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DNA supercoiling by gyrase is linked to nucleoid compaction

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Abstract

The genes of *E. coli* are located on a circular chromosome of 4.6 million basepairs. This 1.6 mm long molecule is compressed into a nucleoid to fit inside the 1-2 μm cell in a functional format. To examine the role of DNA supercoiling as nucleoid compaction force we modulated the activity of DNA gyrase by electronic, genetic, and chemical means. A model based on physical properties of DNA and other cell components predicts that relaxation of supercoiling expands the nucleoid. Nucleoid size did not increase after reduction of DNA gyrase activity by genetic or chemical means, but nucleoids did expand upon chemical inhibition of gyrase in chloramphenicol-treated cells, indicating that supercoiling may help to compress the genome.

Introduction

Bacteria compact their DNA without the aid of nucleosomes. In the compression model proposed in Figure 1A, compaction by macromolecular crowding, DNA-binding proteins, and supercoiling by DNA gyrase (Woldringh, Jensen & Westerhoff, 1995; Woldringh & Odijk, 1999) counteracts expansion by coupled transcription, translation, and protein translocation (transertion) (Norris, 1995; Woldringh, Jensen & Westerhoff, 1995).

Macromolecular crowding compacts isolated nucleoids (Cunha, Woldringh & Odijk, 2001). Supercoiling makes DNA more compact and increases mobility on agarose gels (e.g. Bates & Maxwell, 1993). Whether supercoiling compacts nucleoids *in vivo* is the topic of this paper. Several proteins may affect nucleoid structure. The DNA-binding protein H-NS compacts DNA *in vitro* (Dame, Wyman & Goosen, 2000), and overexpression condenses nucleoids inside *E. coli* (Spurio et al., 1992). Transertion counteracts the compaction forces by transiently linking DNA to the plasma membrane, which may explain why nucleoids contract upon inhibition of transcription or translation (Dworsky, 1973; Van Helvoort, Kool & Woldringh, 1996; Woldringh, 2002).

The compaction forces are described in a mathematical model (Odijk, 1998). We calculated DNA supercoiling versus nucleoid compaction, modulated supercoiling in live *E. coli* cells experimentally, and found that supercoiling can act as a nucleoid compaction force *in vivo*.

Methods

Growth of *E. coli* PJ4271 and its derivative PJ4273 (in which a lacZ promoter drives DNA gyrase expression), tuneable induction and measurement of gyrase expression by Western blotting, and determination of supercoiling from Southern blots of chloroquin-containing agarose gels have been described (Jensen et al. 1999). Both strains contain plasmid pBR322 as supercoiling reporter.

Chloramphenicol (0.2 g/l) and coumermycin A1 (0.1 g/l) were applied for 10 minutes, nucleoids were stained by adding 0.05 μg/ml 4',6-diamino-2-phenylindole to cells 10-15 minutes before harvesting.

Cells were spread on microscope slides coated with 1% agarose in growth medium. Phase-contrast and fluorescence microscopy images were captured and processed with Object-Image 2.08 (Vischer et al., 1999), available at http://simon.bio.uva.nl/object-image.html.

Results and discussion

The nucleoid compaction forces outlined in Figure 1A are included in a mathematical description that links physical properties of DNA, protein, and other cell components to the volume of the nucleoid (Odijk, 1998). Figure 1B shows a slightly modified version of that model. DNA-binding proteins partially relax writhe so that the linking difference σ under normal physiological conditions (σ=-0.06) (Bates & Maxwell, 1993) is reduced to an effective linking difference σ=-0.06-(-0.35)=-0.025. The term G accounts for the presence of more than one genome per nucleoid. In our experiments, the average DNA content per nucleoid was about 1.5 chromosomes. The predicted nucleoid volume as a function of the linking number is plotted in Figure 1C. From the maximum linking number (σ=-0.07) to the minimum (σ=-0.03) attainable *in vivo*, the nucleoids are expected to expand considerably.

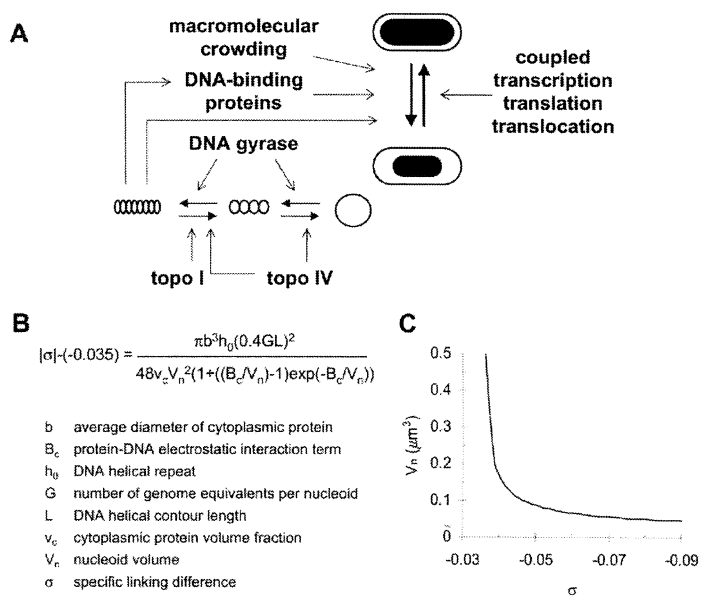

Figure 1. *A. Model to link DNA supercoiling and other compaction and expansion forces to nucleoid size. topo, DNA topoisomerase.* ***B.*** *Mathematical model of nucleoid compaction. Derivation and other details in Odijk (1998).* ***C.*** *Nucleoid size versus supercoiling calculated from the equation in B.*

We developed an electronic ruler to measure compactness from fluorescence microscope images. Because nucleoids have no sharp boundary, their volumes cannot be determined precisely. To estimate their size, we calculated volumes from the length and width of ellipsoids that contained 75% of total nucleoid fluorescence. A similar method successfully quantified the impact of macromolecular crowding on nucleoid compaction *in vitro* (Cunha, Woldringh & Odijk, 2001). Nucleoid volumes were normalized by setting the average volume at normal gyrase activity at 100%. Growth rate, cell size, and average DNA content per nucleoid were similar at all gyrase induction levels used (not shown).

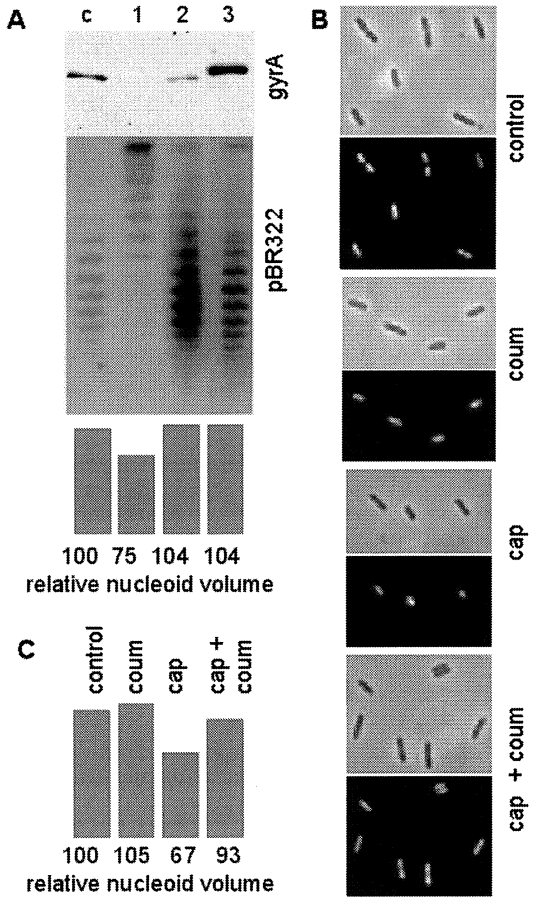

***Figure** 2. A. DNA gyrase expression levels (Western blot with anti-gyrase A (gyrA, top), topoisomer distributions (middle), and relative average nucleoid volumes determined from fluorescence microscope images (bottom) in strain PJ4271 (control, c) and PJ4273 at increasing DNA gyrase induction levels (1, 2, 3). **B.** Phase-contrast (top) and fluorescence (bottom) images of strain PJ4271 treated with chloramphenicol (cap) and coumermycin (coum). **C.** Relative average nucleoid volumes after treatment with cap and coum.*

DNA gyrase supercoils both chromosomal and plasmid DNA. Titration of the enzyme by genetic means (Fig. 2A, top) resulted in various levels of supercoiling of a reporter plasmid, with more negatively supercoiled species running faster on the gel shown in Figure 2A (middle). In contrast with the prediction of our model, nucleoids in cells overexpressing gyrase were not smaller than those in wild type cells, and nucleoids in cells with highly relaxed DNA were smaller instead of larger (Fig. 2A, bottom). The unexpected response of nucleoid volume to supercoiling is possibly due to supercoiling-dependent reprogramming of expression of DNA-binding proteins. Expression of Fis is supercoiling-dependent (Schneider, Travers & Muskhelishvili, 2000), and this is possibly a feature of other DNA-binding proteins as well. To avoid large changes in cellular protein composition we inhibited DNA gyrase with coumermycin to relax supercoiling rapidly. Incubation with coumermycin did not increase nucleoid volumes much (Fig. 2B,C). Bigger nucleoids should leave less space for the surrounding cytoplasm. The backpressure of macromolecular crowding may be too strong to allow expansion. Therefore we inhibited gyrase after making the nucleoids smaller. Inhibition of transertion by chloramphenicol reduced nucleoids to two-thirds of their original volume, but combined with inhibition of DNA gyrase they were almost the same size as in untreated cells (Fig. 2B,C).

Thus, the compaction force of supercoiling can be observed *in vivo* if it is not obscured by other forces acting on nucleoid compactness. For full understanding and a complete model of the nucleoid compression format all forces that contribute to nucleoid size should be quantified.

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References

Bates, A.D. & Maxwell, A., DNA topology, IRL Press, Oxford, UK, 1999.

Cunha, S., Woldringh, C.L. & Odijk, T., Polymer-mediated compaction and internal dynamics of isolated Escherichia coli nucleoids. J Struct Biol, 136 (2001) 53-66.

Dame, R.T., Wyman, C. & Goosen, N., H-NS mediated compaction of DNA visualised by atomic force microscopy. Nucleic Acids Res, 28 (2000) 3504-3510.

Dworsky, P., Einfluß von inhibitoren der protein- und nucleinsäuresynthese auf die gestalt des nucleoids von Escherichia coli. Z Allg Mikrobiologie, 14 (1973) 3-28.

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

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

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

Schneider, R., Travers, A. & Muskhelishvili, G., The expression of the Escherichia coli fis gene is strongly dependent on the superhelical density of DNA. Mol Microbiol, 38 (2000) 167-175.

Spurio, R., Durrenberger, M., Falconi, M., La Teana, A., Pon, C.L. & Gualerzi, C.O., Lethal overproduction of the Escherichia coli nucleoid protein H-NS: ultramicroscopic and molecular autopsy. Mol Gen Genet, 231 (1992) 201-211.

Van Helvoort, J.M.L.M., Kool, J., & Woldringh, C.L., Chloramphenicol causes fusion of separated nucleoids in Escherichia coli K-12 cells and filaments. J Bacteriol, 178 (1996) 4289-4293.

Vischer, N.O., Huls, P.G., Ghauharali, R.I., Brakenhoff, G.J., Nanninga, N. & Woldringh, C.L., Image cytometric method for quantifying the relative amount of DNA in bacterial nucleoids using Escherichia coli. J Microsc, 196 (1999) 61-68.

Woldringh, C.L., The role of co-transcriptional translation and protein translocation (transertion) in bacterial chromosome segregation. Mol Microbiol, (2002) in press

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

Woldringh, C.L. & Odijk, T., Structure of DNA within the bacterial cell: physics and physiology. In Charlebois, R.L. (ed.) Organization of the prokaryotic genome. ASM Press, Washington DC, 1999. pp. 171-187.