Chromosomal Rearrangements in Salmonella enterica Serovar Typhi Strains Isolated from Asymptomatic Human Carriers

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ABSTRACT Host-specific serovars of Salmonella enterica often have large-scale chromosomal rearrangements that occur by recombination between rrn operons. Two hypotheses have been proposed to explain these rearrangements: (i) replichore imbalance from horizontal gene transfer drives the rearrangements to restore balance, or (ii) the rearrangements are a consequence of the host-specific lifestyle. Although recent evidence has refuted the replichore balance hypothesis, there has been no direct evidence for the lifestyle hypothesis. To test this hypothesis, we determined the rrn arrangement type for 20 Salmonella enterica serovar Typhi strains obtained from human carriers at periodic intervals over multiple years. These strains were also phage typed and analyzed for rearrangements that occurred over long-term storage versus routine culturing. Strains isolated from the same carrier at different time points often exhibited different arrangement types. Furthermore, colonies isolated directly from the Dorset egg slants used to store the strains also had different arrangement types. In contrast, colonies that were repeatedly cultured always had the same arrangement type. Estimated replichore balance of isolated strains did not improve over time, and some of the rearrangements resulted in decreased replichore balance. Our results support the hypothesis that the restricted lifestyle of host-specific Salmonella is responsible for the frequent chromosomal rearrangements in these serovars.

IMPORTANCE Although it was previously thought that bacterial chromosomes were stable, comparative genomics has demonstrated that bacterial chromosomes are dynamic, undergoing rearrangements that change the order and expression of genes. While most Salmonella strains have a conserved chromosomal arrangement type, rearrangements are very common in host-specific Salmonella. This study suggests that chromosome rearrangements in the host-specific Salmonella enterica serovar Typhi, the causal agent of typhoid fever, occur within the human host over time. The results also indicate that rearrangements can occur during long-term maintenance on laboratory medium. Although these genetic changes do not limit survival under slow-growth conditions, they may limit the survival of Salmonella Typhi in other environments, as predicted for the role of pseudogenes and genome reduction in niche-restricted bacteria.

M ost of the ~2,600 serovars of Salmonella enterica can infect a variety of animal species, leading to pathologies ranging from a self-resolving gastroenteritis to a life-threatening systemic infection, depending on the particular serovar-host interaction (1, 2). For instance, infection of mice with S. enterica serovar Typhimurium causes a systemic disease that is often lethal, but ingestion of Salmonella Typhimurium by humans typically results in gastroenteritis that usually self-resolves within weeks. In contrast to these broad-host-range S. enterica serovars (generalists), a small number of serovars are either host adapted or host specific. Host-adapted serovars are capable of infecting various animal species but commonly infect a preferred host. For example, S. enterica serovar Choleraesuis primarily infects swine, but can also cause bacteremia in humans. Host-specific serovars such as the fowl-specific S. enterica serovars Pullorum and Typhi, the etiological agent of typhoid fever in humans, only cause disease in one species or closely related species. Even within a serovar, strains may differ in host range. Variants of Salmonella serovar Typhimurium that have been host adapted to pigeons for decades have unique characteristics and belong to specific phage types (3).

The genomes of a number of generalist and host-specific serovars have been sequenced (4–11). A comparative analysis indicates that housekeeping genes are >96% identical and the major pathogenicity islands are shared between serovars (12). Genetic differences between serovars, as well as strains within the same serovar, include indels and various repertoires of Salmonella pathogenicity islands (SPIs) and prophages. While these genetic differences can affect the virulence of a strain (13–17), they do not affect host specificity. The greater number of pseudogenes present in host-specific strains indicates that, compared to generalist strains, host-specific strains are under less-stringent selection for a variety of genetic functions (5, 8–10). This suggests that as they become restricted to their specific host, these Salmonella serovars begin to undergo genome reduction (8), an evolutionary process.

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observed in other niche-restricted bacteria such as the obligate endosymbiont Buchnera aphidicola (18–24).

Chromosomal rearrangements can occur by recombination between multiple copies of sequences, such as insertion (IS) elements and tRNA (rrn) operons. These rearrangements are common in host-specific Salmonella and other niche-restricted bacteria (18, 23, 25–28), suggesting they are associated with the evolution of bacterial pathogens (20, 21, 23, 29, 30). These rrn rearrangements result in inversions, translocations, duplications, or deletions of regions of the genome of various sizes. Most host-specific Salmonella serovars have chromosomal rearrangements that occur by recombination between the seven chromosomal copies of rrn (29, 31–35). In contrast, generalist serovars almost always have a common rrn arrangement (35, 36), the “conserved” rearrangement type designated 1234567 (Fig. 1). While both generalist and host-specific serovars undergo rrn rearrangements at similar frequencies in vitro (37), rearrangements in generalist strains of Salmonella are rare, but have been found in Salmonella Typhimurium strains stored in stab vials for decades (38) and in pigeon-adapted Salmonella Typhimurium strains (39). Recombination events either invert or levitate/translocate the chromosomal regions between the rrn operons, resulting in reordering of the regions. Of the 1,440 possible arrangements, only 32 have been observed in the ~150 Salmonella Typhi strains analyzed to date (26, 31, 33–35, 37), suggesting that there are selective forces that handicap certain arrangement types.

One hypothesis to explain why rrn rearrangements occur in host-specific S. enterica serovars proposes that horizontal gene transfer events, such as transfer of phages or SPIs, made one replichore longer than the other (replichores are the chromosomal halves on either side of the ori-dif axis), and the insertion event caused an imbalance in DNA replication between the two replichores, stimulating chromosomal rearrangements that reestablish balance (29, 31, 33, 34, 40).

However, recent evidence suggests that random rrn rearrangements are more likely to worsen replichore balance, while most naturally occurring rearrangements do not affect replichore balance (26). An alternative hypothesis suggests that some aspect of the lifestyle of the host-specific serovars induces and/or promotes tolerance of the rrn rearrangements (33, 37). For example, one lifestyle difference is that strains of host-specific serovars establish a chronic intracellular carrier state within their animal host that can persist for months to years.

In this study, we describe the arrangement types of multiple colonies derived from 20 Salmonella Typhi strains isolated over a 23-year period from four asymptomatic human carriers who had never been treated with antibiotics. These strains provide a rare opportunity to study changes in chromosome structure of Salmonella Typhi during long-term infections of human hosts. After isolation, these strains were stored on Dorset egg slants at ambient temperature, so the growth of the bacteria was presumably limited during long-term storage. The replichore balance of each resolved arrangement type was estimated (26), and growth rates of strains with various replichore degrees of balance were measured. The results support the hypothesis that unique aspects of their lifestyle are responsible for the chromosomal rearrangements found in host-specific Salmonella serovars.

RESULTS

Chromosome arrangement types. A prediction of the hypothesis that unique aspects of their lifestyle allows rearrangements to accumulate in populations of host-specific serovars is that the rearrangements occur within the host during long-term infections. To test this prediction, the rrn arrangement types were determined for a unique collection of 20 Salmonella Typhi strains that had been isolated over two decades from four human carriers never treated with antibiotics. These strains were stored on Dorset egg slants, and up to seven independent colonies of each strain were analyzed. Strains isolated from the same carrier but in different years often had different arrangement types. In addition, colonies derived from the same slant also had different arrangement types. At least one colony per carrier contained a tandem duplication of one of the small rrn regions (region 4, 5, or 6).

The four strains from carrier 1 were isolated between 1981 and 1998. Most colonies of the 1981 and 1998 strains had a translocation of region 6 into the rrnC operon as well as a symmetrical inversion of region 1 by recombination between the rrnG and rrnH operons (Table 1). In contrast, colonies of the 1993 isolate had arrangement types resulting from interreplichore translocations of region 4 into rrnD in addition to the region 6 translocation. Furthermore, two colonies from 1993 as well as all of the colonies of the 1996 strain contained a second symmetrical inversion via recombination between rrnD and rrnE that switched the replichore locations of regions 2 and 7.

Six strains were isolated from carrier 2 between 1981 and 1999.
The arrangement types identified in these strains were more homogenous, with most colonies showing a translocation of region 6 into rrnC from each of the years they were isolated (Table 2). Strains isolated in both 1985 samples as well as in 1994 and 1998 samples also had an inversion of region 1. This inversion was the only rearrangement found in the single colony analyzed from 1994. One colony from the 1985A strain contained a second asymmetrical interreplichore inversion between rrnD and rrnB.

Three carrier 3 strains were isolated between 1977 and 1985. All of the colonies from the three strains contained the two symmetrical inversions that switch replichore locations of regions 2 and 7 and leave region 1 in its native orientation (Table 3). Colonies of the 1983 strain also had a translocation of region 6 into the hybrid rrnH/G operon between regions 1 and 7.

Seven strains were isolated from carrier 4 between 1976 and 1991. Most carrier 4 strains only had one rearrangement: an inversion of region 1 (Table 4). This was observed in all colonies from strains isolated in 1981, 1983, 1987, and 1989 and in four colonies from the 1976 strain. The three other 1976 colonies as well as most of the colonies from 1986 also contained the second symmetrical inversion resulting from recombination between rrnD and rrnE. Most colonies of the 1991 strain, on the other hand, contained a second asymmetrical inversion by recombination between rrnD and rrnC.

### TABLE 1 Arrangement types and estimated replichore imbalance of Salmonella Typhi strains isolated from carrier 1

| Yr isolated | Arrangement type | Observed occurrence (no. of colonies/total) | Estimated imbalance (°) |
|-------------|-----------------|--------------------------------------------|------------------------|
| 1981        | 1234657         | 2/6                                        | 3.2 CCW                |
|             | 1’236457        | 2/6                                        | 1.1 CW                 |
|             | 1’6234557       | 1/6                                        | 3.4 CW                 |
|             | 17543652        | 1/6                                        | 27.5 CCW               |
| 1993        | 1’2643657       | 3/7                                        | 10.5 CCW               |
|             | 1’243657        | 2/7                                        | 9.0 CCW                |
|             | 1743652         | 1/7                                        | 22.8 CCW               |
|             | 17566342        | 1/7                                        | 28.4 CCW               |
| 1996        | 1765432         | 3/7                                        | 37.1 CCW               |
|             | 1763542         | 1/7                                        | 15.8 CCW               |
|             | 17665432        | 1/7                                        | 17.3 CCW               |
|             | 17653442        | 1/7                                        | 21.4 CCW               |
|             | 1743652         | 1/7                                        | 34.0 CCW               |
| 1998        | 1’236574        | 5/6                                        | 1.1 CCW                |
|             | 1475632         | 1/6                                        | 37.1 CCW               |

* 1’, region 1 is in the inverted orientation.
  CW, clockwise; CCW, counterclockwise.

### TABLE 2 Arrangement types and estimated replichore imbalance of Salmonella Typhi strains isolated from carrier 2

| Yr isolated | Arrangement type | Observed occurrence (no. of colonies/total) | Estimated imbalance (°) |
|-------------|-----------------|--------------------------------------------|------------------------|
| 1981        | 12366457        | 4/6                                        | 1.6 CCW                |
|             | 1234657         | 2/6                                        | 3.2 CCW                |
| 1985A       | 1’236457        | 4/5                                        | 1.1 CW                 |
|             | 1754362         | 1/5                                        | 34.0 CCW               |
| 1985B       | 1’236457        | 6/6                                        | 1.1 CW                 |
|             | 1’234567        | 1/1                                        | 1.1 CW                 |
|             | 1’236457        | 7/7                                        | 1.1 CW                 |
| 1999        | 1236457         | 1/1                                        | 3.2 CW                 |

* 1’, region 1 is in the inverted orientation.
  CW, clockwise; CCW, counterclockwise.

### TABLE 3 Arrangement types and estimated replichore imbalance of Salmonella Typhi strains isolated from carrier 3

| Yr isolated | Arrangement type | Observed occurrence (no. of colonies/total) | Estimated imbalance (°) |
|-------------|-----------------|--------------------------------------------|------------------------|
| 1977        | 1736542         | 3/6                                        | 12.7 CCW               |
|             | 1736442         | 2/6                                        | 7.4 CCW                |
|             | 17366542        | 1/6                                        | 11.0 CCW               |
| 1983        | 1673542         | 6/7                                        | 15.8 CCW               |
|             | 16673542        | 1/7                                        | 17.3 CCW               |
| 1985        | 17366542        | 7/7                                        | 11.0 CCW               |

* CW, clockwise; CCW, counterclockwise.

### TABLE 4 Arrangement types and estimated replichore imbalance of Salmonella Typhi strains isolated from carrier 4

| Yr isolated | Arrangement type | Observed occurrence (no. of colonies/total) | Estimated imbalance (°) |
|-------------|-----------------|--------------------------------------------|------------------------|
| 1976        | 1’234567        | 4/7                                        | 1.1 CW                 |
|             | 1734562         | 3/7                                        | 12.7 CCW               |
| 1981        | 1’234567        | 7/7                                        | 1.1 CW                 |
| 1983        | 1’234567        | 7/7                                        | 1.1 CW                 |
| 1986        | 1734562         | 4/6                                        | 12.7 CCW               |
|             | 1734562         | 1/6                                        | 6.9 CCW                |
| 1987        | 1’234567        | 7/7                                        | 1.1 CW                 |
| 1989        | 1’234567        | 7/7                                        | 1.1 CW                 |
| 1991        | 1765432         | 5/7                                        | 37.1 CCW               |
|             | 17356642        | 1/7                                        | 11.0 CCW               |
|             | 1’543267        | 1/7                                        | 33.2 CCW               |

* 1’, region 1 is in the inverted orientation.
  CW, clockwise; CCW, counterclockwise.

**Vi phage typing.** Numerous phage typing schemes have been developed to characterize strains of the same *Salmonella* serovar based on sensitivity to a set of phages (41). The Vi phage typing scheme developed by Craigie and Yen has been the traditional method for typing *Salmonella Typhi* strains (42, 43) and was the epidemiological tool of choice for studying outbreaks of typhoid fever before the advent of modern molecular approaches such as ribotyping, IS200 typing, and pulsed-field gel electrophoresis (PFGE) (44–50). The Vi phage typing scheme has been used extensively to distinguish outbreak strains, determine sources of infection, identify sporadic cases, and track the status of long-term human carriers. The 20 *Salmonella* Typhi strains analyzed in this study were Vi phage typed before long-term storage on Dorset egg slants and again after their revival. Strains isolated from the same carrier had the same Vi phage type before and after storage regardless of the year isolated (see Table S1 in the supplemental material). The strains from carriers 2 to 4 had defined phage types, while the four strains from carrier 1 had a noncharacteristic phage pattern (51). These results show that the established phage types for each strain are stable and that each carrier acquired *Salmonella* Typhi during different typhoid outbreaks, and they suggest that each carrier was infected only once because *Salmonella Typhi* reinflection typically occurs with strains having other phage types (52, 53).

**Rearrangements occur infrequently during culturing.** While it has been demonstrated that rearrangements by rrn recombination occur infrequently in vitro (37), it remained possible that the rearrangements observed in this study occurred during routine culturing of the strains. To determine if rearrangements occurred during standard culturing conditions, the arrangement types of three strains each from carriers 1, 2, and 4 and two strains from...
carrier 3 were determined following growth under standard laboratory conditions (Table 5). Frozen stocks were prepared from single colonies of each strain streaked from the Dorset egg slants. Subsequently, six single colonies were isolated from the frozen stocks and were used to inoculate broth cultures for genomic DNA isolation. All six colonies isolated from each frozen stock had identical arrangement types, demonstrating that rearrangements do not occur frequently enough under standard culturing conditions to be detected by the PCR screen. These results agree with previously published data showing that while strains belonging to Salmonella serovars Typhi (host specific) and Typhimurium (host generalist) have similar relative rearrangement frequencies in generalist), there have been similar relative rearrangement frequencies to be detected by the PCR screen. These results agree with previous studies reporting that rearrangements occur within a few days of restreaking and culturing of multiple colonies (56).

**Estimated replichore balance.** We used a "replichore calculator" (26) to determine the replichore balance of each of the observed arrangements. While most observed arrangement types had well-balanced replichores, the observed rearrangements caused considerable variations in replichore balance between colonies isolated from the same strain and slant (Tables 1 to 4). The majority of colonies derived from carrier 1 strains isolated in 1981 and 1998 had no more than an imbalance of 3°. Colonies from the 1993 strain were more imbalanced, with one colony having an arrangement type with an estimated imbalance of 23°. Colonies derived from the 1996 strain were even more imbalanced because of a second inversion. In contrast, all of the carrier 2 colonies had an estimated imbalance of ≤3°, except for one colony derived from the 1985A sample that had undergone a second asymmetrical inversion resulting in an estimated imbalance of 34°. Most colonies derived from the carrier 3 strains had an estimated imbalance of between 11 and 17°, and more than half contained a duplication of either region 4 or region 6. In contrast, all of the colonies derived from the carrier 4 strains isolated in 1981, 1983, 1987, and 1989 and four of seven colonies from the strain isolated in 1976 were balanced. The other colonies from 1976 as well as colonies from the 1986 had larger amounts of replichore imbalance (up to 13°), while colonies derived from the 1991 strain had up to 37° imbalance. These results indicate that rearrangements drastically increase the imbalance of previously well-balanced arrangement types, and rearrangements do not improve replichore balance over time.

**Growth rates.** It has been suggested that chromosomal rearrangements and the resulting replichore imbalance may have detrimental fitness effects (54–57). To test this idea, the growth rates of strains isolated from the same carrier but with different arrangement types and degrees of replichore balance were measured. The arrangement types (and degrees of replichore imbalance) of the strains measured were 16673542 (17.3° imbalance) and 17366542 (11° imbalance) for the 1983 and 1985 carrier 3 strains, respectively; 1234567 (1.1° imbalance) for the 1987 and 1989 carrier 4 strains; and 1543267 (33.2° imbalance) for the 1991 carrier 4 strain. Each of the strains tested had identical growth rates regardless of the carrier they were isolated from, their arrangement type, or estimated replichore balance. These results corroborate other evidence (54, 58) that suggests that relative fitness is not adversely affected by chromosomal rearrangements (including duplications and inversions) that imbalance chromosomal replichores up to 50°.

**DISCUSSION**

The pattern of observed arrangement types, in particular the arrangement types found in the strains from carriers 1 and 4, suggests that rearrangements occurred in vivo over time within the carrier. Furthermore, the presence of different arrangement types identified in colonies derived from the same strain/slant implies that rearrangements also occurred on the slants over time. The replichore balance estimations showed that rearrangements often decrease balance and that balance does not improve over time. Finally, colonies with arrangement types having either tandem or interreplichore duplications were found in at least one strain from each carrier. These observations support the hypothesis that aspects of lifestyle, not replichore imbalance per se, are responsible for the multitude of chromosomal rearrangements found in host-specific Salmonella serovars.

Two explanations for this observation are that the carrier was originally infected with bacteria having different arrangement types and that rearrangements occur within the carrier over time. Although the arrangement types of strains isolated from the same carrier at different time points varied depending on the carrier, strains isolated from the same carrier always had the same Vi phage type, suggesting that strains isolated from the same carrier were derived from a single infection. These results may be explained by both the lifestyle of Salmonella Typhi in the carrier state and how Salmonella Typhi is transmitted from a carrier to a new human host. Chromosomal rearrangements occur at the same relatively low frequency in both generalist and host-specific Salmonella serovars (37), but do not become fixed within generalist Salmonella populations because bacteria with inversions are less fit under certain environmental conditions and thus lost from the population. However, when in the carrier state, Salmonella Typhi cells are intracellular and are not under the same types of selective pressures experienced by generalist Salmonella serovars. In addition, because Salmonella Typhi is host specific to humans and has no known environmental reservoir, a genetic bottleneck occurs during human-to-human transmission. In contrast, the generalist salmonellae can infect multiple host species and therefore do not pass through a strict bottleneck during each transmission to a new host. These stochastic processes may explain how chromosomal rearrangements become fixed with Salmonella Typhi populations as well as populations of other host-specific Salmonella serovars.

The replichore balance estimations showed that most common arrangement types were well balanced (<15° imbalance), and the estimated degrees of balance of colonies derived from strains iso-
lated during a particular year were similar if not identical. However, there were notable exceptions. For example, the carrier 1 strain isolated in 1993 and 1996 and the carrier 4 strain isolated in 1991 were estimated to have replichores that are up to 37° imbalanced. Other imbalanced arrangement types were relatively rare, occurring only once within a set of colonies, as in the 1998 strain from carrier 1 and the first 1985 strain from carrier 2. Replichore balance did not consistently improve over time but was random, depending on the strain isolated in a particular year, as clearly exemplified by strains isolated from carrier 4 (Table 1).

Rearrangements occur infrequently during standard culturing conditions, as demonstrated by Helm et al. (37) and our observation that rearrangements did not occur in colonies streaked from frozen stocks. In contrast, rearrangements occurred during long-term storage on slants. Rearrangements have also been observed in *Salmonella enterica* serovar Typhimurium strains stored for decades in stab cultures (38, 59). Most of these rearrangements were tandem duplications of region 4, 5, or 6. Such duplications occur by unequal recombination between *rrn* operons at frequencies as high as 10^-2 (60). Other rearrangements that changed the order or inverted the chromosomal regions between *rrn* operons were observed in colonies derived from long-term storage on agar slants. While duplications may provide a selective advantage in nutritionally limited environments, such as those found during long-term storage on stabs or slants (61, 62), other rearrangements probably do not. Another possibility that explains both types of rearrangements is that during long-term growth on the slant, growth is very slow, so selection is relaxed, allowing rearrangements to become fixed within the slant population.

In conclusion, chromosomal rearrangements were observed in archived slants of *Salmonella* Typhi strains isolated from human carriers. The rearrangements occurred over time during carriage in vivo as well as during storage of the slants. Our results agree with a previous analysis of multiple *Salmonella* Typhi strains isolated from the same outbreaks of typhoid fever in Spain. These strains also had the same Vi phage type but different arrangement types (63), and analysis of isolates from an asymptomatic carrier suggested that one arrangement type was derived from the other (48). These results support the hypothesis that the unique aspects of lifestyle in the host are responsible for these rearrangements in *Salmonella* Typhi and other host-specific *Salmonella* serovars.

**MATERIALS AND METHODS**

**Strains and growth conditions.** The strains used were isolated from fecal samples taken between 1976 and 1999 from four asymptomatic female *Salmonella* Typhi carriers from eastern Germany who had never been treated with antibiotics. Single-colony isolates were serotyped, Vi phage typed, and then streaked and stored on Dorset egg slants in a controlled ambient temperature room at the Robert Koch Institut, Wernigerode, Germany. Colonies for this study were obtained by streaking swabs from the slants onto Endo agar (Oxoid, Wesel, Germany) for single-colony isolation. One colony per slant was phage typed to confirm original results. Bacteria were routinely cultured on Luria-Bertani (LB) medium at 37°C. Solid LB plates were prepared by adding agar to 1.5% (wt/vol). Generation times were calculated by growing strains in triplicate at 37°C with aeration. Readings were taken every 30 min on a Klett-Summerson colorimeter using a 540-nm filter. Time points in early exponential phase were used to calculate the generation time by the formula 1/((log(OD<sub>600</sub> at t<sub>2</sub>) - log(OD<sub>600</sub> at t<sub>1</sub>))/[0.301 × (t<sub>2</sub> - t<sub>1</sub>)]), where OD<sub>600</sub> is the optical density at 600 nm and t is the time in hours.

**Isolation of chromosomal DNA.** Chromosomal DNA was isolated from overnight cultures by using either the Wizard genomic DNA purification kit, as described by the manufacturer (Promega, Madison, WI), or the cetyltrimethylammonium bromide (CTAB)-based bacterial genomic DNA miniprep protocol described in reference 64.

**PCR conditions.** Reactions were performed using Platinum Taq high-fidelity DNA polymerase according to the manufacturer’s instructions (Invitrogen Corp., Carlsbad, CA). Reaction mixtures were heated to 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 7 min and a final step at 68°C for 7 min. The primer sequences and combinations for detecting specific *rrn* combinations were the same as those described by Helm and Maloy (65). The presence of *rrn* PCR products was determined by running 10 µl of each reaction mixture out on a 0.8% agarose–1× Tris-borate-EDTA (TBE) gel, followed by detection using ethidium bromide staining.

**PERL script to estimate replichore balance.** To estimate replichore balance, a PERL-based calculator was used (26). The calculator estimated the replichore balance of each arrangement type from the order of chromosomal regions between the *rrn* operons and the size of each chromosomal region. (The mean length of each region was determined from the sequenced *Salmonella* Typhi strains Ty2 and CT18.) The origin of replication and the *dif* site were used as the replichore endpoints.

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**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.orglookup/ suppl doi:10.1128/mBio.00060-11/-DCSupplemental.

**TABLE S1, PDF file, 0.011 MB.**

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