Chapter 13 Lecture Notes: Microbial Genetics – General Principles

I.   Why microbial genetics?
   A. Gene function tells us about cell function because cell structures and processes are genetically encoded.
   B. Microbes are simple, easily manipulated systems. Haploidy (possessing only one set of genes) allows the expression of mutations immediately.
   C. Cloning of many eukaryotic genes occurs in microbes.
   D. Genetics of organisms used in applied and medical science is important.
   E. Many genetic systems in microbes have counterparts in prokaryotes (i.e. mutagenesis and repair).

II.   General definitions and concepts
   A. Strain = a population of organism that descends from a single cell or isolate
   B. Genome = all the genes present in a cell or organism
        1. Prokaryotes have 1 set of all their genes = haploid
        2. Eukaryotes usually have 2 sets = diploid
   C. Genotype = the precise genetic makeup of an organism
   D. Phenotype = an observable characteristic of an organism
   E. DNA is the genetic material in many organism
   F. The genetic code – codon/anticodon matchup specifies which tRNA and thus which amino acid will be added during translation of mRNA into protein
   G. Gene = linear sequence of nucleotides that codes for a polypeptide, tRNA, or mRNA

III.   Mutations
   A. Some basic lingo…..
        1. Mutation = a process that produces a gene or chromosome that differs from the wild type
        2. Mutation = the gene or chromosome that results from a mutational process; a permanent heritable change in the genetic material which may cause change in the phenotype
        3. wildtype (wt): Wild type is an <u>arbitrary</u> standard for what "normal" is for an organism. Please remember that what is considered wild type today may have been a mutant in the evolutionary past.
        4. forward mutation – mutation from wt to mutant form
        5. reversion – mutation from mutant form to wt
             a) backward – converts DNA sequence to wt sequence
             b) suppression – converts phenotype to wt but not necessarily DNA sequence to wt
   B. A mutant is the organism or cell whose changed phenotype is attributed to a mutation
   C. Frequency in bacteria ranges from 1 mutation per 10^7 cells to 1 mutation per 10^{11} cells.

D. Types - according to phenotype
  1. Morphological - mutations that affect the outwardly visible properties of an organism
  2. Biochemical - mutations that may not be visible or affect a specific morphological characteristic but may have a general affect on the ability to grow or proliferate.

  > Most microorganisms are <u>prototrophs</u> which means that they can grow on a simple growth medium including an energy source and inorganic salts. Biochemical mutations include those that affect proteins or enzymes required to grow on various nutrients or to synthesize various components. Thus, these mutations cause the microorganisms to become <u>auxotrophs</u> (they must be supplied with additional nutrients if they are to grow). For example, the bacterium *Escherichia coli* does NOT require the amino acid tryptophan for growth because it can synthesize tryptophan. However, there are *E. coli* mutants that have mutations in the *trp* genes. These mutants are auxotrophic for tryptophan, and tryptophan must be added to the medium for growth.

  3. Conditional = only see mutant phenotype under certain conditions (i.e. temperature sensitive mutation)
  4. Loss of function vs. gain of function
      a) Loss of function mutations are those that destroy the function of the gene product.
      b) Gain of function mutations are those that produce a new function for the gene product.
  5. Lethal = mutation results in death of the organism when expressed

E. Types – according to genotype   Table 13-2
  1. point mutations – mutations that change one basepair
      a) mutation at the DNA level
          (1) transition purine → purine
          (2) transversions purine → pyrimidine or pyrimidine → purine
      b) mutation at DNA level and classified according to effect at protein level
          (1) silent – mutant codon encodes same amino acid
          (2) neutral – mutant codon encodes functionally equivalent amino acid (sometimes also called silent)
          (3) missense – mutant codon encodes chemically different amino acid
          (4) nonsense – mutant codon results in the generation of a stop codon
  2. insertion mutations – mutations from the insertion of basepairs
      cause frameshift – change in the reading frame for the synthesis of a protein due to insertion or deletion of basepairs in any amount except multiples of three
  3. deletion mutations – mutations from the deletion of basepairs
      also cause frameshifts

F. Mechanisms
   1. Spontaneous
      a) errors in DNA replication due to tautomerism – results in point mutations (Fig. 13-9)
      b) errors in DNA replication due to slippage at direct repeats – results in insertions or deletions (Fig. 13-10)

   2. Induced by chemical mutagens
      a) base analogues
         compounds sufficiently similar to basepair with the correct base during DNA replication → analogues have two modes of H bonding because they easily convert to the incorrect tautomeric form → once they are incorporated they can be mutagenic by causing misincorporation in later rounds of DNA replication if they are in the incorrect tautomeric form.
      b) chemicals that alter bases
         (1) deamination – cause mispairing during DNA replication
         (2) alkylation - cause mispairing during DNA replication
         (3) radiation – cause T-T dimers which block DNA replication resulting in the induction of error prone DNA repair (see below)
      c) intercalating agents
         insert between the stacked bases of the DNA helix causing DNA distortion → causes insertions or deletions

   3. Induced by biological mutagens
      a) transposons and insertion sequences (jumping genes) – insert into genes and disrupt the coding
      b) mutation genes?

   4. Induced by SOS DNA repair (see later)

   5. Adaptive mutation: Some bacteria direct certain mutations to occur so that they can adapt better to their surrounding; mutations occur DURING nonlethal selection and SPECIFICALLY in response to the selective pressure. This is in contrast to #1-4 where the mutations are essentially random and occur BEFORE the selective pressure has been applied.

   6. Site directed mutagenesis: Researchers can engineer practically any mutation that they want in some organisms that are genetically manipulatable.

G. Detection
  1. Importance of the haploid state
  2. Selections vs. screens
      a) Microorganisms allow for the use of <u>selective systems</u> for mutation detection vs. the <u>screening systems,</u> although both can be used. A selective system is one in which the experimenter can DEMAND that the only individuals that grow or survive are the ones that have the mutation of interest. On the other hand, a screening system is one in which the experimenter must examine each individual to see if it has the mutation of interest.

      b) Selections – Fig. 13-16 and attached
          (1) Selection for reversion of an auxotroph to a prototroph: Plate 10^9 adenine auxotrophs on agar plate with no adenine. The only bacteria that grow are those that have a random mutation in the mutant *ad* gene that now reverts it back to the wild type allele.
          (2) Selection for resistance to an environmental factor (bacteriophage, antibiotics).

      c) Screens (Fig. 13-17 and attached)
          (1) Screening for forward mutations from wild type to auxotrophy: Plate bacteria on complete media to form colonies. Replica plate many, many colonies to plates with and without the nutrient you are testing for auxotrophy. Compare the plus and minus nutrient X plates to look for a colony that appears on the plus nutrient plate but not the minus nutrient plate. If you expect the mutation frequency to be 1 in 1,000,000, then you will need to replica plate at least 1,000,000 colonies to get the 1 mutant. Lots of work!!!!
          (2) Enrichments are important in screening because they reduce the number of organisms that you have to screen. Enrichment for auxotrophs works by selectively removing or killing growing microorganisms while they are in medium that allows only the prototrophs to grow. (Penicillin enrichment – see attached)

3. Carcinogenicity testing (The Ames test – Fig. 13-17)
    a) Basis: Many carcinogens are also mutagens
    b) Components:
        (1) *Salmonella typhimurium* strains, each with a different mutation in the *his* biosynthetic operon
            (a) all with a mutation to make the cell wall more permeable to compounds
            (b) all with defects in the UvrABC excision repair DNA repair system
            (c) all with error prone DNA repair genes
        (2) Putative carcinogen
        (3) Liver extract to activate the carcinogen
        (4) Plates with just enough histidine to support growth for only several generations
    c) Method: Plate *S. typhimurium* strains on plate with carcinogen and on a control plate → after 24 hours observe the number of *his* revertants that can now grow without histidine → the more revertants, the more mutation, the more mutagenic the compound
    d) Limits : Carcinogenicity does not have to be mutagenic and vice versa

IV. Repair
  A. Proofreading by DNA polIII

  B. UvrABC system of excision repair (Fig. 13-18)
    1. Recognizes distorted DNA
    2. Mechanism: UvrABC endonuclease cuts DNA backbone to remove the lesion → single stranded gap is filled in by DNA polI → DNA ligase joins the fragments

  C. Other excision repair
    1. AP endonucleases recognizes apurinic and apyrimidinic sites and cuts out a small fragment of the strand that contains them → single stranded gap is filled in by DNA polI → DNA ligase joins the fragments
    2. gycosylases recognize damages bases and excise the bases yield AP sites → AP endonucleases recognizes apurinic and apyrimidinic sites and cuts out a small fragment of the strand that contains them → single stranded gap is filled in by DNA polI → DNA ligase joins the fragments

  D. Photoreactivation
    1. Recognizes thymine – thymine dimers
    2. Mechanisms of action: T-T dimers are split apart using light energy and photolyase

E. Mismatch repair
>   1. Recognized mismatched bases
>   2. Mechanism: Scans the newly replicated DNA for mismatches → excises
>   stretch of DNA on the undermethylated strand → single stranded gap is filled in
>   by DNA polI → DNA ligase joins the fragments

F. Recombination repair (Fig. 13-19)
>   1. Recognizes damaged DNA without a template (both basepairs
>   missing/damaged; gap across from a lesion such as T-T dimers)
>   2. Mechanism: See Fig. 13-9

G. SOS repair
>   1. Recognizes lesions in the DNA
>   2. Mechanism → Error-prone DNA repair proteins bind to DNA polIII and allow
>   it to replicate across the lesion at the expense of DNA fidelity (DNA replication
>   errors is better than no DNA at all)

A final note on repair – transcription of most of these DNA repair genes are negatively regulated by the repressor LexA which binds to operators in their promoters and prevents transcription. The signal to turn on these genes is DNA damage and is thought to occur as follows: RecA binds to damaged DNA which alters the conformation of RecA so that RecA can now mediate cleavage of the LexA repressor. This in turn activates transcription of the DNA repair genes. *uvrABC* are activated before SOS *umuDC* because the SOS system is mutagenic and a last ditch effort to save the cell because the *umuDC* operator binds the LexA repressor much tighter than the *uvrABC* operator.