Location of the unique integration site on an *Escherichia coli* chromosome by bacteriophage lambda DNA in vivo

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The search for specific sequences on long genomes is a key process in many biological contexts. How can specific target sequences be located with high efficiency, within physiologically relevant times? We addressed this question for viral integration, a fundamental mechanism of horizontal gene transfer driving prokaryotic evolution, using the infection of *Escherichia coli* bacteria with bacteriophage λ and following the establishment of a lysogenic state. Following the targeting process in individual live *E. coli* cells in real time revealed that λ DNA remains confined near the entry point of a cell following infection. The encounter between the 15-bp-long target sequence on the chromosome and the recombination site on the viral genome is facilitated by the directed motion of bacterial DNA generated during chromosome replication, in conjunction with constrained diffusion of phage DNA. Moving the native bacterial integration site to different locations on the genome and measuring the integration frequency in these strains reveals that the frequencies of the native site and a site symmetric to it relative to the origin are similar, whereas both are significantly higher than when the integration site is moved near the terminus, consistent with the replication-driven mechanism we propose. This novel search mechanism is yet another example of the exquisite coevolution of λ with its host.

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Target location | establishment of lysogeny | viral transduction

The search for specific sequences along genomic DNA plays a key role in the location of specific sites by transcription factors (1), the repair of DNA lesions (2), and horizontal gene transfer (3). Common to these processes is a search through a very large number of possible sequences because of the long genomes involved. A fundamental question is how specific target sequences can be located with high efficiency, within physiologically relevant times. This question is crucial to understand viral transduction, one of the fundamental mechanisms of horizontal gene transfer driving the evolution of prokaryotes (3, 4). In transduction, a viral genome integrates at a unique site on a bacterial genome following infection, conferring new traits such as pathogenicity (5). A classic example of transduction is furnished by the infection of *Escherichia coli* cells by bacteriophage λ. Infection of an *E. coli* host by the temperate bacteriophage λ begins with the binding of the phage to the *E. coli* maltose porin LamB (6, 7). The phage then injects its DNA into the cell, a process that lasts about 5 min (8). Infection can lead to two possible outcomes, lysis or lysogeny, which reflect alternative pathways of gene expression (9–11). In the lytic pathway, execution of a viral gene expression cascade leads to the replication of the viral DNA, resulting in cell death and lysis to release about 100 phage progeny (12). Alternatively, by establishing lysogeny, the phage shuts off the lytic cycle and locates with high efficiency (13) a unique sequence along the cellular genome where it integrates its DNA by site-specific recombination. This recombination takes place at special attachment sites called attB and attP in the bacterial and phage genomes, respectively, and requires both the phage Int and the bacterial integration host factor (IHF) proteins. Once integrated, the prophage remains in a stable, dormant state, replicating passively with the host genome.

In this study, we followed in real time the search and eventual encounter between the attP site on single λ DNA molecules and the attB integration site on the genome of individual, live *E. coli* bacterial cells, immediately following phage infection. The results shed light on the mechanisms of search and how the encounter is achieved with high efficiency to establish integration and stable lysogeny.

**Results**

The attB Site Moves Toward λ DNA to Establish Lysogeny. We followed the dynamics of search by labeling the bacterial and phage genomes with yellow (yGFP) and red (RFP) fluorescent protein markers, respectively, near their respective att sites, using two types of parS/ParB, which are part of bacterial partitioning systems (14–16). One type of parS sequence (P1 parS) was inserted near attP on bacteriophage λ, while another type of parS sequence (pMT1 parS) was inserted near the attB site in the bacterial genome (Materials and Methods). The two types of parS sequences are recognized by their respective ParB proteins, mCherry-P1Δ30ParB labeling the phage DNA and yGFP-pMT1Δ23ParB labeling the attB locus (Fig. S1). Control experiments measuring the integration frequency show that labeling and ParB polymerization do not affect the process under study (17) (Fig. S2). Upon establishment of lysogeny, the distance between

**Significance**

Viral infection and integration is fundamental in the generation of genetic diversity in prokaryotes. After entering an *Escherichia coli* cell, bacteriophage λ DNA must locate a unique site among ~5 million possible sites on the bacterial genome, with high efficiency and within physiological times, to integrate and establish lysogeny. How does it do it? We show that λ DNA does not carry out an active search. Instead, it remains confined at its entry point where it undergoes limited diffusion, while the process of bacterial DNA replication conveys the bacterial site close to the λ DNA. This mechanism adds to the list of profligate use of host functions by λ, brought about by coevolution of host–phage processes.


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both parS sequences is ∼12 kbp. This distance was chosen to ensure that ParB polymerization from parS sites does not interfere with phage DNA integration (17). Note that due to genome compaction, the physical separation between the two ParB foci is orders of magnitude below the optical resolution limit. We followed the fate of 241 infected cells, 36% of which followed the lysogenic pathway while the rest, 64%, followed the lytic pathway. Typical snapshots of a bacterial cell following infection are shown in Fig. 1A. The snapshots depict a single λ DNA molecule soon after its entry into the cell and subsequent RFP labeling, until successful integration at the attB site is achieved, as evidenced by the coalescence of the red and yellow loci representing the attP and attB sites. In stark contrast with our initial expectation of observing λ DNA motion as part of the search process for the integration site, the snapshots clearly show that λ DNA barely moves over the time it takes for integration to occur and that the attB site is the one to move toward a cell pole. Consistent with previous findings (18), we observe λ DNA soon after infection to be located primarily at cell poles or at midcell (65% and 35%, respectively, of 400 events total; the distribution of λ DNA along the cell axis is discussed further below). When the lytic pathway is followed, replication of λ DNA also takes place primarily at the cell poles (18) (Fig. S3). Fig. 1A also illustrates that in addition to normal cell division (Fig. S4), infection can result in growth arrest, while, in other cases, it can cause cell filamentation (47% of lysogens exhibited growth arrest, 41% normal growth and 12% filamentation). Uninfected cells continue to grow and divide normally during the experiment. Expression of the λ phage is known to alter cell physiology by inducing temporary blocks to both cell division and initiation of new rounds of replication (19). However, such effects on each cell may be dependent upon when during the cell cycle infection occurs, or other variables of infection, e.g., actual multiplicity of infection (MOI).

We show, in Fig. 1B, the distance on the focal plane between RFP and yGFP loci labeling attP and attB sites, respectively, in the same cells, for a number of events such as those illustrated in Fig. 1A. Each trace corresponds to an attP-attB trajectory in a single cell. Integration, indicated by the attainment of a time-independent minimal distance of ∼0.2 μm (Fig. S5), is typically established at times of 77 ± 34 min (mean ± 2 std). A histogram of integration times is depicted in Fig. 1C. Independent measurements of integration in the bulk using PCR methods, which are sensitive and report the earliest integration events, yield a similar although shorter integration time (∼45 min) (Fig. S6), consistent with the large amount of events observed at 45 min in Fig. 1C. Note that 60 min is the cell doubling time of uninfected cells under the conditions of the present experiments. The typical behavior observed in Fig. 1B is not observed after the infection of an immune cell (Fig. S7).

λ DNA Undergoes Constrained Diffusion. To quantify more precisely the extent to which λ DNA moves within a bacterial cell, we calculated the mean square displacement (MSD) between two individual λ DNAs within the same cell, following known procedures (20). This allows us to characterize the displacement of the molecule independently from any cellular marker such as a cell pole, which may exhibit motion due to cell growth. The MSD is a measure of the mean square change in the distance between points, and it is the canonical way to characterize quantitatively Brownian motion (20). We show the mean square displacement as a function of time for λ DNA pairs observed in different cells, as well as the average over all traces in Fig. 2. The average behavior is characteristic of constrained diffusion, with saturation reached around ∼50 s. At saturation, the mean square displacement corresponds to roughly ∼120 nm (SI Materials and Methods).

The Dynamical Behavior of the attB Site Is Not Affected by Infection. Given that λ DNA remains confined to the point of entry while the attB site moves (e.g., Fig. 1A), we asked whether this motion is driven by a phage factor to facilitate lysogeny, or by normal DNA replication. To address this, we compared the dynamical behavior of the attB site in uninfected cells with that of infected cells that eventually become lysogens. The distance between the attB marker and the cell’s center as a function of the time elapsed after the last cell division is plotted in Fig. 3. The averaged traces for infected and uninfected cells are statistically similar, and therefore we conclude that the motion of the attB site is driven by E. coli DNA segregation as it is being replicated. The histogram of λ DNA locations along the cell’s axis on the right shows that the attB site is pushed toward regions enriched with λ DNA. Although infection may block initiation of DNA replication temporarily, replication that has already been initiated is not affected (19).

Integration Frequency Measurements in Strains with the attB Site at Different Genomic Locations Are Consistent with a Replication-Driven Mechanism. To provide further evidence that the movement of the attB site toward the phage DNA is driven by chromosome...
infecting the midcell region may integrate when the
both DNA locations can reach the genomes of phages infecting
and its symmetrical counterpart have a similar frequency, and
\( \lambda \)

Discussion

moved to the terminus.

measured for the different strains (Fig. 4). The native
mycin plates and the number of colonies on LB plates was then
lication (16). The integration frequency defined as the ratio
DNA at the terminus remains close to midcell throughout rep-

segregation, we moved the \( attB \) site at 17 min to two other ge-
omic positions: one at 51 min in a nearly symmetrical position
relative to \( oriC \), and the other at the terminus region. Bacterial
DNA at the terminus remains close to midcell throughout replica-
tion (16). The integration frequency defined as the ratio
between the number of colony-forming units grown on kana-
mycin plates and the number of colonies on LB plates was then
measured for the different strains (Fig. 4). The native \( attB \) site
and its symmetrical counterpart have a similar frequency, and
both DNA locations can reach the genomes of phages infecting
at poles and midcell. In contrast, the frequencies of the native
\( attB \) site and its symmetrical counterpart are significantly higher
than when the \( attB \) site is at the terminus, because only phages
infecting the midcell region may integrate when the \( attB \) site is
moved to the terminus.

Fig. 2. Constrained diffusion of \( \lambda \) DNA. Mean square displacement (MSD)
between pairs of \( \lambda \) DNAs in doubly infected \( E. coli \) cells as a function of time.
Each gray trace represents the mean square distance between a pair of \( \lambda \)
DNAs in the same cell. The average of all traces (blue) shows constrained
diffusion with a confinement radius of \( \sim 120 \) nm. The snapshots, taken at 20
min intervals during one run, illustrate the small motion of both \( \lambda \) spots.

Discussion

In order for bacteriophage \( \lambda \) DNA to integrate and establish
stable lysogeny after infecting an \( E. coli \) cell, a unique 15-bp-long
integration site must be located among \( \sim 5 \) million possible sites
on the bacterial chromosome. In this work, we aimed at elucidat-
ing the strategy used by \( \lambda \) phage to find the integration site
with high efficiencies and within a physiologically relevant time.
We have visualized the dynamics of the \( attB \) and \( attP \) sites in live
\( E. coli \) cells during infection. Contrary to our initial expectations,
it is not \( \lambda \) DNA that carries out a search throughout the cellular
interior, either by diffusion or by exploiting native mechanisms to
move directionally as many viruses infecting eukaryotic cells do
(21, 22), nor by deploying facilitated transport mechanisms as
transcription factors do in their search for their cognate sites (1,
23). Instead, \( \lambda \) DNA remains confined near the point of entry
into an infected cell, and takes advantage of the directed motion
provided by the segregation of the nascent nucleoid driven by
bacterial DNA replication machinery (15, 24), to bring the \( attB \)
site of the bacterium close to the phage \( attP \) site. Once in close
proximity, confined diffusion of both attachment sites facilitates
their final encounter.

What constrains \( \lambda \) DNA to remain near the point of entry into
the cell? Most likely, \( \lambda \) DNA becomes anchored to a site on the
cellular membrane through which the \( \lambda \) DNA entered the cell.

Extant experimental evidence suggests association of the phage
DNA to the membrane is mediated by early gene \( N \) expression
(25–27). Despite \( \lambda \) DNA’s circularization and its likely compac-
tion upon entry into the cell (28), both of which reduce the ef-
fective physical size of the molecule within the cell, reduced
diffusion by crowding is unlikely to hinder \( \lambda \) DNA movement
significantly (29).

To address whether the genomic location of the \( attB \) site
affects the integration frequency, we moved the \( attB \) site to two
different locations on the \( E. coli \) chromosome. The chromosome
in \( E. coli \) is organized with the origin of replication (\( oriC \) site)
and the terminus region at midcell, and the two chromosome
halves located at separate cell halves (16, 30). Chromosomal
replication is initiated at the \( oriC \) site and proceeds in both
directions (31). In slow-growing \( E. coli \) cells, replication and
chromosome segregation are continuous and simultaneous (32).
The replicated \( oriC \) sites are segregated and captured approxi-
mately at each quarter-cell position, which will become the new
cell centers after division, and other loci lie roughly in the po-

Right) A histogram of \( \lambda \) DNA positions measured along the bacterial axis in the same coordinates.

Fig. 3. (Left) Infection does not aeffect the dynamics of the integration site on the \( E. coli \) chromosome. Distance between the \( attB \) site and the cell’s center as a function of the time elapsed after the last cell division, for un-
infected (blue) and infected cells (red). Each plot represents an average over
ten traces measured in individual bacteria, and error bars represent SEs. The
distance is measured along the long cell axis in coordinates in which 0 and 1
correspond to the cell center and pole, respectively. The overlap between
the two plots indicates that within experimental error, the effect of infection
on the motion of the \( attB \) site is not appreciable. (Right) A histogram of \( \lambda \) DNA
Bacillus subtilis nants were selected by a linked and unique binding sites for the two different fluorescently tagged ParB by PCR, and confirmed by sequence analysis. These measured in strains with Tal et al. PNAS (41) was used to create the recombinant derivatives of phage λ functions were supplied by pSIM18. Following isolation of recombinants, each of these sites was assayed by PCR.

These results are consistent with a search driven by DNA segregation during replication.

We believe that this mechanism can be generalized to other temperate bacteriophages infecting different types of bacteria, because many of its key features are preserved. It has been shown that different bacteriophages enter cells preferentially once intracellular, the phage DNA of different bacteriophages through the poles (18). Evidence has also been provided that some segregation, either due to entropic (24) or other forces differences in chromosome organization and segregation, chromosomal loci within the bacterial chromosome [e.g., λ](34), will drive global movement of chromosomal loci within the bacterial chromosome (33). Furthermore, many temperate phages integrate at specific sites on the bacterial chromosome [e.g., φ80 infecting E. coli (34), φ105 infecting Bacillus subtilis (35), and P22, which infects Salmonella typhimurium (36)]. Although different types of bacteria exhibit differences in chromosome organization and segregation, chromosome segregation, either due to entropic (24) or other forces (37), will drive global movement of chromosomal loci within the bacteria, and we anticipate that this movement will assist in the encounter between the bacterial and phage DNA attachment sites.

Materials and Methods

Bacterial Strains and Phage Construction. A general recombineering protocol (41) was used to create the recombinant derivatives of phage λ and MG1655, containing the parS sites used in this paper. To create the MG1655 strains carrying the pMT parS sites on the chromosome, the λ red recombination functions were supplied by pSIM18. Following isolation of recombinants, pSIM18 was removed by growing at 42 °C. The p1 parS (near attP on bacteriophage λ) and pMT parS (near attB in the bacterial genome) recombinants were selected by a linked kan^R or cm^R cassette, respectively, screened by PCR, and confirmed by sequence analysis. These parS sites serve as specific and unique binding sites for the two different fluorescently tagged ParB proteins, P1 ParB and pMT ParB, expressed from the plasmid (see SI Materials and Methods). The sequences of P1 par5-kan and pMT par5-cat are shown in SI Materials and Methods (sequences S1 and S2) and were PCR amplified from a bacterial strain generously supplied by the Stuart Austin laboratory.

The PCR product of P1 par5-kan was made with chimeric primers RG3 and RG4 (Table S1) that contain homologies at their 5′ ends to insert the cassette near attB on λ. To do this, strain LT447, MG1655 cI857Δ parSΔ (41), carrying a single copy of λ in the prophage state was grown to midlog, and Red functions were induced from the intact prophage at 42 °C to carry out the recombination step (41). Following electroporation, cells were left to grow at 42 °C to allow continued λ gene expression and lytic growth. A phage lysate containing recombinants was harvested and used to lysogenize MG1655 at 32 °C. Recombinant λ lysogens were selected as kan^R colonies. A kan^R single-copy lysogen was used to generate a high-titer, pure λΔ par5-kan^R lysate. The P1 par5 site was confirmed by sequence analysis.

A deletion of the native attB site in MG1655 was made using oligonucleotide RG21 (Table S1) following standard recombineering protocols. Colonies were screened for recombinants by mismatch amplification mutation assay PCR (41), and the identified recombinants were purified and confirmed by sequence analysis. This MG1655 derivative carrying ΔattB was used to insert the wild-type attB with the cat-pMT par5 cassette at two different locations on the genome by recombineering as described below.

A PCR product of the pMT par5 site linked to cm^R was made using chimeric primers RG1 and RG2 (within the bioD gene) or RG7 and RG8 (within the bioA gene) that carried homologies at their 5′ ends to target insertion of the pMT parS site near attB at 17 min (Table S1). The PCR product was recombineering into MG1655 using standard protocols, selecting CM^R. We then PCR-amplified the entire attB-cat-pMT par5 cassette at bioA using chimeric primers with 5′ homologies to target the cassette to the chromosome locations at 34 or 51 min. The primer sequences are given in Table S1; RG25 and RG26 were used to make the PCR product targeting the 34-min region, and RG29 and 30 were used for the 51-min region product. The MG1655 ΔattB mutant was the recipient strain so that the newly inserted intact attB site associated with the pMT parS site becomes the only cellular site for λ integration. Each par5 site was verified by sequence analysis. Note that the homology segments of the primers (Table S1) can be used by the reader to determine the exact location of each attB-pMT par5 insertion in the genome sequence (see Sequences S1 and S2 in SI Materials and Methods).

Microscopy. Images were taken with a modified Zeiss Axiovert 135TV microscope. Phase contrast and fluorescence images were collected using a Neoplan 100x 1.3 phase contrast objective (Zeiss) with temperature controlled to 32 °C and an Ixon EMCCD (Andor Technology). The objective lens is placed on a piezo mount that is controlled by a Mipos100 piezo controller (Piezosystem Jena). The piezo is used for z-scanning in a contrast detection autofocus algorithm, before image acquisition. All filters were purchased from Semrock. For RFP and yGFP images, filters FF01-503/572-25 for excitation, FF445/520/590-DI01 as dichroic were used. The yGFP and RFP emissions were split using a FF562-DI02 dichroic mirror, and are then imaged on half of the camera CCD chip with the RFP and yGFP emission filters FF01-628BS2 and FF01-535S2, respectively. Phase-contrast pictures were taken with the 2.5× integration. The microscope is equipped with a XY motorized stage (PI), which enables automatic data acquisition and taking multiple fields of view in every experimental run. Phase contrast and fluorescent images were taken at 10-min intervals.

Experimental Growth Conditions. Cells from the bacterial strain with parS in the bioD region were propagated in LBMM medium (LB supplemented with 0.2% maltose and 10 mM MgSO_4_2 and grown at 32 °C overnight from a single-colony inoculum. The cultures were diluted 1:100 into LBMM medium supplemented with 10 mM MgSO_4_2, 0.2% maltose, 0.4% glycerol, 0.05% casamino acids, 1 μM thiamine, 1 μM uracil, and 100 μM isopropyl β-D-thiogalactopyranoside and grown at 32 °C to OD_600 of 0.6. Cells were then mixed with λ phage particles at a MOI of ~3 for 5 min in room temperature. Cells were then deposited on a low-melting agarose pad (1.5%), prepared with AB medium with supplements, and covered with glass slides before mounting on the microscope.

Measurement of Integration Frequency. Cells from the bacterial strain with parS in the bioA region (WT) and the strains with the attB site at different locations were propagated in LBMM medium supplemented with 50 μM ampicillin at 37 °C overnight from a single-colony inoculum. Cells were then diluted 1:200 into 8 mL TB medium (10 g/L Bacto-tryptone, 5 g/L NaCl, pH 7.2) supplemented with 0.2% maltose and 10 mM MgSO_4_2 and grown for 5 h. Cells were then centrifuged and resuspended in 4 mL, TMG buffer [10 mM Tris (PH 7.4), 10 mM MgSO_4_2, 100 μM gelatin] for 30 min at 30 °C. Cells...
were diluted 1:5 in TM buffer (10 mM Tris (pH 7.4), 10 mM MgSO$_4$), the OD of all strains was set to OD = 0.5 and then mixed with phage lysate at MOI = 3 to a 100-μL final volume at 30°C for 1 h. Four milliliters of LB were then added, and the cells were incubated for 1 h at 30°C before plating. The cells were serially diluted to give single colonies and plated on LB and LB supplemented with 30 μg/mL Kanamycin plates overnight at 30°C. For calculation of integration frequency, the number of lysogens and the number of cells surviving infection, as colonies growing on Kanamycin plates. The integration frequency is defined as 100 times the ratio between the number of colonies growing on Kanamycin plates; the number of cells surviving infection was measured as the number of colonies growing on LB plates. The integration frequency was defined as 100 times the ratio between the number of lysogens and the number of cells surviving infection, as colonies growing on Kanamycin plates.

**Supporting Information**

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**SI Materials and Methods**

**Plasmid Construction.** Plasmid pFHC2973 (1) was the basic plasmid and carried the genes for CFP-Δ30ParB and yGFP-pMT1Δ23ParB. These tagged constructs were a generous gift from Stuart J. Austin. The CFP site was exchanged to mCherry, which was introduced into pFHC2973 as a PCR fragment carrying a Bgl II site in two steps. First, Fw-NarI and Rev-Bgl II primers were used in one reaction and Rev-EcorI and Fw-Bgl II primers were used in a second reaction to amplify fragments from the plasmid pFHC2973. The resulting PCR products were mixed and used as a template for a third PCR that was amplified with Fw-NarI and Rev-EcorI primers. The PCR product of the second step and pFHC2973 backbone were cut with the restriction enzymes NarI and EcorI, and the last cut insert was ligated into the backbone plasmid. A PCR mCherry fluorescent protein (RFP) fragment carrying the restriction sites Eco RI and Bgl II was amplified with Fw-mCherry and Rev-mCherry primers from the plasmid pFPV-mCherry (Addgene). The purified PCR product of the mCherry insert and the pFHC2973 plasmid carrying the Bgl II site were cut with EcoRI and Bgl II, and the insert was ligated into the pFHC2973 backbone. The modified pFHC2973 plasmid carrying mCherry-Δ30ParB and yGFP-pMT1Δ23ParB is termed p2973. The plasmids were transformed into DH5α Escherichia coli competent cells (Invitrogen) and colony PCR was performed to check for correct fragment insertion by using one primer annealing to the insert and the other to the plasmid backbone sequence. Clones were sequenced and plasmids were transformed into the MG1655 derivatives used in the paper. All PCR primers are listed in Table S1.

**Preparation of Δ Stock.** Bacteria were grown overnight in LBMM (LB supplemented with 0.2% maltose and 10 mM MgSO₄). Cells were diluted 1/10 in 2 mL fresh TB (10 g/L Bacto-tryptone, 5 g/L NaCl, pH 7.2) containing 0.2% maltose, 10 mM MgSO₄ and incubated at 32 °C for 2.5 h. Then 0.25 mL of the cells was mixed with 0.3 mL from a single phage plaque suspended in 1 mL of TM (10 mM Tris base, 10 mM MgSO₄, pH 7.4) and incubated at room temperature for 10 min. Top Agar (10 g/L Bacto-Tryptone, 5 g/L NaCl, 7 g/L agar, pH 7.2) at 50 °C was then added to the cells and poured on TB plates supplemented with 0.3% glucose, 75 µM CaCl₂, and 2 mM MgSO₄ and incubated for 3.5 h at 37 °C. When plaques on the plates began to exhibit a lacy appearance, they were harvested by adding 5 mL TM to the surface of each plate. Plates were put overnight in 4 °C, and the next day, the TM from the surface of each plate was collected together with a scrape of the top surface of the agar. Forty microliters of chloroform was added, and the lysate was centrifuged at 8000 × g for 10 min at 4 °C. The supernatant, which contains high-titer, pure phage particles, was collected and stored at 4 °C.

**Image Segmentation.** All image processing and data analysis were performed using Matlab (MathWorks). Cell recognition was performed on phase contrast images of cells using a program developed in our laboratory. The program applies morphological operations, using the Matlab image processing toolbox. The program’s output was checked manually in all experiments and corrected for errors in recognition.

**Fluorescent Spot Recognition and Tracking.** The fluorescent spots were identified and tracked using a version of the IDL Particle Tracking software (www.physics.emory.edu/~weeks/idl/) (2), adapted to Matlab by D. Blair and E. Dufresne (http://physics.georgetown.edu/matlab). Briefly, the images were first spatially filtered with a band-pass filter to eliminate pixel noise. Fluorescent spots were then identified to pixel resolution by finding the maximum of each spot, followed by subpixel centroid identification of the spot. Then the tracking procedure was used to track each spot throughout the experiment.

**Image Registration.** To calculate the distance between two fluorescent foci of different wavelengths, images were registered into the same coordinate system. Control points that were evenly spread across the RFP image were mapped to their appropriate points in the yGFP image. Local weighted mean mapping was then used to correct for registration errors that arise locally without allowing their influence to extend to the rest of the space. This mapping is a weighted sum of second-order polynomials determined locally around each point (3).

**Calculation of the Radius of Confinement from MSD Measurement.** Two fluorescent spots are diffusing in a confined space. To cancel out drift of the frame with time, bacterial movement and growth effects, we use the distance between the spots to calculate the MSD. We denote r1 and r2 as the position of the two spots, and the MSD is defined as:

\[
\text{MSD}(t) = \left( (r_1(t + \tau) - r_2(t + \tau)) - (r_1(t) - r_2(t)) \right)^2
\]

For long enough \( \tau \), there is no correlation between the position of the spots and the MSD reaches a plateau that depends on the radius of confinement of the spots. Because there is no correlation, the probability of a spot to be in a volume \( dV \) inside the confining volume \( V \) is \( dV/V \), and the joint probability of all of the spots is simply the multiplication of the single spot probability:

\[
\text{MSD}(\infty) = \frac{1}{V^2} \int \int \int \int [ (r_1(\infty) - r_2(\infty))^2 + (r_1(t) - r_2(t))^2 - 2(r_1(\infty) - r_2(\infty))(r_1(t) - r_2(t)) ] dV
\]

Because the integral of \( r \) is 0, the last term cancels out. The first two terms are equal:

\[
\text{MSD} = \frac{2}{V} \int \int [ (r_1 - r_2)^2 ] dV
\]

\[
\text{MSD} = \frac{2}{V^2} \int \int [ (x_1 - x_2)^2 + (y_1 - y_2)^2 ] dV = \frac{4}{V^2} \int \int (x_1 - x_2)^2 dV
\]

\[
\text{MSD} = \frac{4}{V^2} \int \int (x^2 + y^2 - 2x_1x_2) dV
\]

Again, the last term equals 0 and the first two terms are equal:

\[
\text{MSD} = \frac{8}{V} \int \int \int r^2 \cos^2 \theta \sin^2 \theta \cos \phi d\phi \sin \phi d\theta d\phi = \frac{8}{V} \int \int \int r^2 dV = \frac{8}{V} \int \int \int r^2 \cos^2 \phi \sin^2 \theta \cos \phi d\phi \sin \phi d\theta d\phi = \frac{8R^2}{V}
\]
We find that the plateau in the MSD is equal to $8/5 R^2$ with the confinement radius $R$.

Sequence S1: The P1 parS-kan Cassette in λ Phage. The λ sequences used as targeting homology are in blue. The kan is in green, the P1 parS fragment is in black.

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TGAATGAACTGGCCGCAGCGCTCGGGAATGATCCAGATTGTTTTGCTACCACCACCATGTTATGGTATTTAATTTAGAATGACTAGTTCTGTGCAAGCAACTTTCATATATTGGAATTTGGGAGTTCCTGGTATGAGTCAGCAACACCTTCTTCACGAGGAGAAAGGTTGTTCCTCAATGCAATCGA TACGTTAGCTGCGAAGTGAACACTATCCCATATCACGCTACACCGTCTTTCATTGCCATACGGAATTCGGAGCATTCATCAGGGCGGGCAAGAATGTTAATTTCTCCTGCGAAGTGATCTTCCGTCACAAGGGCTTCCCGGTATCAACAGGGACACCAAGATTTATTCTGCGAAGTG
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Sequence S2: The pMT parS Cassette. The cat gene is in green, the pMT parS fragment is in black. The primer pairs will add homology at the 5' and 3' end of this cassette to target it for recombination at a specific site on the bacterial genome.

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GAGGTTGAAAAGCGTGGTGATTTCAGTGTGAAAGAA
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Fig. S1. Labeling scheme of λ phage attP and bacterial attB sites. An E. coli strain carrying the pMT parS site near the attB integration site on the bacterial genome and a modified bacteriophage λ containing the P1 parS near attP were constructed by recombineering methods. These parS sites serve as specific and unique binding sites for the two different fluorescently tagged ParB proteins, P1 ParB-RFP and pMT ParB-yGFP, expressed from the same plasmid (p2973) from an inducible promoter. (A) After infection but before integration, the expressed ParB proteins label their respective parS sites, allowing monitoring of the labeled loci through the microscope. (B) After λ phage DNA integration at the attB site, the genomic distance between parS sites is ∼12 kbp and the physical distance is much less than the optical resolution.

Fig. S2. Labeling with parS/ParB systems does not affect integration frequency. Comparison of the integration frequency of λcl857 P1 parS kanR phage DNA into MG1655 strains containing a pMT1-parS site inserted either in the bioD or bioA genes, with the integration frequency of λcl857 bor::kanR (1) into a MG1655 strain that does not contain a parS site. The modified MG1655 strains were transformed with plasmid p2973 (see Materials and Methods). The frequency of integration is defined as 100 times the ratio of lysogens to the number of surviving cells. The methods are the same as for Fig. 4 (see Materials and Methods), except for the addition of 100 μM Isopropyl β-D-1-thiogalactopyranoside to induce ParB expression from the plasmid p2973. Error bars denote SEs from three independent experiments. This control shows that ParB polymerization does not interfere with λ DNA integration. Note that strain λcl857 bor::kanR has been shown to have similar integration frequency as a wild-type λ (1). As in the experiments reported in Fig. 4, within each experiment, the number of colonies grown on LB plates for the different strains did not vary by more than 9%, i.e., the number of survivor cells per total cell input at the start is strain independent.


Fig. S3. Time-lapse images of an infected cell in the lysis pathway. The images are an overlay between the fluorescent channel of the λ DNA (red) and the phase contrast image. Time indicated is the time from infection. As the λ DNA replicate, the fluorescent focus becomes diffuse and eventually the cell lyses.
Fig. S4. Time-lapse images of infection of a cell leading to lysogeny. The images, taken every 10 min, show the ParB-RFP locus (red) marking the attP site and the ParB-yGFP locus (yellow) marking the attB site on the phage DNA. The ParB-RFP and the ParB-yGFP loci are separated by ~12 kbp when lysogeny is established. The scale bar at $t = 0$ denotes 3 $\mu$m.

Fig. S5. Measurements of the distance between the yGFP and RFP foci in lysogenic cells. The distance was measured in lysogenic cells expressing the ParB proteins and grown under the same conditions as in the experiments. The mean distance $197 \pm 67$ nm reflects the accuracy limit of our experimental setup.

Fig. S6. Measurement of integration time in bulk cultures using PCR methods. Cells were grown following the same procedure as for the experiments performed under the microscope, except that DNA samples were extracted at the indicated times with TRIzol (Ambion), following the manufacturer’s instructions. Time is measured after a 10-min incubation of cells with bacteriophages. DNA was extracted from a lysogen culture (+) or from uninfected cells (-) as controls. Insertion of the $\lambda$ phage into the attB site was verified using PCR with primers B-1 and AT-FWR that amplify the boundary between the phage and the bacterial genomic DNA. The expected size of the PCR product is 1150 bp. For primer sequences, see Table S1.
Fig. S7. Infection of a cell with an immunity region present. What happens to λ DNA when a phage infects a lysogenic cell? To address this question, we cloned the cI gene and its control region into the bacterial chromosome lac operon (1), and followed the fate of λ DNA following infection. As shown, the λ DNA does not replicate with the bacterial DNA, and is diluted away by being passed to one of the daughter cells following cell division. On long time scales, the λ DNA foci disappear. We assume that eventually they are degraded. Time-lapse images of immune cells that are infected with λ phage. The images are an overlay between the fluorescent channel of the λ DNA (red) and the phase contrast image. Time indicated is the time from infection. The λ DNA does not replicate, and cells carrying the λ DNA are diluted through divisions, when only one of the daughter cells receives the λ DNA.


Table S1. Sequences of primers used for clone and plasmid preparation

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<th>Primer</th>
<th>Sequence (5’ to 3’)</th>
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