Chapter 6

DNA organisation and DNA segregation in *Escherichia coli*

Both the gradual-segregation model and the 'sticky-jump' model (chapter 4) intend to give a description of observed FISH data. However, they do not tell us what the mechanism of DNA segregation is. In this chapter I have re-examined some of the factors involved in organising DNA (chapter 1), but now more explicitly with respect to their possible role in DNA segregation (section 6.1). Subsequently, I give my view on how and whether knowledge of DNA segregation in eukaryotes could help us to understand DNA segregation in E. coli (section 6.2).

6.1 Factors involved in DNA segregation

6.1.1 DNA

The double-stranded circular DNA-molecule itself has intrinsic structural properties. For instance, it forms supercoils. Supercoiling promotes compaction of DNA to some degree (Sottas *et al.* 1999), and during replication it promotes compaction of daughter DNA's upon themselves, rather than upon each other or upon unreplicated DNA (Alexandrov *et*

al. 1999). Thus, DNA segregation could be the result, at least in part, of DNA compaction by supercoiling after replication.

Also, the distribution of genes around the chromosome of *E. coli* is non-random (Blattner *et al.* 1997; Pedersen *et al.* 2000; Ussery *et al.* 2000). This suggests that transcriptional activity is non-randomly distributed over the chromosome. In addition to genes, structural properties of DNA, such as DNA curvature and DNA bending are also distributed non-randomly over the chromosome (see section 1.1.4; Pedersen *et al.* 2000). For instance, in *E. coli* the intrinsic structure of a region near *oriC* is clearly different from that of a large region of DNA encompassing the terminus. How this may affect DNA organisation or DNA segregation is not yet clear. Nevertheless, the replisome and the segregation mechanism have to deal with DNA with variable structural properties during replication.

6.1.2 Proteins

6.1.1.1 'Mitotic-like' proteins

The most direct involvement of proteins in DNA segregation is predicted by the 'mitotic-like'-segregation model. This model proposes that specific proteins actively pull replicated DNA apart towards opposite cell poles, in a process reminiscent of chromatid segregation during mitosis in eukaryotes. However, to the best of my knowledge these proteins have not yet been found in bacteria (see also section 6.2.2).

6.1.1.2 DNA polymerase

Is the enzyme that replicates DNA also involved in DNA segregation? If replisomes are specifically localised in the centres of (prospective daughter) cells, and if they are stationary with respect to the DNA they are replicating (section 1.1.5.3), then the replisome positions replicating DNA within the cell during the cell cycle. More specifically, DNA polymerase may be an important factor in the movement of unreplicated

DNA towards the replisome, and, possibly, in giving an initial direction to replicated DNA away from the replisome. It probably does not exert a direct pushing force on DNA over long distances, because when replication is inhibited, but not transcription, DNA is still being moved apart (Woldringh *et al.* 1994).

6.1.1.3 Topo-isomerases

Topo-isomerases are involved in maintaining DNA in a negatively supercoiled state during replication, and in decatenating replicated DNA-molecules during termination of replication (see for instance Kornberg and Baker 1992b). Some topo-isomerases are part of the replication machine (Kornberg and Baker 1992a, table 15-4), and replication cannot proceed without any topo-isomerases. Thus, topo-isomerases are likely to have an effect on DNA segregation via their effect on DNA supercoiling (see section 1.1.5.7).

6.1.1.4 Nucleoid-associated proteins

Through dynamic binding to DNA, nucleoid-associated proteins may influence DNA segregation. This influence could be direct, e.g. by actively pulling or pushing DNA, or indirect, e.g. by compacting DNA after replication. MukB, the SMC equivalent of *E. coli*, co-localises with the nucleoid (fig. 1.6; Den Blaauwen submitted), and may thus serve as an example. It has been suggested that MukB actively moves DNA via a presumed motor function (Lockhart and Kendrick-Jones 1998). However, more recent findings suggest that MukB is involved in DNA segregation because of a role in compacting DNA after replication (Sawitzke and Austin 2000; Weitao *et al.* 2000a; Weitao *et al.* 2000b, or see section 1.1.5.4 or, for a review, see Dasgupta *et al.* 2000; Graumann 2001; Holmes and Cozzarelli 2000).

6.1.1.5 RNA polymerase

Are RNA polymerases involved in DNA segregation? Just as DNA polymerase, RNA polymerase is probably stationary with respect to the

DNA it is transcribing (see section 1.1.5.2). RNA polymerase is a very strong motor protein (Yin *et al.* 1995), 5-6 times stronger than kinesin (force of procession between 25 and 30 pN; Wang *et al.* 1998). However, how could this strength be used to segregate DNA?

In bacteria, whilst RNA polymerase is transcribing a gene, ribosomes already translate the transcript, and nascent translation-products may start moving to their destination. This chain of gene
RNA polymerase transcript ribosomes translation-products may anchor DNA to, for instance, the membrane (Norris 1995; Woldringh *et al.* 1995). Through these anchors, RNA polymerase may be able to move DNA within the cell (Woldringh personal communication). In fact, because cytoplasm is viscous, anchoring to the cytoplasm may have a similar effect. Considering that many RNA polymerases are active in a cell, transcription may be an important driving force behind DNA segregation. However, for segregation DNA has to move in specific directions: replicated DNA away from the replisome, unreplicated DNA towards the replisome. It is not yet clear how directional movement of DNA is accomplished by transcription combined with anchoring of DNA to the membrane or the cytoplasm.

6.1.1.6 Transcription factors and other DNA-binding proteins

Transcription factors and DNA-binding proteins that I did not mention before may transiently alter the structure of DNA regions (e.g. AraC in section 1.1.5.1). They may thus be responsible for a certain amount of DNA movement, especially because they are so plentiful. However, we can probably assume that the net contribution to DNA segregation is negligible, because of a lack in directionality. Nevertheless, it is possible that these movements explain part of the observed variation in FISH data.

6.1.2 Membranes

Membranes may not have been given the proper amount of attention in

this thesis. As any other cellular constituent they influence DNA organisation, at least indirectly. For instance, DNA partitioning is related to cell growth, hence, related to membrane growth. In fact, in combination with co-transcriptional-translation-and-insertion of membrane proteins, membrane-growth may even play an essential part in DNA segregation (see previous section). Another example of an important role of the membrane might be the supposed transient sequestering of DNA near the origin, following initiation of replication. It is assumed that sequestering prevents premature re-initiation of replication (Ogden *et al.* 1988, for a review see Crooke 1995), but it would also link a specific DNA region to a growing membrane. However, to the best of my knowledge, sequestering of *oriC* has not been shown by microscopy.

6.1.3 Towards a model of the mechanism underlying DNA segregation in *E. coli*

In the foregoing, a number of factors (DNA, various proteins, membranes) were presented that might contribute to DNA segregation. If we are to make a model of the mechanism of DNA segregation in *E. coli*, we may assume that it should be based on a combination of these factors. So, what is the relative importance of each of these factors and how do they interact to give rise to DNA segregation?

6.1.3.1 The relative importance of specific factors

Even if the mechanism behind a process is unknown, it is possible to determine the relative importance of a specific factor involved. For instance, using a biochemical method (Groen *et al.* 1982; Westerhoff *et al.* 1998), the 'inherent control' of DNA gyrase on DNA supercoiling was measured to be only 0.2 % in *E. coli* (see section 1.1.5.7 and Jensen *et al.* 1999 for details); homeostatic regulation of DNA supercoiling appears to dominate (Jensen *et al.* 1999). Unfortunately, as far as I know, not many factors involved in DNA segregation have been analysed using these kinds of methods.

6.1.3.2 Interactions between factors

To find out which interactions determine DNA segregation, we may define a putative model and predict what kind of data the model would produce. However, if some of the factors in our model interact non-linearly, this becomes a non-trivial task. In contrast, if a model is based on a few linear equations, it is relatively easy to simulate data and compare the predicted data with, for instance, FISH data (chapter 4 gives examples). However, most biological systems are based on non-linear interactions (Westerhoff *et al.* 1998); we have to assume DNA segregation is no exception.

In some cases, the consequences of a 'non-linear' model can be predicted. For instance, by applying a set of biophysical calculations, Odijk showed that DNA supercoiling and depletion forces in *E. coli* can give rise to the nucleoid as a distinct structure (Odijk 1998). Computer simulation can also be used to predict the consequences of a non-linear model. For instance, Brownian-dynamics simulations have been used to predict both the structure and the dynamics of small DNA molecules, such as plasmids (e.g. Klenin, K. *et al.* 1998; Klenin, K. V. and Langowski 2001; Langowski *et al.* 1999). To the best of my knowledge, these methods have not yet been used specifically to study DNA segregation in bacteria. (A further discussion on the use of computer simulation methods is beyond the scope of this thesis.)

6.1.4 Conclusion

The purpose of this section was to review, briefly, some of the factors that may contribute to DNA segregation in *E. coli*. We may assume that none of these factors is much more important than the others; a combination of factors is probably responsible for DNA segregation. Ideally, we would define a model that incorporates all known factors, and then evaluate it by comparing computer-simulated data with experimental data (cf. chapter 4). However, the foregoing makes clear

that both defining this type of model, as well as predicting what kind of data it produces is difficult. Nevertheless, without such an evaluation, models remain non-committal. Some aspects of DNA segregation may be examined using biophysical or biochemical methods, but I think that, in addition, computer-simulation methods should be used to examine possible mechanisms underlying DNA segregation in *E. coli* (see section 5.3).

6.2 DNA segregation in *E. coli* versus DNA segregation in eukaryotes

6.2.1 Introduction

Several aspects of prokaryotes and eukaryotes may be compared. For instance, bipolarity is a common concept for cell division and DNA segregation in both classes (see Nanninga 2001). At the molecular level, many bacterial components that are presumed to be involved in organising DNA are supposed to be similar to eukaryotic counterparts. For example, some nucleoid-associated proteins, such as HU, are called 'histone-like' (e.g. Jaffe *et al.* 1997; Rouviere-Yaniv *et al.* 1979; Shellman and Pettijohn 1991; Yasuzawa *et al.* 1992; see also section 1.1.5.5), others, such as MukB, are called 'SMC-like' (e.g. Graumann 2001; Melby *et al.* 1998), nucleotide sequences that contain *oriC* or are close to *oriC* are called 'centromere-like' (e.g. Gordon and Wright 2000; Marston and Errington 1999; Sharpe and Errington 1999), the terminus region has been called 'telomere-like' (e.g. Gordon and Wright 2000), and the whole nucleoid has even been called 'nucleosome-like' (e.g. Azam *et al.* 2000). Finally, the process of DNA segregation in bacteria has been called 'mitotic-like' (e.g. Begg and Donachie 1991; Lin *et al.* 1997; Moller-Jensen *et al.* 2000; Niki and Hiraga 1998; Sharpe and Errington 1999; Webb *et al.* 1997; Wheeler and Shapiro 1997). Unfortunately, the use of the additive '-like' does not seem to be bound by any definition. For instance, in the case of proteins and sequences, it is unclear if '-like' implies sequence homology, structural similarity, functional similarity, or a combination of these. I suggest restricting the use of '-like' to those cases where there is

distinct sequence-homology. Perhaps we should make an exception for classes of proteins that have a structural definition, such as the class of SMC proteins (section 1.1.5.4).

6.2.2 Size

I will base my comparison of DNA segregation in prokaryotes and eukaryotes on size, and I will do so briefly, as an in-depth treatment is beyond the scope of this thesis (for a more general comparison between DNA organisation in prokaryotes and eukaryotes see for instance Nanninga 2001; Woldringh and van Driel 1999). On the basis of size, DNA segregation in bacteria and mitosis in eukaryotes are of a different order (fig. 6.1). Although the function of both processes is to partition replicated DNA over two prospective daughter-cells, bacteria move their DNA over less than one micrometer, whilst eukaryotes may move their DNA over more than 5 micrometers. Consequently, the cellular structures and forces required to move the DNA are different. Whereas diffusion may be enough to accomplish DNA segregation in bacteria (chapter 4 and next section), in eukaryotes DNA segregation is achieved through a specialised system consisting of large regions of heterochromatin (centromeres*), large protein complexes attached to centromeres (kinetochores), specific motor proteins and microtubules. The microtubules attach to the centromere via the kinetochore, and with the aid of motor-proteins the chromatids are segregated towards opposite cell poles. The centromere may be several thousands base pairs long, and the size of the centromere/kinetochore structure is about half a micrometer (the structure in figure 6.1 is more or less on scale). This is enormous compared to the size of a bacterium and bacterial DNA.

* I use centromere here to denote the region of heterochromatin that gives rise to the visible constriction in metaphase chromosomes, which is involved in chromatid segregation. The underlying nucleotide sequences differ from species to species or even between chromosomes of one species. They contain a variety of heterochromatic sequences (see for instance Vig. 1994. *Mutat Res* 309: 1-10.).

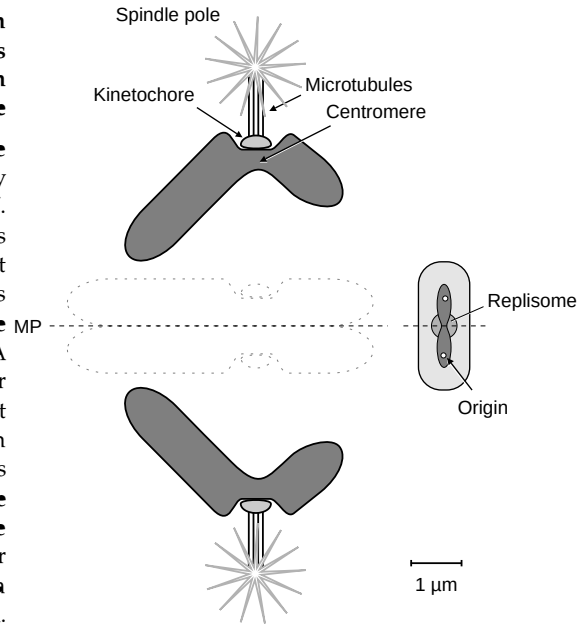


Figure 6.1 - Comparison between DNA segregation in eukaryotes (mitosis) and DNA segregation in *E. coli*, based on size

The sizes are more or less on scale. The eukaryotic chromosome is loosely based on human chromosome 7. During the anaphase stage of mitosis the two chromatids are pulled apart over a distance of about 5 µm towards spindle poles on opposite sides of the cell. The chromatids are copies of DNA molecules that were replicated earlier during *S*-phase and subsequently kept together until mitosis (see text). In *E. coli*, DNA segregates whilst DNA is replicating. Thus, there is no stage during the cell cycle of *E. coli* where chromosomes are held together over their full lengths. Segregation is over a distance less than 1 µm.

Hence, based on size, we cannot expect to find any 'centromere-like' sequences or structures in bacteria. Indeed, typical components of the eukaryotic mitotic-system, such as centromeric DNA-sequences, kinetochores, or microtubules, have not been found in eubacteria.

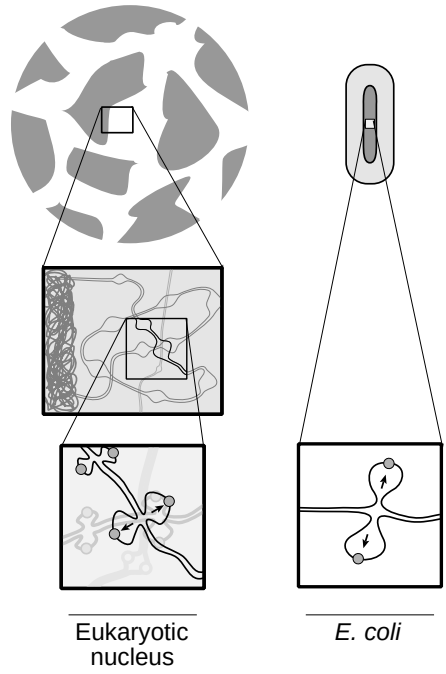
6.2.3 Initial DNA segregation during replication

On the basis of size, a more appropriate comparison may be that between DNA-segregation in bacteria and initial DNA-segregation in eukaryotes during S-phase (fig. 6.2). In both classes of species a complex containing DNA polymerase replicates DNA. Replication initiates from an origin of replication and a replication bubble is created (one per chromosome in bacteria, many per chromosome in eukaryotes). Replicated DNA may initially segregate in opposite directions using a similar mechanism in both cases. In slow-growing bacteria segregation is towards opposite cell

Figure 6.2 -

Comparison of initial segregation of replicated DNA in eukaryotes and *E. coli*

Left: initial segregation of replicated DNA during *S*-phase in eukaryotes; right: segregation of replicated DNA during the *C*-period in *E. coli*. For simplicity, the cartoon depicts a B/r strain with one replisome replicating in the cell centre (cf. Koppes *et al.*, 1999). In both species a complex containing DNA polymerase replicates DNA, thereby forming a replication bubble. Eukaryotes have multiple bubbles per chromosome, because they have multiple origins per chromosome. *E. coli* has one bubble per replicating chromosome, because it has only one origin per chromosome.



poles; in fast-growing bacteria, undergoing multi-fork replication, sister copies do not move towards opposite cell poles, but they may still segregate in opposite directions.

If the above comparisons make sense, the idea that diffusion of DNA is a driving force behind DNA segregation in bacteria may also be applicable to initial DNA segregation in eukaryotes during S-phase. That is, in obtaining a defined distance between sister chromatids. Similarly, the idea that compaction of DNA after replication segregates DNA in bacteria (see section 6.1.2.4; Dasgupta *et al.* 2000; Graumann 2001; Holmes and Cozzarelli 2000 and references therein), may be applicable as well.

Nevertheless, because of important differences at later stages of DNA segregation we should be careful with these comparisons. For instance,

in bacteria sister copies have fully segregated at termination of replication, whilst in eukaryotes, sister copies are cohered together after replication until mitosis (Hirano 2000 and references therein). Cohesion may take place almost immediately after replication. Recent evidence suggests that also in eukaryotes the position of replicating DNA is related to compaction (see section 6.1.2.4 and above). Electron-microscopic data from nuclei from V97 Chinese hamster cells indicated that nascent DNA is positioned in less-dense areas of chromatin, whereas DNA that is not replicating is confined to more-condensed areas of chromatin (Jaunin *et al.* 2000). However, the type of compaction is different in bacteria and eukaryotes. In bacteria, compaction is accomplished mainly through *plectonemic* supercoiling in combination with depletion forces and, presumably, SMC-proteins such as MukB (e.g Holmes and Cozzarelli 2000). In eukaryotes, a much higher level of compaction is achieved through *solenoidal* supercoiling. Histones define this type of structure, although SMC proteins play an important role as part of a protein complex, condensin, that appears to play a central role in chromosome compaction (Hirano 2000 and references therein). Some histones are released during replication, but the majority of histones remain attached (although some biochemical properties of these histones change).

In summary, differences in size, but also differences in some basic properties of DNA-segregation during replication, suggest that prokaryotes and eukaryotes require fundamentally different mechanisms to segregate their DNA.

6.3 Concluding remarks

In chapter 4 we show that two current models, designed to explain FISH data, do not explain all of the variation in our data. From re-examining the assumptions on which these models were based (chapter 5), we concluded that this is so because a number of these assumptions are not met in practice. For instance, the models assume that the position of

DNA is dependent solely on cell length ('a 1-D model'), and that variation between cells, regarding parameters that might influence DNA segregation, is negligible. We might be able to explain more of the variation in our FISH data, if we could define a 3-D model that would allow us to incorporate more variation in these parameters. However, deriving such a model analytically is not a trivial task, and there is little information available about 3-D organisation of DNA in *E. coli*.

Another way to explain DNA segregation is by a model based on factors that organise DNA in the cell (previous sections). An important factor in determining DNA organisation in *E. coli*, put forward in this thesis, is Brownian motion (diffusion) of DNA regions. Our simulations and preliminary data have shown that Brownian motion (i.e. diffusion) within a confined region (cf. Marshall *et al.* 1997) may contribute greatly to the total of observed variation (Roos *et al.* 2001). However, it is not yet clear what its precise function is in positioning DNA during the cell cycle. This is mainly because we were unable to quantify the position and size of the putative confined-regions properly (section 5.2.3).

As a next step, I suggest to test the possibility that diffusion, replication, and cell growth determine how replicated DNA is segregated in *E. coli* (fig. 6.3). I will refer to this model as the 'diffusional-drift' model. Diffusion is the motive force in this model, replication gives initial direction to replicated DNA, and cell growth produces the necessary space for the increasing amount of DNA. Whilst template DNA moves towards the replisome, replicated DNA 'drifts' towards the cell poles as the cell is growing in the length axis of the cell. In addition to these three factors, repulsion between DNA molecules may help in separating the stretches of replicated DNA and the template DNA.

Because it is not immediately clear what mathematical function would represent this model, the method of adding variation to mathematically determined positions, such as described in chapter 4 of this thesis, cannot be applied. An alternative method is to use a Brownian-dynamics procedure. This procedure has already been used successfully to model

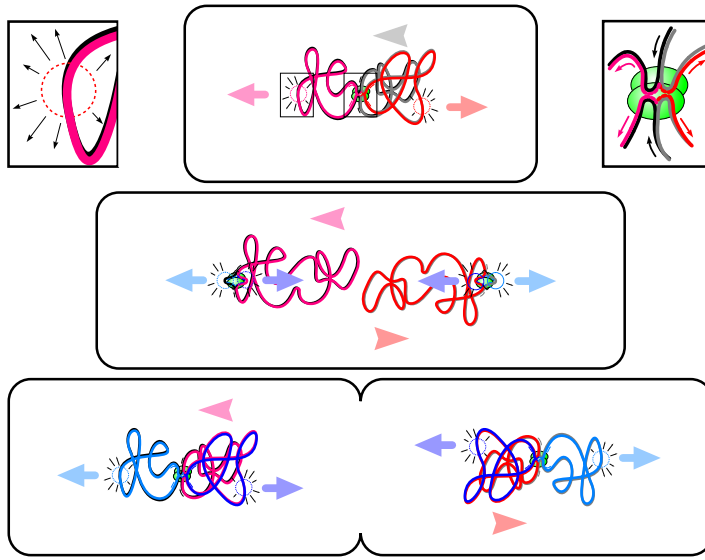


Figure 6.3 - Simplified model of DNA segregation in E. coli based on diffusion and cell growth. The dashed circles indicate the position of an oriC region

Three stages of the cell cycle are depicted. All DNA (the curves) displays Brownian motion (shown for *oriC* by black arrows, see the enlarged image on the left). On average, each *oriC* region moves in the direction of a cell pole (old or new), as the cell is growing (large coloured arrows). In this way segregation of replicated DNA is achieved. Parental DNA moves towards the replisome (large arrowheads). The replisome gives initial direction to daughter strands and pulls parental DNA (arrows in enlarged image on the right). Note that DNA is not drawn on scale and is depicted without twist.

the structure of super helical DNA as a function of salt concentration (in combination with Monte Carlo simulations; Langowski *et al.* 1999), and to model a diffusion-controlled reaction between two DNA regions 470 bp apart on a 2.5 kbp-long supercoiled plasmid (Klenin and Langowski 2001). In these studies, the DNA molecule is represented as a worm-like polyelectrolyte chain. A limited set of parameters is used to define properties, such as bending and twisting elasticity, hydrodynamic diameter, and electrostatic interactions between parts of the molecule (Klenin *et al.* 1998). Shape and dynamics of DNA are further determined by the surrounding fluid, which was represented as a continuous viscous fluid. The simulations in these studies are of DNA up to a few kbp in

length. The whole *E. coli* chromosome is $4.6 * 10^6$ bp long. So, if we are to simulate DNA organisation as a function of replication in *E. coli* within a realistic time period, then we probably need to make some simplifications. For instance, we could use the data of Pedersen to define large-scale structural properties of the DNA molecule (Pedersen *et al.* 2000; section 1.1.4), instead of using the properties of each individual nucleotide.

The process of segregation is a result of the interaction of many factors. One of these is certainly diffusion of DNA regions, as outlined in chapter 4. Presumably, it will not be possible to predict, beforehand, how such a model would result in segregation of replicated DNA, because many (non-linear) interactions are involved. Nevertheless, using sophisticated computer simulation we may be able to gain insight into the process of DNA segregation. For instance, we may ask if the combination of factors that determines DNA segregation in slow growing cells can also explain the more complex DNA movements in fast growing cells. In addition, we may learn about the relationship between DNA segregation, DNA replication and cell division. For instance, we may ask if there is a common principle that positions both replication and cell division at the cell centre, be it at different times. In any case, I think it is important to evaluate such models by comparing simulated data to experimentally obtained data as we did in chapter 4. I see this approach as an important methodological aspect of this thesis. It led us to propose the diffusional-drift model.

6.4 References

Alexandrov, A. I., N. R. Cozzarelli, V. F. Holmes, A. B. Khodursky, B. J. Peter, L. Postow, V. Rybenkov, and A. V. Vologodskii. 1999. Mechanisms of separation of the complementary strands of DNA during replication. *Genetica* 106: 131-140.

Azam, T. A., S. Hiraga, and A. Ishihama. 2000. Two types of localization of the DNA-binding proteins within the *Escherichia coli* nucleoid. *Genes Cells* 5: 613-626.

Begg, K. J., and W. D. Donachie. 1991. Experiments on chromosome separation and positioning in *Escherichia coli*. *New Biol* 3: 475-486.

Blattner, F. R., G. Plunkett, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A.

Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* 277: 1453-1474.

Crooke, E. 1995. Regulation of chromosomal replication in *E. coli*: sequestration and beyond. *Cell* 82: 877-880.

Dasgupta, S., S. Maisnier-Patin, and K. Nordstrom. 2000. New genes with old modus operandi. The connection between supercoiling and partitioning of DNA in *Escherichia coli*. *EMBO Rep* 1: 323-327.

Den Blaauwen, T. submitted. *Mol Microbiol*.

Gordon, G. S., and A. Wright. 2000. DNA segregation in bacteria. *Annu Rev Microbiol* 54: 681-708.

Graumann, P. L. 2001. SMC proteins in bacteria: Condensation motors for chromosome segregation? *Biochimie* 83: 53-59.

Groen, A. K., R. J. Wanders, H. V. Westerhoff, R. van der Meer, and J. M. Tager. 1982. Quantification of the contribution of various steps to the control of mitochondrial respiration. *J Biol Chem* 257: 2754-2757.

Hirano, T. 2000. Chromosome cohesion, condensation, and separation. *Annu Rev Biochem* 69: 115-144.

Holmes, V. F., and N. R. Cozzarelli. 2000. Closing the ring: links between SMC proteins and chromosome partitioning, condensation, and supercoiling. *Proc Natl Acad Sci U S A* 97: 1322-1324.

Huls, P. G., N. O. Vischer, and C. L. Woldringh. 1999. Delayed nucleoid segregation in *Escherichia coli*. *Mol Microbiol* 33: 959-970.

Jaffe, A., D. Vinella, and R. D'Ari. 1997. The *Escherichia coli* histone-like protein HU affects DNA initiation, chromosome partitioning via MukB, and cell division via MinCDE. *J Bacteriol* 179: 3494-3499.

Jaunin, F., A. E. Visser, D. Cmarko, J. A. Aten, and S. Fakan. 2000. Fine structural in situ analysis of nascent DNA movement following DNA replication. *Exp Cell Res* 260: 313-323.

Jensen, P. R., C. C. Van Der Weijden, L. B. Jensen, H. V. Westerhoff, and J. L. Snoep. 1999. Extensive regulation compromises the extent to which DNA gyrase controls DNA supercoiling and growth rate of *Escherichia coli*. *Eur J Biochem* 266: 865-877.

Kido, M., K. Yamanaka, T. Mitani, H. Niki, T. Ogura, and S. Hiraga. 1996. RNase E polypeptides lacking a carboxyl-terminal half suppress a *mukB* mutation in *Escherichia coli*. *J Bacteriol* 178: 3917-3925.

Klenin, K., H. Merlitz, and J. Langowski. 1998. A Brownian dynamics program for the simulation of linear and circular DNA and other wormlike chain polyelectrolytes. *Biophys J* 74: 780-788.

Klenin, K. V., and J. Langowski. 2001. Diffusion-controlled intrachain reactions of supercoiled DNA: Brownian Dynamics simulations. *Biophys J* 80: 69-74.

Kornberg, A., and T. A. Baker. 1992a. Replication mechanisms and operations. Pages 471-510. *DNA replication*. W.H. Freeman, New York.

...1992b. Topoisomerases. Pages 379-402. *DNA replication*. W.H. Freeman, New York.

Langowski, J., M. Hammermann, K. Klenin, R. May, and K. Toth. 1999. Superhelical DNA studied by solution scattering and computer models. *Genetica* 106: 49-55.

Lin, D. C., P. A. Levin, and A. D. Grossman. 1997. Bipolar localization of a chromosome partition protein in *Bacillus subtilis*. *Proc Natl Acad Sci U S A* 94: 4721-4726.

Lockhart, A., and J. Kendrick-Jones. 1998. Nucleotide-dependent interaction of the N-terminal domain of MukB with microtubules. *J Struct Biol* 124: 303-310.

Marshall, W. F., A. Straight, J. F. Marko, J. Swedlow, A. Dernburg, A. Belmont, A. W. Murray, D. A. Agard, and J. W. Sedat. 1997. Interphase chromosomes undergo constrained diffusional motion in living cells. *Curr Biol* 7: 930-939.

Marston, A. L., and J. Errington. 1999. Dynamic movement of the ParA-like Soj protein of *B. subtilis* and its dual role in nucleoid organization and developmental regulation. *Mol Cell* 4: 673-682.

Melby, T. E., C. N. Ciampaglio, G. Briscoe, and H. P. Erickson. 1998. The symmetrical structure of structural maintenance of chromosomes (SMC) and MukB proteins: long, antiparallel coiled coils, folded at a flexible hinge. *J Cell Biol* 142: 1595-1604.

Moller-Jensen, J., R. B. Jensen, and K. Gerdes. 2000. Plasmid and chromosome segregation in prokaryotes. *Trends Microbiol* 8: 313-320.

Nanninga, N. 2001. Cytokinesis in pro- and eukaryotes: common principles and different solutions. *Microbiol Mol Biol Rev*: in press.

Niki, H., and S. Hiraga. 1998. Polar localization of the replication origin and terminus in *Escherichia coli* nucleoids during chromosome partitioning. *Genes Dev.* 12: 1036-1045.

Norris, V. 1995. Hypothesis: chromosome separation in *Escherichia coli* involves autocatalytic gene expression, transertion and membrane-domain formation. *Mol Microbiol* 16: 1051-1057.

Odijk, T. 1998. Osmotic compaction of supercoiled DNA into a bacterial nucleoid. *Biophys Chem* 73: 23-29.

Ogden, G. B., M. J. Pratt, and M. Schaechter. 1988. The replicative origin of the *E. coli* chromosome binds to cell membranes only when hemimethylated. *Cell* 54: 127-135.

Pedersen, A. G., L. J. Jensen, S. Brunak, H. H. Staerfeldt, and D. W. Ussery. 2000. A DNA structural atlas for *Escherichia coli*. *J Mol Biol* 299: 907-930.

Roos, M., R. Lingeman, C. L. Woldringh, and N. Nanninga. 2001. Experiments on movement of DNA regions in Escherichia colievaluated by computer simulation. *Biochimie* 83: 67-74.

Rouviere-Yaniv, J., M. Yaniv, and J. E. Germond. 1979. *E. coli* DNA binding protein HU forms nucleosomelike structure with circular double-stranded DNA. *Cell* 17: 265-274.

Sawitzke, J. A., and S. Austin. 2000. Suppression of chromosome segregation defects of *Escherichia coli muk* mutants by mutations in topoisomerase I. *Proc Natl Acad Sci U S A* 97: 1671-1676.

Sharpe, M. E., and J. Errington. 1999. Upheaval in the bacterial nucleoid. An active chromosome segregation mechanism. *Trends Genet.* 15: 70-74.

Shellman, V. L., and D. E. Pettijohn. 1991. Introduction of proteins into living bacterial cells: distribution of labeled HU protein in *Escherichia coli*. *J Bacteriol* 173: 3047-3059.

Sottas, P. E., E. Larquet, A. Stasiak, and J. Dubochet. 1999. Brownian dynamics simulation of DNA condensation. *Biophys J* 77: 1858-1870.

Ussery, D., H. H. Stærfeldt, and L. J. Jensen. 2000. DNA structural analysis of sequenced prokaryotic genomes. http://www.cbs.dtu.dk/services/GenomeAtlas/

Vig, B. K. 1994. Do specific nucleotide bases constitute the centromere? *Mutat Res* 309: 1-10.

Wang, M. D., M. J. Schnitzer, H. Yin, R. Landick, J. Gelles, and S. M. Block. 1998. Force and velocity measured for single molecules of RNA polymerase. *Science* 282: 902-907.

Webb, C. D., A. Teleman, S. Gordon, A. Straight, A. Belmont, D. C. Lin, A. D. Grossman, A. Wright, and R. Losick. 1997. Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. *Cell* 88: 667-674.

Weitao, T., S. Dasgupta, and K. Nordstrom. 2000a. Role of the *mukB* gene in chromosome and plasmid partition in *Escherichia coli*. *Mol Microbiol* 38: 392-400.

Weitao, T., K. Nordstrom, and S. Dasgupta. 2000b. *Escherichia coli* cell cycle control genes affect chromosome superhelicity. *EMBO Rep* 1: 494-499.

Westerhoff, H. V., P. R. Jensen, J. L. Snoep, and B. N. Kholodenko. 1998. Thermodynamics of complexity: The live cell. *Thermochimica Acta* 309: 111-120.

Wheeler, R. T., and L. Shapiro. 1997. Bacterial chromosome segregation: is there a mitotic apparatus? *Cell* 88: 577-579.

Woldringh, C. L., P. R. Jensen, and H. V. Westerhoff. 1995. Structure and partitioning of bacterial DNA: determined by a balance of compaction and expansion forces? *FEMS Microbiol Lett* 131: 235-242.

Woldringh, C. L., and R. van Driel. 1999. The eukaryotic perspective: Similarities and distinctions between pro- and eukaryotes. Pages 77-90 in Charlebois, R. L., ed. *Organization of the prokaryotic genome*. ASM Press, Washington, D.C.

Woldringh, C. L., A. Zaritsky, and N. B. Grover. 1994. Nucleoid partitioning and the division plane in *Escherichia coli*. *J Bacteriol* 176: 6030-6038.

Yasuzawa, K., N. Hayashi, N. Goshima, K. Kohno, F. Imamoto, and Y. Kano. 1992. Histone-like proteins are required for cell growth and constraint of supercoils in DNA. *Gene* 122: 9-15.

Yin, H., M. D. Wang, K. Svoboda, R. Landick, S. M. Block, and J. Gelles. 1995. Transcription against an applied force. *Science* 270: 1653-1657.