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 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, 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 lacI expression, developed by Alexander Fleming, 16672 Vari, Athens, Greece. Phone: 30-210-9656310-211. Fax: 30-210-9656-563. E-mail: oehler@fleming.gr.

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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 “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 O2 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.

I thank Benno Müller-Hill for providing me with the lacO2 episome and Alexandros Kioupakis for critically reading the manuscript.

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′ lacO1</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′ lacO3</td>
<td>2.7 (0.1)</td>
<td>0.88 (0.14)</td>
</tr>
</tbody>
</table>

a All repression values are the means (± standard errors) of six determinations.

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/g soluble protein of cells grown in the presence of 0.2 mM IPTG divided by fmole LacR/g soluble protein of cells grown in the absence of IPTG. A reparation value of 1 indicates no repression.