Genetic nomenclature

Phenotype: Lac⁻; what's observed

Genotype: lacZ⁰ or lacY⁻
refers to specific gene defect

Particular mutations = “alleles”
e.g., lacZ343

Early knowledge of lactose metabolism:
1. Lactose cleaved by cells (= beta-galactosidase)
2. Lactose taken into cells (= lactose permease)
3. Induction (lactose addition increases lactose cleavage and uptake rates)

Questions answered genetically:

1. How many genes are required for lactose utilization?

A. Isolate hundreds of Lac⁻ mutants (Lac⁻¹, Lac⁻², ...)

B. Determine how many complementation groups are defined by the Lac⁻ mutations.

B. Determine how many complementation groups (= genes) are defined by the Lac⁻ mutations.

General form of complementation test: Does lac⁻₁ / lac⁻₂ strain grow on lactose or not?

Lac⁻ mutant isolation:

\[ \text{F}^-\text{ lac}^+ \text{ cells} \]
\[ \downarrow \]
Mutagenize
\[ \downarrow \]
Plate cells for colonies and screen for mutants
\[ \downarrow \]
lac⁻¹; lac⁻₂; etc
Complementation analysis of lac\textsuperscript{–} mutations

1. Transfer F’lac\textsuperscript{+} into Lac\textsuperscript{–} mutant (e.g., lac\textsuperscript{–}1)
2. Allow recombination to take place to transfer mutation (lac\textsuperscript{–}1) into F’lac
3. Conjugate F’lac carrying mutation (lac\textsuperscript{–}1) into second lac\textsuperscript{–} strain (e.g., lac\textsuperscript{–}2)
4. Examine whether F’ lac\textsuperscript{–}1 / lac\textsuperscript{–}2 cells grow on lactose

<table>
<thead>
<tr>
<th></th>
<th>F’lac\textsuperscript{–}1</th>
<th>F’lac\textsuperscript{–}2</th>
<th>F’lac\textsuperscript{–}3</th>
<th>F’lac\textsuperscript{–}4</th>
</tr>
</thead>
<tbody>
<tr>
<td>lac\textsuperscript{–}1</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lac\textsuperscript{–}2</td>
<td>–</td>
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<td>+</td>
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<td>lac\textsuperscript{–}3</td>
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<tr>
<td>lac\textsuperscript{–}4</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>lac\textsuperscript{–}5</td>
<td>–</td>
<td>–</td>
<td>+</td>
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Complementation group I = lac\textsuperscript{–} 1, 2, 5
Complementation group II = lac\textsuperscript{–} 3, 4

Coding assignments:

One set of mutants were beta-galactosidase\textsuperscript{–}, transport\textsuperscript{+} (= lacZ\textsuperscript{–} lacY\textsuperscript{+})

Second set of mutants were beta-galactosidase\textsuperscript{+} transport\textsuperscript{–} (= lacZ\textsuperscript{+} lacY\textsuperscript{–})
2. Does induction involve a positively- or negatively-acting factor?

A. Alternative models: Negative vs positive

Positive regulation predicts that the most common class of constitutive mutation would permanently activate activator.

Negative regulation predicts that the most common class of constitutive mutation would eliminate repressor.

Models distinguished using constitutive mutants

Steps:
1. Isolate constitutive mutants
2. Determine whether such mutations are dominant or recessive to wild-type

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cleaved by β-galactosidase</th>
<th>Induces λc operon transcription</th>
</tr>
</thead>
<tbody>
<tr>
<td>lactose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenyli-β-D-galactoside (PG)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Isopropyl-β-D-thiogalactoside (IPTG)</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Hfr cell → Conjugation → F- cell

Transient partial diploid cell ("merozygote")
Predictions for positive vs negative regulation (no inducer added)

Why are most common constitutive mutants lacI– rather than lacO–?

How could one selectively isolate lacO– mutants?