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Maintenance of chromosome structure in Pseudomonas aeruginosa

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Abstract

Replication and segregation of genetic information is an activity central to the well-being of all living cells. Concerted mechanisms have evolved that ensure that each cellular chromosome is replicated once and only once per cell cycle and then faithfully segregated into daughter cells. Despite remarkable taxonomic diversity, these mechanisms are largely conserved across eubacteria, although species specific distinctions can often be noted. Here, we provide an overview of the current state of knowledge about maintenance of the chromosome structure in *Pseudomonas aeruginosa*. We focus on global chromosome organization and its dynamics during DNA replication and cell division. Special emphasis is made on contrasting these activities in *P. aeruginosa* and other bacteria. Among unique *P. aeruginosa* features are the presence of two distinct autonomously replicating sequences and multiple condensins, which suggests existence of novel regulatory mechanisms.

Keywords

condensins; MksBEF; SMC; chromosome structure; Pseudomonas aeruginosa; PA4685

In the 1970's Booker and Loutit proposed that *Pseudomonas aeruginosa* strain PAO1 has two chromosomes, based on conjugational linkage studies (Booker & Loutit, 1974). This later proved incorrect. There is, of course, only one chromosome in *P. aeruginosa*, whereas the manifestation of the two linkage groups was caused by the now well-known phenomenon of clustering of housekeeping genes near the origin of replication and accessory genes near the terminus (Nichols, *et al.*, 2011). However, although our understanding of bacterial genome organization greatly improved since then, we still know far less about chromosome maintenance in *P. aeruginosa* than in its more celebrated relatives *Escherichia coli*, *Caulobacter crescentus* and *Bacillus subtilis*. This short review attempts to take stock of our present knowledge about replication and organization of *P. aeruginosa* chromosome with the focus on global chromosome dynamics.

The chromosome of *P. aeruginosa* (6.3 Mb for strain PAO1) is about one third longer than that of a typical laboratory strain of *E. coli* (4.6 Mb) or *B. subtilis* (4.2 Mb). The

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evolutionary origins of the extra sequence are yet to be determined. What is clear, however, is that the greater length of the *P. aeruginosa* chromosome results from genetic complexity rather than gene duplication, which allows this bacterium to colonize diverse niches (Stover, *et al.*, 2000, Silby, *et al.*, 2011). Encoded within this additional DNA is a variety of biosynthetic enzymes, transport systems, transcription factors and signal-response regulators, which allow this bacterium not only to strive in diverse environments but also display a remarkable plasticity of gene expression and ability to differentiate into metastable populations (Stover, *et al.*, 2000, Lee, *et al.*, 2006). Additional layers of complexity can be also found in global chromosome organization and segregation; however, their contribution to the cell physiology is only beginning to emerge.

DNA polymerases

Bioinformatics analysis identifies five DNA polymerases in PAO1. Four of them are homologous to the *E. coli* polymerases I through IV (Table I), whereas no homolog can be found to the Y-family UmuDC translesion polymerase. UmuDC, also known as Pol V, is induced as a part of SOS response and, following RecA-dependent activation, supports DNA synthesis across damaged DNA (Sutton & Walker, 2001, McHenry, 2011). Although this type of replication is highly mutagenic, it also allows cells survive heavy damage to DNA.

The fifth *P. aeruginosa* polymerase, DnaE2 (PA0669), belongs to the Pol IIIα family and is broadly spread among several subdivisions of eubacteria (Timinskas, *et al.*, 2014). DnaE2 is non-essential, at least in planktonic cells, and contributes to error-prone DNA repair (Sanders, *et al.*, 2006). The other two genes in the *dnaE2* operon share high similarity to a Y-family DNA polymerase (PA0670) and an SOS-induced inhibitor of cell division SulA (PA0671). These data indicate that DnaE2 might play the same role in *P. aeruginosa* as UmuDC in *E. coli* and is likely responsible for SOS-induced DNA repair.

Compared to its *E. coli* counterpart, the replicative DNA polymerase Pol III lacks the theta subunit of its core polymerase. In contrast to the rest of the subunits, the psi subunit of the clamp loader (PA4679) displays little homology to its *E. coli* counterpart and is misannotated in the primary databases as is the start codon of the gene (Jarvis, *et al.*, 2005). Based on protein expression and copurification studies, the correct 5′ end of the gene was assigned to the in-frame UUG codon located 135 bp upstream from the database prediction. Both the theta and psi subunits are non-essential in *E. coli* (McHenry, 2011), indicating that their primary function is in coordination of DNA synthesis with other cellular activities rather than in DNA synthesis itself. Accordingly, the rate of DNA replication is not impaired by their absence. Indeed, PAO1 transfers the entire chromosome during conjugation in 75 min (O'Hoy & Krishnapillai, 1987). For comparison, *E. coli* K-12 transfers its chromosome in 100 min. At least in *E. coli*, a similar rate of DNA synthesis is observed during chromosome replication. Given that DNA replication must keep up with DNA transfer, these data imply that, at least during conjugation, DNA synthesis occurs twice as fast in PAO1 that in *E. coli*!

The origin of replication

Several key chromosomal loci have been mapped in *P. aeruginosa* genome by now. These include the origin of replication, *OriC* (Yee & Smith, 1990, Jiang, *et al.*, 2006), *ParS* sites, which are required for correct chromosome partitioning (Bartosik, *et al.*, 2004, Livny, *et al.*, 2007), and the *dif* sites, where XerCD recombinase resolves chromosome dimers. No *ter* system, which ensures termination of chromosome replication, has been described so far. Likewise, no proteins with significant homology to Tus or RTP, which facilitate termination of replication in *E. coli* and *B. subtilis*, respectively, can be found in PAO1 genome (Lewis, *et al.*, 1990, Bastia, *et al.*, 2008). The lack of a close ortholog might not be surprising given the low sequence and even structure similarity between Tus and RTP (Bussiere, *et al.*, 1995, Kamada, *et al.*, 1996) and suggests that pseudomonads employ their own unique system, if any, to limit chromosome over-replication.

Bacterial origins of replication are several hundred base pairs long and located mostly in intergenic regions. Initiation of DNA replication is triggered by the binding of DnaA to its targets followed by loading of the helicase DnaB onto the nearby AT-rich repeats also known as the DNA unwinding element, DUE (Kornberg & Baker, 1992, Mott & Berger, 2007). The consensus sequence for the DnaA box is TTATNCACA with only one or two mismatches in it found in diverse bacteria (Mott & Berger, 2007, Zakrzewska-Czerwinska, *et al.*, 2007). A typical origin contains between two and five closely spaced DnaA boxes and two or three tandemly arranged AT-rich repeats. An exception to this rule is found in alfa-proteobacteria where as few as two DnaA boxes and with significant deviation from the consensus sequence can suffice for initiation of chromosome replication (Ioannidis, *et al.*, 2007, Shaheen, *et al.*, 2009). An inspection of numerous bacterial genomes revealed that the density of DnaA boxes serves as a good predictor of the location of the replication origin (Mackiewicz, *et al.*, 2004). Notably, the DnaA boxes do not have to be continuously located since they are brought together during origin activation via DNA bridging activity of DnaA. The split organization of *OriC* was reported for *B. subtilis*, where elements of the origin can be found both up- and downstream of *dnaA* (Moriya, *et al.*, 1992, Smits, *et al.*, 2011).

Several clusters of DnaA boxes are often found elsewhere on the chromosome. Chromatin immunoprecipitation studies revealed that these clusters indeed serve as a high affinity binding site for DnaA and contribute to correct timing of DNA replication in *B. subtilis* (Smits, *et al.*, 2011, Okumura, *et al.*, 2012).

Aside from the DnaA boxes, the origins of replication are poorly conserved and show detectable homology only among closely related species. Their genomic context displays greater stability. Inspection of multiple genomes reveals existence of two characteristic cassettes that harbor *oriC* regions (Fig. 1). The first of them, found in *E. coli* and closely related γ-proteobacteria, carries *oriC* between *mioC* and *gidA* (glucose inhibited cell division) genes in the *mioC-oriC-gidA-gidB* cassette (von Meyenburg, *et al.*, 1982, Ogawa & Okazaki, 1991). *gidA* and *gidB* are involved in posttranslational modification of, respectively, tRNA and 16S RNA (Okamoto, *et al.*, 2007, Moukadiri, *et al.*, 2009). The mechanism that leads to inhibition of cell division upon disruption of these genes remains unknown. This lays grounds for an intriguing possibility that these proteins are involved in

cell cycle control of DNA replication (although other mechanisms cannot be ruled out). Curiously, some bacteria carry *parA* and *parB* genes within this cassette, whose involvement in chromosome replication and segregation is far better established. Notably, this arrangement of the genes is conserved even in bacteria that initiate chromosome replication from other loci (Fig. 1).

The second cassette contains *oriC* between divergently expressed *rpmH* and *dnaA*, which encode ribosomal protein L34 and replication initiator DnaA. The intergenic space between *rpmH* and *dnaA* can be often found even in species that initiate replication elsewhere (Fig. 1). In many bacteria, including *P. aeruginosa*, the two gene clusters are located together and face in opposite directions (Briggs, *et al.*, 2012). Curiously, a genetic screen for autonomously replicating sequences, ARS, identified both these cassettes as a potential origin of replication (Yee & Smith, 1990). The two elements, named *oriCI* and *oriCII*, contain five DnaA boxes and two (*oriCII*) or three (*oriCI*) AT-rich 13-mer repeats. Although only one of the origins, *oriCI*, is essential in planktonic bacteria, any of them can support propagation of an otherwise origin-less plasmid (Jiang, *et al.*, 2006). In contrast, the *E. coli rpmH-dnaA* intergenic region carries only one consensus DnaA box, which is consistent with previous failures to find alternative autonomously replicating sequences in *E. coli* K-12.

Pseudomonads are not the only bacteria to harbor more than one DnaA box cluster complete with a plausible DUE. A similar arrangement can be found in *Vibrio cholerae* and *Yersinia pestis*, which are presumed to initiate replication from the cluster upstream from *gidA* (Fig. 1). This comparison reveals that migration of *OriC* from the *dnaA* to *gidA* cassette is a process distinct from the large chromosome rearrangement that split the two cassettes apart in enterobacteria. In firmicutes, the origin proximal DnaA box cluster is also found in the vicinity of *trmE*, but not downstream from it as in γ-Proteobacteria but upstream, next to the firmicute specific gene *jag* (Fig. 1).

Notably, the two cassettes do not exhaust potential locations for *oriC*. In *C. crescentus* and other α-proteobacteria, for example, the origin of replication is found between divergent *hemE* (encoding an uroporphyrine decarboxylase; CC_3763) and *cog1806* (a putative PEP synthetase regulatory protein; CC_0001) genes (Shaheen, *et al.*, 2009). It is unclear, however, whether or not this distinction points to independent evolutionary origins of the *C. crescentus Cori*. Indeed, the *hemE-oriC-cog1806* fragment is located only 5 kb upstream from the *trmE-gidAB-parAB* cassette and *dnaA*, and such migration could potentially occur in a single recombination event.

Initiation of DNA replication

The reason why PAO1 does not use *oriCII* is unclear and suggests the existence of additional control elements. Several such systems have been described in various bacteria. The first one involves *dam* DNA methylation coupled to the activity of SeqA (Slater, *et al.*, 1995). The *E. coli oriC* contains multiple GATC sites, which are methylated at N6 position of adenines in both DNA strands by *dam* methylase prior to replication. Curiously, the origin-less intergenic *rpmH-dnaA* region in enterobacteria also contains multiple *dam*

methylation sites. DNA replication converts the fully methylated GATC into their hemimethylated versions (Zyskind & Smith, 1986), which, in turn, recruit SeqA and become sequestered from DnaA and *dam* methylase (Slater, *et al.*, 1995, Brendler & Austin, 1999). This system provides a time delay needed for replication to advance before DNA is fully methylated again and the next round of replication is initiated (von Freiesleben, *et al.*, 2000). As a result, it blocks premature initiation of DNA replication and is essential for coordination of chromosome replication and segregation (Riber & Lobner-Olesen, 2005). Inactivation of this pathway results in excessive initiation of DNA replication, increased DNA damage and asynchronous chromosome replication (Boye, *et al.*, 1996). Notably, this system is unique to enterobacteria and a subset of γ-proteobacteria and is not found in pseudomonads (Brezellec, *et al.*, 2006).

The second system was described in *C. crescentus* and involves cell-type specific control of DNA replication. *C. crescentus* undergoes asymmetric cell division producing a surface-attached stalked cell and a mobile swarmer cell (Domian, *et al.*, 1997, McAdams & Shapiro, 2003). The swarmer cells do not replicate their chromosomes until they differentiate into a stalked cell. This is accomplished with the help of CtrA (cell cycle transcription regulator A) protein, which is expressed in swarmer but not stalked cells (Domian, *et al.*, 1997). The *C. crescentus* origin of replication, *Cori*, contains five CtrA binding sites, which are spread throughout *Cori* (Siam & Marczynski, 2000). Binding of CtrA to *Cori* apparently blocks DnaA binding and initiations of replication. A similar system has been recently identified in *B. subtilis*, where a master regulator of sporulation Spo0A was shown to inhibit DNA replication (Castilla-Llorente, *et al.*, 2006). These examples indicate that control of DNA replication at the level of initiation might be widespread among bacteria undergoing differentiation.

Yet another potential link to cell physiology is implied by the presence of binding sites for histone-like proteins within *oriC*. The *E. coli oriC* contains one each binding site for FIS (factor for inversion stimulation) and IHF (integration host factor) (Filutowicz & Roll, 1990, Gille, *et al.*, 1991) and, similarly, an IHF binding site is found in the *C. crescentus Cori* (Siam, *et al.*, 2003). Besides their initially recognized role in site-specific recombination, these proteins also serve as nucleoid organizing proteins and were also implicated in regulation of gene expression (Browning, *et al.*, 2010, Dillon & Dorman, 2010, Rimsky & Travers, 2011). The ability of IHF and FIS to modulate DNA reactions stems from DNA bending that accompanies their binding to DNA (Pan, *et al.*, 1994, Rice, *et al.*, 1996). Owing to such bending, distant DNA sites could be brought together in a proper orientation that would favor- or preclude- a macromolecular nucleoprotein assembly needed for a given reaction. FIS and IHF indeed contribute to DNA replication in *E. coli* since their inactivation, while not affecting cell viability, disrupts the synchrony of the origin firing (Ryan, *et al.*, 2004). This mechanism gives the cell the means to link DNA replication to its growth stage. Indeed, the abundance of the nucleoid proteins varies depending on growth phase or environmental conditions and could conceivably be used to adjust replication rate to fit the environment (Browning, *et al.*, 2010, Dillon & Dorman, 2010, Rimsky & Travers, 2011). It should be noted here that the control of replication initiation rather elongation is

preferred from the cell fitness point of view since it helps the cell to direct its resources into production of complete genomes and thereby maximize its survival rate.

None of the replication control systems described here has been identified so far in *P. aeruginosa*. It seems very likely, however, that they exist. Indeed, a recent FROS (fluorescent repressor operator system) microscopy study revealed a highly coordinated progression of replication forks in PAO1 (Vallet-Gely & Boccard, 2013), which implies synchronous firing of all replication forks across the cell and, by extension, the existence of mechanisms that preclude premature initiation. Likewise, the intricately controlled propensity of *P. aeruginosa* to differentiate into various planktonic and adherent forms as well as its ability to withstand hostile environment suggests high efficiency of the bacterium in marshalling resources to increase its fitness and persistence.

Local chromatin structure

The global structure of bacterial chromosome is established in concerted action of numerous DNA binding and remodeling activities (Fig. 2). The major nucleoid associated proteins (NAPs) were identified and thoroughly characterized in *E. coli* (reviewed in (Browning, *et al.*, 2010, Dillon & Dorman, 2010, Rimsky & Travers, 2011)). Most of them, including HU (heat unstable nucleoid protein), IHF, FIS, Dps (DNA binding protein from starved cells) and Hfq (host factor for Qβ replicase), have close homologs in *P. aeruginosa* (Stover, *et al.*, 2000). There is no H-NS or StpA in PAO1, although the H-NS-like MvaT and MvaU appear to function as their homologs (Vallet-Gely, *et al.*, 2005). NAPs are typically recognized through their tight DNA association and the resultant copurification with the chromosome during cell fractionation (Murphy & Zimmerman, 1997, Ohniwa, *et al.*, 2011). Contrary to the initial views, NAPs primarily function as global transcription regulators, whereas their contribution to DNA packing owes mostly to their abundance and the ability to bend or bridge DNA. Accordingly, inactivation of NAPs seldom has noticeable effects on cell physiology unless their primary function is affected. For example, a recent report linked synthetic lethality of MvaU and MvaT to activation of Pf4 prophage in the mutant cells (Castang & Dove, 2012).

DNA supercoiling is another major factor that affects compactness and activity of bacterial chromosome (Cozzarelli & Wang, 1990). Owing to its double helical nature, DNA is constantly unwound and rewound by numerous information processing enzymes such as DNA or RNA polymerases. To avoid the ensuing potentially staggering entanglement problems, the cell carries a battery of special enzymes, DNA topoisomerases, which remove the generated topological links (Corbett & Berger, 2004, Wang, 2009). *P. aeruginosa* carries the same full complement of DNA topoisomerases as originally identified in *E. coli* and is expected to exhibit the same regulatory mechanisms. While highly efficient in general, topoisomerases fall behind in highly transcribed regions, especially in the context of divergent promoters, which gives rise to local waves of supercoiling (Wu, *et al.*, 1988, Rovinskiy, *et al.*, 2012).

The net activity of topoisomerases maintains cellular DNA underwound by about 5% throughout the chromosome. About half of the resulting DNA supercoiling is constrained by

the bound nucleoid associated proteins, whereas the rest is absorbed by DNA twisting and writhing (Bliska & Cozzarelli, 1987). The effect of supercoiling on DNA activity is two-fold. First, the altered shape of supercoiled DNA dramatically changes statistics of intersegment collisions, which, in turn, markedly affects activity of many DNA processing enzymes (Vologodskii & Cozzarelli, 1996). Similarly, DNA supercoiling provides a powerful driving force for DNA decatenation and thereby contributes to chromosome segregation (Rybenkov, *et al.*, 1997, Alexandrov, *et al.*, 1999, Jun & Mulder, 2006). Second, DNA supercoiling favors recruitment of proteins that untwist DNA upon binding (Vologodskii & Cozzarelli, 1994). Being a global property, DNA supercoiling affects activity of the entire chromosome. Expression of about 10% of *E. coli* genes changes in response to variations in DNA supercoiling (Peter, *et al.*, 2004). Inside the cell, diffusion of DNA supercoiling is limited by the bound proteins to within ~10 kb stochastically formed topological domains (Postow, *et al.*, 2004). As a result, DNA supercoiling is non-uniformly distributed throughout the DNA and can significantly deviate from the average around actively transcribed genes or the progressing replication fork (Rovinskiy, *et al.*, 2012).

Whereas the local chromatin structure is largely opportunistic and dedicated to support of regulated gene expression within cellular confines, the global folding of the chromosome ensures spatial coordination of chromosome replication with other cellular activities, most notably, cell division. Such coordination is needed to ensure that exactly two copies of genome are produced during each round of replication and then passed one each to the daughter cells. Precise mechanism how this is achieved is yet to be understood. However, some themes are beginning to emerge. Two of such widely spread systems, condensins and ParABS, are discussed below.

Chromosome dynamics during segregation

Examination of several bacteria using FROS microscopy revealed ordered arrangement of the chromosome within the cell. This was observed for *E. coli* (Bates & Kleckner, 2005, Nielsen, *et al.*, 2006, Espeli, *et al.*, 2008), *B. subtilis* (Teleman, *et al.*, 1998), *C. crescentus* (Viollier, *et al.*, 2004) and, recently, for *P. aeruginosa* (Vallet-Gely & Boccard, 2013). In all cases, genomic coordinate of the DNA was found to correlate with its subcellular location. Curiously, two typical arrangements emerged. In non-growing *E. coli*, the origin of replication is found in the middle of the cell, whereas the two chromosome arms are aligned along the two halves of the cell. In *B. subtilis*, *C. crescentus* and *P. aeruginosa*, the alignment is longitudinal, with *oriC* and *ter* located at the opposite poles and the arms linearly stretching along the cell length (Fig. 3A). Thus, global chromosome dynamics in bacteria is decided not by their phylogenetic proximity but should be traceable to the presence or absence of a particular genetic marker.

This distinction becomes less pronounced once replication begins. In all tested bacteria, the two daughter origins move into the opposite halves of the cell soon after their formation. In *E. coli* and *P. aeruginosa*, they settle close to ¼ and ¾ positions (0.2 and 0.8 in *P. aeruginosa*), i.e. the places that will become the middle of the daughter cells (Fig. 3B), In *C. crescentus*, one copy of the origin remains at the cell pole throughout the cell cycle whereas the other, once formed, migrates to the opposite pole (Jensen & Shapiro, 1999). There is no

clarity yet whether this motion is powered by some sort of a mitotic apparatus or simply caused by topological repulsion of two growing unlinked polymer chains. The replicated clockwise and counterclockwise arms of the chromosome follow the origins to be orderly placed in the daughter cells. The *ter* region has to pass through the middle of the cell, where the FtsK DNA translocase is located. The activity of FtsK is required in order to align the *dif* sites on dimeric chromosomes and allow XerCD catalyzed resolution of the dimer (Aussel, *et al.*, 2002).

Despite many similarities, chromosome segregation did not proceed identically in all species. One discrepancy was related to location of the replisomes. The *E. coli* replisomes move around the cell presumably tracking the DNA (Reyes-Lamothe, *et al.*, 2008). In *P. aeruginosa*, replisomes stay in the middle of the cell for most of the replication cycle (Vallet-Gely & Boccard, 2013). In this respect, *P. aeruginosa* behave closer to *B. subtilis* than *E. coli*. Also unlike in *E. coli*, segregation of the replicated regions occurred progressively, without any discontinuity. Thus, the existence of sister chromatid cohesion at the snap regions that was observed in *E. coli* (Joshi, *et al.*, 2011) appears to be a species dependent phenomenon.

Global chromosome architecture

In many bacterial genomes, two systems, condensins and ParABS, are routinely found as the key factors responsible for global folding of the chromosome. The emerging data indicate that the two systems cooperate with each other as well as DNA replication to yield a functional chromosome. Mutations in condensins or ParABS lead to massive chromosome disorganization and are lethal in some species.

Condensins are multisubunit cytoplasmic proteins that link the global and local chromatin dynamics in organisms ranging from bacteria to humans (Cobbe & Heck, 2004, Graumann & Knust, 2009, Gruber, 2011). They contain at their core a dimer of the characteristically V-shaped SMC (structural maintenance of the chromosome) proteins. SMC proteins consist of the ABC type ATPase globular domain connected via a long coiled-coil to the hinge domain (Melby, *et al.*, 1998, Matoba, *et al.*, 2005). Exact architecture of the complex is unclear since both V- and I-shaped molecules can be found in solution (Matoba, *et al.*, 2005). The globular domain undergoes ATP-sandwiched dimerization and is responsible for ATP-modulated interaction with DNA whereas the function of the hinge and the coiled-coil is less clear (Woo, *et al.*, 2009). The accessory subunits interact in a dynamic, ATP-controlled manner with the globular domain of the SMC (Hirano & Hirano, 2004, Lammens, *et al.*, 2004, Petrushenko, *et al.*, 2006, Woo, *et al.*, 2009).

The primary activity of SMCs is ATP-controlled DNA bridging which allows them to act as macromolecular clamps that bring distant DNA segments together (Strick, *et al.*, 2004, Cui, *et al.*, 2008, Petrushenko, *et al.*, 2010), In principle, this activity could give rise to the chromosome scaffold that organizes the DNA into a set of giant loops (Cui, *et al.*, 2008). In this sense, the protein could be viewed as an intermediate between local and global chromatin folding. The actual mechanism of the protein is even more complex. Condensins are not uniformly distributed across the DNA but form distinct foci at the conspicuous ¼

and ¾ positions (Ohsumi, *et al.*, 2001, She, *et al.*, 2007, Minnen, *et al.*, 2011, Badrinarayanan, *et al.*, 2012, Kleine Borgmann, *et al.*, 2013), which points to their possible association with the replication or chromosome positioning machinery. Mutational analysis indicated that the focal localization of condensins is essential for their activity and that the accessory subunits play a central role in it (Shin, *et al.*, 2009, She, *et al.*, 2013). In contrast, the scaffolding activity of SMCs could be accomplished by the SMC core alone (Cui, *et al.*, 2008). Given that both DNA and non-SMC binding to the SMC core is controlled by ATP, a variety of models can be devised that envision coordination between DNA binding by SMCs and their subcellular localization.

Recent data suggested an alternative or, perhaps, supplemental explanation why recruitment of condensins to the quarter foci is essential for chromosome organization. The *E. coli* condensin, MukBEF, was found to associate with the ParC subunit of topo IV, and this association was confirmed as essential and stimulatory for the topo IV activity (Hayama & Marians, 2010, Li, *et al.*, 2010). Perhaps, recruitment of topo IV to replication fork facilitates decatenation of the daughter chromatids and, thereby, promotes both progression of the DNA replication and chromosome segregation.

Intriguingly, *P. aeruginosa* carries several condensins, which sets it apart from the archetypal laboratory strains of *E. coli* and *B. subtilis* (Petrushenko, *et al.*, 2011). Until recently, two families of condensins were known in bacteria. Several orders of γ-proteobacteria, including *Enterobacteriales*, *Vibrionales* and *Pasteurelleles*, encode MukBEF, whereas almost all other eubacteria carry the SMC-ScpAB complex (Hiraga, *et al.*, 2000, Cobbe & Heck, 2004). Despite significant sequence divergence, the two proteins are apparently similar in function and structure. In addition, a third family of condensins, MksBEF (MukBEF-like SMCs), was recently discovered (Petrushenko, *et al.*, 2011). MksBEF has the same operon organization as MukBEF and sometimes can be traced to the *E. coli* MukBEF based on homology. However, MksBs have shorter coiled coil than MukB and display low sequence conservation (Fig. 3). Several families of MksBEFs were identified with barely detectable homology to each other, mostly among outliers, suggesting that the proteins evolved independently.

P. aeruginosa strain PAO1 encodes two condensins, SMC-ScpAB and MksBEF, and the third condensin MksBEFG2 is found in the more virulent strain UCBPP-PA14 (Table II; Fig. 4). Of note, the correct start codon of *mksB2*, GUG, is found 168 bp upstream from its predicted position (unpublished data). Compared to PAO1, UCBPP-PA14 carries about 200 kb of extra genome, which appears to be remnants of a prophage that are now split into several pathogenicity islands (Lee, *et al.*, 2006). Finding MksBEF2 in one of these islands points to potential evolutionary origins of condensins and suggests that the proteins could have been involved in packing of large extrachromosomal genomes. The *P. aeruginosa* condensins perform distinct, partially overlapping functions, although their precise role is under investigation (Petrushenko, *et al.*, 2011). At least in planktonic bacteria, faithful chromosome partitioning requires the SMC-ScpAB complex, whereas MksBEF is expendable. Curiously, a widely known deletion in PA4684 and PA4685 that now spread throughout many subclones of PAO1 (Dotsch, *et al.*, 2009) is located in the *mksBEF* operon and encompasses *mksE* and *mksF*.

Mitotic apparatus

Long being controversial, the bacterial mitotic apparatus has finally materialized in the body of the ParABS system (reviewed in (Szardenings, *et al.*, 2011, Mierzejewska & Jagura-Burdzy, 2012)). This system consists of three elements. ParA protein (known as Spo0J in *B. subtilis*) is a cytoskeletal ATPase highly prone to oligomerization in vitro and in vivo (Fogel & Waldor, 2006, Ringgaard, *et al.*, 2009, Ptacin, *et al.*, 2010). ParB (Soj in *B. subtilis*) is a sequence specific DNA binding protein that serves as an adaptor for ParA. *ParS* is a *cis* acting DNA stretch that recruits ParB. This system is found in genomes of many bacteria, often as a part of the *oriC* cassette, as well as in low copy plasmids both in Gram-negative and Gram-positive bacteria (Livny, *et al.*, 2007). Chromosome (or plasmid) segregation is accomplished with the help of the pulling forces within ParB filament that stretches between the sister *ParS* sites or, perhaps, connects *ParS* to anchor proteins on cell poles. The system is completely portable and can be used to stabilize low copy number plasmids in foreign bacteria.

The consensus *ParS* sequence, TGTTCCACGTGGAACA, is highly conserved in diverse bacteria (Livny, *et al.*, 2007). In *P. aeruginosa*, two of such have been found, both within several kb from *oriC*. When up to two substitutions to the consensus are allowed, 10 putative sites can be found in the PAO1 chromosome (Bartosik, *et al.*, 2004). Four of them are located close to the origin, two each at about 500 kb from *oriC* both counter- and clockwise from it, and two are located in the *ter* region. The functional significance of the perfect matches was verified when the predicted ParABS cassette was found to stabilize plasmids in *E. coli* (Bartosik, *et al.*, 2004).

ParA and ParB are non-essential in PAO1, although their inactivation leads to dramatic chromosome disorganization and increased frequencies of the chromosome partition defects (Bartosik, *et al.*, 2009, Vallet-Gely & Boccard, 2013). It is tempting to speculate that these defects develop due to the loss of the mitotic forces that push sister chromosomes apart. This conclusion, however, needs further verification, since the activity of chromosomally encoded ParABS is integrated into other genome duplication functions. In *B. subtilits*, for example correct loading of the SMC-ScpAB condensin onto the chromosome requires functional ParABS with correctly positioned *ParS* sites (Gruber & Errington, 2009). Similarly, ParABS is involved in correct timing of DnaA-mediated initiation of DNA replication (Murray & Errington, 2008).

Concluding remarks

Most of the key systems involved in replication and segregation of *P. aeruginosa* chromosome have been mapped and at least initially characterized. This makes this bacterium an attractive model system for further studies of chromosome dynamics. A word of caution here is a rather high frequency of misannotated start codons in public databases.

The replication origin region consists of the contiguously located *dnaA* and *gidA* cassettes complete with the DUE-containing DnaA box clusters. Both these elements can serve as an *OriC* in one bacterium or another and support propagation of origin-less plasmids in pseudomonads. Pseudomonads are the only documented bacterial system with two ARS on

the same chromosome. The function of *OriCII*, if any, is unknown as are the structural determinants that render it dormant.

Multiple chromosome maintenance systems are yet unidentified. Prominently missing is the knowledge on systems that ensure synchronous initiation of chromosome replication or its control in response to developmental needs.

Spatial chromosome dynamics bears greater resemblance to *B. subtilis* than *E. coli*. The chromosome layout is longitudinal, not transeversal; the DNA polymerase stays long at midcell during replication. However, no evidence for polar attachment of the origin has emerged so far.

P. aeruginosa encodes multiple condensins, from both the SMC and MukBEF superfamilies'. These condensins apparently play distinct roles. Intriguingly, inactivation of condensins has only mild impact on *P. aeruginosa* viability, which points to existence on redundant mechanisms in global chromosome packing.

Most of research has been focused on planktonic bacteria. Virtually nothing is known about how cell differentiation affects chromosome maintenance. The existence of potentially redundant systems raises questions about their possible role in differentiation.

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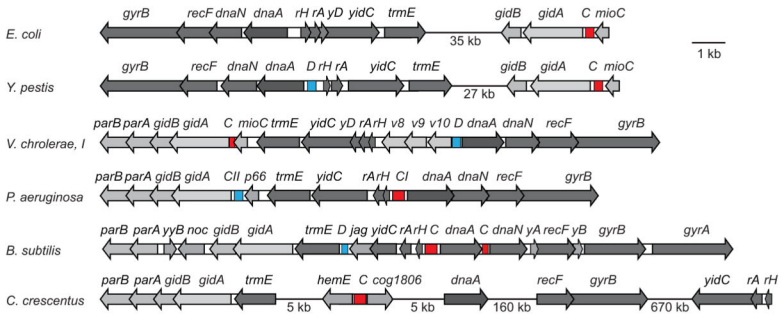

Fig. 1.

Comparison of genomic context for various bacterial origins of replication. The origin of replication (*C*, red) and a DnaA box clusters with DUE (*D*; blue) are often found embedded within the *dnaA* or *gidA* cassettes. *rH*, *rpmH; rA*, *rnpA; yD*, *yidD; v8*, *VC0008; v9*, *VC0009; v10*, *VC0010; p66*, *PA5566; yyB*, *yyaB; yA*, *yaaA; yB*, *yaaB.*

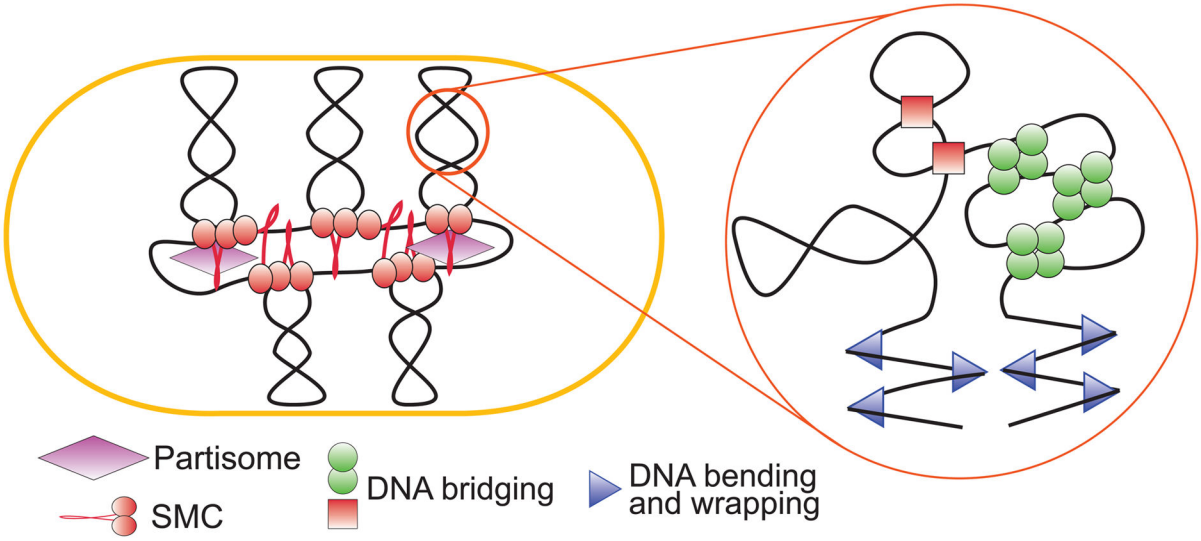

Fig. 2.

A working model of bacterial chromosome. Chromosome structure is stabilized by various NAPs that bend and bridge DNA, supercoiling, which energizes and compacts DNA, and condensins, which stabilize giant loops and tether them to extrachromosomal elements.

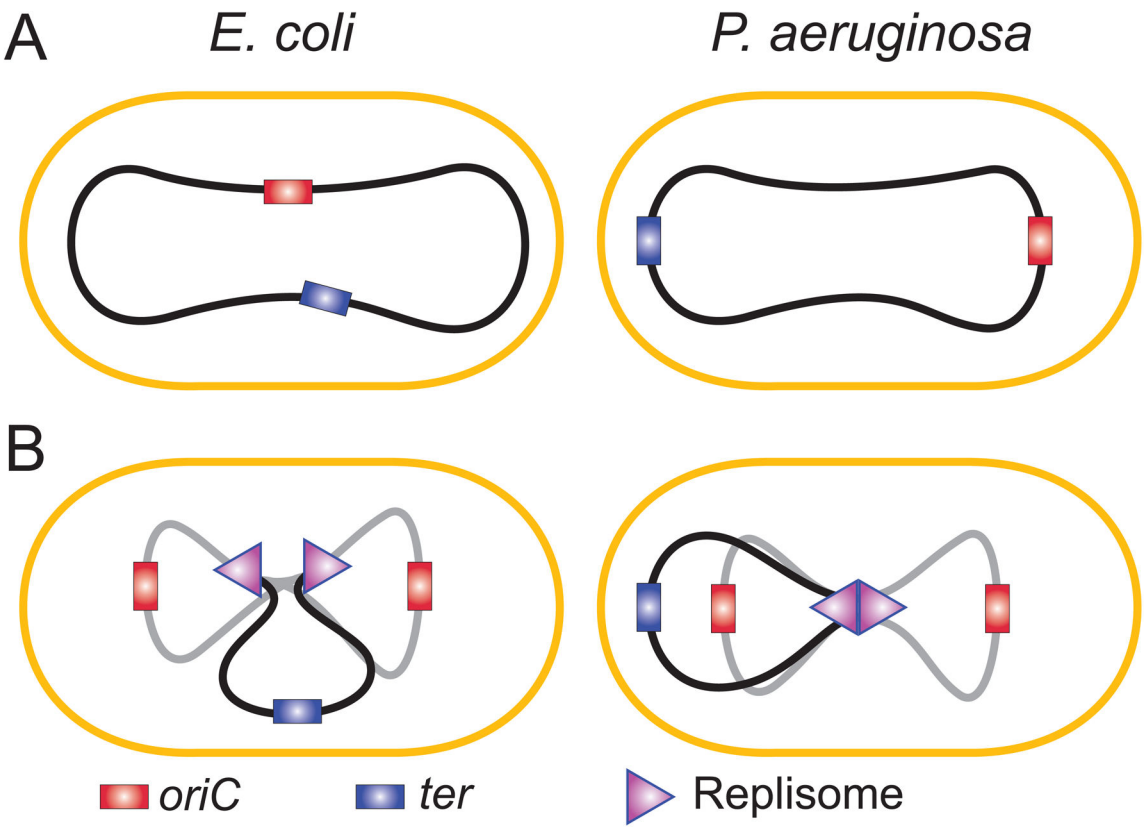

Fig. 3.

Subcellular organization of the chromosome in *E. coli* and *P. aeruginosa* prior to (**A**) or during (**B**) replication. Newly replicated DNA is shown in gray.

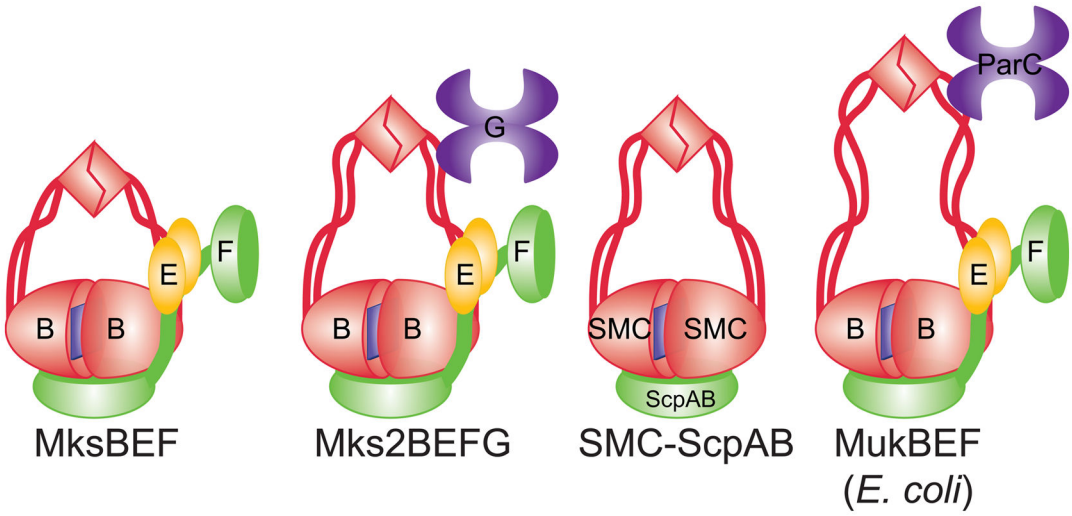

Fig. 4.

Comparison of the *E. coli* and *P. aeruginosa* condensins. The *P. aeruginosa* condensins differ in the length of their coiled coil region, which are all shorter than in MukB. One of the condensins, Mks2BEFG, encodes a Toprim protein MksG and is postulated to form a similar complex with MksB2 as ParC with MukB.

Table I

DNA polymerases in *E. coli* and *P. aeruginosa*

Polymerase	*E. coli*	*P. aeruginosa*
Pol I	PolA	PA5493
Pol II	PolB	PA1886
Pol IIIα	DnaE	PA3640
Pol IV	DinB	PA0923
Pol V	UmuC	none
DnaE2-	none	PA0669

Table II

Condensins in *P. aeruginosa* strains PAO1 and UCBPP_PA14

Condensin	PAO1	UCBPP_PA14
SMC/ScpA/B	PA1527/PA3197/PA3198	PA14_44680/PA14_22840/PA14_22860
MksF/E/B	PA4684/PA4685/PA4686	PA14_61960/PA_61980/PA14_61990
MksF2/E2/B2/G2	none	PA14_03250/PA14_03260/PA14_03270/PA14_03285