Notes

Feedback Regulation of Lac Repressor Expression in Escherichia coli

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Negative feedback regulation, mediated through repressor binding site O3, which overlaps the lacI gene, could explain the robustness of the weak expression of Lac repressor. Significant autorepression of Lac repressor has never been ruled out. In the work presented here, the degree of autoregulation of Lac repressor was determined. It is negligible.

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 repressor (LacR) and positively controlled (activated) by the promoter and operator and negatively controlled (repressed) by the operon-specific transcriptional regulation (9). Expression of the tricistronic lac mRNA is negatively controlled (repressed) by the lacI gene. lacI gene is low but negatively controlled (repressed) by the lacI gene. lacI gene is not a 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, however, did 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, however, were not reproducible enough to detect a small severalfold difference (4). An in vivo experiment with an Escherichia coli strain that is temperature-sensitive for the production of LacR actually indicated a twofold inducibility of LacR expression, but the mutation used was not well enough characterized to allow conclusive interpretation of the data (11). The 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 thus seemed subject to negative feedback regulation, the extent of which could not, however, be determined from these data. This circumstantial evidence was so convincing that it has been stated as a fact that LacR is autorepressed (1).

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

Autorepression was measured by comparing LacR levels of bacteria growing under repressed (LacR binds to its operators) versus induced (the operators are free of LacR) conditions, analogous to repression measurements of β-galactosidase. Three derivatives of lac deletion strain BMH8117 (3), carrying different epimers, were used: (i) the episome from CSH23 (8), containing the wt lac operon; (ii) an episome containing a lac operon with an inactivated O2 (3); and (iii) the episome from CSH47 (8), containing a lac operon with an Oc (severely impaired O1, leading to constitutive lac expression). While the first episome reflects the wt situation, the second will exhibit stronger loop formation between O1 and O3, since O2 no longer competes with O3 for interaction with O1 (Fig. 1), and the third has reduced occupancy of O3 through reduced loop formation.

Cultures of the three strains were grown at 37°C in minimal medium A (8) with 0.4% glycerol, from which two subcultures were inoculated, one without and one with 0.2 mM IPTG. Cells...
were harvested at an optical density at 600 nm of 0.8 to 1.0. Aliquots were used for determining specific β-galactosidase activities (8), and the rest for the preparation of crude extracts, as follows. Pellets from 25 ml of culture were resuspended in 400 μl BB+ (10 mM Tris-HCl, pH 7.2, 3 mM Mg-acetate, 0.1 mM EDTA, 3% dimethyl sulfoxide, 0.1 mM dithiothreitol) and sonicated, insoluble material removed by centrifugation, and the supernatants dialyzed four times for 2 h against a 100-fold excess of BB+; all steps were performed at 4°C or on ice. Protein concentrations were determined with the method of Warburg and Christian (21) after Layne (6).

Because of the low abundance of LacR, the total protein concentration in the binding reaction mixtures 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 radiolabeled 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 previously (13). Figure 2A shows the quantitative evaluation. Percent bound operator is plotted against protein concentration. Binding is initially linear and starts to be inhibited at protein concentrations above 1 mg/ml. 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 values (the quotient of specific activity in the presence of IPTG [induced] and specific activity in the absence of IPTG [repressed]) of β-galactosidase, as well as the analogous repression values of LacR.

Repression of the episomal wt lac operon is close to 2,000-fold. Inactivation of O2 leads to the expected three- to fivefold drop in repression (3, 16), and the O′ mutation nearly abolishes repression. LacR expression itself is, however, not detectably repressed in any of the three strains. While the tmRNA pathway prevents the accumulation of truncated LacR in the cell (1), its effect on LacR expression is apparently small. Autorepression of LacR expression is at most about 10%. It thus appears that the classical 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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REFERENCES


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′ lacac</td>
<td>1,940 (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′ lacO</td>
<td>2.7 (0.1)</td>
<td>0.88 (0.14)</td>
</tr>
</tbody>
</table>

* All repression values are the means ± standard errors of six determinations.

† 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.

‡ Repression is defined as fmoles LacR/g soluble protein of cells grown in the presence of 0.2 mM IPTG divided by fmoles LacR/g soluble protein of cells grown in the absence of IPTG. A repression value of 1 indicates no repression.

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 by 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 BMH8117 carrying an episome containing the wt lac operon) was used. (B) The initial, linear portion of the binding curve with the corresponding linear regression line.