Microbial Genetics

Flow of Genetic Information =

Terminology

- Genetics: The study of what genes are, how they carry information, how information is expressed, and how genes are replicated.
- Gene: A segment of DNA that encodes a functional product, usually a protein.
- Genome: All of the genetic material in a cell
- Genomics: The molecular study of genomes
- Genotype:
- Phenotype:

DNA

- Polymer of nucleotides:
- Double helix associated with proteins
- "Backbone" is deoxyribose-phosphate
- Strands are held together by hydrogen bonds between AT and CG.
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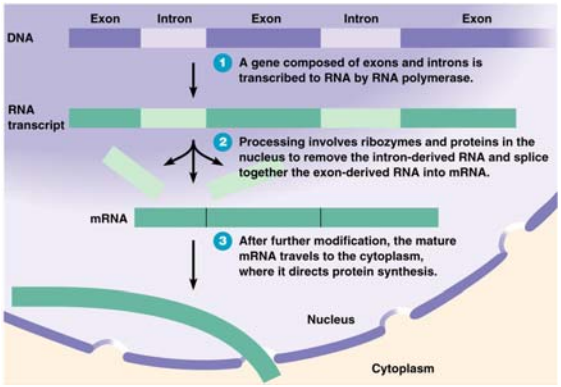
- DNA is copied by DNA polymerase
  -
  - Initiated by an RNA primer
  - Leading strand is synthesized continuously
  - Lagging strand is synthesized discontinuously
  -
  - RNA primers are removed and Okazaki fragments joined by a DNA polymerase and DNA ligase

DNA replication is semiconservative



Transcription

- DNA is transcribed to make RNA (mRNA, tRNA, and rRNA).
- Transcription begins when RNA polymerase binds to the promotor sequence
- Transcription proceeds in the 5' $\rightarrow$ 3' direction
- Transcription stops when it reaches the terminator sequence

Translation

- mRNA is translated in codons (three nucleotides)
- Translation of mRNA begins at the start codon:
- Translation ends at a stop codon:

Regulation of Bacterial Gene Expression

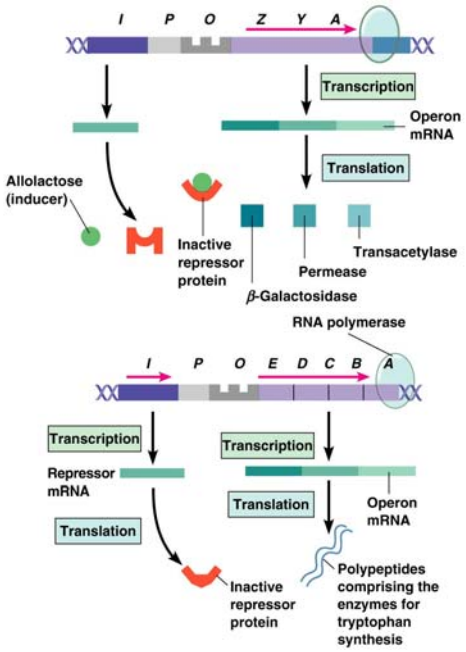
- Constitutive enzymes are expressed at a fixed rate.
  - constantly being transcribed and translated into proteins
- Other enzymes are expressed only as needed.
  - Repressible enzymes
  -

- induction - inducible genes turned on by inducers
  - inducers often substrates required for catabolic pathways
- repression - repressible genes turned off by repressors
  - repressors are end products of anabolic (_____) pathways - when sufficient product is made gene gets turned off
- this regulation often achieved with the operon
  - group of genes that can be induced or repressed

Lac Operon

- Bacteria such as *E. coli* that can utilize lactose require a lag time during which lac operon becomes induced.
  - If medium contains glucose (preferred sugar) and lactose, lac operon <u>not</u> induced until / unless glucose all used up.

- So what if glucose and lactose are present, why not metabolize lactose along with glucose?
  - genes for glucose metabolism are constitutive
  - activating other genes for more pathways requires energy
    - therefore, bacteria conserve energy by using glucose first, then any other energy sources



Mutation

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- Mutations may be neutral, beneficial, or harmful.
- Mutagen:
- Spontaneous mutations: Occur in the absence of a mutagen
- Base substitution (_____)
- Missense mutation
- Change in one base
- Results in change in amino acid

- Nonsense mutation
- Results in a nonsense codon

- Frameshift mutation
- Insertion or deletion of one or more nucleotide pairs

- Ionizing radiation (_____) causes the formation of ions that can react with nucleotides and the deoxyribose-phosphate backbone.
- Nucleotide excision repairs mutations
-
- Light-repair separates thymine dimers.

- Is there such a thing as a healthy tan?

- Should food be irradiated to destroy microbes and increase shelf life?

The Frequency of Mutation

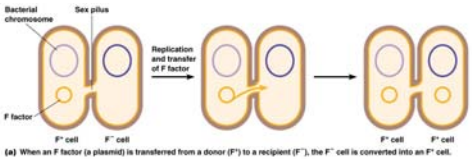
- Spontaneous mutation rate = 1 in 10^9 replicated base pairs or 1 in 10^6 replicated genes
- Mutagens increase to $10^{-}5$ or $10^{-}3$ per replicated gene.

Genetic Transfer and Recombination

- Vertical gene transfer: Occurs during reproduction between generations of cells.
- Horizontal gene transfer: The transfer of genes between cells of the same generation.

Transformation

- **Purpose of transformation?**
  - mechanism of transfer of genetic info among bacteria
  - occurs naturally with linear pieces of DNA
  - under laboratory conditions certain bacteria can be made “competent” and take up plasmids
  - once inside, DNA can undergo recombination with host chromosome and be inserted



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted into an F^+ cell.

Conjugation

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- transfer of DNA from one bacterial cell to another
- gram (-) requires pilus
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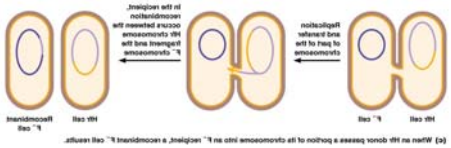

- Gram (-)
  - donor cells contain F plasmid (_____) F^+
  - recipient cells do not contain F plasmid F^-
  - if plasmid is extrachromosomal, then F^+
  - if plasmid is integrated in host chromosome, then Hfr (high frequency of recombination)
  -
  - sex pilus from F^+ or Hfr cell attaches to F^- cell, pilus contracts pulling F^- close

- contrary to text diagram, DNA transfer does not go through the pilus, rather pilus pulls cells close together, cell walls interact and by as yet unknown mechanism, DNA transferred
- recipient cell becomes F^+ if donor was F^+
- recipient cell remains F^- if donor was Hfr
- bacterial genes get transferred, fertility factor is last thing to transfer
- pairing of cells is fragile, usually break apart before end of transfer, therefore recipients remain F-

- Gram (+)
  -
  - other plasmids involved, mechanism unclear

Transduction

- Bacteriophage (_____) mediated transfer of DNA from one bacterial cell (_____) to another bacterial cell (_____).

Genetic Recombination

- Exchange of genes between two DNA molecules
  - Crossing over occurs when two chromosomes break and rejoin

Plasmids

- Conjugative plasmid: Carries genes for sex pili and transfer of the plasmid
- Dissimilation plasmids: Encode enzymes for catabolism of unusual compounds
- R factors:

Transposons

- Segments of DNA that can move from one region of DNA to another (_____).
- Contain insertion sequences for cutting and resealing DNA (_____).
- Complex transposons carry other genes.
- In bacteria, transposons jump between the chromosome and plasmids.

So What?

- **Implications of gene transfer among bacteria?**

- All three types of gene transfer can be manipulated by us. Most important is transformation. We can engineer plasmids to carry a variety of genes and insert them into bacteria with relative ease.

Study Objectives

1. Define gene, genetics, genome, genomics, genotype, phenotype.
2. Describe the overall process of DNA replication in prokaryotes and contrast it with eukaryotic replication.
3. Describe the process of transcription. How is prokaryotic transcription different from eukaryotic transcription?
4. Briefly describe translation.
5. Compare and contrast gene induction with gene repression.
6. Compare and contrast inducible operons with repressible operons.
7. Describe the Lac operon. Why is it inducible and why aren't those genes constitutively expressed?
8. Describe the types of mutations that occur and their possible consequences.
9. Describe how UV and ionizing radiation damage DNA and cause mutations.
10. Compare and contrast horizontal and vertical gene transfer.
11. Describe in detail: transduction, transformation, and conjugation. Differentiate between generalized and specialized transduction.
12. Describe how each of the above methods of gene transfer can be exploited by scientists to manipulate DNA.
13. Compare and contrast the three types of plasmids discussed here.
14. Describe transposons.
15. What are the implications of gene transfer among bacteria?