

BB101

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Spring Semester 2021

1 Introduction and Tour of Cell

- To tackle big Bio Challenges we need inter-disciplinary applications to develop technology
- Nature gives us insights for better efficiency in technological designs eg.
 1. Kingfisher catching fishes from water inspired Bullet Trains
 2. Lotus Leaves have crevices, i.e. rough leaf surface, through which water which trap air and becomes hydrophobic so that water flows out, this inspired Waterproofing by company Green Shield Construction Chemicals for lotus effect in paint.
 3. Architecture, termites' homes' structures are known to have cooling temperature, as it cools it through passive cooling, air comes from holes and hot air goes away from top. Mick Pierce designed East Gate Building in Zimbabwe inspired from this uses only 10 per. for electricity
 4. Dolphins recognize each other by the frequency of calls of their mates by gauging the presence of specific frequencies in them and their scattering properties, EvoLogics used it to make underwater communication and sonar underwater robotics, currently there in indian ocean to detect catastrophe.
 5. Namib Desert Beetle collects water droplets from morning fog and let moisture roll as its meshy, into its mouth same concept was used by an IIT -B Student to create a fence-like mesh could be used in arid conditions to collect water from fog in the morning.
- Google Brain, Google X Smart Contact lens(shaped like eye, as Glucose from tears enter it diabetes could be detected), IBM in Computational Biology, IBM Watson working on BlockChain, AI and also used for AI assistance in stuff like COVID-19
- **IIT-B Research:** Based Portable Diagnostic System for urine analysis and sugar diagnosis, Knee based Prosthetic, Mobile Apps to diagnose Sick Cell Anaemia exploiting differential RBC count.
- **IITB COVID Projects:** Helmet Patient Interface, Portable UVC Germicidal Unit, Self-disinfecting Masks, Tapestry
- **Proteomics at IIT-B for COVID:** To decide from protein analysis whether or not a case could become serious
- **Biological Complexity:** Around 2000, the major Genome Project was processed and now we know a lot about number and types of genes in various organisms, Gene Numbers are more or less same and is no indication of advancement.
- **Central Dogma of Life:** Genome- \rightarrow Chromosomes- \rightarrow DNA (\rightarrow Transcription(RNA)- \rightarrow Translation(Protein)). Genes remain static throughout life, the active component RNA is transcribed, which leads to Proteins. Proteins thus are dynamic and help us to respond to drugs, microbes etc.
- **Life Processes:** Energy Processing, Order, Regulation, Evolutionary Adaption, Environmental Response, Reproduction
- **Cell and its Properties:**

1. Light Microscopy is used for live cells, whereas Electron Microscopy is used mostly for dead cells. Resolution varies inversely with Wavelength.
2. **Prokaryotic Cells:** Earliest Form of life, This is the first life which has evolved through many conditions over the billions of years.
 - Cell wall and capsule composition needs to be studied properly as it decided which form of substances could be allowed to enter our cell
 - Fimbriae are hair like substances to help the cell move through various things.
 - The Flagella helps in motion and different activity. Composed of Motor, Hook and Filament, differs from Eukaryotes
 - Free-floating DNA Material(Nucleoid), lacks nucleus
 - Lacks compartments, no compartments
 - Bacterial Prokaryotes also have some extra chromosomal bacterial known as plasmid(double stranded, covalently closed, circular genetic material, which replicate and inherit independent of Bacterial Chromosomes which are the other component of Genetic Material in Bacteria) used for recombination activities
 - Bacterial Cell Wall has peptidoglycan layer which is sugar cross linked with small polypeptides, helps bacteria to extend on surface, Their thickness and distribution could help decide whether it is gram positive or gram negative bacteria, Gram Positive have thick layer, Gram Negative have less Peptidoglycan and also have an extra membrane. The Gram Negative are actually more complex due to having 3 sandwiched layer with thin peptidoglycan but also have an outer membrane of lipopolysaccharide, This aids Staining Method to differentiate.
 - **Staining Method** Comes out when a scientist was figuring ways to diagnose and treat patients.
 - **Gram Positive/Gram Negative helps in Diagnosis and Treatment:**Penicillin helps peptidoglycan crosslinking especially in Gram Positive Bacteria
 - **Gram Staining Procedure:** Sterilize and Cool loop, take a loop full of culture and make a smear on a clean slide, Then let it heat fixate to slide, then add crystal violet, then wash of stain and add Gram Iodine, which forms complex with crystal violet and gets stuck to wall. then when we wash with acetone-alcohol(ethanol!) decolourizer the gram positive stay violet whereas gram negative are decolourized and later colored with safranin. After that we'll wash off the safranin and let smear be air-dried. Then add emulsion oil and observe under microscope.
3. **Eukaryotic Cell:** True Nucleus, With all genetic material inside, and also has many compartments.
 - Few Genes in Mitochondria as well
 - Ribosomes are organelle which synthesize the Proteins
 - Peroxisomes are organelles to catalyze Hydrogen Peroxide.
 - Micro-villi are there to help movement
 - Micro-tubules which contain Centrosomes
 - Two types: Animal and Plant Cells
 - Both are mostly same, but some differences such as Cell Wall, Plasmodesmata(which provides connectivity with other cells), Plasma Membrane is also there, Central Vacuole for waste materials, Chloroplast is there specific to plants(also having unique genes)[All these are there in Plants but not in animals]
 - Cytoskeleton: Microtubules etc. are part of it, provides structure to cell and thus different organelles.
 - Plasma Membrane also helps taking signals from outside.
 - Golgi Apparatus also helps in exporting waste out of the cell
 - Plastids is the superset of Chloroplasts, Chromoplasts and Leucoplasts
 - **Lysosome:**
 - **Glyoxysome:**
- **Histones:** Characteristic proteins which encode the DNAs in Archaea and Eukaryotes. It is a Homologous Protein.

2 Lecture 1's Interactive Session

- Archaea are capable of growing in extreme conditions such as high temperature, salty environment etc.
- Eukaryotes are known to evolve through various symbiotic relationships between archaea and other bacteria.
- We can play with BioInformatics at [By Getting the Protein Sequences from here](#) and [and Here to compare proteins](#).
- Safranin Dye is cationic which bonds with the DNA in Cell wall and its the DNA which gives color like that
- We Heat fix the smear before observing, so that it denatures the proteolytic enzymes which could cause cell lysis(i.e. Enzymes breaking the cell membrane/Wall)

3 1st Tutorial Section

- A and T are joined by 2 hydrogen bonds whereas C and G are joined by 3 Hydrogen Bonds

4 2nd Lecture

- In Gram Positive Bacteria, We find that the 20-80 nm homogenous layer of peptidoglycan forms the cell Wall
- It contains Lipoteichoic acid which is connected either to the Peptidoglycan or the Plasma Membrane's lipids itself.
- There's a peri-plasmic space separating Plasma Membrane and the peptidoglycan.
- Cell Wall has certain Integral Proteins in addition to a bi-layer formed of Phospholipids forming the polar head of the surface, and the fatty acyl chain towards the inside.
- In Gram Negative Bacteria: Porin Proteins are found about the outer Membrane(formed of lipopolysaccharides) which allow transfer of molecules through it.
- **Ribosomes in Eukaryotes:** composed of Proteins and Ribosomal RNA
 - The Prokaryotic 70S Ribosomes is made of a large 50S ribosome and a small 30S Ribosome.
 - S is Svedberg Coefficient which provides indication about the rate of sedimentation
- Flagella is made of protein flagellin. It has helical structure with a sharp hook. To swim forward, it rotates in counter-clockwise. When the rotation abruptly changes to clockwise, the bacteria tumbles.
- Mitochondria has Porin Channels to help flow of materials from outside. It has ribosomes, ATP synthase, Cristae which are the foldings of its inner structure, Matrix which fills its inside, and two membranes(inner and outer)
- **Ribosomes in Eukaryotes:** composed of Proteins and RNA, they read nucleic acid information from mRNA and convert it to Proteins. They have 80S units consisting of a large 40S subunit which binds to amino acids, and a small 28S subunit which binds to mRNA during protein synthesis
- **Endoplasmic Reticulum:** Rough ER is a major site of protein synthesis, while the SER synthesizes lipids, steroids, metabolites, carbohydrates and regulates calcium concentration in muscles.
- **Golgi Apparatus:** Packages and Transports macromolecules, within and outside the cells
- Lysosome takes care of radicals in cells, and any intracellular debris.
- They contain Hydrolytic enzymes in sacs to do this!

- Peroxisomes also take care of digesting low-chain fatty acids
- They are single cell organisms
- **Endosymbiotic Origin of Double Membrane Structures in Eukaryotic Cells:** Aerobic non-photosynthetic bacteria was engulfed by another prokaryotic cell and became part of it. Chloroplast similarly came as some algae was engulfed in the cell.
- A proof to the previous point is that Genetic Material and Protein Synthetic Machinery is different in other Double Membrane Structures and Nucleus.
- 8 Histone Proteins attach to the DNA Molecules to form Nucleosome, These Nucleosome gets packed to give two different types of Chromatin
- Chromatids are less condensed than chromosomes. A chromosome consists of a single, double-stranded DNA molecule. Chromatids are two molecules of double-stranded DNA joined together in the center by a centromere. Chromosomes have a thin ribbon-like structure.
- **Cell Cycle:** Every cell must have come from the other cell, In the video part we say that
 - **Mitosis:** Identical chromatids, one of which identifies as Father and another as mother (pair of homologous but non-sister chromatids)
 - **Meiosis:** Responsible for shuffling of genes and hence variation in features
 - * In Prophase I in Meiosis, There's a synaptic site between the two non-sister chromatids: Chiasma from where some genes cross over from one bunch of chromatids to the other and hence leading to shuffling of genes
 - * Then the complete thing happens just like Mitosis in Meiosis I and then Meiosis II happens which divided the Chromosomes to 2 further pieces to give 4 haploid daughter cells
- **Regulation of Cell Cycle:** Cyclins (made during G2) are a family of proteins which along with Cyclin-dependent kinases (made during G1). Both are produced during beginning of Reproduction, and the Cyclins get and fit into CDK as a key-lock pair, which is completed only after M-phase and after that, they dissociate, and cyclin is degenerated.
- **External Regulations:** Space, Nutrients, Density-Dependent Inhibition
 - Cancer cells lose dependence on these external as well as Internal factors for Signals for Proliferation.
 - That's why even after Cancer is treated, it remains in meta-static phase in some cases and grows again after few years.
- **Cell Re-programming and Cloning:**

5 Important Stuff from Whatsapp Groups:

- The Rough E.R is continuous with Nuclear Membrane, the SER is comparatively distant.
- Histone forms an octamer around which DNA makes 1 and 3/4th turn to form a nucleosome (like a unit for coiling) and Histone +ve charged DNA negative So it's stuck and won't get uncoiled

6 Lecture 3: Cellular Metabolism

- Mesophyll are a type of Cell which contain Chloroplast.
- They contain stomata.
- **Light Reaction:** Occurs in presence of Sunlight
 - ATP and NADPH are produced.

- takes place in granum of Chloroplast
- ATP and NADPH are further taken to Stroma where dark reaction/Calvin Cycle are performed.
- Chlorophyll absorbs Violet-Blue and Orange-red thus, only green is transmitted
- H_2O gives H^+ reacts with O_2 and consumes some electrons from P680(Photosystem II or PSII), this P680 does so by absorbing light and gets excited to $P680^+$, these electrons are carried through a series of pigments which are plastoquinone and finally goes to Cytochrome-B complex and then Plasto-cytochrome and then transferred to Photo-System I($P700^+$), and the reduction(happens through Ferredoxin Molecule) to $NADP^+$ and H^+ and in presence of $NADP^+$ reductase, NADPH is formed.
- When the Pq-Cytochrome-Pc, that is only where using chemo-osmotic processes ATP Synthesis occurs.
- In certain cases, when the flow becomes cyclic and only PSI is involved, we find that NADPH isn't produced in that case.
- **Dark Reaction:** Sugar/Glucose is produced (Calvin Cycle/ C3 Cycle)
 - Three phases: Carboxylation, Reduction and Regeneration
 - This gives a 3-C product, Triose Phosphate, which gives it its name.
- **C4 Cycle:** In Plants with hot climate regions(some:)
 - First stable compound: OAA(Oxaloacetate 4 Carbon) combines with PEP(Phosphoenol-Pyruvic Acid) which eventually becomes Malic Acid(in presence of Malic de-hydrogenase), which further decomposes to Pyruvic Acid and CO_2 (with the help of H^+ from NADPH breaking to NADP and H^+)[Happening in Bundle Cells]. The Pyruvic acid re-enters mesophyll cell and helps in generating PEP.
 - CAM(Crassulacean acid metabolism) plants have stomata closed in morning and open in night. CO_2 stored in Night is used in day time for photosynthesis. (Found in Plants living in Arid Conditions)
 - Both CAM and C4 are found in hot and arid climates.
- **Metabolism:**
 - **Cellular Respiration (Catabolism):**
 - * Pyruvic Acid formed after Glycolysis, undergoes Aerobic/Anaerobic Processes depending upon the availability of CO_2 . Aerobic leads to Krebs Cycle.
 - * **FAD, $FADH_2$:** Electron transfer agents like NAD^+ and NADH.
 - * Energy yield depends on what Electron Transfer Process is taken.
 - Cancer Metabolism: The Observations of Warburg point that Intrinsic and Extrinsic Factors, could change Biosynthesis, Bioenergetics, Redox State of cell and have correlation with Cancer.
 - ATP generation in glycolysis is required for the cancer cells to grow and proliferate in unrestricted manner.(PKM2 shows that this pathway isn't the only source of energy for Cell Proliferation, and if it's changed by Extrinsic factors then it could have profound implications.)
 - FDG-PET test goes over the entire body and see where there is a high uptake of Glucose to detect tumours.
 - Other Tests look at Metabolic substances(Metabolites) to detect Cancer.
 - **Cancer Research Highlights:** Proteomic Investigation: See the grade of proteins which are present in various fluids in the brain. To appreciate the variations between the sick and healthy people. In order to get an idea about what's causing it and how to prevent it. Using PCA to see if we could separate clusters of these proteins to different categories, which could give us comprehensive inferences to the proteins' importance. We also look at their numbers and also radiology data. Isocitrate Dehydrogenase Mutations are quite common in Gliomas patients. Gliomas are the most basic of cancers.

7 Live Lecture 3

- **Mitochondrial Diseases:** Mitochondria use process known as phosphorylation to generate energy. It takes thousands of proteins for it to function, A single one wrong could cause problems. Only 13 of the Proteins could get got from Mitochondria, other come from DNA. The Mitochondrial diseases could hamper any organ, tissue and happen anytime in life. eg. Leigh Syndrome happens in Children could lead to many problems like mental retardation and later to loss in Movement abilities, but the disease can be stopped before it passed on. Mitochondrial DNA comes from mother, if its found, it could be transplanted from healthy mothers. This technique was recently approved in UK.
- **Cell Cycle and Totipotency Review:**
 - Morphulis gave the Model for Endosymbiotic Origin for double membrane structures.
 - G1 phase is where DNA synthesis happens and makes sure that all conditions is favourable for the Chromosome Replication in S phase.
 - G2 Phase covers growth, to further make sure that the cell is ready for dividing and reproduction.
 - In Meiosis, Interphase happens only once.(Before Meiosis I)
 - The Checkpoints are presents somewhere before the end in G1, as well as the beginning and end of M phase.
 - Cytokinin and Gibberelin helps the totipotent cells proliferate to form the callus.
 - When Cyclin gets conjugated with CDK, and it forms a complex MPF(Mitosis Promoting Factor) which induces phosphorylation and helps in the process of Mitosis.
 - Cloning has also benefitted a lot from the concepts of Checkpoints in the Cell Cycle.
 - iPS: Induced Pluripotent Stem
 - Allele are different version of gene on corresponding loci of chromosomes(Loci of genes which contain information about a particular trait)
 - In Chromosome Duplication, the single-stranded Chromosome when they replicate, two of them remain attached at their centromere(with kinetochore proteins over them!), which are known as Chromatids.
 - Prometaphase happens in Meiosis I?
- Endomembrane Systems include ERs(SER and RER) besides Golgi Apparatus.

8 Tutorial 2

- Stacked Thylakoids are called Granums.
- Chlorophyll is found inside Thylakoids
- The Inner Square in the PS diagram represents the reaction-centre, i.e. We find various complexes which aid the reaction around here.
- At the end of C3 Cycle, we find that RuBP gets converted to Rubisco, which is used in the next phase of the cycle(As an enzyme aiding Carbon fixation).
- NADPH is involved in fixing/reducing 1,3- Biphosphoglycerate to G3P.
- Electron Shuttle refers to the protein involved in transporting Pyruvate.
- The halving of chromosomes happens at the end of Meiosis I, Meiosis II is simply a copy of Mitosis.
- Even a Proton Gradient with artificial source would give us a feasible ATP-generation mechanism.
- (In General)DNA Content: 2 times the No. of Chromosomes, as each chromosome has two strands of DNA defining it completely.
- (In Mitosis)The Chromosome Number remains 2 times throughout during Mitosis, except of Anaphase, when the kinetochore connection between the two chromatids joined together breaks.

9 Live Lecture: DNA and Genetic Tools

- Each type of ddNTP is coded with a fluorescent material, so that it could be seen.
- In Sanger Method: We see that the ddNTP stops at the the next element, in the sequence and when it attached to that, it gives fluorescence.
- After that using a fluorescent Laser, we could find which bases are at what point.
- Genome Sequencing allows for differential treatment of Cancer.
- Conventional Techniques simply fill the patient with all medicines, hoping it would stop cancer.
- If I see more Red in Microarrays, that means Cancer Cell Population is high, (To update after seeing the lecture recording!)
- Polymerization Reaction(PCR) is used to make many copies of a partiular gene, so that it could be put in other places.
- These Copies are then taken back into the bacteria by transformation(by making the memebrene permeable so that the plasmid could be inserted back), then let it reproduce and multiply in proper environment.
- Then you can take the copies of gene from here, as well as the proteins made due to these genes, then put it somewhere else.
- The Restriction Enzyme HindIII cuts only between two AAs in the Gene sequence.
- In Extension phase of PCR, we add DNA ReCombinase or something like that, so that we length could increase. Tag-Polymerase also play a role in this length increase!
- The Tm value gives the temperature of Annealing.
- GEL:
- RNAs have Characteristic poly-A tail, poly-dT is the primer which is the inverse of this one.

10 Recorded Lecture 5: Genetics

- The sequences of different allele, need to change only one base, and the features could change.
- True Breeding Varieties: Homozygous, We get them by having a particular Phenotype with itself and as long as we don't get something else, that means its true-breeding varieties.
- In order to identify the heterozygous genotype, he carried out TEST CROSS, i.e. by crossing them with the homozygous recessive, in order to find which is homozygous would have all dominant traits expressed only.
- Are there other patterns of inheritance then Mendelian?
- As the number of genes increase, it becomes difficult for traits to follow Mendel's Laws.
- But some Traits still follow the Mendelian Laws.
- The 6 finger allele is dominant, but still its less prevalent(Natural Selection could be to plain!)
- Blood Grouping System: A test to know the Blood Type:
 - Put three isolated drops of blood on a slide.
 - Pour a drop Anti-A to the first drop, Anti-B to the Second Drop and, Anti-D(used to detect Rh Antigen) on the third drop
 - Mix the antigens with the respective drops(use a new toothpick each time!)
 - The drops which Agglutinate will tell us which Carbohydrates are actually present.

11 Live Discusison on Genetics Class

- The initial high temp. nears boiling.
- Taq-polymerase(a type of thermostable DNA Polymerase) binds to the primers after annealing and adds nucleotides to make a new double-strand.
- Alleles are actually the factors coming from both parents which combine to express a trait.
- Primers are taken depending upon which gene we want to search for.
- 3' and 5' are the starting and stading Carbons of the Sugar Moiety or its branches.
- Standard: Primers are always kept from 5' to 3' direction.
- DNAs are predominantly negatively charged and move towards anode, in gel electrophoresis, The ones with greater size move less far, the ones with smaller lengths move greater lengths towards the anode.
- Cy3 and Cy5 are dyes to label the RNA from different Cells(Cancerous/Normal)

12 Tutorial

- If primers are very short, specificity is lost as now the same primer could be found in different templates.
- Long Primers react with themselves.
- The Tempature for PCR should be almost same for forward and reverse temperature.
- For 5'-3' DNA Sequence, The Forward Primer is same as the starting sequence of the actual DNA, and the reverse primer is reverse complimentary derived from this sequence.
- If Annealing Temperature is below optimal, they would react anywhere in the sequence. Specificity is lost, as now the Primer won't bind where we want them to.
- If temperature is high, Annealing may not happen at all.
- In DNA Sequencing, using Sanger's Method, d(base), runs from 5'-3'(th ddNTP end is thus the 3') from bottom to top, and thus the real strand is complementary to it and the 3' becomes 5' and also, Whenever we write the sequence, we write from 5' to 3'.
- The ABO-grouping aren't three separate genes, but rather three type of alleles, and O is recessive, and AB means both A and B.
- Autosomal Traits: Pertaining to the Chromosome which is not the Sex Chromosome.
- X-linked means Pertaining to X Chromosome, and Y means their linked with the Y-Chromosome, and we'll have to study the pedigree to decide if they are recessive or dominant.
- A cross in a shape in the pedigree diagram shows that if a person was deceased.