DNA Supercoiling and Genomic Sequence Organization

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The bacterial growth cycle was described as a closed, self-reproducing, or autopoietic circuit, reestablishing the physiological state of stationary cells initially inoculated in the growth medium. In batch culture, this process of self-reproduction is associated with the gradual decline in available metabolic energy and corresponding change in the physiological state of the population as a function of “travelled distance” along the autopoietic path. This directional alteration of cell physiology is both reflected in and supported by sequential gene expression along the chromosomal OriC-Ter axis. During the E. coli growth cycle, the spatiotemporal order of gene expression is established by coupling the temporal gradient of supercoiling energy to the spatial gradient of DNA thermodynamic stability along the chromosomal OriC-Ter axis.

bacterial growth cycle growth phase-dependent gene expression DNA supercoiling

1. Coupling of Chromosomal Gene Order and Transcription

The continuous information underpinning the operation of the bacterial growth cycle is apparently of a hereditary nature and, therefore, it must be encoded (either itself or its mechanism of production) in the cellular genome. In order to vary concertedly with bacterial growth, this information also has to be dynamic. The genetic information encoded in the form of unique genes is of a discontinuous or digital nature and is static. Therefore, it cannot readily satisfy the demands of continuity and dynamics. However, during the *E. coli* growth cycle the genes, especially those encoding the major regulators of growth and adaptation, are expressed in temporal succession corresponding to their spatial order along the chromosomal replication origin-to-terminus (OriC-Ter) axis. The latter was shown to serve as a coordinate system for genetic regulation [1][2]. Thus, although the underlying gene order is static, the gene expression is subject to control by a dynamic, continuous variable determining the sequential, chromosomal position-dependent gene expression pattern. Researchers argue that the continuous variable governing the patterns of gene expression at the most fundamental level is the changing genomic distribution of the effective DNA superhelicity [3], defined as the torsional energy available for the untwisting of gene promoters and transcription initiation.

The bacterial chromosome (aka nucleoid) has been proposed to behave as a “smart polymer”, capable of undergoing large conformational transitions (e.g., reversible collapse) in response to small changes in environmental factors such as pH, temperature, or ionic strength [4]. Furthermore, the nucleoid was shown to undergo cycles of compaction-decompaction under the manipulation of crowding conditions [5], in line with the proposed switch-like conformational transition model for chromatin folding [6]. The regulatory role for “pre-

programmed" phase transitions [4] was suggested by observations indicating that the nucleoid structure and gene expression are interdependent [1][7][8][9][10][11]. However, the tight coupling of chromosome structural transitions with gene expression dynamics appears at variance with the notion of gene expression as a fundamentally "noisy" stochastic process [12]. The deterministic average gene expression pattern observed at the cell population level emerges gradually from noisy single-cell expressions [13]. Yet, even though the gene expression patterns in individual bacterial cells might appear stochastic, the chromosomal gene order is not. Furthermore, temporally the gene transcription is correlated with the gene order along the OriC-Ter axis at the population level [7][14] and with the timing of gene replication at a single-cell level (see below).

2. Coupling of DNA Structure to Function Using Two Types of DNA Information

The CODOs ("coherent domains of gene expression" denoted formerly as "functional domains") [7] emerge as growth condition-dependent, transient, spatially extended gene expression patterns. These spatially confined patterns are thought to manifest the structural-functional organization of the bacterial genome. In *D. dadantii,* the CODOs comprising thermodynamically variable DNA sequences are associated with distinct genetic traits; in other words, the CODOs, which emerge in various constellations depending on the applied stress, integrate the chromosomal transcriptional response to the stress-induced peculiar changes of supercoiling with the expression of stress-specific virulence and adaptation genes [15][16][17]. This coupling of the DNA physicochemical properties and the supercoiling response with particular genetic function within the CODOs, underscores once more the role of genomic sequence organization (i.e., the spatially ordered distribution of DNA thermodynamic stability) in coordinating the global transcriptional response. Also, in *Streptococcus pneumoniae* the sequence composition of chromosomal domains harboring the distinct adaptation and virulence traits was found to be determinative for peculiar supercoiling response [18][19], again underscoring the role of genomic sequence organization in coordinating the bacterial genetic response. In *E. coli*, this strategic coupling of the supercoiling response and the genetic function in genomic sequence organization is made conspicuous in the selective enrichment of the relatively G/C-rich OriC and relatively A/T-rich Ter chromosomal poles for anabolic and catabolic genes, respectively [20][21]. More compellingly, the bacterial genomic sequence organization reveals how during the growth cycle the environmentally determined availability of superhelical energy can be fittingly coupled to a genetic adaptive response. While in *E. coli* this directional supercoiling response is encoded in and enabled by the peculiar genomic sequence organization along the OriC-Ter axis, in other examined bacteria (especially in pathogens having a relatively complex lifestyle) the genomic organization patterns may be more nuanced yet represent variations on a common theme [16][17]. As mentioned above, in general, both the local and global transcriptional responses to supercoiling vary, with relatively G/C-rich and G/C-poor sequences responding to high and low levels of negative superhelicity, respectively [22][23].

The patterns of the sequence-dependent supercoiling responses of genomic transcription demonstrate that in addition to static genetic information (digital code), the chromosomal DNA polymer also provides dynamic information manifested in the variable distribution of the available DNA torque. The latter determines the

organization of functionally meaningful spatial transcript patterns in the genome. Essentially, this dynamic information depends on the distinct thermodynamic stabilities of the consecutive DNA base steps, which overlap and thus provide information of a continuous or analogue type [21][24]. The DNA analogue information encoded in various arrangements of base steps stabilizing distinct DNA conformations facilitates the binding of proteins involved in DNA transactions including transcriptional control [25][26][27][28]. More specifically, the genome encodes both the amino acid sequences of DNA-binding proteins and enzymes governing DNA transactions, as well as the dynamic, supercoiling-dependent structures, serving as recognition signatures recruiting these enzymes and DNA-binding proteins to particular genomic loci [20]. This notion is consistent with the proposed evolutionary "multiplexing" of DNA genetic and structural information into the same molecular context [29]. Thus, by operating with these two—discontinuous (digital) and continuous (analogue)—types of information interwoven in the very same genomic sequence, the DNA appears to communicate with itself. Researchers propose that this "self-communication" represents the global feedback mechanism endowing the growing cell population with the capacity to monitor its status quo, a capacity that is reflected, in part, in the interdependence of chromosome structural dynamics and spatial patterns of gene expression [1][10][11].

3. Coupling of DNA Topology, NAP Binding Effects, and Holoenzyme Sigma-Factor Composition: Major Regulatory Events during the *E. coli* Growth Cycle

On nutritional shift-up, the expression of the *gyrA* and *gyrB* genes encoding DNA gyrase—an enzyme introducing negative supercoils into the DNA in an ATP-dependent manner,—increases [30][31] concomitantly with an increase in the ATP/ADP ratio [32][33][34]. It is likely that at the chromosomal OriC pole negative superhelicity rapidly attains high densities of σ~−0.07 to −0.08, consistent with both the enrichment of the OriC pole for the gyrase binding sites and the preferential binding of gyrase downstream of the strongly transcribed genes organized predominantly around OriC including the exceptionally strong rRNA operons [35][36]. Increased negative superhelicity both facilitates replication initiation and strongly activates the OriC-proximal *fis* gene expression [37][38][39], whereas the accumulation of FIS in turn maintains activated ribosomal RNA transcription [40]. Structurally, the increase in negative superhelicity leads to a branching of plectonemically coiled DNA, thus multiplying the tightly bent apical loops and facilitating the wrapping of DNA by the RNAP σ^{70} holoenzyme [41][42][43][44], which prefers highly supercoiled templates for transcription [45]. Notably, the RNAP σ^{70} holoenzyme demonstrates an OriC-Ter gradient of binding site frequency distribution correlating with that of the gyrase binding sites [1]. Concomitantly with activation of the *fis* gene, the expression of the *rpoZ* gene encoding the RpoZ (ω)subunit of RNA polymerase is also strongly increased [20][31]. RpoZ stabilizes the polymerase σ^{70} holoenzyme assembly [46] and is also directly involved in mediating the response to the "alarmone" nucleotide ppGpp [47][48]. FIS activates the *hupA* gene [49] such that at the early growth stage, the high levels of negative superhelicity and the RNAP σ^{70} holoenzyme coexist with increased levels of the two major "early" NAPs—FIS and HUα. High FIS levels boost rRNA synthesis, whereas HU supports the organization of rRNA operons in "transcription factories" engaging hundreds of RNAP molecules [35][50]. The latter effect requires high levels of negative superhelicity [51]. Accordingly, both FIS and HU stabilize negative supercoils [52][53][54].

On transition to the stationary phase associated with a shortage of resources, the rapid increase in ppGpp levels (ppGpp spike) decreases both rRNA transcription and the supercoil density around OriC, thus precluding replication initiation [55]. The *gyr*A/*B* gene expression and the gyrase levels subside [30] and the total superhelicity drops to σ~−0.03. The down-regulation of the chromosomal OriC pole at this stage is likely supported by the binding of the global repressor H-NS and the "late" NAPs such as Lrp and Dps [1][56], whereas the concomitant activation of the Ter pole is attributable to the accumulation of the RNAPσ^S holoenzyme and IHF, the binding sites for both of which are strongly enriched around the chromosomal Ter pole [1]. Notably, the "late" NAPs as well as the RNAPσ^S holoenzyme, preferentially bind relaxed DNA [20]. Thus, in the stationary phase, the RNAPσ^S holoenzyme responsible for the maintenance function predominates, whereas the globally relaxed DNA is organized by the binding effects of three abundant NAPs including IHF, which stabilizes planar bends without the substantial constraints of negative superhelicity, Lrp, which constrains positive supercoils, and Dps, which packages the relaxed DNA in a protective crystalline lattice [57][58].

What happens to the nucleoids of cells enriched for these abundant stationary-phase NAPs on nutritional shift-up? After nutritional shift-up, the compaction state of the nucleoid undergoes marked alterations adopting a more open conformation [59], presumably due to competition between the early and late growth stage NAPs, respectively, FIS and Dps [56][60]. The DNA binding of IHF and Dps was shown to depend on environmental factors [61]. Under conditions of the increase in DNA negative superhelicity after nutritional shift-up, both IHF and Dps likely start to dissociate from genomic binding sites, perhaps accelerated in part by "facilitated dissociation"—a general mechanism thought to modulate gene expression by assisting in the local removal of DNA binding proteins from cognate sites [62][63][64]. However, IHF remains stably bound at the chromosomal origin of replication, its dissociation being prevented by the replication initiator protein DnaA cooperating with IHF in initiating chromosomal replication [65]. This latter process is facilitated by FIS protein produced at high levels on nutritional shift-up [66].

4. Genomic Transcription during the Bacterial Growth Cycle Is Steered by Supercoil Energy

Changes in DNA supercoiling are assumed to mediate the transmission of environmental changes to the chromosome, responding not only to the altered availability of metabolic resources during the growth cycle [67] but also to the various stress factors including suboptimal oxygen tension, temperature, and osmolarity [32][68][69][70]. This mediation by supercoiling is due to the capacity of the DNA double helix to sense the environmental conditions and to respond by adjusting accordingly the superhelical density on the one hand, and by channeling the available superhelical energy into corresponding gene expression patterns on the other. During the bacterial growth cycle, changes in DNA topology modulate the binding of DNA architectural proteins and the activity of transcription machinery concertedly, resulting in orchestrated directional alterations of chromatin architecture and gene expression. Several lines of evidence are consistent with this notion.

First, the continuous alteration of DNA superhelical density during the growth cycle is dependent on the availability of nutritional resources [67]. As in the progression of the population along the growth cycle, the superhelical density decreases, the tightness of apical loops and DNA interwindings also decreases, and so the accessibility to the RNA

polymerase changes. As mentioned above, from the early exponential phase to the early stationary phase dominated by the RNAPσ^{70} holoenzyme, the number of total superhelical turns per 1 kb would reduce from ~8 to ~3, with a reduction in unconstrained turns likely to be greater and hence, the available DNA superhelical torque lower. Under these conditions, the RNAPσ^{S} holoenzyme preferring relaxed DNA substrates for transcription becomes active [45][71]. At the early growth stage the RpoZ-dependent stabilization of the RNAPσ^{70} holoenzyme facilitates the utilization of negatively supercoiled templates, whereas the lack of RpoZ leads to a global DNA relaxation and an increased activity of the RNAPσ^{S} holoenzyme, switching the global transcription preferences to the utilization of the relaxed DNA templates [72]. Importantly, the impact of accumulated σ^{S} could not be detected until the superhelical density of the reporter plasmids subsided to relatively low levels (ΔLk of ~3–4 between the late exponential phase and the early stationary phase, where the σ^{S} impact was observed). It was thus inferred that the variations in DNA supercoiling as a function of the growth phase act as a checkpoint, precluding the shift from the RNAPσ^{70} to the RNAPσ^{S} transcriptional machinery until the growth conditions become unfavorable enough to cause entry into the stationary phase [73].

Second, the superhelical density and the supercoiling response of the genomic transcription change coordinately both during the growth cycle of *E. coli* [74] and during the circadian cycle of gene expression in cyanobacteria. A combination of topological analyses with transcriptomics data in *Synechococcus elongatus* suggested that each topological state corresponded to a unique state of gene expression, indicating that supercoiling plays a primary role in regulating circadian gene expression [75]. Notably, the DNA sequence characteristics of genes monotonically activated and repressed by chromosomal relaxation during the circadian cycle were similar to those of the supercoiling-responsive genes in *E. coli*.

Third, the sequential activation of sets of primary and downstream regulatory genes was observed in response to the long-term supercoiling imbalance achieved by modulating the *topA* gene expression in *Streptomyces coelicolor*, whereby increased negative superhelicity modified the levels of topoisomerases and NAPs coordinately [76]. Another relevant observation comes from experimental evolution studies, which identified mutations in genes encoding the NAPs and topoisomerases. These mutations induced inheritable adaptive changes of supercoiling and also provided fitness gains, thus revealing the pivotal role of NAPs and topoisomerases in organizing the global transcription program during adaptation [77][78].

Fourth, supercoiling can impose directionality by rendering the structural transitions in DNA both deterministic [79] and coordinated [80][81][82][83], whereas the nucleoprotein structures stabilized by NAPs have been implicated in the directional channeling of torsional energy toward the transcription initiation sites [84][85][86]. Available data indicate that the NAP-dependent alterations of gene expression during the growth cycle involve directional and coordinated transitions in the composition of regulatory nucleoprotein structures associated with gene promoter regions [87][88][89][90]. These directional transitions of nucleoprotein complexes associated with individual gene promoters are paralleled by the coordinated redistribution of transcription machinery during the growth cycle at the global scale of the entire chromosome [51][91].

Finally, it is noteworthy that the regulation of gene transcription by supercoil energy is also modulated by gene organization in the genome. The processes of DNA supercoiling and transcription are interdependent [92] and in addition to the specific regulation of gene promoters, global transcriptional responses to changes in DNA supercoiling depend on constraints imposed by the local orientation of genes and the supercoil diffusion induced by the transcription process itself [93][94][95][96]. Ongoing transcription generates negative and positive supercoils, respectively, upstream and downstream of the translocating RNAP and this transcription-coupled diffusion of supercoils (TCDS) modulates the activity of neighbouring gene promoters. The TCDS effect exerted on a particular gene depends on the mutual orientation (either convergent, divergent, or tandem) of its surrounding transcription units. For example, a gene embedded between two divergently oriented transcription units will experience high levels of negative superhelicity (negative TCDS), and vice versa in the case of flanking convergent units, high positive superhelicity (positive TCDS). Although the transcriptional regulation shaped by local genomic architecture can be modulated by changes in global supercoiling and by NAPs [97], these local constraints provide additional means for fine-tuning the supercoiling-dependent impacts on gene expression during the growth cycle. In particular, the measurement of TCDS during the growth cycle of *E. coli* shows that the genes responding to high negative superhelicity (*hyp* genes) also experience negative TCDS from their neighbors, whereas the genes responding to DNA relaxation (*rel* genes) experience positive TCDS. TCDS varies noticeably with growth time and chromosomal region. These observations are fully consistent with the notion of local genomic architecture providing additional means for modulating and fine-tuning the effects of changing DNA superhelicity on gene expression during the bacterial growth cycle.

5. Spatially Shifting Superhelicity Optimum Determines the Temporal Gene Expression

There is little doubt that supercoiling regulates global gene expression, but this by itself does not explain how the spatiotemporal order of gene expression is established. The latter has been correlated with gene replication [98] and thus with the cell cycle, but what about the gene expression order during the growth cycle of the bacterial population?

It is conceivable that the genomic sequence organization, in conjunction with the temporal gradient of supercoiling, determines the spatiotemporal gene expression order during the bacterial growth cycle. As mentioned above, the genomic sequence of *E. coli*, and Gamma-proteobacteria in general, demonstrates a gradient of DNA thermodynamic stability (approximated by G/C-richness) along the OriC-Ter axis of the chromosome, whereas in general, the relatively more A/T-rich and more G/C-rich sequences respond to low and high levels of superhelicity, respectively [22][23][99].

In particular, the G/C composition of the promoter discriminator sequence (the sequence between the -10 promoter element and the transcription startpoint) was shown to be determinative for the supercoiling response [99][100]. Individual gene promoters for which the supercoiling response optima were studied in detail, are shown in **Table 1**. Although the sample of supercoiling-sensitive promoters provided in **Table 1** is far from comprehensive, it shows a trend suggesting that the closer the promoter is located to OriC, the higher the optimum superhelical density for its

transcription, consistent with the gradual decline in the average DNA thermodynamic stability (~G/C content) as a function of distance from OriC. This general trend—a positive correlation between the transcriptional response to high negative superhelicity, high negative melting energy content (~G/C-richness), and proximity to OriC of the genes—is corroborated by time-resolved analyses of global genomic transcription during the growth cycle.

Table 1. Optimal superhelical density for promoter activity as a function of distance from OriC (OriC at 3.92 Mbp; Ter at 1.59 Mbp).

Gene Promoter	Distance from OriC (bp)	Condition	Optimal * Superhelical Density (σ)	#References
hisR	62,500	In vitro	~−0.08 to −0.1	[101]
rrnAP1	120,000	In vitro	~−0.076	[102]
fis	508,500	In vitro & in vivo	~−0.07-to −0.08	[38]
tyrT	1,967,600	In vitro	~−0.05–0.06	[100]
osmE	2,097,900	In vitro	~−0.03–0.04	[73]

roceeding directionally from OriC toward Ter imposes a supercoiling asymmetry with higher negative superhelicity accumulated at the OriC pole of the chromosome [1][35]. At the early growth stage under conditions of high negative superhelical density, the OriC proximal G/C-rich sequences are likely to be transcribed optimally. However, it is conceivable that the gradual decrease in the global superhelical density during the growth cycle [67] would shift the optimum of the promoter supercoiling response toward genomic regions with lower average G/C content and hence, away from OriC toward Ter. Thus, with the passage of time and the associated decrease in global negative superhelicity, progressively more OriC-distal genes with lower G/C content and lower optima of superhelical density for transcription would become maximally active. Researchers propose that the coupling of the temporal growth-phase-dependent gradient of chromosomal DNA supercoiling with the spatial gradient of genomic DNA thermodynamic stability along the chromosomal OriC-Ter axis acts as a timing chain determining the spatiotemporal order of gene transcription during the bacterial growth cycle.

6. The Temporal Gradient of Superhelicity Reflects the Gradient of Ion Composition and Intracellular pH

The temporal gradient of superhelicity apparent during the growth cycle and its correlation with the energy requirement for promoter opening, suggest that both these effects are a response to changes in energy availability. A primary response to shift-up is an almost immediate doubling of the intracellular energy charge from the stationary phase level [103]. On a similar time scale, there is a rapid influx of K^+ mediated by the proton-dependent K^+ transporting P-type ATPase accompanied by an efflux of H^+ and Na^+ resulting in an excess of K^+ [104]. The net effect of these changes in the ion composition is an increase in intracellular pH [105]. The change in energy charge could also promote DNA gyrase activity [33]. During the subsequent growth cycle, the relative concentrations of K^+

and Na^+ are rebalanced [104] whereas the energy charge falls from the onset of the stationary phase [103], and the intracellular H^+ concentration increases [106]. The observed changes in the intracellular ion composition during the growth cycle are likely relevant to transcriptional regulation and DNA compaction. The efficient in vitro transcription of supercoiled DNA is K^+ (and not Na^+) dependent [107], whereas during the stationary phase the relative DNA binding of the major NAPs, IHF and Dps, depends on the pH, K^+, and Mg^{++} concentrations, with that of Dps being favored by low pH and low $[K^+]$ [61]. Researchers suggest that the temporal gradient of superhelicity reflects a corresponding temporal gradient in the ion composition and intracellular pH, which in turn would influence energy availability via the F_0F_1 ATPase.

References

1. Sobetzko, P.; Travers, A.; Muskhelishvili, G. Gene order and chromosome dynamics coordinate spatiotemporal gene expression during the bacterial growth cycle. Proc. Natl. Acad. Sci. USA 2012, 109, E42–E50.
2. Kosmidis, K.; Jablonski, K.P.; Muskhelishvili, G.; Hütt, M.-T. Chromosomal origin of replication coordinates logically distinct types of bacterial genetic regulation. NPJ Syst. Biol. Appl. 2020, 6, 1–9.
3. Travers, A.; Muskhelishvili, G. DNA supercoiling—A global transcriptional regulator for enterobacterial growth? Nat. Rev. Microbiol. 2005, 3, 157–169.
4. Scolari, V.F.; Sclavi, B.; Cosentino Lagomarsino, M. The nucleoid as a smart polymer. Front. Microbiol. 2015, 6, 424.
5. Pelletier, J.; Halvorsen, K.; Ha, B.Y.; Paparcone, R.; Sandler, S.J.; Woldringh, C.L.; Wong, W.P.; Jun, S. Physical manipulation of the Escherichia coli chromosome reveals its soft nature. Proc. Natl. Acad. Sci. USA 2012, 109, E2649–E2656.
6. Barbieri, M.; Chotalia, M.; Fraser, J.; Lavitas, L.M.; Dostie, J.; Pombo, A.; Nicodemi, M. Complexity of chromatin folding is captured by the strings and binders switch model. Proc. Natl. Acad. Sci. USA 2012, 109, 16173–16178.
7. Sobetzko, P.; Glinkowska, M.; Travers, A.; Muskhelishvili, G. DNA thermodynamic stability and supercoil dynamics determine the gene expression program during the bacterial growth cycle. Mol. BioSyst. 2013, 9, 1643–1651.
8. Benza, V.G.; Bassetti, B.; Dorfman, K.D.; Scolari, V.F.; Bromek, K.; Cicuta, P.; Lagomarsino, M.C. Physical descriptions of the bacterial nucleoid at large scales, and their biological implications. Rep. Prog. Phys. 2012, 75, 076602.
9. Travers, A.A.; Muskhelishvili, G. DNA thermodynamics shape chromosome organization and topology. Biochem. Soc. Trans. 2013, 41, 548–553.

10. Verma, S.C.; Qian, Z.; Adhya, S.L. Architecture of the Escherichia coli nucleoid. PLoS Genet. 2019, 15, e1008456.
11. Amemiya, H.M.; Schroeder, J.; Freddolino, P.L. Nucleoid-associated proteins shape chromatin structure and transcriptional regulation across the bacterial kingdom. Transcription 2021, 12, 182–218.
12. Raj, A.; van Oudenaarden, A. Nature, nurture, or chance: Stochastic gene expression and its consequences. Cell 2008, 135, 216–226.
13. Piras, V.; Selvarajoo, K. The reduction of gene expression variability from single cells to populations follows simple statistical laws. Genomics 2015, 105, 137–144.
14. Brinza, L.; Calevro, F.; Charles, H. Genomic analysis of the regulatory elements and links with intrinsic DNA structural properties in the shrunken genome of Buchnera. BMC Genom. 2013, 14, 73.
15. Jiang, X.; Sobetzko, P.; Nasser, W.; Reverchon, S.; Muskhelishvili, G. Chromosomal “stress-response” domains govern the spatiotemporal expression of the bacterial virulence program. mBio 2015, 6, e00353-15.
16. Muskhelishvili, G.; Forquet, R.; Reverchon, S.; Meyer, S.; Nasser, W. Coherent Domains of Transcription Coordinate Gene Expression During Bacterial Growth and Adaptation. Microorganisms 2019, 7, 694.
17. Meyer, S.; Reverchon, S.; Nasser, W.; Muskhelishvili, G. Chromosomal organization of transcription: In a nutshell. Curr.Genet. 2017, 64, 555–565.
18. De la Campa, A.G.; Ferrándiz, M.J.; Martín-Galiano, A.J.; García, M.T.; Tirado-Vélez, J.M. The Transcriptome of Streptococcus pneumoniae Induced by Local and Global Changes in Supercoiling. Front. Microbiol. 2017, 8, 1447.
19. Martín-Galiano, A.J.; Ferrándiz, M.J.; de la Campa, A.G. Bridging Chromosomal Architecture and Pathophysiology of Streptococcus pneumoniae. Genome Biol Evol. 2017, 9, 350–361.
20. Travers, A.; Muskhelishvili, G. Chromosomal Organization and Regulation of Genetic Function in Escherichia coli Integrates the DNA Analog and Digital Information. EcoSal Plus 2020, 9.
21. Nigatu, D.; Henkel, W.; Sobetzko, P.; Muskhelishvili, G. Relationship between digital information and thermodynamic stability in bacterial genomes. EURASIP J. Bioinform. Syst. Biol. 2016, 2016, 4.
22. Muskhelishvili, G.; Sobetzko, P.; Mehandziska, S.; Travers, A. Composition of Transcription Machinery and Its Crosstalk with Nucleoid-Associated Proteins and Global Transcription Factors. Biomolecules 2021, 11, 924.

23. Peter, B.J.; Arsuaga, J.; Breier, A.M.; Khodursky, A.B.; Brown, P.O.; Cozzarelli, N.R. Genomic transcriptional response to loss of chromosomal supercoiling in Escherichia coli. Genome Biol. 2004, 5, R87.
24. Travers, A.A.; Muskhelishvili, G.; Thompson, J.M. DNA information: From digital code to analogue structure. Philos. Trans. A Math. Phys. Eng. Sci. 2012, 370, 2960–2986.
25. Whitson, P.A.; Hsieh, W.T.; Wells, R.D.; Matthews, K.S. Supercoiling facilitates lac operator-repressor-pseudooperator interactions. J. Biol. Chem. 1987, 262, 4943–4946.
26. Higgins, N.P.; Collier, D.A.; Kilpatrick, M.W.; Krause, H.M. Supercoiling and integration host factor change the DNA conformation and alter the flow of convergent transcription in phage Mu. J. Biol. Chem. 1989, 264, 3035–3042.
27. Pontiggia, A.; Negri, A.; Beltrame, M.; Bianchi, M.E. Protein HU binds specifically to kinked DNA. Mol. Microbiol. 1993, 7, 343–350.
28. Wei, J.; Czapla, L.; Grosner, M.A.; Swigon, D.; Olson, W.K. DNA topology confers sequence specificity to nonspecific architectural proteins. Proc. Natl. Acad. Sci. USA 2014, 111, 16742–16747.
29. Babbitt, G.A.; Schulze, K.V. Codons support the maintenance of intrinsic DNA polymer flexibility over evolutionary timescales. Genome Biol. Evol. 2012, 4, 954–965.
30. Schneider, R.; Travers, A.; Kutateladze, T.; Muskhelishvili, G. A DNA architectural protein couples cellular physiology and DNA topology in Escherichia Coli. Mol. Microbiol. 1999, 34, 953–964.
31. Sobetzko, P.; Glinkowska, M.; Muskhelishvili, G. GSE65244: Temporal Gene Expression in Escherichia coli. Gene Expression Omnibus. 2017. Available online: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65244 (accessed on 20 June 2021).
32. Hsieh, L.S.; Burger, R.M.; Drlica, K. Bacterial DNA supercoiling and /. Changes associated with a transition to anaerobic growth. J. Mol. Biol. 1991, 219, 443–450.
33. Van Workum, M.; Dooren, S.J.M.; Oldenburg, N.; Molenaar, D.; Jensen, P.R.; Snoep, J.L.; Westerhoff, H. DNA supercoiling depends on the phosphorylation potential in Escherichia coli. Mol. Microbiol. 1996, 20, 351–360.
34. Snoep, J.L.; van der Weijden, C.C.; Andersen, H.W.; Westerhoff, H.V.; Jensen, P.R. DNA supercoiling in Escherichia coli is under tight and subtle homeostatic control, involving gene-expression and metabolic regulation of both topoisomerase I and DNA gyrase. Eur. J. Biochem. 2002, 269, 1662–1669.
35. Berger, M.; Farcas, A.; Geertz, M.; Zhelyazkova, P.; Brix, K.; Travers, A.; Muskhelishvili, G. Coordination of genomic structure and transcription by the main bacterial nucleoid-associated protein HU. EMBO Rep. 2010, 11, 59–64.

36. Sutormin, D.; Rubanova, N.; Logacheva, M.; Ghilarov, D.; Severinov, K. Single-nucleotide-resolution mapping of DNA gyrase cleavage sites across the Escherichia coli genome. Nucleic Acids Res. 2018, 47, 1373–1388.
37. Ninnemann, O.; Koch, C.; Kahmann, R. The E. coli fis promoter is subject to stringent control and autoregulation. EMBO J. 1992, 11, 1075–1083.
38. 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. 2000, 38, 167–175.
39. Ball, C.A.; Osuna, R.; Ferguson, K.C.; Johnson, R.C. Dramatic changes in Fis levels upon nutrient upshift in Escherichia Coli. J. Bacteriol. 1992, 174, 8043–8056.
40. Zhang, X.; Bremer, H. Effects of Fis on ribosome synthesis and activity and on rRNA promoter activities in Escherichia Coli. J. Mol. Biol. 1996, 259, 27–40.
41. Maurer, S.; Fritz, J.; Muskhelishvili, G.; Travers, A. RNA polymerase and an activator form discrete subcomplexes in a transcription initiation complex. EMBO J. 2006, 25, 3784–3790.
42. Bednar, J.; Furrer, P.; Stasiak, A.; Dubochet, J.; Egelman, E.H.; Bates, A.D. The twist, writhe and overall shape of supercoiled DNA change during counterion-induced transition from a loosely to a tightly interwound superhelix. Possible implications for DNA structure in vivo. J. Mol. Biol. 1994, 235, 825–847.
43. ten Heggeler-Bordier, B.; Wahli, W.; Adrian, M.; Stasiak, A.; Dubochet, J. The apical localization of transcribing RNA polymerases on supercoiled DNA prevents their rotation around the template. EMBO J. 1992, 11, 667–672.
44. Rivetti, C.; Guthold, M.; Bustamante, C. Wrapping of DNA around the E. coli RNA polymerase open promoter complex. EMBO J. 1999, 18, 4464–4475.
45. Kusano, S.; Ding, Q.; Fujita, N.; Ishihama, A. Promoter selectivity of Escherichia coli RNA polymerase Eσ70 and Eσ38 Holoenzymes. Effect of DNA supercoiling. J. Biol. Chem. 1996, 271, 1998–2004.
46. Minakhin, L.; Bhagat, S.; Brunning, A.; Campbell, E.A.; Darst, S.A.; Ebright, R.H.; Severinov, K. Bacterial RNA polymerase subunit omega and eukaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly. Proc. Natl. Acad. Sci. USA 2001, 98, 892–897.
47. Zuo, Y.; Wang, Y.; Steitz, T.A. The mechanism of E. coli RNA polymerase regulation by ppGpp is suggested by the structure of their complex. Mol. Cell 2013, 50, 430–436.
48. Gentry, D.; Xiao, H.; Burgess, R.; Cashel, M. The omega subunit of Escherichia coli K-12 RNA polymerase is not required for stringent RNA control in vivo. J. Bacteriol. 1991, 173, 3901–3903.

49. Claret, L.; Rouviere-Yaniv, J. Regulation of HU alpha and HU beta by CRP and FIS in Escherichia Coli. J. Mol. Biol. 1996, 263, 126–139.
50. Cabrera, J.E.; Jin, D.J. The distribution of RNA polymerase in Escherichia coli is dynamic and sensitive to environmental cues. Mol. Microbiol. 2003, 50, 1493–1505.
51. Weng, X.; Bohrer, C.H.; Bettridge, K.; Lagda, A.C.; Cagliero, C.; Jin, D.J.; Xiao, J. Spatial organization of RNA polymerase and its relationship with transcription in Escherichia coli. Proc. Natl. Acad. Sci. USA 2019, 116, 20115–20123.
52. Schneider, R.; Lurz, R.; Lüder, G.; Tolksdorf, C.; Travers, A.; Muskhelishvili, G. An architectural role of the Escherichia coli chromatin protein FIS in organising DNA. Nucleic Acids Res. 2001, 29, 5107–5114.
53. Schneider, R.; Travers, A.; Muskhelishvili, G. FIS modulates growth phase-dependent topological transitions of DNA in Escherichia Coli. Mol. Microbiol. 1997, 26, 519–530.
54. Broyles, S.S.; Pettijohn, D.E. Interaction of the Escherichia coli HU protein with DNA. Evidence for formation of nucleosome-like structures with altered DNA helical pitch. J. Mol. Biol. 1986, 187, 47–60.
55. Kraemer, J.A.; Sanderlin, A.G.; Laub, M.T. The Stringent Response Inhibits DNA Replication Initiation in E. coli by Modulating Supercoiling of oriC. mBio 2019, 10, e01330-19.
56. Antipov, S.; Tutukina, M.N.; Preobrazhenskaya, E.V.; Kondrashov, F.A.; Patrushev, M.V.; Toshchakov, S.V.; Dominova, I.; Shvyreva, U.S.; Vrublevskaya, V.V.; Morenkov, O.S.; et al. The nucleoid protein Dps binds genomic DNA of Escherichia coli in a non-random manner. PLoS ONE 2017, 12, e0182800.
57. Frenkiel-Krispin, D.; Ben-Avraham, I.; Englander, J.; Shimoni, E.; Wolf, S.G.; Minsky, A. Nucleoid restructuring in stationary-state bacteria. Mol. Microbiol. 2004, 51, 395–405.
58. Dadinova, L.A.; Chesnokov, Y.M.; Kamyshinsky, R.A.; Orlov, I.A.; Petoukhov, M.V.; Mozhaev, A.A.; Soshinskaya, E.Y.; Lazarev, V.N.; Manuvera, V.A.; Orekhov, A.S.; et al. Protective Dps-DNA co-crystallization in stressed cells: An in vitro structural study by small-angle X-ray scattering and cryo-electron tomography. FEBS Lett. 2019, 593, 1360–1371.
59. Hammel, M.; Amlanjyoti, D.; Reyes, F.E.; Chen, J.H.; Parpana, R.; Tang, H.Y.; Larabell, C.A.; Tainer, J.A.; Adhya, S. HU multimerization shift controls nucleoid compaction. Sci. Adv. 2016, 2, e1600650.
60. Ohniwa, R.L.; Morikawa, K.; Kim, J.; Ohta, T.; Ishihama, A.; Wada, C.; Takeyasu, K. Dynamic state of DNA topology is essential for genome condensation in bacteria. EMBO J. 2006, 25, 5591–5602.

61. Lee, S.Y.; Lim, C.J.; Dröge, P.; Yan, J. Regulation of Bacterial DNA Packaging in Early Stationary Phase by Competitive DNA Binding of Dps and IHF. Sci. Rep. 2015, 5, 18146.
62. Graham, J.S.; Johnson, R.C.; Marko, J.F. Concentration-dependent exchange accelerates turnover of proteins bound to double-stranded DNA. Nucleic Acids Res. 2011, 39, 2249–2259.
63. Kamar, R.I.; Banigan, E.J.; Erbas, A.; Giuntoli, R.D.; Olvera de la Cruz, M.; Johnson, R.C.; Marko, J.F. Facilitated dissociation of transcription factors from single DNA binding sites. Proc. Natl. Acad. Sci. USA 2017, 114, E3251–E3257.
64. Tsai, M.Y.; Zhang, B.; Zheng, W.; Wolynes, P.G. Molecular Mechanism of Facilitated Dissociation of Fis Protein from DNA. J. Am. Chem. Soc. 2016, 138, 13497–13500.
65. Kasho, K.; Oshima, T.; Chumsakul, O.; Nakamura, K.; Fukamachi, K.; Katayama, T. Whole-Genome Analysis Reveals That the Nucleoid Protein IHF Predominantly Binds to the Replication Origin oriC Specifically at the Time of Initiation. Front. Microbiol. 2021, 12, 697712.
66. Flåtten, I.; Skarstad, K. The Fis protein has a stimulating role in initiation of replication in Escherichia coli in vivo. PLoS ONE 2013, 8, e83562.
67. Balke, V.L.; Gralla, J.D. Changes in the linking number of supercoiled DNA accompany growth transitions in Escherichia Coli. J. Bacteriol. 1987, 169, 4499–4506.
68. Higgins, C.F.; Dorman, C.J.; Stirling, D.A.; Waddell, L.; Booth, I.R.; May, G.; Bremer, E. A physiological role for DNA supercoiling in the osmotic regulation of gene expression in S. typhimurium and E. Coli. Cell 1988, 52, 569–584.
69. Hsieh, L.S.; Rouviere-Yaniv, J.; Drlica, K. Bacterial DNA supercoiling and / ratio: Changes associated with salt shock. J. Bacteriol. 1991, 173, 3914–3917.
70. Tse-Dinh, Y.C.; Qi, H.; Menzel, R. DNA supercoiling and bacterial adaptation: Thermotolerance and thermoresistance. Trends Microbiol. 1997, 5, 323–326.
71. Hengge, R. Stationary-phase gene regulation in Escherichia Coli. EcoSal Plus 2011, 4.
72. Geertz, M.; Travers, A.; Mehandziska, S.; Sobetzko, P.; Janga, S.C.; Shimamoto, N.; Muskhelishvili, G. Structural coupling between RNA polymerase composition and DNA supercoiling in coordinating transcription: A global role for the omega subunit? mBio 2011, 2, e00034-11.
73. Bordes, P.; Conter, A.; Morales, V.; Bouvier, J.; Kolb, A.; Gutierrez, C. DNA supercoiling contributes to disconnect σS accumulation from σS-dependent transcription in Escherichia coli. Mol. Microbiol. 2003, 48, 561–571.
74. Blot, N.; Mavathur, R.; Geertz, M.; Travers, A.; Muskhelishvili, G. Homeostatic regulation of supercoiling sensitivity coordinates transcription of the bacterial genome. EMBO Rep. 2006, 7, 710–715.

75. Vijayan, V.; Zuzow, R.; O'Shea, E.K. Oscillations in supercoiling drive circadian gene expression in cyanobacteria. Proc. Natl. Acad. Sci. USA 2009, 106, 22564–22568.
76. Szafran, M.J.; Gongerowska, M.; Małecki, T.; Elliot, M.; Jakimowicz, D. Transcriptional Response of Streptomyces coelicolor to Rapid Chromosome Relaxation or Long-Term Supercoiling Imbalance. Front. Microbiol. 2019, 10, 1605.
77. Crozat, E.; Philippe, N.; Lenski, R.E.; Geiselmann, J.; Schneider, D. Long-term experimental evolution in Escherichia coli. XII. DNA topology as a key target of selection. Genetics 2005, 169, 523–532.
78. Heyde, S.A.H.; Frendorf, P.O.; Lauritsen, I.; Nørholm, M.H.H. Restoring Global Gene Regulation through Experimental Evolution Uncovers a NAP (Nucleoid-Associated Protein)-Like Behavior of Crp/Cap. mBio 2021, 12, e0202821.
79. Yan, Y.; Xu, W.; Kumar, S.; Zhang, A.; Leng, F.; Dunlap, D.; Finzi, L. Negative DNA supercoiling makes protein-mediated looping deterministic and ergodic within the bacterial doubling time. Nucleic Acids Res. 2021, 49, 11550–11559.
80. Ellison, M.J.; Fenton, M.J.; Ho, P.S.; Rich, A. Long-range interactions of multiple DNA structural transitions within a common topological domain. EMBO J. 1987, 6, 1513–1522.
81. Kelleher, R.J., 3rd; Ellison, M.J.; Ho, P.S.; Rich, A. Competitive behavior of multiple, discrete B-Z transitions in supercoiled DNA. Proc. Natl. Acad. Sci. USA 1986, 83, 6342–6346.
82. Aboul-ela, F.; Bowater, R.P.; Lilley, D.M. Competing B-Z and helix-coil conformational transitions in supercoiled plasmid DNA. J. Biol. Chem. 1992, 267, 1776–1785.
83. Kim, S.H.; Jung, H.J.; Lee, I.B.; Lee, N.K.; Hong, S.C. Sequence-dependent cost for Z-form shapes the torsion-driven B-Z transition via close interplay of Z-DNA and DNA bubble. Nucleic Acids Res. 2021, 49, 3651–3660.
84. Travers, A.; Muskhelishvili, G. DNA microloops and microdomains: A general mechanism for transcription activation by torsional transmission. J. Mol. Biol. 1998, 279, 1027–1043.
85. Muskhelishvili, G.; Buckle, M.; Heumann, H.; Kahmann, R.; Travers, A.A. FIS activates sequential steps during transcription initiation at a stable RNA promoter. EMBO J. 1997, 16, 3655–3665.
86. Sheridan, S.; Benham, C.J.; Hatfield, G.W. Activation of gene expression by a novel DNA structural transmission mechanism that requires supercoiling-induced DNA duplex destabilization in an upstream activating sequence. J. Biol. Chem. 1998, 273, 21298–21308.
87. González-Gil, G.; Kahmann, R.; Muskhelishvili, G. Regulation of crp transcription by oscillation between distinct nucleoprotein complexes. EMBO J. 1998, 17, 2877–2885.
88. Nasser, W.; Schneider, R.; Travers, A.; Muskhelishvili, G. CRP modulates fis transcription by alternate formation of activating and repressing nucleoprotein complexes. J. Biol. Chem. 2001,

276, 17878–17886.

89. Browning, D.F.; Cole, J.A.; Busby, S.J. Transcription activation by remodelling of a nucleoprotein assembly: The role of NarL at the FNR-dependent Escherichia coli nir promoter. Mol. Microbiol. 2004, 53, 203–215.
90. Duprey, A.; Muskhelishvili, G.; Reverchon, S.; Nasser, W. Temporal control of Dickeya dadantii main virulence gene expression by growth phase-dependent alteration of regulatory nucleoprotein complexes. Biochim. Biophys. Acta. 2016, 1859, 1470–1480.
91. Cameron, A.D.S.; Dillon, S.C.; Kröger, C.; Beran, L.; Dorman, C.J. Broad-scale redistribution of mRNA abundance and transcriptional machinery in response to growth rate in Salmonella enterica serovar Typhimurium. Microb. Genom. 2017, 3, e000127.
92. Dorman, C.J. DNA supercoiling and transcription in bacteria: A two-way street. BMC Mol. Cell. Biol. 2019, 20, 26.
93. Fulcrand, G.; Zhi, X.; Leng, F. Transcription-coupled DNA supercoiling in defined protein systems and in E. coli topA mutant strains. IUBMB Life 2013, 65, 615–622.
94. Meyer, S.; Beslon, G. Torsion-mediated interaction between adjacent genes. PLOS Comput. Biol. 2014, 10, e1003785.
95. Sobetzko, P. Transcription-coupled DNA supercoiling dictates the chromosomal arrangement of bacterial genes. Nucleic Acids Res. 2016, 44, 1514–1524.
96. El Houdaigui, B.; Forquet, R.; Hindré, T.; Schneider, D.; Nasser, W.; Reverchon, S.; Meyer, S. Bacterial genome architecture shapes global transcriptional regulation by DNA supercoiling. Nucleic Acids Res. 2019, 47, 5648–5657.
97. Reverchon, S.; Meyer, S.; Forquet, R.; Hommais, F.; Muskhelishvili, G.; Nasser, W. The nucleoid-associated protein IHF acts as a ‘transcriptional domainin’ protein coordinating the bacterial virulence traits with global transcription. Nucleic Acids Res. 2021, 49, 776–790.
98. Wang, M.; Zhang, J.; Xu, H.; Golding, I. Measuring transcription at a single gene copy reveals hidden drivers of bacterial individuality. Nat. Microbiol. 2019, 4, 2118–2127.
99. Forquet, R.; Pineau, M.; Nasser, W.; Reverchon, S.; Meyer, S. Role of the Discriminator Sequence in the Supercoiling Sensitivity of Bacterial Promoters. mSystems. 2021, 6, e0097821.
100. Auner, H.; Buckle, M.; Deufel, A.; Kutateladze, T.; Lazarus, L.; Mavathur, R.; Muskhelishvili, G.; Pemberton, I.; Schneider, R.; Travers, A. Mechanism of transcriptional activation by FIS: Role of core promoter structure and DNA topology. J. Mol. Biol. 2003, 331, 331–344.
101. Figueroa-Bossi, N.; Guérin, M.; Rahmouni, R.; Leng, M.; Bossi, L. The supercoiling sensitivity of a bacterial tRNA promoter parallels its responsiveness to stringent control. EMBO J. 1998, 17, 2359–2367.

102. Rochman, M.; Aviv, M.; Glaser, G.; Muskhelishvili, G. Promoter protection by a transcription factor acting as a local topological homeostat. EMBO Rep. 2002, 3, 355–360.
103. Chapman, A.G.; Fall, L.; Atkinson, D.E. Adenylate energy charge in Escherichia coli during growth and starvation. J. Bacteriol. 1971, 108, 1072–1086.
104. Schultz, S.G.; Solomon, A.K. Cation transport in Escherichia coli. I. Intracellular Na and K concentrations and net cation movement. J. Gen. Physiol. 1961, 45, 355–369.
105. Kashket, E.R.; Barker, S.L. Effects of potassium ions on the electrical and pH gradients across the membrane of Streptococcus lactis cells. J. Bacteriol. 1977, 130, 1017–1023.
106. Castanie-Cornet, M.P.; Penfound, T.A.; Smith, D.; Elliott, J.F.; Foster, J.W. Control of acid resistance in Escherichia coli. J. Bacteriol. 1999, 181, 3525–3535.
107. Wang, J.Y.; Drlica, K.; Syvanen, M. Monovalent cations differ in their effects on transcription initiation from a sigma-70 promoter of Escherichia Coli. Gene. 1997, 196, 95–98.