Cell and Molecular Biology - Lecture 1: Cells and Organelles

Cell theory

- Three tenets of the cell theory:
  1. All living organisms are composed of one or more cells
  2. The cell is the basic unit of structure and organization in organisms
  3. Cells arise from pre-existing cells

- Generalized properties of cells:
  - Genetic information: genetic information is stored as DNA
  - Protein synthesis: proteins are stored on ribosome
  - Plasma membrane: a selectively permeable plasma membrane encloses every cells

Units of measurement used in microscopy		
Name	Unit	Magnitude (in metres)
Centimetre	cm	10^{-2} m
Millimetre	mm	10^{-3} m
Micrometre	µm	10^{-6} m
Nanometre	nm	10^{-9} m
Picometre	pm	10^{-12} m

Microscopy

- 3 important parameters in microscopy:
  - Magnification: enlargement of the image
  - Resolution: measure of the clarity of the image
  - Contrast: enables you to distinguish between different parts of the cell

Types of microscope			
	Light microscope	Electron microscope	
	Fluorescence microscope	Scanning EM	Transmission EM
Maximum resolution (nm)	200 nm	10 nm	2 nm
Function	Used to visualize whole cells and large subcellular organelles	Used to study cell surface and generate 3D images	Used to study internal cell structure, organelles, proteins
Characteristics of view	Cells/organelles cannot be distinguished without aid; require fluorescence staining for contrast		
Mechanism	Light is shined through a specimen into the objective lens and eyepiece lens for magnification	SET focuses a beam of electrons onto the surface of the specimen	TEM focuses a beam of electrons through the specimen

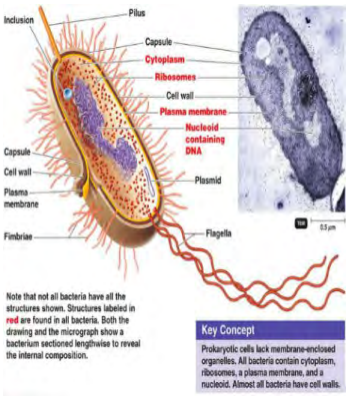
- Bright-field microscopy (light): only white light is used for illumination
- Fluorescence microscopy (light): fluorescence dies are used for resolution

- Light microscopes are limited by the wavelength of light (400-700 nm), and therefore is difficult to resolve smaller objects
- Beams of electrons have a much smaller wavelength so electron microscopes have a much higher resolution

Prokaryotic cells

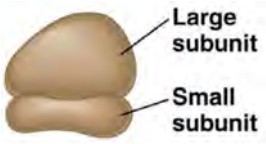
- Prokaryotic cells: cells that do not have compartmentalized structure (cell contain membrane-bound organelles)

Central prokaryotic organelles	
Nucleoid	DNA concentrated regions not enclosed by a membrane
Cell wall	Rigid structure that protects the cell and maintain the shape to prevent excessive swelling and bursting
Plasma membrane	Allows for passive and active transport of molecules
Capsule	Jelly-like coating covering the cell wall
Cytoplasm	Present in one uninterrupted chamber and contains many enzymes for metabolism
70s ribosome	Smaller than those of eukaryotes; fundamental for RNA translation
Circular DNA	Only one is present in a circular model; does not contain introns
Additional prokaryotic organelles	
Flagellum	Used for locomotion
Fimbriae	Used for attachment to other cells or surface
Mesosomes	Infolding of plasma membrane during cell division
Plasmids	Small circle of DNA that contains additional information



- Notable characteristics of a prokaryotic cell:
  - Lack of membrane bound organelles: there are little or no internal structure or organelles
  - Circular DNA: has one circular DNA with no introns

Ribosomal RNA (rRNA)

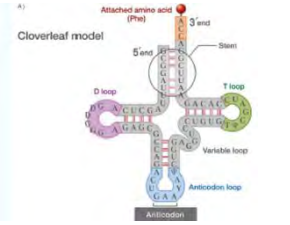


- rRNA: RNA component of the ribosome used during protein synthesis (translation)
  - Location: synthesised in the nucleolus from highly repetitive DNA
  - Function: helps support structure of ribosomes
  - Composition in ribosome: consists 60% of ribosome (40% consists of proteins)

Messenger RNA (mRNA)

- mRNA: RNA molecule that conveys information from DNA in the nucleus to the ribosome
  - Location: synthesised in the nucleus (product of transcription)
  - Features: synthesised at a fast rate, degraded rapidly once entering cytosol, and so present in small amounts
  - Function: conveys information from DNA to the ribosome

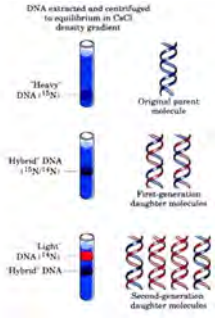
Transfer RNA (tRNA)



- tRNA: RNA molecule that serves as the physical link between the mRNA and the amino acid sequence of proteins during translation
  - Location: synthesised in the nucleus (product of transcription)
  - Features: anticodon loop binds to codon in mRNA
  - Function: translates nucleotide sequence in mRNA into amino acids
  - Types: there is at least one specific tRNA for each of the 20 amino acids
  -
  - Structure of tRNA:
    - Double stranded section: naturally created by base pairing
    - Anticodon loop: triplet of bases that binds to codon of mRNA
    - D loop: aminoacyl tRNA synthetase recognition
    - T loop: involved in ribosome recognition
    - 3’ end base sequence of CCA: site for amino acid attachment

DNA replication

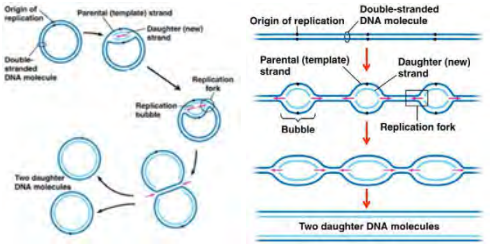
Semi conservative nature of DNA



- Meselson and Stahl devised an experiment that showed DNA replication is semi-conservative
- The density of the DNA through generation got lighter after generations in a light solution

- Process of the experiment
  - Culture in radioactive nitrogen: bacterium was culture for fourteen generations in a medium with ^{15}N as the only nitrogen source
  - Transfer to lighter solution: bacteria were transferred to a medium with ^{14}N only
  - Sample collection: DNA samples were collected from the bacterial culture for several hours from the time when it was transferred
  - Measurement: they extracted the DNA and measured its density by caesium chloride density gradient centrifugation; DNA with both strand containing 14N travel further down

Process of DNA replication

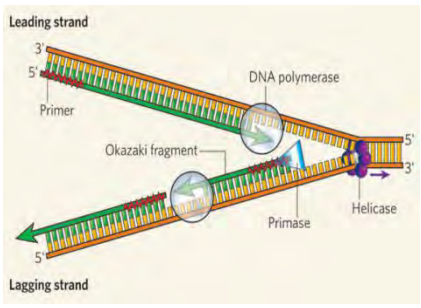


- Origin of replication: is a particular nucleotide sequence in a genome at which DNA replication is initiated
  - Prokaryotes: only one origin of DNA replication
  - Eukaryotes: many origins of DNA replication

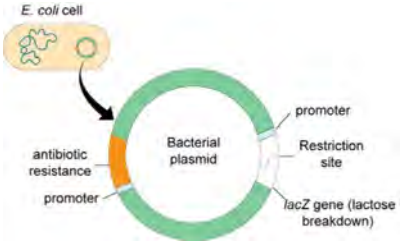
- Replication on different strands (due to bi-directional)
  - Leading strand: strand replicated continuously; polymerase replicates towards the replication fork
    - Direction of helicase propagation: 3’ → 5’ direction
    - Direction of DNA being read: 3’ → 5’ direction
    - Polymerase replication: continuous

  - Lagging strand: strand replicated discontinuously; polymerase replicates away from the replication fork
    - Direction of helicase propagation: 5’ → 3’ direction
    - Direction of DNA being read: 3’ → 5’ direction
    - Polymerase replication: needs to disassociate, move further up the molecule then reattach

    - Okazaki fragment: DNA fragments that are formed on the lagging template strand



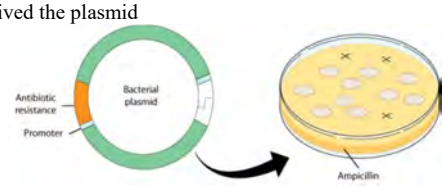
- Cloning vectors: small piece of DNA in which a foreign DNA fragment can be inserted (e.g. bacterial plasmids, bacteriophages)
  - Convenient removal/insertion into cell: enable researches to easily isolate DNA from host, modify in vitro and reintroduce into the host
  - Easy of amplification of DNA: allow large amount of recombinant DNA to be produced by replication of the vector
  - Plasmids: circular autonomously replicating DNA molecule which can replicate inside a host bacterial cell



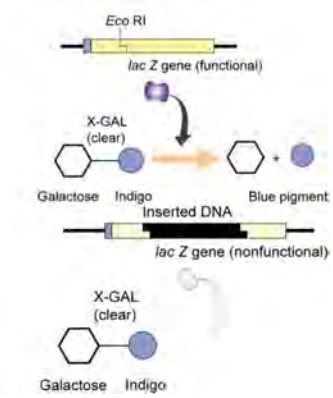
- Host bacteria (mainly E. coli): bacteria used during amplification of recombinant DNA
  - Easy growth: bacteria should be able to be grown easily
  - Convenient removal/insertion of vectors: has a large number of vectors available for E. coli
  - Common: DNA isolation and transformation procedures are well established

- Gene transfer methods (transformation): used for introducing plasmid DNA into bacterial cells
  - Treatment of bacteria and uptake of plasmids: cells are treated to make them competent to take up plasmid DNA (e.g. through calcium and heat-shock treatment or electroporation)
  - No need to recombine with host chromosomes: while plasmids enter cell, it is not required for them to recombine with host chromosomes

- Selectable markers: allows distinguishing of cells that have received the plasmid
  - Antibiotic resistance marker: market that makes sure bacterium has taken up plasmid
    - Marker contains gene resistant to antibiotic: cells uptake marker that contain ampicillin resistance gene
    - Resistance in cell: cells with the marker are resistant to ampicillin
    - Spread transformants on plates containing antibiotic: when cultured on plates with ampicillin, cells that did not uptake plasmids are killed



  - Insertion marker: market that makes sure the plasmid has an insert or foreign gene
    - Inactivation of functional gene: through insertion of DNA, lac Z gene becomes non-functional
    - Inability to breakdown X-GAL: due to absence of functional lac Z gene, X-GAL cannot be broken down to release blue pigments
  - Result explained through example using E. coli, ampicillin (antibiotic resistance marker) and X-GAL (insertion marker):
    - Colonies: all colonies that grow have plasmids
    - White colonies: all white colonies have insert DNA



Genetics - Lecture 6: Genetics: the chromosomal basis of inheritance II

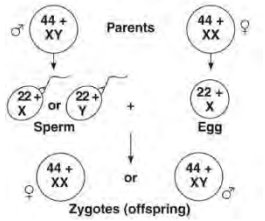
Chromosomal basis of sex

- Utilizing the chromosomal basis of sex:
  - Provides understanding of disorders: provides an understanding of disorders associated with sexual phenotypes
  - Provides model to study organogenesis and genetic control: gonadal development provides an excellent model to study organogenesis and genetic control involved

- Types of sex:
  - Heterogametic sex: the sex that produces two kinds of gametes; determines the sex of offspring (e.g. XY)
  - Homogametic sex: the sex that produces one kind of gamete (e.g. XX)

Sex chromosomes in humans

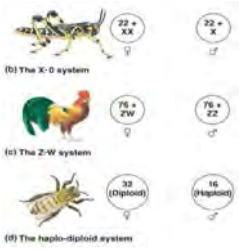
- Sex determination in humans: determined by the types of sex chromosome inherited
  - Old belief for sex determination: until beginning of the 20th century, sex determination was ascribed to environmental factors (e.g. nutrition, heat of passion during coitus)



- Human karyotype: has 46 chromosomes; 22 pairs of autosomes and two sex chromosomes
  - Heterogametic sex: males are heterogametic sex (XY)
  - Homogametic sex: females are homogametic sex (XX)

Chromosome status and sexual phenotype in humans			
Sex chromosome	Human phenotype		Drosophila phenotype
XO	Sterile female	Turner's syndrome	Sterile male
XX	Normal female		Normal female
XXX	Fertile female		Sterile female
XY	Normal male		Normal male
XXY	Sterile male	Klinefelter's syndrome	Fertile female
XYY	Fertile male		Fertile male

- The sex of organisms of different species may differ for a similar chromosomal disease; the "default sex" depends on the organism varies with organism (e.g. XO gives sterile females for humans, but sterile males for Drosophila)

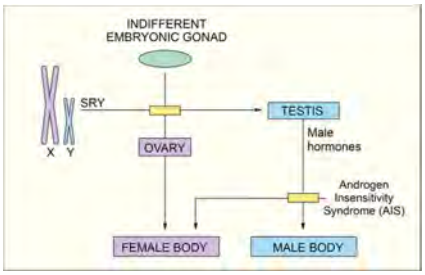


Chromosomal basis of sex in different organisms		
Organism	Male	Female
Crickets	XX	X
Birds	ZW	ZZ
Bees	Diploid	Haploid

- Sry gene: single gene (in the sex-determining region Y) which is responsible for maleness by triggering testicular development; codes for transcription factors that bind to sites on the DNA and triggers cascade of reactions (identified by Andrew Sinclair)

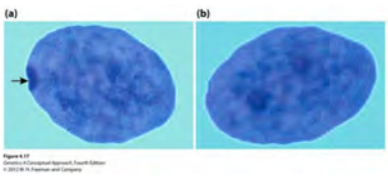
- Androgen insensitivity syndrome (AIS): mutation in the androgen receptor gene that result in partial or a complete inability of the cells to respond to androgens

- Process of sex determination:
  - Sex chromosomes: organism contain either XX or XY
  - SRY gene: if Y chromosome present, SRY gene in chromosome triggers testicular development
  - Testicular development: embryo with testes develop with primary male characteristics
  - Androgen insensitivity syndrome (AIS): if embryo with XY gene has AIS, the cells are resistant to male hormones and secondary male characteristics do not appear



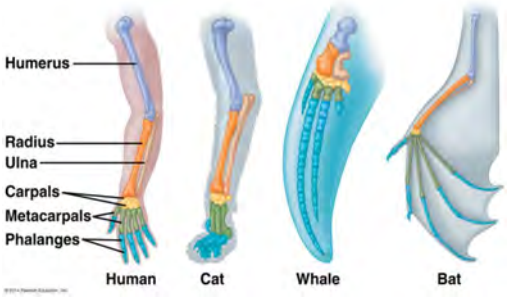
X chromosome inactivation

- Barr body: the inactivated X chromosome in females (proposed by Mary Lyon)
  - Random nature: each embryonic cell randomly inactivates one of the two X chromosomes
  - Expression of genes: most Barr body genes are not expressed
  - Physical traits: densely stained objects in the nuclei of females
  - Limited reactivation: Barr bodies are stable and inactive through mitosis but the X is reactivated in the cells that give rise to ova

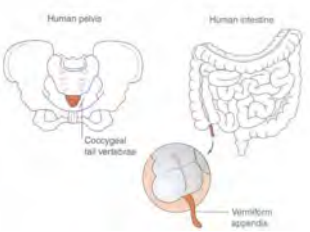


Evidence for macroevolution

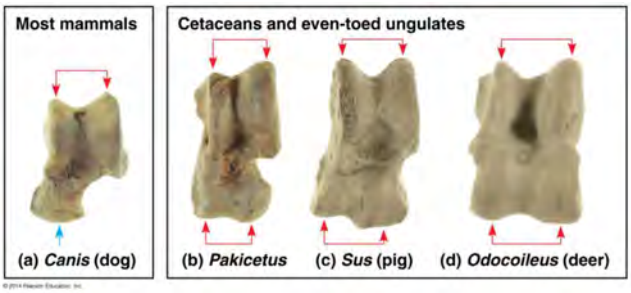
- Macroevolution: evolution on a scale at or above the level of species
- Homologous structures: structures that show a similarity in characteristic derived from a common ancestor
  - Underlying similarities: related species can have characteristics that have underlying similarities
  - Different functions: despite similarities, the functions may be very different
  - Target traits: can be seen in morphological, genetic or behavioural characteristics
  - Evidence for evolution: some shared features make little sense except in the context of evolution – us sharing a common ancestor



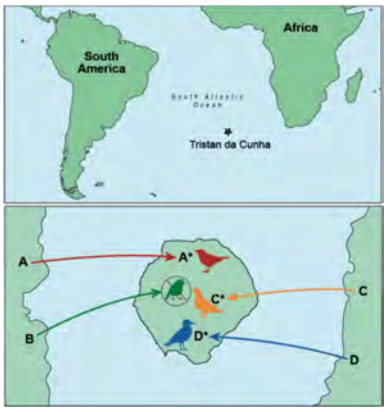
  - Example: mammalian forelimb; underlying skeleton of the arm, forelegs, flipper and wing of mammals
- Vestigial structures: structures of apparently little or no importance to the organism
  - Functionless and rudimentary: vestigial structures rudimentary version of a body part that had an important function in other closely related species
  - Evidence for evolution: producing these structures who appear to be a waste, and will make no sense if the animal/plant had been 'designed'
  - Example: North Island brown kiwi; a flightless bird but still has a tiny stubble wing (ancestors with a fully developed/functional wing)



- Fossil records: documents the pattern of evolution; shows succession and continuity of species
  - Succession in fossils: fossils in aged strata showed that fossils of most primitive forms of life are the oldest
  - Ecological sequence: the sequence in which they appear matches the sequence they are expected to evolve
  - Extinction and origin of new groups: trends of extinction and progression of new groups (e.g. cetaceans)

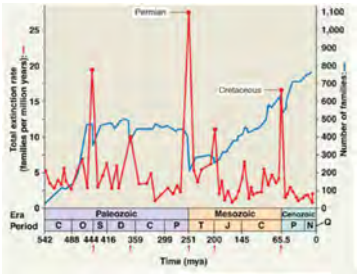


  - Example: cetaceans (mammalian order that include wales, dolphins, porpoise) – comparing fossils and present-day examples of the astragalus (type of ankle bone) indicates that cetaceans are closest related to even-toed ungulates not odd-toed ungulates (e.g. meat eating animals such as dogs) as previous palaeontologists expected
- Biogeography: study of the distribution of species and ecosystems in geographic space and through geological time
- Darwin and the Galapagos islands:
  - Galapagos islands: a group of young volcanic island located near the equator ~900km west of South America
  - Endemic but resembling mainland: animals on the island resembled mainland species but mostly were mainland
  - Variation in species on each island: mocking birds and tortoise varied from island to island
  - Suggestion of evolution: Darwin suggested that Galapagos had been colonized by organisms from South America then diverged giving rise to new species
- Voyage of Beagle: Darwin's five-year (1831–1836) circumnavigation aboard the HMS Beagle is often credited as being the inspiration for his theory of evolution



Extinction

- Extinction: disappearance of a species from the earth
  - Rate of extinction: the fossil record reveals a low steady rate of extinction interrupted periodically by mass extinction
  - Majority of species are extinct: overwhelming majority of species that ever lived are now extinct
  - Reason for extinction: species can go extinct for a number of reasons (e.g. habitat destruction or environmental change)



- Mass extinction events: the fossil record shows five mass extinction events
  - Period: five accepted mass extinction events in the past 500 myr
  - Target for evaluation: data obtained from the fossil record of the hard bodied animals that lived in the sea
  - Implication: evidence from extinction events interrupt the overall increase in the number of marine animals over time

- Note that two mass extinctions, the Permian and the Cretaceous have received the most attention
- The most recent mass extinction (65.5 myr) ended the era of the dinosaurs and initiated the era of mammals

- Glucose use in average resting male:
  - Overall glucose usage: entire body uses 160g/day glucose (brain uses 120g/day glucose)
  - Glucose storage: glucose stores 20g blood glucose, 190g in glycogen
  - Glucose storage usage: 90% of glucose in storage used / day (57% by brain) – only 10% at the end of day
  - Need for gluconeogenesis: body is not always in rest, and thus we must make glucose

- Glucose in body:
  - Highly dependent tissues: certain tissues of the body are highly dependent on glucose (e.g. brain)
  - Constant blood glucose: blood glucose is held constant ~80 mg/dl (~5 mM)

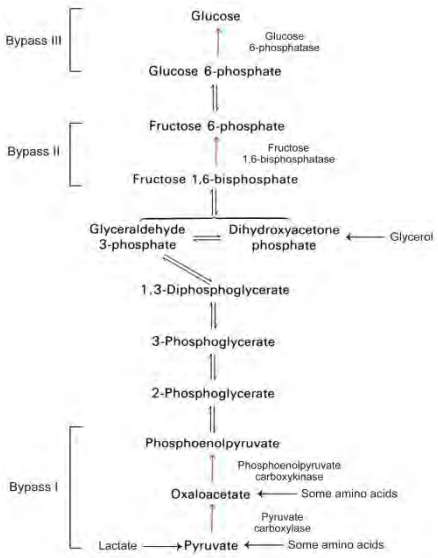
Pathway of the gluconeogenesis

- Reversing glycolysis: three bypasses are required for the irreversible kinases in glycolysis
  - Irreversible glycolysis enzymes: hexo/glucokinase, phosphofructokinase (PFK), pyruvate kinase (PK)
  - Reversible glycolysis enzyme: note that phosphoglycerate kinase (PGK) in first energy harvest is reversible

- Bypass I: bypasses irreversible reaction of pyruvate kinase (2 step)
  - Pyruvate carboxylase: enzyme converts pyruvate to oxaloacetate in matrix
  - Phosphoenolpyruvate carboxykinase (PEPCK): converts oxaloacetate to phosphoenolpyruvate in cytosol
  - Energy input: 2 ATP equivalents are used overall, and a NADH as oxaloacetate needs NADH for export

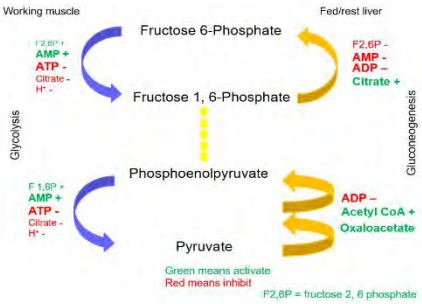
- Bypass II: bypasses irreversible reaction of PFK (1 step)
  - Fructose-1,6-biphosphatase: enzyme converts fructose-1,6-biphosphate to fructose-6-phosphate in cytosol
  - Lost ATP: phosphate group is lost with no ATP reform

- Bypass III: bypasses irreversible reactions of hexo/glucokinase (1 step)
  - Glucose-6-phosphatase: enzyme converts glucose-6-phosphate to glucose
  - Lost ATP: phosphate group is lost with no ATP reform



Control of gluconeogenesis and glycolysis

- Mismatch of ATP when simultaneously occurring: gluconeogenesis uses 11-12 ATP while glycolysis makes 2 ATP (10 mismatch)
  - Implication: two pathways must not run at the same time
  - Control through inhibition activation:
    - Glycolysis: activated through [AMP], [F1,6P] while inhibited by [ATP], [H+], [citrate]
    - Gluconeogenesis: activated through [citrate], [oxaloacetate], [AceCoA] while inhibited by [AMP] and [ADP]



- PEPCK over expression in mouse muscle: mutation to PEPCK coding gene led to over expression of PEPCK, increasing magnitude of gluconeogenesis; made mouse to be more durable at running with very low lactate level compared to wildtype