RESEARCH ARTICLE

The ParB-*parS* Chromosome Segregation System Modulates Competence Development in *Streptococcus pneumoniae*

Laetitia Attaiech,[a] Anita Minnen,[b] Morten Kjos,[a] Stephan Gruber,[b] Jan-Willem Veening[a]

Molecular Genetics Group, Centre for Synthetic Biology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands[a]; Max Planck Institute of Biochemistry, Martinsried, Germany[b]

ABSTRACT **ParB proteins bind centromere-like DNA sequences called *parS* sites and are involved in plasmid and chromosome segregation in bacteria. We previously showed that the opportunistic human pathogen *Streptococcus pneumoniae* contains four *parS* sequences located close to the origin of replication which are bound by ParB. Using chromatin immunoprecipitation (ChIP), we found here that ParB spreads out from one of these *parS* sites, *parS*(−1.6°), for more than 5 kb and occupies the nearby *comCDE* operon, which drives competence development. Competence allows *S. pneumoniae* to take up DNA from its environment, thereby mediating horizontal gene transfer, and is also employed as a general stress response. Mutating *parS*(−1.6°) or deleting *parB* resulted in transcriptional up-regulation of *comCDE* and *ssbB* (a gene belonging to the competence regulon), demonstrating that ParB acts as a repressor of competence. However, genome-wide transcription analysis showed that ParB is not a global transcriptional regulator. Different factors, such as the composition of the growth medium and antibiotic-induced stress, can trigger the sensitive switch driving competence. This work shows that the ParB-*parS* chromosome segregation machinery also influences this developmental process.**

IMPORTANCE ***Streptococcus pneumoniae*** **(pneumococcus) is an important human pathogen responsible for more than a million deaths each year. Like all other organisms, *S. pneumoniae* must be able to segregate its chromosomes properly. Not only is understanding the molecular mechanisms underlying chromosome segregation in *S. pneumoniae* therefore of fundamental importance, but also, this knowledge might offer new leads for ways to target this pathogen. Here, we identified a link between the pneumococcal chromosome segregation system and the competence-developmental system. Competence allows *S. pneumoniae* to take up and integrate exogenous DNA in its chromosome. This process plays a crucial role in successful adaptation to—and escape from—host defenses, antibiotic treatments, and vaccination strategies. We show that the chromosome segregation protein ParB acts as a repressor of competence. To the best of our knowledge, this is the first example of a ParB protein controlling bacterial competence.**

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Address correspondence to Jan-Willem Veening, j.w.veening@rug.nl.

Streptococcus pneumoniae (pneumococcus) is an opportunistic human pathogen with high morbidity and mortality rates which causes several invasive diseases, such as pneumonia, septicemia, and meningitis. For instance, for only the disease pneumonia, it was recently estimated that *S. pneumoniae* is annually responsible for more than 400,000 deaths in children under the age of 5 (1).

The evolutionary success of *S. pneumoniae* strongly depends on its ability to colonize its host and adapt to changing environments within the host. A crucial adaptation system in pneumococci is the competence system, which allows genetic transformation, a horizontal gene transfer mechanism in which the cells are able to take up and integrate exogenous DNA into their genome (2). During competence, cells activate expression of about 100 genes, fewer than 20 of which are required for transformation (3–8). Moreover, development of competence responds to changes in the environment and is activated by certain antibiotics (9–11). Thus, competence is considered a general stress response (6, 12). It has also been suggested that certain cell cycle cues such as DNA replication can serve as regulatory inputs for competence development (11, 13), but it remains unclear how this works under non-stressed conditions.

Chromosome segregation is an essential process during the bacterial cell cycle, and studying the molecular mechanisms underlying chromosome segregation in *S. pneumoniae* might offer new leads for targeting this important human pathogen. We have shown previously that chromosome segregation in *S. pneumoniae* is promoted by at least two proteins, ParB and SMC (structural maintenance of chromosomes) (14). ParB binds to a 16-bp DNA sequence called *parS* (*S. pneumoniae* D39 consensus sequence, tG TTTCACGtGAAACa; bases in lowercase can deviate), four of which can be found in the pneumococcal genome: *parS*(−19.2°), *parS*(−3.7°), *parS*(−1.6°), and *parS*(+2°) (the numbers indicate the relative distance in degrees from the origin of replication as a

function of the circular chromosome). Interestingly, ParB recruits the conserved condensin SMC complex to these *parS* sites, thereby enriching SMC near the origin of replication (*oriC*) (14–16). In the absence of ParB or SMC, chromosome segregation is perturbed, leading to a significant fraction of anucleate cells. Time-lapse microscopy of the pneumococcal cell cycle has revealed that SMC is involved in organizing and splitting of newly replicated *oriC*s, and in the absence of SMC, chromosomes are frequently guillotined by the cell division machinery (17). In addition to ParB-*parS* and SMC, several other processes, such as transcription and replication, might also drive chromosome segregation in *S. pneumoniae* (17, 18). For recent reviews on chromosome segregation in other bacteria, see references 18 to 27.

Besides having active roles in chromosome segregation, chromosomal ParB proteins were also shown to influence the bacterial cell cycle in other ways. For instance, ParB proteins affect DNA replication either directly or indirectly in a number of different bacteria, including *Bacillus subtilis* and *Vibrio cholerae* (28, 29). Moreover, plasmid ParB proteins can act as transcriptional regulators by binding *parS* and "spreading" into nearby plasmid promoters (30). Spreading is the ability of ParB proteins to bind nonspecific DNA, presumably via oligomerization that nucleates at a *parS* site, and via DNA looping, even distant sites can be bound (27). Interestingly, ParB oligomers occupying DNA near chromosomal *parS* sites generally do not affect gene expression (27, 31). However, there are exceptions; it was recently shown that the chromosomal ParB1 protein of *V. cholerae* can indeed control transcription (32), and, directly or indirectly, deletion of *Pseudomonas aeruginosa parB* leads to global transcriptional changes (33). Together, these observations raise the question of whether *S. pneumoniae* ParB also has additional roles besides recruiting SMC to *oriC*. In particular, we noticed that one of the *parS* sequences, *parS*(−1.6°), is located only 5 kb away from the *comCDE* operon (Fig. 1). The *comCDE* operon lies at the heart of the competence development cascade in pneumococcus (Fig. 1), and small differences in *comCDE* expression can activate competence because of the positive feedback loop built into the regulatory network (11, 34, 35).

To test whether ParB binds the *comCDE* operon and influences its expression, we performed chromatin immunoprecipitation (ChIP) and real-time luciferase assays. We show that ParB binds to *parS*(−1.6°) and from there spreads and/or loops into *comCDE*, thereby reducing expression of *comCDE* and of downstream competence genes. The data suggest that the autocatalytic nature of the competence system makes ParB regulation unique, since ParB does not influence global gene transcription in *S. pneumoniae*. Together, these results suggest that cell cycle events provide molecular cues for the entry into pneumococcal competence development.

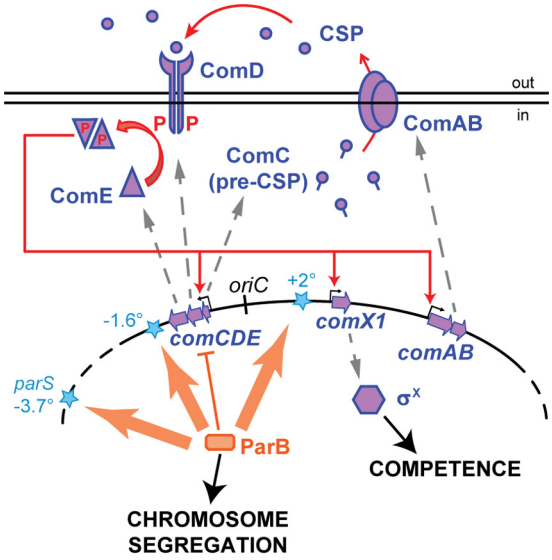

FIG 1 Interplay between chromosome segregation and regulation of competence in *S. pneumoniae*. ParB (orange rectangle) binds to *parS* sites (blue stars), which are located close to the origin of replication (*oriC*). Basal levels of *comCDE* transcription are required to build up the extracellular levels of competence-stimulating peptide (CSP), encoded by *comC*. Once a threshold level of CSP is reached and sensed by ComD, an autocatalytic loop is activated in which phosphorylated ComE (purple triangles) stimulates further expression of *comCDE* and *comAB* (encoding the CSP exporter), leading to expression of the alternative sigma factor σ^X and downstream competence genes. By spreading from *parS* and/or forming DNA loops, ParB binds the *comCDE* locus, effectively reducing CSP accumulation and thereby controlling competence development.

RESULTS

ParB represses competence development in *S. pneumoniae*. Competence is a tightly regulated physiological state in which bacteria become able to take up exogenous DNA and use it for genetic transformation. In *S. pneumoniae*, different factors, such as the composition of the medium, the pH of the growth medium, and certain stresses caused by antibiotics, have been shown to trigger competence (6). However, the molecular mechanisms influencing initiation of competence remains poorly understood. We recently showed that *S. pneumoniae* ParB binds to four *parS* sequences located near *oriC*. Intriguingly, all the operons implicated in the development of natural competence (i.e., *comCDE*, *comAB*, and *comX*) are also located near *oriC* (Fig. 1). In particular, *parS*(−1.6°) is located within 5 kb of the *comCDE* operon, suggesting that binding of ParB to this chromosomal region might influence *comCDE* expression and thus competence development. To test this, we first constructed a reporter strain in which we integrated the gene encoding firefly luciferase behind *comCDE* (Fig. 2A). This reporter (*comCDE_luc*) allows real-time observation of *comCDE* expression, since the amount of light detected (resulting from the activity of luciferase) is directly proportional to the expression level of *comCDE*. Moreover, as firefly luciferase is a very labile protein, this transcriptional fusion is a very sensitive reporter of any changes in expression (36). To check whether changes in *comCDE* expression actually alter competence development, we also introduced a second reporter (P_{ssbB}_*mKate2*) (Fig. 2A), where expression of the red fluorescent protein mKate2 is controlled by the promoter of the *ssbB* gene (P_{ssbB}). P_{ssbB} activity is dependent on the competence-specific alternative sigma factor σ^X and can thus serve as a reporter for competence development (Fig. 1). Wild-type and *parB* mutant cells were grown in C+Y medium at multiple competence-permissive pHs in microtiter plates to measure the optical density, luminescence, and fluorescence at intervals. As shown in Fig. 2 and in Fig. S1 and S2 in the supplemental material, expression of both *comCDE* and *ssbB* was increased in the absence of ParB. Specifically, their expression not only reached higher levels but also was activated at lower cell densities in the *parB* mutant than in the wild type (Fig. 2C and D).

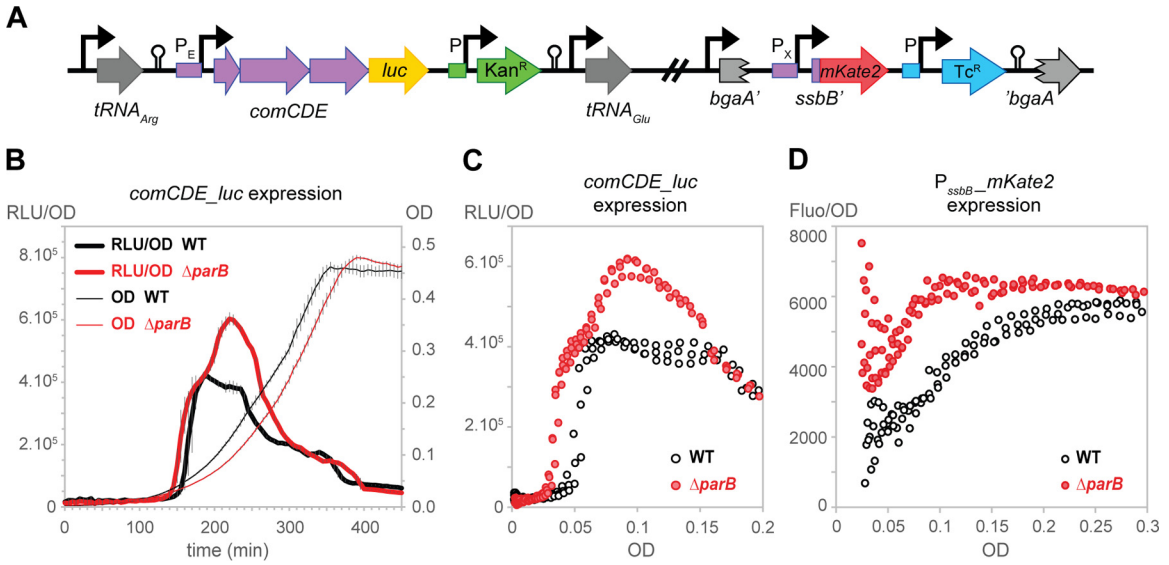

FIG 2 ParB attenuates competence development. (A) Representation of the transcriptional fusions used to follow competence development in the double-labeled strains DLA82 (WT) and DLA84 (Δ*parB*). For the *comCDE_luc* fusion, the *luc* open reading frame (ORF) preceded by its ribosome binding site (and a kanamycin resistance gene with its own promoter) was inserted right after the *comE* ORF. A transcriptional fusion between the promoter of *ssbB* (late competence gene) and the *mKate2* ORF was stably integrated at the nonessential *bgaA* locus. (B to D) The WT strain (DLA82: *comCDE_luc bgaA*::P_{ssbB}*_mKate2*) and the *parB* mutant (DLA84: Δ*parB*::*spc comCDE_luc bgaA*::P_{ssbB}*_mKate2*) were grown in C+Y medium; optical density at 595 nm, luciferase activity (in relative luminescence units [RLU]), and mKate2 production (fluorescence detection using 590 nm and 612 nm as excitation and emission wavelengths, respectively) were determined every 5 min. (B) Cell density (OD) and *comCDE_luc* expression (RLU/OD) as a function of time (averages of three replicates with the standard errors of the mean [SEM] are plotted). (C) Data points are from the same 3 replicates, but *comCDE_luc* expression (RLU/OD) is plotted as a function of OD, which allows direct comparison between strains with different growth kinetics (45). (D) Relative expression of P_{ssbB}*_mKate2* (Fluo/OD) as a function of OD (see Fig. S2 in the supplemental material for the representation of Fluo/OD as a function of time).

Although subtle, these results were highly reproducible and show that the *parB* mutant displays a competence-up phenotype.

We previously showed that competence development is promoted by certain antibiotics that perturb DNA replication (11). When these antibiotics stall replication elongation while new rounds of replication initiate, it causes an increased gene dosage for the genes located close to the origin and consequently an increased expression of *comCDE*. To test whether competence is activated in the absence of ParB due to an increased *comCDE* gene dosage, we performed quantitative real-time PCR to determine the ratio between *oriC* and the terminus region (*ori-ter* ratio). This analysis showed that the *comCDE* gene dosage is slightly reduced in the *parB* mutant (see Fig. S3 in the supplemental material), suggesting that another mechanism must be responsible for the ParB-dependent competence-up phenotype.

***parS*(−1.6°) contributes to the control of competence development.** As mentioned above, *parS*(−1.6°) is located within 10 kb of *oriC* and within 5 kb of *comCDE* (Fig. 3A). To test whether ParB could be a direct repressor of *comCDE* by binding and spreading from *parS*(−1.6°), we mutated this site. As the *parS*(−1.6°) site is situated within a gene (*SPD_2058*), we introduced six silent point mutations to reduce homology to the *parS* consensus sequence [*parS*(−1.6°)$_{mut}$] while keeping the amino acid sequence of *SPD_2058* unaltered (Fig. 3B). To directly compare the impact of the presence or absence of a functional *parS* site at this locus, we complemented the *parS*(−1.6°)$_{mut}$ strain by reinserting the functional *parS*(−1.6°) sequence downstream of *SPD_2058*, resulting in the *parS*(−1.6°)$_{mut}$C strain (Fig. 3C), and we also made a non-complemented mutant derivative, the *parS*(−1.6°)$_{mut}$NC strain (Fig. 3D).

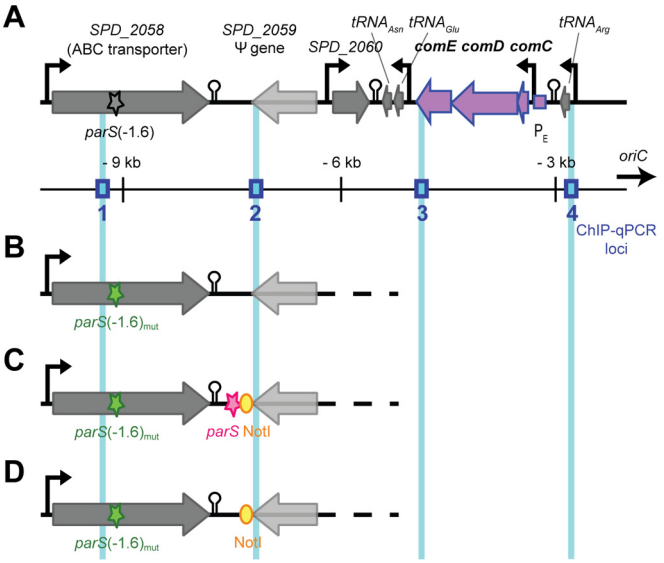

FIG 3 Organization of the *parS*(−1.6°) locus. (A) Schematic representation (proper scale is preserved, and distance from *oriC* is indicated) of the chromosome between *parS*(−1.6°) and the *comCDE* loci in the wild-type strain. Blue boxes show the localization of the four loci tested by qPCR after ChIP experiments (Fig. 5). (B) Chromosomal organization in the *parS*(−1.6°)$_{mut}$ strain. To be able to properly assess the influence of *parS*(−1.6°) on *comCDE* expression, a complemented (C) and a non-complemented (D) derivative of the *parS*(−1.6°)$_{mut}$ strain were constructed. In the *parS*(−1.6°)$_{mut}$C (complemented) strain, the functional *parS*(−1.6°) sequence and a NotI restriction site were inserted at 1.7 kb from the *parS*(−1.6°) locus (between *SPD_2058* and *SPD_2059*), whereas in the *parS*(−1.6°)$_{mut}$NC (non-complemented) strain, only the NotI site is present.

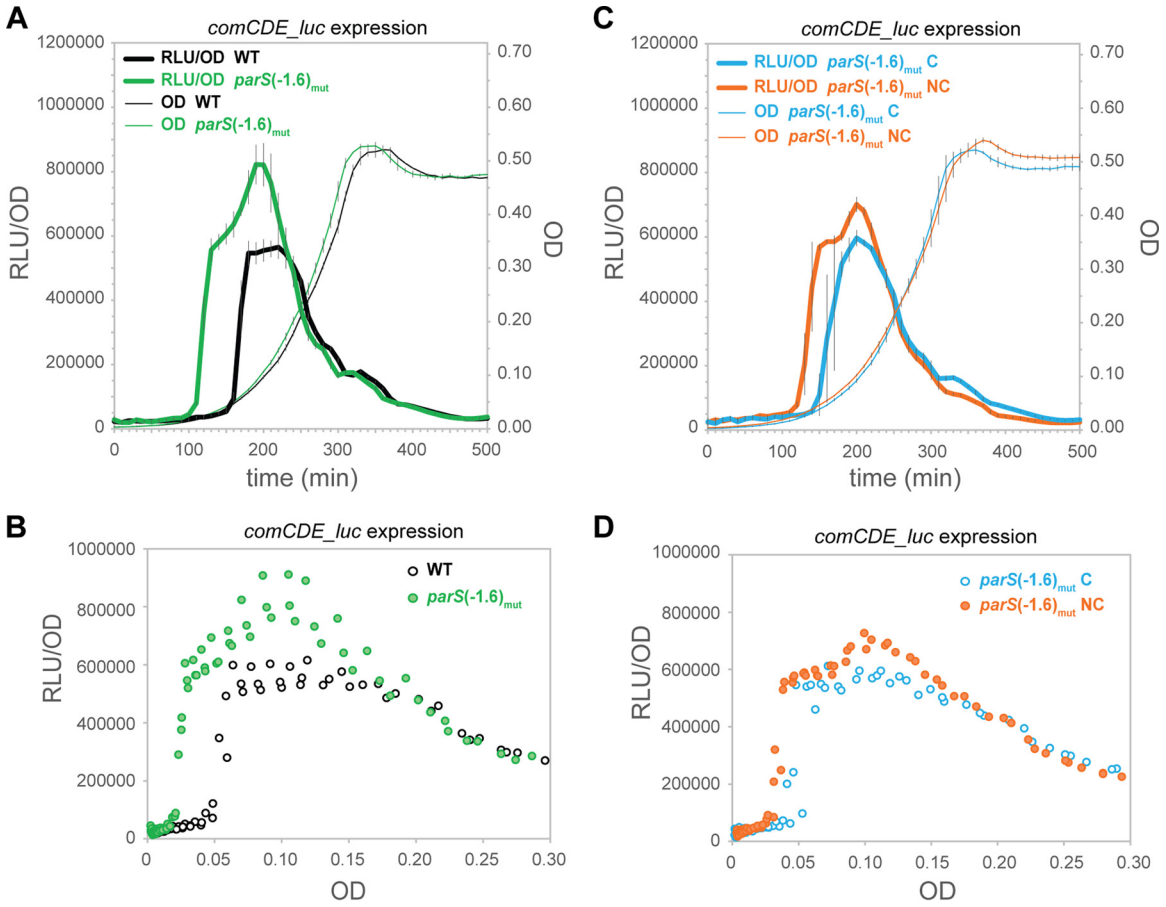

FIG 4 Mutating *parS*(−1.6°) derepresses competence development. Strains with the *comCDE_luc* reporter fusion were grown in C+Y medium; optical density at 595 nm and luciferase activity (in relative luminescence units [RLU]) were determined every 10 min. (A and B) The WT strain (DLA18) versus the *parS*(−1.6°)$_{mut}$ strain (DLA28). (C and D) The *parS*(−1.6°)$_{mut}$C strain (DLA65) versus the *parS*(−1.6°)$_{mut}$NC strain (DLA66). (A and C) Cell density (OD, right axis) and *comCDE_luc* expression (RLU/OD) are presented as a function of time in minutes (averages of three replicates with the SEM are plotted). (B and D) The data points are from the same 3 replicates as in panels A and C, respectively, but *comCDE_luc* expression (RLU/OD) is plotted as a function of OD.

To test the effect of the *parS*(−1.6°) site on competence development, we studied the expression of *comCDE* and *ssbB* by luciferase assays. As shown in Fig. 4A and B and in Fig. S4A to C in the supplemental material, *comCDE* and *ssbB* expression was upregulated and activated earlier when *parS*(−1.6°) was mutated. Note that mutating *parS*(−1.6°) did not lead to the growth defects observed in the *parB* mutant (Fig. 2B) (14), indicating functional redundancy of the *parS* sites and suggesting that the competence phenotype in *parB* and *parS* mutants is not due to a defect in ParB-dependent chromosome segregation (Fig. 4A). Our results show that reintroducing a functional *parS* site close to the mutated one is able to repress *comCDE* (and *ssbB*) (Fig. 4C and D; also, see Fig. S4D to F in the supplemental material). Hence, the presence of a *parS* sequence near *comCDE* is sufficient to delay competence development.

ParB binds to *comCDE* by spreading from *parS*(−1.6°). ParB proteins are thought to organize chromosomes by forming nucleoprotein complexes, nucleating from *parS* sites (27). To test whether *S. pneumoniae* ParB could spread from *parS*(−1.6°) into the nearby *comCDE* operon, and in that way control competence, we performed ChIP assays followed by quantitative real-time PCR of different loci [see Fig. 3 for the localization of the tested loci around *parS*(−1.6°)]. First, we replaced wild-type ParB with a fully functional green fluorescent protein (GFP)-tagged ParB that allows ChIP using anti-GFP antibodies (14). As shown in Fig. 5A, ParB-GFP was highly enriched at the two *parS* sites closest to *oriC*, *parS*(+2°) and *parS*(−1.6°), and was virtually absent from loci located close to the terminus region (T1 and T2, at −175.7° and −174.2° of the chromosome, respectively), as was established before (14). ParB-GFP was also enriched at the 3′ end of the *comCDE* locus and even present, although less so, at the *comCDE* promoter, which is 6.5 kb away from *parS*(−1.6°) (Fig. 5B). Importantly, mutating *parS*(−1.6°) led to a significant reduction of ParB-GFP at *comCDE* (Fig. 5B), strongly suggesting that *parS*(−1.6°) is the main contributor to the presence of ParB at *comCDE*. Introducing a *parS* sequence downstream of the mutated *parS*(−1.6°) locus restored ParB-GFP binding at *comCDE* (Fig. 5C and D). Together, these data show that ParB binds at *parS*(−1.6°) and from there spreads and/or forms DNA loops, thereby binding for more than 6.5 kb into the *comCDE* locus.

ParB is not a global transcriptional regulator in *S. pneumoniae*. The data so far show that *S. pneumoniae* ParB can bind DNA as far as 6.5 kb away from *parS* sequences and that it acts as a repressor for *comCDE* expression and in that way fine-tunes

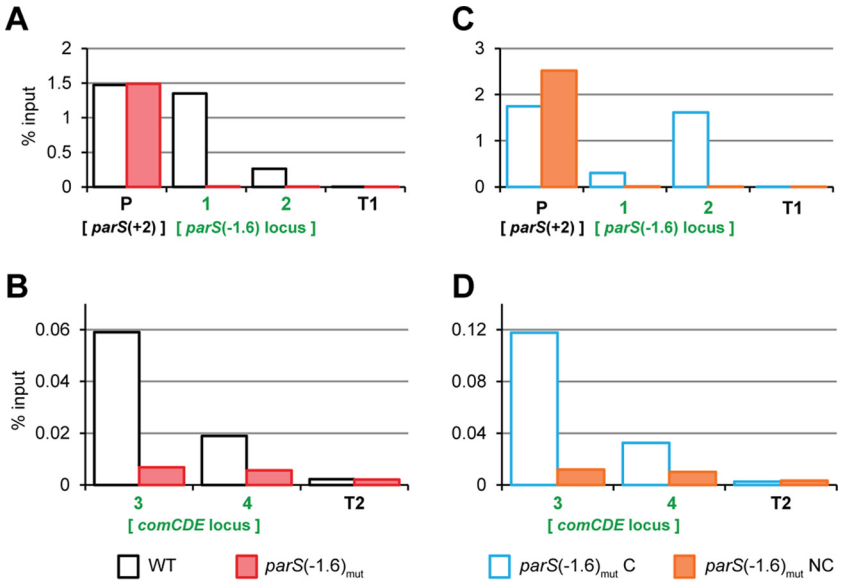

FIG 5 ParB spreads from *parS*(−1.6°) into *comCDE*. In the strains of interest, ParB was replaced by a ParB-GFP fusion. Exponentially growing cells were subjected to chromatin immunoprecipitation (ChIP) using anti-GFP antibodies, and the pulled-down DNA was subsequently analyzed by qPCR. (A and B) The WT (DLA42) versus the *parS*(−1.6°)$_{mut}$ strain (DLA43). (C and D) The *parS*(−1.6°)$_{mut}$C strain (DLA77) versus the *parS*(−1.6°)$_{mut}$NC strain (DLA80). The loci amplified by primer pairs 1, 2, 3, and 4 are shown in blue in Fig. 3A. The primer pair P amplifies another *parS* site [*parS*(+2°)] situated +11 kb from *oriC*. Primer pairs T1 and T2 amplify 2 different loci in the terminus region. The graphs show pulldown efficiency (ChIP-DNA/input DNA × 100) for each primer pair. Note the different *y* axis scale between panels A and C and panels B and D.

competence development. To test whether ParB affects transcription globally, we performed DNA microarray experiments under non-competence-permissive conditions (pH 7.0). Total RNA of mid-exponential-phase wild-type and *parB* mutant cells was isolated and used for DNA microarray experiments (see Materials and Methods for details). As shown in Table 1, expression of only 9 genes was significantly altered (>2-fold change; $P < 0.005$). Importantly, none of these differentially expressed genes was located close to *parS* sequences. Together, these data suggest that pneumococcal ParB is not a global transcriptional regulator and that, in general, transcription of genes located in close proximity to *parS* sites is not strongly affected.

Expression of a constitutive synthetic promoter is not influenced by the presence of *parS*. ParB influences competence de-

TABLE 1 Significantly differentially regulated[a] genes in strain MT3 (Δ*parB*::*spc*) compared to wild-type D39

Gene category and locus tag	Description	Fold change	*P* value	Nearest *parS* site	Distance (kb) from *parS*[b]
Upregulated genes					
SPD_0731	Topology modulation protein	3.6	0.004	*parS*(+2°)	733.4
SPD_1874	LysM domain-containing protein	2.7	2.41E-08	*parS*(−19.2°)	85.2
SPD_1871	Hypothetical protein	2.5	0.002	*parS*(−19.2°)	86.2
SPD_0978	Hypothetical protein	2.3	0.005	*parS*(+2°)	945.9
SPD_1933	*malQ* (4-alpha-glucanotransferase)	2.2	0.001	*parS*(−19.2°)	31.7
Downregulated genes					
SPD_2069	*parB*[c]	−21.1	9.07E-11	*parS*(−1.6°)	8.6
SPD_0114	Hypothetical protein	−4.1	3.05E-09	*parS*(+2°)	104.4
SPD_0115	Hypothetical protein	−3.9	2.46E-06	*parS*(+2°)	104.9
SPD_0116	Hypothetical protein	−3.8	2.79E-08	*parS*(+2°)	106.4
Competence genes[d]					
SPD_2065	*comC*	1.6	0.144	*parS*(−1.6°)	6.4
SPD_2064	*comD*	−1.2	0.123	*parS*(−1.6°)	6.3
SPD_2063	*comE*	−1.1	0.572	*parS*(−1.6°)	5.0
SPD_0049	*comA*	1.5	0.157	*parS*(+2°)	30.5
SPD_0050	*comB*	1.4	0.104	*parS*(+2°)	32.7

[a] Genes were considered differentially regulated when the change was >2-fold and *P* was <0.005. Note that data originated from two biological replicates, one dye-swap, and three technical replicates (9 data points for each gene).
[b] Distance from the gene to the closest *parS*.
[c] Note that *parB* is missing in strain MT3, but due to cross-hybridization, some residual signal was detected in strain MT3.
[d] The competence genes were not significantly differentially regulated ($P > 0.005$) under these non-competence-permissive conditions (pH 7.0).

velopment by binding and repressing *comCDE* but does not significantly affect expression of other genes located close to *parS* sites. If regulation of *comCDE* by ParB is indeed unique, then transcription of a synthetic constitutive promoter should not be affected by the nearby presence of a *parS* site. To test this, we constructed a number of reporter constructs. First, we introduced the constitutive synthetic P_{32} promoter (37) fused to luciferase close to *oriC* at +5° of the chromosome. Next, we compared the expression of P_{32}_*luc* in a wild-type strain to that in *parB* mutant (see Fig. S5A and B in the supplemental material) and also between the *parS*(−1.6°)$_{mut}$NC mutant and its complemented counterpart, the *parS*(−1.6°)$_{mut}$C strain (see Fig. S5C and D in the supplemental material). In all cases, transcription of luciferase from P_{32} was unaffected by the absence of ParB or by the presence of *parS* (see Fig. S5A to D in the supplemental material). However, the closest *parS* sequence in these strains is *parS*(+2°), approximately 18 kb away from P_{32}_*luc* at 5°; thus, it could be that ParB spreading or looping is insufficient to efficiently alter transcription of the P_{32}_*luc* fusion. Therefore, we introduced the P_{32}_*luc* construct, together with either an intact or mutated *parS* sequence [*parS*(−1.6°)] at the ectopic *bgaA* locus. As shown in Fig. 6, luciferase expression from this synthetic construct is identical whether or not it is preceded by a functional *parS* sequence.

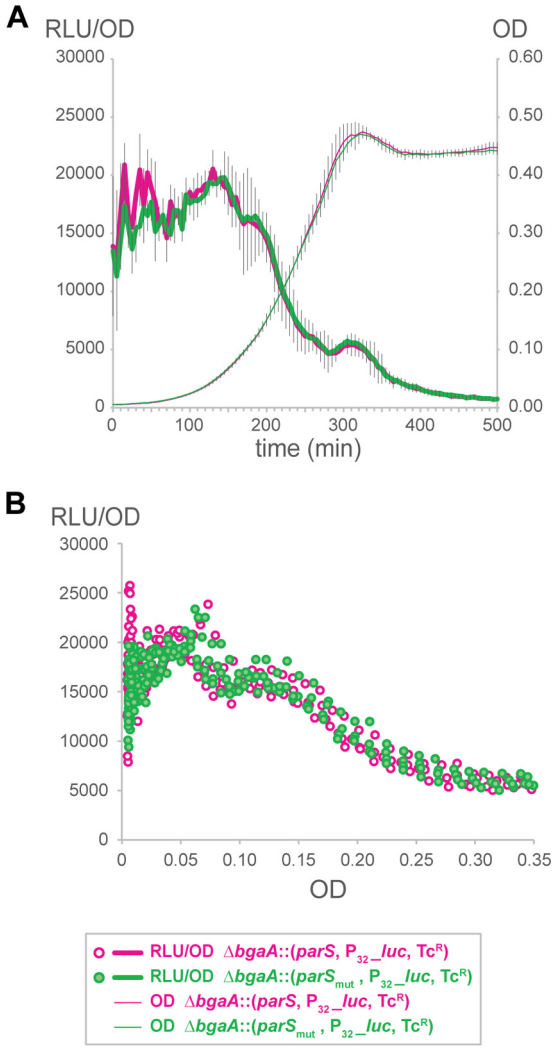

FIG 6 The ParB-*parS* repressor effect is not detected on a strong synthetic promoter. Strains were grown in C+Y medium; optical density at 595 nm and luciferase activity (in relative luminescence units [RLU]) were determined every 5 min. The P_{32}_*luc* reporter fusion was inserted at the *bgaA* locus and preceded either by a functional *parS* sequence (strain DLA106) or by a mutated *parS* sequence (strain DLA107). (A) Cell density (OD) and P_{32}_*luc* expression (RLU/OD) as a function of time (averages of three replicates with the SEM are plotted). (B) Data points are from the same 3 replicates, but P_{32}_*luc* expression (RLU/OD) is plotted as a function of OD.

DISCUSSION

Competence development by the *comCDE* regulatory system is highly conserved in clinical isolates of *S. pneumoniae* (38). The system relies on the tight genetic regulation of CSP production and export, and as this regulation involves a positive feedback loop, competence activation spreads throughout the population (Fig. 1) (39). Moreover, competence development is influenced by several parameters (40). For example, the composition and pH of the medium affect induction of competence (9), and certain antibiotics promote competence (10) either by inducing the production of misfolded proteins (41) or by altering the gene dosage of *comCDE* (11). Here, we show that the ubiquitous chromosome segregation protein ParB also influences competence development: it binds to *parS*(−1.6°) and from there modulates expression of *comCDE* (Fig. 2, 4, and 5). Whether ParB spreads from *parS*(−1.6°) into *comCDE* or loops DNA to bind *comCDE* is currently not clear. Also, we cannot exclude an indirect effect of the *parS*-mediated ParB effect on competence. In any case, *comCDE* is, under our experimental conditions, the only *parS*-dependent ParB-regulated operon, and gene expression in general is not affected by binding of ParB (Fig. 6; Table 1). This highlights the unique sensitivity of the competence developmental system: due to its autocatalytic nature, even slight imbalances in *comCDE* expression can trigger competence development. The biological function of ParB-controlled competence is at this point unclear and currently under investigation by us, but it is tempting to speculate that cell cycle control of competence is crucial for the maintenance of genome integrity and for coping with stress (6, 12). While competence allows pneumococci to take up exogenous DNA and in that way rapidly acquire new traits, such as antibiotic resistance markers and altered capsule serotypes, competent cells also present a higher fitness in certain stressful conditions, even in the absence of DNA (42). Furthermore, our finding that ParB affects competence development also suggests that cell cycle events, such as chromosome segregation, can provide molecular cues for the entry into competence. Interestingly, the chromosomal location of *comCDE* near *oriC*, and thus near *parS* sites, is highly conserved in *S. pneumoniae* (11), suggesting that ParB control of competence is also conserved. It will be interesting to see if ParB proteins in other organisms can also control sensitive genetic switches and can link chromosome segregation with bacterial development.

MATERIALS AND METHODS

Strains, plasmids, and growth conditions. Bacterial strains and plasmids used in this study are listed in Table S1 in the supplemental material. Growth conditions are described in detail in the supplemental material, as well as the construction of the plasmids and strains used. *S. pneumoniae* strains were grown as standing cultures in complex C+Y medium at 37°C. Blood agar plates were made from Columbia agar containing 4% defibrinated sheep blood (Johnny Rottier, Kloosterzade, the Netherlands).

Escherichia coli EC1000 was grown at 37°C in a shaking incubator in TY broth (Bacto-tryptone [1%], Bacto-yeast extract [0.5%], and 1% NaCl).

Growth curves, luminescence, and fluorescence assays. C+Y medium (pH adjusted with HCl) was complemented with 340 μg/ml luciferin and inoculated with mid-exponential-phase frozen cultures diluted 100 times. Cells were grown at 37°C in 96-well plates (Polystyrol, white, flat, and clear bottom; Corning) in a microtiter plate reader (Tecan Infinite F200 Pro). Throughout growth, absorbance (optical density at 595 nm [OD_{600}]), luminescence (expressed in relative luminescence units [RLU]), and mKate2 signal (590 nm/612 nm, 50% dichroic mirror) were measured every 5 or 10 min with 3 to 5 replicates for each condition. Expression of the *luc* gene results in the production of luciferase and thereby in the emission of light when the medium contains luciferin (36). Due to high background fluorescence of the C+Y medium and a growth-dependent decrease in background fluorescence during bacterial growth, normalization of fluorescence values was done by using a strain identical to the one studied, except for the absence of the P_{ssbB}-*mKate2* reporter in the *bgaA* locus (DLA18 for DLA82 and DLA20 for DLA84). The average mKate2 signal normalized to the OD_{600} of 3 replicates of the control strain was subtracted from the mKate2 signal normalized to the OD_{600} of each of the tested strain replicates to obtain the fluorescence/OD values plotted in Fig. 2, calculated as follows: $[(mKate2_{reporter} - mKate2_{medium})/(OD_{reporter} - OD_{medium})] - [(mKate2_{control} - mKate2_{medium})/(OD_{control} - OD_{medium})]$.

Recombinant DNA techniques and oligonucleotides. Procedures for DNA purification, restriction, ligation, agarose gel electrophoresis and transformation of *E. coli* were carried out as described previously (43). The oligonucleotides used in this study are listed in Table S2 and were obtained from Biolegio (Nijmegen, the Netherlands). *S. pneumoniae* chromosomal DNA was isolated using a Promega Wizard genomic DNA purification kit. DNA-modifying enzymes were purchased from Roche (Mannheim, Germany), New England Biolabs (Ipswich, MA, USA), Bioline (London, United Kingdom), and Fermentas (Burlington, Canada) and used as described by the manufacturer.

ChIP-qPCR. The chromatin immunoprecipitation-quantitative PCR (ChIP-qPCR) protocol is detailed in reference 14 and in the supplemental material. Briefly, cells were grown to mid-exponential phase (OD_{600} = 0.2) in acid C+Y medium (pH = 6.8) at 37°C, and 70 ml of culture was mixed by inverting with 7 ml of fixing solution (50 mM Tris [pH 8.0], 100 mM NaCl, 0.5 mM EGTA, 1 mM EDTA, 11% [vol/vol] formaldehyde) and incubated at room temperature for 30 min. Cells were washed and sonicated, and then immunoprecipitation was performed using anti-GFP antibodies (rabbit serum, polyclonal; Invitrogen A-6455) and protein G-coupled Dynabeads (Invitrogen). The pulled-down DNA was purified and analyzed by qPCR as described in Text S1 in the supplemental material.

DNA microarrays, RNA isolation, and cDNA preparation. *S. pneumoniae* D39 (wild-type) and MT3 (Δ*parB*::*spc*) cells with an OD_{600} of 0.2 were diluted 100-fold in 28 ml C+Y medium (pH 7.0) and grown without aeration to an OD_{600} of 0.2. Cells were collected by centrifugation at 8,000 × *g* for 5 min at 4°C. RNA was isolated using the macaloïd method and the High Pure RNA isolation kit (Roche), as previously described (44). RNA concentration and quality were determined using NanoDrop ND-1000 (Thermo Scientific) and capillary electrophoresis with an Agilent 2100 Bioanalyzer (Agilent Technology). cDNA synthesis and labeling with either DyLight550 or DyLight650 (Thermo Scientific) were performed as described in reference 11. For microarray methods and data analysis, procedures described in reference 44 were used. Genes were considered significantly affected when the absolute change was greater than 2-fold, with a *P* value cutoff of 0.005.

DNA microarray accession number. The DNA microarray data have been submitted to ArrayExpress with accession no. E-MTAB-3300.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00662-15/-/DCSupplemental.

Text S1, PDF file, 0.2 MB.
Figure S1, TIF file, 0.8 MB.
Figure S2, TIF file, 0.5 MB.
Figure S3, TIF file, 0.2 MB.
Figure S4, TIF file, 0.8 MB.
Figure S5, TIF file, 0.6 MB.
Table S1, DOCX file, 0.1 MB.
Table S2, DOCX file, 0.1 MB.

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