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MiniReview

Structure and partitioning of bacterial DNA: determined by a balance of compaction and expansion forces?

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Abstract

The mechanisms that determine chromosome structure and chromosome partitioning in bacteria are largely unknown. Here we discuss two hypotheses: (i) the structure of the *Escherichia coli* nucleoid is determined by DNA binding proteins and DNA supercoiling, representing a compaction force on the one hand, and by the coupled transcription/translation/translocation of plasma membrane and cell wall proteins, representing an expansion force on the other hand; (ii) the two forces are important for the partitioning process of chromosomes.

Keywords: DNA supercoiling; Nucleoid structure; Coupled transcription/translation; Protein translocation

1. Introduction

In eukaryotic cells DNA is first replicated at multiple origins during the S-phase before the linear chromosomes are partitioned. The final separation occurs with the help of unique centromere DNA sequences in the process of mitosis during which transcription stops. In contrast, the circular chromosome of *Escherichia coli* is replicated bidirectionally from a unique origin, and transcription continues throughout the cell cycle.

Three important properties distinguish bacterial DNA from eukaryotic chromatin: (i) bacterial DNA is associated with relatively little protein; (ii) in bacteria, the processes of transcription and translation can occur simultaneously, as there is no nuclear membrane; (iii) bacteria host a unique enzyme, DNA gyrase, that actively supercoils the DNA at the expense of free energy from ATP hydrolysis. As a result of these properties, the bacterial chromosome constitutes a comparatively open and expanded structure, accessible throughout the cell cycle to DNA-binding proteins, DNA and RNA polymerases and ribosomes [1], but occupies a confined region in the cell centre distinct from the cytoplasmic phase. In bacteria, no mitosis-like phase such as found in higher eukaryotic cells exists in which the chromosomes condense prior to their partitioning.

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2. Compaction forces affecting nucleoid structure

One basic property of the DNA in bacterial nucleoids is a deficit in the linking number of the double helix, which results in a negative superhelical tension (reviewed in [2]). The observation that multiple nicks in the DNA are required to relax supercoils in vitro suggests the presence of some 40 topologically independent domains [3]. Analysis of the degree of superhelicity in vivo suggests that the negative supercoils are in part restrained by histone-like proteins (compare nucleosome structure in eukaryotic chromatin; reviewed in [4]). The superhelical tension is maintained through the combined action of topoisomerases. *E. coli* has been shown to contain four such enzymes: topoisomerase I (encoded by the *topA* gene) and topoisomerase III (*topB*) are type-1 topoisomerases, which change the linking number in steps of 1 by passing DNA segments through transient single-strand breaks; DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*topC* and *topE*) are type-2 topoisomerases, changing the linking number in steps of 2 by passing loops of double-stranded DNA through transient double-strand breaks (reviewed in [5]).

At the expense of ATP, DNA gyrase reduces the linking number even against negative superhelical tension in the DNA molecule. Topoisomerases I, III and IV only relax the superhelical tension. Consequently, *E. coli* DNA is held at a negative superhelical tension that depends on the relative activities of DNA gyrase and topoisomerase I and on the ATP/ADP concentration ratio ([6–9]; Jensen et al., in preparation). Transcription may also affect supercoiling [10,11]. When there is highly active transcription, DNA gyrase activity in front of the transcription complex can lead to additional negative supercoiling. Conversely, topoisomerase I activity behind that complex can lead to reduced negative supercoiling or, in extreme conditions, even to positive supercoiling [12]. The latter two phenomena are strong when membrane protein encoding genes are transcribed or when two operons are antiparallel and close together [13,14]. Under physiological conditions the transcription-independent activities of the topoisomerases appear to control most of the superhelical tension [7,13,15].

The number of times the two strands of a DNA molecule are intertwined (the linking number) equals the number of times the ribbon formed by the two single strands winds around its axis both in terms of its secondary structure (its twisting within the helix structure) and in terms of its tertiary structure (its writhing; the coiling of the helix axis through space). In the latter case the DNA can assume the plectonemic (interwound) or the solenoidal supercoiled form [16]. In *E. coli* most of the DNA is solenoidal, presumably due to interactions with DNA binding proteins such as HU [17]. Absence of the HU protein in *hupA hupB* double mutants of *E. coli* has been observed to lead to an unfolding of the chromosome and to the induction of filamentation [18]. Likewise, another abundant DNA-binding protein, H-NS, has been implicated in compaction of the *E. coli* chromosome [19]. Depending on the concentration of DNA-binding proteins and on the extent of superhelicity, the writhing induced by the superhelical tension may represent a compaction force which folds and concentrates the DNA within the structure of the nucleoid.

3. Expansion forces affecting nucleoid structure

Another property, which we here propose to affect nucleoid structure, is the process of coupled transcription/translation/translocation. Although translocation and translation of membrane proteins are not necessarily coupled in *E. coli*, such coupling does occur, depending on the hydrophobicity of the signal peptide [20]. Significant co-translational translocation may occur in the FtsY-dependent protein export system [21]. Cook et al. [13] and Lynch and Wang [14] obtained evidence that membrane anchoring of DNA (e.g. through *tet* and *pho* genes on plasmid) through coupled transcription/translation/translocation did take place. Due to the lipophilic nature of (parts of) many proteins, some anchoring of nascent protein may occur independently of any signal peptide. The *atp* operon encodes a significant fraction of membrane protein, the F_0 protein, which may already insert into the membrane during synthesis [22]. The location of the *atp* operon close to the origin of replication [22] might then bestow it with a role in chromosome partitioning.

If protein synthesis is inhibited by chloramphenicol, the usually extended nucleoid contracts into a sphere (Fig. 1B; see also [1]). This suggests that in actively growing cells the process of protein synthesis pulls the nucleoid into its elongated and more complicated shapes. This is supported by the observation that, in the absence of DNA replication, the nucleoid continues to be moved in the growing filament and is pulled apart into small domains along the length of the cell. When these cells are then treated with chloramphenicol, the extended complexes still contract into one or two spherical nucleoids [23]. It can thus be envisaged that loops of chromosomal DNA encoding inner membrane or secreted proteins become transiently and indirectly anchored to the plasma membrane through the process of co-translational export [14,21]. As this will occur at multiple points on the chromosome, it may represent an expansion force on the nucleoid that opposes the compaction force through DNA supercoiling (Fig. 2).

4. Nucleoid partitioning

Several authors have recently expressed the view that, in *E. coli*, chromosome partitioning occurs after replication, by a mechanism similar to the one operating at eukaryotic mitosis (reviewed in [24]). However, it is well known that DNA replication in *E. coli* under rapid growth conditions can take place throughout the cell cycle [25]. Additionally, in slowly growing cells, it has been shown that the two daughter nucleoids have structurally separated immediately after termination of DNA replication [26] and that the nucleoid borders move gradually along with the poles of the growing and elongating cell [27]. These observations suggest that the process of partitioning takes place during replication and not after replication as it does in eukaryotes. This implies that the forces which compact or expand the structure of the nucleoid may also operate during partitioning and suggests that these forces may be instrumental to the partition process itself. It can thus be envisaged that,

Fig. 1. Structure of the *E. coli* nucleoid before and after treatment with chloramphenicol. Cells were grown in broth with a doubling time of 30 min (**A**) and (**B**) treated with 50 $\mu g\ ml^{-1}$ chloramphenicol for three generations. Cells were fixed with 2.5% glutaraldehyde and 1% OsO_4 before embedding in Vestopal.

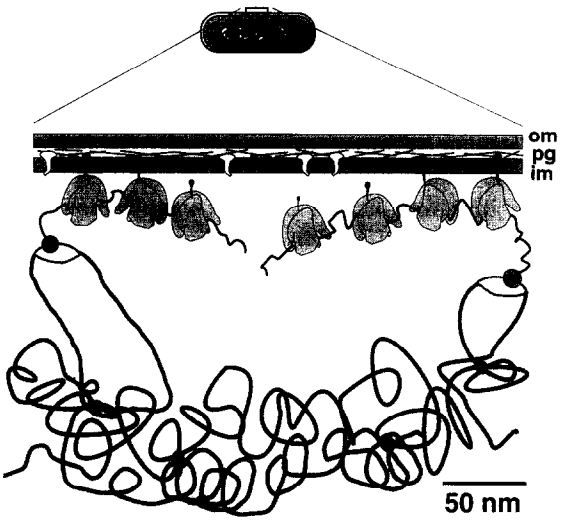

Fig. 2. Schematic representation of co-transcriptional translocation of membrane proteins along a 300-nm stretch of the cell envelope. Dimensions of cell envelope, ribosomes and RNA polymerase were taken from Woldringh and Nanninga [34]. Transmembrane proteins (white), involved in the synthesis of the peptidoglycan layer (pg) between the outer membrane (om) and the cytoplasmic membrane (im), may hinder lateral diffusion of proteins (grey) that are being translocated. These exert a pulling force on supercoiled DNA, held in a solenoidal conformation by DNA binding proteins (black circles). If all envelope proteins would be co-transcriptionally translocated, a rough estimate (see text) shows that a cell would need the continuous action of 1 translocation site per 40×40 nm surface to double its mass.

soon after initiation of a round of replication, the newly replicated daughter strands are already partitioned to their prospective cell halves and that this occurs sequentially for the remainder of the chromosome (as suggested in [28]).

Such continuous partitioning would allow for an important phenomenon that distinguishes the bacterial cell cycle from that of the eukaryotic cell: multifork replication. Through this process, *E. coli* is able to start new rounds of replication, for instance every 20 min, in spite of the 40 min it takes for each fork to finish a round of replication. If multifork replication is coordinated with immediate and continuous partitioning of newly replicated DNA, the unique origins (or sequences nearby) could act as centromere equivalents and are moved gradually apart along with the elongating cell.

What remains necessary in this mechanism is an initial separation of the two daughter origins of replication. It was found that initiation of chromosome replication is inhibited for about 10 min through sequestering of the hemimethylated origin by association with the membrane [29]. However, there is no definitive evidence that the origin of replication itself plays a decisive role in chromosome partitioning. The origin sequence, for instance, has not been found to stabilize plasmid partitioning [30]. A continued separation through attachment of the origins to the membrane and subsequent displacement because of membrane synthesis taking place between the origins in the middle of the cell wall was originally proposed by Jacob et al. [31]. A role of the outer membrane or murein cell wall was also considered [32]. This has become less likely because of the dispersed mode of synthesis of the peptidoglycan layer [33].

As an entirely different mechanism for the initial partitioning, movement in the cytoplasm independent of direct membrane attachments and based on the formation of microcompartments for ribosome assembly has been proposed [34]. In this initial phase, ribosomal RNA genes close to the origin (like *rrnC*) may play a centromeric role by the formation of a nucleolus-like structure which moves the origins apart by enlargement of the ribosomal assembly centres. Alternatively, a motor protein like MukB [35] could be involved in the early partitioning of sister origins.

The possibility that DNA supercoiling affects DNA partitioning makes it relevant to ask whether DNA supercoiling varies during the cell cycle. Recently, DNA supercoiling was observed to be involved in the expression of *nrd*, encoding ribonucleotide reductase [36]. This expression is cell cycle regulated in parallel with the initiation of DNA replication [36].

5. A balance of forces

We propose that during DNA replication the two daughter strands are compacted by supercoiling and by DNA binding proteins and partitioned continuously through a large number of individually transient but collectively persistent anchors to the plasma membrane (Fig. 2). The latter result from coupled transcription–translation and insertion of membrane proteins or translocation of exported proteins. Here it

may be important that some of the transmembrane proteins will be hindered in their two-dimensional membrane diffusion either due to specific interactions with the peptidoglycan layer or by a general squeezing against this wall layer by the turgor pressure. Such hindrance in the movement of membrane proteins may also explain the observation of low diffusion rates of membrane proteins in experiments with prokaryotes [37] and of proteins sequestered in specific regions of the *E. coli* cell membrane. An example or possible reflection of such protein sequestration is the polar deposition of the methyl accepting chemotaxis proteins [38].

It should be emphasized that the individual contacts between DNA and membrane through coupled transcription/translation/translocation will be transient. As synthesis and translocation of an individual polypeptide is finished, other such synthesis and translocation is initiated. At steady state, many such transient contacts will be present: assuming that the cell envelope of a glucose-grown cell represents 15% of the cell volume [34], and that the protein concentration in the envelope is the same as in the cytoplasm, the number of envelope proteins with an average size of 40 kDa will be about 5×10^5. If each of these envelope proteins would be co-transcriptionally translated and translocated in 20 s (16 amino acids per s), the cell will need the continuous action of 4000 translocation sites to double the number of envelope proteins in one generation (45 min). Taking the surface of an average cell to be 6.6 μm^2, there will be one translocation site per 40×40 nm^2 (compare with Fig. 2). Previously, it was estimated that for outer membrane proteins only, the cell would need one translocation site per 60×60 nm^2 [39]. Indeed, it has already been suggested that the insertion of outer membrane proteins might play a role in chromosome segregation [40].

Defects in the process of protein export may impair early nucleoid partitioning as they reduce the expansion force necessary for the movement of the nucleoid. This is supported by the fact that both transport (*secA*; [41]) and secretion (*tolC*; [42]) mutants have been observed to form filaments, and also by the aberrant nucleoid partitioning in *ftsH* mutants depleted for the signal recognition particle of *E. coli* [21], and by the filamentation phenotype of the putative *E. coli* signal recognition particle mutants (*ffh*; [43]). It remains to be determined whether in all these cases the primary cause of filamentation lies in the defect of nucleoid partitioning, since a more specific reason could be the failure to export proteins needed for division, such as PBP3.

Fig. 3 illustrates the forces we propose to be important compaction forces, effected by DNA gyrase and DNA binding proteins, and expansion forces, brought about by coupled transcription–translation–translocation of membrane proteins. In our view, while DNA loops are pulled to the membrane at multiple points, the DNA is compacted by the association with DNA binding proteins and by transcription-driven, gyrase-mediated supercoiling. We conjecture that it is the balance between compaction and expansion forces that determines the size and shape of the nucleoid and controls nucleoid partitioning.

6. Predictions and implications

This hypothesis holds a number of predictions: relative to the wild-type cell (Fig. 4A), chromosomal supercoiling can be increased (Fig. 4B,D) or de-

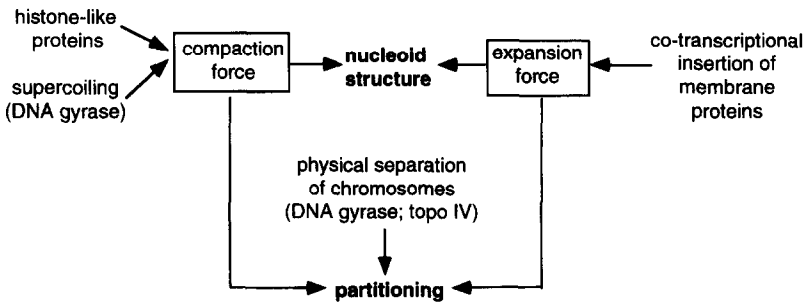

Fig. 3. Diagram illustrating the two forces proposed to determine the structure of the nucleoid and to influence nucleoid partitioning.

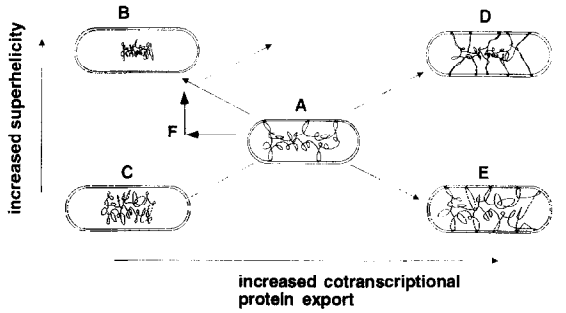

Fig. 4. The wild-type situation (A) and four theoretical states of nucleoid structure as determined by supercoiling and co-translational protein export. Horizontally and vertically, the two relevant driving forces are indicated, i.e., as resulting from coupled transcription translation translocation and from DNA supercoiling respectively. Arrows A → B and A → E indicate the resultant (in the vectorial sense) balanced forces that compact or expand, respectively. The resultant in the direction C-A-D (dashed arrow) indicates improved partitioning. (F) represents a rescue experiment (see text).

creased (Fig. 4C,E) by modulating the energy state of the cell and the activity of DNA gyrase and we predict that the nucleoid structure should change accordingly. Cells growing under conditions of low osmotic stress, which may lead to relaxation of DNA supercoiling (cf. [44]), should exhibit over-expanded nucleoids and postponed partitioning and cell division (in correspondence with unpublished observations). DNA compaction should also be modulated, independent of the activity of DNA gyrase, by varying the expression of histone like DNA binding proteins or the production of topoisomerase I (*topA*). At any state of supercoiling, nucleoid structure should become more confined when inhibiting transcription/translation activity (e.g. with chloramphenicol) or in transport-defective mutants (Fig. 4B,C). In addition, the pulling force resulting from coupled transcription/translation and membrane translocation of proteins should be modulated by the overexpression of membrane proteins (Fig. 4D,E). Partitioning might be obstructed even further by lowering the expression of membrane proteins (Fig. 4C).

It should be noted that although both reduced DNA supercoiling and increased coupled transcription/translation/translocation are proposed to enlarge the nucleoid, they will do so in different ways (Fig. 4). Increased coupled transcription/translation/translocation will lead to a stronger pulling of the nucleoid at more points towards the membrane. When the DNA is fairly relaxed, this should lead to an extended, loose structure of the nucleoid (Fig. 4E). When the DNA is highly supercoiled, the structure should become more tight, with a condensed central part and thin connections to the membrane; because of the expected compaction force, the DNA is both pulled towards the centre and pulled towards the membrane (Fig. 4D). When the coupled transcription/translation/translocation is halted, the latter connections should disappear, the nucleoid condensing into a single ball (Fig. 4B). These different conformations of the nucleoid will exert an effect on chromosome partitioning and either promote or postpone division, thereby changing the shape of the cells.

The proposition that nucleoid structure results from a balance between processes that compact and expand the DNA and that the same processes are responsible for DNA partitioning could be tested by the following rescue experiment: if partitioning would be inhibited by a decreased insertion of membrane proteins as, for instance, in transport mutants, it should be possible to rescue the partition process by adjusting the degree of supercoiling (Fig. 4, arrows at F; increased compaction of less membrane-attached DNA may assist the pulling of the DNA by the membrane). A similar rescue phenomenon has already been observed with plasmids: the inheritance of mini-F (*sop*) plasmids, deficient in their Par function [30,45] and also of minichromosomes [46] has been found to recover or improve by *topA* mutations, which increase DNA superhelicity. Of course, plasmid and chromosome partitioning are so different that different dependencies on the balance of forces should be expected.

7. Outlook

The structure of DNA does not end with the double helix. In the living cell, the double helix is twisted, supercoiled and folded. In part this is due to interaction with DNA binding proteins, in part it derives from active processes that affect DNA topology. Here we have added coupled transcription/translation/translocation to the processes that may affect nucleoid structure. Gene expression is not just

determined by the primary sequence of the DNA, but also by its higher order structure. Accordingly, the hypothesis formulated here may be relevant for the coordination of gene expression with the process of the translocation of membrane and export proteins. The importance of this new type of global regulatory mechanism, remains to be assessed.

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