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Chromosome arrangement within a bacterium

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Background: The contour length of the circular chromosome of bacteria is greater than a millimeter but must be accommodated within a cell that is only a few micrometers in length. Bacteria do not have nucleosomes and little is known about the arrangement of the chromosome inside a prokaryotic cell.

Results: We have investigated the arrangement of chromosomal DNA within the bacterium *Bacillus subtilis* by using fluorescence microscopy to visualize two sites on the chromosome simultaneously in the same cell. Indirect immunofluorescence with antibodies against the chromosome partition protein Spo0J were used to visualize the replication origin region of the chromosome. Green fluorescent protein fused to the lactose operon repressor LacI was used to decorate tandem copies of the lactose operon operator *lacO*. A cassette of tandem operators was separately inserted into the chromosome near the origin (359°), near the replication terminus (181°), or at two points in between (90° and 270°). The results show that the layout of the chromosome is dynamic but is principally arranged with the origin and terminus maximally apart and the quarter points of the chromosome in between.

Conclusions: The use of cytological methods to visualize two chromosomal sites in the same cell has provided a glimpse of the arrangement of a bacterial chromosome. We conclude that, to a first approximation, the folding of the bacterial chromosome is consistent with, and may preserve, the linear order of genes on the DNA.

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Background

All cells face the challenge of packing their genome into a small space. Genomes are DNA molecules of extraordinary length, often hundreds of times longer than the cells in which they are contained, and must be highly compacted to form the chromosomes of higher cells and the nucleoids of bacteria. At the same time, the genome must remain functional and accessible to the DNA replication, chromosome segregation, and gene transcription machinery of the cell. In eukaryotes, this problem is solved, in part, by wrapping DNA around nucleosomes and then wrapping the nucleosomes into higher-order structures to create fibers of approximately 30 nm in diameter. Despite the high degree of compaction, the nucleosome structure remains dynamic. Chromatin is, for example, subject to reversible covalent and non-covalent modifications that render specific genes accessible to the transcription machinery in response to specific regulatory signals.

Bacteria, on the other hand, do not have nucleosomes, and how the circular chromosome of many prokaryotes is folded in the nucleoid is largely unknown [1]. Some compaction is achieved by maintenance of the DNA in a supercoiled state as a result of underwinding [2,3]. Abundant proteins that bind to DNA and cause it to bend are

likely to achieve most compaction; these proteins include HU, HNS, FIS and IHF [4–6]. Of special significance is the discovery in bacteria of homologs of a family of eukaryotic 'structural maintenance of chromosomes' proteins (SMCs), which are involved in chromosome folding [7]. Mutants of *smc* in *Bacillus subtilis* exhibit decondensed chromosomes and defects in chromosome segregation [8,9]. Mutants of *Escherichia coli mukB*, which is a functional analog of SMC, exhibit similar defects in chromosome structure and segregation [10].

We are concerned here with the architecture of the chromosome in the bacterial cell. How is the circular genome organized within the 'nucleoid' and what is the location of specific regions on the chromosome within the cell? This problem has been difficult to investigate because of the small size of bacteria (~2 μm in length) and the absence of methods for visualizing specific regions of the chromosome within cells. Recently, however, the application of cytological methods in conjunction with fluorescence microscopy has made it possible to localize specific chromosomal segments within the intact cell. These methods include the use of fluorescence *in situ* hybridization (FISH) [11,12], the insertion into the chromosome of tandem copies of the lactose operon operator, which can

be visualized by the use of green fluorescent protein (GFP) fused to the lactose operon repressor LacI (GFP–LacI) [13–17], and the use of GFP fused to, or antibodies raised against, chromosome partition proteins that bind to the replication origin region of the chromosome in certain bacteria [18–20]. In the case of *B. subtilis*, the partition protein known as Spo0J binds to a cluster of at least eight sites spread out over a distance of about 400 kb around the origin of replication [21] and is believed to cause the origin region to fold into a higher-order structure [8,21]. The use of these methods has led to the conclusion that during chromosome segregation the replication origin region of the chromosome tends to be located towards the cell pole at the outer edge of the nucleoid and that the terminus tends to be located towards the cell center. In newborn cells with a single origin, the replication origin region is generally near one pole of the cell and the terminus near the other.

To investigate the organization of the chromosome within the cell, we visualized two sites simultaneously on the chromosome of *B. subtilis*. To visualize the replication origin region, we used antibodies against the Spo0J protein [18,19,21,22] and immunofluorescence microscopy. The second site was visualized by insertion into the chromosome of a *lacO* cassette, which was decorated with GFP–LacI [15,16]. Because the *lacO* cassette could be inserted at various sites, we reasoned that we would be able to compare simultaneously the position of the replication origin region with that of any other site on the chromosome at which the cassette had been inserted.

Results and discussion
### Co-localization of Spo0J and the replication origin region
First, we investigated whether Spo0J would co-localize with a *lacO* cassette that had been inserted into the chromosome (at 359°) near the origin of replication in a strain (AT63) that had been engineered to produce GFP–LacI. A complication was that the fixation procedure employed to detect Spo0J by immunofluorescence lowered the level of fluorescence from GFP–LacI (compared with the level in unfixed living cells). Nonetheless, sufficient signal was retained for both Spo0J (red) and GFP–LacI (green) to be detected in the same cell. Cells were grown at 25°C in defined minimal medium with glucose as the carbon source. The low temperature increased the intensity of the GFP fluorescence and the relatively slow growth rate (doubling time of ~100 min) reduced the number of initiation events per cell.
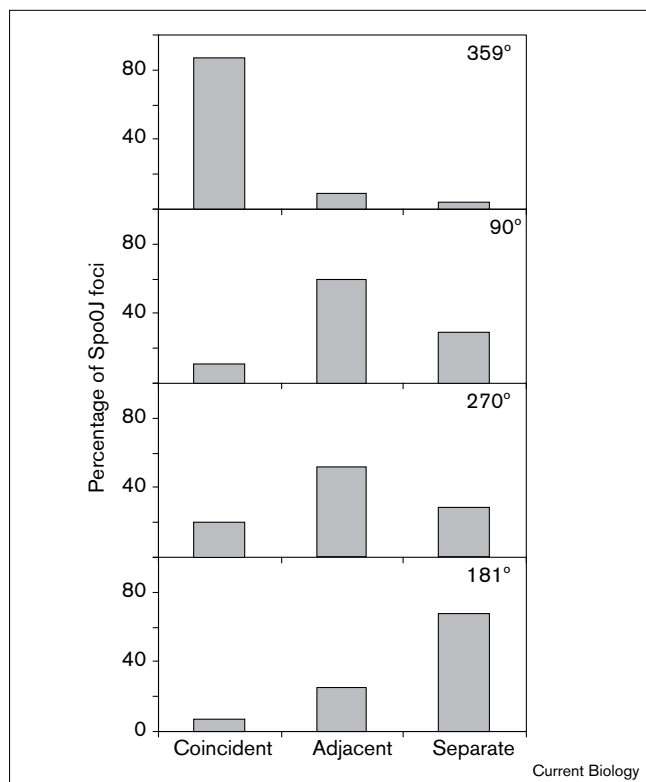
The field of cells displayed in Figure 1a shows that fluorescence from Spo0J partially or substantially coincided with that from the GFP–LacI-decorated *lacO* cassette (the yellow color in the overlay panel indicates coincidence of GFP fluorescence and immunofluorescence). The position of the fluorescent foci relative to the boundaries of the cells can be seen in the Nomarski image, although, because of the lysozyme treatment in the immunofluorescence procedure, the images are less clear than for untreated living cells.

Figure 1

Simultaneous localization of Spo0J and a *lacO* cassette that has been inserted at one of four different sites on the chromosome. **(a)** Cells harboring a *lacO* cassette at 359° (origin; strain AT63). **(b)** Cells harboring a *lacO* cassette at 90° (strain AT64). **(c)** Cells harboring a *lacO* cassette at 270° (strain AT61). **(d)** Cells harboring a *lacO* cassette at 181° (terminus; strain AT62). Each row contains four images of the same field; for (b–d), the two rows show two separate sample fields. The first panel in each row is an immunofluorescence image of Spo0J, the second panel is an image of fluorescence from GFP–LacI bound to the operator cassette, the third panel is a Nomarski differential interference contrast (DIC) image, and the last panel is a color-coded overlay of the GFP fluorescence (green) and Spo0J immunofluorescence (red). Spo0J immunodetection micrographs were deconvoluted by Exhaustive Photon Reassignment software (Scanalytics) to remove out-of-focus background light.

lacO position — Spo0J — GFP — Nomarski — Overlay
(a) 359°
(b) 90°
(c) 270°
(d) 181°

Current Biology
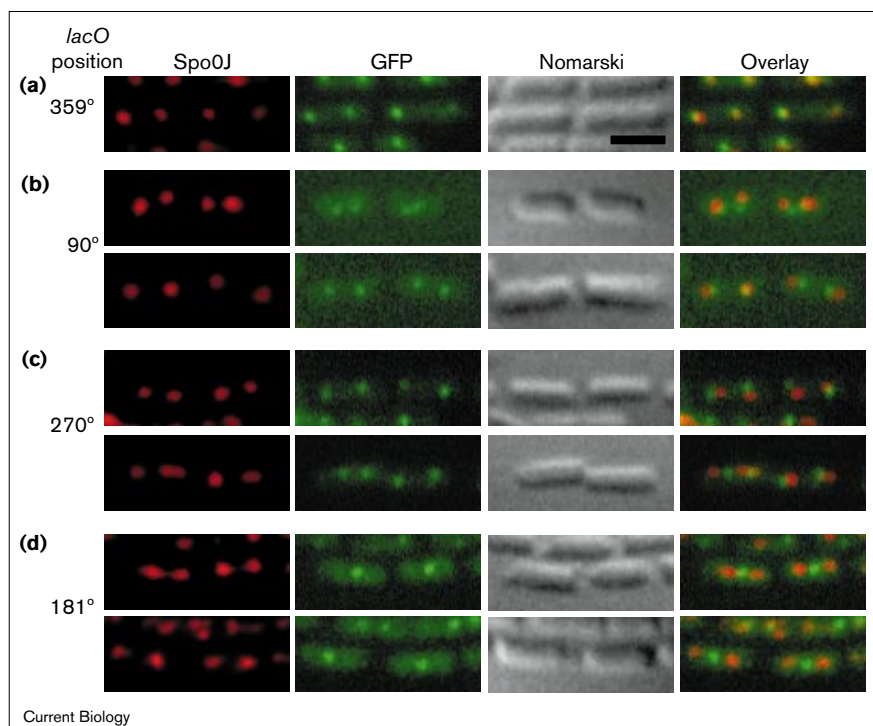
Figure 2

Distribution of distances between Spo0J foci and *lacO* cassette foci. Immunofluorescence microscopy using Spo0J-specific antibodies was used to detect Spo0J localization in strains producing GFP–LacI and containing a *lacO* cassette that had been inserted into the chromosome at 359°, 90°, 270°, or 181°. All Spo0J foci were divided into three classes: those with a spatially coincident GFP signal (referred to in the figure as 'Coincident'), those with an overlapping but not quite coincident GFP signal ('Adjacent'), and those that could be resolved and separated from any GFP signal ('Separate'). The total number of Spo0J foci counted for 359°, 90°, 270° and 181° were 123, 175, 270 and 164, respectively.

Tabulating the results for 123 Spo0J foci, we found that 87% of the foci were coincident with a *lacO* cassette. A further 9% overlapped with, but were not substantially superimposable with, the *lacO* cassette (Figure 2); in such cases, the *lacO* cassette seemed to lie 'adjacent' to the Spo0J focus. In the remaining 4% of the cells there was no visible signal from the GFP–LacI, and so we could not determine whether Spo0J was closely associated with the *lacO* cassette. In summary, the cytological evidence indicates that, within the resolution limits of light microscopy, Spo0J molecules principally localized at or near the replication origin region of the chromosome, a finding that is fully consistent with the results of previous investigations of the subcellular distribution of Spo0J [18,19,21,22].

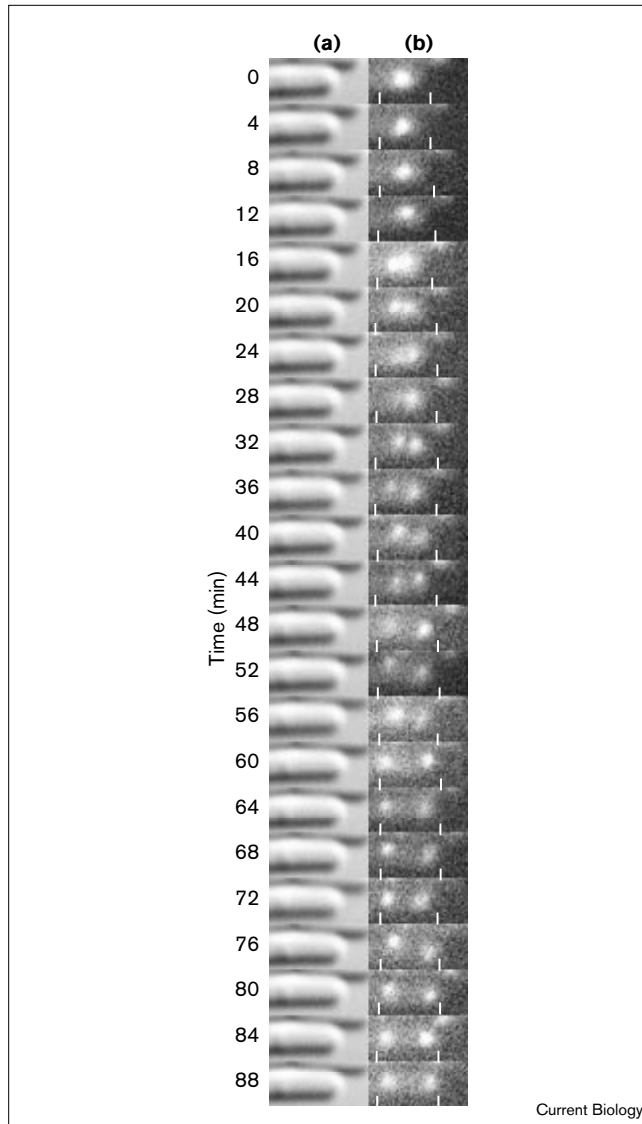### Spo0J movement during the cell cycle

To investigate further the association of Spo0J with the replication origin, we examined the behavior of Spo0J

during the cell cycle. Using time-lapse fluorescence microscopy and the *lacO* cassette decorated with GFP–LacI, we had previously reported that, after duplication, replication origin regions move towards opposite poles of the cell, often separating abruptly during a brief period of the cell cycle [16]. We found that Spo0J behaves in a similar fashion. We recorded the movement of Spo0J in living cells using a GFP fusion and time-lapse microscopy. Starting with a cell having a single Spo0J focus at the mid-cell position, we observed duplication of the focus and movement of the resulting foci towards opposite poles of the cell (Figure 3).

The speed of separation from the time (12–16 min) when two Spo0J foci were first resolvable to the time (60 min) when they stopped moving apart was 0.025 µm/min. From 11 such time-course experiments, we calculated an average speed of separation of about 0.08 µm/min (s.d. = 0.06 µm/min) and an average distance of separation of 1.1 µm (s.d. = 0.4 µm) when measured between the centers of the two foci.
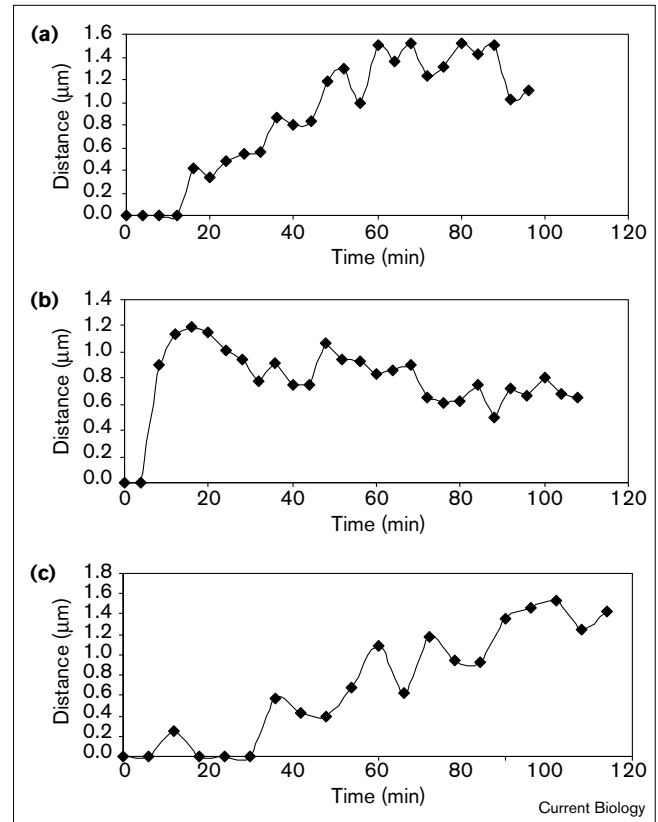
These measurements underestimate the maximum velocity because a substantial portion of the total separation of foci was often observed to occur over a short period of the cell cycle (although this was not the case in the example of Figure 3). Figure 4 plots the separation of Spo0J foci as a function of time for three time-course experiments. Figure 4a corresponds to the time-course of Figure 3, whereas Figure 4b documents a case in which most separation occurred over a short time. Measuring the rate of movement during the period of most rapid separation of Spo0J foci for 11 time-course experiments, we calculated an average peak velocity of separation of 0.17 µm/min (s.d. = 0.07 µm/min) and a maximal velocity of 0.31 µm/min. *In toto*, these results are similar to those obtained previously using the *lacO* cassette to monitor movement of the origin region. We conclude that Spo0J co-localizes with the replication origin region of the chromosome and that it remains associated with, and behaves in a manner similar to, the origin region during the entire course of the cell cycle.

The velocities that we observe for Spo0J–GFP and GFP–LacI are approximately an order of magnitude more rapid than those recently reported by Sharpe and Errington [23] for Spo0J–GFP. One possible explanation for the discrepancy is that our measurements were collected for cells that were undergoing a duplication of a single origin focus whereas those of Sharpe and Errington [23] were collected from cells undergoing a duplication of two foci. A second explanation could be that our measurements were collected from individual living cells over the course of the cell cycle whereas those of Sharpe and Errington [23] were collected from separate fixed cells of a DNA replication mutant at various times after a temperature shift.

Figure 3

Time-lapse images of Spo0J–GFP movement during the cell cycle. Cells producing a Spo0J–GFP fusion (strain RL1746) were grown to mid-exponential phase in defined minimal glucose medium and then placed on a slide coated with minimal medium that had been solidified with 1% agarose. Time-lapse photography was used to follow **(a)** cell growth and **(b)** Spo0J–GFP movement in a single cell. Pictures of (a) Nomarski DIC and (b) GFP fluorescence were taken at 4 min intervals. Time elapsed from the start of the sequence is indicated on the left. Cell boundaries are indicated for reference by white ticks on the fluorescence image.

Figure 4

Graphical representation of the time-course of separation of Spo0J–GFP foci during the cell cycle. **(a)** Plot corresponding to the time-course of Figure 3 showing gradual separation of Spo0J–GFP foci. **(b)** Plot showing abrupt separation of Spo0J–GFP foci. **(c)** A second example of more gradual Spo0J–GFP separation, as in (a).

Although the use of Spo0J–GFP and of the *lacO* cassette decorated with GFP–LacI yielded similar results, some differences were noted between the two procedures. For example, the percentage of cells with a single fluorescent focus from the origin region was higher with the *lacO* cassette method (29%) than with Spo0J–GFP (10%; A.A.T., Undergraduate Honors Thesis, Harvard Univer-

sity, 1998). Conversely, the percentage of cells with two replication origin foci was lower with the *lacO* cassette method (67%) than with Spo0J–GFP (77%). We suspect that insertion of the *lacO* cassette in the origin region and its decoration with GFP–LacI causes a slight perturbation of the normal processes of chromosome replication and segregation. If, as postulated previously, Spo0J causes the origin region of the chromosome to fold up into a higher-order structure [8,21], then perhaps the insertion of tandem copies of *lacO* decorated with GFP–LacI interferes with the proper assembly of this structure. In addition, the presence of the *lacO* cassette bound by GFP–LacI might also delay separation of newly replicated origin regions.

Finally, we note that Spo0J foci visualized either by indirect immunofluorescence (Figure 1) or by use of a GFP fusion (data not shown) appeared to be somewhat larger than the foci from *lacO* cassettes decorated with GFP–LacI. Conceivably, the larger size of Spo0J foci reflects the fact that the partition protein binds to multiple sites dispersed over a wide region around the replication origin [21].

Visualizing the origin and the terminus regions of the chromosome in the same cell

Knowing that Spo0J could be reliably used to visualize the replication origin region of the chromosome, we visualized the replication origin and terminus regions of the chromosome simultaneously in the same cell. For this purpose we used antibodies against Spo0J to visualize the origin region and GFP–LacI to visualize the terminus region in a strain (AT62) in which a cassette of tandem *lacO* repeats had been inserted into the chromosome at 181°. Examples of cells that had been fluorescently labeled in this way at the origin and the terminus regions are shown in Figure 1d. In confirmation and extension of previous results with cells that were singly labeled at the origin or the terminus [15,16], the images show that Spo0J foci were generally localized near the cell poles and that fluorescence from GFP–LacI was principally visible near the mid-cell position. Most often, only a single focus of GFP–LacI was present, which presumably represented either one terminus region which had not yet replicated or two newly replicated terminus regions which had not separated sufficiently to be resolved. Sometimes, however, two distinct GFP–LacI foci could be seen near the mid-cell, indicating that the terminus region had replicated and the two sister chromosomes had moved apart sufficiently to be resolved by light microscopy. These results are entirely consistent with those of Niki and Hiraga [12], who recently used FISH to visualize the origin and terminus regions of the *E. coli* chromosome.

Tabulating the separation of origin and terminus regions for 164 images (Figure 2), we found that in most cases (68%) Spo0J foci were clearly resolved and separated from the GFP–LacI foci. In a minority (25%) of the images the terminus signal partially overlapped with, but was not coincident with, the origin signal; that is, the Spo0J and GFP–LacI foci seemed to be adjacent. Finally, in a small number of cases (7%) the Spo0J focus was coincident with the GFP–LacI signal. Thus, in sharp contrast to the distribution pattern observed with cells harboring the operator cassette in the origin region, these results indicate that the origin and terminus regions generally occupy distinct regions in the cell.

Resolving the replication origin region from 90° and from 270° on the chromosome

Knowing that the replication origin and the terminus are generally well separated from each other in the cell, we investigated the disposition of two sites lying at intermediate positions on the chromosome. For these experiments we created GFP–LacI-producing strains harboring the *lacO* cassette at positions corresponding to 90° (strain AT64) and 270° (strain AT61) and used Spo0J as a marker for the origin region of the chromosome. We observed three principal patterns of localization (Figure 2). In the first pattern, representing the predominant class (60% and

52%, respectively, for the 90° and 270° cassettes; Figure 2), GFP–LacI foci were seen at positions immediately adjacent to the Spo0J foci. Examples of this pattern are shown in Figure 1b,c. In the second pattern (representing 29% and 28%, respectively, for the 90° and 270° cassettes; Figure 2), Spo0J foci were clearly separated from GFP–LacI foci. Often, in these cases, the Spo0J foci were located near the cell poles and one or two GFP–LacI dots were observed at or near the mid-cell position. In the third pattern (representing 11% and 20%, respectively, for the 90° and 270° cassettes), the GFP–LacI foci were coincident with the Spo0J foci. We conclude that the 90° and 270° sites on the chromosome are generally situated at distinct positions in the cell from the replication origin region. Also, because GFP–LacI foci at the 90° and 270° loci were predominantly located 'adjacent' to Spo0J foci whereas GFP–LacI foci at the terminus region were predominantly 'separated' from Spo0J foci, we infer that 90° and 270° in the chromosome are generally located at intermediate positions in the cell between the origin and terminus regions (Figure 5).

Another way to analyze the images is to count the number of GFP–LacI dots in cells exhibiting two polar Spo0J foci. When the *lacO* cassette is located near the terminus, cells are frequently observed that have two Spo0J foci near opposite poles and only one GFP–LacI focus. As discussed above, this pattern corresponded to cells in which the terminus region of the chromosome had not yet undergone replication or cells in which the chromosome had been completely duplicated but the termini had not separated sufficiently to be resolved by microscopy. Tabulating the results for cells with bipolar foci of Spo0J, we found that 77% of cells harboring a *lacO* cassette at 181° had one GFP–LacI dot, whereas only 28% and 20% of cells harboring a *lacO* cassette at 90° and 270°, respectively, had this localization pattern. Most cells (that is, the

Figure 5
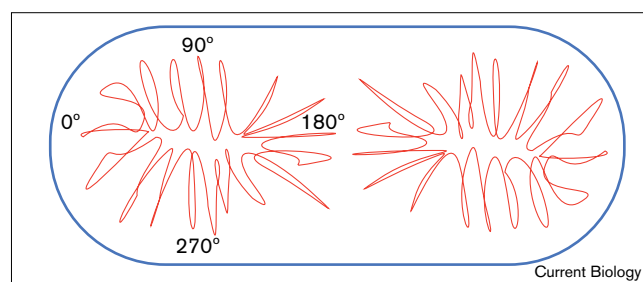
A model of chromosome arrangement in the cell. The cartoon shows the nucleoid as a dense core with loops extruding towards the cell membrane. In general, the DNA is locally compacted (that is, it doesn't string back and forth between the poles of the cell), and the chromosome is usually arranged such that origin regions are towards the cell poles, termini are towards the mid-cell position and the 90° and 270° regions are in between.

remaining 72% and 80%, respectively) containing bipolar Spo0J foci and harboring a *lacO* cassette at either 90° or 270° had two GFP–LacI dots. Our interpretation of these results is that the 90° and 270° regions of the chromosome separate earlier than does the terminus region, which indicates that segregation propagates from the origin through the 90° and 270° regions towards the terminus.

Time-lapse microscopy of strains in which the *lacO* cassette was integrated into the 181° region of the chromosome showed dynamics of separation similar to the origin regions, though not as fast and as far apart [16]. In this study, the 90° and 270° regions were found to separate in a rapid fashion similar to the origin and terminus regions of the chromosome (data not shown), which suggests that the force that separates the *B. subtilis* chromosomes is applied not only at the origin but also, at least, at the most distant and the intermediate regions.

The arrangement of the chromosome is not fixed

Our findings are consistent with the view that the chromosome is often arranged in the nucleoid with the origin at one end, the terminus at the other end, and 90° and 270° sites in between (Figure 5). It is important to emphasize, however, that this is a preferred arrangement, and that the chromosome can, and frequently does, exhibit other patterns. For example, when the *lacO* cassette was near the terminus, which is usually near the mid-cell position, 12% (*n* = 315) of the cells exhibited a localization pattern in which the GFP–LacI signal was closer to the cell pole than was the Spo0J focus. Similarly, when the *lacO* cassette was at 90° or 270°, fluorescent dots from GFP–LacI were found at a more polar position than Spo0J in 23% and 21% of the cases respectively (*n* = 92 and *n* = 147 respectively). These observations reinforce the findings from time-lapse studies indicating that the chromosome is dynamic and undergoes substantial changes in location and organization during the course of the cell cycle.

Conclusions

We have used fluorescence microscopy to visualize two sites on the chromosome simultaneously in the same cell. We used indirect immunofluorescence with antibodies against the partition protein Spo0J to visualize the replication origin region of the chromosome and GFP–LacI to visualize tandem copies of *lacO* that had been separately inserted into the chromosome at four quarter points (359°, 90°, 181° and 270°). We draw two principal sets of conclusions, one concerning the location of Spo0J on the chromosome and the other concerning the overall architecture of the chromosome.

First, in confirmation and extension of previous work [18,19,21,22], we conclude that Spo0J binds near the origin of DNA replication and that it remains associated with the origin region throughout the cell cycle. This conclusion is

based on the high proportion of Spo0J foci that were closely associated with foci from a *lacO* cassette that had been inserted at the origin and on the similarity in the behavior of Spo0J (as visualized by use of a GFP fusion) in time-lapse images of living cells to that previously observed for *lacO*. We also note that Spo0J foci (as visualized either by indirect immunofluorescence or by use of a fusion to GFP) were somewhat larger than *lacO* foci. This observation is consistent with, and could be a reflection of, the fact that Spo0J binds to multiple sites spread out over a large distance around the origin of DNA replication [21].

With regard to the architecture of the chromosome, we conclude that the arrangement of the chromosome is dynamic and that individual sites can be found at a variety of locations in the cell. Nonetheless, in the main, the origin and the terminus regions of the chromosome tend to be maximally apart whereas the 90° and 270° positions on the chromosome tend to exhibit an intermediate level of separation from the origin (Figure 5). Thus, the use of cytological methods to visualize two chromosomal regions simultaneously has given us a glimpse of the arrangement of the chromosome in a bacterial cell. To a first approximation, the folding of the chromosome is consistent with, and may preserve, the linear order of genes on the DNA.

Materials and methods

Strain constructions
RL1746, which corresponds to DCL233 [18], produces Spo0J–GFP and contains a *spo0J* null mutation. The Spo0J–GFP fusion protein is functional and the strain is phenotypically Spo0J+ [18].

An integration plasmid (pAT19) for directing the synthesis of GFP–LacI under the control of the promoter for the *veg* gene in the chromosome was described previously [16]. In the present work, a derivative of pAT19 was created by site-directed mutagenesis using the primers ATO19 (5′-CCAACACTTGTCACTACTCTCACTTATGGTGTTCAA-TGC-3′) and ATO20 (5′-GCATTGAACACCATAAGTGAGAGTAGT-GACAAGTGTTGG-3′). This created pAT27, which contained a Phe to Leu change at codon 64 of *gfp–lacI* in addition to the Ser to Thr change already present at codon 65. The resulting F64L, S65T double-mutant GFP was several-fold brighter in our experiments than the S65T single-mutant protein. The pAT27 plasmid was introduced into the chromosome of *B. subtilis* strain PY79 by transformation followed by selection for resistance to erythromycin (Erm^R; 5 µg/ml) to yield strain AT60.

Four plasmids were created to insert a cassette of *lacO* tandem repeats at either 359°, 90°, 181° or 270° on the *B. subtilis* chromosome. Each plasmid was derived from pAT12 [15], which contains a chloramphenicol-resistance gene and 256 copies of *lacO*, by insertion of a copy of a segment of DNA from each of the four chromosomal positions. Plasmids pAT14 and pAT15 contain sequences from the **181°** (*cgeD*) and 359° (*spo0J*) regions of the chromosome, respectively, as described previously [15]. pAT26 (90°) was created by insertion of a 0.7 kb segment of DNA from *yheH* between the *Aat*II and *Bam*HI sites of pAT12. The 0.7 kb DNA fragment was amplified by PCR from the chromosome of strain PY79 using primers ATO17 (5′-TCTAGACGTCAAGGCGAAACCGTAG-3′) and ATO18 (5′-TCTAG-GATCCTGGGTTTTTGAGCGT-3′) and was then digested with *Aat*II and *Bam*HI. Similarly, pAT25 (270°) was created by introducing into the *Hind*III site of pAT12 a 1 kb DNA from *cotS* that was amplified from

chromosomal DNA by PCR using as primers ATO13 (5′-TCTAG-GATCCATCGACCATGTGGCG-3′) and ATO14 (5′-TCTAGGATC-CGTCAATTCGCCTCCC-3′) and then treated with *Hind*III.

To generate strains harboring *lacO* cassettes at 270°, 90°, 181° and 359°, plasmids pAT25, pAT26, pAT14 or pAT15 were used to transform wild-type PY79 followed by selection for chloramphenicol resistance (5 µg/ml) to create AT52, AT53, AT54 and AT55. These were then transformed with chromosomal DNA from AT60, selecting for Erm^R, to generate strains AT61, AT64, AT62 and AT63, respectively, which contain a *lacO* cassette and produce GFP–LacI. Finally, to increase the number of *lac* operators in these strains, AT61, AT62, AT63 and AT64 were grown at 30°C on Luria–Bertani (LB) plates containing an elevated concentration of chloramphenicol (25 µg/ml), a procedure that selected for tandem duplications of the integrated *lacO*-cassette-containing plasmids.

Growth conditions and fluorescence microscopy
To obtain reasonably bright GFP–LacI signals, strains were grown in the following manner before fixation for immunofluorescence microscopy. Cell cultures (2 ml) were prepared by inoculating L broth with single colonies from each strain and allowed to grow for 6 h at 37°C. This culture was then diluted at various concentrations into defined minimal glucose medium [24] and allowed to grow overnight (~12 hr) at 25°C. During growth, fluorescence from GFP–LacI was dim. As cells reached stationary phase, the GFP–LacI signal became brighter, probably as a result of more and more GFP molecules reaching maturation, which is a slow process. Later, as the cells became older, the intensity of GFP–LacI signal decreased. The culture with the brightest signal was diluted 1:10 into minimal medium (2 ml) and allowed to grow at 25°C for 110 min. Time-lapse photomicroscopy showed that a subpopulation of cells began to grow immediately after dilution and that approximately half of the cells were growing by 75 min after dilution [16]. The GFP–LacI signal become less bright 110 min after dilution, perhaps due to the dilution of mature GFP–LacI among progeny cells.

Cells were fixed for immunofluorescence using methanol (MeOH) as modified from a previously described procedure [25]. This was found to preserve GFP fluorescence better than fixation with glutaraldehyde and paraformaldehyde [15]. Culture (1 ml) growing in minimal medium was collected by centrifugation and resuspended in 30–50 µl of its own medium. This volume of cells was then injected into 1 ml 99% MeOH previously equilibrated at −20°C, gently rocked back and forth, and then rapidly placed back at −20°C for at least 10 min. These cells were then placed on a poly-lysine coated slide and allowed to dry fully (~5 min) before lysis and incubation with antibodies. Cells were blocked using 2% BSA/PBS overnight at 4°C. Polyclonal rabbit anti-Spo0J antibodies [18] were used at a 1:16,000 dilution for 1 h at room temperature. Anti-rabbit Cy3 secondary antibodies (Molecular Probes) were used at a 1:300 dilution and also incubated for 1 h at room temperature.

Nomarski and GFP images were acquired and processed as previously described [16]. Spo0J images were deconvoluted to remove out-of-focus light as follows. A piezoelectric device was installed on the objective lens of the microscope to move the plane of focus by 0.1 µm increments in the direction perpendicular to the microscope stage. An electric shutter was also installed to electrically control emission of the excitation light from the ultraviolet bulb of the fluorescence microscope. Image acquisition, shutter control and voltage on the piezoelectric device were all controlled by Metamorph image acquisition software. A stack of 40 images was acquired for each field of interest with a vertical spacing of 0.1 µm. The stack was then deconvoluted by the Exhaustive Photon Reassignment software package (Scanalytics) to a convergence of 1×10^{-4} with a smoothing factor of 10.

Time-lapse images of Spo0J–GFP movement
Time-lapse images of Spo0J–GFP movement were obtained using strain RL1746 as described previously [16], except that the images were collected during the mid-exponential phase of cell growth.

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