MINIREVIEW

Maintenance of chromosome structure in *Pseudomonas aeruginosa*

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Abstract

Replication and segregation of genetic information are the activities 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 eu-bacteria, 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.

Introduction

In the 1970s, Booker and Loutit proposed that *Pseudomonas aeruginosa* strain PAO1 has two chromosomes, based on conjugal 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 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 thrive 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 1), 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 III family and is broadly spread among several subdivisions of eubacteria (Timinskas et al., 2014). DnaE2 is nonessential, 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 nonessential 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 as 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

| Table 1. DNA polymerases in Escherichia coli and Pseudomonas 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           |
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 (oriCI) or three (oriCII) 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 hemE (encoding an uroporphyrin 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 OriC. Indeed, the hemE-oriC-cog1806 fragment is located only 5 kb upstream from the trmE-gi

dAAB*–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. Caulobacter 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 & Marczyński, 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 factor for inversion stimulation (FIS) and integration host factor (IHF) (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 as 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 as 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 fluorescent repressor operator system (FROS) microscopy study revealed a highly coordinated progression of replication forks in PAO1 (Vallet-Gely & Bocard, 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 marshaling 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 QB 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 topoisomerase, which remove the generated topological links (Corbett & Berger, 2004; Wang, 2009). Pseudomonas 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 NAPs, whereas the rest is absorbed by DNA twisting and writhing (Bliska & Cozzarelli, 1987). The effect of supercoiling on DNA activity is twofold. 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 ca. 10 kb stochastically formed topological domains (Postow et al., 2004). As a result, DNA supercoiling is nonuniformly 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, 2012).
In all cases, genomic coordinate of the DNA was found to correlate with its subcellular location. Curiously, two typical arrangements emerged. In nongrowing *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*), that is, 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 structural maintenance of the chromosome (SMC) 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 as 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., 2006; Petrushenko 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 1/4 and 3/4 positions (Ohsumi et al., 2001;
MukBEF based on homology. However, MksBs have shorter coiled-coil than MukB and display low sequence conservation (Fig. 3). Several families of MksBEF2s were identified with barely detectable homology to each other, mostly among outliers, suggesting that the proteins evolved independently.

*Pseudomonas aeruginosa* strain PAO1 encodes two condensins, SMC-ScpAB and MksBEF, and the third condensin MksBEF2 is found in the more virulent strain UCBPP-PA14 (Table 2; Fig. 4). Of note, the correct start codon of mksB2, GUG, is found 168 bp upstream from its predicted position (A. Clevenger and V.V. Rybenkov, 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.

### Table 2. Condensins in *Pseudomonas aeruginosa* strains PAO1 and UCBPP_PA14

<table>
<thead>
<tr>
<th>Condensin</th>
<th>PAO1</th>
<th>UCBPP_PA14</th>
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<tbody>
<tr>
<td>SMC/ScpAB</td>
<td>PA1527/PA3197/PA3198</td>
<td>PA14_44680/PA14_22840/PA14_22860</td>
</tr>
<tr>
<td>MksF/E/B</td>
<td>PA4684/PA4685/PA4686</td>
<td>PA14_61960/PA61980/PA14_61990</td>
</tr>
<tr>
<td>MksF2/E2/B2/G2</td>
<td>None</td>
<td>PA14_03250/PA14_03260/PA14_03270/PA14_03285</td>
</tr>
</tbody>
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![Fig. 4. Comparison of the Escherichia coli and *Pseudomonas aeruginosa* condensins.](https://academic.oup.com/femsle/article-abstract/356/2/154/541847)
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-Burdzcy, 2012)]. This system consists of three elements. ParA protein (known as SpoOJ 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, TGTCCACGTGGAA CA, 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 counterclockwise 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 nonessential 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, as the activity of chromosomally encoded ParABS is integrated into other genome duplication functions. In B. subtilis, 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 transversal; the DNA polymerase stays long at midcell during replication. However, no evidence for polar attachment of the origin has emerged so far.

Pseudomonas 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 of 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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References


for entrance into sporulation, is an inhibitor of DNA replication. EMBO J 23: 3890–3899.


