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Quantitation of the DNA tethering effect in long-range DNA looping in vivo and in vitro using the Lac and λ repressors

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Efficient and specific interactions between proteins bound to the same DNA molecule can be dependent on the length of the DNA tether that connects them. Measurement of the strength of this DNA tethering effect has been largely confined to short separations between sites, and it is not clear how it contributes to long-range DNA looping interactions, such as occur over separations of tens to hundreds of kilobase pairs in vivo. Here, gene regulation experiments using the Lac and λ repressors, combined with mathematical modeling, were used to quantitate DNA tethering inside Escherichia coli cells over the 250- to 10,000-bp range. Although Lac and λ loop DNA in distinct ways, measurements of the tethering effect were very similar for both proteins. Tethering strength decreased with increasing separation, but even at 5- to 10-kb distances, was able to increase contact probability 10- to 20-fold and drive efficient looping. Tethering in vitro with the Lac repressor was measured for the same 600- to 3,200-bp DNAs using tethered particle motion, a single molecule technique, and was 5- to 45-fold weaker than in vivo over this range. Thus, the enhancement of looping seen previously in vivo at separations below 500 bp extends to large separations, underlining the need to understand how in vivo factors aid DNA looping. Our analysis also suggests how efficient and specific looping could be achieved over very long DNA separations, such as what occurs between enhancers and promoters in eukaryotic cells.

Significance

Proteins bound to DNA often interact with proteins bound elsewhere on the same DNA to regulate gene expression. The intervening DNA tethers the proteins near each other, making their interaction efficient and specific, but the importance of this tethering effect is poorly understood at large DNA separations. We quantitated tethering inside bacterial cells, using two different proteins at separations up to 10,000 bp, to show that tethers are strong enough to drive efficient interactions over these distances. The same interactions were ~10-fold weaker outside cells, implying that cellular factors enhance tethering. However, tethers were lost at a DNA separation of 500,000 bp inside bacteria, indicating special mechanisms inside eukaryotic cells to provide efficient and specific interactions over such distances.


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L2 concentration of LacI in solution at 242 bp to 1.8-fold K1 was measured in the absence of Lac repressor (Oid) or at two different Lac concentrations (L1 and L2), expressed from PlacI/lacI or PlacP/lacI constructs integrated at a separate chromosomal site (Fig. 24). The results showed, as expected, that the presence of OI or Oid at O3 increased repression substantially, depending on the operator combination and the LacI concentration (Fig. 2B).

By fitting the data to a statistical-mechanical model of LacI repression (Fig. 2C), we were able to estimate a number of in vivo parameters (Fig. 2D). The model is similar to Han et al. (9), with the addition of RNA polymerase (RNAP) binding to PlacUV5 to compete with LacI (24). The cell populations were obtained from the WT lacI promoter has been estimated at 18 nM (25) (11 tetramers in a 1-FL volume) and was used for L1. The model does not permit determination of absolute binding constants, and K1 was arbitrarily fixed to 1 nM. The relative dissociation constants obtained for the four reporters are similar to those found by Garcia and Phillips (25) (0.22, 1, 4.5, and 335 for Kid, K1, K2, and K3, respectively). Western blotting and PlacI+ and PlacI− activities (Fig. S4) supported the estimate of the ratio of the high and low Lac repressor concentrations of L2/L1 ≈ 10.9.

Other reporter measurements (SI Materials and Methods) supported the estimate of 18.1 background LacZ units from the PlacUV5/lacZ reporter (Fig. 2D).

The fitting gives an estimate of jloop/L1 that is independent of the fixed values chosen for K1 or L1. Assuming L1 = 18 nM gives jloop = 1,400 nM (for this 300-bp spacing), similar to estimates of ~1 μM for short lac operator spacings in vivo (6, 8, 19).

Effect of DNASpacing on Lac Looping in Vivo. To measure jloop over longer distances, we used Oid−O2 reporters with operator spacings ranging from 242 to 5,600 bp. The sequence of spacer DNA can affect the efficiency of short DNA loops (26), so we made two series of spacers: series 1 (300–5,600 bp) and series 2 (242–3,200 bp). We also inserted Oid or O′ in the chromosome 500 kb away from O2 (SI Materials and Methods).

The upstream operator aided repression most strongly at the shortest spacing (242 bp). Although its effect weakened with increasing spacer length, there remained a significant effect of the upstream operator at 5,600 bp at the L1 concentration (Fig. 3A). The operator placed 500 kb away had no effect on repression. The reporter data were fitted using the model and the parameters from Fig. 2D but allowing jloop/L1 to vary for each operator spacing (SI Materials and Methods). These jloop/L1 values (Fig. 3B and Fig. S5) show that the effective concentration of the operators relative to each other ranges from 180-fold the L1 concentration of LacI in solution at 242 bp to 1.8-fold L1 at 5,600 bp. Thus, even at a 5,600-bp separation, a Lac repressor at the distal lac operator is seen by the promoter almost two times more frequently than all of the other Lac repressors in the cell combined. In contrast, the data for the 500-kbd spacing was fitted with a jloop/L1 value of 0.09 (Fig. 3C); a Lac repressor bound 500 kb away contacts the proximal operator no more frequently than any of the other ~11 Lac repressors in the cell.

The jloop/L1 values allow calculation of the fraction of looping for Oid and O2 at each separation and [LacI] (Fig. 3C). LacI looping is sensitive to concentration; at low concentrations, neither operator is occupied, whereas at high concentrations, looping is obstructed by the formation of doubly bound species (species 6; Fig. 2C). Maximal looping decreases with increasing spacing, but even at separations of 5,600 bp, Oid−O2 are looped at least 30% of the time over a 100-fold range of [LacI] (0.2–20 nM; Fig. 3C).

Effect of Separation on Long-Range Looping by λ CI Repressor in Vivo. We used λ CI to obtain an independent measurement of the relationship between jloop and DNA separation in vivo. DNA looping by λ CI can be detected by repression of the λ PRM

![Diagram](image)
promoter (7, 23, 27). At low concentrations, CI dimers form tetramers on OR1.OR2, activating PRM, and these tetramers can interact to form an octamer-bridged DNA loop (Fig. 4A; free energy $\Delta G_{\text{oct}}$). The loop allows a dimer bound at OR2 to help a dimer bind to the weak OR3, forming a trans-tetramer and repressing PRM (Fig. 4A). dimer-dimer interaction free energy $\Delta G_{\text{tet}}$. Thus, repression at moderate CI concentrations requires the distal OR2 site. With a statistical-mechanical model for CI regulation (7, 23), the free energy of the DNA looping reaction in vivo, $\Delta G_{\text{loop}}$, can be extracted from a comparison of PRM activity $\pm$ OL at high CI concentrations.

The looping reaction comprises the unfavorable DNA looping energy $\Delta G_{\text{loop}}$ and a favorable protein–protein interaction $\Delta G_{\text{PRM}}$ between DNA-bound CI tetramers: $\Delta G_{\text{OC}} = \Delta G_{\text{loop}} + \Delta G_{\text{PRM}}$. Estimating $\Delta G_{\text{PRM}}$ from a measure of the free energy of CI octamerization in vitro (−9.1 kcal/mol) (28, 29), it is possible to derive $\Delta G_{\text{loop}}$ from $\Delta G_{\text{OC}}$ (7) (SI Materials and Methods).

We made a series of OR.PRM.lacZ reporters with OR (or an OR sequence) at distances from 150 to 10,000 bp upstream of OR (Fig. 4A, Figs. S1 and S2, and SI Materials and Methods). A chromosomally integrated CI gene (or vector only) provided 3.3 WT lysogenic units (WLUs) of CI (or no CI) (23). OL-dependent repression of PRM was strongest for the 250-bp spacing and became weaker with increasing distance (Fig. 4B and Fig. S6). However, even at 10,000 bp, the presence of OL aided repression of PRM. In contrast, OL 500 kb away on the chromosome gave no enhancement of PRM repression (Fig. S7).

The reporter data were fitted by allowing a different $\Delta G_{\text{OC}}$ for each spacing but holding all other parameters fixed to the values obtained in our previous detailed analysis of OR–OR looping at 2,300-bp spacing (23) (SI Materials and Methods and Fig. S6). However, we could not achieve good fits to the data for the shorter spacers unless we decreased $\Delta G_{\text{OC}}$ to −3.4 kcal/mol, below our previous estimate of −2.4 kcal/mol. The 2,300-bp data (23) are still reasonably well fitted using this revised value for $\Delta G_{\text{OC}}$ and this value is in better agreement with the estimate of $\Delta G_{\text{OC}} = −3.2$ kcal/mol obtained by single cell imaging of looping at 2,300 bp by CI in vivo (20).

The resulting $\Delta G_{\text{OC}}$ estimates (Fig. 4C) have large errors, primarily reflecting uncertainty in the value for $\Delta G_{\text{OC}}$, which has the effect of shifting the whole $\Delta G_{\text{OC}}$ vs. separation curve up or down. The lower $\Delta G_{\text{OC}}$ value causes our $\Delta G_{\text{loop}}$ estimates to be some 10-fold lower than before (7, 23) (Fig. 4C). However, our new 2,300-bp $\Delta G_{\text{OC}}$ estimate agrees well with that derived from single cell imaging (20) (Fig. 4C). The $\lambda$ CI values are also remarkably similar to those obtained for LacI with $L_1 = 18$ nM (Fig. 4C). We also used our Lac model to analyze existing LacI looping data for 60- to 1,500-bp separations (21) and found a good match with the $\Delta G_{\text{loop}}$ values from our LacI and CI data (Fig. S8).

These $\Delta G_{\text{loop}}$ values produce efficient looping in the CI system, with OL–OR looped over 50% of the time at the 10,000-bp separation at 3.3 WLUs CI (Fig. 4D). Unlike the Lac system, the looped fraction increases with increasing CI concentrations (Fig. 4D) because the CI tetramer in the looped complex, the octamer, does not form readily in solution, and loop-blocking species are only likely to form at considerably higher CI concentration (29).

Long-Range Looping by lac Repressor In Vitro. Previous studies indicate that LacI looping with short DNA tethers (<500 bp) is less efficient in vitro than in vivo. To test whether this difference holds for longer DNA tethers, we examined LacI looping in single molecules by TPM (9, 30–32), where looping of a DNA molecule attaching a bead to a surface can be detected as a restriction in the Brownian motion of the bead (Fig. 5A). The fragments were the same as the 600-, 900-, 1,200-, and 3,200-bp spaces in vivo constructs, but with $O1$ at $O3$ (Figs. 5A, 5E, and SI Materials and Methods).

Looped and unlooped states were followed over time over a range of LacI concentrations. Excursion values from the motion records of all selected beads in one experimental condition were assembled in a histogram from which average looping probabilities were determined (Fig. 5B). As expected, the probability of looping goes through a maximum with respect to [LacI] (Fig. 5C). At LacI concentrations at which $O2$ is fully occupied, the decrease in looping with increasing [LacI] allows estimation of $\Delta G_{\text{loop}}$ independently of the affinity of $O1$ (Fig. 5D), because looping or unlooping is a simple binding competition between bound or free LacI (9).

The 600-bp $\Delta G_{\text{loop}}$ value obtained by TPM is 5.7-fold lower than the LacI and CI in vivo estimates (Fig. 5E), consistent with previous studies. Interestingly, this difference increases at longer separations: 11-fold at 1,200 bp and 45-fold at 3,200 bp (Fig. 4D). The TPM measurement of $\Delta G_{\text{OC}} = 1.7$ kcal/mol for CI looping at 2,300 bp (17) gives a $\Delta G_{\text{loop}}$ value that is also approximately sevenfold lower than the in vivo CI and LacI estimates (Fig. 4D).

Discussion

DNA Can Foster Long-Range Interactions Even at Large Site Separations. We used two well-characterized bacterial systems where transcription is regulated by DNA looping to obtain measurements of $\Delta G_{\text{loop}}$ in vivo for DNA tether lengths beyond 5 kb. The results for LacI

Fig. 2. Using regulation by LacI looping to measure $\Delta G_{\text{loop}}$ in vivo. (A) Chromosomal PlacUV5.lacZ reporters with a lac operator at the promoter (O1) and 300 bp upstream (O2), with Lac supplied from a chromosomal lac gene with plac’ (low [Lac] = $L_1$) or plac” (high [Lac] = $L_2$) (SI Materials and Methods and Figs. S1–S3). (B) Data and model fits for the four O1–O2 combinations tested, assuming $L_1 = 18$ nM. The numbers on the plots are data (model) in the absence of LacI. Data errors are 95% confidence limits; n ≥ 8. (C) Statistical mechanical model of regulation by LacI tetramers. $L$ is the concentration of LacI tetramers, $K_s$ are dissociation constants, and $R$ is a dimensionless constant describing RNAP occupation of the promoter. (D) Parameters obtained from data fitting. $K_1$, $K_2$, and $K_3$ are dissociation constants for Oid, O1, O2, and O3, respectively. $Z_{\text{max}}$ is the Lac2 activity obtained if the promoter were to be fully occupied by RNAP (average shown; SI Materials and Methods). $b_{\text{kg}}$ is the Lac2 units obtained if the promoter is completely repressed. Errors are SDs from 100 data fittings (SI Materials and Methods).
and λ CI were in remarkable agreement, especially considering the differences in looping mechanism, with formation of a LacI loop primarily a DNA–protein interaction (Figs. 1A and 2C) and formation of the CI loop dominated by a protein–protein interaction (Figs. 1B and 4A). The different modeling approaches and the distinct additional parameters needed to extract information of the CI loop dominated by a protein–protein interaction (Figs. 1A and 2C) and formation of the CI loop dominated by a protein–protein interaction (Figs. 1B and 4A). The different modeling approaches and the distinct additional parameters needed to extract information of the CI loop dominated by a protein–protein interaction (Figs. 1A and 2C) and formation of the CI loop dominated by a protein–protein interaction (Figs. 1B and 4A). The different modeling approaches and the distinct additional parameters needed to extract information of the CI loop dominated by a protein–protein interaction (Figs. 1A and 2C) and formation of the CI loop dominated by a protein–protein interaction (Figs. 1B and 4A). The different modeling approaches and the distinct additional parameters needed to extract information of the CI loop dominated by a protein–protein interaction (Figs. 1A and 2C) and formation of the CI loop dominated by a protein–protein interaction (Figs. 1B and 4A). The different modeling approaches and the distinct additional parameters needed to extract information of the CI loop dominated by a protein–protein interaction (Figs. 1A and 2C) and formation of the CI loop dominated by a protein–protein interaction (Figs. 1B and 4A). The different modeling approaches and the distinct additional parameters needed to extract information of the CI loop dominated by a protein–protein interaction (Figs. 1A and 2C) and formation of the CI loop dominated by a protein–protein interaction (Figs. 1B and 4A).

At separations <300 bp, \( J_{\text{LOOP}} \) was >1 \( \mu \text{M} \) and decayed with separation roughly as a power law with exponent \(-1.2\) (Fig. S10). In the 5- to 10-kb separation range, \( J_{\text{LOOP}} \) was still \(-20\) to 40 \( \text{nM} \), substantially higher than the 1.7 \( \text{nM} \) concentration of a single molecule within a cell volume of 1 fl (1 \( \mu \text{m}^3 \)). Thus, as long as the concentration of a protein in the cell is reasonably low, even a distant operator can strongly increase its effective local concentration. As a result, even at these distances, the fraction of time that the DNA sites spend looped can be considerable. For OidI-O2 at 5,600-bp separation, the fraction looped could be almost 40\% (Fig. 3C). For λ OL-OR at 10-kb separation, the fraction looped can be at least 50\% at CI concentrations >3 WLU (Fig. 4D).

This reasonably strong tethering effect at 5-10 kb disappears at a separation of 500 kb, where the distal operator has no detectable effect on regulation by LacI or CI. Thus, even at large separations, distance matters: a DNA site 10 kb away is much closer than a site 500 kb away. A question remains whether there is a gradual decline in \( J_{\text{LOOP}} \) over the 10- to 500-kb range or whether tethering is lost at separations well short of 500 kb. Simple extrapolation of the in vivo data in Fig. 5E (Fig. S10) suggests that substantial tethering could exist at separations of ~100 kb. Previous measurements of relative contact efficiencies using sites for recombinases in bacteria have revealed a steady drop in relative contact efficiency over the 10- to 90-kb range (13). It may be possible to use our λ CI reporter system to quantify \( J_{\text{LOOP}} \) at 100-kb separations, because \( J_{\text{LOOP}} \) values as low as 3 \( \text{nM} \) (\( \Delta G_{\text{act}} \approx -3 \text{kcal/mol} \)) should be measurable (Fig. 4A).

**In Vivo Factors Increase \( J_{\text{LOOP}} \) at Long Separations.** The in vitro \( J_{\text{LOOP}} \) values obtained for LacI looping by TPM were ~5- to 45-fold lower and appeared to decay faster with separation than the in vivo values (Fig. 5E), fitting a power law with an exponent of ~1.5 (Fig. S10). Our TPM estimates are roughly comparable with published in vitro values. Our \( J_{\text{LOOP}} \) was 75 \( \text{nM} \) for the 600-bp LacI loop is higher than the ~10- and ~30-nM values obtained for LacI by TPM for ~100- and ~300-bp loops, respectively, by Han et al. (9), but this is consistent with the expected increase in \( J_{\text{LOOP}} \) over these separations due to relief of enthalpy costs (5). The TPM results of Johnson et al. (26) indicate even poorer looping with short spacers, with \( J_{\text{LOOP}} \) values of 0.3-4 \( \text{nM} \) for different ~100-bp LacI loops. Our 900-bp estimate of \( J_{\text{LOOP}} \) is 54 \( \text{nM} \) similar to the \( J_{\text{LOOP}} = 37 \text{nM} \) obtained for an 870-bp loop formed by Cre recombinase (15).

At the 3,200-bp spacing, our in vitro \( J_{\text{LOOP}} \) fell to ~8 \( \text{nM} \), which is comparable to 18 \( \text{nM} \) at 3,044 bp (9). For DNA cyclization at 4,000 bp (14), TPM analysis of CI looping at 2,300 bp gave \( J_{\text{LOOP}} = 24 \text{nM} \) (17), somewhat higher than our TPM values (Fig. 5E); however, this may be an overestimate because a low value of \( \Delta G_{\text{act}} \) was used.

Thus, we are confident that the in vivo–in vitro difference is real and applies over two orders of magnitude of the separation between sites (100–10,000 bp).

In vivo factors that increase flexibility or compaction of DNA such as DNA supercoiling and nonspecfic DNA-binding proteins that bend or bridge DNA, such as the nucleoid protein HU, are thought to enhance short-range DNA looping (8,33–36). It is not clear whether these factors also act at distances over which DNA bending and twisting are not limiting. However, increased DNA flexibility due to random binding of bend-inducing proteins such as nucleoid proteins or histones (35) should make the DNA more likely to wind back on itself, keeping it more compact and making it less likely that sites far apart on the DNA will be far apart in space. Unconstrained DNA supercoiling is implicated in aiding long-range looping in vivo (13). We note that our in vivo data can be reasonably well fitted by assuming that the DNA is a flexible polymer with an apparent persistence length of 23 nm (66 bp; Fig. S10). Relative FLP recombination rates over separations of 70–15,000 bp in human cells indicated a similar apparent persistence length of 27 nm (12).

Our data do not identify which factors are responsible for improved long-range looping in vivo. However, our work provides a quantitative target for the in vivo/in vitro difference—an ~10-fold effect—and provides an experimental system to measure the effects of candidate factors by addition of factors in vitro or removal of factors in vivo.

**Creating Efficient Long-Range Looping.** Given a fixed \( J_{\text{LOOP}} \) between any two DNA sites, how can DNA looping be maximized? The LacI and λ CI proteins represent extremes of a biochemical continuum (Fig. 1). In the case of LacI, the protein–protein...
interactions that connect the two DNA-binding ends of the complex are strong, and the DNA-looping multimer, the tetramer, forms at exceedingly low concentrations (37). For CI, the assembly of dimers into tetramers, and tetramers into the loop-forming octamer is relatively weak so that the DNA looping complex does not form readily in solution (29).

Efficient LacI looping requires the protein concentration to be substantially below $j_{\text{LOOP}}$ and to lie close to the $K_D$ of both DNA sites (9, 26). At very long separations, where $j_{\text{LOOP}}$ is small, efficient looping by LacI is thus likely to be limited by difficulties in achieving reliable low protein concentrations inside cells due to gene expression noise.

Fig. 4. Effect of DNA separation on λ CI looping in vivo. (A) Approach for measuring $j_{\text{LOOP}}$ with λ CI. Structure of the OL-spacer-OR.PRM.lacZ reporters. CI activates PRM at low concentrations and represses it at high concentrations. Repression is dependent on the presence of OL and the free energy of loop formation between OL and OR, $\Delta G_{\text{oct}}$. (B) Decreased repression of PRM with increasing OL-OR separation. OR.PRM.lacZ reporters ± OL placed various distances upstream were assayed at 3.3 WT lysogenic units (WLU) of CI and in the absence of CI (SI Materials and Methods and Fig. S7). Also shown are previous $j_{\text{LOOP}}$ estimates for in vivo CI looping (square, ref. 7; triangle, ref. 23; red diamond, ref. 20); as well as the $j_{\text{LOOP}}$ values for LacI (Fig. 3C), assuming $L_1 = 18$ nM (blue circles). (D) Predicted fraction of looping for OL and OR at different separations up to 3.3 WLU, using $j_{\text{LOOP}}$ estimates from C.

Fig. 5. Measurement of $j_{\text{LOOP}}$ by TPM. (A) The TPM setup. (B) Examples of excursion vs. time traces for single beads, showing transitions between looped and unlooped states (left) and mean excursion probability distributions (right) for tethers with lac operators separated by 600–3,200 bp at 10 nM LacI. Different Lac loop conformations (two peaks) were distinguishable for the 900-bp loop. (C) The probability of looping vs. LacI concentration for the 900-bp separation. (D) $j_{\text{LOOP}}$ for each DNA was determined from the average fraction of time the DNA is looped ($p_{\text{looped}}$) at each [LacI], using the [LacI] dependence of loop blockage at [LacI] 0.5–20 nM (Oid was filled at 0.1 nM), according to the equation $p_{\text{unlooped}}/p_{\text{looped}} = 2K_1/j_{\text{LOOP}} + 2[LacI]/j_{\text{LOOP}}$ (9) ($K_1$ was fixed at 1 nM). The slope of each $p_{\text{unlooped}}/p_{\text{looped}}$ vs. [LacI] plot equals $2/j_{\text{LOOP}}$. (E) Comparison of the TPM $j_{\text{LOOP}}$ values with the in vivo values (LacI, blue; CI, brown). The red square shows a previous TPM estimate for λ CI (17).
For CI, efficient looping requires the free energy of the protein–protein interaction, $\Delta_G_{PTN}$, to compensate for the unfavorable $\Delta_G_{LOOP}$. The interaction of two CI tetramers is estimated to provide $-9.1$ kcal/mol to drive the looping reaction (28, 29). However, this octamerization reaction alone would fail to give more than 50% looping for DNA separations above $-1,000$ bp, where $\Delta_G_{LOOP} > +9.1$ kcal/mol ($G_{LOOP} < 384$ Nm). One way to improve CI looping would be to strengthen $\Delta_G_{PTN}$ by increasing the strength of the interactions between protein subunits. However, such changes would tend to increase the formation of larger complexes in solution, which would block looping.

The efficiency of looping by $\lambda$ CI is increased by increasing the number of interacting proteins at each site. The additional bridge formed by the interaction of a CI dimer at each site, to form a tetramer, effectively means that $\Delta_G_{PTN}$ (octamerization) and $\Delta_G_{tet}$ (tetramerization) combine to counterbalance $\Delta_G_{LOOP}$. As suggested by Dröge and Müller-Hill (38), this approach of using a DNA scaffold to assemble at each site a large complex of proteins that individually interact weakly but in combination provide a strong protein–protein interaction seems optimal for creating strong looping at very long separations, such as in eukaryotic genomes. A similar method could also be used to make LacI-style looping more efficient; placement of additional LacI binding sites at each site could permit a DNA loop to be bridged by two Lac tetramers.

However, strategies that use multiple interacting sites for the same protein to strengthen looping will eventually be limited by the formation of short DNA loops within each set of binding sites. A way around this limitation could be to combine different looping proteins. This kind of approach for making looping efficient has the advantage that specificity of looping could be provided by using a relatively small number of different looping proteins in different combinatorial patterns. Such a strategy may be necessary to provide both efficiency and specificity for the very long-range DNA interactions in eukaryotic genomes.

**Materials and Methods**

**Reporter and Expression Constructs.** The lacZ reporter and LacI and CI expression constructs were chromosomally integrated using the OSIP system and its precursors (23, 39) into MG1655 rph ΔlacIΔZYA (SI Materials and Methods). Cells were grown at 37 °C in minimal medium (LacI looping strains) or in rich medium (CI looping strains) and assayed by a modified LacZ microtiter plate method (7) (SI Materials and Methods).

**Mathematical Modeling.** Data fitting to extract $\Delta_G_{max}$ values from the $\lambda$ CI reporter data was as previously described (23) with some modifications. Details of this and the LacI modeling are given in SI Materials and Methods.

**TTP.** TTP experiments were conducted as previously described (30–32) with some modifications (SI Materials and Methods). Drift correction was improved by subtracting the motion of one to five stuck beads in the same field of view. Stringent selection of motion records based on symmetry and unlooped excursion amplitude was used. The mean square excursion of the bead was used to determine tether length by reference to a calibration curve with tethers of known length.

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