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Single-cell Analysis of \( \lambda \) Immunity Regulation

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We have examined expression of the \( \lambda cl \) operon in single cells via a \( \text{rex}^{\pm} \) \( \text{gfp} \) substitution. Although average fluorescence agreed with expectations for expression of \( \lambda \)-repressor, fluorescence fluctuated greatly from cell-to-cell. Fluctuations in repressor concentration are not predicted by previous models and are tolerated in part by a regulatory response to DNA damage.

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**Keywords:** bacteriophage lambda; repressor; GFP; single-cell analyses

**Introduction**

When phage \( \lambda \) infects a sensitive *Escherichia coli*, it responds to a variety of intracellular signals and enters one of two developmental pathways. If it enters the lytic pathway, after about an hour the bacterium lyses releasing on the order of 100 progeny phage. If it enters the lysogenic pathway, most phage genes become repressed and the phage genome is integrated into the bacterial chromosome where it is passively replicated. The decision-making process by \( \lambda \) was the first epigenetic switch to be deciphered and has been the subject of extensive study.\(^1\)

The lysogenic state is maintained by the continued synthesis of a prophage-encoded repressor, the product of the \( \lambda cl \) gene. Repressor binds to two operators, \( cl \) and \( oR \) and thereby turns off transcription from the lytic promoters \( pL \) and \( pR \), respectively. Binding of repressor to \( oR \) also stimulates the promoter directing repressor synthesis in a lysogen, \( pRM \).\(^1\) Departure from the lysogenic state and entry into lytic development generally occurs only through the SOS response, the *E. coli* system for responding to DNA damage. Also according to the model, the majority of regulatory events maintaining proper repressor concentration are mediated by repressor acting at \( oR \), which is a constellation of three operators, \( oR1, oR2 \) and \( oR3 \) (Figure 1). These operators overlap two divergent promoters, the lytic promoter, \( pR \), and the promoter directing synthesis of repressor in the lysogen, \( pRM \). The \( pRM--oR--pR \) region is compact. The distance separating the start sites of the two transcripts is 82 base-pairs. Binding of repressor to \( oR \) is asymmetric; that is, repressor binds preferentially to \( oR1 \), which overlaps \( pR \), and only at higher concentrations to \( oR3 \), which overlaps \( pRM \). Binding of repressor to \( oR1 \) blocks expression of \( pR \) and cooperative binding to \( oR2 \)

Abbreviations used: GFP, gene fusion protein; UV, ultraviolet light; IPTG, isopropyl thiogalactoside; m.o.i., multiplicity of infection; PFU and CFU, plaque and colony-forming units, respectively.

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stimulates expression of cl from pRM. Thus, the asymmetry of oR readily explains the synthesis of repressor and the repression of lytic functions in a lysogen.

Since the early measurements of cl expression were made on gene fusions separated from the other elements of the immunity region such as oL, features intrinsic to the context of the immunity region were not investigated. Recent reports suggest more features regulate the immunity of phage \( \lambda \) than previously proposed. First is the participation of oL in repression at oR,\(^{10} \) consistent with the crystal structure of repressor.\(^{11} \) oL overlaps pL, the lytic promoter for the left operon of \( \lambda \) (Figure 1). A second and more surprising observation is the lack of a requirement for asymmetry at oR.\(^{12} \) Models relying on asymmetry are difficult to reconcile with the observations by Little and co-workers\(^{12} \) that phage with a symmetric oR form stable lysogens that can induce and grow lytically. The ability to form stable lysogens for mutants with symmetric operators suggests three possible scenarios for control of pRM. First, although all or nearly all transcription of cl in a lysogen is initiated at pRM,\(^{13} \) there may be a second promoter responsible for a small amount of cl in a lysogen. Second, pRM may be substantially repressed in a wild-type lysogen and thus regulation of pRM tolerates or perhaps relies on repression. The second model is especially attractive in light of the observation that pRM is at least 60% repressed in a lysogen.\(^{14} \) A third model would rely on a higher-order complex including oL to generate the asymmetry at oR, reducing the requirement for asymmetry at oR itself.

**Results and Discussion**

**Role of pRE in stability of lysogens**

We tested the contribution of another promoter to synthesis of repressor in a lysogen. The most likely candidate for a second promoter expressing cl is pRE, the promoter for repressor establishment, and pRM, the promoter for repressor maintenance. The three operators of oL and oR are shown as are the lytic promoters pL and pR. rexA and rexB are co-transcribed with cl. B. The substitution replacing parts of rexA and rexB with gfp. Features in this Figure are not drawn to scale.

Figure 1. Immunity region of phage \( \lambda \). Promoters for cl transcription are pRE, the promoter for repressor establishment, and pRM, the promoter for repressor maintenance. The three operators of oL and oR are shown as are the lytic promoters pL and pR. rexA and rexB are co-transcribed with cl. B. The substitution replacing parts of rexA and rexB with gfp. Features in this Figure are not drawn to scale.
phage examined, all complemented at least 100-fold less efficiently than the \( \lambda^+ \) parent. Surprisingly, all 20 of the turbid plaque-forming phage examined, although able to form turbid plaques at both 37 °C and 42 °C, were inefficient in complementing c\( \text{I} \). That is, the released phage which form turbid plaques are not wild-type but are mutated in their ability to produce repressor in lysogens. Our results are consistent with findings of R. A. Roberts & J. W. Little (University of Arizona) that, among the turbid-plaque-forming phage released from a recA lysogen, the vast majority are not wild-type but carry a down-promoter mutation in pRM which destabilizes the lysogenic state (personal communication). Since the released phage able to form turbid plaques are not wild-type, their release is not due to failure of the lysogenic regulatory circuit. Therefore, the lysogenic regulatory circuit must be much more stable than previously believed.

Table 1. Measurement of phage release and ability to complement a temperature-sensitive repressor

A. Frequency of released phage from \( \lambda \text{RE} \) lysogens

<table>
<thead>
<tr>
<th>Strain</th>
<th>Prophage</th>
<th>Turbid</th>
<th>Clear</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB12</td>
<td>( \lambda^+ )</td>
<td>11</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.9</td>
<td>6.9</td>
<td>10.8</td>
</tr>
<tr>
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<tr>
<td></td>
<td></td>
<td>3.1</td>
<td>8.1</td>
<td>11.2</td>
</tr>
<tr>
<td>KB13</td>
<td>( \lambda c_{\text{y42}} )</td>
<td>25</td>
<td>25</td>
<td>50</td>
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<tr>
<td></td>
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<td>16</td>
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<tr>
<td></td>
<td></td>
<td>6.5</td>
<td>6.5</td>
<td>13.0</td>
</tr>
<tr>
<td>KB14</td>
<td>( \lambda c_{\text{y2001}} )</td>
<td>24</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>22</td>
<td>44</td>
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<tr>
<td></td>
<td></td>
<td>17</td>
<td>17</td>
<td>34</td>
</tr>
<tr>
<td>KB15</td>
<td>( \lambda c_{\text{iII68}} )</td>
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<td>6.6</td>
<td>13.2</td>
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<tr>
<td>KB16</td>
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<td></td>
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<td>15</td>
<td>15</td>
<td>30</td>
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B. Ability of released phage to complement a c\( \text{I857} \) lysogen for growth at 42 °C

<table>
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<tr>
<th>Lysate</th>
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<th>Max.</th>
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<tr>
<td>( \lambda^+ ) phage</td>
<td></td>
<td></td>
<td>1445</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>cul. A</td>
<td></td>
<td>21</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cul. B</td>
<td></td>
<td>23</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2100</td>
<td>1363</td>
<td></td>
</tr>
<tr>
<td>Turbids released from KB12</td>
<td>10</td>
<td>cul. A</td>
<td>6.0</td>
<td>0.64</td>
<td>12.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cul. B</td>
<td>6.0</td>
<td>0.42</td>
<td>17.2</td>
</tr>
<tr>
<td>Clear released from KB12</td>
<td>5</td>
<td>cul. A</td>
<td>7.5</td>
<td>0.7</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cul. B</td>
<td>5.0</td>
<td>1.5</td>
<td>11</td>
</tr>
</tbody>
</table>

Measurement of phage release and ability to complement a temperature-sensitive repressor are described in Materials and Methods.

Gene fusion to measure transcription of \( \text{cI} \) in situ

To examine \( \text{cI} \) transcription in single cells, we prepared a gene fusion to \( \text{gfp} \). Since the presence of \( \text{lO} \) increases repression at \( \text{oK} \), we wanted to retain the surrounding prophage DNA in case other structures participated in regulation of \( \text{cI} \) transcription. To this end, we prepared a gene fusion that maintained the wild-type spacing and surrounding DNA at the immunity region. To retain these features we substituted most of \( \text{rexA} \) and \( \text{rexB} \), two genes of the \( \text{cI} \) operon, with the mut2 allele of \( \text{gfp} \) rendering \( \text{gfp} \) cotranscribed with \( \text{cI} \) (Figure 1B; Materials and Methods). In the \( \text{rex} \text{::} \text{gfp} \) phage, \( \text{lo} \) and \( \text{oR} \) are separated by 2.2 kb compared with a wild-type spacing of 2.3 kb.

\( \text{GFPmut2} \) is stable and matures rapidly. Once protein synthesis was blocked (Materials and Methods), we observed no change in
fluorescence during the course of our measurements. Therefore, fluorescence represents GFP concentrations in the cells upon withdrawal from the growing culture.

### Regulation of rex::gfp

In order to test whether gfp expression in individual cells reflected repressor concentrations, we first verified that gfp showed the regulation expected for cl. We supplied excess repressor from a multicopy plasmid expressing repressor from the lac promoter, pcl+. Both pcl+ and the control plasmid bearing the defective cl60 allele, pcl60, are derivatives of pUC19 and maintained with at least 50 copies per cell. Transformants were grown to mid-log phase and the fluorescence of individual cells compared (Table 2).

We observed decreased fluorescence in the presence of multicopy cl. We interpret the reduced fluorescence of KB126pcl+ when compared with the cl− control, KB126pcl60, to reflect repression of pRM. We cannot assess the degree of repression, since the residual fluorescence produced by the cl− control approaches our threshold of detection, the auto-fluorescence observed with the KB124 which lacks gfp. Repression of pRM by multicopy expression of repressor was reported earlier. Therefore the rex::gfp fusion showed proper regulation.

We also examined the response of a lysogen lacking ol, SS122 (Table 2). Its fluorescence was also strongly repressed by pcl+, indicating proper regulation of pRM. The unrepRESSED fluorescence observed with SS122pcl60 is substantially higher than that of the ol− strain, KB126pcl60. Increased expression from pRM in the absence of ol has been described (Figure 2).

Intriguing results were obtained with pSS5. Like pcl+, pSS5 has the lac promoter placed upstream from cl. However, pSS5, unlike pcl+, retains or. In the majority of our experiments a fraction of the cells harboring pSS5 fluoresced as strongly as the pUC18 control (Table 2). We suggest the strongly fluorescing cells result from an interaction between or retained on pSS5 and the operators of the prophage. In support of this conclusion we note that when KB126 or SS122 was made lacI+ by transcription, the fluorescence of the weakly fluorescing cells decreased with added IPTG but the fluorescence of the strongly fluorescing cells was insensitive to IPTG (data not shown). Thus, the bimodal distribution observed with pSS5 is not due to repressor becoming insoluble in a fraction of the cells due to a high concentration. We suggest or of pSS5 formed an aberrant complex with repressor and or of the prophage and interfered with the ability of excess repressor to inhibit pRM. Lastly, we observed a bimodal distribution of gfp expression in the absence of recombination (the recA strain, SS74pSS5; data not shown) indicating recombination is not necessary to form the de-repressed population.

### Distribution of fluorescence in single cells

We measured the fluorescence of individual cells with the goal of inferring features of cl regulation from the cell-to-cell distribution of fluorescence. A micrograph of one strain examined, SS122, is shown in Figure 3. In Figure 4 we show the distribution of intensity for KB126 (Materials and Methods).

To better understand the distribution we compared it with a computer simulation of the production of GFP molecules (Materials and Methods). Since gfp is preceded by an efficient site for translation initiation and cl by an inefficient site for translation initiation, we assume there is at least as much GFP as repressor produced from each transcript initiated at pRM. In terms of our model, we make the conservative assumption that

---

**Table 2.** Plasmids and hosts used in this study

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Average fluorescence pRM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pcl60 cl−&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Host</td>
<td></td>
</tr>
<tr>
<td>KB124 (aex&lt;sup&gt;+&lt;/sup&gt;)</td>
<td>1.2 2.2 1.4 2.5 &lt;1</td>
</tr>
<tr>
<td>KB126 (aex::gfp)</td>
<td>1.2 2.2 1.4 2.5 &lt;1</td>
</tr>
<tr>
<td>SS122 (aex::gfp Δol)</td>
<td>1.2 2.2 1.4 2.5 &lt;1</td>
</tr>
</tbody>
</table>

Average fluorescence for each strain is described. aex::gfp lysogens harboring pSS5 showed a bimodal distribution of fluorescence. The average fluorescence of the minority population is shown in parentheses. The highly fluorescing population varied from 10% to 40% in various experiments.
one GFP molecule is produced per repressor molecule. Furthermore, our model assumes that production of GFP and repressor is a random stochastic process, and that dilution of both GFP and repressor only takes place upon cell division due to their observed stability.\textsuperscript{15,20} That is, at cell division each molecule in the cell randomly segregates between the two daughter cells. The predicted distribution of GFP concentrations is also shown in Figure 4.

It can be seen that the model predicts a distribution of GFP that is much narrower than observed. If KB126, a $\text{rec}^+$ strain, was partially induced it would display a broader distribution than an isogenic $\text{recA}$ strain. We examined the distribution of GFP in the isogenic $\text{recA}$ strain, SS74, and found the relative distribution of fluorescence intensities was very similar in the two strains, but slightly broader in the $\text{rec}^+$ strain ($\text{KB126} \ \sigma/\text{mean} = 0.29$; $\text{SS74} \ \sigma/\text{mean} = 0.26$).

Although if no feature of a $\text{rec}^+$ strain broadens the distribution, gene expression in $\text{recA}$ strains is expected to vary more than in the isogenic $\text{rec}^+$ strain,\textsuperscript{21} we believe the differences observed between the $\text{recA}$ and $\text{rec}^+$ strains are minor. We suggest the resistance to perturbations caused by altering the $\text{recA}$ gene, as with the tolerance of symmetric operators,\textsuperscript{12} is due to the regulatory capacity of the $\text{cl}$ expression system.

To test whether the observed distribution was an artifact of the microscopy we only analyzed bacteria that were precisely in the focal plane of the image and conducted the experiment with different pinhole diameters. Since depth of field varies with pinhole diameter, a variation due to imaging of the cells would show a dependence on pinhole diameter. We observed similar distributions with different pinhole diameters indicating the distribution was unlikely to be an artifact of the microscopy. Another potential source of broad fluorescence distribution could be fluctuations in background autofluorescence. We measured fluorescence in strains isogenic to SS74 and KB126 but lacking $\text{gfp}$, S2463 and KB124, and found autofluorescence could not account for the observed breadth of the distribution. Therefore, the observed breadth of the distribution was a property of the lysogens.
In our simulation, the stability of the prophage would not be substantially reduced by the fluctuations in repressor concentrations per se, such as observed for GFP concentrations. This is because failure of cl expression would have to occur for several generations in order to deplete the intracellular pool of repressor. Thus, the observed fluctuations of GFP concentrations are compatible with the observed stability of the prophage. However, if the observed fluctuations were due to an effect of Cro, an antagonist of repressor synthesis, then in the absence of additional mechanisms to control cl expression, the predicted instability is incompatible with the observed stability of the prophage.

**UV experiment**

To search for a mechanism to maintain cl expression in the presence of the large fluctuations, we examined the response of the rex::gfp expression to reductions in repressor concentrations such as those induced by DNA damage. We expected increased repressor synthesis in response to DNA damage could compensate for the cell-to-cell variation and permit the prophage to regulate departure from lysogeny. To investigate this possibility, we examined the response of the cl operon expression to UV irradiation in the Rec⁺ lysogen.

Cultures of KB126 were exposed to various doses of UV irradiation and both GFP production and phage induction were measured (Materials and Methods). The results of this experiment are shown in Figure 5. It can be seen that fluorescence increased with doses of UV that induced only a small fraction of the lysogens to enter lytic development. The greatest increase in fluorescence was approximately twice the uninduced level. With higher UV doses, more lysogens were induced but further increase in fluorescence was not observed. De-repression of cl expression at very low doses of UV coincides with the depression of the initial portion of the phage release versus UV dose curve (summarized in Figure 5C). This break in the phage release curve was observed with both the rex::gfp lysogen, KB126, and the rex⁺ lysogen, KB124 (Figure 5A) although the rex::gfp lysogen was less sensitive to UV. An interaction between Rex and repressor was suggested earlier.²² The coincidence of the break in the phage release curve and the plateau in gfp expression suggests the likelihood phage will depart lysogeny in response to small amounts of DNA damage is reduced by de-repression of cl.

To investigate the contribution of the oL–CI–oR complex⁹,¹⁰ (Figure 2) to the UV-mediated de-repression of pRM, we deleted oL from the prophage of KB126. If the prophage contained a large deletion that included oL (SS122; Figure 5B) the induction of GFP synthesis by UV was not observed. We suggest the differences in GFP induction between KB126 and SS122 was due to

![Figure 5](image-url)
the presence of ol and the induction in KB126 was due to opening of the ol–Cl–or complex. The average fluorescence of SS122 in this experiment was 31 units while the fluorescence of KB126 following UV exposure peaked at 23 units. Thus, in spite of induction by UV, the prophage retaining ol did not attain the high level of expression observed for the prophage lacking ol.

\( \lambda \) repressor can exist as a monomer, a dimer and a dimer bound to the \( \lambda \) operators. Exchange between these states is rapid compared with the generation time of \( E.\ coli \) (review^3). The form of repressor, which degrades via activated RecA in response to DNA damage, is the monomer form (review^4). We suggest the depletion of repressor monomers results in opening the ol–Cl–or complex increasing transcription of cl from pRM (Figure 2). The increased expression of the cl operon in response to low exposure to a DNA-damaging agent prevents premature departure from the lysogenic state. This increase in cl operon expression helps the prophage tolerate fluctuations in repressor concentrations while maintaining lysogeny. However, greater increases in cl expression would prevent the prophage from departing lysogeny after severe DNA damage. The limited increase of GFP content observed in KB126 permits the prophage to efficiently depart lysogeny after higher doses of UV.

**Conclusion**

The \( \lambda \) prophage is remarkably stable. In \( recA \) strains, the majority of phage that have departed lysogeny are mutated in the repressor, i.e. they form clear plaques. Furthermore, turbid plaques, those previously assumed to be wild-type and result from failure of the lysogenic regulatory network, are also the result of mutation. The turbid plaques released from \( recA \) strains are defective in the maintenance of repression. Thus, the \( \lambda \) prophage is vastly more stable than previously assumed. Furthermore, the stability prevails in the absence of the second known promoter for cl transcription, \( pRE \).

The profound stability of the lysogenic state is not due to exceptionally precise control of repressor concentrations. In fact, the concentration of repressor varies widely from cell-to-cell. The fluctuation in repressor concentrations does not lead to instability of the prophage, since the prophage regulatory network can compensate for substantial decreases in repressor concentrations. The response to low doses of UV presumably occurs through this compensatory response, since the prophage remains profoundly stable in \( recA \) strains, which cannot mount an SOS-response. The response to low doses of UV required the presence of ol and we suggest the response occurs through the ol–Cl–or complex. In spite of a UV-induced increase in repressor synthesis, the prophage can effectively respond to greater DNA damage and depart lysogeny. The network retains its ability to respond to DNA damage because of the limited induction ratio for repressor synthesis.

**Materials and Methods**

**Plasmids**

The immunity region (base-pairs 34,500 to 39,173) was cloned from \( \lambda^+ \) as the BamHI-EcoRI fragment replacing the BclI-EcoRI fragment of pACYC184 to create pSS1. The Psfl-PstI fragment of \( rex\text{-}ex\text{R} \) (base-pairs 37,006–36,124) was replaced with the Xbal-HindIII fragment containing \( gfp \) from pGFPmut2 to create pSS2. The fragment ends were made blunt by T4 DNA polymerase for the portion from pSS1 and by Klenow fragment of \( E.\ coli \) DNA polymerase for the portion from pGFPmut2. pSS5 places cl under the control of the lac promoter and contains the BglII-PstI fragment of \( \lambda^+ \) (base-pairs 38,107–37,001) replacing the BamHI-PstI fragment of pUC18.

A fragment containing 508 bp internal to the galK gene was amplified using pCP44 as a template and primers 5’GGTGAAACATCTGCAGCTGCGTAACAAC 3’ and 5’ATATGACGCACGCGTTTTGCC 3’. The fragment was introduced into pSS2 replacing the AhdI-PstI fragment. The PstI site is located immediately downstream of the stop codon of \( gfp \). A Psfl fragment from pSB590 encoding kanamycin resistance was inserted at the Psfl site between \( gfp \) and the \( galK \) fragment to yield pKB85.

**Strains and media**

All bacterial strains are derivatives of \( E.\ coli \) K12 and are described in Table 3. The parental strains were MC4100, MG1655, S971 and SY822. The \( rex::gfp \) fusion was crossed with \( lmmh \) by transforming SS16 with pSS2 and mating with SS15. Zygotically induced phage were purified on SS10. Single lysogens were identified by the PCR assay described by Powell et al. using primers: \( bv \) side of attP, GTATGCAATGGTGGGTGG GAA; \( int \) side of attP, CGGCCACGAAAAAGCATCAGTT; \( gal \) side of attP, CCCAGACATCCGCGGGAAGAAG; and \( bio \) side of attB, CTCTCTGCGCAAGGCGCTCGATT. All \( lmmh \) strains, except S2245 were determined to be single lysogens. Bacteria were grown in YT medium unless stated. Ampicillin was added to 200 \( \mu \)g/ml, chloramphenicol to 25 \( \mu \)g/ml, kanamycin to 30 \( \mu \)g/ml, streptomycin to 100 \( \mu \)g/ml and tetracycline to 10 \( \mu \)g/ml as required.

SS122 was constructed by recombination with pKB85 as described. pKB85 was transformed into S971 lysogenized with \( \lambda \text{rex}::gfp \) and mated with KB126 selecting for streptomycin and kanamycin resistance. P1vir was grown on the resistant population and used to transduce KB126 to kanamycin resistance. SS122 was identified as a Gal+, tetracycline-sensitive transductant that did not release phage.
Table 3. *E. coli* strains used in this study

<table>
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<tr>
<th>Strain</th>
<th>Genotype (Genotype)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB12</td>
<td>F- rps srl::Tn10 Δ(srl-recA)306 lamB (λ·&lt;sup&gt;·&lt;/sup&gt;)</td>
<td>This study</td>
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<tr>
<td>KB13</td>
<td>F- rps srl::Tn10 Δ(srl-recA)306 lamB (acy42)</td>
<td>This study</td>
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<td>KB14</td>
<td>F- rps srl::Tn10 Δ(srl-recA)306 lamB (acy2001)</td>
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<td>KB16</td>
<td>F- rps srl::Tn10 Δ(srl-recA)306 lamB (AcI2002)</td>
<td>This study</td>
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<tr>
<td>KB124</td>
<td>F- Δ(lac-ZY)M455 his arg rpsL31 lamB (λ·&lt;sup&gt;·&lt;/sup&gt;)</td>
<td>This study</td>
</tr>
<tr>
<td>KB126</td>
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<td>S2463</td>
<td>F- Δ(lac-ZY)M455 Δ(srl-recA)304 his arg rpsL31 lamB (λ·&lt;sup&gt;·&lt;/sup&gt;)</td>
<td>This study</td>
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<td>S2480</td>
<td>F- rps srl::Tn10 Δ(srl-recA)306</td>
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<tr>
<td>S910</td>
<td>F- Δlacx74 araD139 thi rpsL (km&lt;sup&gt;4434&lt;/sup&gt;)</td>
<td>This study</td>
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<tr>
<td>S15</td>
<td>F- Δlacx74 araD139 thi rpsL (km&lt;sup&gt;434&lt;/sup&gt;)</td>
<td>This study</td>
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<tr>
<td>S16</td>
<td>HfrH lac&lt;sup&gt;+&lt;/sup&gt; lamB (km&lt;sup&gt;434&lt;/sup&gt;)</td>
<td>This study</td>
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<td>S774</td>
<td>F- Δ(lac-ZY)M455 Δ(srl-recA)304 his arg rpsL31 lamB (λ·&lt;sup&gt;·&lt;/sup&gt;: gfp)</td>
<td>This study</td>
</tr>
<tr>
<td>S822</td>
<td>F- Δ(lac-ZY)M455 his arg rpsL31 lamB (λ·&lt;sup&gt;·&lt;/sup&gt;: gfp) Δ(rex-galK):kan</td>
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</tr>
<tr>
<td>SY822</td>
<td>F- Δ(lac-ZY)M455 Δ(srl-recA)304 his arg rpsL31</td>
<td>37</td>
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</table>

All strains are derivatives of *E. coli* K12.

**Complementation analysis**

Liquid lysates were made from individual plaques as described by Silhavy et al. and titers of the lysates were determined. A saturated culture of S2245 grown at 30°C was spread at several concentrations onto T agar and allowed to dry. Dilutions of the lysates were spotted in 10 µl drops on the lawn of bacteria and colonies were counted after overnight incubation at 42°C. To calculate multiplicity of infection (m.o.i.) the area of the spots was measured to determine PFU/cm², which was divided by CFU/cm². Bacteria and phage were combined to generate m.o.i. values between 10⁻⁴ and 10⁻¹³. Efficiency of transduction was determined from the linear portion of the relationship of colonies formed at 42°C to input PFU.

**Phage release**

Overnight cultures were washed with M63 salts and resuspended in YT supplemented with 10 mM MgSO₄. The washed cultures were diluted 1000-fold into 20 ml of YT supplemented with 10 mM MgSO₄ and grown to 5 x 10⁷ CFU/ml. Aliquots were removed and viable cells quantified by colony formation. The remainder of each culture was sterilized with CHCl₃ and filtered through a 0.2 µm pore size filter. The filtrates were titrated on MC4100 to quantify phage released.

**Microscopy and data collection**

Cultures in mid-log phase were mixed 1:1 with 100 µg/ml of spectinomycin in 10 mM sodium phosphate, 0.1 M NaCl (pH 7). Samples (14 µl) were transferred to washed, agarose-coated slides. Samples were examined as wet-mounts by laser scanning confocal microscopy. Fluorescence was quantified by measuring the average gray scale value in the fluorescent channel along the midline extending the length of the cell. When only the distribution of fluorescence among cells was examined, the data from different images were pooled by setting the average fluorescence intensity of cells in each image to 1.

**UV irradiation**

Cells were grown to mid-log phase and washed in pre-warmed 0.1 M MgSO₄. Washed cells were irradiated with a 254 nm UV source at 0.09 J/m² second in dim ambient light. After UV irradiation cell suspensions were diluted 1:1 in pre-warmed YT and shaken in the dark. After 30–40 minutes an aliquot was removed for microscopy and treated as described. Incubation was continued for four hours at 37°C. The cultures were sterilized with CHCl₃, cell debris removed by centrifugation and the released phage titered. The concentration of viable cells was measured immediately before UV treatment.

**Fluctuation analysis and computer simulation**

To translate the observed level of fluctuations into cellular mechanisms we employed an extended version of the stochastic model described by Aurell et al., taking into account both *oL*–*oR* interactions for negative auto-regulation of *pRM* developed to fit the *pRM* activity data by Dodd et al., and supplemented by a special treatment for GFP production and segregation into daughter cells. In practice this involves the inclusion of 27 different patterns of CI and Cro bindings to *oL* bindings, 40 different bindings of RNA polymerase, CI and Cro to *oR*, and inclusion of *oL*–CI–*oR* binding of, respectively, –1 kcal/mol (1 cal = 4.184 J) for octamer association, and a –3 kcal/mol for a full 12-mer binding of CI on all *oL* sites to CI at all *oR* operator sites. Finally, from the *pRM*–CI data by Dodd et al., and the *pR*–Cro data by Pakula et al., as discussed by Reinitz & Vaisnys, we estimate CI and Cro non-specific binding to both be –3.5 kcal/mol. GFP is subsequently introduced as a passive ingredient in the model, and is always produced in conjunction with CI but is not negatively self-regulated. Thus, the fluctuations of GFP are slightly larger than for CI levels.

The basic ingredient in a stochastic model for protein production is a production term given by a number of mRNA transcripts and a fluctuating number of proteins per transcript (see Aurell et al.). In addition, when the
cells divide all molecules should be randomly distributed to the two daughter cells. The resulting fluctuations in GFP concentration will be of order \( N_{\text{rep}} \sqrt{N_{\text{mRNA}}} \) where \( N_{\text{reps}} \) is the number of independent events that control the amount of GFP in the cell. However, because each cell division limits the overall meandering of concentration of any protein in a cell, a more precise estimate of fluctuations needs a simulation. However, in practice we find that the spread over mean for the GFP signal is well fitted by:

\[
\frac{\sigma}{\text{mean}} = \frac{1}{\sqrt{N_{\text{mRNA}}}}
\]

where \( N_{\text{mRNA}} \) is the number of CI–GFP mRNA transcript produced in a cell generation. The simulation assumed 70 transcripts per cell cycle were initiated at \( pRM \) to generate 170 repressor monomers. This efficiency of translation of \( cl \) is derived from the ratio of translation of \( cl \) from \( pRM \) to \( lacZ \) from its own ribosome-binding site and the efficiency of translation of \( lacZ \). The number of repressor monomers per cell is derived from the concentration of repressor in lysogens and the molecular mass of the \( \lambda \)-repressor.

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References


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