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DNA segregation in enterobacteria

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Abstract

DNA segregation ensures that cell offspring receive at least one copy of each DNA molecule, or replicons, after their replication. This important cellular process includes different phases leading to the physical separation of the replicons and to their movement toward the future daughter cells. We review these different phases and processes in enterobacteria with emphasis on the molecular mechanisms at play and their controls.

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Introduction

DNA segregation encompasses the processes ensuring that cell offspring receive at least one copy of each DNA molecule (replicon) composing the genome after DNA replication and before the completion of cytokinesis. Compared to Eukaryotes, bacteria typically lack a phase of visible chromosome condensation, attachment to a mitotic spindle and a defined segregation phase distinct in time from other phases by checkpoints in the cell cycle. Indeed, segregation is concomitant with the replication and the cytokinesis phases in most bacteria, particularly in enterobacteria. In addition, enterobacterial main chromosomes lack a dedicated partition system, as found in most other bacteria. These lacks largely delayed the understanding of segregation at the cellular level. Post-genomics approaches coupled to the advent of modern cell biology techniques and more recently biophysics and mathematical modelling are now closing the gap with the best described organisms.

DNA segregation includes two different phases that may overlap in time (Fig. 1): the physical separation of the newly replicated (sister) replicons and their displacement towards - and their positioning to - the cell compartments corresponding to the future sister cells. The physical separation phase includes two different processes: unlinking, which consists in the removal of physical links that persist between sister replicons after replication - *i.e.*, catenation links and eventually dimeric forms when recombination has occurred between sisters - and pairing release, consisting in the separation of sister loci paired by non-covalent links, *i.e.* protein bridges. The final segregation of sister replicons, once separated, is referred to as partitioning. Partitioning involves different processes depending on the replicon types (see below). Replicons may be randomly distributed, confined to a subcellular location or actively displaced using specific partitioning (Par) systems. We will start by reviewing the different types of replicons found in enterobacteria, their global organization and the segregation systems they carry, then describe our current knowledge of the different steps of segregation and end with the integration of segregation with other events of the cell cycle.

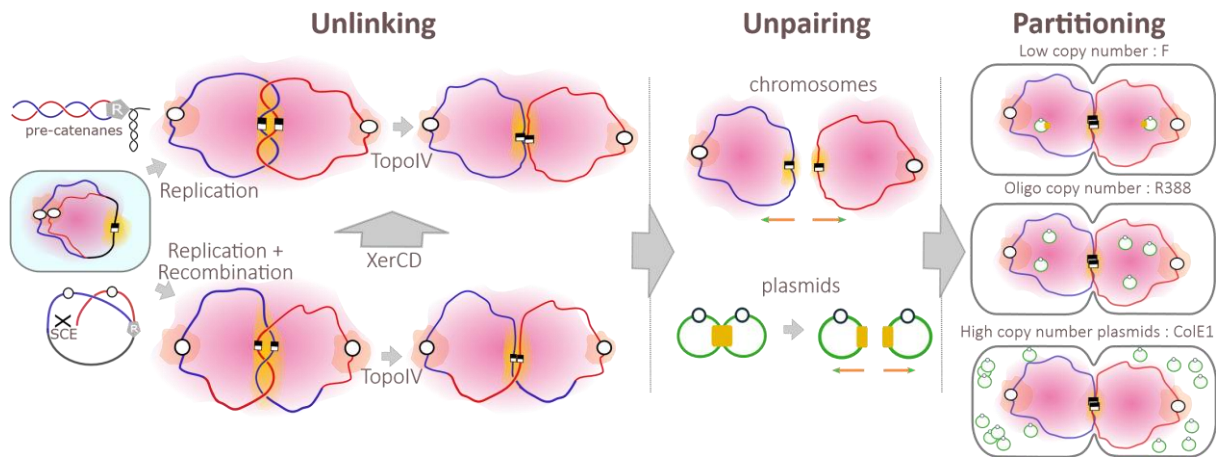


Fig. 1: The different steps of DNA segregation.

The left drawing represents a bacterium (rod with smoothed angles) replicating its chromosome (black line embedded into a pink nucleoid volume). The replication origin, *oriC* is represented as an open circle contained into the *ori* region (red halo) and the *dif* site as a black and white square, representing the XerC and XerD-binding sites, contained into the *ter* region (yellow halo). Top path: pre-catenanes and catenanes are formed by diffusion of the topological constraints created by progression of the replication forks (the 'R' gray arrow represents the replisome), leading to catenated sister chromosomes after termination of replication. Bottom path: dimers are formed by recombination between sister chromatids (SCE, here during recombinational repair of a double strand break associated with collapsing of a replication fork), leading to the fusion of the sister chromosomes as a single DNA molecule. Unlinking step: Topoisomerase IV (TopoIV) unlinks catenanes and unknots dimers whereas XerCD-mediated recombination between *dif* sites resolves dimers. Unpairing step: after catenane and dimer resolution, chromosomes remain paired by protein-protein and protein-DNA interactions until separated during cell division (see text for details). Plasmids may remain paired for a few minutes before separation via their replication initiator protein (*e.g.*, RepA or RepE) and/or their ParB protein. Partitioning step: dividing cells are drawn with their sister chromosomes at the unpairing step. Depending on their copy number and the partitioning system they carry, plasmids may display different subcellular positioning corresponding to different modes of partitioning: (i) partitioning from mid-cell to the cell quarter positions; (ii) positioning into the nucleoid volume or (iii) exclusion from the nucleoid volume.

Replicons of enterobacterial genomes

Bacterial genomes usually contain more than one replicon (Fig. 2). Of these, the main chromosome is easy to identify, being the largest replicon of the genome. It is almost in every case replicated from a single, well defined origin (*oriC*), acted on by conserved proteins, of which DnaA, the replication initiator (1). The main chromosome hosts the major part of the core genome, containing housekeeping genes. These genes are highly homologous between the different strains belonging to the same species (*e.g.*, more than 95% between *E. coli* strains (2)) and display similar and homogeneous codon usage (3), together specifying a species sequence signature. Chromosomes are replicated bidirectionally from *oriC* to the opposite region, named the terminus region (*ter*). Bidirectional replication has a profound impact on gene orientation and sequence biases (4, 5). Genes are preferentially oriented to be transcribed and replicated in the same direction, particularly essential genes (4, 6). The sequence composition of the two strands is also biased according to the replicative organization. Sequences running 5' to 3' from *oriC* to the *ter* region (*i.e.*, the templates of lagging strands) are richer in purine, particularly in Gs, than the complementary sequences. This sequence bias, called GC skew, inverts at *oriC* and inside *ter*, usually at or close to the *dif* chromosome dimer resolution site (see below). GC skew is accompanied by orientation biases

of numerous sequence motifs following the *oriC-ter* axis (5, 7–9). Replication terminators, recognized by the Tus termination protein, are oriented to stop replication forks progressing towards *oriC*, creating a replication fork trap opposite *oriC* (Fig. 2). Two purine-rich octameric motifs have also received particular attention and proved to play functional roles. Chi sites elicit recombination when reached from their 3' end by the RecBCD complex (5), promoting repair of broken replication forks. The second motif, the KOPS (*ftsK* Orienting Polar Sequences), is directly involved in segregation (see below). In contrast to Chi sites, KOPS are not significantly over-represented, with about 1 site per 12 kb on the *E. coli* chromosome. Their orientation is strongly biased, particularly in the *ter* region, where they strictly point to the *dif* site. Taken together, sequence biases determine two *ori*-to-*dif* replichores, constituting the main and most conserved global organization feature of bacterial chromosomes.

Beside replichores, other levels of global chromosome organization have been inferred from experimental approaches. Topological domains, defined as regions whose topological constrain cannot be transmitted to other ones, were long considered as resulting from a specific chromosome scaffold (10). They now appear as local (about 10 kb) organization features, whose borders are randomly positioned in individual cells (11, 12). Chromosome insulated domains (CIDs) have been defined from genome-wide contact maps between DNA loci using the chromosome conformation capture technique and its derivatives (13–18). They consist of domains whose loci interact more frequently with loci of the same CID than with those of other CIDs. Their borders appear to involve long highly transcribed genes. In *E. coli*, CIDs range from 40 to 300 kb (17), in agreement with CIDs detected in other bacteria. Both their significance and roles, if any, remain elusive. Lastly, large chromosome domains, called macrodomains (MDs), were uncovered first from the subcellular positioning of their loci (19), then by monitoring the interaction between remote loci using a genetic assay (20). Of these, the one encompassing the *ter* region received particular interest. It is specifically bound by the MatP protein, at a few tens of *matS* binding sites scattered along an 800-kbp region centered on the *dif* site. MatP insulates the *ter* region from the rest of the chromosome and delays its segregation until a mature divisome is assembled (21–23). MatP interacts with the MukBEF cohesin complex and appears to exclude MukBEF from the *ter* region (17, 24–27). It also interacts with the ZapB protein, itself interacting with the cell division apparatus (the divisome) through the ZapA protein (21, 28, 29). These two activities make *ter* highly specific compared to the rest of the chromosome concerning the way it is positioned and segregated. In addition, *ter* is devoid of binding sites for the SImA protein, a cell division inhibitor that binds other chromosome regions, ensuring they are segregated away from mid-cell before divisome assembly - *ter* is thus the only region allowed to be in the vicinity of the divisome in a normal cell cycle (30–33). One may thus argue that the *E. coli* chromosome is composed of two regions differing in the way they are positioned and segregated: *ter*, of which MatP is the main determinant and the rest of the chromosome which relies on MukBEF-dependent processing.

Secondary replicons, composing the genome along with the main chromosomes, are highly diverse (Fig. 2) (34–40). In enterobacteria, they usually account for 2 to 10 % of the

genomic DNA. The vast majority of them are circular, most often replicating via a theta-type mechanism, and thus must carry dimer resolution systems (see below). Most small plasmids (*i.e.*, less than 30-kbp (34)) are present in high copy-numbers, *i.e.*, more than 10 copies per chromosome. They replicate throughout the cell cycle by a random copy choice mechanism (41, 42). They lack partitioning systems, are excluded from the central DNA mass volume (the nucleoid) and rely on the random positioning of their multiple copies for their stability (Fig. 1). They are often mobilizable, referring to their capacity to be transferred between cells by a helper plasmid despite lacking their own complete transfer apparatus (34, 38). Larger plasmids, ranging from 30 kb to more than 1 Mb, and mostly around 100 kb long, are prominent in enterobacteria. They most often are mobile genetic elements that can transfer between strains and species by conjugation and thus encode the complex machinery for their transfer (34, 43–45). Low copy-number plasmids, when known, do not replicate at a specific cell cycle stage (46–48). They are maintained at more than one and up to 8-10 copies per chromosome. It might be useful to draw two categories when discussing their segregation functions, since their copy number appears associated with the maintenance function they carry (49): true low copy-number plasmids, maintained at less than 3-4 copies and the other ones, ranging from 4 to 10 copies. We will refer to this last category as oligo copy-number plasmids in the following. In all cases, low and oligo copy-number, random positioning is not sufficient and the presence of partitioning systems is essential for replicon maintenance (49, 50).

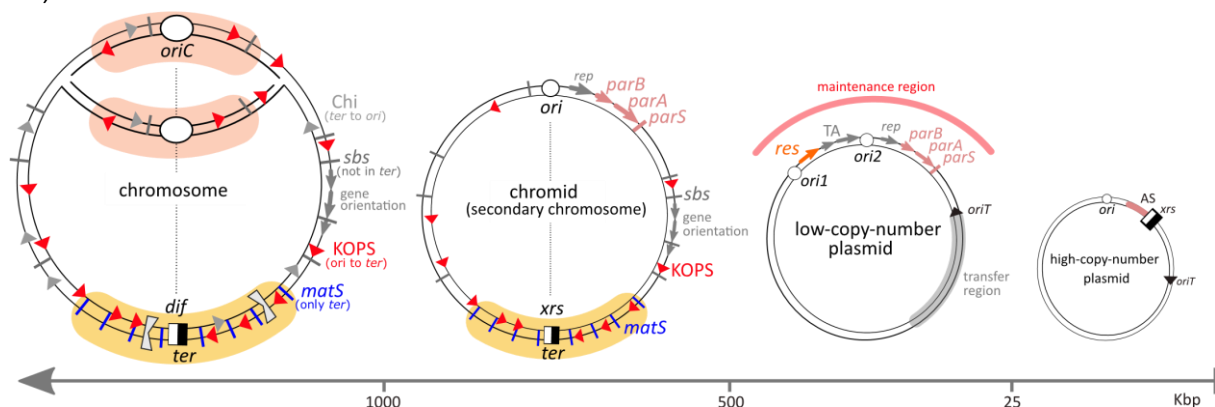


Fig. 2: Enterobacterial replicons.

A- The main chromosome is drawn during replication from its origin of replication *oriC* (white circle), contained into the *ori* region (red zone), to the opposite termination region (yellow zone), delimited by a series of replication terminators (gray flags, only the two inner sites, defining the replication fork trap, are shown here) and containing the *dif* site (black and white square). Features relevant to the global polarization of the chromosome as replichores and to segregation are indicated. Leading strands are purine-rich so that the G/C content of the two DNA strands differs. This GC skew changes at *oriC* and around the *dif* site. Essential genes are preferentially oriented to be transcribed and replicated in the same direction. Chi sites, recognized by RecBCD, preferentially point towards *oriC* whereas KOPS sites, recognized by FtsK, point towards *dif*. MatP binding sites (*matS*) are specific for the *ter* region whereas SlmA-binding sites (*sbs*) are excluded from *ter*.

B- Chromids, or secondary chromosomes, carry a replication origin (*ori*) that differs from that of the main chromosome and uses a specific replication initiator (Rep), along with a type I DNA partition system (*parABS*). Except for these two elements, the organization of chromids resembles that of the main chromosomes.

C- Low-copy-number plasmids are highly diverse. They may carry one or more replication origins with associated genes (*rep*), one or more DNA partition systems of any type (here *parABS*), a dimer resolution system, most often with a plasmid-encoded recombinase (here *resD*) and most often toxin/antitoxin systems (TA), clustered in a

maintenance region. They are often conjugative, encoding the transfer apparatus from a clustered dedicated region and a transfer origin (*oriT*).

D- High copy-number plasmids harbor a single replication origin and a dimer resolution system, often using a Xer recombination site (*xrs*) recognized by the cellular XerCD recombinases, controlled by accessory sequences (AS). They may possess a transfer origin, allowing conjugation using the apparatus encoded by other plasmids.

The term megaplasmids is usually used for very large plasmids (>350-kbp) that show no important chromosome-like feature, like the presence of essential genes (39). They frequently have mosaic structures, carrying evidence of plasmid co-integration (*e.g.*, multiple replication origin and/or partition loci) and of extensive gene transfer (51). Lastly, chromids, often called secondary chromosomes, are large replicons displaying chromosome-like features (40, 52). About 10% of bacterial genomes harbor at least one chromid. They are usually larger than megaplasmids (*i.e.*, >500 kb), may carry essential genes and appear more adapted to their host than plasmids and megaplasmids since they display nucleotide composition and codon usage closer to the main chromosome. Chromids are rare in enterobacteria, as are secondary replicons longer than 250 kb. They nevertheless appear present in some species like *Pantoea* (53) or *Rahnella* (54) often containing replicons above 500 kb. Since no functional study of enterobacterial chromids exist, we will consider the *Vibrio cholerae* secondary chromosome as a model chromid in the following. Based on their replication functions, chromids likely originate from plasmids. They nevertheless harbor KOPS biased-replicihores along with chromosome-like dimer resolution, a MatP-bound region opposite *oriC*, SlmA binding sites except in the MatP-bound region and CIDs as main chromosomes (55, 56). Importantly, when known, they replicate in a cell-cycle-controlled manner so that their termination, not initiation, of replication is synchronized with that of the main chromosome (55, 57, 58), further highlighting the importance of *ter*-specific events in cell-cycle control (see below).

Choreography of the replicons during segregation

In *Escherichia coli*, replicating chromosomes display a choreography mostly involving the successive movement of loci to mid-cell, from where they are progressively segregated (Fig. 3) (19, 59–65)(reviewed in (66, 67)). In new-born cells, *ter* is located close to the newest pole of the rod-shaped cell while the rest of the chromosome occupies a large fraction of the remaining cell volume. Replication is initiated and occurs in the mid-cell zone (59, 68–70). Sister loci stay together during a post-replicative co-localization period (see below), then segregate towards opposite cell poles. Segregation of loci follows a universal dynamic, arguing against a specific transport mechanism acting at a particular locus (*e.g.*, close to *ori*; (68)). Bilobed (split) nucleoids appear with mid-cell zones cleared off the bulk DNA while replication still proceeds (59). Meanwhile, *ter* loci migrate progressively to mid-cell, crossing the bulk chromosome along the width of the cell (21, 29, 59, 71). Upon relocation of *ter* at mid-cell, pre-divisional cells acquire the nucleoid conformation of newborn cells (Fig. 3).

A sequential segregation pattern equivalent to that of the main chromosome is thought to apply to chromids. Indeed, the *V. cholerae* model chromid replicates once per cell cycle at a specific time and segregates progressively as the main chromosome does (55, 58,

72, 73). Its *ter* region colocalizes most of the time with the *ter* region of the main chromosome while the rest of the chromid aligns along the terminal third of the main chromosome (55). This segregation pattern depends on *matS* sites on the chromid (74). Whether the same applies to other chromids or megaplasmids in enterobacteria remains to be established.

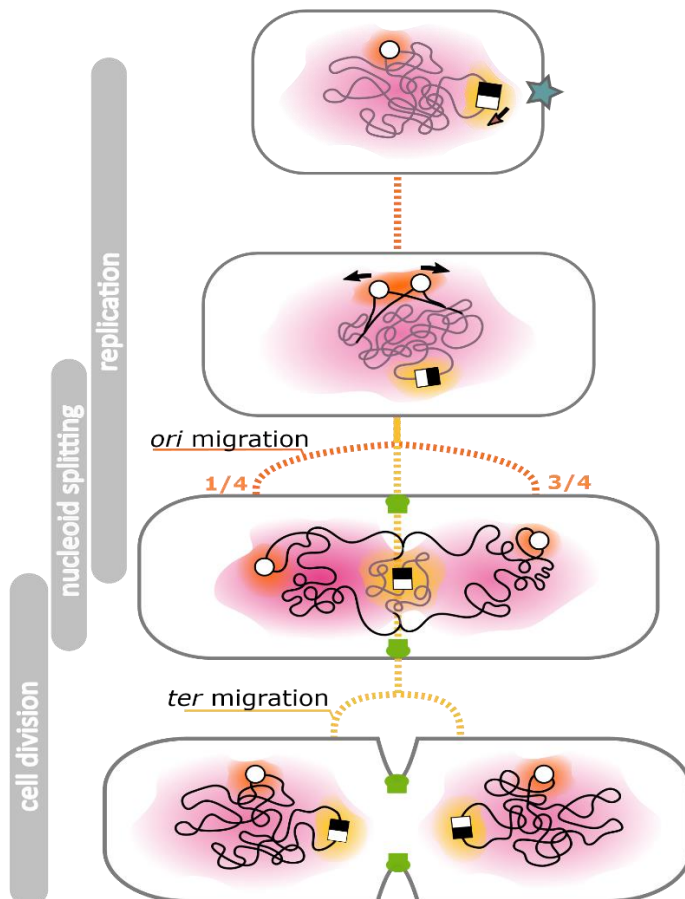


Fig. 3: Dynamics of the *E. coli* nucleoid during the cell cycle.

Symbols are the same as in figure 1 and 2. In newborn cells, the *ori* region is at mid-cell and *ter* near the newest cell pole (indicated by the star). The *ter* region then migrates to mid-cell during replication and sister *ori* migrate to the $\frac{1}{4}$ - $\frac{3}{4}$ positions after their replication. The bulk of the chromosome migrates towards *ori* after replication, while sister *ter* regions stay localized at mid-cell until segregated during cell division. Dotted arrows represent *ori* and *ter* migrations following a ballistic-type movement (see text).

At the resolution times it has been studied, plasmids segregate as a whole, though we are not aware of any study of the subcellular positioning of different plasmid loci, which may be informative in the case of large plasmids. When known, plasmid copies are replicated at any time during the cell cycle and at their subcellular position, which depends on the partition function they carry: ParABS and ParRM systems mediate specific positioning of plasmid copies, either unreplicated copies, linked sister copies or segregated copies, ensuring efficient partitioning to sister cells (Fig. 1 & 5; see below). On the other hand, high-copy-number plasmids replicate following a random copy choice mode (*i.e.*, one copy may be replicated several times during a cell-cycle) and at any time during the cell-cycle. They tend to localize as clusters, preferentially positioned at the cell poles and around mid-cell and are mostly excluded from the nucleoid volume (Fig. 1; (41, 75, 76)). This hybrid distribution ensures that, at cell division, many plasmid copies are positioned on both sides of the cell, allowing their stable maintenance without the need of a partition system. Indeed, random distribution well describes the high stability level of plasmids with more than 10 copies per chromosome (77).

A third mode of segregation adapted to oligo-copy-number plasmids has been proposed recently for the broad-host range plasmid R388 belonging to the widespread PTU-W family (78–80) and for the staphylococcal plasmid pSK1 (81, 82). These plasmids use unrelated segregation systems that both involve a single plasmid-encoded DNA binding protein. R388 is an oligo-copy-number plasmid (4-8 copies per cell), which appears to localize at random over the nucleoid area. This localization depends on a single plasmid-encoded protein, StbA, which binds to a *cis*-acting site located next to *oriT*, the transfer origin used for plasmid conjugation. StbA, together with its partner protein StbB, also controls conjugation, which was proposed to reflect an interplay between the subcellular positioning and the capacity of plasmid copies to be transferred (78). The StbAB system and its particular localization next to *oriT* are conserved and widespread among enterobacterial plasmids, particularly on medium-sized plasmids (49, 78). This system appears preferentially found on oligo-copy-number plasmids, creating a new paradigm of replicon segregation. However, the mechanism of the particular nucleoid location mediated by StbA and how it ensures stable maintenance remain to be established.

Unlinking: resolving catenanes

Two kinds of links persist between sister chromosomes: catenanes, corresponding to interlinked sister chromosomes and dimers corresponding to chromosome fusions (Fig. 1). Catenanes are formed during replication (83). Opening and copying the DNA double helix requires reducing the topological links, *i.e.*, one link per helix turn. Most of this constraint is moved ahead of the progressing fork, counteracting negative and creating positive supercoiling ahead of the fork, and is resolved there by the gyrase type II topoisomerase. A part of the constraint, however, leads to interlinked nascent chromatids behind the fork, either because the replisome slowly rotates while opening the DNA helix or because the replisome allows some of the stress accumulated upstream to diffuse behind the fork, potentially during pauses and/or partial dissociation periods (84). Replicating plasmids with interlinked sister chromatids, referred to as precatenanes, were detected and characterized (85). Upon termination of replication, precatenanes are converted to catenanes (Fig. 1) (84, 86, 87). Two particularities accompany termination: (i) gyrase may not be able to act ahead of converging forks, or of a fork converging to its termination point, which may render the creation of precatenanes compulsory at this step; (ii) the final closing of sister chromatids prohibit their decatenation by type I – requiring instead the action of type II topoisomerases.

The type II topoisomerase TopoIV, a heterotetramer of the ParC (carrying the DNA cleaving - rejoining activity) and ParE (carrying the ATPase and control domains), is the main decatenase in enterobacteria. It is essential for growth and the inactivation of either subunit leads to a phenotype characterized by centrally located unseparated sister chromosomes, the inhibition of cytokinesis and the formation of filaments (88). Gyrase may also resolve precatenanes and catenanes *in vivo* and *in vitro*, although far less efficiently than TopoIV (86, 89, 90). Of the two type I topoisomerase present in enterobacteria, only TopoIII can resolve pre-catenanes and catenanes provided they contain a single stranded gap (91).

Two independent assays led to the establishment of precatenanes and catenanes as the major cause of post-replicative cohesion in *E. coli* and of TopoIV as the major activity resolving them (Fig.4): both the colocalization and the interactions between sister loci raise upon TopoIV inactivation (92–94). Whole genome contact mapping (Hi-C experiment) confirmed an increase of contacts between remote loci when TopoIV is impaired, suggesting increased interactions between paired sister chromatids (95). The same study, together with the observation that TopoIII is associated with replication forks (96), suggested a role for TopoIII in removing part of the precatenanes. Other studies established the SeqA protein as a key factor behind replication forks that control the resolution of pre-catenanes (Fig. 4). SeqA binds hemi-methylated 5'-GATC sites in the DNA, produced by replication of the fully methylated DNA and, by doing so, delays their re-methylation by the Dam methylase (97). The interplay between SeqA and Dam controls post-replicative colocalization of sister loci via topolV activity (92). Consistently, SeqA oligomerizes to form large structures lagging behind the replication forks (98–101). The extent of the cohesive zone is currently estimated around 0.3 to 0.5 Mb (*i.e.*, loci segregate about 8 min after their replication (Fig. 4) (70, 93). How SeqA controls TopoIV activity is currently disputed. In one study, SeqA was reported to interact with TopoIV and to stimulate its activities of supercoil relaxation and decatenation, contrasting with the effect observed *in vivo* (102). SeqA structures can modify DNA conformation and constrain supercoiling *in vitro* (103). Building on this observation, SeqA was proposed to create a DNA conformation inhibiting TopoIV decatenation activity (92).

Beside SeqA, the major function controlling TopoIV activity is the MukBEF condensin complex (104). This complex is proposed to dynamically organize the chromosome in loops (105), and shows a preferred association with an ill-defined region containing *oriC*, the *ori* region (106) by an unknown mechanism (see below). MukB interacts with ParC and stimulates relaxation of negative supercoiling by TopoIV *in vitro* (107–109). The decatenation activity of TopoIV is not activated by MukB, showing that MukBEF is not a direct activator of precatenanes and catenanes removal (27, 110). It was nevertheless suggested that the MukB-ParC interaction directs a part of the TopoIV to the *ori* region and promotes its segregation (106, 111). Inactivation of MukB nevertheless proved only little effect, if any, on the segregation of the *ori* region in an other study (92). The role of MukBEF in chromatid unlinking remains thus far elusive and its interaction with TopoIV may be more related to the extrusion of loops compacting the chromosome (112) than to segregation *per se*.

Current models for sister chromatid unlinking during replication (Fig. 4A) posit that : (i) sister chromatids stay colocalized after replication fork passage; (ii) this cohesion stage lasts 8 to 10 min and depend on the growth conditions and strains used, concerning about half a megabase behind the forks; (iii) cohesion is maintained by the binding of SeqA structures to the nascent DNA containing hemimethylated GATC sites; (iv) this somehow prevents the action of TopoIV, allowing the persistence of precatenanes between sister chromatids; (v) meanwhile, TopoIII acts in the fork-proximal part of the cohesion zone, still containing single strand gaps between Okazaki fragments, to resolve a part of the pre-catenanes; (vi) away from

the forks, remethylation by the Dam methylase finally displaces SeqA, allowing resolution of the remaining pre-catenanes by TopoIV.

Whatever the way precatenane removal is controlled in the bulk chromosome, it does not apply to the *ter* region. This is in part due to the fact that *ter* is the only region where catenanes are formed (since it hosts termination of replication). The density of catenanes linking sister *ter* regions may be higher than that of precatenanes along the chromosome (see above), hence a higher TopoIV activity is required inside *ter* (113, 114). In addition, the combined action of the MatP and FtsK proteins determine a specific processing for *ter* (Fig. 4B). Post-replicative co-localization is longer for sister *ter* loci than for non-*ter* loci (23, 59, 115), which appears to be due to a longer period of inhibition of decatenation by TopoIV, depending on MatP (24, 116). MatP excludes MukBEF from *ter* (17, 24, 105), which was proposed to involve a direct interaction between the two proteins (25, 26)). This may exclude TopoIV along with MukBEF, explaining the decatenation delay (24). This however contrasts with the high need for TopoIV activity during sister *ter* unlinking. Clearly, exclusion of TopoIV from *ter*, if it exists, cannot be permanent. Rather, MatP may release TopoIV from MukBEF while excluding MukBEF from *ter*, as both proteins compete for the same interface in MukB (26), which may provide MukBEF-free TopoIV to the *ter* region. This would make sense considering MukBEF does not activate the decatenation activity of TopoIV (110). This scenario assumes that MukBEF-free TopoIV is kept inactive during the cohesion period by an unknown mechanism. This could be achieved by a peculiar DNA structure as proposed in the case of SeqA-bound precatenanes. A high density of catenanes may also inhibit their resolution by TopoIV (117).

Several lines of evidence indicate that sister *ter* cohesion is released in an FtsK-dependent manner. FtsK is an ATP-powered hexameric DNA translocase associated with the divisome (118, 119). It loads onto the DNA located in the vicinity of or spanning the division septum. It then translocates in the direction dictated by the KOPS motifs, *i.e.*, most often towards the *dif* site (Fig. 4B; (120, 121)). FtsK preferentially translocates DNA located into an about 0.5 Mb region centered on *dif* (115) and is required for the accurate progressive segregation pattern of this region, ending at *dif* (23). Both the regional preference of FtsK and the progressive segregation pattern of *ter* also depend on MatP (23). FtsK is active for translocation only when tethered to the divisome and at a time corresponding to the segregation of *ter* loci. The mechanism of FtsK activation involves unknown features of divisome maturation (122–124), favoring the formation of hexamers (125). FtsK interacts with TopoIV and activates its decatenation activity (126). This stimulation is specific to the processing of positive DNA crossings, as the one found in catenanes (127). FtsK may thus activate catenane resolution by TopoIV while translocating towards *dif*. However, catenanes appear preferentially resolved at *dif*. Indeed, *dif* or its immediate surrounding sequences are preferentially bound and cleaved by TopoIV and these activities depend on XerCD, the recombinases acting at *dif*, the *dif* site, and to a lesser extent on MatP (114). In addition, XerCD-catalyzed recombination at *dif* has been shown to resolve catenanes as a backup system, at least in certain genetic backgrounds, and this activity depends on oriented

translocation by FtsK (128, 129). It is thus likely that FtsK pushes most catenanes to *dif* while translocating.

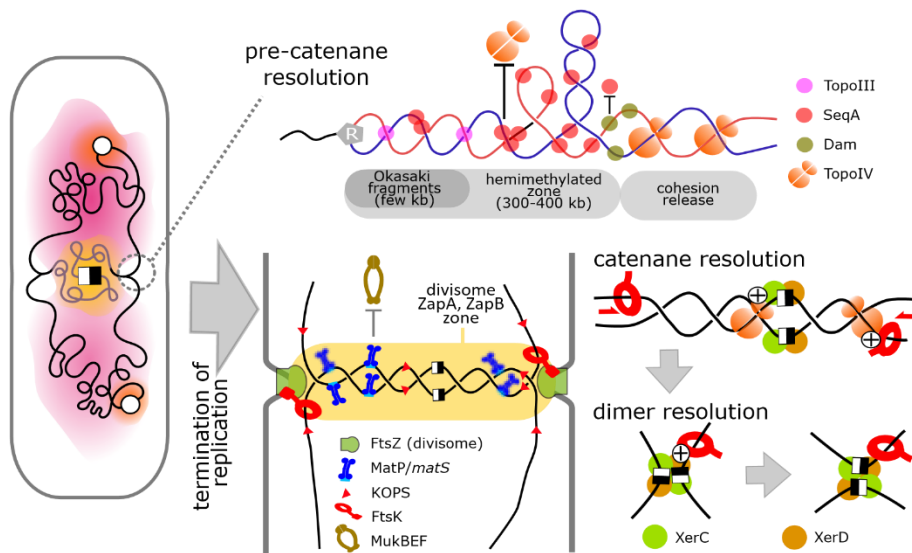


Fig. 4: Models of chromosome unlinking.

Symbols are the same as in figure 1 and 2, or indicated on the figure. Left: a cell towards the end of chromosome replication. Top: precatenanes are formed behind replication forks and concern three zones: i) a zone containing Okazaki fragment into which TopoIII can act to resolve a part of the pre-catenanes; ii) a zone not containing Okazaki

fragment but still containing hemimethylated GATC sites to which SeqA binds, inhibiting the resolution of at least a part of the pre-catenanes by TopoIV; iii) a zone where Dam re-methylates the GATC sites, allowing resolution by TopoIV. Bottom: after termination of replication, catenanes persist between sisters *ter* regions located at mid-cell in a MatP-dependent manner. Catenane's resolution is inhibited by an unknown mechanism. Translocation by FtsK is activated during cell division and oriented towards the *dif* sites by KOPS recognition. This may directly activate TopoIV and/or push the catenanes towards *dif* where interaction with XerC activates TopoIV (middle right). If chromosomes are dimerized, recombination between *dif* sites is required to complete unlinking, which is activated by a direct contact between FtsK and XerD (bottom right).

Unlinking: resolving dimers

In *E. coli*, chromosome and plasmid dimers mostly form by homologous (RecA-mediated) recombination (130–132). Both RecBCD- and RecFOR-driven recombination can dimerize chromosomes (122, 133), while small plasmids mostly recombine via the RecFOR pathway (134, 135). The RuvABC complex, which processes Holliday junction-containing recombination intermediates, appears to be biased towards avoiding the formation of dimers (136, 137). A rate of 15% of chromosome transiting through a dimeric state - inferred from the rate of cells requiring XerCD/*dif* to have a viable progeny - was measured in growing *E. coli* strains (122, 133). Inactivating either or both *dif* sites of *V. cholerae* main chromosome and chromid suggested that the rate of dimerisation primarily depends on the size of the replicon (138). In *E. coli*, unresolved chromosome dimers lead to a specific phenotype. Cell division proceeds, trapping the sister *dif* regions spanning the mid-cell zone (139). The trapped DNA is sheared by an unknown mechanism, inducing DNA degradation (140), high frequencies of RecBCD-dependent homologous recombination (141) and induction of the SOS system (132, 139, 142). Cells then continue growing and form filaments that finally die. From studies of the *V. cholerae* model chromid, unresolved chromid dimers are thought to be processed the same way as chromosome dimers (138). Little is known about the fate of low copy number plasmid dimers. In the case of iteron-based replication systems, *i.e.*, most enterobacterial plasmids, handcuffing between replication origins mediated by the Rep initiator protein has been

detected, and shown to lower the copy number of dimers (143). Lowering the number of segregating units at division below 2 copies would affect the stable inheritance of plasmids, even if actively partitioned. The effects of dimerization are best documented for high copy number plasmids. Dimerization and higher multimerization occur at low frequencies. However, multimers have a higher probability to be replicated than monomers, provoking the so-called 'dimer catastrophe' into which a small portion of cells containing only multimers explain the necessity of multimer resolution systems for stability despite high-copy number (144, 145).

Dimers are resolved by site-specific recombination (131). Site-specific recombination systems fall into two main classes named after the protein catalyzing recombination : Y-recombinase and S-recombinases, the former being prominent in enterobacteria (146). A limited number of systems have undergone in-depth analysis thus far, although in great detail. Recombination occurs at specific sites, called 'core sequences', about 30 bp in length, containing inversely repeated binding sites for the recombinase separated by a 2 to 8 bp central region where exchange takes place. Two general behaviors exist concerning the way they are integrated with other replicon features to ensure dimer resolution (131). Recombination may be controlled by local 'accessory' elements. Few hundreds of base pairs adjacent to the core sequence contain additional binding sites for the recombinase, allowing the assembly of a complex trapping negative supercoiling crosses between two recombination sites. These complexes, referred to as 'topological filters', form more efficiently on dimers than on separated monomers, ensuring a preferred direction of recombination towards dimer resolution. Systems using accessory elements are often found close to the replication origins of plasmids, into the regions dedicated to plasmid maintenance, as exemplified by the *ResD/rfs* system of the F plasmid ((147, 148); Fig. 2). On the other hand, some systems are devoid of local accessory sequences, their DNA site consisting only of the core sequence. This is the case of the *Cre/loxP* system of the plasmidic prophage P1 (149–151). In this case, the recombination site is not located in the maintenance region, but in the opposite replication termination region (152) and recombination *per se* does not ensure directionality, which relies on the global organization of the replicon and its processing during the cell cycle. The best described dimer resolution system is the Xer system, which can adapt to both types of control depending on the replicon.

In enterobacteria, the Xer system consists of two Y-recombinases encoded by the chromosome, XerC and XerD (153, 154). It recombines 28 to 30 bp core sequences organized as binding sites for XerC and XerD flanking a central region at the edges of which DNA strands are cut and exchanged. Xer recombination sites are found on high-copy-number plasmids and on chromosomes. Plasmid-borne Xer sites contain two types of accessory sequences adjacent to the XerC binding site of the core sequence (153). These are recognized by PepA, an aminopeptidase, which plays a structural role independent from its catalytic activity (155, 156). PepA binds DNA with poor specificity and appears directed to the accessory sequences by either the ArgR (*e.g.*, for the *cer* site of ColE1 plasmid; (157–159)) or the ArcA protein (in the case of the *psi* site of pSC101; (158, 160)). Assembly of the topological filter complex

activates recombination between otherwise inactive or poorly active core sequences, allowing XerC to catalyze the exchange of the first pair of strands. This forms a Holliday-junction-containing intermediate, resolved either by XerD-mediated exchange of the second pair of strands (between *psi* sites) or by replication (between *cer* sites). Lastly, a small RNA is expressed from the *cer* site of plasmid dimers and appears to delay cell division of dimer-containing cells by an unknown mechanism, further counteracting dimer maintenance in growing populations (161). This additional control is however restricted to ColE1 and few related plasmids for which dimer resolution is poorly efficient (due to a non-optimal 8bp central region in the case of *cer*; (144)).

On the *E. coli* chromosome, the *dif* site is opposite *ori*, at the center of *ter*, surrounded by the *matS* sites and at the point where KOPS orientation converges (Fig. 2). This particular location is required for efficient recombination and for dimer resolution (133, 142, 162–165). Recombination between *dif* sites requires neither PepA nor accessory sequence in addition to the core sequence (157). Consistently, it both creates and resolves dimers and multimers when inserted in a high-copy-number plasmid. Recombination at *dif* strictly depends on FtsK (123, 166, 167). It is activated via a direct interaction between the extreme C-terminal domain of FtsK, FtsKy, which also recognizes the KOPS, and XerD (168–171)), and activates XerD-mediated exchange of the first pair of strands, producing a Holliday junction-containing intermediate subsequently resolved by XerC-mediated catalysis (166). Although FtsKy alone can induce recombination, it is much more efficient when linked to the translocating FtsK motor, suggesting FtsK loading onto the DNA and translocation are prerequisite for efficient activation (172, 173). Indeed, FtsK has to reach and stop at the XerCD/*dif* complex to activate recombination (174–178). It has long been thought that FtsK loads only on dimers, ensuring directionality of recombination at *dif* (*i.e.*, bias towards resolution; (133, 165, 167, 179). This, however, proved false since FtsK segregates monomers as well as dimers (23). Nevertheless, the fact that recombination between two *dif* sites inserted as direct repetitions at the *dif* position depends on RecA suggests that recombination is indeed directional (133). To reconcile these observations, one may propose that assembled XerCD/*dif* recombination complexes are either structurally different and/or persist for a longer time on dimeric chromosomes. Either or both would be required for FtsK-mediated recombination activation. Consistent with this view, recombination activation appears to involve a structural change of the XerCD/*dif* complex (178). FtsK would thus segregate monomeric chromosomes without activating Xer recombination in most cases.

Pairing and release

In enterobacteria, as certainly in all living organisms, chromosomes and other replicons segregate following a “pairing and release” mode : sister copies remain paired after replication so that their separation can be controlled in time and space (Fig 1) (180). In the case of chromosomes, pairing appears mostly mediated by pre-catenanes and catenanes, while the release of pairing is controlled by their resolution (see above). It is however often difficult to assess whether differences in segregation times of chromosome regions or loci

result from different unlinking speed or from pre-catenane-independent pairing mechanisms. For example, late segregating 'SNAP' regions (181) were later shown to depend on SeqA, pointing to a prominent role of TopoIV (92). The appearance of divided nucleoids, associated with a global reorganization called nucleoid splitting (see above), was proposed to involve the persistence of tethers between sister chromatids (59, 182). The nature of these tethers and their relation to pre-catenanes has not been further investigated. Catenanes do not appear to be the principal cause of high-copy-number plasmids pairing, since they decatenate shortly after replication (41, 86). The fates of low-copy-number plasmids and chromids are unknown as very few studies have addressed this question. Two pairing mechanisms, considered not involving catenation, were described: the first one concerns the *ter* region of the chromosome and involves the MatP and ZapB proteins, the second one concerns low copy-number plasmids and involves ParABS systems.

Pairing of the *ter* region of the *E. coli* chromosome depends on MatP (22) and release is FtsK-dependent (23). It was recently suggested that MatP-dependent pairing involves both catenane-dependent and independent mechanisms (116). While TopoIV overproduction accelerates segregation of non-*ter* loci (92, 94, 183), this is not sufficient at a *ter* locus but requires concomitant inactivation of the formation of MatP tetramers, its interaction with ZapB or both, via the removal of its extreme C-terminal residues (116). It was thus proposed that MatP mediates both a catenane-mediated pairing involving a control of TopoIV activity, possibly via its interaction with MukB (17, 24, 25), and a catenane- and TopoIV-independent pairing via its interaction with ZapB. Note that the nature of this pairing, *i.e.*, the composition of the bridges, and the distance between the paired regions are still to be established. As the catenane-mediated pairing, it is released by FtsK translocation after divisome assembly.

In the case of low copy-number plasmids, the ParABS systems appears to play a specific role in pairing release, distinct from its role in partitioning (see below). The ParB protein assembles a nucleoprotein complex gathering the sister *parS* centromere site after replication (184). The ParA ATPase then becomes prone to initiate the physical separation of the paired sister ParB/*parS* partition complexes. It was estimated that this separation occurs within 5 minutes after replication (184, 185). This step was shown to require the stimulation of the ParA ATPase activity by ParB (186). How ATP hydrolysis by ParA separates the partition complexes is still unknown.

To note, the timing of pairing release relative to the cell cycle appears important for segregation success. This has been exemplified in the case of late replication leading to late and inefficient partitioning in the context of the severe incompatibility when the same centromere is present on two low-copy-number plasmids (187). Although replication of low-copy-number plasmids is precisely regulated to control the copy-number (188), it may occur at any time in the cell cycle (47, 48). The probability of replication gradually increases as the cell cycle progresses. Plasmids thus have a significant probability of replicating late in the cell cycle, *i.e.*, near the time of cell division, which could impair the partition process.

Mechanism of main chromosomes partitioning

In Enterobacteria, by contrast to a large number of bacteria and to plasmids, the main chromosome does not rely on a dedicated partition system for segregation, as the ParABS or ParRMC systems (see below). The mechanisms of positioning and partitioning of the *ori* region and the rest of the chromosome has thus been puzzling for a long time. The gradual movement of the duplicated DNA in opposite direction was proposed to be the result of the combined actions of several general mechanisms such as, for instance, DNA compaction by nucleoid associated proteins (NAPs) and topoisomerases, DNA replication, and transection (coupled transcription, translation and insertion of the nascent protein in the membranes; *e.g.*, (189)). Entropic forces were also proposed to drive chromosome segregation by spontaneously de-mixing the daughters DNA strands (67, 190–195). Indeed, entropy-driven de-mixing is now emerging as a major player in chromosome segregation, while specific mechanisms (see below) act to control it in time and space (for a complete discussion on entropy-driven segregation (196)). Consistent with a general segregation mechanism, loci around the chromosome move with similar mode and speed during their partitioning (68). Loci may move faster than cell growth (59, 61, 197–199). They move at maximum speed right after unpairing then slow down with increasing distance (68). This ballistic-like behavior may involve the accumulation of stress at mid-cell due to the rising quantity of replicated DNA (*i.e.*, unseparated sister chromatids) in a radially confined volume, which may act as a spring (182). Unlinking and release of sister chromatid pairing would then drive loci partition with the observed characteristics.

As the first replicated and segregated, the *ori* region, containing the replication origin *oriC*, has received particular attention in the search for mechanisms involved in its partitioning. Two independent studies led to the discovery of the *migS* site (200, 201). This 25-bp site, located inside the *wecB* gene (*i.e.*, 45-kbp from *oriC*), is the first locus segregated in most cells and is required for the early migration of the *ori* region. Another approach led to the identification of the *maoS* site as a *cis*-acting site involved in the organisation of the *ori* region as a macrodomain, in particular in constraining its mobility (202). This 17-bp site is located upstream of the *maoP* gene, identified as a *trans*-actor for the same function, between *oriC* and *migS*. Inactivation of the *MaoP/maoS* system also changes the order of segregation of *ori*-proximal loci. However, neither deletion of *migS* nor *maoS* have important effects on chromosome segregation. This suggests these systems have only local effects, for instance in controlling their own timing of pairing release, and reinforce the view that no partition system specific of a chromosome region exist in enterobacteria, except for the *ter* region (see above), but that chromosome loci partition by a universal mechanism. The *ori* region and *oriC* nevertheless play a prominent role in chromosome organization and segregation. First, the constraint on mobility and insulation of non-*ter* chromosome regions primarily depends on their position relative to *oriC*, *i.e.*, their timing of replication in the cell cycle (203). This observation highlights the importance of the replication pattern on chromosome conformation and changes our view of non-*ter* macrodomains, which may not be chromosome structuration elements *per se* but rather the consequences of the dynamic

organization of the chromosome. Second, the *ori* region is preferentially associated with the MukBEF condensin complex, a major player in both organization and segregation of the *E. coli* chromosome (104, 204) - its positioning, unpairing and partitioning being concomitant with those of the clusters formed by MukBEF (106, 205, 206). MukBEF forms a few dynamic clusters located at the mid- or quarter-cell positions (205, 207, 208). This localization was proposed to arise from self-organization of MukBEF complexes resulting from a reaction-diffusion mechanism (209). Why are *ori* regions preferentially associated with MukBEF clusters remains a mystery, as no specific DNA sequence for this interaction has been uncovered (24). Consistent with this, MukBEF clusters are not compulsorily assembled at *ori* (106). Loci of the *ori* region were nevertheless shown to move more efficiently towards MukBEF clusters than towards mid-cell (106, 209). A physical model was shown to recapitulate MukBEF-dependent *ori* positioning from known properties, proposing a new paradigm of chromosome segregation assisted by self-organization of a protein complex (209). Yet, the model predicts a preferential loading of MukBEF into the *ori* region for accurate positioning. Strikingly, the combination of a preferential loading with entropic repulsion, as would experience two closed loops (*i.e.*, akin nascent sister chromatids), permits accurate partitioning of *ori* with a dynamic behavior similar to experimental data.

Even if a lot of questions remain unanswered, a global picture may be proposed for the mechanism of chromosome segregation in enterobacteria : (i) the *ori* region is located at mid-cell in a MukBEF-dependent manner; (ii) the MukBEF cluster splits and move to the quarter positions due to its self-organizing properties; (iii) the splitting of MukBEF clusters and the stress created by paired sister chromatids promote partitioning of the *ori* region toward the quarter positions; (iv) the rest of the chromosome partitions, after unpairing, and package onto the quarter-proximal *ori* regions; (v) meanwhile, the polarly-located *ter* region crosses the bulk nucleoid along the cell periphery towards mid-cell to get replicated; (vi) *ter* stays outside the nucleoid bulk due to the exclusion of MukBEF by MatP and at mid-cell due to its interaction with the divisome.

Partition using dedicated Par systems

By contrast to high-copy-number plasmids and enterobacterial chromosomes, low-copy-number plasmids critically rely on active partition mechanisms to prevent plasmid loss at cell division. Three types of partition systems have been identified, each involving two proteins and centromere sites (reviewed in (50)). They are characterized by the type of partition NTPase that interacts with the partition complex assembled on the centromere sites. Type I systems, or ParABS, employ a Walker-type ATPase. They are the most prevalent in sequenced plasmid genomes. To be noted, large secondary replicons (megaplasmids and chromids) above 200 kb all encode a type I system (49). Chromosomally-encoded Par systems, found in most bacterial families but enterobacteria, are also exclusively type I. Type II partition systems, or ParMRC, use an actin-like ATPase, and Type III partition systems encode a tubulin-like GTPase. So far, type III systems, which use a dynamic polymerization mechanism, have not been found in enterobacteria and will not be described here. Lastly, a new kind of partition

system involving only one plasmid-encoded protein and a centromere site has been reported on oligo-copy plasmids (78). This suggests that efficient partition does not strictly require an NTPase protein and highlights that the replicon copy number determines different requirements for the segregation functions they carry.

Some natural plasmids were found to carry more than one partition system (49, 210, 211). To our knowledge, they are always of different types. In the cases of plasmids pB171 and R27 from *E. coli*, the two encoded-partition systems are of type I and II. They both contribute to plasmid inheritance although the type I system makes the larger contribution to plasmid stabilization (49, 210, 211).

Importantly, several low-copy-number plasmids encoding the same type of partition system often coexist in the same cell. However, they could not harbor an identical one, otherwise they would be excluded from each other - a phenomenon called plasmid incompatibility (reviewed in (50)). The three components of the partition systems - the centromere, the centromere binding protein and the NTPase - provokes by themselves an incompatibility with another replicon carrying a partition system with an identical component. The mechanisms driving these partition-mediated incompatibility are diverse depending on the partition component involved (212).

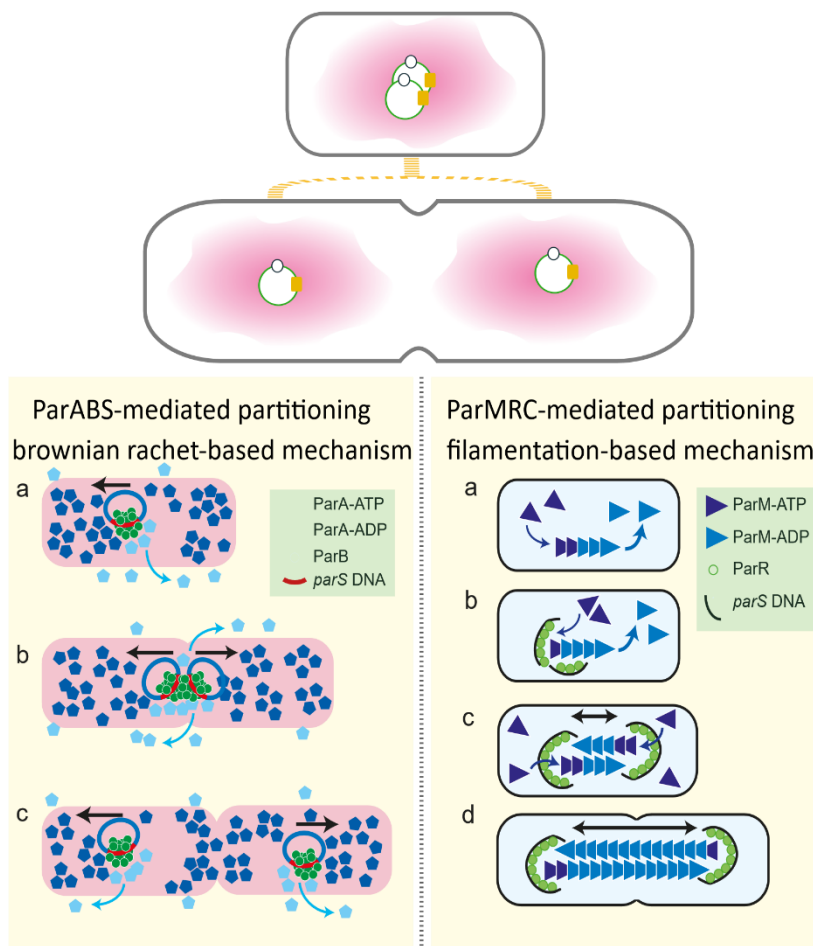


Fig. 5: Mechanisms of partitioning using active partition systems.

Top: partition systems act on low-copy number plasmids (here two copies) and migrate sister copies to cellular location (here from mid-cell to the $\frac{1}{4}$ - $\frac{3}{4}$ positions), ensuring sister cells receive at least one copy each after cell division.

ParABS-mediated plasmid partition. Plasmids are depicted by blue circles and the nucleoid is represented by a pink oval, other symbols are indicated on the figure. Plasmids carrying ParABS systems are located around mid-cell, quarter cell positions or equi-positioned in the cell length when present at one, two or more copies, respectively. (a) Their positioning relies on the tethering of the partition complex via ParA, preferentially bound to high-density region (HDR) within the nucleoid. Their partitioning relies on a Brownian ratchet mechanism

mediated by the ATP-dependent nonspecific DNA binding of the ParA ATPase, which binds within the bacterial nucleoid mass. The plasmid, via the ParB/*parS* partition complex, interacts with nucleoid-bound ParA proteins.

This interaction stimulates ATP hydrolysis and/or a conformational change that releases ParA from DNA. Due to the slow ParA rebinding to the nucleoid, a void of ParA is created on the nucleoid, which serves as a barrier to the partition complex motion so that the plasmid (ParB/*parS* complex) moves towards the remaining ParA. (b) After plasmid replication, ParA hydrolysis is involved to separate the duplicated partition complexes, which are then directed toward opposite directions by following the ParA tracks. (c) the void of ParA created close to the partition complexes prevents their fusion so that they are located mostly away from each other until the nucleoid split before cell division, thus ensuring that each daughter cell receives at least one copy of the ParABS-carrying replicon.

ParMRC-mediated plasmid partition. Ovals represent the cell wall, nucleoids are not shown and only *parC* DNA is shown for clarity. Plasmids are preferentially located close to cell poles or mid-cell. After replication, plasmid copies are driven to opposite cell poles by the polymerization of the ParM ATPase, which relies on dynamic instability. (a) ParM-ATP polymerizes as a parallel double helical filament. ATP hydrolysis is activated within the filament and propagated to adjacent ParM subunits. When a ParM-ADP reaches one extremity, the filament is rapidly disassembled. (b) ParR proteins bind to the *parC* centromere composed of direct repeats forming a curved nucleoprotein complex. This partition complex is inserted at the growing end of the polar filaments. This capping at the "barbed" end of the ParM filament is not sufficient to prevent the ParM depolymerisation when ParM-ADP reaches the uncapped extremity. (c) After replication, partition complexes assemble on the duplicated *parC* centromeres. ParM filaments assemble and slide in an antiparallel manner to form doublets. The two "pointed" ends are capped by the partition complexes preventing the disassembly of ParM doublets that can grow and push the plasmids, via the partition complexes, to opposite cell poles.

Mechanism of ParABS-mediated partitioning

Plasmids encoding a ParABS system display a preferential mid-cell and quarter-cell positioning in newborn and older cells, respectively (Fig. 5) (197, 213). Plasmids move around these positions (214, 215), which are located within the nucleoid mass as revealed by 3D fluorescence microscopy (216). This positioning relies on a higher-order structure, called partition complex, assembled by ParB at and around the *parS* centromere site, and on the interaction of ParA proteins, which dynamically pattern the nucleoid, with the partition complex.

The assembly of Type I partition complexes depends on whether the DNA binding domain of ParB is composed of an helix-turn-helix (HTH) or a ribbon-helix-helix (RHH) domain (reviewed in (50)). The higher-order structure formed with RHH-ParB dimer is not known. HTH-ParB partition complexes are composed of hundreds of ParB dimers. In the case of the prototypical system of plasmid F, over 90% of intracellular ParB are present in clusters nucleated at *parS* (217). Such higher-order assemblies are initiated at, and uniquely at, the *parS* site by ParB, involving contacts with residues located both inside (218) and outside the HTH domain (219, 220). In a few cases, as for the plasmid P1 ParB and its relatives, an additional motif within the dimerization domain is responsible for the specific binding to a second DNA sequence motif present within the cognate *parS* sites (218).

Upon interacting with *parS*, ParB dimer binds to CTP, which induces its clamping over the DNA and the subsequent release from *parS* of the clamped form (221–224). The clamped-ParB is then able to slide over the DNA proximal to *parS*, along several Kb on naked DNA (224). This sliding was proposed to be restricted to short distances *in vivo* by proteins bound to DNA (184) and by the CTP turnover (225). Interestingly, the partition complex behaves as a droplet-like assembly (226): *in vivo*, the hundreds of ParB assemble in condensates of spherical shape that are able to fuse, and with ParB able to diffuse rapidly between different *parS*-dependent condensates. *In vitro*, ParB dimers were shown to intrinsically forms droplets, whose

nucleation are enhanced both by *parS* and CTP (227), which would promote ParB-ParB interactions leading to DNA compaction in the partition complex (228).

ParA proteins belong to the deviant Walker A family, with intrinsically low ATP hydrolysis activity (229). Inside the cell, ParA exhibits several behaviors: it concentrates in a few patches linked to some DNA-dense regions (High Density Regions, HDR), forms dynamic gradients and oscillates within the volume of the nucleoid between its two edges (186, 210, 230, 231, 232, 216). The fraction of ParA colocalized with the HDR is responsible for the intracellular positioning of the partition complexes within the nucleoid, while the oscillating ParAs are implicated in the displacement of the partition complex by attraction toward the highest ParA concentration. Several models for ParA-dependent partition are currently proposed, sharing the basic principle of a Brownian ratchet (233–236). The differences between the mechanisms proposed may reflect variations between the partition systems and conditions modeled.

The Brownian ratchet imposes a directionality in the movement of the partition complexes provoked by the formation of a barrier, *i.e.*, the depletion of ParA induced by the partition complex (Fig. 5). The molecular properties governing these displacements, established for several ParAs, include (i) an ATP-dependent non-specific DNA binding activity of ParA, (ii) a stimulation of ParA ATPase activity by ParB and the DNA, and (iii) a high concentration of ParB molecules in partition complexes (reviewed in (237)). ATP-bound ParA binds to the nucleoid DNA. ParB, concentrated around *parS*, interacts with ParA-ATP (238), inducing the release of ParA from the DNA. This occurs by two pathways, one dependent and one independent of the stimulation of ParA ATPase activity by ParB (186, 239). The displaced ParA rapidly diffuses in the cytoplasm until it rebinds ATP to regain, upon a time delay, its DNA-binding activity (239). This time delay leaves a void of ParA on the nucleoid and creates the collective and dynamic behavior of ParA (Fig. 5). The partition complex, via ParB-ParA interactions, then follows the gradient of ParA that it causes, moving away from the ParA depleted zone. Notably, this mechanism has been partially recapitulated *in vitro* and shown to create movement (240–242). Upon partition complex duplication following replication, a bidirectional motion of the separated partition complexes would occur since they would move apart once pairing is released (see above), because the concentration of ParA would be lowest between them.

Mechanism of ParRMC-mediated partitioning

The visualization by fluorescence microscopy of plasmid R1, relying on a type II partition system, revealed a different intracellular positioning pattern compared to the one mediated by type I systems (summarized in Fig. 5). Plasmids present as a single copy are essentially found near one cell pole. When duplicated and segregated, they are most often located at both cell poles (243, 244). In the prototypal R1 plasmid-encoded system, the *parC* centromere site contains direct repeats forming a helical shaped structure to which ParR binds, forming the partition complex (Fig. 5) (245, 246). The actin-like ParM forms polymers with the plasmids present on both tips, indicating that the movement of plasmid R1 to opposite cell

poles is driven by ParM polymerization between plasmids (247, 248). Biochemically, ParM polymerization follows the actin paradigm, relying on dynamic instability (249). ParM-ATP monomers spontaneously nucleate and a ParM-ATP parallel double helical filament elongates. ATP hydrolysis is activated within the filament and propagates rapidly toward the filament tips, leading to ParM-ADP polymer disassembly. Only when both tips are capped by partition complexes, the ParM-ADP polymer remains stable and continues to grow by insertion of ParM-ATP monomers at the interface with ParR-*parC* (250–252). The current model propose that two ParM parallel double helical filament assemble together and slide in an antiparallel manner to form doublets, so that plasmid-mediated filament growth occurs bidirectionally, at both ends of the bundle, effectively separating the plasmids (250).

Integration with other cell cycle events

The DNA cycle, replication and segregation of the replicons, is highly integrated with cell growth and cell cycle events. A growing number of examples reporting links with features involved in metabolism and resource allocation have been reviewed elsewhere (253), though the mechanisms remain elusive. A main point of integration is with cell division (254). Cell division involves the assembly of the divisome in successive spatiotemporally-controlled steps, then cytokinesis *per se* (255). At early steps, a ring of FtsZ assembles at mid-cell, accompanied and stabilized by early divisome proteins, of which ZapA and ZapB (Fig. 6). This step is primarily controlled by patterning of the cell membrane by the MinCDE system, prohibiting FtsZ polymerisation at the cell poles, and by patterning of the chromosome by the SlmA protein (30). When bound to DNA, SlmA is an efficient inhibitor of FtsZ polymerisation (32, 256). It binds to specific sites on the *E. coli* chromosome that are absent from *ter*, thereby licensing divisome assembly by segregation of the non-*ter* DNA (32, 256). Inactivation of both MinCDE and SlmA is synthetic lethal (30). Such strains, however, show residual growth in poor synthetic media, revealing other controls of divisome positioning. A positive control, depending on MatP and ZapB, was proposed to mediate a preferred assembly of the divisome in the vicinity of *ter* (257). This involves the *ter*-MatP-ZapB-ZapA-FtsZ interaction string called the *ter*-linkage (Fig. 6) (29, 257, 258). An additional control coupling the speed of cytokinesis to the progression of *ter* segregation was proposed to be mediated by FtsK (259). In this hypothesis, translocating FtsK would inhibit septum closure. Such a control, although so far poorly documented, is particularly appealing to explain how chromosome trapping into the closing divisome and potentially the associated DNA damages are avoided despite the long-lasting pairing of sister *ter* at mid-cell determined by MatP. The observation that the division septum closes faster in the absence of MatP (260) is consistent with this view.

Lastly, the nucleoid, its dynamics during the cell cycle and functions involved in DNA segregation may be co-opted for other purposes. For example, ParAB-like systems are used by some bacteria to control the localization of protein complexes involved in chemotaxis (261) or carbon fixation (262). Indeed, the nucleoid, as the largest cytoplasmic structure, is emerging as a major actor in cell patterning and we expect more examples yet to emerge.

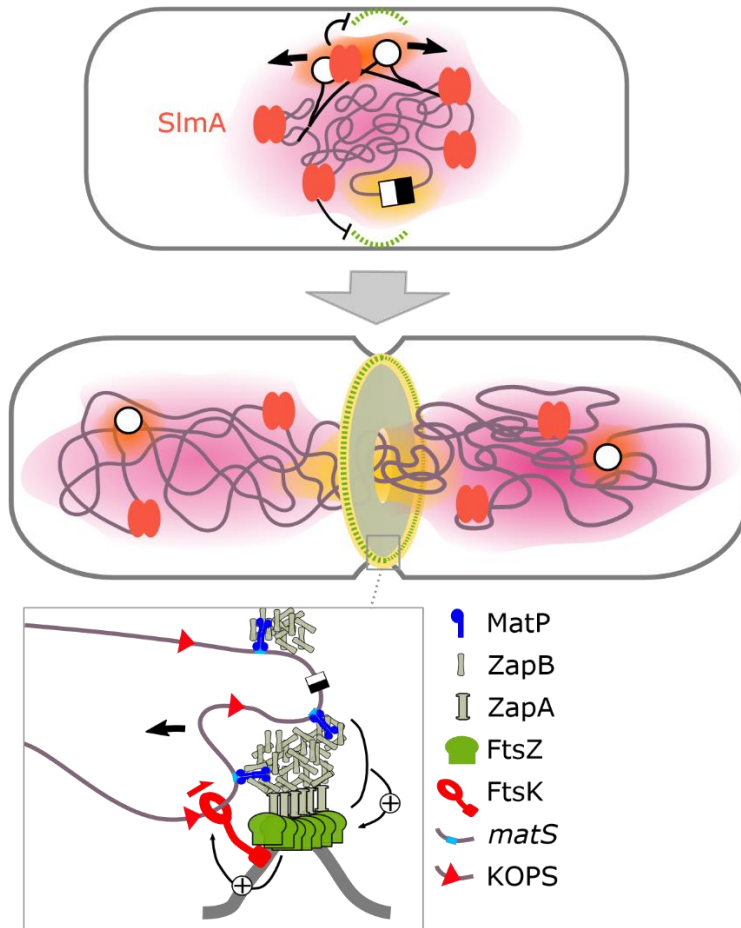


Fig. 6: Coupling chromosome segregation to cell division.

Top: a cell after initiation of replication. The SlmA protein binds specific sites scattered around the chromosome except in the *ter* region, and inhibits divisome (the dotted line represents FtsZ polymers) assembly. Middle: segregation of the non-*ter* chromosome regions deplete the mid-cell zone of SlmA, allowing divisome assembly. Bottom: In the mid-cell zone, sister *ter* regions are tethered to the divisome via the *ter* linkage (MatP-ZapB-ZapA-FtsZ interaction string). This facilitates the early steps of divisome assembly, which in turn activates FtsK translocation. Translocating FtsK removes MatP from the DNA, allowing *ter* segregation.

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