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Exam IV Review – Genetics

- Bacteria
  - One of the most diverse and abundant domains, but we know very little about it
  - Single circular chromosome, 10^6-10^7 base pairs
    - Plasmids (circular), 10^3-10^4 base pairs
  - Can grow bacteria on a petri dish and a series of colonies will form
    - These are clones and every member could hold millions and millions of organisms (clonal: population of organisms with the same genome)
    - The plate has a complete media that contains all the necessary ingredients for bacteria to grow
    - Replica plating: take one plate and very carefully center it on the next plate and press down, can transfer the plate to a minimal media
    - Minimal media: can be manipulated so that substances are lacking
    - Results show that colonies can only grow in certain conditions
    - Auxotrophic colonies will not grow – they are deficient, others are prototroph and can synthesize everything that they need
    - Make another replica plate on different media to identify another auxotroph
    - The point is to identify genetic variants that are deficient in their ability to synthesize an amino acid, vitamin, etc.
    - Take the clone (auxotroph mutants) and grow it in a liquid culture to propagate it
    - Pour the liquids together and grow on a plate that is lacking both substances
    - This results in a small number of clones that are able to grow despite the deficiency – these bacteria can have sex!
      - Can transfer DNA from one clone to another, prototrophic/wild type)
  - 3 ways bacteria can exchange information – transformation, conjugation, transduction
  - Recombination is not eukaryotic specific, it is a property of DNA
    - Transformation: bacteria takes up foreign DNA and puts in itself
      - Ability to do this is referred to as competence
        - Varies as a function of bacterial species, different natural competencies
      - Can be treated in lab to allow them to take up foreign DNA
      - The process:
        - Chromosome is associated with the cell membrane
        - Identified some bacteria that is deficient in its synthesis
        - Other cell dies and its chromosome breaks up into small pieces
        - Cell takes up the sequence from another chromosome, the foreign DNA, and for that locus they become diploid
        - Wild type copy and the mutated copy combine

        - Gives rise to a bacterium with a mixed chromosome
    - Can use this process to map genes
        - Look for the ability of co transformation rates
        - Examine 3 different auxotrophic mutants, one bacterium is deficient in all 3
        - Mix with prototroph and measure the consequence of this
        - How often does co transformation occur
            - Between a and b – 6 clones
            - Between a and c – 36 clones (must be the closest, recovering more of them, close relative to the others)
            - Between b and c – 28 clones
            - Map: b…….c…a
        - Close together clones are more likely to be co transformed, way to establish and examine the proximity of genes
            - This is the most used process to engineer bacteria
                - Use plasmids (small DNA molecule that is separate from and can replicate independently of chromosomal DNA, independent, circular)
                - Make clones, transfer antibiotic resistance
                - Superbugs that are resistant to multiple antibiotics are made this way
    - Conjugation: bacterial sex
        - U tube experiment
            - Screen in bottom of U let small molecules pass through but not bacteria
            - The molecule was unable to grow phototrophic bacteria
            - Needed to be direct bacterial connection in order to generate phototrophic colonies
        - Led to the discovery of bacterial sex
            - Direct bacterium to bacterium transfer of genetic information that requires a direct connection
            - By virtue of association, had to have an F+ donor and an F- acceptor…there is some asymmetry in this process
            - F- accepts and then becomes new F+, now a donor
            - Bacteria that are F+ have sex villi that emanate from the cell membrane
            - During association, a specific sex villi grows and connects with an F- to create a conjugation tube
                - Replicates and forces DNA through the conjugation tube into the recipient bacteria
                - Given sufficient time, you are left with a bacterium that possesses the original plasmid
            - The F factor is a plasmid that is in the F+ donor bacterium
                - Rolling circle replication
- High frequency recombination strains (HFr+)
    - Donor: thr+ leu+ azi+ ton+ lac+ gal+ strep^s

- Recipient: thr- leu- azi' ton' lac- gal - strep+
- Typical plasmids have less than 7 genes
- Conversion of chromosomal genes, converting too many genes for it just to be a transfer
- F plasmid has incorporated and recombined into the chromosome, modified bacterial chromosome
- Episome: plasmid that can be incorporated into the bacterial chromosome
- Replication begins and the entire bacterial chromosome is sent through the conjugation tube
- Entire chromosome is transferred and can recombine with recipient chromosome, can change genotype

▪ Experiment: blend them, break the conjugation tubes, and study what is recovered
- Plate them on deficient plates after certain time periods to see when you recover a recombinant
- Tells which recombinants you get and when
- Characteristic amount of time for recombination; use this to map the entire bacterial chromosome
- Also acts as a clock, process takes 100 (for E. coli) hours total; map bacterial
- Relative to the origin, the closest gene is the recombinants you get first and fastest…the next gene is the second fastest and so on

▪ Take advantage of the diversity of different strains
- Insert episomes at different points and change the sequence around the circle

o Transduction: to carry across
▪ Viral: mediated genetic exchange
▪ Bacteriophages: bacteria eater
- Protein capsid coat, viral genome inside, double or single stranded RNA
- Virus throws genome into a bacterial cell
  - Can go in and sit there, piggy back on replication of the cell and whenever genome is replicated it gets replicated too
  - Any stress, viral genome will pop out and enter the lytic phase, replicates and is transcribed
  - Takes over the machinery and more viral genomes are replicated until the cell bursts and releases new viruses

▪ Lysogenic phase – viral genome is inserted in the bacterial genome/host chromosome
- Quiescent for a while, every time the bacteria replicates, it gets replicated
- No active infection of other bacteria by the virus, just obligatory making of copies of the viral genome by virtue of its own replication

▪ Lytic phase – requires DNA damage, cellular stress to the bacterium