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review

New genes with old *modus operandi*

The connection between supercoiling and partitioning of DNA in *Escherichia coli*

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The process of partitioning bacterial sister chromosomes into daughter cells seems to be distinct from chromatid segregation during eukaryotic mitosis. In *Escherichia coli*, partitioning starts soon after initiation of replication, when the two newly replicated *oriC*s move from the cell centre to quarter positions within the cell. As replication proceeds, domains of the compact, supercoiled chromosome are locally decondensed ahead of the replication fork. The nascent daughter chromosomes are recondensed and moved apart through the concerted activities of topoisomerases and the SeqA (sequestration) and MukB (chromosome condensation) proteins, all of which modulate nucleoid superhelicity. Thus, genes involved in chromosome topology, once set aside as 'red herrings' in the search for 'true' partition functions, are again recognized as being important for chromosome partitioning in *E. coli*.

Introduction

Our view of chromosome partitioning in bacteria has traditionally been based upon our understanding of mitosis in eukaryotic cells. After completion of replication, duplicated chromosomes are condensed, aligned as pairs in the middle of the cell, and pulled apart by contractile fibres that emanate from centrioles near the two poles of the cell, and attach themselves to specific regions on the chromosomes called centromeres. Intuitively, we have been trying to create models for bacterial chromosome partitioning along similar lines, incorporating modifications that accommodate the bacterial replication and cell cycle characteristics that are distinct from those of eukaryotic cells.

A number of essential differences that must be accounted for in any model of bacterial partitioning include the following. First, in contrast to eukaryotic cells where genome duplication (S) and mitosis (M) are distinctly separated by clear gap periods (G_1 and G_2), bacterial cells undergoing rapid growth may not only abolish the gap G_1 (B-period in the bacterial cell cycle), but may even sustain overlapping rounds of replication on the same genome (Helmstetter, 1996). This would result in continuous chromosome replication, precluding the separation of distinct S and M periods. Bacterial cells must therefore have the ability to undergo mitosis while replication is in progress. Secondly, bacterial replication distinguishes itself from eukaryotic replication in that daughter chromosomes do not undergo concerted condensation after replication is complete. Studies with fluorescence *in situ* hybridization (FISH) and/or green fluorescent protein (GFP) fusions have shown that the chromosomal segments move from origin to terminus (Niki and Hiraga, 1998; Roos *et al.*, 1999; Niki *et al.*, 2000) through the replication machinery localized at mid-cell (Lemon and Grossman, 1998). However, post-replicative condensation has not been detected for whole bacterial chromosomes. Thirdly, bacteria seem to lack a visible spindle apparatus from which contractile fibres could pull the chromosomes apart as during the transition from interphase to prophase for eukaryotic chromatids. Sister chromosomes never even align themselves for post-replicative segregation.

Prokaryotic centromeres

The first evidence for the presence of centromere sites on prokaryotic genomes was obtained for plasmids with active partitioning mechanisms. These include P1, F and R1, all of which possess a *trans*-acting partition factor that binds at a specific sequence on the plasmid genomes and pairs them through interactions among the DNA-bound proteins. The sequences are considered to be eukaryotic centromere analogues (Abeles *et al.*, 1985; Gerdes and Molin, 1986; Mori *et al.*, 1986). Such sites have recently been detected on the *Bacillus subtilis* chromosome near its replication origin (Lin and

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Grossman, 1998), and in *Caulobacter crescentus* (Mohl and Gober, 1997), although the mechanisms of chromosome positioning in these organisms too have yet to be worked out.

No centromere analogue has yet been detected for the *Escherichia coli* chromosome, although the demonstration of active partitioning of plasmids in *E. coli* hosts suggests that they possess the machinery necessary for centromere-mediated segregation. Furthermore, although the *E. coli* chromosome itself has all the characteristics of active partitioning, *oriC* minichromosomes do not exhibit the controlled dynamics of an ordered separation (as visualized by GFP or FISH) unless associated with the partition region from a plasmid such as F or P1 (Niki and Hiraga, 1999). Hence, if a chromosomal centromere is present in *E. coli*, it does not lie close to the replication origin *oriC*, and *oriC* itself cannot function as the centromere analogue. Nevertheless, as in *B. subtilis* and *C. crescentus*, the *E. coli* replication origin displays ordered dynamics during replication and cell division: the single chromosomal *oriC* of the pre-replicative cell moves from its polar location to a mid-cell position just before initiation, and the duplicated *oriC*s move to quarter-length positions during early stages of replication (Niki *et al.*, 2000). The dynamics of chromosomal *oriC* positioning thus appears to be independent of centromere site(s), suggesting the possibility of an alternative mechanism for the movement and anchoring of the chromosome domains.

The search for partition functions

The earliest efforts to identify partition genes in bacteria involved screening for conditional lethal mutations that cause defects in the segregation of bacterial nucleoids, the folded nucleoprotein structure of the *E. coli* genome. A large number of segregation-defective mutants were identified and designated as *par* (partition) mutants. Most of these mapped in genes coding for gyrases or topoisomerases (*parA* = gyrase B, *parD* = gyrase A, *parC* = topoisomerase IVA, *parE* = topoisomerase IVB). With the exception of *parB*, which turned out to be an allele of the DNA primase gene *dnaG* (Norris *et al.*, 1986), most of the Par products were found to be involved in elongation and/or termination of replication through their influence on the superhelicity of the chromosome (Schaechter, 1990). Loss-of-function of *parA* and *parD* resulted in the presence of large nucleoid masses in the middle of filamentous cells, suggesting failure in decatenation of sister chromosomes as the cause of the partition defects (Kato *et al.*, 1990). Similarly, *parC* and *parE* loss-of-function mutants gave rise to cells with unseparated bacterial nucleoids. *In vitro* and *in vivo* investigations showed that ParC and ParE can function as type II topoisomerases capable of relaxing negative and positive supercoils, and of resolving knotted DNA. Conditional lethality caused by mutations in these genes was also associated with incomplete chromosome replication. Thus, the Par$^-$ phenotype actually resulted from defects in replication elongation, replication termination, and/or daughter chromosome resolution by decatenation, all of which were believed to be upstream activities needed to be completed prior to partition. These results were considered unavoidable artifacts that would be generated in any general screen for partition defects. A new genetic approach was therefore thought to be necessary for the identification of genes responsible for positioning of the replicated chromosomes without affecting replication, termination or resolution.

muk genes: positioning nucleoids and plasmids

In the late 1980s, S. Hiraga developed an ingenious genetic screen for the isolation of *par* genes and was successful in identifying a group of positioning mutants termed *muk* (Hiraga *et al.*, 1989). These mutations caused a subpopulation of the cells to produce one anucleate and one diploid daughter cell. The *mukA* gene codes for an outer membrane protein previously known as *tolC* (Hiraga *et al.*, 1989), and *mukB* was a hitherto unknown gene that codes for a very large protein (170 kDa) containing both a DNA-binding domain and a domain with ATPase activity (Niki *et al.*, 1991; Yamanaka *et al.*, 1994). Its native form in solution is dimeric, and the homodimer has a rod and hinge structure similar to some of the motor proteins found in eukaryotic cells (Niki *et al.*, 1992). The two other *muk* genes, *mukE* and *mukF*, code for proteins that form complexes with the MukB protein, and it was believed that the Muk proteins form a spindle analogue (Yamazoe *et al.*, 1999). FISH was used to compare the localization of *oriC* and other chromosomal segments in exponentially or synchronously growing wild-type and *muk* mutant strains. It was shown that the intracellular positions of chromosome segments follow a reproducible pattern during cell cycle progression in wild-type cells, but that this pattern is seriously perturbed in *ΔmukB*::*kan* strains (Niki *et al.*, 2000; Weitao *et al.*, 2000). Nevertheless, plasmid replicons carrying their own partition system were stably maintained in *mukB* hosts (Ezaki *et al.*, 1991; Funnell and Gagnier, 1995) although their positioning in the cell was affected (Weitao *et al.*, 2000).

The MukB$^-$ phenotype: suppression by regulating nucleoid structure

Not all *muk* genes fulfil the expected criteria for 'true' partitioning genes. For example, null mutations in the *mukB* gene caused conditional lethality associated with filamentous cells and irregular nucleoid distribution. Thus, not only the positioning but also the separation of the nucleoids was affected. Nucleoids in cells of a *ΔmukB* strain had lost their position as well as compact shape and were spread out through the interior of the cell (S. Dasgupta, unpublished observation; Gullbrand and Nordström, 2000). They were found to sediment more slowly than those from a wild-type strain in a sucrose gradient. When the isolated nucleoids were stained with DAPI and examined by fluorescence microscopy, they looked flaccid and spread-out rather than compact and rounded as was the case for wild-type nucleoids (Weitao *et al.*, 1999). Clearly, inactivation of the *mukB* gene had a profound effect on the hydrodynamic properties of the nucleoid, consistent with unfolding of the bacterial chromosome. Spontaneously arising suppressors of the temperature-sensitivity phenotype of *mukB*-null mutant strains were isolated and mapped near the *topA* locus (Sawitzke and Austin, 2000). This was the first genetic evidence that the loss of MukB function can be compensated by the loss of a topoisomerase activity, which influences nucleoid structure by altering its average superhelical density. It had already been shown that *mukB* mutation renders cells more sensitive to the gyrase-inhibiting drug novobiocin (Weitao *et al.*, 1999). Also, inactivation of SeqA [a factor involved in sequestration of newly replicated hemi-methylated DNA and in imposition of synchrony in initiation from *oriC* (Crooke, 1995)], which in itself

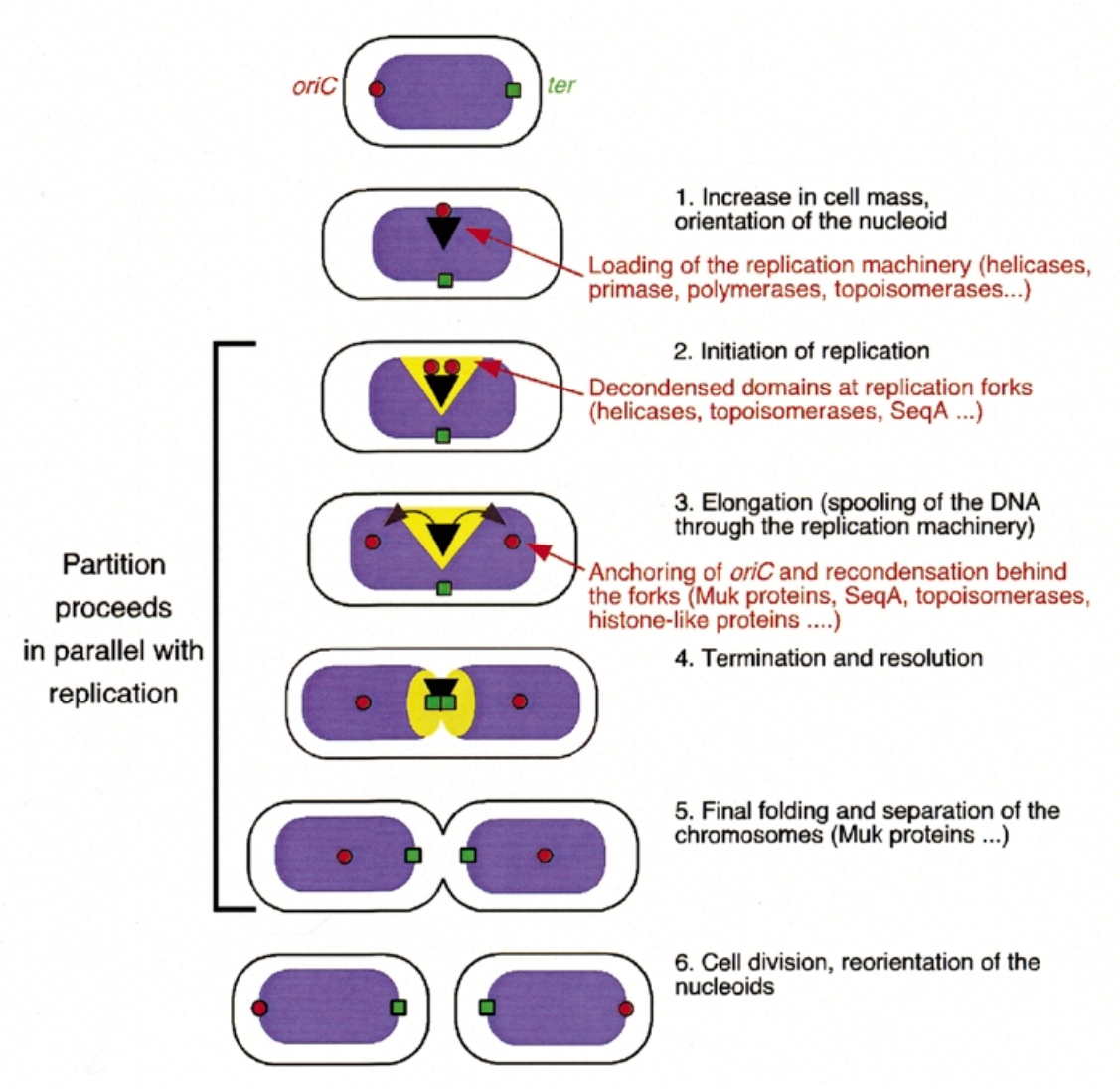

Fig. 1. Model for nucleoid segregation through decondensation/condensation and supercoiling during the *E. coli* cell cycle. Red circles represent the origin of replication (*oriC*), green squares the terminus, and the black triangle the replication machinery. Blue represents the condensed, and yellow the decondensed, domains of the nucleoid or the bacterial chromosome. Refolding and partitioning of the chromosome occur continuously and in parallel with replication. According to this model, DNA decondenses in the vicinity of the replicating segment. Duplicate copies of *oriC* move rapidly apart from the centre of the cell to quarter positions on opposite sides. The replicated DNA behind the fork recondenses and positions itself within the cell.

leads to a significant compaction of *E. coli* nucleoids, suppressed the segregation defects and conditional lethality of *mukB* null mutants (Weitao *et al.*, 1999). Direct measurement of the superhelicity of the bacterial nucleoids in the wild-type strain and its *mukB* and *seqA* derivatives showed that the chromosomes from the mutant strains have lower and higher negative superhelicity, respectively, compared with that of the wild-type strain (T. Weitao, K. Nordström and S. Dasgupta, in preparation). Plasmid pBR322 was used as a probe for the level of supercoiling and it was confirmed that *mukB* and *seqA* alter the general supercoiling potential of the cells. Thus, their effect on nucleoid structure is not necessarily due to direct interaction with the bacterial chromosome. Taken together, these results indicate that phenotypic suppression of *mukB* can be brought about by any activity that leads to condensation of the bacterial nucleoids, and that such condensation can be generated by increased negative superhelicity of the nucleoids. The idea that MukB-mediated condensation might at least in part derive from altered levels of supercoiling has recently gained broader acceptance (Holmes and Cozzarelli, 2000).

Partitioning: condensation and superhelicity

The data discussed above suggest that MukB, through its effect on the superhelicity of *E. coli* nucleoids, might provide the tensile force necessary to condense and partition daughter chromosomes. Structural studies have now shown that MukB is an analogue of the SMC (structural maintenance of chromosomes) protein family whose members are involved in

promoting DNA condensation and chromosome segregation in both eukaryotes and prokaryotes (Britton *et al.*, 1998; Moriya *et al.*, 1998; Jensen and Shapiro, 1999). Dimeric forms of these gigantic coiled-coil proteins, which have DNA-binding domains at each extremity and a centrally located hinge, could be conceived to bind distant segments of DNA (Melby *et al.*, 1998; Hirano, 1999), bringing them closer and folding the chromosome into a condensed state. It is already known that MukB maintains the condensation state of the nucleoids of stationary cells and also of cells in pre-replicative or replication run-out stages (S. Dasgupta, unpublished observations; Gullbrand and Nordström, 2000). During replication, a large number of SeqA molecules (and possibly additional unknown factors) bind co-operatively to the hemimethylated DNA near the centrally located replication machinery behind the replication fork (Hiraga *et al.*, 1998; Onogi *et al.*, 1999). There, they hold the replicating chromosome in an unfolded state, thereby preventing topoisomerase-mediated rewinding (Torheim and Skarstad, 1999) which would result in a tangled mess. As the replicated DNA is remethylated, the SeqA–DNA complex dissociates and MukB can re-establish the folded, condensed state of the newly replicated domain, perhaps in concert with the histone-like proteins (Jaffé *et al.*, 1997). According to this scenario, partitioning does not wait until replication is complete, but starts soon after initiation and continues in parallel with their replication and cell growth, transporting chromosomal domains to their proper intracellular locations in order of their replication (van Helvoort and Woldringh, 1994; Roos *et al.*, 1999; Niki *et al.*, 2000).

While eukaryotic replication (S) and mitosis (M) are clearly separated events, bacterial replication and partition are *parallel* processes. As the compartmentalization of replication and partitioning disappears, so do the distinctions between the classical and 'true' Par functions, including those performed by the Muk genes. All the *par* genes, *muk* genes and *seq* gene(s) work together in parallel to coordinate not only chromosome replication, but also separation and partitioning of sister chromosomes into the daughter cells (Figure 1).

Interestingly, all of these genes seem to modulate the superhelical state of the chromosome directly or indirectly. Hence, prokaryotic partitioning distinguishes itself from its eukaryotic counterpart by not having any external partitioning apparatus that pulls the chromosomes apart. The moving force may come from the torsional energy introduced by negatively supercoiling the bacterial chromosome (Pettijohn, 1996). This feature is absent from the linearized histone-coated DNA complexes that are found in eukaryotic nuclei. It is possible that the evolution of the elaborate mitotic apparatus, complete with spindles and microtubules, occurred concomitantly with the transition of genomes from free-floating, naked DNA to DNA–histone complexes confined within a nuclear membrane. It would be interesting to find organisms where remnants of both partitioning mechanisms co-exist. Some extremophiles are known to maintain their chromosomes in either relaxed or positively supercoiled states (Forterre *et al.*, 1996). Examination of the partitioning mechanisms in such organisms might shed further light on the role of superhelicity in partition.

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