Construction of *lacI*ts and *lacI*qts expression plasmids and evaluation of the thermosensitive *lac* repressor

Noaman Hasan Waclaw Szybalski (1995),
Gene 163, p.35 - 40
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Outline

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The *lac*-operon and the *lacI*-repressor

**The *lac*-operon**

http://www.guidobauersachs.de/bc/lac2.jpg
The *lac*-operon and the *lacI*-repressor

The *lacI*-repressor

- Tetrameric DNA-binding protein
- Three distinct regions
- Induction by IPTG and lactose
- *lacI*\(^q\) and *lacI*\(^ts\)

[Image: http://proteopedia.org/wiki/images/b/bb/Lac_repressor_snapshot.png]
Objectives of the experiments

• Characterize the lacIts gene

• Characterize the thermosensitive lacI product

• Construct two sets of plasmids derived from pACYC184 and pKO3 that overexpress the lacIts gene

• Show that gene expression from the lacZop vectors is inducible by heat rather than by IPTG
Experiments: Mapping and sequencing the \textit{lacI}ts mutation

- Preliminary analysis of the \textit{lacI}ts fragment
  \( \rightarrow \) HaeIII site (GGCC) is missing

- Sequence analysis of the affected fragment (BstEII-HincII) and Substitution of the terminal fragments (EcoRI-BstEII and HincII-EcoRI) by wt \textit{LacI} DNA

  \( \rightarrow \) \textit{wt lacI}+: 5`- CGGGCCCC
  \textit{lacI}ts : 5`- CGAGCCCC
  \( \rightarrow \) G to A substitution results in Gly187 to Ser substitution and loss of HaeIII site
Construction of plasmids containing either \textit{lacI}ts or \textit{lacI}qts

**Plasmid pKO3**

- High copy number (hcn) plasmid
- Contains \textit{lacI}ts oder \textit{lacI}q gene
- Expression of the repressor gene controlled by \textit{lacI} (wt) or \textit{lacI}q promoter

http://www.genetik.uni-bayreuth.de/download/page_34/teach_85/Publikationsdateiern/Themen_Schumann/05_HasanGene163-35.pdf
Construction of plasmids containing either \textit{lacI}ts or \textit{lacI}qts

Plasmid pACYC184

- medium copy number (mcn) plasmid

- pNH39/lacI\textsubscript{ts} and pNH41/lacI\textsubscript{q}ts are transcribed by \textit{lacIp}/\textit{lacIq}p and a \textit{Cm}R-promotor (higher expression)

- pNH38/lacI\textsubscript{ts} and pNH40/lacI\textsubscript{q}ts are transcribed by \textit{lacIp}/\textit{lacIq}p and the \textit{Cm}R-promotor in the antisense direction

http://www.genetik.uni-bayreuth.de/download/page_34/teach_85/Publikationsdateien/Theme_n_Schumann/05_HasanGene163-35.pdf
Heat inactivation of lacIts

• pACYC184 derived plasmids were tested in strain LBG1081 (deficient in lacI)

• Cells were plated on LB agar + XGal plates (with Tc and IPTG) at 30°C and 42°C

XGal

Lactose

http://www.biology.lsu.edu/introbio/tutorial/multicellular/X-gal.jpg

http://www.uni-duesseldorf.de/MathNat/Biologie/Didaktik/zucker/bilder/lactose_gr.jpg
## Heat inactivation of lacIts

### Results:

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>IPTG+XGal 30°C</th>
<th>IPTG+XGal 42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>blue</td>
<td>blue</td>
</tr>
<tr>
<td>pNH38/lacIts</td>
<td>white</td>
<td>blue</td>
</tr>
<tr>
<td>pNH39/lacIts</td>
<td>white</td>
<td>white</td>
</tr>
<tr>
<td>pNH40/lacIqts</td>
<td>white</td>
<td>blue</td>
</tr>
<tr>
<td>pNH41/lacIqts</td>
<td>white</td>
<td>white</td>
</tr>
</tbody>
</table>

→ Gene dosage plays important role in *lacZop* repression/depression in cells with mcn plasmids
Heat inactivation of \textit{lacI}ts

- Chromosomal expression of \textit{lacZ} in the presence of pNH258 (carrys \textit{lacZop}) was measured at 30°C and 42°C
- Used plasmids: pNH38\textit{lacI}ts, pNH39\textit{lacI}ts, pNH40\textit{lacI}qts, pNH41\textit{lacI}qts

→ Full thermal induction of chromosomal \textit{lacZ} gene
Heat inactivation of lacI{sub}ts

- GalK expression in strain C600 including pNH455-1 (plasmid that contains galK gene) was measured.
- Used plasmids: pNH38/lacI{sub}ts, pNH39/lacI{sub}ts, pNH40/lacI{sup}q{sub}ts, pNH41/lacI{sup}q{sub}ts
- Results:

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>GalK (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30°C</td>
</tr>
<tr>
<td>pNH38/lacI{sub}ts</td>
<td>24</td>
</tr>
<tr>
<td>pNH39/lacI{sub}ts</td>
<td>8</td>
</tr>
<tr>
<td>pNH40/lacI{sup}q{sub}ts</td>
<td>2</td>
</tr>
<tr>
<td>pNH41/lacI{sup}q{sub}ts</td>
<td>0.5</td>
</tr>
</tbody>
</table>

→ Complete induction of galK gene at 42°C
Heat inactivation of lacI ts

- Chromosomal expression of the lac Z gene was measured at 30°C and 42 °C

- Used plasmids: pMCts (contains lacZ operon), pNH34/lacI ts (contains lacZ operon), pNH35/lacI ts, pNH36/lacI qts, pNH37/lacI qts
Heat inactivation of *lacI*ts

**Results:**

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>βGal induction (%) at 30°C</th>
<th>βGal induction (%) at 42°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMCts</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>pNH34lacI*ts</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>pNH35lacI*ts</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pNH36lacIqts</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>pNH37lacIqts</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

→ *lacI*ts gene dosage is important in *lacZop* repression/depression
IPTG and lactose induction of βGal

• Expression of lacZ was measured at 30°C/42°C in the presence or absence of IPTG

• pNH38/lacI\text{ts}, pNH39/lacI\text{ts}, pNH40/lacI^\text{qts}, pNH41/lacI^\text{qts}, pACYC184, pMCts, pNH34/lacI\text{ts}, pNH35/lacI\text{ts}, pNH36/lacI^\text{qts}, pNH37/lacI^\text{qts}, pKO3
IPTG and lactose induction of βGal

• Results for plasmids with ts repressor:

→ no expression of βGal at 30°C in absence or presence of IPTG
→ expression of βGal at 42°C in absence or presence of IPTG

• Results for plasmids with wildtype repressor:

→ expression of βGal whenever IPTG is present
IPTG and lactose induction of βGal

• **Results for dihybrid plasmids** (*lacI*ts and *wt lacI*):

  → induction by IPTG at 30°C and 42°C

  → *ts* phenotype is recessive

  → hybrid tetrameric repressor can be inactivated by IPTG
IPTG and lactose induction of βGal

• \textit{lacZ} expression was measured in presence of lactose by growing cells on a MacConkey plate and a Tetrazolium plate

• Used strains: LBG1081 (does not contain \textit{lac\textbackslash ts} plasmids), D1210 (contains \textit{lac\textbackslash q})

• Used plasmids: pNH38\textit{lac\textbackslash ts}, pNH39\textit{lac\textbackslash ts}, pNH40\textit{lac\textbackslash q\textbackslash ts}, pNH41\textit{lac\textbackslash q\textbackslash ts}
IPTG and lactose induction of βGal

**Results:**

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>MacConkey + lactose 30°C</th>
<th>Tetrazolium + lactose 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>D1210</td>
<td>D1210</td>
</tr>
<tr>
<td>red</td>
<td>LBG1081</td>
<td>LBG1081</td>
</tr>
<tr>
<td>red</td>
<td>white</td>
<td>white</td>
</tr>
<tr>
<td>white</td>
<td>red</td>
<td>red</td>
</tr>
<tr>
<td>white</td>
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<tr>
<td>white</td>
<td>red</td>
<td>red</td>
</tr>
<tr>
<td>white</td>
<td>red</td>
<td>red</td>
</tr>
</tbody>
</table>

→ no induction by lactose (hybrid tetramer is not being inactivated)
Conclusions

• Two sets of plasmids carrying the lacI\textsubscript{ts} gene were constructed

• Level of lacI\textsubscript{ts} produced can sometimes completely repress lacZ\textsubscript{op} present on hcn compatible plasmids

• Derepression of the lacZ\textsubscript{op} is only achieved by a temperature of 42°C if ts mutant is present

• Mutant repressor is insensitive to IPTG or lactose

• ts plasmids eliminate the need for chemical inducers and are not sensitive to contaminants
Take home message

*lac*Its repressor establishes an easy possibility to control the gene expression of the *lacZ*-operon without chemical inducers
Discussion

• On which concept is the ts mutation based on?

• How many subunits does the active form of the lacI repressor contain?