

Genetics of Bacteria and Phage

Bacteria - Model Genetic Systems:

Short generation time (< 1 hour)

Small haploid genomes – many sequenced

Select for rare mutants / recombinants

Screen: Examine many individuals

Look for desired class

Select: Only mutants or recombinants live

Example: Select for antibiotic resistance

Favorite Model: Escherichia coli (E. coli)

Bacterial Plasmids:

Extrachromosomal ds DNA molecules

Some (episomes) integrate into chromosome

Example: F factor important in conjugation

Often contain antibiotic resistance genes

Widely used in molecular biology / biotechnology

Asexual Reproduction:

Liquid Cultures in Tubes or Flasks:

- 1. Initial lag phase*
- 2. Log / Exponential*
- 3. Stationary Phase*

Solid Medium in Agar Plates

Start with single cell ----> Colony of descendant cells

Start with many cells ---> Lawn of bacterial growth

Add virus to lawn -----> Clear region (plaque)

Results from lysis of bacterial cells by phage

Examples of Bacterial Mutants:

Auxotrophs: Require essential nutrient for growth

Example: bio⁻ Requires supplemental biotin

bio⁺ Wild type (makes own biotin)

Sensitivity / Resistance to Antibiotics:

Example: str^r : Streptomycin resistant
 str^s : Streptomycin sensitive

Response to Phage: Sensitive vs resistant to infection

Origin of Variation in Bacterial Populations:

Resolved by Luria-Delbruck experiment

Adaptation: Response to environment

Mutation: Spontaneous mutations that become apparent after selection

Genetic Recombination (Conjugation) in Bacteria

Discovered by Lederberg and Tatum (1940's)

Involves cell contact between different mating types

F factor plasmid plays central role

Nature of F factor in Different Mating Cell Types:

F^- Absent
 F^+ Separate from chromosome
 Hfr Integrated into chromosome
 F' Combined with chromosome fragment

Nature of Bacterial Strains:

F^- Stable and pure (all F^- cells)
 F^+ Mostly F^+ cells but a few Hfr
 Hfr Mostly Hfr cells

Conjugation Between Strains:

F^+ x F^- strains: Low frequency of recombinants
Rare Hfr cells in F^+ strain responsible

Hfr x F^- strains: High frequency of recombinants

F' x F^- strains: Sexduction (more complex results)

Conjugation at Cell Level:

F^+ cell x F^- cell: Copy of F factor transferred to F^- cell changed into an F^+ cell
 F^+ cell remains unchanged
No chromosomal recombination

Hfr cell x F^- cell: Genetic recombinants produced
Unidirectional gene transfer
 Hfr (donor) to F^- (recipient)
 F^- cell becomes recombinant
 Hfr cell remains unchanged

Example: Mating = $Hfr a^+ \times F^- a^-$
Recombinants = $F^- a^+$ (not $Hfr a^-$)

Methods for Genetic Mapping in Bacteria:

Conjugation: Time of entry of gene into F^- cell
Results in map based in minutes

Conjugation: Recombination frequency in F^- cell

Transformation: Frequency of simultaneous uptake and incorporation of 2 linked genes

Example: DNA from $a^+ b^+$ strain + $a^- b^-$ cells

If a / b close, most a^+ transformants also b^+
If a / b distant, most a^+ transformants not b^+

2 genes co-transformed often if closely linked

Transduction: Phage-mediated transfer of bacterial genes from donor to recipient cells
Use frequency of co-transduction of markers

Discovery of Bacterial Conjugation:

Strain A: $met^- bio^- thr^+ leu^+ thi^+$ (F^-)

Strain B: $met^+ bio^+ thr^- leu^- thi^-$ (F^+)

Mix 2 strains in enriched medium and plate on basal
A few prototrophic colonies observed

Not found when parental strains plated on basal media

Conclude: Prototrophic cells result from recombination

Recombination Requires Cell Contact:

*U-tube experiment
Porous filter separated A / B strains
Recombinants no longer obtained*

Mapping with Interrupted Matings

Example of Strategy: Hfr str(s)a(+) x F- str(r)a(-)

*Plate on basal medium with streptomycin
Parentals die and recombinants survive*

<i>Hfr str(s)a(+)</i>	<i>Parental</i>
<i>F- str(r)a(-)</i>	<i>Parental</i>
<i>F- str(r)a(+)</i>	<i>Recombinant</i>

Example of Mating Used for Mapping Genes:

Hfr str(s)a(+)b(+)c(+)d(+) x F- str(r)a(-)b(-)c(-)d(-)

*Conjugation in liquid enriched medium (non-selective)
Disrupt at different times with blender (stop mating)
Plate exconjugants on various selective media
Determine time of entry of marker gene into F- cell
Creates map based on time (~90 minutes for E. coli)
Good for approximate map location on chromosome*

Repeat with different Hfr Strains:

#1 F-a-c-b-d #2 F-b-c-a-d #3 F-b-d-a-c

*Conclusions: Bacterial chromosome is circular
F factor inserts at different sites*

Mapping Based on Recombination % in F- Cell

*Fine structure mapping of closely-linked genes
1 minute = About 20% recombination
Even number of crossovers (2) required*

Example: Hfr str(s)a(+)c(+) x F- str(r)a(-)c(-)

Select for c(+) recombinants

Include nutrient A and streptomycin in medium

Surviving cells of 2 genotypic classes:

F- str(r)a(-)c(+) and F- str(r)a(+)c(+)

Replica plate to determine relative numbers

*Use velvet "stamp" to transfer colonies
to same position on plate lacking nutrient A*

Surviving colonies: a(+)

Missing colonies: a(-)

Formation of c(+)a(+) Recombinants:

Common if 2 genes are very close

Formation of c(+)a(-) Recombinants:

Common if 2 genes are not close

Recombination Frequency: $\frac{[c(+)a(-)]}{[c(+)a(-) + (c(+)a(+)]}$

*c(+) = selected marker c(-) = unselected marker
a(+) vs a(-) distinguished by replica plating*

Example of Mating with 3 Markers

Used to determine gene order and relative distances

Hfr str(s)a(+)b(+)c(+) x F- str(r)a(-)b(-)c(-)

Select for c(+) recombinants

Plate exconjugants on A, B, streptomycin

Replica plate to other types of selective media

Determine full genotypes for all c(+) recombinants

<i>Results:</i>	<i>c(+)a(+)b(+)</i>	<i>320</i>	
	<i>c(+)a(-)b(+)</i>	<i>30</i>	
	<i>c(+)a(-)b(-)</i>	<i>6</i>	
	<i>c(+)a(+)b(-)</i>	<i>0</i>	<i>(4 c.o. required)</i>

Mapping with Transducing Phage

Overview of Phage Infection Pathways:

- A. *Lytic:* *Bacterial cell lysed (burst)*
 Many new phage produced
- B. *Lysogenic:* *Bacterial cell survives*
 Phage chromosome integrates
 Resulting prophage may later excise
- C. *Phage genome degraded by bacterial endonucleases*

Formation of Transducing Phage:

Generalized: Formed during lytic pathway
Random bacterial fragment surrounded by phage coat

Bacterial DNA

Specialized: Formed at transition from lysogenic to lytic pathways
Partial phage genome, adjacent chromosome fragment surrounded by phage coat
Maximal length of bacterial fragment: ~1% chromosome

Bacterial DNA

Phage DNA

Mapping with Generalized Transducing Phage

Does not involve conjugation, F factor, or cell contact

Approach: Donor $a(+)$ $b(+)$ $c(+)$ \times Recipient $a(-)$ $b(-)$ $c(-)$

Select for $a(+)$ transductants
Determine frequency of unselected markers
Adjacent genes co-transduced at high frequency

Results: 50% of transductants are also $c(+)$
2% of transductants are also $b(+)$

Conclusion: "a" is closer to "c" than to "b"

Then: Start over and select for $b(+)$ transductants
Results: 3% are $a(+)$; 0% are $c(+)$

No $a(+)$ $b(+)$ $c(+)$ transductants found

Phage Genetics

Rapid replication cycles
Selection strategies possible
Mutants available (no auxotrophs)

Examples of Mutants:

r = rapid lysis ---> altered plaque morphology
h = host range ---> altered bacterial specificity

Mapping strategy:

Mixed infection: Add both mutants to culture
Select for rare recombinants

Example: Add $r(+)$ $h(-)$ + $r(-)$ $h(+)$ phage to culture

Select for $r(+)$ $h(+)$ recombinants

If common: r and h distant
If rare: r and h close

Intragenic Mapping in Phage

Extremely fine structure mapping
Not mapping distances between genes
Map positions of mutations within 1 gene
Use alleles with different mutation sites
Extremely rare events - distances are short
Powerful selection strategies required

Benzer (1950's): rII locus of T4 phage

Classic work on intragenic recombination

rII = Complex locus with 2 cistrons (A, B)
Each cistron coded for different polypeptide
Term not used much in modern genetics
Some genes defined by mutation studies contain > 1 coding region (cistron)

Benzer experiments involved 2 concepts:

*Intragenic mapping (recombination)
Genetic complementation (allelism test)*

Benzer Mapping Studies:

Formation of wild-type intragenic recombinants

Benzer used 2 different bacterial strains:

*B: rII and rII(+) distinguished by plaque morphology
Mutant phage form small plaques
Used to maintain mutant phage*

K: Mutant phage cannot form plaques (reproduce)

*Isolated many independent rII mutant alleles
Many different mutation sites involved
Co-infect strain K with 2 rII mutants*

Count number of plaques resulting from rare intragenic recombinants

*If 2 mutation sites are VERY close – small number
If 2 mutation sites are more distant – larger number*

Also used deletion mapping strategy for rough location

Resolution of mapping method: A few base pairs

Rare recombinants were found using rII mutants with mutation sites separated by a few base pairs

Benzer Complementation Tests

Demonstrated whether 2 rII mutants were defective in same cistron or different cistrons

*Did NOT involve recombination or selection
Utilized recombination-defective bacterial strains*

*If different cistron: Plaques formed
2 mutants complement*

*If same cistron: No plaques formed
2 mutants fail to complement*

Complementation Tests in Eukaryotes:

*Used to determine whether 2 recessive mutants are allelic
Particularly useful when mutants have similar phenotypes
Also used when mutants map to same chromosome region*

*Cross 2 different homozygous recessives
Analyze phenotype of F1 progeny*

If all mutant - 2 mutants fail to complement - allelic - defective in same gene - homozygous at mutant locus

If all normal - 2 mutants complement - not allelic - defective in different genes - heterozygous at each locus