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Identification and Characterization of a Bacterial Chromosome Partitioning Site

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Summary

We have identified a DNA site involved in chromosome partitioning in *B. subtilis*. This site was identified in vivo as the binding site for the chromosome partitioning protein Spo0J, a member of the ParB family of partitioning proteins. Spo0J is a site-specific DNA-binding protein that recognizes a 16 bp sequence found in *spo0J*. Allowing two mismatches, this sequence occurs ten times in the entire *B. subtilis* chromosome, all in the origin-proximal ~20%. Eight of the ten sequences are bound to Spo0J in vivo. The presence of a site on an otherwise unstable plasmid stabilized the plasmid in a Spo0J-dependent manner, demonstrating that this site, called *parS*, can function as a partitioning site. This site and Spo0J are conserved in a wide range of bacterial species.

Introduction

Efficient chromosome partitioning ensures the stable inheritance of genetic material to progeny cells. In eukaryotes, the spindle pole body, the bipolar mitotic spindle, motor proteins, the centromere, kinetochore, and cohesion proteins are parts of the mitotic apparatus that function in concert to ensure faithful segregation of chromosomes. In contrast to the situation in eukaryotes, the underlying components and mechanisms governing chromosome partitioning in prokaryotes have not been well defined (reviewed in Hiraga, 1992; Wake and Errington, 1995). Whereas several bacterial genes have been identified that are involved in chromosome partitioning, *cis*-acting DNA sequences have yet to be defined. By determining the binding site for the chromosome partition protein Spo0J, we have identified a DNA sequence in the *Bacillus subtilis* chromosome that functions as a partitioning, or centromere-like, site.

The *B. subtilis spo0J* gene product is required for efficient chromosome partitioning during vegetative growth and sporulation. Approximately 1.5% of the cells in a growing culture of a *spo0J* mutant are anucleate, a frequency ~100-fold greater than that of wild-type cells (Ireton et al., 1994). Spo0J and Soj (encoded by the gene upstream from and cotranscribed with *spo0J*) are similar to the ParB (SopB) and ParA (SopA) family of plasmid-encoded partition proteins, respectively (Ogasawara and Yoshikawa, 1992). Chromosomally encoded homologs of ParB have been found in a wide range of bacterial species, including *Pseudomonas putida*, *Caulobacter crescentus*, *Streptomyces coelicolor*, *Streptococcus pneumoniae*, *Mycobacterium leprae*, *Helicobacter pylori*, and

Streptococcus pyogenes. *C. crescentus* ParB is involved in chromosome partitioning (Mohl and Gober, 1997), and it is likely that the basic mechanism by which ParB homologs function in partitioning is conserved.

Most of what is known about the biochemical function of the ParB family of proteins comes from work with ParB from the P1 prophage and SopB from the F plasmid of *E. coli* (reviewed in Nordström and Austin, 1989; Austin and Nordström, 1990; Hiraga, 1992; Williams and Thomas, 1992). ParB (SopB) binds to a centromere-like sequence, *parS* (*sopC*), located immediately downstream of the *parB* gene. *parA* (*sopA*), immediately upstream of *parB* (*sopB*), encodes an ATPase that interacts with ParB. All three components, ParA, ParB, and *parS*, are required for plasmid partitioning. One predominant model for plasmid partitioning proposes a pairing function for ParB proteins (Nordström and Austin, 1989; Austin and Nordström, 1990; Williams and Thomas, 1992). It is thought that plasmids are paired via interaction between ParB–*parS* complexes from two plasmids. Concurrent with or subsequent to pairing, positioning occurs such that each daughter cell receives a plasmid. Recent experiments have shown that the Par system is required for proper subcellular localization (positioning) of the plasmid. During the course of the *E. coli* cell cycle, the P1 and F plasmids move from midcell to the 1/4 and 3/4 positions along the length of the cell (Gordon et al., 1997; Niki and Hiraga, 1997). Loss of *sopABC* results in improper positioning of F plasmids (Niki and Hiraga, 1997).

Recent work has indicated that the origin of replication (*oriC*) of the *B. subtilis* (and *E. coli*) chromosome is in a defined orientation for most of the bacterial cell cycle (Glaser et al., 1997; Gordon et al., 1997; Lin et al., 1997; Webb et al., 1997). In newborn cells, the origin region is positioned near the pole of the nucleoid body, oriented toward a cell pole. After replication of this region, one of the two origins rapidly moves toward the opposite pole of the nucleoid. This movement indicates the function of a mitotic-like apparatus for separating sister origin regions (Gordon et al., 1997; Webb et al., 1997).

Studies with *C. crescentus* and *B. subtilis* have shown that the chromosomally encoded ParB/Spo0J proteins are needed for proper chromosome partitioning (Ireton et al., 1994; Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997; Mohl and Gober, 1997). The existence of a partitioning site(s) bound by Spo0J has been inferred from the similarity to the family of plasmid ParB proteins and the subcellular localization of Spo0J (Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997; Mohl and Gober, 1997). The subcellular localization of Spo0J is similar to that of the origin region, and Spo0J appears to colocalize with the origin-proximal ~30% of the *B. subtilis* chromosome (Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997). These localization experiments led to the idea that Spo0J associates with a site(s) in the origin-proximal region of the chromosome that functions in chromosome partitioning.

In this paper, we describe the identification and characterization of the binding site for Spo0J. This site,

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named *parS*, is a 16 bp sequence containing an imperfect 8 bp inverted repeat and is found in the *spo0J* gene. Cloning a single *parS* into an otherwise unstable plasmid stabilizes the plasmid in a Spo0J-dependent manner. A search of the recently completed *B. subtilis* genome (Kunst et al., 1997), allowing for two mismatches from the site in *spo0J*, revealed the existence of ten potential binding sites, which are all located in the origin-proximal ∼20% of the chromosome. Eight of these sites are bound to Spo0J in vivo. We propose that the binding of Spo0J to these multiple *par* sites is involved in pairing newly replicated origin regions before the rapid separation mediated by a mitotic-like apparatus.

Results

Identification of the Spo0J Binding Site In Vivo

By analogy to the plasmid partition systems, we suspected that the Spo0J binding site would be near the *spo0J* gene, approximately 10 kb from the *B. subtilis* origin of replication. To identify DNA associated with Spo0J in vivo, we used a chromatin immunoprecipitation assay (Solomon and Varshavsky, 1985; Hecht et al., 1996; Strahl-Bolsinger et al., 1997) (see Experimental Procedures). In brief, formaldehyde was added to cells during exponential growth to cross-link protein and DNA, cells were lysed, and the DNA sheared to an average size of approximately 500–1000 bp. The Spo0J–DNA complexes were then immunoprecipitated using affinity-purified polyclonal antibodies against Spo0J, the cross-links reversed, and the precipitated DNA analyzed by PCR.

Four sets of primers were used in the PCR assay to test for the presence of different chromosomal regions in the immunoprecipitate. Each primer set specifically amplified a different size fragment, ranging from ∼200 to ∼620 bp. DNA from the *spo0J* region was specifically immunoprecipitated, while little or no DNA was detected from three other chromosomal regions (Figure 1). Furthermore, in parallel experiments, no DNA was detected from a *spo0J* null mutant (Figure 1, lane 3). These results indicate that Spo0J, or protein closely associated with Spo0J, binds to a site(s) in or near the *spo0J* gene.

The Spo0J binding site was defined more precisely by cloning DNA fragments into a multicopy plasmid and testing in vivo for binding to Spo0J using the same approach. Plasmid pIK219 contains an ∼760 bp restriction fragment that includes the 3′ end of *spo0J* and extends ∼540 bp downstream (Figure 2A). Spo0J was able to bind to this plasmid in vivo (Figure 2A), but not to the parent vector (Figure 2C), indicating that the insert contains a Spo0J binding site(s). Subclones of pIK219 were constructed to further define the binding site (Figure 2A). A 55 bp fragment, contained in pDL90A, was sufficient to confer binding to Spo0J (Figure 2). This 55 bp fragment is internal to *spo0J*, indicating that the Spo0J binding site was located in *spo0J*.

Further analysis defined a 16 bp sequence in pDL90A, composed of an imperfect 8 bp inverted repeat, that is able to bind Spo0J in vivo. Several derivatives of pDL90A were constructed and tested for binding to Spo0J (Figures 2B and 2C). Deletion of the inverted repeat (pDL105)
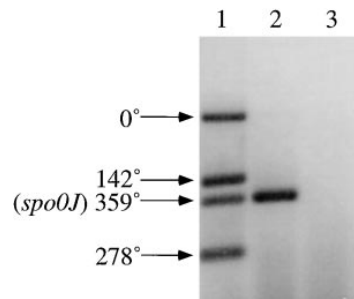

Figure 1. In Vivo Association of Spo0J with DNA from Near the *spo0J* Gene

Affinity-purified antibody was used to immunoprecipitate Spo0J from cell extracts after formaldehyde cross-linking in vivo (see Experimental Procedures). After reversal of the cross-links, DNA in the immunoprecipitate was amplified by PCR using four sets of primer pairs from four different regions of the chromosome: 0° (∼620 bp), 142° (∼380 bp), 278° (∼200 bp), and 359° (∼330 bp, in *spo0J*). (The ∼4200 kbp *B. subtilis* chromosome is 360°, and the origin of replication is at 0°/360°. One degree is ∼11.7 kbp). The PCR products were separated on an agarose gel. The four primer pairs were used together in PCR with total chromosomal DNA (lane 1); DNA from the immunoprecipitate from wild-type cells (lane 2); and DNA from the immunoprecipitate from a *spo0J* null mutant (lane 3). The chromosomal location of the PCR products are indicated to the left.

or base changes in seven positions of the inverted repeat (pDL106) greatly reduced or eliminated the ability of Spo0J protein to cross-link to the plasmid in vivo (Figure 2C). In contrast, pDL104, which contains only a 16 bp insert with the 8 bp imperfect inverted repeat could be cross-linked to Spo0J (Figure 2C). Thus, the 16 bp sequence, 5′-TGTTCCACGTGAAACA-3′, interacts with Spo0J in vivo, either directly or indirectly.

Spo0J Is a Site-Specific DNA-Binding Protein

The in vivo cross-linking immunoprecipitation results did not address whether the specificity of Spo0J for the DNA site was due to Spo0J or another factor. Since formaldehyde is capable of cross-linking protein to protein and protein to DNA, the possibility remained that Spo0J was interacting with another protein, which provided the specificity of interaction with the 16 bp site. To address whether Spo0J itself binds specifically to the site, we performed gel mobility shift assays with Spo0J protein.

Purified Spo0J protein was able to bind, in vitro, to a DNA fragment containing the 16 bp site identified in the in vivo experiments. Hexa-histidine-tagged Spo0J, which functions in vivo, was purified (Experimental Procedures) and tested for binding to a radiolabeled 24 bp DNA fragment containing the wild-type site from within the *spo0J* gene (Figure 3A). Half-maximal DNA binding was observed at a Spo0J concentration of ∼300 nM, and there was one major shifted band (Figures 3A and 3B). Use of a larger DNA fragment as a probe resulted in multiple slower-migrating bands, indicating that several molecules of Spo0J were binding per DNA fragment (data not shown). Formation of these larger shifted species appeared to be cooperative (data not shown).

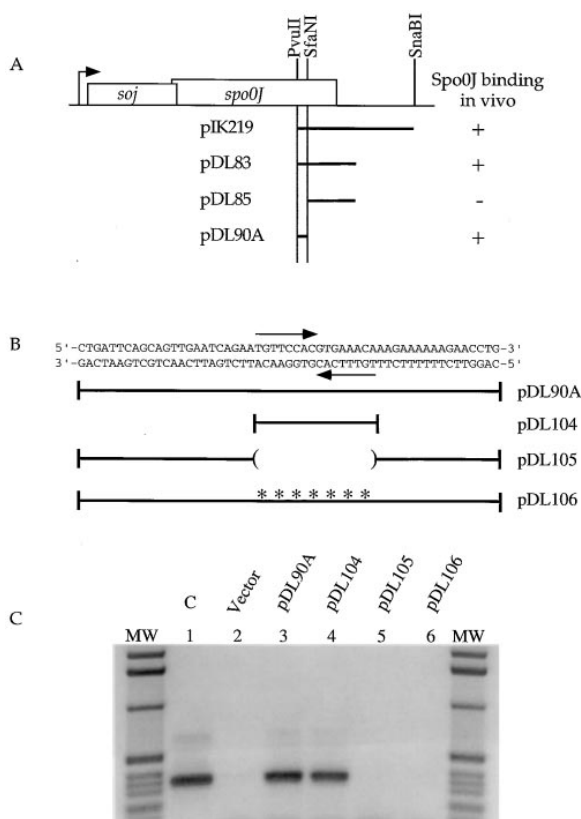The specificity of Spo0J binding to the 16 bp site was

Figure 2. In Vivo Identification of the Spo0J Binding Site Located in the *spo0J* Gene

Plasmids containing different inserts from the *spo0J* region were tested in vivo for binding to Spo0J using formaldehyde protein–DNA cross-linking and immunoprecipitation. Immunoprecipitated DNA was analyzed by PCR using plasmid-specific primers.

(A) The *soj-spo0J* operon is drawn schematically, and the inserts contained in different plasmids are indicated below. The presence (+) or absence (−) of the four different plasmids in the immunoprecipitate is indicated. The Spo0J binding site was contained in the 55 bp PvuII–SfaNI fragment in plasmid pDL90A.

(B) The 16 bp sequence containing an 8 bp imperfect inverted repeat is indicated by arrows above and below the sequence of the 55 bp insert in pDL90A. The inserts contained in plasmids pDL104, pDL105, and pDL106 are drawn schematically. pDL106 contains seven changes from the wild type (5′-CGTGCCCAGGGAGACC-3′; underlined bases are mutant).

(C) The 16 bp sequence is the Spo0J binding site. PCR reactions with plasmid-specific primers and the indicated DNA. Lane 1: control with purified vector DNA. Lanes 2–6: immunoprecipitated DNA from strains containing the indicated plasmid. MW = molecular weight markers.

demonstrated by competition experiments with different unlabeled DNA fragments. A 24 bp fragment containing 7 changes in the 16 bp site (mutant) was not an efficient competitor compared to the 24 bp fragment with the wild-type site (Figures 3C and 3D). Approximately 45-fold more of the mutant fragment was needed to compete to the same extent as the wild type (Figure 3D). Similar results were obtained with a different competitor with a completely unrelated sequence (data not shown). Taken together, these results demonstrate that Spo0J binds directly to DNA in a site-specific manner.

Spo0J Binds to Multiple Sites in the Origin-Proximal Region of the Chromosome

We identified a total of ten potential Spo0J binding sites (including the one in *spo0J*) in the entire *B. subtilis* chromosome by inspection of the published genomic sequence (Kunst et al., 1997). The genome was searched with the sequence in *spo0J*, and up to two mismatches were allowed. All ten sites are located in the origin-proximal ~20% of the chromosome. Using PCR with primers specific for each region, eight of the ten potential binding sites were detected in the Spo0J immunoprecipitate, although at varying levels (Figure 4A). The map location of each of these eight sites is indicated in Figure 4B.

To test the relative affinity or occupancy of Spo0J binding to these sites, we compared the relative amounts of each site in the immunoprecipitate. This was accomplished by comparing the amount of PCR-amplified DNA from serial dilutions of the Spo0J immunoprecipitate to that from dilutions of the total input DNA before the immunoprecipitation reactions. Six of the sites, located at 4°, 359° (in *spo0J*), 356°, 355°, 354°, and 330°, were most abundant in the immunoprecipitate (Figure 4A) and therefore are designated as "strong" sites. The amount of DNA from each of these sites was approximately 5- to 25-fold greater than that for the sites located at 15° and 40° (Figure 4A). Potential sites at 31° and 347° were not detected in the Spo0J immunoprecipitate (data not shown).

A consensus Spo0J binding sequence, 5′-TGTTNCAC GTGAAACA-3′, was derived from alignment of the eight sites (Figure 4A). Four of the strong sites, 4°, 359° (in *spo0J*), 354°, and 330°, contained perfect matches to the consensus. Two strong sites, 356° and 355°, differ from consensus in a single position, one weak site, 15°, differs from consensus in one position, whereas the other weak site, 40°, differs in two positions (Figure 4A). The two potential sites that are not bound detectably in vivo both differ in two positions (Figure 4). Additional potential binding sites, some outside of the *oriC* region, can be found in the *B. subtilis* genome by allowing for more mismatches. We have not tested for Spo0J binding to these other sequences.

Mutations in Six Sites Cause Increased Binding to a Weak Site

Because a null mutation in *spo0J* causes an ~100-fold increase in the frequency of anucleate cells (Ireton et al., 1994), we reasoned that elimination of most (or all) of the Spo0J binding sites might also cause an increase in the frequency of anucleate cells. To test this, we deleted five of the sites (4°, 40°, 330°, 354°, and 356°) that are not located in open reading frames. Each site was replaced in the chromosome with a different drug resistance cassette (Experimental Procedures). In addition, 7 bp (of 16) in the site in *spo0J* were changed without affecting the amino acid sequence of the gene product (see Experimental Procedures). This is the same 7 bp mutation that does not bind Spo0J in vivo (Figure 2) and that does not compete well in the in vitro binding assay (Figures 3C and 3D).
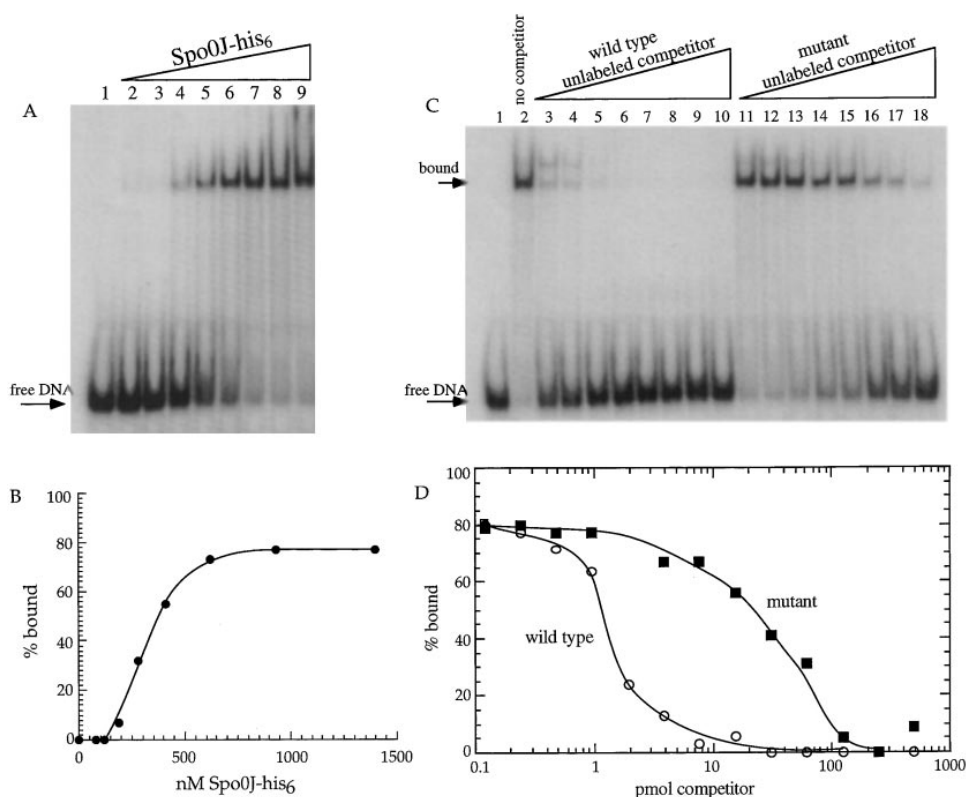
Figure 3. Site-Specific Binding of Spo0J to DNA In Vitro

Gel mobility shift assays were used to measure binding of purified Spo0J-his$_6$ protein to DNA. In all cases, a radiolabeled 24 bp DNA fragment, containing the 16 bp Spo0J binding site (as determined in vivo) was used as a probe.

(A) Gel shift assays were performed with ~1.5 fmol radiolabeled DNA mixed with various concentrations of purified Spo0J protein in a reaction volume of 15 μl: no protein (lane 1), 80 nM (lane 2), 120 nM (lane 3), 190 nM (lane 4), 280 nM (lane 5), 410 nM (lane 6), 620 nM (lane 7), 930 nM (lane 8), and 1400 nM (lane 9).

(B) Percent of radiolabeled DNA bound (100 − % free) is plotted as a function of the concentration of Spo0J protein. At the highest protein concentrations, ~80% of the probe DNA was bound. Half-maximal binding was at a protein concentration of ~300 nM. Data are from the exeriment in (A). Similar results were obtained in several experiments.

(C) Competition experiment with mutant and wild-type sites. ~1.5 fmol of the radiolabeled 24 bp DNA fragment was incubated in 15 μl reactions with either no protein (lane 1) or 710 nM Spo0J (lanes 2–18). Competition assays were performed with increasing amounts of unlabeled DNA fragments of 24 bp containing either the wild-type (lanes 3–10) or mutant (lanes 11–18) Spo0J binding sites. The mutant contained the 7 bp changes indicated in Figure 2. Amounts of competitor DNA were: none (lane 2), 1.93 pmol (lanes 3 and 11), 3.85 pmol (lanes 4 and 12), 7.7 pmol (lanes 5 and 13), 15.4 pmol (lanes 6 and 14), 30.8 pmol (lanes 7 and 15), 61.6 pmol (lanes 8 and 16), 123 pmol (lanes 9 and 17), and 246 pmol (lanes 10 and 18).

(D) Percent of radiolabeled DNA bound (100 − % free) is plotted as a function of the concentration of unlabeled wild-type competitor (open circle) or unlabeled mutant competitor (closed square). Data are from an experiment similar to that shown in Figure 3C.

The strain with six sites inactivated, DCL484, had only a small increase (~5-fold) in the frequency of anucleate cells compared to wild type, far less than that of a *spo0J* null mutant (data not shown). This lack of a strong partitioning defect appears to be due to compensation by other Spo0J binding sites. DNA from the Spo0J binding site at 15° was 10- to 20-fold more abundant in immunoprecipitates from the multiple-site mutant compared to that from wild type (Figure 5). These results indicate that at least some of the Spo0J binding sites are occupied more often in the absence of other sites. We suspect that this increased occupancy compensates for loss of multiple binding sites.

The Spo0J Binding Site Is a Partitioning Site

A single Spo0J binding site was able to confer a partition function to a heterologous replicon. We cloned the binding site into an unstable, low-copy *B. subtilis* plasmid and tested for effects on plasmid stability. pDL110 contains a chloramphenicol resistance marker for selection in *B. subtilis*, and the origin of replication and the gene encoding the replication initiation protein from pLS32, a plasmid originally isolated from *B. natto* (Hassan et al., 1997). To test for plasmid stability, we measured the fraction of cells containing a plasmid after several generations of growth in the absence of selection (Experimental Procedures).

A plasmid with a functional Spo0J binding site was much more stable than plasmids without the binding site. When grown in the presence of chloramphenicol to select for the presence of the plasmid, the percentage of cells containing the vector without a Spo0J binding site (pDL110) was only 10%–15%, compared to ~60% for the plasmid (pDL125) with a Spo0J binding site (Table 1; Figure 6A). During growth without selection, the vector was rapidly lost; after ~20 generations <0.1% of the

A

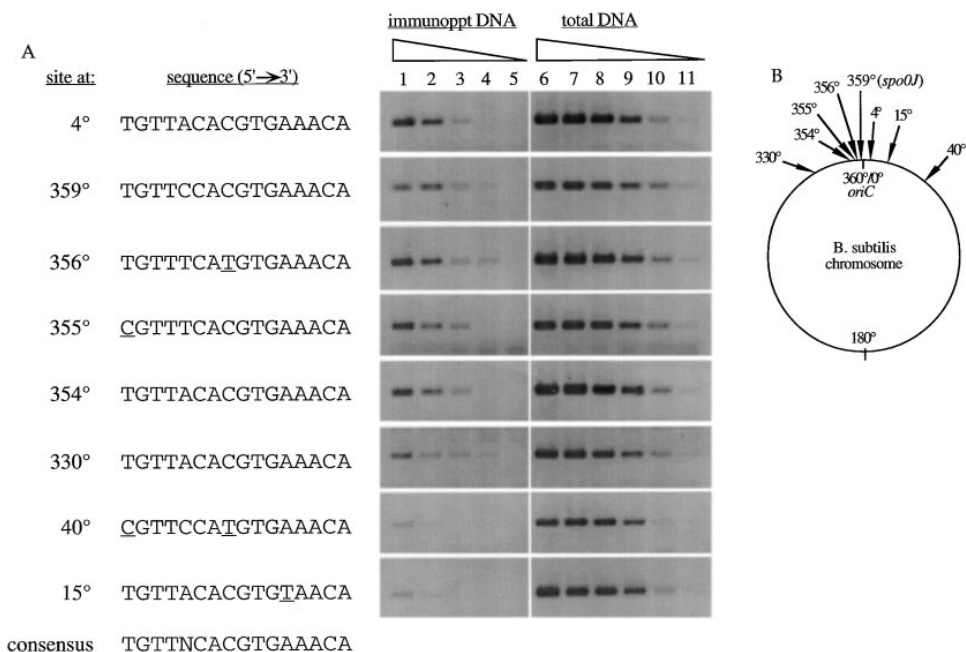| site at:	sequence (5'→3')
4°	TGTTACACGTGAAACA
359°	TGTTCCACGTGAAACA
356°	TGTTTCATGTGAAACA
355°	CGTTTCACGTGAAACA
354°	TGTTACACGTGAAACA
330°	TGTTACACGTGAAACA
40°	CGTTCCATGTGAAACA
15°	TGTTACACGTGTAACA
consensus	TGTTNCACGTGAAACA

Figure 4. Multiple Spo0J Binding Sites in the *B. subtilis* Chromosome

Ten potential sites were identified from the complete *B. subtilis* genomic sequence. Eight of these sites were associated with Spo0J in vivo and their sequence (A) and approximate map position are indicated (A and B). The consensus sequence derived from comparison of the eight sites is shown. In each site, differences from consensus are underlined.

(A) Serial 5-fold dilutions of the immunoprecipitated DNA (lanes 1–5) or the total input DNA (lanes 6–11) were amplified by PCR with primers specific to sequences flanking each site. Separate PCR reactions were done for each primer pair. Dilutions of 1/5 (lane 1), 1/25 (lane 2), 1/125 (lane 3), 1/625 (lane 4), and 1/3125 (lane 5) of the immunoprecipitated DNA and dilutions of 1/5 (lane 6), 1/25 (lane 7), 1/125 (lane 8), 1/625 (lane 9), 1/3125 (lane 10), and 1/15625 (lane 11) of the total DNA before immunoprecipitation were used. Potential sites at 31° (5′-TGATCCTCGTGA AACA) and 347° (5′-TGTTCCGAGTGAAACA) differ from consensus in two positions (underlined) and were not detected in the immunoprecipitates (data not shown).
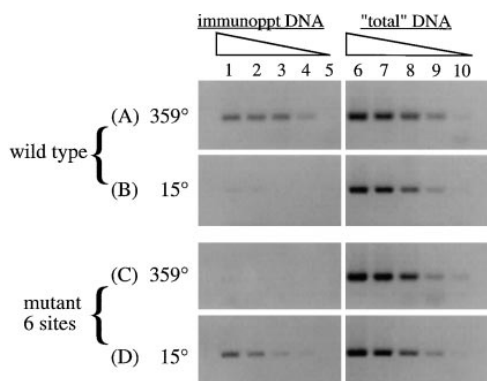

Figure 5. Increased Occupancy of the Spo0J Binding Site at 15° in the Mutant with Six Sites Inactivated

Binding of Spo0J to the sites at 359° (in *spo0J*) and 15° in wild-type cells (A and B) and in the strain (DCL484) with six sites inactivated (C and D) was measured as in Figure 4. Dilutions of 1/5 (lane 1), 1/20 (lane 2), 1/80 (lane 3), 1/320 (lane 4), and 1/1280 (lane 5) of the immunoprecipitated DNA and dilutions of 1/25 (lane 6), 1/125 (lane 7), 1/625 (lane 8), 1/3125 (lane 9), and 1/15625 (lane 10) of the total DNA before immunoprecipitation were used in PCR.

cells contained a plasmid (Figure 6A). In contrast, the plasmid with a single Spo0J binding site (pDL125) was much more stable; after ~20 generations ~20% of the cells still had a plasmid (Figure 6A). A plasmid (pDL126) that has the same insert but with the 7 bp mutation in the Spo0J binding site was not stabilized (Figure 6A), demonstrating that the increased stability of pDL125 was due to the Spo0J binding site. Quantitative Southern blot experiments indicated that the Spo0J binding site did not affect the copy number of the plasmid (Table 1). Together, these results indicate that the Spo0J binding site acts as a partitioning site, and we call each chromosome partition site *parS*.

The increased stability of the plasmid containing a single *parS* (pDL125) was dependent on *spo0J*. pDL125 was no longer stable in cells containing a null mutation in *spo0J* (Figure 6B). Unexpectedly, *soj*, the gene immediately upstream from *spo0J*, was also required for plasmid stability: a nonpolar null mutation in *soj* (Ireton et al., 1994) prevented stabilization of pDL125 (Figure 6B). The instability of pDL125 was similar in cells containing a mutation in either *soj* or *spo0J* and was no worse in cells containing mutations in both *soj* and *spo0J* (Figure 6B). The *soj* gene product is a member of the ParA family of partition proteins, a putative ATPase, and an inhibitor of sporulation (Ireton et al., 1994; Grossman, 1995). An *soj* null mutation has relatively little effect on chromosome partitioning (Ireton et al., 1994). In contrast, in the P1 and F plasmid systems, the ParA (SopA) protein is required for partitioning. Although an *soj* null mutant does not have an obvious chromosome partitioning defect, our results indicate that Soj plays some role in

Table 1. Copy Number Comparison of Plasmids with and without a Spo0J Binding Site

Plasmid	Fraction of Cells with Plasmid[a]	Plasmid/Chromosome in Total Population of Cells[b]	Plasmid/Chromosome per Plasmid-Containing Cell[c]
pDL110 (vector)	0.13	0.21	1.6
pDL110 (vector	0.14	0.27	1.9
pDL125 (parS)	0.74	1.56	2.1
pDL125 (parS)	0.59	1.09	1.8

[a] Data are shown from two experiments with each of two plasmids, pDL110 (the vector without a Spo0J binding site) and pDL125 (pDL110 with a Spo0J binding site). Cells were grown in chloramphenicol to select for the presence of the plasmid. Even with selection, only a fraction of the cells actually contained a plasmid as judged by plating efficiency in the presence and absence of chloramphenicol.
[b] DNA was prepared from exponentially growing cells containing the indicated plasmid. Numbers are the ratio of the intensity of the plasmid band to the chromosomal fragment, as determined by phosphorimager analysis of a Southern blot. Since two different DNA probes were used, one for the plasmid and one for the chromosomal fragment, these ratios are not an absolute indication of copy number.
[c] The plasmid/chromosome ratio was divided by the fraction of cells containing a plasmid.

partitioning, and this function may be redundant in the context of chromosome partitioning.

Possible Chromosome Partition Sites in Other Organisms

Chromosomally encoded homologs of Spo0J/ParB are widespread. Database searches reveal that homologs are found in at least 15 different bacterial species, and we suspect that a DNA binding-site for many of these will be located in or near the structural gene. In fact, ten organisms that have a Spo0J/ParB homolog also have a potential binding site that matches the consensus sequence of parS of B. subtilis. In five of these organisms, this sequence is in or near the structural gene (Table 2). In others, including Deinococcus radiodurans, Pseudomonas aueruginosa, Vibrio cholerae, Treponema pallidum, and Neisseriae gonorrhoeae, the location of these sites with respect to the parB/spo0J gene is not clear from the available sequence information. We postulate that, in at least several of these organisms, these sites are chromosome partition sites and that the role of ParB/Spo0J in chromosome partitioning is conserved.

Discussion

We have identified a family of chromosome partitioning sites (parS) from B. subtilis. Each parS is the binding site for the chromosome partition protein Spo0J and is a 16 bp sequence composed of an imperfect 8 bp inverted repeat. The presence of a single site, on an otherwise unstable plasmid, stabilizes the plasmid, indicating that the sequence functions as a partition site. There are at least eight parS sites in the B. subtilis genome that are occupied in vivo. All are located in the origin-proximal ~20% of the chromosome (Figure 4B). The subcellular localization of Spo0J to the poles of the bacterial nucleoid (Glaser et al., 1997; Lin et al., 1997) is probably a direct reflection of the coordinate binding of Spo0J to these sites.

Spo0J and parS contribute to the efficiency of chromosome partitioning. The multiple sites appear to be redundant, and cells compensate for loss of several sites with increased binding of Spo0J to other sites. Null mutations in spo0J cause a 100-fold increase in the frequency of anucleate cells (Ireton et al., 1994). This is a significant increase that would probably be lethal in nature in competition with wild-type organisms. That

~98% of the cells of a spo0J mutant manage to get an intact genome indicates that other mechanisms contribute to efficient partitioning.

One additional component required for efficient chromosome partitioning in B. subtilis, and probably in most other organisms, is the smc gene product. A homolog of the eukaryotic Smc proteins (structural maintenance of chromosomes) has been identified in B. subtilis (Oguro et al., 1996). Like those in eukaryotes (Peterson, 1994; Hirano et al., 1995; Koshland and Strunnikov, 1996; Heck, 1997; and references therein), B. subtilis Smc is required for efficient chromosome partitioning and condensation (R. Britton, D. C.-H. L., and A. D. G., submitted). An smc null mutant has nucleoids that appear less condensed than those in wild-type cells. In addition, ~10% of the cells in a growing culture of the smc mutant are anuclate. Most strikingly, an smc spo0J double mutant has a synthetic phenotype; there is a severe growth defect, and ~25% of the cells in a culture are anucleate (R. Britton, D. C.-H. L., and A. D. G., submitted). This phenotype is discussed below in the context of models for Spo0J function.

The Role of Spo0J in Chromosome Partitioning

One possible function of Spo0J bound to multiple parS sites might be to help position the origin region of the chromosome. The partition system of the E. coli F plasmid is required for proper plasmid positioning. A plasmid containing the sopABC system is localized at midcell in newborn cells and at the 1/4 and 3/4 positions in older cells preparing to divide (Gordon et al., 1997; Niki and Hiraga, 1997). In contrast, a plasmid missing the sop system is localized randomly in the cytosolic space (Niki and Hiraga, 1997).

During most of the cell cycle in B. subtilis, the oriC region is positioned near the pole of the nucleoid, oriented toward a cell pole (Glaser et al., 1997; Lin et al., 1997). A null mutation in spo0J causes the oriC region to be mislocalized in a small fraction of cells, but in the majority of mutant cells the origin region appears properly positioned (D. C.-H. L. and A. D. G., unpublished data). Preliminary experiments indicate that proteins required for DNA replication may be involved in establishing the position of the oriC region (K. P. Lemon and A. D. G., unpublished data). Thus, it appears that Spo0J is not required to establish, but might be involved in maintaining, chromosome orientation.
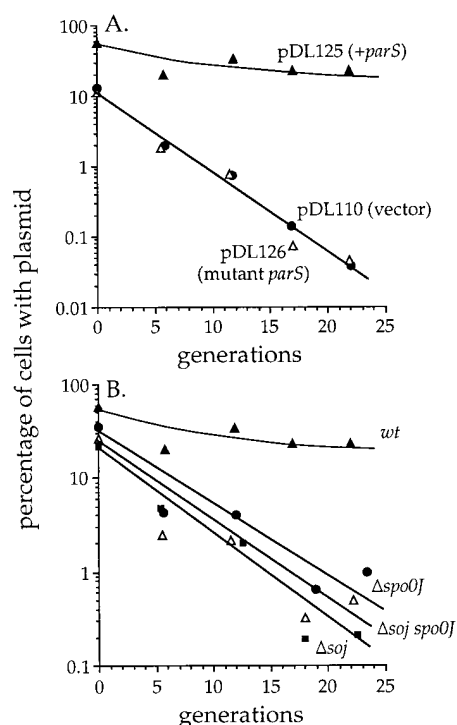
Figure 6. The Spo0J Binding Site Can Stabilize a Plasmid

Cells were grown for several generations in the absence of selection and tested for the presence of the plasmid. Zero (0) generations is the time at which selection for the plasmid was removed. For wild-type strains with pDL125, 58% of the cells grown with selection actually contained a plasmid, as judged by the fraction of colonies resistant to chloramphenicol. For strains containing pDL110 or pDL126, ∼10%–15% of the cells grown with selection contained the plasmid.

(A) Wild-type cells containing the parental plasmid (pDL110, closed circle) and a plasmid containing a mutated Spo0J binding site (pDL126, open triangle) are rapidly lost in the absence of selection. In contrast, a plasmid with a wild-type Spo0J binding site (pDL125, closed triangle) is stabilized.

(B) Stabilization of pDL125 depends on *spo0J* and *soj*. pDL125 was stabilized in wild-type cells (closed triangle) (same data as in Figure 6A), but not in cells containing a mutation in *spo0J* (closed circle) or *soj* (closed square). The instability was no worse in the *soj spo0J* double mutant (open triangle).

In growing cells, sister origins are rapidly separated and become positioned at opposite ends of the nucleoid, oriented toward opposite cell poles (Glaser et al., 1997;

Gordon et al., 1997; Lin et al., 1997; Webb et al., 1997). We speculate that the function of Spo0J bound to the multiple *parS* sites is to pair sister origin regions for recognition by components involved in separation and movement (Figure 7). This origin-pairing model for Spo0J is an extension of models for sister chromatid pairing or cohesion in eukaryotes (Miyazaki and Orr-Weaver, 1994; Bickel and Orr-Weaver, 1996; Guacci et al., 1997; Heck, 1997; Michaelis et al., 1997) and plasmid pairing in prokaryotes (Nordström and Austin, 1989; Austin and Nordström, 1990; Williams and Thomas, 1992).

We propose that after the origin region is duplicated, a Spo0J–*parS* complex on one chromosome contacts a Spo0J–*parS* complex on the other chromosome, pairing the sister origins for part of the cell cycle. We suspect that this pairing function may serve to indicate that two sister origins exist and are ready to be partitioned, and may help to distinguish sister origins from nonsisters during rapid growth when there are several overlapping rounds of replication. Pairing may also help to orient the origin regions such that one is "selected" to be moved toward the opposite pole. The Spo0J–*parS* complex seems to persist during most (all?) of the cell cycle (Glaser et al., 1997; Lewis and Errington, 1997; Lin et al., 1997), indicating that disruption of sister origin pairing is not mediated by degradation of Spo0J. The putative ATPase Soj (ParA) might be involved in disruption of sister origin pairing, but if so, its function appears to be redundant.

The postulated pairing of sister origin regions by Spo0J–*parS* complexes is somewhat analogous to the function of sister chromatid cohesion proteins in eukaryotes (Miyazaki and Orr-Weaver, 1994; Bickel and Orr-Weaver, 1996; Heck, 1997; and references therein). These proteins are involved in pairing the sister chromatids at centromeric regions and along the length of the chromosomes until the metaphase–anaphase transition, ensuring that the sister chromatids are not separated precociously.

The pairing model for Spo0J–*parS* function helps to explain the synthetic phenotype of a *spo0J smc* double mutant. In wild-type cells, with highly condensed, compact nucleoids, it seem likely that newly replicated origin regions might remain near each other. In the *smc* mutant, defective in chromosome condensation, the pairing function of Spo0J becomes much more important to maintain proximity of the newly replicated origins before they are actively separated. Whereas other models are

Table 2. Possible Spo0J/ParB Binding Sites in Other Bacteria that Are Similar to the *B. subtilis* Consensus

Organism	Sequence	Distance from spo0J/parB Gene
Bacillus subtilis	TGTTNCACGTGAAACA	Consensus[a]
Mycobacterium leprae	TGTTTCATGTGAAACA	∼0.9 kb
	TGTTTCACGTGAAACA	∼1.8 kb
Mycobacterium tuberculosis	TGTTTCACGTGAAACA	∼2 kb
	TGTTTCACGTGAAACA	∼1.1 kb
Streptomyces coelicolor	CGTTTCACGTGAAACA	∼1 kb
	GGTTTCACGTGAAACA	Internal
Borrelia burgdorferi	TGTTCCACGTGGAACA	∼0.1 kb
Streptococcus pyogenes	TGATTCACGTGAAACA	∼7 kb

[a]One *parS* is found internal to *spo0J*; seven others are in the origin-proximal ∼20% of the chromosome (Figure 4B).
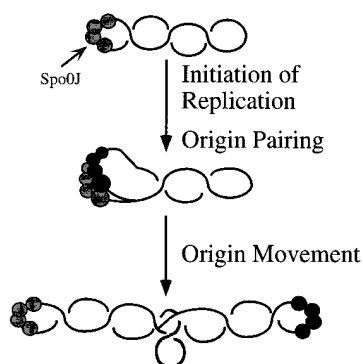
Figure 7. Spo0J May Be Involved in Pairing Newly Replicated Origin Regions

Gray and black circles represent Spo0J binding to *par* sites in the origin region of the chromosome (not drawn to scale). We postulate that Spo0J is involved in pairing the newly replicated origins. After origin pairing, separation of origins is governed by as yet uncharacterized proteins. Spo0J may also be involved in maintaining the polar localization of the origin region, perhaps by interacting with proteins near the poles of the cell.

also consistent with the phenotype of the *spo0J smc* double mutant, we currently favor the pairing model, especially in light of recent findings that SMC and SMC-associated proteins are involved in chromosome cohesion (pairing) in yeast (Guacci et al., 1997; Michaelis et al., 1997).

Chromosome Dynamics

Our current view of chromosome partitioning in bacteria involves orientation and active movement of the origin region, and continuous condensation and compaction of the entire chromosome. Regions of the chromosome near and including the origin of replication are positioned at an end of the nucleoid toward a cell pole. We propose that newly replicated origins are paired by

Table 3. *B. subtilis* Strains Used

Strain	Relevant Genotype
AG174 (JH642)	*trpC pheA*
AG1468	Δ*spo0J::spc* (Ireton et al., 1994)
AG1505	Δ(*soj spo0J*)::*spc* (Ireton et al., 1994)
KI1944	Δ(*soj spo0J*)::*spc thr*::(Δ*soj spo0J*⁺) (Ireton et al., 1994)
DCL108	pHP13
DCL352	pIK219
DCL365	pDL83
DCL367	pDL85
DCL381	pDL90A
DCL430	pDL104
DCL431	pDL105
DCL432	pDL106
DCL438	pDL110
DCL484	Sextuple *parS* mutant, *parS-6* (see Experimental Procedures)
DCL490	pDL125
DCL491	pDL126
DCL492	Δ(*soj spo0J*)::*spc*; pDL125
DCL494	Δ*spo0J::spc*; pDL125
DCL497	Δ(*soj spo0J*)::*spc thr*::(Δ*soj spo0J*⁺); pDL125

Spo0J until the segregation machinery separates them and repositions one origin toward each pole. Condensation, partly by Smc, facilitates pairing. Condensation also is likely to provide a mechanism to move the bulk of the chromosome mass away from midcell and toward the position where the origin has been established. Division at midcell creates two cells, each with an intact genome. The continuing challenge is to identify the remaining components involved in chromosome partitioning and to determine their mechanisms of action.

Experimental Procedures

Strains and Plasmids

B. subtilis strains are all derivatives of AG174 (JH642) and contain the *trpC* and *pheA* mutations. Standard procedures (Harwood and Cutting, 1990) were used for transformations and strain constructions. Strains and relevant genotypes are listed in Table 3. Plasmids are described in Table 4 or in the text below.

Construction of Strain DCL484

Strain DCL484 contains mutations in six of the eight known Spo0J binding sites. Five of the sites were deleted and a drug resistance cassette inserted. For each mutation, DNA (∼400 bp) from upstream and downstream of the Spo0J binding site was amplified by PCR and cloned upstream and downstream of a drug resistance cassette. A different drug resistance marker was used for each mutation. Sequences of all oligonucleotides used in the PCR are available upon request.

Each mutation was introduced by transformation into the *B. subtilis* chromosome by double crossover, selecting for resistance to the specific marker. Each mutation was confirmed by PCR analysis. The following plasmids were used: pDL112 replaces 32 bp, removing the Spo0J binding site at 330°, with a phleomycin resistance cassette; pDL113 replaces ∼140 bp, removing the Spo0J binding site at 356°, with a erythromycin resistance cassette; pDL114 replaces 19 bp, removing the Spo0J binding site at 4°, with a kanamycin resistance cassette; pDL116A replaces 117 bp, removing the Spo0J binding site at 354°, with a tetracycline resistance cassette; pDL122 replaces ∼60 bp, removing the Spo0J binding site at 40°, with a spectinomycin resistance cassette.

Of the 16 bp in the Spo0J binding site in *spo0J*, 7 were changed so as not to alter the gene product. In order to create a strain with the 7 bp changes in *spo0J*, strain AG1468 (Δ*spo0J::spc*) (Ireton et al., 1994) was transformed with pDL107 (which contains *spo0J* with the 7 bp site mutation, Table 4), and chloramphenicol-resistant transformants, which arise by single crossover at the *spo0J* locus, were selected. As expected, two classes of transformants were obtained, Spo⁺ for crossovers upstream and Spo⁻ for crossovers downstream of the *spc* insertion in *spo0J*. A Spo⁻ transformant was chosen (strain DCL440). Excision of pDL107 from DCL440 by a single crossover created a strain (DCL468) that is Spo⁺, chloramphenicol- and spectinomycin-sensitive, and has the 7 bp mutation in the Spo0J binding site. The presence of the mutation was confirmed by PCR and DNA sequencing.

Formaldehyde Cross-Linking and Immunoprecipitations

Cells were grown at 37° for several generations in defined minimal medium (Vasantha and Freese, 1980; Jaacks et al., 1989) containing 1% glucose, 0.1% glutamate, 40 μg/ml tryptophan, 40 μg/ml phenylalanine, trace metals, and appropriate antibiotics when necessary, and samples were taken during exponential growth (OD$_{600}$ ∼0.6). Cross-linking and sample preparation were based on chromatin immunoprecipitation assays (Solomon and Varshavsky, 1985; Hecht et al., 1996; Strahl-Bolsinger et al., 1997). Samples were treated with NaPO₄ (final concentration 10 mM) and formaldehyde (final concentration 1%) for 10 min at room temperature followed by 30 min at 4°C. Cells (10 ml) were pelleted and washed twice with 10 ml of 1× phosphate-buffered saline (pH 7.3) (Ausubel et al., 1990). Cells were resuspended in 500 μl of solution A (10 mM Tris [pH 8], 20% sucrose, 50 mM NaCl, 10 mM EDTA) containing 20 mg/ml

Table 4. Plasmids Used

	Description
Plasmid Vectors	
pHP13	Cm, MLS; *B. subtilis* and *E. coli* shuttle vector (Harwood and Cutting, 1990).
pBPA23	Contains the replicon from pLS32 from *B. natto* (Hassan et al., 1997).
pGEM*cat*	Ap, Cm; integrative vector (Harwood and Cutting, 1990).
pJH101	Ap, Tet, Cm; integrative vector (Harwood and Cutting, 1990).
Other plasmids	
pIK219	Contains an ~760 bp fragment, extending ~540 bp downstream of *spo0J* (Figure 2A), cloned into pHP13. Used to define the Spo0J binding site in vivo.
pDL83	Contains an ~310 bp fragment, extending ~100 bp downstream of *spo0J* (Figure 2A), cloned into pHP13. Used to define the Spo0J binding site in vivo.
pDL85	Contains an ~255 bp fragment (Figure 2A) cloned into pHP13. Used to define the Spo0J binding site in vivo.
pDL90A	Contains the 55 bp fragment from PvuII to SfaNI in *spo0J* (Figure 2A) cloned into pHP13. Used to define the Spo0J binding site in vivo.
pDL104	Contains the 16 bp Spo0J binding site (Figure 2B) cloned into the SmaI site of pHP13. Single stranded oligomers 5′-TGTTCCACGTGAAACA-3′ (LIN-73) and its complement 5′-TGTTTCACGTGGAACA-3′ (LIN-74) were annealed, phosphorylated with polynucleotide kinease, and cloned into pHP13. The plasmid was verified by DNA sequencing.
pDL105	Contains a 38 bp fragment, missing the Spo0J binding site (Figure 2B), cloned into the SmaI site of pHP13. Single-stranded oligomers 5′-CTGATTCAGCAGTTGAATCAGAAAA GAAAAAAGAACCTG-3′ (LIN-75) and its complement 5′-AGGTTCTTTTTTCTTTTCTGATTC AACTGCTGAATCAG-3′ (LIN-76) were annealed, phosphorylated with polynucleotide kinase, and cloned into pHP13. DNA sequencing revealed that the plasmid is essentially pDL90A with a 17 bp deletion removing the Spo0J binding site.
pDL106	Contains a 55 bp fragment with 7 bp changes in the Spo0J binding site, cloned into pHP13. Single strand oligomers 5′-CTGATTCAGCAGTTGAATCAGAAC̲GTG̲CCC̲AGGGAG̲ACC̲AA GAAAAAAGAACCTG-3′ (LIN-77) and its complement 5′-CAGGTTCTTTTTTCTT̲GGT̲CTC̲C C̲TG̲GGC̲AC̲GTTCTGATTCAACTGCTGAATCAG-3′ (LIN-78) were annealed, phosphorylated with polynucleotide kinase, and cloned into pHP13. The annealed oligomers contain the 55 bp insert in pDL90A except that 7 bp in *parS* have been changed (underlined above). The plasmid was verified by sequencing.
pDL107	Contains all of *spo0J*, with the 7 bp mutation in *parS*, cloned into pGEM*cat*. Used to construct the multiple *parS* mutant strain, DCL484.
pDL110	Contains the ~1.5 kb EcoRI-XbaI fragment from pBPA23 (containign the replicon of pLS32) cloned between the EcoRI-NheI sites in pJH101. Used in the plasmid stability experiments.
pDL125	Contains the 55 bp fragment of *spo0J* from pDL90A, with *parS*, cloned into pDL110. The ~60 bp EcoRI-HindIII fragment from pDL90A was cloned between the EcoRI-HindIII sites of pDL110. Used in the plasmid stability experiments.
pDL126	Contains the 55 bp fragment of *spo0J* from pDL106, with the mutant *parS*, cloned into pDL110. The ~60 bp EcoRI-HindIII fragment from pDL106 was cloned between the EcoRI-HindIII sites of pDL110. Used in the plasmid stability experiments.

lysozyme and incubated at 37°C for 30 min. Five hundred microliters of 2× IP buffer (100 mM Tris [pH 7], 300 mM NaCl, 2% Triton X-100) and PMSF (final concentration 1 mM) was added, and the cell extract was incubated an additional 10 min at 37°. The DNA was sheared by sonication to an average size of ~500–1000 bp. Insoluble cellular debris was removed by centrifugation, and the supernatant was transferred to a fresh microfuge tube. In order to determine the relative amount of DNA immunoprecipitated to the total DNA before immunoprecipitation, 75 μl of supernatant ("total" DNA control) was removed and saved for later analysis.

Protein and protein–DNA complexes were immunoprecipitated (1 hr, room temperature) with affinity purified polyclonal anti-Spo0J antibodies (Lin et al., 1997) followed by incubation with 30 μl of a 50% protein A-Sepharose slurry (1 hr room temperature). Complexes were collected by centrifugation and washed five times with 1× IP buffer and twice with 1 ml TE (10 mM Tris [pH 8], 0.1 mM EDTA). The slurry was resuspended in 50 μl of TE. The 75 μl "total" DNA control was treated with *S. griseus* protease (final concentration 0.1 mg/ml) for 10 min at 37°C, and SDS was added to 0.67%. Formaldehyde cross-links of both the total DNA and the immunoprecipitate were reversed by incubation at 65°C for 6 hr, and samples were used in PCR without further treatment.

PCR was performed with Taq DNA polymerase using serial dilutions of the immunoprecipitate and the total DNA control as the template. Oligonucleotide primers were typically 20–25 bases in length and amplified an ~300–450 bp product. Sequences of all primers are available upon request. PCR products were separated on agarose gels and stained with ethidium bromide. Relative affinities of Spo0J to different *par* sites were determined by comparing the intensity of bands in the linear range of the PCR from both the immunoprecipitate and "total" DNA control. Gels were photographed onto Polaroid 665 film, and the negatives were scanned using Adobe Photoshop software.

Spo0J-his₆

Spo0J with a hexa-histidine tag at the C terminus is functional in *B. subtilis*, both in sporulation and chromosome partitioning (Lin et al., 1997). Spo0J-his₆ was purified from *E. coli* strain DCL128, a BL21 (lambda DE3) strain carrying a plasmid, pDL3, with *spo0J-his₆* under the control of the T7 promoter in pET21(+) (Lin et al., 1997). An extract from the overproducing strain was loaded onto a metal chelating column (Pharmacia) that had been charged with NiSO₄, according to instructions from the manufacturer. Spo0J-his₆ was eluted with a linear gradient of imidazole (60 mM–1 M) in buffer containing 20 mM Tris (pH 8), 500 mM NaCl. Fractions containing Spo0J-his₆ were pooled and dialyzed into buffer containing 20 mM Tris (pH 8), 250 mM NaCl, 1 mM EDTA, 1 mM DTT. Following dialysis,

glycerol was added to 10%, and protein concentration was determined with Bio-Rad protein assay kit using BSA as a standard. Spo0J-his$_6$ was ~90% pure as judged by SDS polyacrylamide gel electrophoresis and staining with Coomassie blue.

DNA Binding Assays

A 24 bp DNA fragment containing the Spo0J binding site was used as the probe in gel mobility shift assays. Two oligonucleotides, 5′-AGAATGTTCCACGTGAAACAAAGA-3′ (LIN-71) and its complement 5′-TCTTTGTTTCACGTGGAACATTCT-3′ (LIN-72), were annealed and radiolabeled using polynucleotide kinase and gamma-^{32}P-ATP. The radiolabeled 24 bp fragment was gel-purified and resuspended in TE. Binding reactions (15 μl) were performed in 20 mM HEPES [pH 7.6], 292 mM NaCl, 5% glycerol, 1 mM DTT and contained approximately 1.5 fmol of DNA. Reactions were incubated for 15 min at 32°C and then loaded onto a prerun 8% polyacrylamide (29:1) gel in 0.5× TBE. Gels were run at 4°C at 150 V, dried, and exposed to a phosphorimager cassette (Molecular Dynamics). Bands were quantitated using ImageQuant software.

Competition assays were performed with both the unlabeled 24 bp fragment containing a wild-type Spo0J binding site, and a 24 bp fragment containing seven base pair changes in the Spo0J binding site. The mutant fragment was made by annealing the oligomers 5′-AGAACGTGCCCAGGGAGACCAAGA-3′ (LIN-120) and its complement 5′-TCTTGGTCTCCCTGGGCACGTTCT-3′ (LIN-121).

Plasmid Stability Assays

Cells containing the indicated plasmids were grown in defined minimal medium containing 1% sodium succinate, 0.1% glutamate, 40 μg/ml tryptophan, 40 μg/ml phenylalanine, 100 μg/ml threonine (when needed), and trace metals. Cells were grown first for several generations with chloramphenicol to select for the plasmid. At generation time zero, cells were removed from chloramphenicol-containing medium by centrifugation, resuspended, and used to inoculate fresh medium in the absence of antibiotic. Cells were maintained in exponential growth by dilution into fresh medium when the culture reached mid-to-late exponential phase. The percentage of cells containing a plasmid was determined by measuring the fraction of cells that were resistant to chloramphenicol, as determined by colony-forming ability on LB plates with and without antibiotic.

Determination of Relative Plasmid Copy Number

The relative copy number of plasmids with and without *parS* was determined by quantitative Southern blots using probes specific to plasmid and chromosomal sequences. The plasmid-specific probe was an ~1500 bp EcoRI-AlwNI fragment from pDL110. The chromosomal-specific probe was an ~1200 bp EcoRI-XhoI fragment from pDL20 that extends from the 3′ end of *dnaA* into the 5′ end of *dnaN* (immediately downstream of *dnaA*). The *dnaA-dnaN* fragment in pDL20 was cloned from PCR products amplified from chromosomal DNA. Probes were labeled with α-^{32}P-dATP using random priming with a hexanucleotide mix (Pharmacia) according to the manufacturer's instructions.

Total DNA was prepared from cells in exponential growth in defined minimal medium, as for the plasmid stability assays. DNA was digested with EcoRI, separated on a 0.8% agarose gel in 1× TAE buffer, and transferred to a nitrocellulose membrane. Hybridization was done essentially as described (Ausubel et al., 1990) using both plasmid and chromosome-specific probes simultaneously. Results were visualized with a phosphorimager, and band intensity was quantitated used ImageQuant software. The ratio of the plasmid-specific band to the chromosome-specific band was determined.

References

Austin, S., and Nordström, K. (1990). Partition-mediated incompatibility of bacterial plasmids. Cell *60*, 351–354.

Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., and Struhl, K. (1990). Current Protocols in Molecular Biology (New York: John Wiley and Sons).

Bickel, S.E., and Orr-Weaver, T.L. (1996). Holding chromatids together to ensure they go their separate ways. Bioessays *18*, 293–300.

Glaser, P., Sharpe, M.E., Raether, B., Perego, M., Ohlsen, K., and Errington, J. (1997). Dynamic, mitotic-like behavior of a bacterial protein required for accurate chromosome partitioning. Genes Dev. *11*, 1160–1168.

Gordon, G.S., Sitnikov, D., Webb, C.D., Teleman, A., Straight, A., Losick, R., Murray, A.W., and Wright, A. (1997). Chromosome and low-copy plasmid segregation in *E. coli*: visual evidence for distinct mechanisms. Cell *90*, 1113–1121.

Grossman, A.D. (1995). Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis*. Annu. Rev. Genet. *29*, 477–508.

Guacci, V., Koshland, D., and Strunnikov, A. (1997). A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerevisiae*. Cell *91*, 47–57.

Harwood, C.R., and Cutting, S.M. (1990). Molecular Biological Methods for *Bacillus* (Chichester, UK: John Wiley and Sons).

Hassan, A.K.M., Moriya, S., Ogura, M., Tanaka, T., Kawamura, F., and Ogasawara, N. (1997). Suppression of initiation defects of chromosome replication in *Bacillus subtilis dnaA* and *oriC*-deleted mutants by integration of a plasmid replicon into the chromosomes. J. Bacteriol. *179*, 2494–2502.

Hecht, A., Strahl, B.S., and Grunstein, M. (1996). Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. Nature *383*, 92–96.

Heck, M. (1997). Condensins, cohesins, and chromsome architecture: how to make and break a mitotic chromosome. Cell *91*, 5–8.

Hiraga, S. (1992). Chromosome and plasmid partition in *Escherichia coli*. Annu. Rev. Biochem. *61*, 283–306.

Hirano, T., Mitchison, T.J., and Swedlow, J.R. (1995). The SMC family: from chromosome condensation to dosage compensation. Curr. Opin. Cell Biol. *7*, 329–336.

Ireton, K., Gunther, IV, N.W., and Grossman, A.D. (1994). *spo0J* is required for normal chromosome segregation as well as the initiation of sporulation in *Bacillus subtilis*. J. Bacteriol. *176*, 5320–5329.

Jaacks, K.J., Healy, J., Losick, R., and Grossman, A.D. (1989). Identification and characterization of genes controlled by the sporulation regulatory gene *spo0H* in *Bacillus subtilis*. J. Bacteriol. *171*, 4121–4129.

Koshland, D., and Strunnikov, A. (1996). Mitotic chromosome condensation. Annu. Rev. Cell Dev. Biol. *12*, 305–333.

Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessiéres, P., Bolotin, A., Borchert, S., et al. (1997). The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. Nature *390*, 249–256.

Lewis, P.J., and Errington, J. (1997). Direct evidence for active segregation of *oriC* regions of the *Bacillus subtilis* chromosome and colocalization with the Spo0J partitioning protein. Mol. Microbiol. *25*, 945–954.

Lin, D.C.-H., Levin, P.A., and Grossman, A.D. (1997). Bipolar localization of a chromosome partition protein in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA *94*, 4721–4726.

Michaelis, C., Ciosk, R., and Nasmyth, K. (1997). Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell *91*, 35–45.

Miyazaki, W.Y., and Orr-Weaver, T.L. (1994). Sister-chromatid cohesion in mitosis and meiosis. Annu. Rev. Genet. *28*, 167–187.

Mohl, D.A., and Gober, J.W. (1997). Cell cycle–dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. Cell *88*, 675–684.

Niki, H., and Hiraga, S. (1997). Subcellular distribution of actively partitioning F plasmid during the cell division cycle in *E. coli*. Cell *90*, 951–957.

Nordström, K., and Austin, S.J. (1989). Mechanisms that contribute to the stable segregation of plasmids. Annu. Rev. Genet. *23*, 37–69.

Ogasawara, N., and Yoshikawa, H. (1992). Genes and their organization in the replication origin region of the bacterial chromosome. Mol. Microbiol. *6*, 629–634.

Oguro, A., Kakeshita, H., Takamatsu, H., Nakamura, K., and Yamane, K. (1996). The effect of Srb, a homologue of the mammalian SRP receptor α-subunit, on *Bacillus subtilis* growth and protein translocation. Gene *172*, 17–24.

Peterson, C.L. (1994). The SMC family: novel motor proteins for chromosome condensation? Cell *79*, 389–392.

Solomon, M.J., and Varshavsky, A. (1985). Formaldehyde-mediated DNA-protein crosslinking: a probe for in vivo chromatin structures. Proc. Natl. Acad. Sci. USA *82*, 6470–6474.

Strahl-Bolsinger, S., Hecht, A., Luo, K., and Grunstein, M. (1997). SIR2 and SIR4 interactions differ in core and extended telomeric heterochromatin in yeast. Genes Dev. *11*, 83–93.

Vasantha, N., and Freese, E. (1980). Enzyme changes during *Bacillus subtilis* sporulation caused by deprivation of guanine nucleotides. J. Bacteriol. *144*, 1119–1125.

Wake, R.G., and Errington, J. (1995). Chromosome partitioning in bacteria. Annu. Rev. Genet. *29*, 41–67.

Webb, C.D., Teleman, A., Gordon, S., Straight, A., Belmont, A., Lin, D. C.-H., Grossman, A.D., Wright, A., and Losick, R. (1997). Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. Cell *88*, 667–674.

Williams, D.R., and Thomas, C.M. (1992). Active partitioning of bacterial plasmids. J. Gen. Microbiol. *138*, 1–16.