MID Lecture #3
"Genetics 2"

Buzz Marcovici
pm2017@columbia.edu

Lecturer: Dr. Figurski

Started with a review of last lecture: Genetic changes

1) Pt. Mutations
    -small genotypic change with possibly large phenotypic change

2) DNA rearrangements
    -large, structural DNA changes
    -can get new genetic combinations and functions

3 GENE TRANSFER MECHANISMS

1) Transformation
2) Transduction
3) Conjugation

1. DISCOVERY OF TRANSFORMATION

Discovery: Griffith saw both smooth and rough variants of Streptococci. Smooth means its virulent (due to capsule being anti-phagocytic). Smooth kills mice. Rough has no capsule, no killing of mice. He found that if you put smooth strep that was heat killed with rough strep, the rough strep could later kill mice.

Transfer: Shown to be the DNA. First evidence that DNA used as genetic material!

CURRENT THEORY OF TRANSFORMATION

-active bacterial process
-involves 20-30 genes
-the proteins of these genes do lots:
    1. allow DNA to bind to surfaces
    2. allow DNA to get inside cell
    3. process DNA and incorporate into host

-cells can be competent one of two ways: inducible or constitutively

-Inducible Competent Bacteria

a) Strep is example. Induced when there are enough around. Otherwise, won't transform. Need certain concentration of protein they make.

b) Hemophylis is another example. Induced when stressed out.

-Constitutively Competent Bacteria

-Niseria is example. Can always uptake DNA.

-Normally, some bacteria can't at all

-E. coli is example. In nature, not competent.

How is the DNA stabilized one in the bacteria?

-Naked DNA outside cell is vulnerable to damage
-needs to be taken up quickly
-can be bound and taken up as either double stranded or single stranded DNA (depends on the bacteria species)
-DNA recombines in one of two ways: homologous or reciprocal

Homologous Recombination

-DNA replaces a piece that is similar to it in some sections
-Needs at least some homology
-Very efficient
-Universal (all bacteria do it)
-RecA: protein used to initiate

Sequence: One strand is nicked. RecA binds the single strand and searches for a homologous piece on host. Once found, second nick made, and ligated into place. Need to finish job by nicking/ligating other end to complete cross-over event. Don't worry about details.

Reciprocal Recombination

-maintains circular genome, but adds more than just homologous sections of DNA
-DNA is similar at ends, but may have intervening "new" sections, with new genes
-similar idea with RecA, etc.

Examples of transformations events in nature

1) Strep: acquired new Penicillin resistance. New Pen. Binding Protein (PBP) created, with lower affinity for Pen. Causes antibiotic (AB) resistance.

2) Niseria: Uses pili to attach and invade epithelium. Made up of pilin protein. These are immunogenic. But avoids immune response. How?

Ag variation: changes expression of pili over time

-spontaneous occurs on 1 out of 10^3 - 10^6 cells
-Pilin protein: C-terminal region varies across pilin. Same function though.

What leads to variation in Pili?

-Niseria have 10-20 gene copies for pili
-Divided into two classes: pilE (expressed) and pilS (silent)
-PilE has active promoter upstream of it, causes trx
-homologous recombination allows the PilE gene to change, create new pilin

Mechanisms of Pilin Change

1) Intrachromosomal recombination
-rare
-pilE and pilS just exchange spots

2) Transformation
-more common
-uptake of dead pili genes from reservoir of dead bacterial DNA
-generates new pilE gene, but other pilS genes have nothing changed

3) Phase Variation
-turn pili on/off
-can be due to recombination w/in cell or transformation
-generate a pilin that is not expressed
-some stages of lifecycle NEED no pili, so this helps them

2. TRANSDUCTION

Bacteriophages

-viruses that infect bacteria
-ds or ss DNA or RNA

-packaged in protein coat
-allows to be stable in env.
-3 to several hundred genes encoded
-all bacteria have phages

Lytic Cycle

-virus gets into bacteria
-replicates its DNA
-genes are trx
-proteins: head, tail, packaging, then lyses cell
-release new viruses
-can all happen in as little as 15 minutes!

Gene exchange possible

-when bacterial DNA is broken down, some may be packaged into new viruses
-such particles of bacterial DNA are then injected into new hosts
-this may then be incorporated

"Generalized Transduction"
-Not specific to a gene
-any DNA can be transferred
-virus is therefore spreading bacterial genes
-tends (not always) to occur in related bacteria because the phage uses the same receptor to get into next host

Temperate Phage and Lysogenic Cycle

-Viral DNA gets into bacteria
-DNA incorporates into host chrom.
-viral DNA itself carries these recombination enzyme genes
-"pro-phage" is the bacteria with the viral DNA in chrom.
-repressor protein is made from viral DNA that inhibits the lytic sequence
-if cell is in trouble, SOS type signal degrades repressor
-lytic genes trx
-viral DNA is excised
-may associate with some bacterial DNA when leaving, and spread this to next host

Examples of phages

1) Corynebacterium diphtheria
    -"B-phage" needed to make bacteria virulent
    -phage encodes the toxin when in chrom
    -"lysogenic conversion"

2) Vibrio cholerae (double phage!)
- first phage encodes pili
- 2nd phage carries toxin, which actually uses the above pili to gain access to cell!

3) Toxic Shock Syndrome Toxin
-when bacteria senses a phage, it excises its TSST gene
-this then copies itself like a phage
-when cycle reaches lytic stage, heaps of toxic shit is released too

4) Treating Anthrax with Phages?
-AB era is ending due to resistance
-now: looking at using phage lytic proteins to kill bacteria
-useful b/c detects target well

*NEXT LECTURE TOPIC: Conjugation

Piece,

Buzz