

## ***Regulation of gene expression***

- a. *Expression of most genes can be turned off and on, usually by controlling the initiation of transcription.*
- b. *Lactose degradation in E. coli (Negative Control)*

***Lac Operon contains three structural genes and is controlled by the lac repressor:***

- (1) ***LacY protein*** transports lactose into the cell.
  - (2) ***LacZ protein*** ( $\beta$ -galactosidase) cleaves lactose (a disaccharide) into glucose and galactose.
  - (3) ***LacA protein*** is a nonessential enzyme.
- c. **Operon:** *several genes are transcribed as a single unit, usually encode proteins involved in a common process (not common in eukaryotes).*
    - ***Operon:*** *The sequences of bases in DNA that contains one or more structural genes together with the operator controlling their expression.*
    - ***Operator:*** *The segment of DNA to which the repressor protein binds; it controls the expression of genes adjacent to it.*
  - d. *Organization of the lac operon*
  - e. *When lactose is added to the culture medium, transcription of the lac operon is induced. In the absence of lactose, transcription is repressed.*

- f. Regulation of transcription (rate of mRNA synthesis) is controlled by the lactose **repressor protein** which has a
- (1) lactose binding site
  - (2) DNA binding site (specific for operator sequence)
- g. **When lactose is absent**, repressor binds to DNA at the lac operator and prevents RNA polymerase from binding to the lac promoter. Transcription is blocked.
- h. **When lactose is present**, repressor binds to it and is then unable to bind to DNA. RNA polymerase can now bind to lac promoter and transcription begins.

## **BACTERIAL GENETICS**

*Why study bacterial genetics?*

### *A. Mutations and mutagenesis*

#### *1. Definitions*

- a. Genotype: the total genetic information that an organism possesses (i.e., what genes are there whether they are silent or expressed).*
- b. Phenotype: the characteristics (appearance and behavior) that an organism displays (depends on the genes being expressed under the defined conditions).*
- c. Mutation: a change in the base sequence of DNA.*
  - (1) A mutation always changes the genotype. It may or may not change the phenotype.*
  - (2) Mutations are a source of genetic variation.*

#### *2. Types of mutations*

##### *a. Single base-pair change*

###### *(1) substitution*

*Example:            -CCA-CTG-GCT-GGA-TAC-  
                          -pro -**leu** -ala -gly -tyr -*

*↓*

*-CCA-CAG-GCT-GGA-TAC-  
-pro -**gln** -ala -gly -tyr -*

###### *(2) deletion or addition (frameshift mutation)*



- b. *Mutations increase genetic variability and result in altered phenotypes.*
- c. *Mutations occur at random in a population and are not directed towards an evolutionary goal.*
- d. *Natural selection determines whether mutated organisms are more or less fit to survive (reproduce) in a constantly-changing environment.*

7. *Bacterial genetics includes:*

a. *generating and screening (or selecting for (ex antibiotic resistant mutant)) for a mutant that is lacking a certain phenotype an investigator is interested on identifying the genes involved in (ex: screening for a nutritional mutant which has a particular requirement = auxotroph created from prototroph – detected by replica plating **FIG 9.2**)*

b. *that mutant is “complemented” by placing back in pieces of normal DNA (cloned “wild-type genes”) into the mutant and screening for restoration of the original phenotype, the DNA fragment that restores the original phenotype usually carries the gene that had the mutation in it*

**B. Molecular basis of gene transfer**

1. *Three mechanisms of gene transfer in bacteria*

- a. **Conjugation:** *DNA transfer when donor and recipient cells are in direct contact.*
- b. **Transformation:** *donor DNA (naked DNA from medium)in solution is taken up by recipient cells.*
- c. **Transduction:** *DNA transfer between donor and recipient cells mediated by viruses.*

2. *In all three mechanisms, homologous recombination may occur after donor DNA has entered the cytoplasm of the recipient cell: nucleotide*

*sequences are exchanged between the two DNA molecules to form recombinant DNA products which contain new combinations of genes.*

### **Conjugation:**

#### **1. Donor versus recipient cells**

a. *Donor cells contain an accessory genetic element called a plasmid. Recipient cells lack the plasmid.*

b. *Plasmids are circular DNA molecules that replicate independently of the bacterial chromosome and carry the following:*

(1) *always, genes for their own replication*

(2) *sometimes, genes that confer new phenotypic characteristics on the host cell*

(a) *antibiotic resistance*

(b) *toxin production*

(c) *new metabolic capabilities*

(3) *usually, genes that mediate plasmid transfer to new cells.*

2. *Conjugation is the mechanism by which plasmids transfer themselves between bacterial cells.*

*In the discovery of conjugation: The ability to carry out conjugation was originally called fertility and the plasmid responsible was named the fertility plasmid or F plasmid. Donor cells were also called "males" and recipient cells were called "females."*

(1) *The F plasmid carries genes for the synthesis of sex pili. Sex pili on the donor cell surface make initial contact with the recipient cell and are then retracted into the donor cell cytoplasm to bring the two cells into direct contact.*

- (2) *F* plasmid DNA is transferred into the recipient cell as a linear single strand, where it is made double-stranded by DNA replication and becomes circular.
- (3) The donor cell retains one copy of the plasmid, while recipient cells inherit a second copy and are converted into donor cells themselves.

3. The *F* plasmid can mediate transfer of donor chromosomal genes

a. First way: formation of Hfr donors

- (1) Cells that contain the *F* plasmid are called  $F^+$ . Cells that do not contain the *F* plasmid are called  $F^-$ .
- (2) In about 1 in  $10^5$   $F^+$  cells, the *F* plasmid integrates into the bacterial chromosome (at a semi-random location).
  - (a) Cells that contain an integrated *F* plasmid are called Hfr (“high frequency of recombination”).
  - (b) When conjugation occurs, chromosomal and plasmid DNA are both transferred to the recipient cell as a linear single strand.
  - (c) The donor DNA fragment does not become circular and eventually will be lost unless it undergoes homologous recombination with the recipient chromosome. In this case, it will be the recipient genes that are lost and the donor genes become a permanent part of the recipient cell’s new genotype.
- (3) Hfr donors transfer chromosomal genes into the recipient in a linear order. Conjugation can be used to construct genetic maps of the bacterial chromosome.
- (4) By Hfr mapping, the genetic map of the *E. coli* chromosome was shown to be circular.

b. *Second way: formation of F' donors*

- (1) *An integrated F plasmid can excise from the bacterial chromosome.*
- (2) *Imprecise excision occurs on rare occasions. The F plasmid carries a small portion of the host cell's chromosome with it and is called an F' (F-prime) plasmid.*
- (3) *In a later mating with a recipient cell, the F' plasmid is transferred in the same manner as an F<sup>+</sup> plasmid.*
- (4) *The recipient acquires the donor genes as a part of the plasmid, which will become circular and be replicated independently of the recipient chromosome.*

**Transformation:**

1. *Definition: transfer of soluble DNA molecules between bacterial cells.*
2. *Uptake of transforming DNA by recipient cells*
  - (A.) *naturally transformable bacteria ("competent") (ex: Bacillus subtilis)*
    - a. *Binding of free dsDNA (e.g. DNA fragments) to specific sites on the surface of the recipient cell.*
    - b. *Random cleavage of DNA into small fragments (6,000 -8,000 base pairs in length).*
    - c. *Penetration of one DNA strand into the cytoplasm of the recipient cell and simultaneous degradation of the other strand.*
    - d. *Homologous recombination of the single strand with the recipient chromosome.*
  - (B.) *Bacteria can be made artificially competent for DNA uptake (ex: E. coli treated with cold CaCl.) Transformation of free DNA is not as efficient as in naturally competent bacteria.*

- *Component: cell able to take up DNA and be transformed*

*(C.) Plasmids can also be transformed into artificially competent and naturally competent bacteria by a different mechanism where they may remain as plasmids.*

***Transduction:***

- 1. Definiton: transfer of DNA between bacterial cells mediated by viruses.*
- 2. Most bacterial viruses (bacteriophages) are not able to transduce bacterial DNA, but a few can.*
- 3. Two types:*
  - *generalized transduction*
  - *specialized transduction.*

**Generalized transduction:** *a consequence of lytic growth*

***Lytic Cycle:***

- *bacteriophage infects the host cell*
  - *takes controls and forces the host to make many copies of the virus*
  - *host bacterium bursts or lyses*
  - *releases new phage*
- a. Two types of virus particles formed when a transducing bacteriophage grows lytically on susceptible bacterial (donor) cells.*
    - (1) Majority are **normal phage particles** that contain viral DNA.*
    - (2) Minority are **transducing particles** in which bacterial DNA has been packaged in place of viral DNA by mistake.*
  - b. When a **transducing particle** attaches to a new host (recipient) cell, the **donor bacterial DNA is injected into the cytoplasm.***

- c. *Donor DNA fragment can then be incorporated into the recipient chromosome by homologous recombination.*
  - *A carrier of genetic information from the original bacterium to another cell and does not initiate a lytic cycle.*

**Specialized or Restricted transduction:**

- *a consequence of lysogenic growth*
- *the transducing particle carries only specific portions of the bacterial genome.*
  - a. *DNA of some (not all!) bacteriophages can integrate into the bacterial chromosome at a specific site (somewhat similar to the F plasmid).*
  - b. *Imprecise excision results in phage DNA molecules that also carry adjacent bacterial (donor) genes.*
  - c. *Hybrid phage-bacterial DNA is packaged into virus particles.*
  - d. *Transducing particle attaches to a new host (recipient) cell, and the hybrid DNA is injected into the cytoplasm.*
  - e. *Hybrid DNA (including the donor bacterial genes) is incorporated into the recipient chromosome, although defective, it can receive help from a normal phage called the helper phage which aids in the integration and reproduction of the defective phage.*
- *Any donor gene can be transferred to the recipient cell by the process of generalized transduction.*
- *Only certain genes can be transferred to the recipient cell by specialized transduction.*