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1 Intracellular positioning systems limit the entropic eviction of 2 secondary replicons toward the nucleoid edges in bacterial cells

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13

14 **Abstract**

15 Bacterial genomes, organized intracellularly as nucleoids, are composed of a main chromosome
16 coexisting with different types of secondary replicons. Secondary replicons are major drivers of
17 bacterial adaptation by gene exchange. They are highly diverse in type and size, ranging from less
18 than 2 to more than 1000 kb, and must integrate with bacterial physiology, including to the nucleoid
19 dynamics, to limit detrimental costs leading to their counter-selection. We show that large DNA
20 circles, whether from a natural plasmid or excised from the chromosome tend to localize in a
21 dynamic manner in a zone separating the nucleoid from the cytoplasm at the edge of the nucleoid.
22 This localization is in good agreement with in silico simulations of DNA circles in the nucleoid volume.
23 Subcellular positioning systems counteract this tendency, allowing replicons to enter the nucleoid
24 space. In enterobacteria, these systems are found in replicons above 25 kb, defining the limit with
25 small randomly segregated plasmids. Larger replicons carry at least one of the three described family
26 of systems, ParAB, ParRM and StbA. Replicons above 180 kb all carry a ParAB system, suggesting this
27 system is specifically required in the cases of large replicons. Simulations demonstrated that replicon
28 size profoundly affects localization, compaction and dynamics of DNA circles in the nucleoid volume.
29 The present work suggests that presence of partition systems on the larger plasmids or chromids is
30 not only due to selection for accurate segregation but also to counteract their unmixing with the
31 chromosome and consequent exclusion from the nucleoid.

1 Introduction

2 Bacterial genomes are compact and highly organized both at the genetic and physical levels. Genome
3 organization results from the need of a compromise between folding chromosomes much longer
4 than bacterial cells and favoring the interaction between DNA and machineries implicated in
5 processes such as transcription, replication and segregation. Bacterial genomes are composed of one
6 main chromosome and most often contain secondary replicons. Main chromosomes range from few
7 hundred to over ten thousand kilobases (kb) and are the largest replicon in the cell. Secondary
8 replicons comprise are highly variable in type and size (Smillie *et al*, 2010). It is generally admitted
9 that they fall into three categories: small multicopy plasmids, larger low copy-number plasmids and
10 chromids, also called secondary chromosomes that are large replicons (generally > 500 kb) found in
11 genomes across a species (Harrison *et al*, 2010). Plasmids carry numerous adaptive functions,
12 including numerous virulence factors, and are the major drivers of the transfer of antibiotic
13 resistance genes. Although most plasmids are relatively small (less than 150 kb), they may together
14 account for up to 30% of the genome. Chromids are frequent and appeared independently in several
15 taxa. They originate from plasmids – from different plasmid families depending on species - as judged
16 by the sequence of their replication origins (e.g. Ramachandran *et al*, 2017). Large plasmids and
17 chromids often carry complex traits involved in bacteria-eukaryote interaction in infections and
18 symbiosis processes (e.g. Marchetti *et al*, 2010). However, how and why some plasmids are
19 domesticated as chromids is currently poorly understood.

20 Depending on the plasmid and its interaction with its genetic background, plasmids can be costly
21 because of the metabolic burden imposed by plasmid replication, the consumption of resources for
22 the expression of plasmid-encoded genes, synthesis of the plasmid conjugation apparatus, alteration
23 in the expression of host genes or fine-tuning cellular pathways, and other metabolic effects such as
24 the introduction of efflux pumps transporting important biomolecules (Baltrus, 2013). Incoming
25 plasmids can be badly adapted to host physiology, imposing a high fitness cost that can be
26 compensated by mutations in the plasmid and/or the host chromosome, rapidly selected for by their
27 effect on growth (Carroll & Wong, 2018; Bouma & Lenski, 1988). How secondary replicons impact the
28 global nucleoid dynamics at the cellular scale and has to adapt to it has received so far poor
29 attention.

30 Small multicopy plasmids are thought to both replicate and segregate following a "random copy
31 choice" model (Reyes-Lamothe *et al*, 2014; Wang, 2017). Other natural (non-engineered) bacterial
32 plasmids are maintained at low copy numbers, rendering their maintenance by random segregation
33 inefficient. These low copy number plasmids necessitate active systems to avoid loss during
34 proliferation . Systems acting as poison-antidote (toxin/antitoxin or restriction/modification systems)
35 enhance their persistence by killing plasmid-free cells (Zielenkiewicz & Cegłowski, 2001). Systems
36 acting in active segregation (partition (Par) systems) reduce the rate of plasmid loss by thousand
37 folds (Bouet & Funnell, 2019). Active plasmid segregation follows DNA replication and involves the
38 separation and transportation of the two copies of the replicon in opposite directions along the
39 longitudinal cell axis, ensuring every daughter cell receives at least one copy of the plasmid. Almost
40 all Par systems identified to date consist of three components: a "centromere" cis-acting site, a
41 centromere-binding protein and an NTPase (ATPase or GTPase). Another system acting on the
42 subcellular positioning and the maintenance of some plasmids but consisting only of a cis-acting

1 element and a protein binding this element, StbA, has been described more recently (Guynet *et al*,
2 2011).

3 Numerous studies have reported the intracellular localization of plasmid DNA using FROS systems or
4 fluorescent versions of their Par proteins. Small plasmids such as ColE1-type plasmids tend to form
5 clusters at the nucleoid periphery (Reyes-Lamothe *et al*, 2014). In contrast, low copy number
6 plasmids like F, P1, RK2 and R388 are preferentially found around mid- or quarter-cell positions,
7 colocalized with the bacterial nucleoid (Gordon *et al*, 1997; Niki & Hiraga, 1997; Erdmann *et al*, 1999;
8 Sengupta *et al*, 2010; Drew & Pogliano, 2011; Guynet *et al*, 2011). This depended on Par or StbA
9 systems, confirming these systems act in the subcellular positioning of their replicons. Plasmids
10 belonging to different families are nevertheless tethered to different sites within the cell, and
11 segregate at different times relative to one another and to the bacterial cell cycle (Ho *et al*, 2002).
12 evidences indicate that the bacterial chromosome, compacted as a nucleoid, contributes both
13 passively (as the major structure occupying space inside a bacterial cell) and actively (as a support
14 and/or matrix for plasmid movement) to plasmid partition mechanisms (Le Gall *et al*, 2016; Derman
15 *et al*, 2008). The F, P1 and R388 plasmids devoid of their Par or StbA systems are excluded from the
16 nucleoid space (Niki & Hiraga, 1997; Erdmann *et al*, 1999; Le Gall *et al*, 2016; Guynet *et al*, 2011). The
17 reason why plasmids deleted for their partition system are excluded from the nucleoid is not
18 understood.

19 In the present work we analyzed the completely sequenced genomes of enterobacteria to survey the
20 presence of partition systems encoded by secondary replicons. We observed that partition systems
21 are present in replicons with a characteristic size above 25kb, and lacking in smaller plasmids. We
22 postulated therefore that the size of a plasmid is a critical determinant of its cost independently of
23 the genes that it encodes. To experimentally test this hypothesis, we analyzed the localization, the
24 dynamics and the loss frequency of the large R27 plasmid from which we perturbed the partition
25 systems. As observed for other plasmids localization of the R27 plasmid in the nucleoid depends on a
26 functional ParABS system, we therefore wanted to test if such nucleoid exclusion is not the default
27 localization of circular DNA elements in *E. coli*. To test this hypothesis we monitored the localization
28 of a large DNA circle excised from the chromosome and performed in silico simulation of polymer
29 circles localization within nucleoid.

30 Our results indicate that the R27 plasmid lacking its ParAB system is more frequently localized
31 towards the pole of the cell at the edge of the nucleoid. We observed a similar localization and
32 mobility characteristics for large DNA circles excised from the chromosome. Simulations suggest that
33 this polar exclusion can be explained by the sole force of entropic repulsion of the polymers. We
34 observed that circles and plasmid do not occupy DNA free regions but rather localized at the
35 transition between the cytoplasm and the nucleoid, suggesting that large DNA molecules do not get
36 access easily to the cytoplasm of *E. coli*. Localization at the nucleoid – cytoplasm frontiers can be
37 disadvantageous for various DNA metabolic processes such as replication, transcription or
38 recombination that happen in the nucleoid core. This leads us to propose that, in addition to assist
39 the correct segregation of replicated plasmids, partition systems have the function of targeting
40 plasmids to the nucleoid. We further propose that this is advantageous for them.

41

42

1 Results

2 The size of the plasmid determines the presence and the type of partition systems

3 To study the distribution of subcellular positioning systems in secondary replicons, we selected 971
4 secondary replicons from sequenced Enterobacterial genomes (Table S1). These were representative
5 of the different replicon families as judged by the genus they belong to (Table S2), the diversity of
6 their replication origins and initiator proteins (Rep types) as well as their transfer functions (MOB
7 types). They ranged from 1.3 to 794 kb in size and show a clear bimodal size distribution with a
8 separation between peaks at around 25 kb (Figure 1). Twenty five percent of these plasmids were less
9 than 25 kb and were predicted to have either a rolling-circle type or a theta type replication. These
10 theta type small plasmids are supposed high copy number based on their described homologs. The
11 repartition of sizes was then monotonous from 30 to 120 kb except for a large over-representation of
12 90 to 100 kb plasmids (11% of total); 23% were longer than 130 kb but only 1% were longer than 200
13 kb and 7 were chromids (> 500 kb). Very large secondary replicons are thus rare in Enterobacteria.
14 We searched for three types of subcellular positioning / segregation systems: ParAB, ParRM and StbA
15 (Figure 1). The well-described ParAB and ParRM systems were searched using appropriate HMM
16 profiles (Table S1, text S1), (Cury *et al*, 2017)). The StbA protein, experimentally studied in R388, was
17 first searched by similarity using the six *stbA* alleles previously identified (Guynet *et al*, 2011), a HMM
18 profile was then constructed using the conserved N-terminal domains of the StbA homologs detected
19 (Material and Methods). Figure 1 shows the presence of these three systems in plasmids ranked by
20 the size of the latter. The vast majority of small plasmids lack any system (Table S3). In contrast, most
21 larger plasmids and chromids possess at least one system. The transition between these two types of
22 replicons occurs around 25 kb, consistent with the minimum of plasmid size distribution. The ParAB
23 system was more frequent than the ParRM and StbA systems. Strikingly, all plasmids above 180 kb,
24 including the chromids, had a ParAB homolog. StbA was mostly present in medium-sized plasmids
25 (34-148 kb). ParRM systems were more evenly distributed than StbA. However, their occurrences in
26 replicons above 180 kb were always accompanied by the presence of a ParAB system.

27 The more detailed analysis of the 79 plasmids longer than 25 kb and lacking partition systems
28 revealed potential ParAB or ParMR systems that did not score well enough in the automatic
29 annotation or contained frameshifts. Some of the latter may result from sequencing errors (60
30 plasmids, Sup Table 2). We found that many of the remaining large plasmids lacking partition systems
31 contained ribosomal operons, were almost entirely composed of bacteriophages or mobile genetic
32 elements. We concluded that: (i) partition systems are effectively essential for plasmids above 25kb
33 long in Enterobacteria; (ii) a ParAB system is specifically required for plasmids above 180 kb, and this
34 includes all the chromids.

35

36 Localisation and dynamics of the R27 megaplasmid strongly depends on ParAB but not on ParRM

37 The above data suggest that large plasmids specifically require a ParAB system for their maintenance.
38 To study the role of ParAB in a large plasmid, we use R27, a 180 kb long natural IncHI2 plasmid
39 carrying resistance to tetracycline (Taylor, 1983) . As all IncHI2 plasmids, R27 codes for both a ParAB
40 and a ParRM system. Both systems were reported to be able to stabilize an unstable cloning vector
41 and involved in R27 maintenance and subcellular positioning, although the ParAB system had a larger

1 effect (Lawley and Taylor, 2003). We first analyzed the R27 sequence and detected a toxin-antitoxin
2 (TA) system not previously annotated, which may impair stability assays. This TA system was deleted
3 and replaced by the *lacI* gene, allowing a colored detection of plasmid less colonies on agar plates in
4 an otherwise $\Delta(lacI)$ strain. Using this system, we confirmed the previous findings on the role of the
5 ParAB and ParRM systems on R27 maintenance (SupFig 1). R27 Δ (TA) was stable over 60 generation
6 of growth. Its $\Delta(parAB)$ derivative was lost whatever the growth conditions (L-broth or M9 broth
7 supplemented with glycerol and casamino acids), although loss was more frequent during rapid
8 growth (L-broth). This contrasted with the $\Delta(parRM)$ derivative, which was lost only during rapid
9 growth. This confirms that ParAB is the prominent system for R27 stability.

10 When tagged with a FROS system, R27 derivatives were shown to localise plasmids (Lawley and
11 Taylor, 2003). Single foci tended to localize close to mid-cell, whereas two or more foci localized
12 symmetrically along cell length. These localizations depended on the ParAB system and to a lesser
13 extent on the ParRM system. To further explore the role of the two Par systems, we tagged our R27
14 derivatives with a *parS_{p1}* site and transfer them into strains producing a GFP-ParB fusion protein from
15 a $\Delta(lacZ)::gfp-parB_{p1}$ chromosomal construct (Stouf *et al*, 2013). R27 Δ (TA) localized in the midcell
16 zone when present as a single focus and towards the cell quarters in cells with two foci. The
17 $\Delta(parAB)$ derivative do not present this localization whereas no defect was detectable for the
18 $\Delta(parRM)$ derivative (Figure 1B - D). We thus confirm that R27 localizes as the smaller plasmids F, P1
19 and R388 plasmids, showing its large size does not preclude localization inside the nucleoid and that
20 this effect depends on ParAB.

21 Taken together, our data suggest that large plasmids strictly need a ParAB system for their
22 maintenance. ParAB maintain plasmid copies inside the nucleoid whatever their size, whereas ParRM
23 systems don't. When devoid of ParAB systems, large plasmids exit the nucleoid and localize in a
24 dynamic manner at the polar nucleoid edge. Localizing inside the nucleoid may thus be especially
25 important for very large plasmids and chromids. To understand why this should be, we performed
26 simulations of secondary replicon dynamics of different sizes.

27

28 **Polymer modeling shows that plasmids are evicted from the nucleoid**

29 In silico simulations of DNA and chromosome polymers have been extremely useful to evaluate
30 physics of chromosome and plasmid conformation and segregation (Vologodskii & Cozzarelli, 1993;
31 Vologodskii *et al*, 1992; Jun & Mulder, 2006; Junier *et al*, 2014). Monte Carlo simulations revealed
32 that bacterial chromosomes unmix in the confined environment formed by the nucleoid shell (Jun &
33 Mulder, 2006). We used simulations to evaluate if the localization and conformation of plasmids
34 lacking partition systems was a consequence of entropy-mediated unmixing of polymers. We
35 simulated a 3D nucleoid containing one chromosome and one plasmid over tens of millions of
36 iterations (simulation steps). The nucleoid volume was defined by a cylinder with a long axis of 2000
37 nm and a diameter of 800 nm (Figure 2A). The chromosome was modeled using a 40 nm thick semi-
38 flexible chain accounting for the nucleoprotein structure that has been observed in rapidly dividing
39 cells with a 100bp/nm base-pair density along the main axis of the chain and a persistence length of
40 100 nm that is much smaller than the diameter of the nucleoid. In practice, the simulations were
41 performed by discretizing the chromosome and plasmid chains using 30 nm long cylinders (1392
42 cylinders in total for the chromosome). Plasmid size was varied from 30 kb (9 cylinders) to 1400 kb

1 (423 cylinders). Finally, to quantify the impact of chromosome on the location properties of plasmids
2 we used, as a control, simulations where only the plasmid was present in the nucleoid shell.
3 Localizations of plasmids in the absence of a chromosome dramatically differed from that of plasmids
4 evolving in a chromosome crowded nucleoid cell (Supplementary Figure S2 and S3).

5 We first quantified the localization of plasmids along the long axis of the nucleoid as a function of
6 their size (L_p). Plasmids with less than 100 kb, localized at approximately similar frequencies inside
7 and outside of the area defined by the chromosome edges, whereas large plasmids resided
8 preferentially on the outside of the chromosome (Figure 2B and Figure 3A). Along the short axis of
9 the nucleoid, the barycenter of small plasmids was found with equal probability on the edge or near
10 the center of the nucleoid volume while the barycenter of large plasmid was mainly observed closer
11 to nucleoid center (Figure 3A). This behavior was significantly different than what we observed for
12 plasmid evolving in an empty nucleoid shell (Supplementary Figure S1). The results of these
13 simulations are in good agreement with the polar localization observed for F (Le Gall *et al*, 2016) or
14 R27 plasmids lacking a partition system (Figure 1B - D). This suggests that partition systems may
15 counteract entropy mediated unmixing of plasmid and chromosomes and to allow plasmids to enter
16 in the main nucleoid volume. Even if the unmixed state is frequent, we observed plasmids localized in
17 between chromosome edges when they were traveling from one side to the other of the nucleoid
18 (Figure 2B). Observation of few examples suggested that plasmid traveling across the nucleoid pass
19 on the side of the chromosome (Figure 3B). To check how frequently plasmid and chromosome
20 actually overlap, we measured the distance of the plasmid barycenter from the nucleoid center on
21 the short cell axis as a function of their position along the long nucleoid axis (Figure 3C). We observed
22 the same trend for small (100kb) and large (600kb) plasmids: when they localized over the
23 chromosome on the long axis of the nucleoid, they were excluded from the nucleoid center on the
24 short axes (Figure 3C). Interestingly, small plasmids were also pushed toward lateral edges of the
25 nucleoid when at the pole, while large plasmids were not. This positioning was dramatically different
26 in a chromosome free shell (Supplementary Figure S2). We conclude that plasmids and
27 chromosomes are mostly unmixed whatever their size and position in the nucleoid.

28

29 **The length of the plasmid determines its compaction within nucleoid**

30 The simulations allowed us to question several aspects of the interplay between plasmids and the
31 chromosome. First, we tested if plasmid and chromosome compaction was modulated by plasmid
32 size. We used plasmid and chromosome spreading, i.e. the longitudinal or lateral extension of
33 plasmids and chromosome as a proxy of their compaction within nucleoid. Our simulations were
34 performed with a fixed nucleoid volume. First, plasmid spreading was measured along the short or
35 the long axis of the nucleoid. Plasmid spreading was directly dependent on the size of the plasmid
36 and the presence chromosomal DNA (Figure 4A and Supplementary Figure S2). Plasmid spreading
37 decreased following a power law with plasmid size. Longitudinal chromosome spreading was only
38 modestly affected by the presence of plasmid. Large plasmids (>1000 kb), however, imposed a
39 reduction of the chromosome extension by 15% (Figure 4A). Interestingly, in the presence of the
40 chromosome, plasmid compaction (the longitudinal spread of plasmid normalized by its size, NLS)
41 was dramatically dependent on plasmid size. NLS follows a power law, $NLS = A * L_p^{-0.5}$, where L_p the
42 length of the plasmid in kb and A the NLS value when L_p tends towards 0 (Figure 4B). Plasmids above

1 700 kb were compacted to the level of the chromosome while smaller plasmids were much less
2 compacted (Figure 4B). For plasmids below 100 kb the compaction was not significantly changed by
3 the presence of chromosome compared to the empty nucleoid shell (Figure 4B). These results
4 suggest that if the nucleoid volume is maintained constant in the cell, plasmid larger than 100 kb will
5 be compacted to fit in the nucleoid volume; this compaction will be severe for the largest plasmids.

6

7 **Mobility of plasmid polymers**

8 We observed plasmid trajectories crossing the nucleoid. Because Monte-Carlo simulations are
9 dedicated (in principle) to quickly reach equilibrium, they may not be suitable for dynamical analyses.
10 However, accepted motions in our model correspond to small, local deformations of the chain, and
11 thus often provide a good estimation of the resulting large-scale dynamics of systems. Simulation
12 traces show that plasmid localizations were highly similar between short interval iterations (,
13 suggesting that localization at iteration N was not completely independent from the localization at
14 iteration N-1. As a consequence, we can analyze plasmid trajectories. In this context, we first
15 measured how frequently plasmids cross the chromosome territory along the long axis of the cell
16 (Figure 4C). This parameter might be important when considering random segregation of plasmids
17 without partition machinery. We observed that crossing frequency exponentially decayed with
18 plasmid size. In the length of our simulations ($>5e10^7$ steps) plasmids above 1MB extremely rarely
19 crossed the chromosome territory. When plasmids were smaller than 600 kb, both the frequency
20 (Figure 4C) and the speed (Figure 4D) of crossing events were strongly dependent on their size. We
21 measured the average velocity of nucleoid crossings in the 200 kb plasmid simulation and compared
22 it to the reptation of an individual chromosomal locus (Figure 4E). Plasmid crossings were much
23 faster than reptations of chromosomal loci. This suggests that unmixing of polymers is much more
24 efficient than reptation inside a single polymer. Rapid unmixing has been already observed for 2
25 chromosome polymers of equal size (Jun & Mulder, 2006) but, to our knowledge, this was not
26 described for two polymers of different sizes in a nucleoid like confinement. Finally, we measured
27 mobility of plasmids at their polar position; it showed that mobility of small plasmids was higher than
28 that of large plasmids and chromosome (Figure 4F). The velocity of plasmids that crossed the
29 nucleoid (Figure 4E) was comparable (9 nm / 1000 iterations for a 200 kb plasmid) to that of plasmid
30 at their polar home position (8.2 nm /1000 iterations for a 200 kb plasmid). Therefore during
31 nucleoid crossing the directionality is imposed by a higher frequency of correlated movements but
32 not by an acceleration (Figure 4E).

33

34 **Localization of excised DNA circles is akin to the localization of R27 plasmids lacking the ParAB** 35 **system**

36 In the purpose to confirm R27 experiments and simulations with a heterologous system, we used an
37 assay designed by F. Boccard and colleagues that allows excision of large DNA segments from the
38 chromosome as DNA circles (Valens *et al*, 2016). The excised segment is tagged with a *parS*/*parBGFP*
39 system to image its localization and dynamics. The strain also expresses a HupA-mCherry fusion to
40 visualize the nucleoid. We have chosen to excise a 137 kb segment of the chromosome 400kb away
41 from *oriC*. Before excision, this locus presented the typical patterns of localization of the *oriC* region,

1 cells with 2 to 4 foci localized at the quarters and 1/8 of the cell. Following induction of the
2 recombinase for 30 min (a sufficient induction to nearly reach 100% of excision) the localization of
3 the ParB-GFP foci dramatically changed, most cells presented one focus localized at the edge or at
4 the middle of the nucleoid (Figure 5A). This polar localization is akin to the localization observed with
5 the R27 plasmid lacking the partition system. The frequency of polar foci was higher for the excised
6 circles (75%) compared to the R27 plasmid lacking ParAB system (35%), suggesting that R27 $\Delta parAB$
7 keeps some nucleoid targeting elements, may be the ParRM system. These results demonstrate that
8 DNA circles lacking positioning system tend to be positioned at the nucleoid edge.

9 **Long range movements of R27 and excised DNA circles**

10 We used time lapse microscopy to evaluate the mobility of the excised circle and R27 plasmid (Figure
11 5B). Kymographs of plasmid lacking ParAB and excised foci differed significantly from that of
12 chromosomal foci (Figure 5B). At long time scale (2h with 10 min intervals) we observed, with the
13 excised circles and the R27 $\Delta parAB$ plasmid, frequent long, rapid and oriented movements. These
14 movements frequently produced merging of foci (Figure 5B). These movements consisted in foci
15 moving from the nucleoid toward the cell pole or toward the nucleoid free mid-cell zone (Figure 5C).
16 Chromosomal loci also presented ballistic like movements but they mainly corresponded to
17 segregation events and they were confined to the nucleoid area. We measured that average velocity
18 of ballistic movements of excised circles and chromosomal loci segregating. Velocity of nucleoid
19 crossing by excised circles toward cell pole is $142 \pm 80 \text{ nm}\cdot\text{min}^{-1}$ (N=50), this is 3 times faster than
20 the average velocity of chromosomal loci segregating from quarter positions, $50 \pm 30 \text{ nm}\cdot\text{min}^{-1}$
21 (N=50). Using the same localization system, P. Wiggins and colleagues observed that most
22 chromosomal loci present a maximal drift velocity, $\approx 300 \text{ nm}\cdot\text{min}^{-1}$, immediately after foci splitting
23 (Cass *et al*, 2016). Two minutes later drift velocity has already slowed down to $20 - 50 \text{ nm}\cdot\text{min}^{-1}$ to
24 finish segregation in 20 min. Here we observed prolonged high velocity movements of excised circles,
25 they might correspond to the ballistic movements observed in the simulation; therefore they can
26 give an estimation of the efficiency of entropy mediated unmixing in these experimental conditions.

27 **Dynamics of R27 and excised DNA circles**

28 At shorter time scale (3 min with 6 sec intervals) ballistic movements of chromosomal loci, R27 or
29 excised circles were rarely detected (Figure 5B). After reaching the nucleoid edges, excised circles
30 and R27 plasmid lacking the ParAB system unfrequently escaped from this positioning. By contrast
31 we observed significant longitudinal movement of the R27 and chromosomal locus (Figure 5B). We
32 measured Mean Squared Displacement (MSD) to obtain quantitative analysis of the mobility of foci
33 (Figure 5D - G). MSD curves were characteristic of subdiffusive motions, following a power law: $\text{MSD} = D \cdot \Delta T^\alpha$
34 where D is coefficient of diffusion, ΔT the time interval and α the scaling exponent. MSD
35 confirmed that excised foci present a different mobility compared to chromosomal loci (Figure 5D
36 and 5E). For time intervals around 2 sec, the MSD α coefficient of excised foci (0.43) is reduced
37 compared to chromosomal foci (0.48). The coefficient of diffusion of excised foci is also reduced
38 (0.0044 vs $0.0053 \mu\text{m}^2 \cdot \text{sec}^{-1}$). The cage surrounding the movements of excised foci is also reduced by
39 a factor of 2. Finally, the average distance that excised foci travel in one interval is 20 % reduced
40 compared to the chromosomal foci. Mobility of WT R27 plasmid ($\alpha = 0.66$ and $D = 0.0026 \mu\text{m}^2 \cdot \text{sec}^{-1}$)
41 differed from that chromosomal loci or excised circles, Figure 5F and 5G). The absence of the ParAB
42 system dramatically reduces the mobility of R27. It was particularly striking for the coefficient of

1 diffusion that dropped to $0.001 \mu\text{m}^2 \cdot \text{sec}^{-1}$. Our observations demonstrate, for every dynamics
2 parameters, that R27 plasmid lacking ParAB behavior is akin but not strictly identical to the excised
3 circle.

4

5 **Nucleoid edges serves as a platform for DNA circle surfing**

6 Differently from the simulations we observed that plasmids lacking partition systems and excised
7 circles were less mobile than chromosomal loci. This suggests that the nucleoid edge is not a
8 constrain-free localization. To understand what is constraining the mobility of excised circles and
9 plasmids at the nucleoid edges, we investigated this localization in more details. Fluorescence
10 quantification suggested that excised circle foci localized precisely at the edges of the nucleoid but
11 do not freely navigate in the DNA free zone at the pole of cell (Figure 6A). As illustrated for one
12 example, we frequently observed, for excised loci, that mobility along the short axis of the cell is
13 higher compared to mobility along the long axis of the cell (Figure 6B). We deconvoluted and
14 performed a three dimensional reconstruction of the Z stack over time (see material and methods) to
15 evaluate the dynamics of these foci at the nucleoid edge, we observed that excised foci moved
16 rapidly on the surface of the nucleoid but keep nucleoid contact for most of their moves (Figure 6C).
17 Deconvolution process allowed the transient resolution of 2 foci at the nucleoid edge, confirming
18 that polar foci are frequently clusters of two or more excised circles. These results suggest that large
19 DNA circles and plasmids lacking partition systems are expelled from the nucleoid core, in agreement
20 with our simulations, and find a home position at the nucleoid edge where they are free to surf on
21 the nucleoid surface but cannot escape it. Excised circles might be unmixed from the nucleoid on the
22 sole force of entropy but concomitantly they are maintained at the transition between the DNA
23 compartment and the cytoplasm.

24

25 **Localization and dynamics of R27 plasmid and excised circles when nucleoid and cell organization** 26 **are perturbed**

27 Finally we tested if R27 and the excised DNA circle localizations at the nucleoid edge were
28 maintained when chromosome confinement was changed. First, we used chloramphenicol to
29 condense the nucleoid immediately after excision. We observed profound changes in the localization
30 and dynamics of excised foci in these conditions (Figure 6D and 6E). The number of circle foci
31 increased significantly (2 to 3 per cell compared to 1 to 2 in the regular condition). The localization of
32 these foci was changed, they were not only localized to the edge of the nucleoid, they were also
33 observed on the side of the nucleoid. Even if the DNA free zone at the pole of the cell was enlarged
34 by chloramphenicol treatment the excised circles did not explore it, they always kept contact with
35 the nucleoid. Time-lapse experiments revealed that excised circle foci travel from the edge to the
36 side of the nucleoid and vice versa (Figure 6E). In the presence of chloramphenicol, R27 plasmid
37 localized to the side of the nucleoid and did not explore nucleoid free regions (Figure 6F). Similar
38 results were observed with WT and ΔparAB plasmids suggesting that chloramphenicol is altering the
39 ParAB positioning system. These observations suggest that localization at the nucleoid pole is
40 dependent on the volume occupied by the nucleoid or might eventually be created by transcription-
41 translation-secretion (transertion) barriers (Roggiani & Goulian, 2015). We used cephalixin to block

1 cell division and create large DNA free zones between segregated nucleoids. In these conditions
2 filamenting cells presented multiple foci on the edge, the side and on the nucleoid (Figure 6G). The
3 nucleoid-foci association was maintained for most cells. We only observed one focus crossing a
4 nucleoid free space out of 200 cells monitored by time lapse over a 2h period (Figure 6G, right
5 panel). We tested the impact of transcription inhibition by rifampicin (Figure 6H); this antibiotic has
6 for consequence to expand the nucleoid. In the presence of rifampicin, we observed foci of the
7 excised region at the edges of the nucleoid. These polar foci were frequently fuzzier than the one
8 observed in regular conditions. Time lapse imaging revealed that they corresponded to 2 - 3 excised
9 circles that cohabited in the same region of the cell but did not overlap completely as frequently than
10 in the absence of the drug (Figure 6I). These results confirmed that DNA circles lacking a partition
11 system are unmixed from the chromosome but are maintained in the nucleoid space at the transition
12 between the nucleoid and the cytoplasm.

13

14 **Discussion**

15 **Partition machinery and localization of plasmid**

16 Our work extends to R27 the observation that plasmid lacking partition system are not localized with
17 the nucleoid core. Using excised chromosomal circles and simulation we confirmed that this
18 localization relies on the biophysical nature of large DNA circles in a crowded nucleoid and cytoplasm
19 environment. Since the “natural” positioning of plasmids lacking partition systems is on the edge of
20 the nucleoid, one could ask why partition systems do target plasmids inside the nucleoid? What
21 could be the benefit for plasmid segregation to happen within the nucleoid? Could plasmid hijack
22 entropy? In this scenario partition systems might serve as a localization system to the nucleoid to
23 allow the plasmid to benefit from the entropic flow. Directionality will be given by protein-protein
24 interactions and reaction-diffusion process of the ParB and ParA ATPase (Walter *et al*, 2017).

25

26 **How much plasmid DNA can be hosted in the nucleoid ?**

27 Simulations indicate that for a define nucleoid volume the carrying capacity of plasmid DNA in the
28 presence of a chromosome is limited. With the simulation parameters that we used, the normalized
29 longitudinal spread of 700 kb plasmid reached that of the chromosome (Figure 5B). This limit is also
30 observable in the frequency of nucleoid crossing that is extremely rare above 700 kb (Figure 6A).
31 Above this size the plasmid, the nucleoid and perhaps cell morphology must adapt to this foreign
32 DNA. Obviously this threshold is strictly dependent on the parameters that we used to define the
33 polymers. Changing the persistence length, the diameter of the chromatin cylinders or the nucleoid
34 volume will modify these limits. Nevertheless, it is tempting to compare this 700 kb threshold with
35 the size of plasmid and secondary chromosomes observed in bacteria. Secondary replicons above
36 700 kb are secondary chromosomes. The presence of a large secondary replicon will therefore
37 necessitate important chromosome conformation changes. Such changes could be detrimental and
38 add an extra cost, in addition to the cost for replication and transcription, to secondary large
39 replicons. Alternatively, the nucleoid volume might change to accommodate the extra replicon but
40 this also has a significant cost in term of cell elongation for example.

1 The second critical size that we observed is much lower, under 200kb the probability to observe
2 plasmid crossing the chromosome increased dramatically and the spread of plasmids approximate
3 the values observed for plasmid residing in a chromosome free nucleoid shell. It is tempting to
4 compare these values with the presence of segregation machinery encoded by plasmid genomes.
5 Plasmids below 25-30 kb can cross the chromosome frequently and rapidly, ensuring efficient
6 segregation by random partition. Above this value, the mobility of replicons becomes limiting and
7 decrease exponentially with increasing size, rendering the presence of a positioning system required
8 for stability. Above 200 kb, both the probability of crossing the chromosome and the crossing speed
9 become extremely low, rendering compulsory the presence of a ParAB system, which is the only one
10 of the three types of positioning systems ensuring both positioning of the replicons inside the
11 nucleoid and separation of their sister copies after replication. Lastly, the presence of replicons
12 above 700 kb in the nucleoid impact chromosome dynamics. These large replicons must thus adopt
13 chromosome like behaviors. Consistent with this, the chromid of *V. cholerae* has an accurately
14 regulated timing of replication, spreads in the long cell axis and couples segregation of its *ter* region
15 with cell division as chromosomes do.

16

17 **Plasmid and excised circles are maintained at the nucleoid –cytoplasm transition**

18 We observed that large plasmids and DNA circles lacking partition systems were expelled from their
19 original localization toward nucleoid edges but did not invade the cytoplasmic space. This is in good
20 agreement with our simulations suggesting that expulsion relates to entropic mediated unmixing of
21 polymers in a confined space. However, entropy does not explain why these molecules do not invade
22 large nucleoid free regions at the pole of the cell or in between nucleoids of filaments. One possible
23 explanation for this could be that large circular DNA, differently, from small plasmid cannot diffuse in
24 a glass-like cytoplasm (Parry *et al*, 2014). This has already been observed for other type of cytoplasm
25 components that become disproportionally constrained with increasing size. Remarkably, small
26 plasmids lacking partition system appeared to diffuse rapidly within cytoplasm even when they form
27 clusters (Reyes-Lamothe *et al*, 2014). A systematic analysis of plasmid or DNA circles mobility as a
28 function of their size would be required to determine the critical size. However, we observed that
29 DNA circles can slide rapidly on the surface of the nucleoid, this might suggest that the nature of the
30 cytoplasm is not the only element contributing to their localization. Nucleic acid charges may also
31 contribute to the control of diffusion at the transition between nucleoid and cytoplasm. Negatively
32 charged ribosome surfaces may impose this limitation on the diffusion of proteins and DNA
33 (Schavemaker *et al*, 2017). Interestingly the fluidity of the cytoplasm can be modulated by drugs
34 affecting metabolism (Parry *et al*, 2014) or antimicrobial peptides (Zhu *et al*, 2019), our results
35 suggest that plasmid localization and dynamics might also be affected by environmental conditions,
36 this would influence their stability and expression and eventually change their cost for the host cell.

37 **Could partition of genetic material imposes constrains on genome dynamics.**

38 In bacteria, horizontal gene transfer (HGT) is the main source of genome dynamics. Recent studies
39 revealed that the size of horizontally acquired DNA in one single event is limited to 25-30kb (Pang &
40 Lercher, 2017, 2019). Plasmids and lysogenic phages are the main drivers of HGT. Interestingly this 25
41 kb limit also corresponds to the presence of partition systems on plasmids. Our simulations show
42 that the ability of plasmid to cross the nucleoid is exponentially decreasing with increasing size

1 (Figure 5). Experiment with R27 plasmid or excised circles clearly show that nucleoid crossing is very
2 rare for 130 kb -200 kb DNA circles. Entropic unmixing might therefore limit the ability of a newly
3 acquired replicon to find a target sequence for integration. In good agreement with this hypothesis it
4 appeared that DNA internal motion likely accelerates protein target search in a packed nucleoid
5 (Chow & Skolnick, 2017)

6

7 **Material and methods**

8 **Annotation of subcellular positioning systems**

9 Partition systems ParAB and ParMR were detected using HMM profiles used in our previous work
10 (Cury *et al*, 2017). Briefly, systems were reported when the two hits of a given system were
11 contiguous in the replicon. A hit was considered significant if the e-value was smaller than 10^{-3} and
12 the coverage of the profile was above 50%. For annotation of *stbA*, tBLASTN searches were first
13 performed against the 971 plasmids using the *stbA* sequences previously reported (Guynet *et al*,
14 2011). Each plasmid with a positive result was annotated in SnapGene. oriTDB
15 (<http://202.120.12.134/oriTfinder/oriTfinder.html>) was used to determine possible *oriT* site,
16 relaxases and type IV secretion system genes. We found a *stbA* or *stbA* like sequence in 136
17 plasmids. A HMM profile was then constructed using the conserved N-terminal domains of the StbA
18 homologs and used to search again the whole plasmid collection (Figure 1A). To further annotate
19 plasmids with no detected systems, *parA* and *parM* genes were searched using tBLASTN in the 79
20 plasmids without any segregation system found. We used the *parA* sequence found in NC_012961,
21 the *parM* sequences described by Jakob Møller-Jensen and colleagues (van den Ent *et al*, 2002) and
22 the *parM* sequence of JCW3 (*Clostridium perfringens*). Using this strategy, we found 54 plasmids with
23 a *parA*-like sequence (including 5 with a frameshift and 1 partial sequence) and 6 plasmids with
24 *parM*-like sequence (including 3 with a frameshift). The 19 remaining plasmid sequences with no
25 system detected were all annotated in SnapGene and inspected “by eyes”. Two sequences were
26 obviously partial sequences of larger plasmids. Mobile genetics elements (MGE) were annotated
27 using ISfinder (<https://www-is.biotoul.fr/>)(Siguier *et al*, 2006), bacteriophage with PHASTER
28 (<http://phaster.ca/>) (Arndt *et al*, 2016). Four plasmids carried ribosomal operons, others were mostly
29 constituted by MGE or bacteriophages.

30 **Strains and plasmids**

31 R27 is a 180 kb plasmid of the IncHI family, naturally resistant to tetracycline (Phan & Wain, 2008;
32 Lawley & Taylor, 2003). We detected a potential toxin-antitoxin system consisting of gene *stm* and an
33 upstream RNA, which we deleted from positions 91,174 to 91,481 bp on the R27 sequence and
34 replaced by the *lacI* gene. For intracellular localization, we inserted a P1 *parS*-Kn cassette (Stouf *et al*,
35 2013) in the intergenic region between the two converging genes R149 and R150 (position 138,435).
36 The ParAB and ParRM systems (R0019-R0020 and R0013-StbA, respectively) were deleted from the
37 beginning of the first gene to the end of the second gene (23,081 to 25,338 and 18,079 to 19,834,
38 respectively), replaced by a FRT-Kn-FRT cassette that was subsequently resolved to remove the KnR
39 determinant. Note that the ParM homolog is called StbA in R27 annotation but is not a StbA
40 homolog. R27 derivatives were introduced into a $\Delta(lacI)$ strain (Deghorain *et al*, 2011) for stability
41 assays and in a $\Delta(lacZ)::gfp-parBP1$ strain (Stouf *et al*, 2013) for microscopy. For the excision of the

1 137 kb we used the FBG150 *trkD*^{attL} R719 strain (Valens *et al*, 2016) containing attL from λ at the *trkD*
2 locus (3928826 bp) and *attR* at the position 4067141bp. Excision is produced by the Int and Xis
3 protein from λ produced from the plasmid pTSA29-CXI (Valens *et al*, 2004). The *parS* P1 tag was
4 inserted at the Ori-7 position at 4024865 bp.

5 **Imaging of excised DNA circle and R27.** Excision was obtained as described before (Valens *et al*,
6 2016) with a 30 min heat shock at O.D. 0.1. Bacteria were immediately spread on M9-casaminoacids
7 and glucose agarose pad supplemented or not with chloramphenicol (30 $\mu\text{g/ml}$) or rifampicin (50
8 $\mu\text{g/ml}$). When required, cephalexin (20 $\mu\text{g/ml}$) was added to the culture medium 1 h before heat
9 shock. Imaging was performed on a Zeiss inverted microscope equipped with a Yokogawa W1
10 spinning disk head and an Orca-Flash 4 CMOS camera. The incubation is maintained to 30°C during
11 imaging. Time-lapse acquisition was controlled by Metamorph software. Deconvolution was
12 performed with Huygens and 3D reconstruction with Imaris softwares. Image analysis was performed
13 with Object J (Vischer *et al*, 2015), Microbe-J (Ducret *et al*, 2016), Track-mate (Tinevez *et al*, 2017)
14 and Particle Tracker (Sbalzarini & Koumoutsakos, 2005).

15 **Simulations.** We simulated *E. coli* chromosomes using a worm-like chain (WLC) model, i.e. a flexible
16 fiber model, of the bacterial chromosome (Junier *et al*, 2014; Lepage & Junier, 2017). One
17 chromosomes and one plasmid were embedded in a volume whose dimensions corresponded to the
18 nucleoid that is observed *in vivo*: a diameter equal to 800 nm and a length from 2.0 μm (G1 phase).
19 We excluded the possibility of the fibers to overlap (self-avoidance constraint) by using hard-core
20 diameters of the nucleoproteic fiber of 35 nm. The base-pair density along the fiber was fixed at 100
21 bp/nm and the persistence length at 100 nm, a value that is much smaller than the diameter of the
22 nucleoid. Simulations and thermodynamic analysis were performed as described previously (Junier *et al*,
23 2014). Briefly the chromosome and plasmid consisted of a semi-flexible polymer composed of a
24 succession of N impenetrable cylinders (three cylinders per persistence length)— $N = 1392$ for the 4.6
25 Mb chromosome. The state space of our polymer models was sampled using a standard Monte-
26 Carlo procedure (Metropolis accept/rejection rule). For each plasmid we performed three
27 simulations (two in the presence of the chromosome and one in its absence). Each simulation
28 consisted of two stages: (i) an initialization stage (see above) and (ii) a thermodynamic analysis. The
29 initial polymer conformations were obtained by first equilibrating the system in the presence of all
30 forces and without confinement (large initial embedding volume). The cell volume was next slowly
31 reduced down to the nucleoid volume, and the thermodynamic properties were eventually
32 computed. The slow reduction of the embedding volume aimed at ensuring that the conformations
33 were the most likely from a thermodynamic point of view (by preventing as much as possible the
34 formation of long-living metastable kinetic conformations). The X, Y, Z position of each plasmid and
35 chromosome monomer and the barycenter of the replicon were recorded every 15 000 iterations
36 (Figure 2A).

37

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3

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3

4

5 Legend of the figures

6 **Figure 1. A)** Distribution of subcellular positioning systems in enterobacterial secondary replicons.
7 970 replicons were ranked according to their size (x-axis; the top panel shows a cumulative plot of
8 replicon sizes indicative of size density). The bottom four panels show event plots of the presence of
9 the indicated system (vertical line) or absence of system detected (none). B) Localization of the R27,
10 R27 Δ parAB, R27 Δ parRM plasmids (*parS* /parBGFP, green) and nucleoid (Hu-mCherry, red) C)
11 Localization along the long axis of the *E. coli* cell of the R27 tagged with a *parS* /ParB-GFP tag. Cells
12 with one focus of the WT R27, R27 delta parAB, R27 delta parRM (N =1000). D) Distribution of R27
13 localization as in C for cells with two R27 foci.

14 **Figure 2. A)** Description of the in silico simulation of plasmid and chromosome and the main
15 parameters that we collected during the simulations. B) In silico simulation of the localization pattern
16 of plasmids (green circles) and chromosome edges (red traces) along the longitudinal axis of the
17 nucleoid . Plasmid size (L_p) ranging from 30 kb to 1000 kb are represented. The simulations were run
18 for at least 3×10^7 iterations.

19 **Figure 3. A)** In silico simulations of the localization of plasmid barycenter along the longitudinal axis
20 of the nucleoid (histograms) and the short axes of the nucleoid (scatter plot). The barycenter of the
21 chromosome is plotted in red on the scatter plots. The black ovoid line map the position of the 70 kb
22 plasmid the most distant from the cell center, the same line is drawn on the 600kb scatter plot to
23 illustrate the fact that barycenters of large plasmids are more frequently localized toward nucleoid
24 center. B) Examples of 600 kb plasmid (green polymer) localization according to the positioning of its
25 barycenter along the longitudinal axis of the nucleoid. Plasmids crossing chromosomes (purple
26 polymer) tend to occupy the lateral side of the nucleoid. C) Histograms of the localization of 200 kb
27 and 600kb plasmid barycenters.

28 **Figure 4. A)** Analyzes of plasmid and chromosome spatial extension along the longitudinal axis
29 (Zspread) of the cell. Red circle represent the median chromosome extension according to the size of
30 the plasmid present in the same nucleoid. The green circles represent the extension of the plasmid in
31 the presence of a chromosome. Open green circles represent the extension of plasmid in an empty
32 nucleoid shell. Data were fitted with power laws. B) Normalized spreading (NS) of plasmids and
33 chromosome along the longitudinal axis of the nucleoid. $NS = Zspread / \text{Size of the replicon (kb)}$. Data
34 were fitted with power laws. C) Frequency of plasmid crossing events according to plasmid size. Data
35 were fitted by a double exponential decay [Freq crossing] = $25 * e^{(-0.03 * L_p)} + 9 * e^{(-0.004 * L_p)}$; $R^2 = 0.98$. D)
36 Average speed of chromosome crossing for small plasmids. Data were fitted by a double exponential
37 decay [Crossing speed] = $1822 * e^{(-0.15 * L_p)} + 12 * e^{(-0.007 * L_p)}$; $R^2 = 0.99$. E) Traces of the 200 kb plasmid
38 simulation. The positioning of a plasmid locus (green) and a chromosome locus (red) were
39 represented. The velocity of movement of the loci along the longitudinal axis of the nucleoid is
40 measured for crossing events of the plasmid (orange lines) and the chromosome (black lines). The
41 average velocity of the detected crossing events was plotted. F) Distribution of mobility of plasmid

1 loci at the polar position (longitudinal position <500 nm or >1500 nm). The 3D displacement of the
2 locus is measured for intervals of 15 000 iteration.

3 **Figure 5.** A) Examples of the localization of the *parS*/ParB-GFP foci (green) at the Ori-7 position of the
4 chromosome (top) and on a 137 kb excised circle (bottom). The nucleoid is labeled with the HU-
5 mCherry fusion (red). The positioning of *parS*/ParB-GFP foci according to cell size were recorded
6 before or 1h after induction of the Xis and Int recombinase to promote excision. B) Kymographs
7 representing the movement of *parS*/ParB-GFP foci at the chromosomal locus, on the excised circle,
8 R27 and R27 Δ *parAB* plasmids. Duration and frame intervals are indicated on top of each kymograph.
9 C) Montage of a representative ballistic movement of the excised circle. D) Mean Squared
10 Displacement analysis of *parS*/ParB-GFP focus at the chromosomal location or on the 137 kb excised
11 circle. E) Log -Log representation of the MSD analysis presented in D. Power law fitting of the data
12 $MSD=D*\Delta T^\alpha$. F) Mean Squared Displacement analysis of *parS*/ParB-GFP focus on the R27 and R27
13 Δ *parAB* plasmids. E) Log -Log representation of the MSD analysis presented in F Power law fitting of
14 the data $MSD=D*\Delta T^\alpha$.

15 **Figure 6.** A) Average normalized fluorescence intensity of *parS*/ParB-GFP foci on excised circles and
16 nucleoid labeled with HU-mCherry along the longitudinal axis of the cell. Cell border is determined as
17 the half maximum of the phase contrast signal. B) Lateral and longitudinal displacements (60 x 120
18 sec) of an excised *parS*/ParB-GFP focus at the nucleoid edge. The nucleoid (red shade) is drawn for
19 illustration, its exact contour changes from frame to frame. C) 3D timelapse imaging of the
20 movement of excised foci at the edge of the nucleoid. Imaging was of *parS*/ParB-GFP and HU-
21 mCherry was performed with 5 Z planes (interval 200 nm each) at 20 second intervals. Images were
22 deconvoluted and 3D reconstructed. D) Examples of the localization of the *parS*/ParB-GFP foci (green)
23 on a 137 kb excised circle in the presence of chloramphenicol to condense the nucleoid (HU-
24 mCherry, Red). The arrow indicates the movement of excised foci toward lateral edges of the
25 nucleoid. Right panel, kymograph along the longitudinal axis of the cell marked with the arrow. E)
26 Same as in A with a shorter imaging time interval (6 sec). Movement of excised foci toward lateral
27 edges of the nucleoid is also observable at this time scale. Kymograph (along the white line) showing
28 the movement of the polar *parS*/ParB-GFP foci toward the cell center via nucleoid edges. F)
29 Localization of the R27 and R27 Δ *parAB* in the presence of chloramphenicol. G) Examples of
30 the localization of the *parS*/ParB-GFP foci (green) on a 137 kb excised circle in the presence of
31 cephalixin to block division and create large cytoplasmic regions in between nucleoids (HU-mCherry,
32 Red). The arrow on the right panel indicates the only *parS*/ParB-GFP focus crossing the cytoplasmic
33 space observed among 200 cells. H) Examples of the localization of the *parS*/ParB-GFP foci (green) on
34 a 137 kb excised circle in the presence of rifampicin to decondense nucleoids (HU-mCherry, Red). I)
35 Timelapse (360 sec with 6 sec intervals) of dynamics of excised foci in the presence of rifampicin
36 (top); bottom Kymograph of the cell presented on the top panel.

37

38 Legend of the supplementary figures

39 **Supplementary Figure S1.** Measure of the rate of loss of R27, R27 Δ *parAB* and R27 Δ *parMR* plasmids

40 **Supplementary Figure S2.** A) Traces of the positioning along the longitudinal axis of the nucleoid of a
41 200 kb plasmid in the presence (green dots) or absence of chromosome (blue circles) within the

1 nucleoid. B) Distribution of the positioning of the 200 kb plasmid along the longitudinal axis of the
2 nucleoid in the presence (green) or absence (orange) of the chromosome. C) Distribution of the
3 positioning of the 200 kb plasmid along lateral axis of the nucleoid in the presence (green) or
4 absence (orange) of the chromosome. D) Traces of the positioning along the longitudinal axis of the
5 nucleoid of a 600 kb plasmid in the presence (green dots) or absence of chromosome (blue circles)
6 within the nucleoid. E) Distribution of the positioning of the 600 kb plasmid along the longitudinal
7 axis of the nucleoid in the presence (green) or absence (orange) of the chromosome. F) Distribution
8 of the positioning of the 600 kb plasmid along lateral axis of the nucleoid in the presence (green) or
9 absence (orange) of the chromosome.

10 **Supplementary Figure S3.** A) Distribution of the 200kb plasmid spatial extension along the
11 longitudinal axis of the nucleoid according to the positioning of its barycenter in the presence of a
12 chromosome. B) Distribution of the 200kb plasmid spatial extension along the longitudinal axis of
13 the nucleoid according to the positioning of its barycenter in the absence of a chromosome. C)
14 Distribution of the 200kb plasmid spatial extension along the lateral axis of the nucleoid according to
15 the positioning of its barycenter in the presence of a chromosome. B) Distribution of the 200kb
16 plasmid spatial extension along the lateral axis of the nucleoid according to the positioning of its
17 barycenter in the absence of a chromosome.

18

19 Table S1 : Annotation of segregation systems for 973 plasmids from enterobacteria

20

21 TableS2: Plasmid count per genus

22

23 Table S3: Plasmids with no segregation systems

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25 Text S1: HMM profile constructed using the conserved N-terminal domains of the StbA homologs
26 detected.

27

28

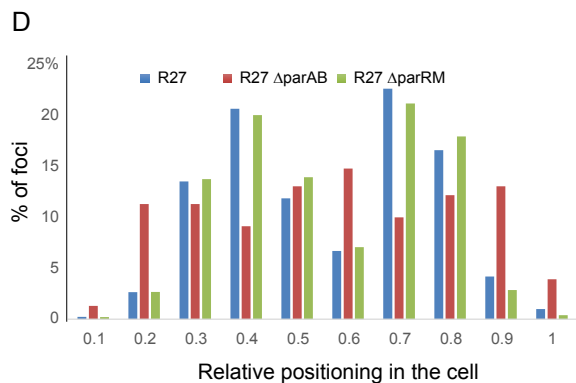
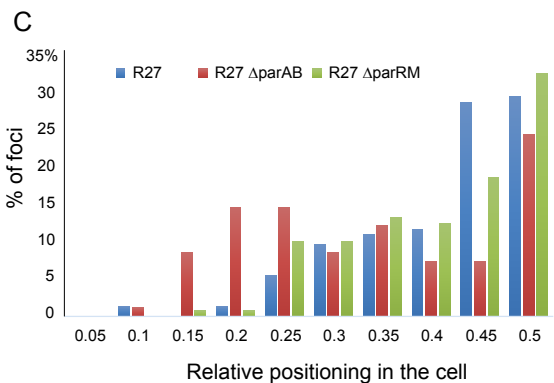
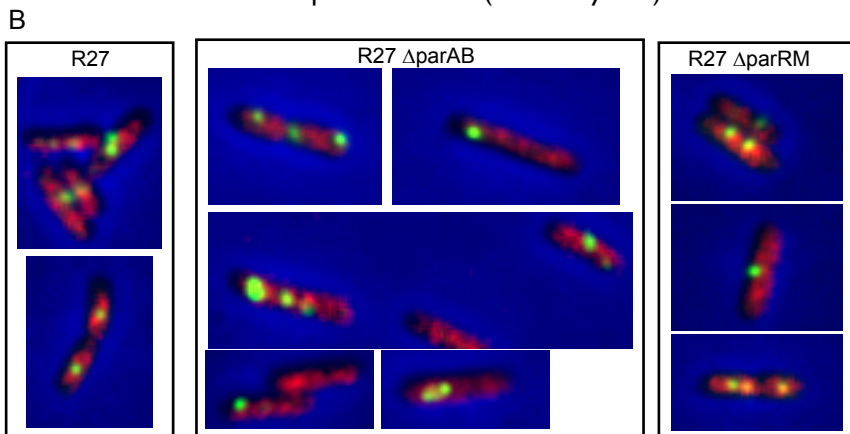
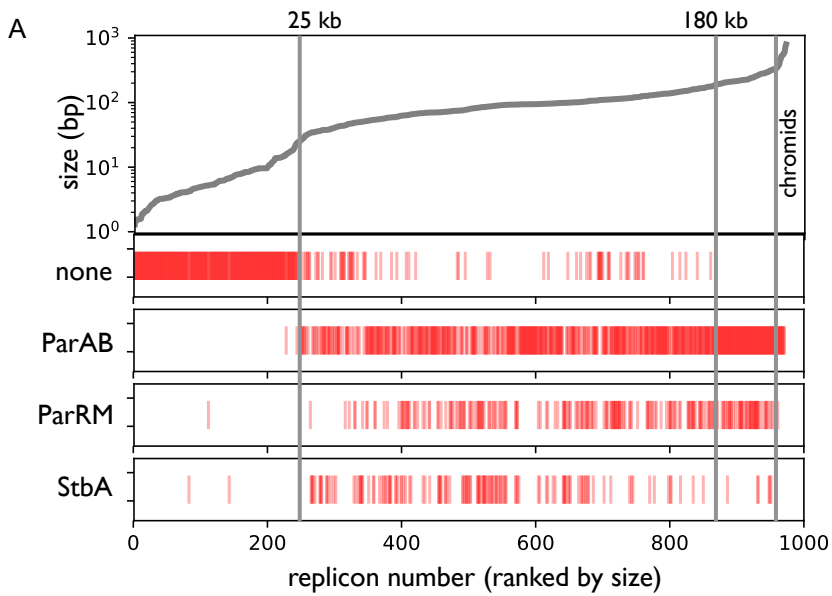
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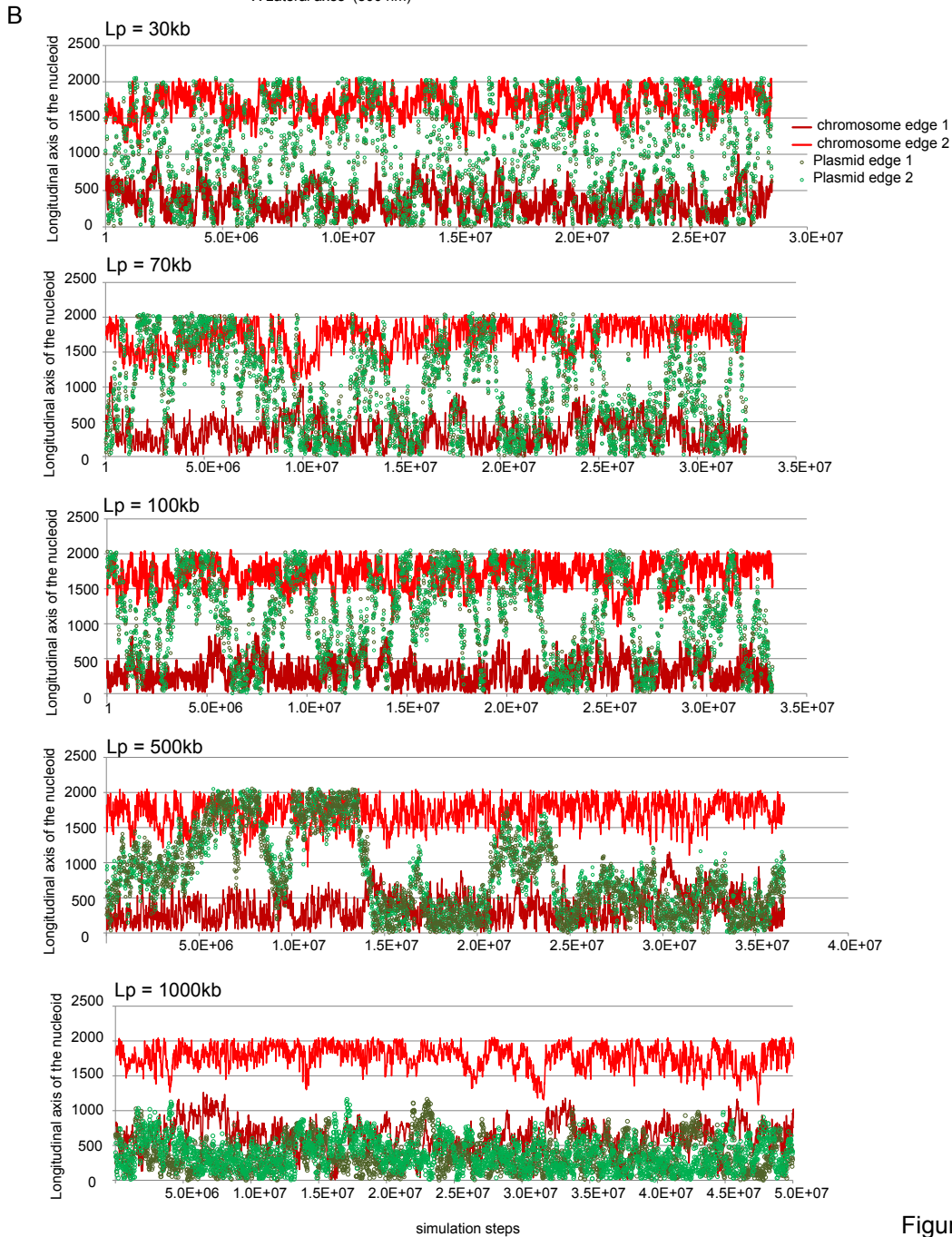
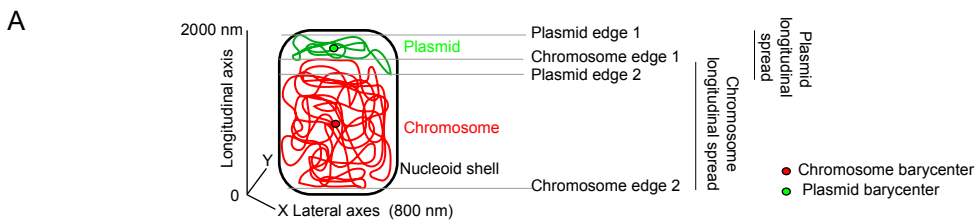


Figure 2

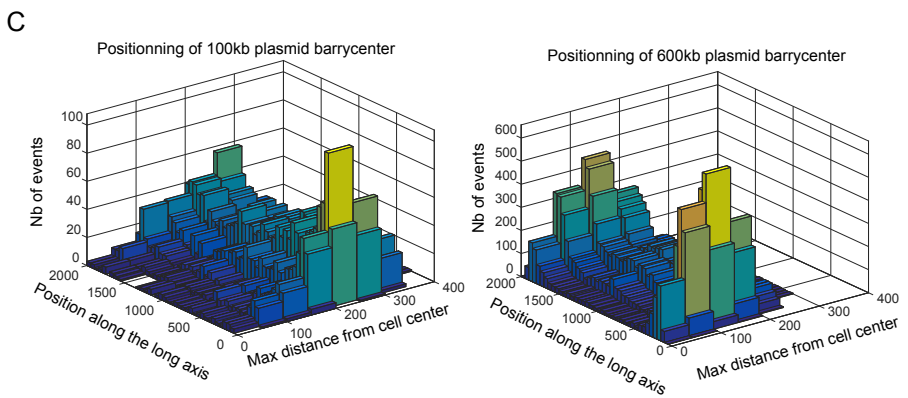
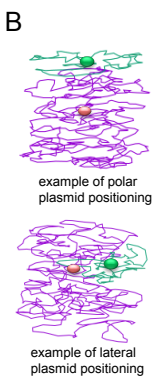
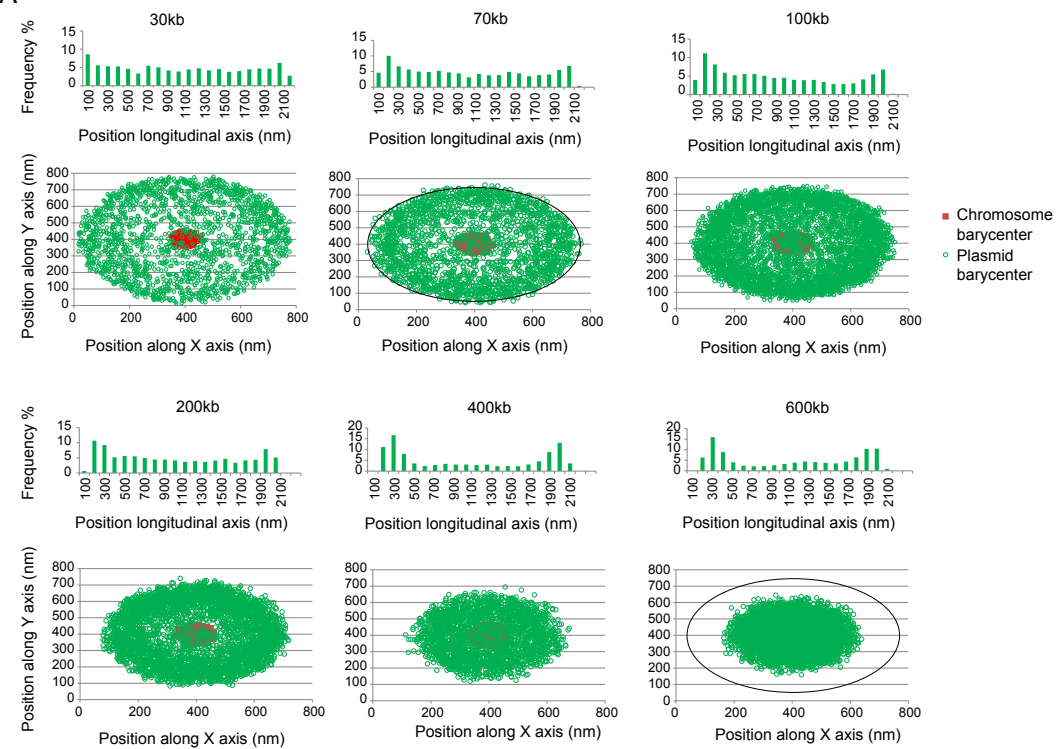


Figure 3

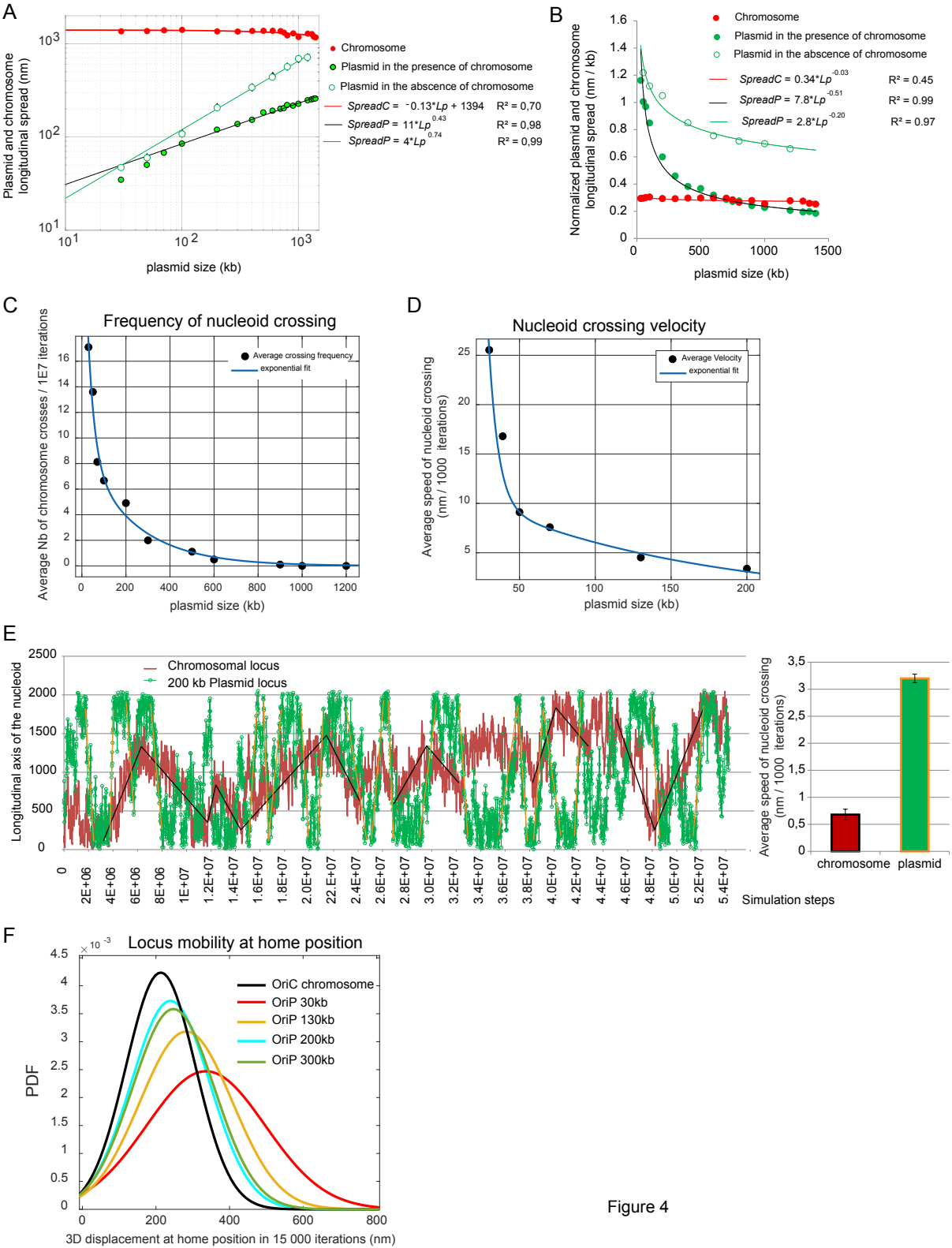
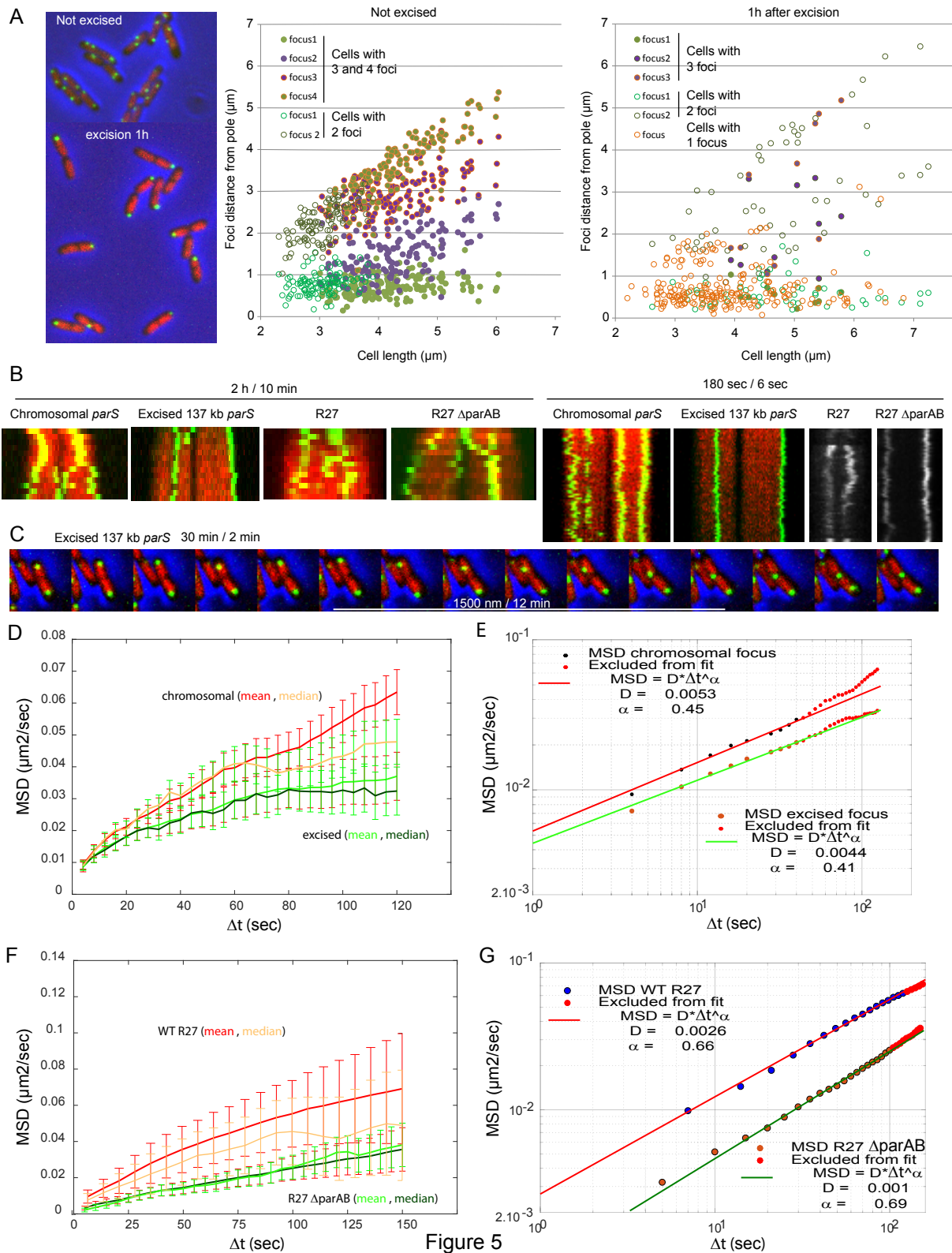


Figure 4



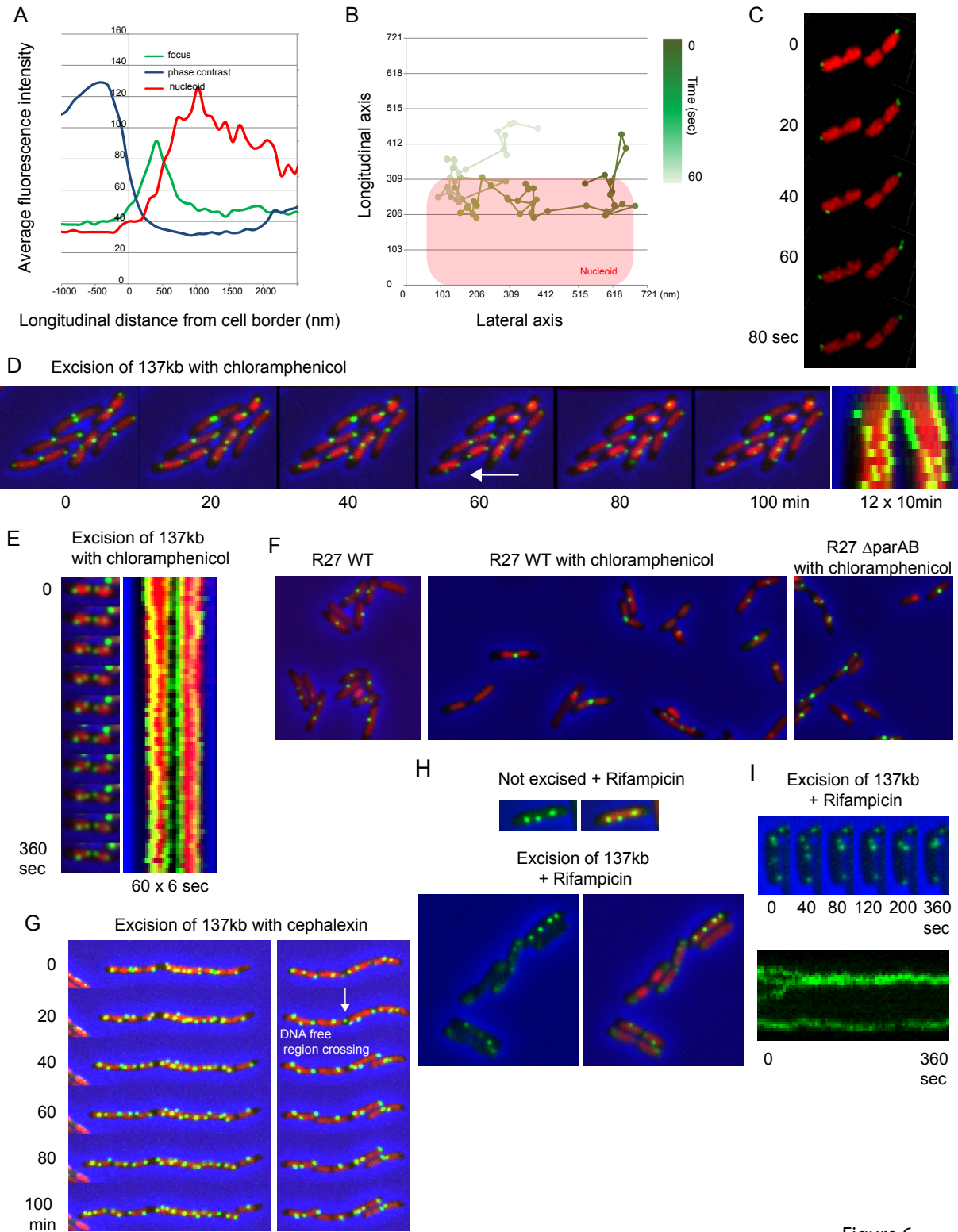


Figure 6