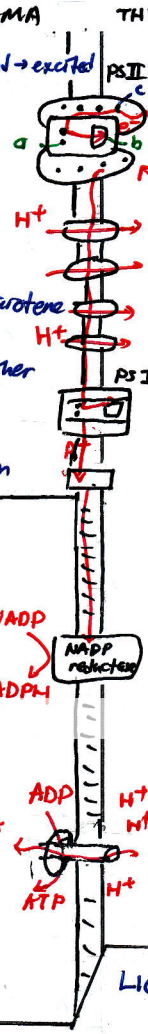


Action spectrum
- Eff of diff λ in stim photosyn

Rxn Ctr
(a) 2 special chlorophyll a molec
PSII: P680, PSI: P700
(b) 1° e^- acceptor

Light harvesting cplx
(c) Pigmt: chlorophyll a, b
(d) Accessory pig: carotenoids eg carotene
→ broaden λ abs for P
- Photons strike & relay e to other pigmt until red. P680
→ Photoprotectn: dissipate light E
→ Add col to flower/fruit - pollin

(c) Photosyn : Photophosrylatn



① Env: H_2O (source) → $2H^+ + 2e^- + \frac{1}{2}O_2\uparrow$ (splitg) in thylakoid sp | supply to P680

② hν strike pigmt in light-harvesting cplx energy relayed to other pigmt, reach P680 & rx ctr

③ excite P680 e^- to ↑E state — capture by 1° e accept replace w/ e^- fr H_2O

④ Photoexcited e^- pass fr 1 e^- accepta → PSI via electron tpt chain: carriers of ↑EN
e^-: downhill in energy terms ~couple to ATP syn

⑤ hν transfer via hν-harvesting cplx → P700 excite e^- hole in P700 filled by ETC e^-

CYCLIC: excited P700 e^- pass to mid of ETC1
prod only ATP - noncyclic prod ATP:NADPH (1:1)
Need 9:6 - cyclic make diff.

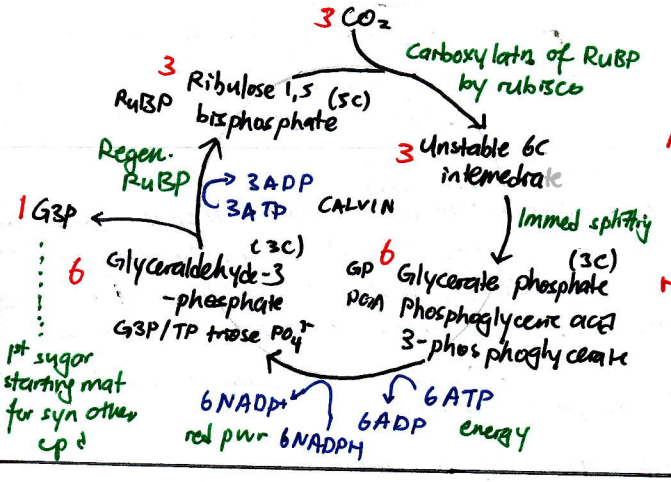
⑥ Pass down 2nd ETC (no ATP)

⑦ NADP reductase - transf e^- to NADP+ (final acceptor)

FUNC:
- coenz
- reduced, carry H^+ & e^- (↑E) fr thylakoid → stroma
- prov reducing pwr fr Calvin cyc
- regen

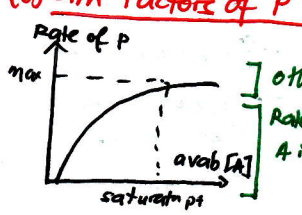
⑧ As e^- travel down ETC: energy rel pump H^+ matrix → thylakoid sp
Thylakoid sp ↑[H^+] | Matrix ↓[H^+]
H^+ diffuse down grad via ATP synthase ADP → ATP

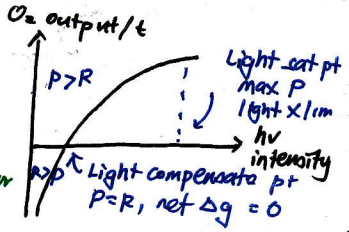
(d) Calvin cycle



1 G3P → pt sugar starting mat for syn other cpd

(e) Lim Factors of P





LIGHT	Norm X lim unless shade/winter Too much light ~bleach chlorophyll - thick cuticle prot
[CO_2]	Maj lim F ... atm CO_2 0.03%, opt 0.1-0.5% Greenhouse: pump in CO_2
TEMP	Enz rx, temp affect. Rate x2 for 10°C, max 35°C
[O_2]	Comp inhibitor fr rubisco RuBP+O_2 → 3C+2C → CO_2 esp bright hot day stomata close to ↓H_2O loss ↓ATP, ↓P o/p

(f) Respiratn : Glycolysis & link rx

CYTOSOL

Glucose (6C) ← Allos inhb by ATP & citrate, ↓ rate of resp
2ATP → 2ADP (phosphofructokinase — Acts by AMP, ADP)
Fructose-1,6-bisphosphate (6C)
↓
2 Glyceraldehyde-3-phosphate (3C) — G3P/TP triose PO_4^{3-} / PGAL
NAD+ → NADH
2 1,3-bisphosphoglycerate
2×ADP → 2×ATP
2 Glycerate phosphate
2ADP → 2ATP
2 Pyruvate

Phosph. of glu
- ↑ rate, committed
- trap in cell ion X pass thru P. mem

Lysis
- 2 isomers interconv

Ox by dehydrogen. energy used to phospholy. G3P

Substrate-lvl phosphorylatn
- ATP prod by dir enz acts on subst

---TPT TO MITOCHON MATRIX---

LINK RX
CoA → CO_2 + NAD+ → NADH
Acetyl-CoA ← other substrates eg. fats

Decarboxylatn
Ox by dehydrog.
- attach CoA

(g) Krebs cy.

* 2 rounds per glu

acetyl-CoA combine w/ oxaloacetate to form citrate

Oxaloacetate (4C) —CoA→ Citrate (6C)

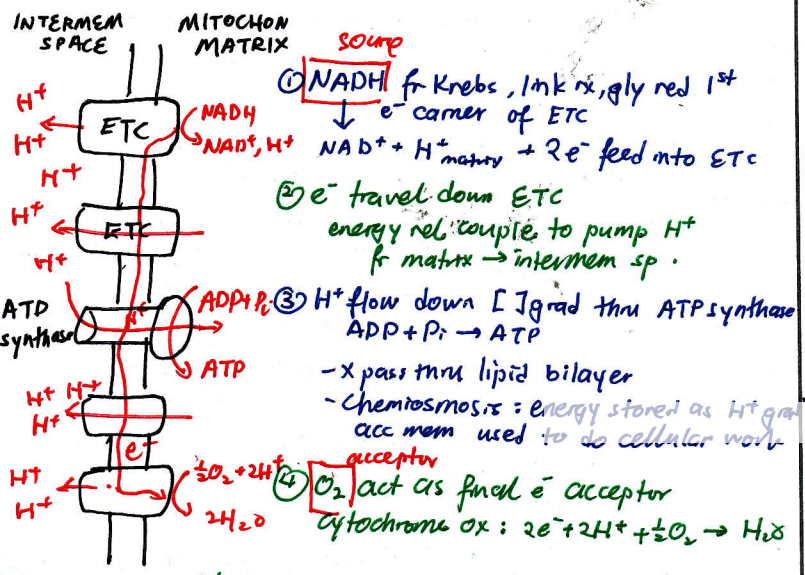
KREBS

3× ox. by dehydrog
- $FADH_2$ ← FAD
- 2 NADH ← 2 NAD^+
substrate-lvl phosphorylatn
- ADP → ATP
- CO_2 (decarboxylatn)

Citrate (6C) → α-Ketoglutarate (5C)
- CO_2 ← Decarbox
- NAD+ → NADH ← Oxy by dehydro

Decarbox ox. decarbox

(h) Resp : ox phosphorylatn



source
① NADH fr Krebs, link rx, gly red 1st e⁻ carrier of ETC
↓
$NAD^+ + H^+_{matrix} + 2e^-$ feed into ETC

② e⁻ travel down ETC
energy rel couple to pump H^+ fr matrix → intermem sp.

③ H^+ flow down [ ]grad thru ATP synthase
ADP + Pi → ATP
- x pass thru lipid bilayer
- Chemiosmosis : energy stored as H^+ grad acc mem used to do cellular work

acceptor
④ O_2 act as final e⁻ acceptor
cytochrome ox : $2e^- + 2H^+ + \frac{1}{2}O_2 \rightarrow H_2O$

IMPTCE OF NAD^+/NADH
- coenzyme
- red, carry ↑ e⁻ & H^+ fr org mol in matrix to ETC in mit cristae
- Des how NADH formed
- Des how re-ox
- Why regen
- 1 NADH = 3 ATP
- H^+ fr NADH + $O_2 \rightarrow H_2O$

IMPTCE OF O_2
- Final e⁻ acceptor, allow ETC reox NADH/$FADH_2$ cont don e⁻ to ETC
Allow ox phos to cont gen ATP
- NAD^+/FAD^+ regen, pick up more H^+ & e⁻ fr gly, link, Krebs
- Remove H^+ fr matrix

IMPTCE OF ETC
- Proton motive F → ATP
- Regen NAD^+, FAD

(i) Anaerobic Resp

→ Prod ATP even w/o O_2
- 1° cycle, ox P X — X reox NAD^+ by O_2.
- only glycolysis cont (2 ATP vs 38 fr aerobic)

→ Regen NAD^+ fr NADH (no energy prod)
└ gly only : NADH accum, X don e⁻ to ETC
- pyruvate/ethanal : final e⁻ accept, reduced
- regen NAD^+ so can cont accept e⁻ @ gly

ALCOHOL FERMENTATN (Yeast/bacteria)

Glucose —2ADP, 2ATP→ 2 Pyruvate
2 Pyruvate —Pyruvate decarbox→ 2 Ethanal (2C) + $2CO_2$
2 Ethanal (2C) —2NAD+, 2NADH [R]→ 2 Ethanol (2C)
Alcohol dehydrogenase

- Wine/beer prod.

LACTIC ACID FERMENTN (muscle cells)

Glucose —2ADP, 2ATP→ 2 Pyruvate
2 Pyruvate —2NADH, 2NAD+ (lactate dehydrogenase)→ 2 Lactate (3C)

- ↑ ATP$_{DD}$, ox P X replenish fast
- ↑ lactic acid : muscle fatigue
- lactate tpt to liver → conv back to pyruvate (ATP)
- Cheese/Yoghurt prod

② HOMEOSTASIS

(a) Defn & need

HOMEO = Ability of org to maintain dynam. stable int env indep of ext env, thru self-reg & -ve feedback

NEED
- Fluc ext env may affect T, pH, [metabolite]
- Homeo ensure cells maintained in int env w/ opt. cond for efficient & proper func
- Buffer against fluc. fr opt

SELF-REG • Org reg int physio env, indep of ext ctrl
-VE FEEDB • Δ physio var trigger resp to counteract init Δ (remov dev fr set pt)

→ STIMULUS — disturbance int env
↓
RECEPTOR — detect stim, send sig to ctrl ctr
↓
CTRL CTR — eval incoming info wrt set pt
↓
EFFECTOR — Signal fr ctrl ctr to gp of cells to bring response — bring cond back to set pt

ENDOCRINE GLAND
- Ductless, secrete hormones dir into blood (eff in ↓ [ ])
- Supported by rich blood SS
- Maintain homeostasis via slow LT ctrl

(b) Blood glu reg

Def: Diabetes

	HYPERGLYCEMIA - INSULIN	HYPOGLYCEMIA - GLUCAGON
Stim	↑ blood glu abv set pt (90 mg/dl)	↓ blood glu below set pt( )
Recep	Detect by β-cell of Islets of Langerhans in pancreas	Detect by α-cell of Islets of Langerhans in pancreas
Sig	β-cells secrete ↑ insulin by exocytosis into blood	α-cells secrete ↑ glucagon
Eff	Insulin tpt by blood to targets bind to cell surf recep - cascade 1. Vesicles w/ glut 4 tpt prot fuse w/ mem : ↑ glu uptake 2. ↑ glycolysis, ↑ lipid syn 3. ↑ glycogenesis (glu → gly) Insulin ↓ to set pt : -ve fdbk, ↓ sec	1. ↑ glycogenolysis 2. Gluconeogenesis (non-carb → glu)

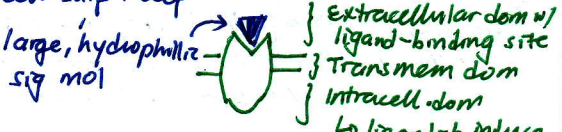
③ CELL SIGNALLING

(a) Ligand-receptor intx

Binding of ligand prod by sig cell to sp, comp prot recep to form ligand-rep cplx

- ligand: steroid, prot etc that can bind to ligand-b site
- prot recep: prot. that bind to sig mol, transduce sig into cellular resp

1. Intracellular recep. – small, hydrophob sig mol
   - can diffuse thru hydrophob bilayer
2. Cell-surf recep



G-prot coupled receptors

7-pass transmem prot w/
- extracel lig-b site
- intracel G-prot binding site

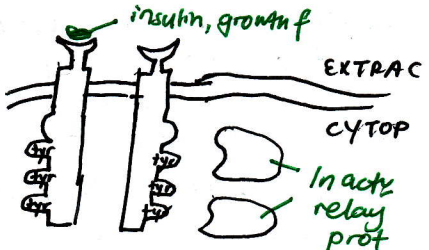
All 3 prot on p-mem ↓ Proximity ↑ chance of intx

GTP binding prot w/ intrinsic GTPase

Inactv: GDP bind
Actv: GTP bind

GTPase: GTP quick hydrolyse, term resp
Ensure cont pres of sig rqd for resp

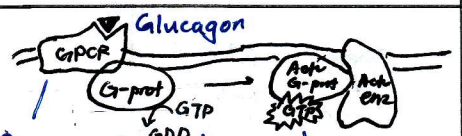
Receptor tyrosine kinase



RTK – xterse w/ intrinsic kinase actv on cytop domain
- cytop. act as tyr kinase transfer PO_4^{3-} of ATP to tyrosine on substrate

(b) Signal transduction

- Binding of sig mol (1° msg) to lig-b site of recep
  - ↳ Δ conf in intrac dom of recep
  - ↳ actv recep – can intx w/ other cellul m.
- Actv anr prot → anr mol → → Final prot @ each stage transduc into diff form, Δ conf
- 2° messenger – small, water sol
  - diffuse thruout cell
- Phosphorylatn cascade (1)
  - ↳ sig transduced by seq. PO_4^{3-} of prot kinase each mol add PO_4^{3-} to next mol in line
  - 2 post-translational mod.



Recep Δ conf bind to inactv G prot

GTP rep GDP → Actv G prot

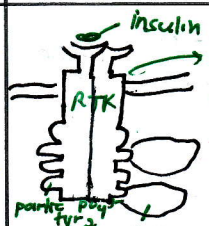
Intrinsic GTPase hydrolyse GTP → return to inactv state dissoc, reuse...

Dissoc fr recep, move along cytop side → Bind to, actv adenyl cyclase → Trigger next stp

ATP → cAMP

2° MESSENGER

- Adenyl cyclase ATP → cAMP
- Actv prot kinase A
- Phosphorylatn cascade (1)



- Recep subu. dimerise
- Autophosphorylate: kinase of each subu X-phosp tyr on other
- Recog by specific relay prot bind to / phosp by RTK – actv
- May actv 10+ intrac relay prot trigger diff sig transd pathways (2)

(d) Advantage of many steps

(1) sig amplif – small no extracell sig → ↑ cellular response
- some mol transm sig to many

(2) 1 sig mol → many cellular resp

(3) ↑ checkpt for reg – 1° msg degraded by extrac enz
- endocytosis lig-recep cplx
- ↑ phosphatases inactv relay mol

(c) Cellular response

Actv glycogen phosphorylase
Glycogen → Glucose
[↑ blood glucose]

Actv glycogen synthase
Glucose → Glycogen
[↓ blood glucose]

④ NERVOUS SYS

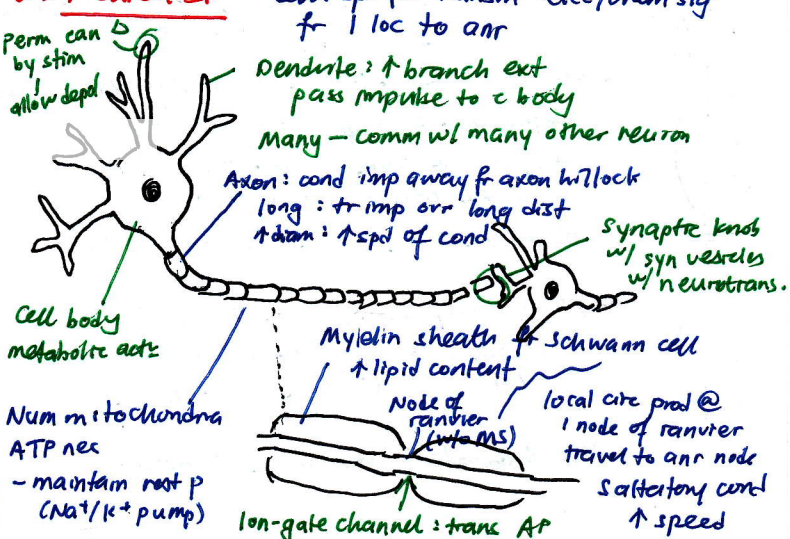
↳ need to trans info bet diff body part w/ diff func to coord body func

(a)

	Endocrine	Nervous
INFO	Chem sig i.e. hormone	Elect sig – neuron + Chem sig – neurotransm / synapse
TRANSM	Endocrine gland secrete hormone into bloodstream ↳ carry to tgt organ	Via neuron network ↳ branch thruout body
SPEED	Slow trans, min/hr for resp	Instant 100m/s transm
DURATN OF RESP	Sustained LT reg & homeostasis	Instant ST rxn ↳ eg knee jerk
EXTENT OF RESP	Usually systemic (eg growth)	Usually localized
SPECIFIC.	Non-sp: horm tpt Target specific	Specific pathway of interconnec neurons
CPLXITY	↓ str cplx – X integ	Cplx – allow integratn of info to elicit resp

(b) Neuron str

= cells sp. for transm elec/chem sig fr 1 loc to anr



(c) Resting pot

-70mV maintain wrt extracell – uneq dist ions

1. Na^+/K^+ pump : $2K^+$ in, $3Na^+$ out → ↑$[Na^+]_{out}$, ↑$[K^+]_{in}$ → -ve inside
2. More K^+ leak channels than Na^+ leak
   2 net flow ions out of cell → -ve
3. Fixed anions (prot, nuc acid) within cell
   X diffuse out due to size → -ve, large

Depolariz = ↑+ve Hyperpol = ↑-ve

(d) Action Pot = all/nothing rapid Δmem pot

PHASE	POTENTIAL	Na^+ V-GATE	K^+ V-GATE	ION MVMT
Resting	-70mV	Close	Close	
Depolariz ↓ Rising	-70 → -55 threshold ↓ -55 → +40	Some open ↓ trigger AP All open.	Close Close	Na^+ in Na^+ in
Repolariz	+40 → -90	Close	Open ↓ Close	K^+ out
Undershoot	hyperpolariz			Restore by Na^+/K^+ pump

(e) Refractory period

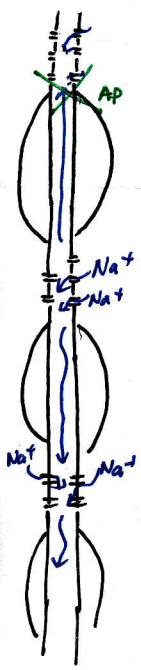
DEF = time during AP during which normal stim X cause anr AP

ABSOLUTE RP → no stim cause AP
RELATIVE RP → req larger than norm stim to gen AP

CAUSE → Amt of time needed for V-gated Na^+ to reset aft AP → V-gated Na^+ inactivated, need reactiv

CONSEQ
1. AP propagated only 1 dir – once reg depol, enter RP – but reg ahead can depol
2. AP X overlap
3. Lim freq AP, X over stim.

(f) Transmission of nerve impulse

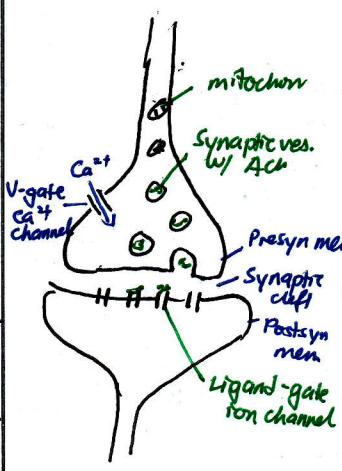


1. Na^+ influx @ AP create loc current of Na^+ which diffuses sideways within axoplasm, down echem grad
2. Bring wave of depol > threshold pot when reach next node of Ranvier: V-gate Na^+ open, AP
3. ∴ Self-prop of AP → one dir only
   ↳ tho Na^+ can travel upstream, V-gate Na^+ upstream in refractory period, X resp to stim.
4. Myelin sheath – ↑lipid: elec insulate, X Na^+, K^+ mvmt
   ↳ prevent leakage of Na^+ out of axon
   ↳ speed up transmission of impulse by SALTATORY CONDUCTION
   ↳ ensure depol @ next node > threshold

* AP can only gen. @ node of Ranvier
  - exposed to extracellular fluid
  - ↑ Na^+ or K^+ V-gated channels

+ Also, ↑diameter ↓resistance ↑speed.

(g) Synaptic transmission = Junc bet 1 axon & dendrite/cell body of anr neuron, muscle/gland cell



1. AP depol pre-synaptic axon term
   Open V-gated Ca^{2+}, Ca^{2+} diffuse in
2. ↑$[Ca^{2+}]$ in ⇒ synaptic vesicles w/ neurotransm fuse w/ presynap mem
   ↓
   Release neurotransm (acetylcholine) into synaptic cleft – exocytosis
3. ACh diffuse across cleft, bind to ligand-G ion chnl on postsynaptic m
   ↓
   Δconf ligand-G: open – Na^+ in
   Depolariz postsynaptic mem
   ↳ AP if reach threshold
4. AChesterase degrade ACh → choline + acetic acid
   Choline tptr actively take back ACh into presynap term – reform ACh

H2 Bio ⑥ MENDALIAN GENETICS

NIGEL FONG -

① DEFS & INTRO

(a) Chrom.

HOMOLOGOUS CHROMOSOME	Sim in size, shape, centrom pos, stain patts Genes for same traits @ corres loci
LOCUS	Pos of gene within chromosome
GENE	Ordered seq. nuc. @ partic. pos @ partic ch that encodes sp. func pdt
ALLELE	Pair/series of alt forms of gene w/ unique seq but occupy same locus on chromosome

(b) Genes

GENOTYPE	Combi of alleles @ homolog ch @ corres loci that det sp. trait
PHENOTYPE	Obs xtenstics – intx bet genotype & env
GEN → PHEN	Diff allele diff nuc seq → diff combi allele Diff genotypes → spec combi allele → sp. prot Sp visible traits → phenotype

(c) Variation

DISCONT VAR	Few, clearly defined phenotypes – discrete Caused by diff allele of single/few g, X env eff
CONT. VAR	Diffs slight & vary along continuum – bell curve No distinct groupings Polygenic inheritance + env. effects

(d) Alleles

HOMOZYGOUS	Alleles identical @ given locus diploid. = PURE/TRUE BREED
HETEROZYGOUS	Alleles diff @ given locus, diploid
DOMINANT	Fully exp in phenotype of heterozygote, mask rec
RECESSIVE	Inf appearance of phen. only w/ anr rec allele
CODOMINANT	2 diff allele. both exp in heterozygote phen

(e) Genes v env

Phenotype result of genotype + env cond

1. Uterus imp embryo dvp – e.g. o.t. malnutrit, disease
2. Phenylketonuria ctrl by ↓phenylalanine diet, X accum
3. Himalayan rabbit coat col. affected by temp
   └ heat X dvp black pigmt.

(f) Inheritance

TEST CROSS	Cross w/ homozyg recessive. ~reveal genot of org w/ dom. trait
INDEP ASSORT	Dihybrid inheritance shows indep inheritance.
LINKED G	Same chrom, close enough – inherited together X show indep assortment
RECOMBINANTS	Chiasma disrupt linkage grp, X-over
RECOM FREQ	% of recom in total offspring → X over freq ↑recom freq, further apart (1 centiMorgan = 1% RF)
SEX-LINK	Genes on X ch, recessv on non-homolog of X appear in males – no dom allele to mask
EPISTASIS	Allele @ 1 locus inhibits exp of allele/genotype @ anr loc. epistatic gene suppress hypostatic gene
└ recessive epi	suppress hypostatic g only when both epi alle rec
└ dom epi	" when homozy/heterozy

② PROBLEM SOLVING

(a) Format

Parental phenotype: Purple × White

Parental genotype: CCPP × c'c'p'p'

Meiosis

Gametes: (CP) (c'p')

Random fertilization

F1 genotype: All Cc'Pp'

F1 phenotype: All purple

Meiosis

Gametes

Random fertiliz.

Circle gametes

(CP) (Cp') (c'P) (c'p') × (CP) (Cp') (c'P) (c'p')

Show phentgen in punnet sq

	(CP)	(Cp')	(c'P)	(c'p')
(CP)	CCPP Purple	CCPp' Purple	Cc'PP Purple	Cc'Pp' Purple
(Cp')	CCPp' Purple	CCp'p' White	Cc'Pp' Purple	Cc'p'p' White
(c'P)	Cc'PP Purple	Cc'Pp' Purple	c'c'PP White	c'c'Pp' White
(c'p')	Cc'Pp' Purple	Cc'p'p' White	c'c'Pp' White	c'c'p'p' White

c'c' mask P

p'p' mask C

(epistasis)

F2 genotype: 9 C–P– : 3 C–p'p' ; 3 c'c'P– : 1 c'c'p'p'

F2 phenotype: 9 Purple : 7 White

(b) Common ratios

3 : 1	Monohybrid	Aa × Aa
1 : 1	Monohybrid	Aa × aa
All dom	Monohybrid	AA × aa or Aa × aa
9:3:3:1	Dihybrid	AaBb × AaBb
1:1:1:1	Dihybrid	AaBb × aabb
Var on rat.	Epistasis (e.g. 9:7, 9:3:4 etc) – add to 16	
↑parental	Linkage	
↑P(male) rec	Sex-link (eg. haemophilia, col blindness) └ use reciprocal cross (rev sexes, same g) to test	
1:2:1	Codominance	RR × WW → RR + 2RW + WW
2:1	Lethal gene	1 CC dre : 2 Cc' creeper : 1 c'c' norm └ does not add to 4/16

(c) Pedigree

M F

Write out possible genotypes

Each allele must come from somewhere

(d) χ^2 chi square

1. H_0: No sig diff bet obs & exp, any diff due to chance
   Find exp. value
2. Calc $\chi^2 = \sum \frac{(obs-exp)^2}{exp}$ ... add up χ^2 of each cat. of phenotype data.
3. Calc deg of freedom = n − 1 (n = no of classes/phenotype)
4. Fr χ^2 table, $0.001 < p < 0.005$
5. At level of sig 5%, since $p < 0.05$, reject H_0
   Conclude diff bet obs & exp not due to chance alone

   At 5% sig, since $p > 0.05$
   Do not reject H_0
   Insuff ev to reject H_0
   No sig diff bet obs & exp
   Diff due to chance alone

BIO H2 ⑦ EVOLUTION

NIGEL FONG

① SPECIES & CLASSIFICATN

(a) Species concepts

A group of organisms ....

BIOLOGICAL	capable of interbreeding to prod fertile & viable offspring
ECOLOGICAL	sharing same ecological niche
PALEONTOLOGICAL	sharing sim. morphology based on fossil records
MORPHOLOGICAL	sharing sim body shape/str. feat
PHYLOGENETIC	Smallest grp of ind w/ common ancestor

Populatn = Gp of ind of 1 sp. interbreed... V & F offsp

(b) Taxonomic classificatn

Grouping & naming org into meaningful divisions based on common xteristics (x account evol hist)

Kingdom Phylum Class Order Family Genus Species →

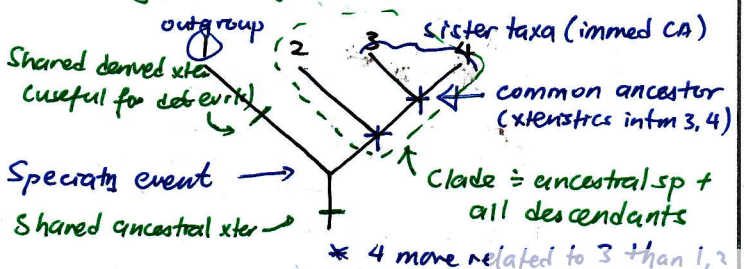
- ↑ closely related
- ↓ no of species
- Write Genus species
- use morpho sim (≠ homolg str)

(c) Phylogeny

Evolu. hist based on ancestor-descendent rls.

Rep. hypothesis abt evol rls — mol seq & morpholg data

→ Classf. not based on common xteristics lead to wrong classf based on superficial sim L e.g. convergent evol.



Allow inference of
- how closely related 2 sp are
- hist. speciatn events
- common ancestors

② SPECIATN & EVOL

(a) Gene flow

= Exchange of alleles bet. pops — interbreeding
— imm/emigratn

↳ extent ∝ geo. proximity
no. inds
amt interbreeding

(b) Allopatric

1 Genetic isol fr. geo. isol
- e.g. island biogeo.
  Darwin's Finches

Sympatric

Gen. isol fr physiological/behavioural isol
- mutn
- Δ mating proj
- Δ habitat
- Δ eco. niche
- Δ food

2. No gene flow bet. 2 pops
3. Indep. evol of diff pop — accum of g diff/random mutn
4. Adaptive radiatn, diff selv pressures
5. Accum of g. diff → phenotypic diff
6. Reproductv isol

premating : habitat, temporal, behv isol } pre-zygotic
mating : mechanical/gamete isol
fert : zygote mortality, hybrid sterility, F_2 ↓ fitness } post-zygotic

⇒ X interbreed to prod V & F young ∴ new sp.

(c) Darwinian evol./homologies

NTL SELN	Differential succ. in reprod of diff phenotp resulting fr. intx org w/ env
EVOLN	Δ allele freq of pop.

1. Overprod of offspring
2. Constancy of no ~ stable pop/env. carrying cap
3. Struggle for survival → name selv pressure
4. Var within pop ~ morphol, physio, biochem, g-diff w/ mem of same sp
   - (in hom. traits)
   IMPT → var allow survival/reprod. differential (raw mat for selv)
   → ensure perpetuatn/safeguard sp fr extinc.
5. Surv. of fittest : org w/ heritable traits env. favour ↑ survival & reprod. — V & F offspring
6. Adv. homolog. xteristics passed down (like prod like)
   ↳ % indv w/ adv trait ↑

→ Directional selv	: select for 1 extreme	Env Δs, new habit	Favour speciatn
→ Disruptv selv	: select for both extreme	Env cond varied	Sub-pop speciate
→ Stabilising selv	: Act against extremes	cnst env	No evol Δ

7. Formatn of new species over time
   - # Modified form of ancestral adv homologous trait | DESCENT W/ MODIF

(d) Agents of evol. Δ

1. NTL selv
2. Mutatn ~ source of new alleles
   indv w/ mutant al may ↑ no. offspring
   } Only heritable mut counts
3. Gene flow ~ interbreedg
   → emigratn/immig
4. G. drift - random sampling & Δ allele
   - Founder effect — colonz by few ind, X carry all source alle
   - Bottleneck effect — ↓ pop e.g. unfav Δ env
   } Rare alleles may bec ↑ freq
   Allele loss
5. Non-random mating ~ X random mixing

(e) Pop. smallest unit that evol

1. Fate of indv org insig, collectv g. resp det surv of sp.
2. LT effect of nH selx @ level of gene & pop.
   └ only pop interbreed, exchg g., pass on.
   → Ev. measured as Δ allele freq, can only measure at pop level

③ EVIDENCE / EVOL

(a) Homology → descent w/ mod

ANALOGY | Sim due to convergent evol, X common ances
Sim selx pressure → evol sim xteristics
e.g. fish fin / dolphin flipper

vs

HOMOLOGY | Sim inherited fr common ancestor
Dup into diff form: selx, Δg, Δg freq

- **ANATOMICAL** | Morpholog str fr. CA
  Modified fr nH selx, serve diff func, look diff or degenerate
  - Pentadactyl limb in vertebrates
  - 2 diff func
  - Vestigial str no func but sim CA e.g. hum tail bone
- **EMBRYOLOGICAL** | Diff in adult but sim in early embryonic dup
  └ basic emb body plan fr. CA modif in descd.
  └ ↑ closely rld, ↑ t emb dup rem similar
  - Hum gill ridges → ev fr aquatic anim w/ gill slit
  - Emb. vertebrates 2-chambered heart
  - Verteb. emb hv notochord
- **MOLECULAR** | Sim genes & aa seq
  ↑ closely rld, ↓ diff
  Fr CA but mod
  Some g in diverse array of species
  - p53
  - Haemoglobin
  - Cytochrome C

(b) Biogeography = study geo dist of extinct & modern sp.

CONCLN | 1. Patterns of past evol found in geo dist of rld sp
2. Unique sp on islands etc ~ evol in isol fr rest of world

CONTINENT | 1. Lungfish hv CA that spread widely when continents merged.
c. drift → diverge & evol: allop. speciatn

2. Marsupial mammals isol in Aus due to c. drift
   └ indep evol... convergent, occupy same niche
   → Anatom sim ∵ occup sim env
   ⇒ nH selx prom adaptn to given env

ISOL. ISLAND | 1. Darwin's finches ~ galapagous is
- closely rld. to sp fr mainland / neighb is
- geo isolated: X gene flow
- Unique selx p: modif homolg str speciatn

(c) Fossil record

FOSSIL = relics / impressions of extinct org preserved in rock.
└ Shell / bone / tissue replaced w/ anr mineral.

1. Shows succession of org.
   - Each stratum has uniq gp of fossil sp
   - Deeper strata = older = ↓ sim to modern life
   - Comparison of fossils show how homolog str modified over t (descent w/ mod)

EG horses
- ev illus by homolg str e.g. forefeet, teeth
- fossil rec = arr of homolog str in chrono.
- show maj evol transitn
  └ widespread grassland: selx for fast grazers

2. Transitional forms link older fossils to mod species thru intermediate xteristics (illustrate evo. transition). - mod of homolg str.

EG archaeopteryx: intern bet birds & dino
- some traits like birds (feathers)
- others like dinos (teeth, bony tail)

EG tiktaalik
- illus. tetrapod ev. fr. fish
- fish-like: gills & scales
- tetrapod-like: pentadactyl limb, skull, lungs...

3. May be incomplete — hard shells / skeletons ↑ pres

(d) Preserv. of recessive alleles

HETEROZYG. PROTECTN | Dominant allele masks eff. of recessive
Recess allele exposed to nH selx only when ind is homozy. rec
↓
Maintain large pool of alleles
Can bring benefit when Δ env

HETEROZYG ADVANTAG | Heterozygote may ↑ fitness than both homoz.
Occur in reg of endemic malaria
- Defects Hb RBC sickle & die when inf w/ plasmodium → trap parasite, ↓ infectn
- Sickle cell trait, X sickle cell, X malaria
  └ surv & reprod — both Hb A & Hb S rem in pop
- otherwise harmful Hb S maint @ ↑ freq
  └ surv. adv to heterozygotes
- Selectn fv sickle cell trait

INBREEDING | Breeding bet close relatives: ↑ homozygosity
Inbre. depression — ↑ freq of harmful rec traits
↑ Disease phenotypes — g. disorders mostly rec
If X inbreedg, ↑ diversity, ↓ P inh rec allele

④ Molecular Methods

(a) Principle

- All org same univ. g code, can compare sim in homolg seq of DNA to infer evol. rls
- When ancestral sp split into 2 lineage, initial descendants – sim DNA seq over time – seq diverge, differences accum.
- ↑closely rtd (more recent common ancestor) ↑seq sim
- G. dist can calc fr sim, draw phylogenetic tree to visualise rls

(b) Techng

1. Choose gene — homologous gene/non-coding seq
   - closely rtd sp: choose fast ev. gene (eg mtDNA)
     ↳ easier to resolve diff bet closely rtd indv
   - mtDNA useful: inh only fr mother ~ direct trace g line
     : No X-over/meiosis which g mes g. recon bet mat/pat DNA
     Diff show only mut since dev fr CA
2. Amplify & sequence gene – choose diff fwd/rev primers
3. Align seq ⇝ conv data into sim/dist matrix
4. Generate evol tree.

(c) Advantages

1. Study any taxa since all life based on nuc acid
   ↳ can comp morphologically indistinguishable or phylogenet. distant sp w/ few morpho. sim
2. Objective & quantitative – quantif °relatedness ∝ less nuc. Unambiguous & detailed – vs morphology hard to dist
3. Offer limitless of xteristics to consider – each nuc.
   ↳ esp. molec diff Xvis phenotypically – eg introns
   single expt can provide ↑info on ↑xters
   ↳ quick gen. large datasets
4. Can store in electronic databases & used fr classif
   Can conv to num form → Statistical analysis

(d) Applicatn

1. Reclassif of org based on evol rls → ext revision
   - Elucidate evol rls … infer phylogeny
   - Superior: actn of evol reflect in g. seq
2. Form basis of dating using molec clock
   est t of speciatn
3. Rapid accum of seq data ⇝ docum of ev. history
4. Ans qns – origin of mod. hum ("out of Africa")
   ↳ mtDNA & Y-ch: trace mitochondrial Eve, Adam
   – Origin of AIDS ⇒ began w/ ↓no viruses
   – fr SIV of chimps
   – start date of spread

(e) Neutral theory

WHY Disadv mut quickly remov by ntl seln
NEUT Adv mut quickly brought to fixatn / are rare
Most mut neutral
- silent mut (e.g. 3rd degenerate bp)
- conservative subst: chem sim aa.
- introns (non-reg) mut, X effect

CONSEQ Little/no effect on fitness — X act on by ntl s
Most ev@molec lvl driven by random drift rather than ntl seln

CLOCK Random proc ⇒ mut const rate like clock
Use to measure evol time:
°diff bet 2 seq ∝ rate of ev / how long diverg

Calibrate wrt fossil record
CA_{AB} 13mya … if 10 mut,
rate of subst $= \frac{10}{2 \times 13}$ sub/yr

BI H2 (8) DNA TECHNIQUES

— NIGEL FONG —

(1) GENE CLONING

= method to prep gene-sized DNA pieces in multip. identical copies (to prod prot ptt)

(a) Rest. Enz

NTL FUNC
- Prod in bacteria — cleave foreign DNA (viral inv) into non-infectv frag, restricting vir replicatn
- Methylate of host DNA — Δ conf, ✗comp to RE
  - ↳ protect bact DNA, ✗ cleave

MECH
- Recog sp. seq in dsDNA, hydrolyse phosphodiester Bs
- Restrictn sites: usu ~6bp, palindromic

Sticky ends: RE staggered cut w/ ss ends
EcoRI — Will anneal w/ comp ss DNA (H-b) cleaved w/ same RE

Blunt ends: RE simple cut acr both str@1 pt
HaeIII — Comp bp ✗ req to join: ↓specific

RECOMB DNA
- RE recog & cut sugar-phosphate backbone@r-site
- DNA frag fr anr source cut w/ same RE - mix
- Comp. sticky ends anneal by comp bp ( )
- Incubate w/ DNA ligase: cov joining — recomb.

(b) Vectors & Host

HOST
- Eg E Coli: Rapid reprod, Easy maint, ↑ cov plasmid

VECTOR
- DNA molec into which DNA frag may be ins
  - ↳ transfer & carry DNA frag into host cell
  - ↳ replicate w/ DNA-syn mach in host

PLASMID = Circ xtrachromosomal DNA in bact, 1 ori
- → Rep indep of bact ch: recomb DNA pass to daught
- → w/ selectable marker (selectb phenotypic trait)
  - select host C that hv taken up vect
  - ID host C that hv taken up recomb vect (insertional inactivatn)
- → Size Lim size of DNA frag ins
- → Copy no: ↑ copy no, ↑ Q recomb DNA obtained

(c) Genomic v cDNA lib

	GENOMIC	cDNA
Contains...	Entire DNA content	Prot-coding DNA exp in cell
How...	Genom DNA —[RE]→ Clone	mRNA exp —[rev transcp]→ cDNA
Req...	Chromosomal DNA	Total mRNA isolate
Can clone...	Coding & non-coding seq w/ intron: ✗ splice in E Coli	Coding seq. w/o intron: ✓ exp in E Coli
Start mat...	Any tissue	Tissue w/ target mRNA ↑↑
Issues...	Many false colonies (non-c) Frag may be cut by RE ↳ non-func pieces	
App...	Clone gene w/ unknown exp Study introns	Study coding seq Study Δ gene exp Prod euk prot in E Coli

(d) Process

PREP OF CDNA.
- Isolate mRNA
- Free oligo-dT hyb. poly-A — serve as primer
- Rev trans syn cDNA using mRNA template → hybrid
- + RNase: break down RNA — ss DNA leave some mRNA ~ primers
- DNA pol. syn 2nd DNA, replace primers

PREP PLASMID

Terminal transferase + dCTP add C to 3' (w/o template)
↳ ✗ self anneal, recirc (not comp)

Choose rest site within marker — linearise w/ RE leave blunt end

Term transferase add G to 3'

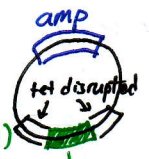
Mix — exposed C & G: H-b, cohesive ends anneal
- nicks sealed w/ DNA ligase → recomb
- insertional inactivatn of marker

TRANSFORMATION (host cell assim ext DNA)

Bact made competent (can take up DNA):
↳ Ca^{2+} + heat shock ~ transient pores

SCREENING → identify recomb host c w/ recom DNA w/g

1. Plate on plate w/ selectv agent (eg. amp)
   Cells that grow must hv taken up plasm Amp-resist marker conf antiB-resist.



2. Replica plating: velvet press master plate transf to replica plate (tet)

Recomb plasmid: amp-r ✓ tet-r ✗ → (insertional inactivatn)
Non-recom: amp-r ✓ tet-r ✓

↳ term transferase may not prod overhang: ✗ ins
Some plasm may recirc / form concatemers

* Or: blue-white selctn: insertional inactv of lacZ.
w/o β-galactosidase: X-gal ✗→ Blue, rem White

PROBING

Press nitrocellulose mem on plate — prod replica
NaOH lyse cells, denature → ssDNA bind to mem
Wash w/ saline — remove cell debris. 80°C fix ssDNA
+ Probes (ssDNA, comp to gene of int, radioactv)
Hybridise to comp DNA seq.
Wash to remove xs unhyb probe
Lay on photo film — pos of probe loc } autoradiography

PROD
Pick up target cell — transfer
Cells grow & div — recom plasm rep. to prod ↑g. inherited by progeny cells
✓ Grow in bioreactr — induce prot prod

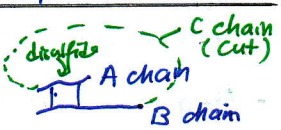
(e) Case study

(1) Somatotrophin (growth hor)
↳ ✗ agromegaly, dwarfism

mRNA fr anterior pituitary
cDNA — segmt remov & rep w/ anr to prov trans sig in E. coli

Use lac promoter to ↑ exp

(2) Insulin



→ Syn A & B sep, extract. form disulfide Bs in vitro

→ Prod pro-insulin w/ C-chain Enz use to cut out C chain

② DNA ANALYSIS

(a) PCR : *amplify segmt of DNA

DENATURATN 95°c
sep 2 str DNA

+ template DNA

PRIMER ANNEALG 64°c
xs primers sp. anneal to ss DNA comp.
x reanneal.

+ Primers (20bp ssDNA)
- nec to init DNA syn
- 1 fwd & 1 rev primer
- need to know flanking seq

EXTENSION 72°c
Taq pol syn cDNA f 3'OH of primer

+ Taq pol ~thermostable
+ dNTP ~substrates
+ Mg^{++} ~cofactor for Taq

REPEAT
Exponential ↑ no of DNA.

(b) Apps/adv of PCR & Lim

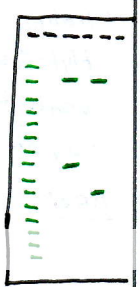
ADV
: Only minute amt DNA req — can ↑ exponentially
: Fast & automated

APPS
1. Clinical – prenatal screen g. diseases
   – detect infects b/f symptoms app (eg HIV)
2. Forensic – match crime scene DNA
3. Evol – Compare DNA frag ~study evol rls

LIM
1. Taq X proofreading — errors compounded, exp ↑
2. If flanking seq unknown, PCR X be used
3. DNA frag amp lim. to 3 kb — pol tends to fall off
4. Contaminant DNA also amplified

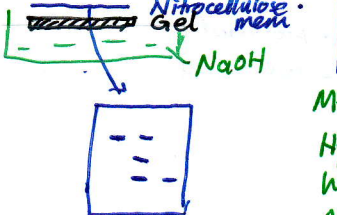
(c) Gel electrophoresis

GEL
- Agarose gel cast w/ wells, place in conducts soln
- DNA mixed w/ loading buffer – dense so DNA sinks
  ↳ load onto wells – monitor prog (dyes)
- Markers loaded – DNA of known size (comparison)
- Current: -ve DNA (PO_4^{3-}) attr twds anode (+ve)
- Agarose meshwork: shorter DNA move faster
  travel further
  ↓
  DNA sep into bands
- Curr turn off b/f dye reaches gel ends
  Stain w/ DNA-binding dye (ethidium Br)
  ↳ UV light: dye fluoresce — visualize bands



⊕ Dist ∝ size
Thickness ∝ amt DNA

SOUTHERN BLOT



(capillary actn)
Alkaline soln drawn upwards ↑
- Denature ds DNA → ss DNA
- Bind to nitrocellulose in same pos

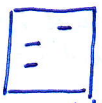
Mem incubate w/ radioactv probe
Hybridize w/ target seq – comp. bp
Wash to remove xs
Autoradiography: X-ray film exposed
img comes to loc of band

(d) Rest. frag length polymorphism

* Examine DNA polymorphisms — small Δ nuc seq
  – Δ no. tandem repeats.
  ↓
Rest digest: diff size rest patterns
diff length — distinguished by gel & s blot

① SNP (single-nuc pol : diff in 1 bp)

- Alter seq. recog by RE → norm ↑MstII, sickle ✗
- Digest genomic DNA w/ MstII
- Gel & s blot w/ comp probe (β-globin)
  ↓
  presence of Hbs seen for bands


norm sickle

⊕ Fast
Specific
⊖ Need to isol PCR amp.
↓ amt DNA

② Flanking DNA (PKU, huntington)

- RFLP in flanking region, tightly linked to disease gene
- Comparison of rest pat identifies ind w/ disease.
- *Possibility of X-over.

③ DNA fingerprinting)

- Diff inds w/ diff STR (microsatellites) VNTR (mini)
- RE cut on either side of STR/VNTR locus
- Δ no. VNTR/STR — unique genetic profile (rest pat)
- Heritable: tell rls

④ Genomic mapping.

- RFLP serve as marker for locus, ≡ allele of gene
- To calc g dist btw 2 loci, obs recomb bet ē 2
- Recom freq = centiMorgan distances

③ HUM GENOME PROJ

(a) Goals

1. Det seq of hum 3 mil bp
2. ID 20-30k hum genes
3. Store info in public databas
4. Improve data analysis tools
5. Transf tech to private sect
6. Address ethic, legal, soc iss

(b) Benefits

1. Impr acc & spd of disease diagnosis ~ eg. SNP, PKU...
2. Possibility of tailor drugs to ind pharmacogenomic profile ~ g diff effect rxn to drug
3. Kn of g. diseases — risk assessmt, ↑ treatmt
   — studies to ID alleles linked to diseas
   — possibility of g therapy
4. Comparative genomics — study evol (see molec methods)
5. Und hum bio → ↑ info abt prot seq
6. Forensics: ID suspects, paternity rls, match organ donor

(c) Issues

1. Affordability of designer drugs
2. Risk profiles: discriminatory uses — eg insurers, employers
3. Reprod issues (pre-implantn g screening) ~ rights?
4. Free will vs g determinism — esp epigenetics
5. Genetic info becoming private IP

BI H2 ⑨ GENETIC ENGINEERING

NIGEL FONG

① STEM CELLS

(a) Features

① Unspecialized
- no tissue-specific structures

② Self-renew
- SYMMETRICAL DIV
- 1 SC → 2 daughter SC
- Can div & renew by mitosis ↑times
- Daughter cells w/ same dup & rep pot

③ Differentiate
- ASYMMETRIC DIV
- 1 SC → Daughter SC (maintain pool) + Progenitor cell (renew specialize)
- -Sig: act° & repressor
- - Diff g exp, others off

(b) Types & Func

MULTIPOTENT	PLURIPOTENT	TOTIPOTENT
Can diff into limited range of cells	Can diff into all cells except placenta	Can diff into all cells inc placenta
	X form entire org	Can form entire org
	eg Inner cell mass of blastocyst	eg Fertilized egg

Adult stem cells

neural glial	haematopoietic	epidermal	intestinal
↓	↓	↓	↓
nerve c glial c	RBC WBC platelet	skin shed	absorptive globet

FUNC OF ADULT SC
1. Replace cells lost to damage/injury
   - renew pop of spec C
2. Ensure const pool of SC

② GENE THERAPY

* CFTR/SCID - notes 3.

(a) Intro

Addn of g mat to correct g defect/disease

SOMATIC inc SC	GERMLINE
X heritable	Heritable

CONDITIONS FOR GT
- Kn of defect
- Avail of g.
- Eff deliv sys
- Access of c.
- X harm
- X alt therapy

GENE AUGMENTN
- Norm func allele ins
- Exp prot suff to replace
- Treat rec disorder
- Dom allele mask

GENE DELIVERY
- Deliver DNA frag.
  - └ corr cell @ corr site
  - └ w/o aff func of other g

IDEAL:
- ↑ uptake eff of norm allele
- ↓ intracellular nuclease deg
- Sust exp enough to allev.

(b) Ex Vivo vs In Vivo

Ex. • Cells remov fr body	– Cells must be easily remov (eg blood)
• Culture in vitro	– Must be amenable to culture
• Norm copy of g transf in vitro	– Can screen for ins mutagenesis
	– Ensure corr exp
• G-corr cell return to patient	– own cells, X adverse immune rxn

IN • Transfer g dir into cells in body	– Nec suff no. c for transfectn
	– Success X dpd on ability of IV cells
• Use suit g deliv sys - target corr c type	– Nec for organs that X remove (CFTR)
	– Need suitable g deliv sys

(c) Viral-mediated / SCID

1. Select vir vector: target sp cell type (sp glycoprot)
2. Disable vir: remove/inactv disease-causing g
3. Ins norm, func alleles
4. Use vir to transfect extracted cells in/ex vivo

	+	-
RETROVIR RNA → ds DNA Int into host g.	• Perm mod: stable exp • ↑ transfectn eff	• Only <8kb g • X ins @ fully diff c • Ins mutagenesis(ol)
ADENOVIR DNA exp, X ins	• Can inf all c. specifical • X ins mutagenesis • ↑ g. exp	• vDNA diz carded aft 1-2 wks • Cause immune rx
ADENO-ASSOC ssDNA int into spec ch19 reg	• X pathogenic, X imm rx • Can inf ↑ range of sp • X ins mutagenesis (sp specific int)	• Max 4kb

SCID
- stem C transplant
- Enz replacemt inj ADA
- GENE T.
  - Remove T cell
  - Ins norm allele (retrov)
  - Transplant back
  - Repeat: reg infusions → transient ADA prod

Once recom T die, replacemt w/o ADA

(d) Non-viral mediated / CFTR.

1. Dir intro
   - micro J
   - gene gun
   - electroporatn
   - heat shock
2. Liposomal vect
   - Artf lipid sphere can fuse w/ cell m
   - Aq core
   - dsDNA/plasmid

+	-
↑ QDNA X imm rx X ins mut Can target sp c - mod mem	↓ transf eff ↓ integratn ↓ g exp Toxicity

CFTR
- Liposome
- CFTR g. plasmid vect

Deliver to lung aerosol spray

Lipofectn - fuse w/ mem release DNA

Int into g: exp CFTR – norm.

(e) Problems

① SHORT LIVED	② TUMOURS	③ IMMUNE RX
Int prob - allele X func	Ins mutagenesis random int vDNA	Vir: imm rx
Death & rep of transf C	- disrupt tum-supp g	Subseq rounds foster imm rx
Need multiple rounds	• vir prom upreg proto-onc	Vect destroy b/f deliv
SOL: Transf SCs		

④ MULTIGENE PROB	⑤ G-REG	⑥ MISC
Hard to treat	eg thalassemia	Need diff vir for diff c
Combined eff of ↑ g	overexp: imbal of globin g	may recover ab to c. disease

(f) ELSI

	+	-
SOMATIC	Treat desperately ill – no alt X impact offspring	Unreliable Pot for abuse Expense – out of reach
GERM	True cure for fut gen ↓ expense of multiple gen GT X aborts etc Only way to access inacc areas – eg brain	LT effects unknown LT follow up hard Poss of g enhancemt └ soc discrim Generates of unconsentg research subjs

③ PLANT CLONING

PTC = asex. prop of plants / in vitro place ↓ amt undiffc in art stemle nutrient med → prod root/shoots later plant normally

Micropop → mass prod of clone w/ PTC

(a) Gen technique

Donor plant
- actv grow, ✗ disease
- desirable xtemstics
- young : ↑ div act° more resp to callus ∫

① COLLECT EXPLANT = porta taken fr intt culture
- Shoot / leaves / storage org / cotelydon ; fr which refers whose plant

② SURF STERILZ : NaClO
- ↳ remove bact / fungi (w/ contam)
- div faster than plant ∴ use up nutrients

EXPLANT

NUTRIENT MEDIA
1. INORG macronut (casein for aa ∴ rec) — N : S : P : K : prot : nuc acid : Ca : Mg : cell w : chlorophyll
   micronut — Fe, Zn, Co · enz cofactors
2. VIT : B, thiamine, niacin, ascorbate
3. C : sucrose, glucose
4. PGR auxin · cell elong / div esp roots
   cytokinin · " esp shoots
   · callus form
5. Activ charcoal : ↓ toxins
6. Antibiotics

③ OBTAIN CALLUS
- seal, stemle cont
- agar
- Low auxin / cytokinin : Infrtate callus (totipot) amorphous mass undiffc
- Prolif : slightly ↑ A
- Calli useful :
  - Fastest mtd for multp
  - Pot dup norm rt / sht
  - Protoplast source
  - Screen eg herbicide r

aux ↓ cyt ↓ ; A ↓ C

NH₃ fr resp to plant inj ∴ rapid div

④ ESTABLISH PLANTLET
- Shoots : ↑ eJA, ↓ sugar ↑ light
- Roots : ↑ A ↓ C
- ↓ Diffn → plantlet

Take out Wash agar

Soak in fungicide

⑤ ACCLIMITASATN
- Wean fr ↑ humidity ↓ light ↑ warm to norm env.
- → cuticle, stomata dup
- → ↑ photosyn

stemle soil

support porous : drain & aerate

⑥ PLANT ORGAN C
- Take plantlet tissue
- Subdiv & multiply

(b) Somatic embryogenesis

= Cultured som-cells ind to f som embryo fr veg-tissue — no gamete fert / zygote f

Est callus / susp cult.
- ↳ hypocotyl, cotelydon
- ↳ leaves, rts, flower
- cells must be competent

asparagus, begonias, celery

Induce embryo formatn ↑ A, ↓ N

fr cells @ / near surf. of small cell cluster (not single cell)

Encapsulate artificial seeds
- nutrient, PGR, fungicide } prot. gel

(c) Protoplast culture

① CHOOSE EXPLANT
- ↑ meristematic cell
- ↳ undiff : can switch to diff pathway
- ↳ ✗ vir

Steriliz w/ NaClO

② ISOL PROTOPLAST
- Plasmolyse : mannitol, sorbitol, sucrose
- free cytoplasm : break plasmodesmata
- Disrupt : Pectinases, Cellulase, Hemicellulase

③ PROTOPLA. FUSION
- Ca^{2+}, polyethylene glycol ↳ make sticky
- Electrofusion
- Screen for tracts

collect protoplast : centrifuge at 100 (cell debris pellet, protoplast float)

isotonic culture sol

Nutrient culture
- Syn new c. wall
- Cell div
- PGR
- organogen
- new plant

ADV.
1. No cell wall — can transform protoplast ∴ hence plant
2. Protoplast fusion
   - Overcome reprod barriers
   - Combine good traits of 2 plant
   - Polyploids : ↑ vigour

(d) Anther culture

Pollen grain → Explant → Haploid anther calli → Stemle haploid plants (& ↓ vigour)

① Can select : phen eff of all gene no masking of rec

② Lethal g elim : rec allele exp

③ Can induce mutatn (chem / uv) & select des

Colchicine disrupt mitotic spindle (×2 ch no)
✗ sep chromatid @ anaphase → Diploid
2n Isogenic P : Homozygous @ all loci

(e) CBA of micropropagatn

⊕ REPROD
1. ↑ fecundity vs sexual.
2. Can multp which produce ↓ Q seeds. banana, stemless; orchid hard to germinate
3. Indep of Δ clim
4. Less space
5. Tpt : air freight

⊕ BETTER OFFSP
1. Genet. uniformity
2. Disease-free
3. Rooted, ready to grow
4. ↑ Robust
5. Gen eng.
6. Sela & gen transgenic plants (protoplast)

USES
1. Prod pathogen-free stock plants
   ↳ aseptic env
   ↳ sela fr explant
2. Maintain g. bank esp for p. with ↓ Q seeds
3. Prod 2° metabolite / syn in immob cell sys (eg codeine, menthol, quinine)

⊖ DISADV . . .
1. $$ seeds of
2. Infected plant ∴ infected progeny
3. ↑ somatic var
4. Losses acclimtz step.

④ GMO

GE = manip of g. mat : Δ DNA ← supplenc gene / combino diff spp
Due to univ g code, org can hv DNA fr unrel org
↳ transgenic org

(a) Significance

Traditional selt° breeding
- slow, random
- intens° labour
- ✗ random

↓

↑ yield, quality
Disease resistance
↓ pesticide
↑ prop arable land

→ PLANT
- ✗ lim of species barriers
- pest / disease resistance
- ↑ Q & nut val of food
- stress / env tolerance
- imp. post-harvest xtemstics

→ Protoplast culture
→ Agrobacterium
→ Viral
→ Liposomes

ANIMAL
- ↑ speed / range
- can transfer g. across sp
- ec impt traits
- pharmac polts

→ DNA micro J
→ Retrovir
→ Recom in embryo sc

(b) EGs

	WHY NEC	HOW	ADV
Bt Corn	Insect pests caterpillar fly larvae	Bt gene-agrobac. Recomp exp toxin Specific to certain pests break down by dig enz → toxic	✗ $ & ① - apply pesticide Specific vs broad spec ↑ Pesticide ✗ accum in env ✗ acc : food chain Safely ↑ yield
Golden rice	3° W need ↑ nutritn β-carotene ↓ Vit-A def blindness & disease	• Ins enz to prod β-carotene (agrobac) • Endosperm promoter ↳ exp in rice.	• Rice ntl prod β-c. • Prod only in endosperm (✗ wasteful) • Adapt to local env (cross w/ loc rice)
Fast-grow salmon	Salmon ✗ grow in winter Prized food source	• Antifreeze promoter fr arctic flounder • Couple to growth hormone - exp cont • Micro J into eggs • Inbreedg - stable transgenic lines	• 3x-6x faster growth • Growth hormone even in winter • Heightened feed conversion efficiency

(c) ELSI

① ENV SAFETY
1. Bt plant : sela for resistance eventual uselessness
2. Effects of toxin on non-target org unknown — harm ecosys (vs chem insecticide ?)
3. Gene flow - rel transgenes → superweeds
4. GM outgrow wild → disrupt ecosys

buffer zones, sterile plants

② HUM SAFETY
1. Toxicity ?
2. Allergy to new prot
3. Δ nut quality
4. Antibiotic res. genes pass to gut E Coli

food safety std ↑ edu - make choices

③ ETHICS
1. Exploits of animals
   ↳ growth horm ↓ health
   ↳ expt stress
2. Cloning tech
   ↳ poss app to hum
3. Relgy implic.
   - apple w/ animal gene
   - w/ pig genes ?