The Feedback Regulation of Lac Repressor Expression in *Escherichia coli*

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Abstract

Negative feedback regulation, mediated through repressor binding site $O3$, which overlaps the $lacI$ gene, could explain robustness of the weak expression of Lac repressor. Significant autorepression of Lac repressor has never been ruled out. In the presented work, the degree of autoregulation of Lac repressor was determined. It is negligible.
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The lac operon is one of the classical model systems for transcriptional regulation (9). Expression of the tricistronic lac mRNA is negatively controlled (repressed) by the operon-specific Lac repressor (LacR) and positively controlled (activated) by the global regulator CAP (17). In recent years, the lac operon has become a focus and testing ground for Systems Biology analyses (19). Such modeling can only deliver meaningful results when it incorporates all relevant features of the system. The textbook picture of the lac operon has sustained substantial change over the years (9), and despite the wealth of information, many questions are still unanswered.

One of the traditional views that have become questionable holds that the lacI gene, encoding LacR, is constitutively expressed at low levels (in wild-type (wt) cells, LacR is present at about 10 tetramers per genome (4)). It was established in the 1960s that Lac repressor expression is not coordinately controlled with the three genes of the lac operon (5). This did however not exclude autoregulation of LacR altogether. Indirect methods suggested that LacR autoregulation cannot be large. Gilbert and Müller-Hill did not find an obvious difference between the yields of LacR purified from uninduced and induced cultures. Their recoveries were, however, not reproducible enough to detect a small severalfold difference (4). An in vivo experiment with an E. coli strain temperature-sensitive for the production of LacR actually indicated a 2-fold inducibility of LacR expression, but the mutation used was not well characterized enough to allow conclusive interpretation of the data (11). Expression of the lacI gene is low but robust: there appears to be little stochastic fluctuation of LacR (12). Feedback regulation is a mechanism that would suppress such fluctuations (7). It was found that the third lac operator, O3, which lies upstream of the lac promoter and overlaps with the last nucleotides of the lacI gene, is not a pseudooperator (15, 16). It contributes through DNA loop formation to repression at the first lac operator, O1 (Fig. 1). It was later reported that transcription of lacI stalls at an occupied O3 and that the incompletely translated protein is tagged for degradation through the tmRNA pathway (1). LacR expression seemed thus subject to negative feedback regulation, the extent of which could, however, not be determined from these data. This circumstantial evidence was so convincing that it has been
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1 stated as a fact that LacR is autorepressed (1).

2

3 Even an induction of 2 or 3-fold, small compared to that of the lac promoter, would invalidate
4 conclusions drawn from induction studies and quantitative modeling ignorant of it. To obtain data
5 sufficiently accurate to detect an effect in this range, a direct assay of LacR levels in cell extracts
6 seemed most appropriate. The traditional method for LacR determination is equilibrium dialysis with
7 radioactive inducer (2, 4). Because inducer binding is relatively weak - the equilibrium dissociation
8 constant (K_d) is ~7 µM for isopropyl-β-D-thiogalactopyranoside (IPTG) (13) - and repressor
9 concentration in wt cells is low (~10 nM), it can only be used for strains overexpressing LacR or for
10 preparations enriched in repressor (4, 10). On the other hand, the affinity of repressor to operator is
11 high (K_d of ~ 10 pM in vitro for symmetric “ideal” lac operator (13, 18, 20), suggesting stoichiometric
12 titration of lac operator as an alternative approach.

13

14 Autorepression was measured by comparing LacR levels of bacteria growing under repressed (LacR
15 binds to its operators) versus induced (the operators are free of LacR) conditions, analogous to
16 repression measurements of β-galactosidase. Three derivatives of lac deletion strain BMH8117 (3),
17 carrying different episomes, were used. A) the episome from CSH23 (8), containing the wt lac operon
18 B) an episome containing a lac operon with an inactivated O2 (3), C) the episome from CSH37 (8),
19 containing a lac operon with an O' (severely impaired O1, leading to constitutive lac expression).
20 While A reflects the wt situation, B will exhibit stronger loop formation between O1 and O3, since O2
21 no longer competes with O3 for interaction with O1 (see Fig. 1), and C has reduced occupancy of O3
22 through reduced loop formation.

23 Cultures of the three strains were grown at 37 ° C in minimal medium A (8) with 0.4% glycerol, from
24 which two subcultures were inoculated, one without and one with 0.2 mM IPTG. Cells were harvested
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at an OD\textsubscript{600} of 0.8 to 1.0. Aliquots were used for determining specific β-galactosidase activities (8), the rest for the preparation of crude extracts (pellets from 25 ml culture were resuspended in 400 μl BB+ (10 mM Tris-HCl, pH 7.2; 3 mM Mg-acetate; 0.1 mM EDTA; 3% DMSO; 0.1 mM DTT), sonicated, insoluble material removed by centrifugation, and the supernatants dialysed 4 times for 2 h against a 100-fold excess of BB+; all steps at 4 °C or on ice; protein concentrations were determined with the method of Warburg and Christian after Layne (6)).

Because of the low abundance of LacR, the total protein concentration in the binding reactions had to be higher than usual (14). Therefore, the linear range of operator binding by LacR as a function of protein concentration was determined first. An extract of BMH8117 carrying the wt lac episome and a radiolabelled 257 bp DNA fragment containing the “ideal” lac operator (13) were used. All binding reactions were done on ice for 15 min in volumes of 10 or 20 μl BB+ with 1 nM of the DNA fragment. The products of the binding reaction were assayed with band shift assays as described (13). Figure 2A shows the quantitative evaluation. Percent bound operator is plotted against protein concentration. Binding is initially linear and starts to be inhibited above 1 mg/ml protein. Figure 2B shows the linear portion of the binding curve with the corresponding linear regression line. The correlation coefficient is 0.996. LacR quantitations were performed in this range (0.33 to 0.55 mg/ml).

Table 1 gives the repression (the quotient of specific activity in the presence of IPTG (induced) and specific activity in the absence of IPTG (repressed)) values of β-galactosidase as well as the analogous repression values of LacR.

Repression of the episomal wt lac operon is close to 2000-fold. Inactivation of O2 leads to the expected 3 to 5 fold drop of repression (3, 16), and the O\textsuperscript{c} mutation nearly abolishes repression. LacR expression itself is, however, not detectably repressed in any of the three strains. While the tmRNA pathway prevents that truncated LacR accumulates in the cell (1), its effect on LacR expression is apparently small. Autorepression of LacR expression is at most about 10%. It appears thus that the classical
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picture prevails. There seems to be no biologically meaningful autoregulation of LacR. If there is a mechanism that ensures robust expression of LacR, it has to be sought elsewhere.

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Figure legends

FIG. 1.

The organization of the lac operators. Not drawn to scale. The first lac operator, O1, lies immediately downstream of the lac promoter, lacP, and is the site of lac operon repression through LacR. Tetrameric LacR bound to O1 also binds to either O2, in the coding region of lacZ, or O3, upstream of the lac promoter, leading to the formation of DNA loops (indicated by dotted arrows). The open reading frames (ORFs) of the lacI and lacZ genes are indicated with the thin lines below. The end of the lacI ORF is marked with an arrowhead and the beginning of the lacZ ORF is marked with a short vertical line. Part of O3 overlaps with the last bases of lacI, which codes for LacR.

FIG. 2.

A. Operator binding by Lac repressor as a function of the concentration of total soluble protein. A crude extract of strain StAAa1 (lac deletion strain BMH817, carrying an episome containing the wt lac operon) is used.

B. The initial linear portion of the binding curve with the corresponding linear regression line.
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Table 1

Repression values of β-galactosidase and Lac repressor

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pertinent genotype</th>
<th>Repression of β-galactosidase</th>
<th>Repression of Lac repressor</th>
</tr>
</thead>
<tbody>
<tr>
<td>StAAa1</td>
<td>Δlac, F’ lac⁺</td>
<td>1940 (± 60)</td>
<td>1.06 (± 0.09)</td>
</tr>
<tr>
<td>StAAa2</td>
<td>Δlac, F’ lacO²</td>
<td>450 (± 8)</td>
<td>1.17 (± 0.10)</td>
</tr>
<tr>
<td>StAAa3</td>
<td>Δlac, F’ lacO²</td>
<td>2.7 (± 0.1)</td>
<td>0.88 (± 0.14)</td>
</tr>
</tbody>
</table>

a All repression values are the means of 6 determinations (± standard error).
b Repression is defined as specific activity of cells grown in the presence of 0.2 mM IPTG divided by specific activity of cells grown in the absence of IPTG.
c Repression is defined as fmole LacR/µg soluble protein of cells grown in the presence of 0.2 mM IPTG divided by fmole LacR/µg soluble protein of cells grown in the absence of IPTG. A repression value of 1 indicates no repression.