Published in final edited form as:

Chem Rev. 2013 November 13; 113(11): . doi:10.1021/cr4001429.

Role of RNA Polymerase in the Organization of the Bacterial Nucleoid

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1. Introduction

One of the major structural differences that provides a taxonomic distinction between eukaryotic and prokaryotic organisms is that prokaryotes lack the nuclear envelope, with exceptions such as planctomycetes¹, and genomic organization mechanisms found in eukaryotes. Instead, the prokaryotic genome is partitioned into a somewhat amorphous region of the cell known as the nucleoid²⁻⁴. In both prokaryotes and eukaryotes, the genome must be compacted to fit into its allotted space while maintaining a level of organization that allows efficient functionality. In eukaryotes, this is accomplished by well-defined histone proteins that form nucleosomal and chromatin structures and yet can be remodeled to allow for the decondensation critical for gene expression⁵⁻⁹. Although the bacterial genome must also be densely compacted, more than one-thousand-fold, and also organized for optimum functionality (chromosome replication/segregation, recombination and transcription)¹⁰⁻¹⁴, the mechanisms by which this is accomplished remain unclear. The lack of “beads-on-a-string” morphology indicates different and/or multiple roles for nucleoid-associated proteins. Since transcription is a major cellular function and involves protein-DNA interaction and DNA topological domain structures, the transcription machinery themselves may play a role in nucleoid organization. Conversely, the nucleoid structure and organization may influence gene expression in a manner analogous to chromatin decondensation in eukaryotes. The interactions involved in nucleoid structure and gene expression continue to be challenging issues in the post-genomic era.

Escherichia coli is a preferred model system for prokaryotic research due to the extensive studies into its genetic, physiology, biochemistry, and molecular biology mechanisms¹⁵. The study of nucleoid organization is intrinsically difficult mainly due to the small size of bacteria in comparison to the limits of optical resolution of conventional instrumentation. Until very recently, images of the nucleoid have not changed much over the last four decades and reveal no structural details¹⁶⁻¹⁸. Consequently, most of the earlier studies were focused on the biochemical properties and morphologies of isolated nucleoids and electron microscopic images after fixation^{3-4,19-24}. However, any preparation or fixation procedure will potentially introduce different organizations of the nucleoids or even artifacts. For example, different fixation procedures produced different nucleoid shapes of electron microscopic images³. Additionally, growing evidence indicates that the organization of the nucleoid in the cell is plastic and sensitive to changes in growth and stress. The challenge of the research is to capture the “true” organization of the nucleoid reflecting the dynamic states of the nucleoid in living cells.

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Extensive studies have primarily emphasized the “histone-like” proteins including FIS, HU, H-NS, and IHF for their architectural roles in bending, looping, bridging and compacting DNA^{25–36}. The first three and RNA polymerase (RNAP) are the major nucleoid-associated proteins (NAPs) isolated from exponentially growing cells. Compared to the “histone-like” proteins, the binding of RNAP to DNA is much stronger. A prevailing view is that bacterial “histone-like proteins” are primarily responsible for the nucleoid organization and compaction in growing cells.

Recent advances in the cell biology of *E. coli* RNAP and the nucleoid have shown that the distribution of RNAP, which is coupled to cell growth, plays an important role in the nucleoid dynamic structure. Emerging evidence indicates that formation of the transcription foci centered at the putative nucleolus structure is critical in nucleoid remodeling and influencing global gene expression. As other recent comprehensive reviews have dealt with the roles of other factors in bacterial nucleoid organization^{37–43}, this review provides an alternative and/or complementary perspective to the traditional views on bacterial nucleoid compaction and expansion.

2. The Nucleoid

The bacterial nucleoid differs from the eukaryotic nucleus in two important ways. The foremost is that the nucleoid lacks a membrane envelope, thus, it interfaces with the cytoplasm intimately. Another is that the mass ratio of protein to DNA of the nucleoid in growing cells appears to be lower than the value of 1:1 for the eukaryotic counterpart^{20,44–45}. Such low protein density is also revealed by a general smooth appearance of the nucleoid from chromatin spread images^{46–47} in contrast to the eukaryotic nucleosomal “beads-on-a-string” morphology^{48–50}. The reason(s) for the apparent low protein to DNA ratio could be attributed to a low content of proteins in the nucleoid and/or, more likely, to an overall low binding affinity of most of nucleoid-associated proteins compared to histones. These features are critical for bacterial nucleoid structure in the cell.

2.1. Environment

E. coli is a gram negative, rod shaped bacterium which changes size depending on growth conditions. A rigid cell wall maintains osmotic pressure inside the cell and is sandwiched by outer and inner (cytoplasmic) membranes; between the cell wall and the cytoplasmic membrane is the periplasmic space⁵¹. The nucleoid is usually located near the center of the cytoplasm along the long axis of the cell⁵² and occupies less than ¼ of the intracellular volume⁵³. Thus, the interaction between the nucleoid and cytoplasm is extensive at the interface. One example of this interaction is the coupling of transcription and translation^{46,54}. A special process of such coupling, known as transertion, involves an additional translocation of transmembrane proteins^{55–58}. Although most of the ribosomes are located in areas outside of the nucleoid, recent superresolution imaging reveals that some ribosomes and RNAP are associated with the nucleoid periphery and cell membrane^{3,59}.

The cytoplasm is filled with proteins, RNAs and ribosomes⁶⁰ (Figure 1). Unlike typical biochemical reactions *in vitro*, which are usually performed at diluted concentrations, the concentration of the macromolecules in the cell is estimated to be 0.3 to 0.5 g/ml⁶¹. This macromolecular crowding is proposed to condense the bacterial nucleoid and influence the interactions between the nucleoid and cytoplasm^{62–65}. Modeling biological interactions in macromolecular crowding environments have provided many new insights into the forces operating in the cell^{66–71}. One hypothesis is that genome organization is entropy-driven by depletion attraction^{72–73}. The *E. coli* chromosome is also suggested to be a polymer with large-scale coiled organization⁷⁴.

Recent experiments using a microfluidic device, which allows manipulation and visualization of expansion and condensation of released nucleoids from lysed cells, indicate that the nucleoid behaves as a cross-linked polymer and entropic force (depletion) influences the nucleoid dynamic structure and segregation in the crowded intracellular environment ⁷⁵.

2.2. Composition

Compact nucleoids isolated from *E. coli* lysates comprise a folded DNA-RNA-protein complex ²⁰. The complex is approximately 80% DNA by weight; less than 10% is protein and the remaining is RNA and lipids ^{76–77}.

2.2.1. Nucleic Acid

2.2.1.1. Genomic DNA: The *E. coli* K12 genome is a circular molecule containing ~4.6 million base pairs (bp) ⁷⁸ and, weighs ~3GDa. When fully extended, it has a contour length of ~1600 μ m. The *E. coli* genome has a single origin (*oriC*) from which DNA replication ⁷⁹ proceeds bidirectionally through somewhat stationary “replication factories” ^{80–83} stopping at the terminus (*ter*), followed by segregation. The newly synthesized DNA strand is unmethylated and the resulting hemimethylated DNA at each replication fork is bound by SeqA protein ^{84–85}. Sister chromatids are held together (cohesion) for some time during the replication and segregate into two halves of the cells ^{86–92}. The replication-segregation time is ~60 to 90 min in *E. coli*. Thus there is only one nucleoid in very slow growing cells which have a doubling time > 100 min ^{93–94}.

However, *E. coli* is capable of very rapid growth, and under optimal growth conditions the doubling time is only ~20 min. When the cell’s doubling time is shorter than the replication-segregation time, there are additional initiation(s) from the *oriC* before the previous round is completed ensuring that each of the two newly divided daughter cells has at least one completed chromosome ⁹⁵. Thus, the total DNA content, the number of *oriC*s connecting sister chromatids and the *oriC* organization are growth-rate dependent ^{84,96–97}. Multiple *oriC*s in fast growing cells may be either co-localized ⁹⁸ or segregated ^{91,99–100}.

2.2.1.2. Nascent RNA: RNA is associated with intact isolated nucleoids and is ~10% by weight. These are nascent RNA molecules attached to elongating RNAP that can “run off” and terminate when NTPs are supplemented ¹⁰¹. About half of the nascent RNAs are rRNA molecules, elongating at the *rnm* operons in nucleoids isolated from late log phase cells grown in minimal medium supplemented with 0.2% of casamino acids ¹⁰². A similar value was obtained from total RNA molecules synthesized in cells under the same growth conditions, demonstrating that the distribution of RNAP in isolated nucleoids is similar to that in living cells.

2.2.2. Nucleoid-Associated Proteins (NAPs)—Primarily four NAPs are identified from nucleoids isolated from lysates of growing cells ^{20,103}. RNAP (core) is the predominant protein, accounting for 50% or more by weight, and the remaining are three small (9 to 16 kDa) positively charged “histone-like” proteins ^{104–105}, including FIS, H-NS and HU. In early log phase cells, FIS, H-NS and HU are the major small NAPs ^{35,106}. However, there are nine other small growth-phase dependent NAPs ^{33,107} and two unknown proteins ¹⁰⁸. Additionally, there are 100–150 transcription factors ¹⁰⁹, most of which also bind to DNA influencing transcription by RNAP. Together, these observations suggest that RNAP and transcription play an important role in nucleoid organization.

2.2.2.1. The “Histone-Like” Proteins: FIS (factor for inversion stimulation) ¹¹⁰, H-NS (histone-like nucleoid structuring protein) ¹¹¹, HU (heat-stable nucleoid protein) ¹¹² and IHF (integration host factor) ¹¹³ have been extensively studied biochemically and structurally *in*

vitro primarily owing to their potential “histone-like” functions in nucleoid compaction and they have been studied genetically because they are also transcription factors^{114–116} and are involved in recombination^{110,117–121}. Collectively, they have an architectural role by bending, bridging or wrapping DNA. These properties are important in DNA looping and/or stabilization of supercoiled loops. They also provide mechanisms for regulation of gene expression and recombination by modulating local DNA configurations. As there are several recent reviews on these small NAPs^{27,32,34,39,114,122–124}, this review highlights other aspects of the “histone-like” proteins relevant to their role in nucleoid organization.

The affinities of the “histone-like” proteins to DNA are significantly weak compared with that of RNAP²⁸ and histones. Among them, HU has the lowest affinity to DNA while the DNA affinities of FIS, H-NS and IHF are comparable¹²⁵. Their relatively weak affinities to DNA, coupled with low coverage of DNA⁴⁴, are inconsistent with the notion that the “histone-like” proteins play a major and similar role as histone proteins in eukaryotes¹²⁶. This feature instead suggests that the “histone-like” proteins are “on” and “off” the nucleoid dynamically. FIS binding is influenced by DNA breathing dynamics (i.e. local transient DNA “melting” of DNA duplex)¹²⁷ based on simulations¹²⁸ and by the purine 2-amino group of DNA minor groove¹²⁹. In principle, binding of other NAPs could also be regulated by thermal fluctuation of “opening and re-closing” of DNA duplex.

The DNA binding sites for the “histone-like” proteins across the genome have been analyzed by Chip-chip and Chip-seq techniques. These techniques require crosslinking by formaldehyde which only crosslinks stably-bound proteins to DNA¹³⁰. Unlike RNAP which is totally crosslinked, ~30 to 50% of H-NS and IHF are not crosslinked to the nucleoids in the cell¹³¹, reflecting their relatively weak affinities to DNA. These properties should be taken into consideration when performing analyses of the genome-wide binding sites for the “histone-like” proteins. For example, these results could explain why HU binding sites are mainly non-specific across the genome¹³² as HU has the weakest affinity to DNA among these small NAPs. From these analyses, two features are noticeable. First, H-NS binds to significantly longer tracks of DNA than FIS, suggesting linear H-NS spread along DNA¹³³. This is consistent with H-NS being a global silencer¹¹⁶. In addition, H-NS is the only “histone-like” protein that forms “clusters” visualized by superresolution imaging¹³⁴. Second, many regions associated with FIS and H-NS are also associated with RNA polymerase³¹. The overlapping binding sites for FIS and H-NS are consistent with the reports that these two proteins have antagonizing functions at certain promoters^{135–138}. The apparent strong association of the binding sites for FIS, H-NS and RNAP in the genome is significant as it indicates that the “histone-like” proteins behave like other transcription factors modulating RNAP binding and/or activity^{126,139–140}, either positively or negatively.

2.2.2.2. RNA Polymerase (RNAP): Eukaryotes have three RNA polymerase species in their nucleus, each of which is dedicated to the synthesis of specialized RNA species¹⁴¹. For example, Pol I is responsible for the synthesis of rRNA at the nucleolus while Pol II performs mRNA synthesis in other parts of the chromosome. Pol III makes small RNAs including tRNAs. In contrast, prokaryotes have only one RNAP responsible for transcription of all RNA species^{142–144}. *E. coli* RNAP consists of two forms: core ($\alpha_2\beta\beta'$, ~400KDa) and holoenzyme ($\alpha_2\beta\beta'\sigma$, ~450KDa)¹⁴⁵. It is a large molecule with dimensions of $17 \times 13.5 \times 13.5$ nm for the holoenzyme^{144,146–147} and $8.5 \times 10.5 \times 14$ nm for core RNAP¹⁴⁸. Only the holoenzyme recognizes a promoter and initiates transcription. In general, σ is released shortly after initiation and elongating RNAP (core) stops at the terminators. Transcription factors including NAPs regulate RNAP activities mainly at initiation, but some influence elongation, termination and RNAP recycling^{109,149}.

Unlike HU, HN-S and IHF, RNAP binds to DNA strongly. The RNAP binding site detected by DNA footprinting varies, ranging from 62–81bp for the initiation complexes¹⁵⁰ to ~23 bp for the elongation complex¹⁵¹. Most RNAP molecules are likely engaged in synthesizing nascent RNAs in fast growing cells. Single molecule analyses reveal that RNAP is a powerful molecular motor during transcription^{152–154}. Remarkable transcription processivity is reflected by the fact that elongating RNAP remains tightly associated with the isolated nucleoid even in the presence of 1.0 M NaCl¹⁰¹.

In addition, both holoenzyme and core RNAP bind to DNA non-specifically with high affinity¹⁵⁵. The fact that the genome contains a vast excess of non-specific DNA suggests that there is minimum unbound RNAP in the cell. Indeed, all RNAP molecules appear to be crosslinked to DNA in the cell in contrast to H-NS and IHF¹³¹. Single molecule analyses show that bound RNAP is able to rapidly slide along DNA for a long distance^{156–157} or hop around three-dimensionally¹⁵⁸. Together, such remarkable thermodynamic movements of bound but transcriptionally disengaged RNAP are likely important for searching for promoters and/or maintaining dynamic organizations of transcription machinery.

2.3. Organization

For more than four decades, *E. coli* nucleoids have been characterized in detail at several levels from isolated nucleoids *in vitro* to topological domains and other structures *in vivo*. The vast amount of information accumulated has provided a solid foundation toward understanding the “whole” picture of the nucleoid in living cells. A review of the extensive literature points to the importance of growth conditions, RNAP and transcription, particularly rRNA synthesis in the organization of the nucleoid in the cell.

2.3.1. Isolated Nucleoids

2.3.1.1. Membrane Association: Many methods have been developed to isolate intact nucleoids that maintain their “native” folded compact state¹⁰². A “high-salt” lysis protocol, incorporating 1.0 M NaCl, lysozyme (4 mg/ml) and detergent, is particularly useful for preparation of highly-folded (condensed) nucleoids²⁰. Both membrane-associated and membrane-free nucleoids have been isolated depending on the temperatures used during lysis⁷⁷ and the two forms of the isolated nucleoids confirmed by electron microscopy^{159–161}. With a “low-salt-spermidine” lysis protocol¹⁰³, which contains lysozyme (0.4 or 4 mg/ml), 0.1 M NaCl and 10 mM spermidine, only membrane-associated nucleoids are recovered. Several inner membrane proteins of unknown nature are crosslinked to 5-bromodeoxyuridine -substituted DNA¹⁶².

However, no specific DNA sequences responsible for the membrane association have been identified¹⁶³. The nature of the membrane-DNA interaction is unclear although both replication initiation and chromosome segregation have been suggested to be associated with the membrane^{22,164–167}. One candidate for membrane-associated proteins is FtsK (~147 kDa), an integral membrane protein and a potent RecA-like dsDNA translocase¹⁶⁸. FtsK is essential for the last steps of chromosome segregation at the *dif* site of the *ter* region of chromosome^{90,169}. Additionally, the transertion process may also contribute to membrane association¹⁷⁰.

2.3.1.2. Effects of Growth Media and Antibiotics: Isolated nucleoids are characterized using sucrose gradients. The nucleoids isolated from nutrient-rich medium have a sedimentation coefficient of 3200 S²⁰, whereas those isolated from minimal medium have an estimated value of 1700 S²¹. In addition, the nucleoids isolated from lysates of amino-acid starved cells have a further reduced sedimentation coefficient of 1300 S compared with those from the unstarved cells (1700 S). Although the reduced S value could be due to a

reduced DNA content, it is also possible that the nucleoids in cells grown in minimal medium and in amino-acid starved cells are decondensed.

Nucleoids isolated from cells treated with different antibiotics also exhibit different relative sedimentation velocities compared to those of untreated cells⁷⁶. For example, inhibition of transcription by rifampicin reduces the relative sedimentation coefficient more than 10-fold. Addition of rifampicin to the condensed nucleoids *in vitro* causes no such change, indicating that inhibition of transcription *in vivo* is critical for reduction of sedimentation⁷⁷. In contrast, inhibition of translation by chloramphenicol increases the relative sedimentation coefficient two-fold, suggesting condensation of the nucleoids. Inhibition of supercoiling by the gyrase inhibitor nalidixic acid has no effect on the relative sedimentation velocities⁷⁶.

2.3.1.3. Supercoiled loops: The condensed nucleoids are negatively supercoiled and the number of the supercoiled loops varies dependent on the method used. An estimate of 12 to 80 loops is suggested by changes in the relative sedimentation coefficient after ethidium bromide intercalation and DNA single-strand nicking with DNase²¹. Each supercoiled loop is an independent domain; eliminating one loop has no effect on the others. However, extensive nicking results in an unfolded nucleoid without supercoiled loops. Electron microscopy measurements of membrane-free nucleoids indicate 98 to 194 supercoiled loops of variable sizes in a rosettes-like morphology for every chromosome¹⁶⁰. These results are consistent with the hypothesis that supercoiling compacts the nucleoid. More recent studies indicate that there are about 500 supercoiled loops in growing cells^{171–172} (see below).

2.3.1.4. RNA Core: RNase treatment converts a condensed nucleoid into a decondensed structure⁷⁷, and it was proposed that RNA binds to DNA and defines the barriers separating the supercoiled loops. Electron microscopy of membrane-free folded nucleoids shows that they seem to have a central core consisting mostly RNA surrounded by ordered DNA fibers and supercoiled loops¹⁶⁰. RNase treatment of the nucleoid also appears to alter the nucleoid structure by atomic force microscopy analysis¹⁷³. However, the RNA molecules responsible for the nucleoid compaction have not been identified. Additional *in vivo* experiments have as yet found no role of nascent RNA in stabilization of supercoiled domains¹⁷⁴. Thus, the implied role of RNA in nucleoid compaction is likely an artifact introduced during the procedures for nucleoid isolation (see below).

2.3.1.5. Lysozyme effect: Nucleoids released from *E. coli* spheroplasts after low osmolarity shock in the presence of very low concentration of lysozyme (3 µg/ml) appear as cloud-like structures; however, a relatively high concentration of lysozyme (30 µg/ml) condenses the nucleoid significantly, reducing the volume by 3-fold¹⁷⁵. Lysozyme is a strongly positive charged molecule with an isoelectric point (pI) of 11.16¹⁷⁶. Thus, lysozyme could compact the nucleoids by interactions with DNA loops and/or nascent RNA. Note that all the protocols mentioned above use high levels of lysozyme and, indeed, the isolated nucleoids from those protocols contain significant amounts of lysozyme¹²⁵. However, RNase treatments have no effect on the structure of the nucleoids isolated in the presence of a very low level of lysozyme. Apparently, organization of isolated nucleoids is influenced greatly by the conditions used during preparation.

2.3.2. Topological Domain Structures in vivo

2.3.2.1. Supercoiled domains—The negative supercoiling of the *E. coli* chromosome is maintained by topoisomerases^{177–179} and other factors¹⁸⁰. DNA gyrase is a critical factor because it introduces negative supercoiling¹⁸¹. RNAP is also important because transcription and supercoiling are coupled¹⁸². Particularly, elongating RNAP generates positive supercoils ahead and negative supercoils behind¹⁸³. The twin domain introduced

by RNAP suggests that DNA topoisomerases are actively involved in the elongation step of transcription¹⁸⁴. Gyrase appears to be co-localized with highly expressed genes¹⁸⁵, reflecting the need for gyrase action ahead of the active elongating RNAP at those genes.

The state of supercoiling of the *E. coli* chromosome is thus likely to be strongly influenced by transcription locally and globally. The extremely high expression of ribosomal RNA operons (*rnn*) in fast growing cells is proposed to generate extraordinary high density of negative supercoiling near those regions¹⁸⁶. It is also suggested that supercoil densities in fast growing cells are higher than those in slow growing cells¹⁸⁷. Changes in supercoiling are associated with transitions in growth phases and the antibiotic rifampicin, which inhibits transcription initiation, causes DNA relaxation¹⁸⁸. Small NAPs could modulate supercoiling by either affecting the expression of gyrase and topoisomerase I¹⁷³ or regulation of global transcription^{189–192}.

The diffusion of supercoiling generated from distant transcription can be followed by monitoring the expression of supercoiling-sensitive promoters. The *leu-500* promoter is activated by negative supercoiling¹⁹³ and without nearby transcription activity is not functional¹⁹⁴; however, it can be activated if there is remote transcription spreading negative supercoiling¹⁹⁵. Thus, supercoiling diffusion has the potential to modulate the expression of other genes within a supercoiled domain.

Transcription-induced supercoiling barriers are demonstrated by inhibiting recombination *in vivo*^{196–197}. Moreover, higher transcription activities introduce stronger barriers¹⁹⁸ and R-loops¹⁹⁹. Results obtained by probing the spread of relaxation from double strand DNA breaks near supercoiling sensitive genes *in vivo* have led to the suggestion that the supercoiled domains are flexible both in size and position in the chromosome, and that there are about 500 supercoiled loops having an average size of ~10 kb¹⁷¹. A similar predominantly stochastic supercoiled domain structure has also been reported in *Salmonella*¹⁷². Supercoils can be stabilized by coupled transcription and translation²⁰⁰. In addition, DNA binding proteins can confine DNA supercoils to localized regions²⁰¹. It seems likely that other NAPs and transcription factors could act as barriers or insulators of supercoiled domains²⁰².

2.3.2.2. Macrodomains—Using fluorescence in situ hybridization (FISH) technique with probes covering different regions of the *E. coli* chromosome, two major domains have been identified²⁰³. One is the Ori domain which encompasses a large region of the genome including *oriC*, and the other is the Ter domain which includes a large segment of the genome around the *dif* of the terminus region. These two domains tend to be positioned at different locations in the cell during the cell cycle. Subsequently, using the lambda *att* sites-mediated recombination system²⁰⁴, long-range DNA interactions of the *att* sites located at different positions of the chromosome have been determined; based on the frequencies of the recombination events, the macrodomain organization of the *E. coli* chromosome was proposed²⁰⁵. In this model, the nucleoid is organized into four major domains, including the Ori, Ter, Right and Left and two other minor less-structured regions.

Many processes and factors, such as replication and transcription machineries, influence the formation of macrodomains. One of these factors, MukB (~170 kDa), is implicated in the macrodomain organization because the Ori and Ter domains are abnormally positioned in a *mukB* mutant²⁰⁶. MukB is a member of the SMC (structural maintenance of chromosome) protein family²⁰⁷. MukB consists of an N-terminal ATP/GTP binding domain, two long segments of coiled-coil separated by a hinge and a C-terminal DNA binding²⁰⁸. The structure of MukB shows two thin rods with globular heads at the ends with a flexible center hinge in an “open” or “closed” configuration²⁰⁹.

The *mukB* mutation results in accumulation of anucleated cells and cells with expanded nucleoids^{203,210}. Mutations in the topoisomerase I encoding gene, *topA*, suppress the segregation defect of the *mukB* mutant, indicating that suppression is due to an increase of negative supercoiling²¹¹. The *mukB* chromosome exhibits reduced negative supercoiling compared to wild type, whereas, another cell cycle mutation, *seqA*, has increased supercoiling²¹². In contrast to MukB, SeqA appears to decrease the supercoiling in the cell²¹³. How MukB and SeqA modulate supercoiling and affect DNA condensation during the cell cycles at different growth conditions remains to be determined. Other macrodomain associated proteins, such as SImA and MatP, have been described²¹⁴.

2.3.3. Hyper-condensed Structures during Extreme Physiology

2.3.3.1. In Late-Stationary-Phase Starved Cells: Global expression pattern changes accompany growth transitions²¹⁵. In particular, Dps (19 kDa), a DNA-binding protein that protects DNA against multiple stresses during the stationary phase^{216–217}, accumulates rapidly during the stationary phase and is one of the few proteins still being expressed in late stationary phase when cells are starved²¹⁸. Intriguingly, DNA-Dps complexes are extremely stable *in vitro* and have similar crystalline structures both *in vitro* and in 2-day cultured cells in which Dps is overproduced²¹⁹. Formation of DNA-Dps crystalline structures may have evolved to isolate damaging agents from the genome. Similarly, RecA-DNA coaggregates reveal lateral assembly morphology in SOS-induced cells²²⁰. The link between stress and highly ordered nucleoid structures has been discussed elsewhere²²¹.

2.3.3.2. High Salt Shock during Initial Osmotic Stress Response: Osmotic response is essential for cell survival in both eukaryotes and prokaryotes²²². It induces sequential changes in physiology and gene expression in *E. coli*^{223–226}. The effects of high salt shock on the nucleoid structure and RNAP distribution *in vivo* are visualized in time-course experiments¹³¹. Shortly (< 20 min) after the hyper-osmotic stress response was induced by the addition of 0.5 M NaCl into fast growing cells (LB, 37°C), the nucleoid became hyper-condensed when cytoplasmic K⁺ accumulated transiently (Figure 2). During a subsequent osmoadaptation phase when K⁺ levels decreased accompanying accumulations of organic osmoprotectants, the nucleoid gradually de-condensed approaching the pre-stress state. In parallel, RNAP dissociated from the nucleoid during the initial high shock followed by re-association during the adaptation, demonstrating that the interaction between RNAP and DNA is governed by the same thermodynamic principle *in vivo* as it is *in vitro*^{155,227–228}. It is speculated that shortly after salt shock the transient dissociation of RNAP from the genome with concurrent nucleoid condensation could be an effective way to silence genome-wide gene expression. During the early adaptation phase, active expression of osmotic stress response genes presumably occurs at the surface of the hyper-condense nucleoid¹³¹.

3. Dynamic Nucleoid Organization by RNAP

Many processes and/or factors influence dynamic nucleoid organization. The contribution of chromosome replication and segregation to nucleoid organization during a single cell cycle in very slow growing cells is well recognized^{12–13,88–89,229–231}. In rapidly growing cells, the replication also play a critical role in nucleoid organization through SeqA-mediated interactions and such interactions could promote cohesion of the newly synthesized chromatin²³². Topological domain structures are important for DNA condensation and the formation of macrodomains. The architectural roles of “histone-like” NAPs have been extensively studied; their implications in nucleoid compaction are almost exclusively extrapolated from *in vitro* analyses. Growing evidence indicates that their primary function *in vivo* is transcription regulation^{126,139–140,233}.

The intertwined relationship between transcription and supercoiling suggests a critical role for RNAP in modulating the dynamic nucleoid structure in response to changes in nutrient status and growth conditions. Co-imaging of RNAP and the nucleoid provides new insights on the role of RNAP in the organization of the *E. coli* nucleoid as the quality of images improves. By studying the distribution of RNAP under different growth conditions, evidence has emerged that the dynamic genome-wide distribution of RNAP, which is predominantly determined by the status of rRNA synthesis, influences the nucleoid compaction/expansion in the cell^{14,234–235}.

3.1. Imaging RNAP Distribution in the Cell using RNAP-Fluorophore Fusion

E. coli strains containing derivatives of *rpoC-gfp* fusion in its normal location in the chromosome of the prototype K-12 strain MG1655 have been constructed to study the cell biology of RNAP by fluorescence microscopy^{234–237}. The distribution of RNAP is dynamic and sensitive to environmental cues²³⁴. The strains containing the *rpoC-gfp* fusion are temperature sensitive for growth at > 30°C likely owing to the GFP protein folding problem. Unlike with most of GFP-fusion proteins, the true states of RNAP and the nucleoid are affected due to perturbations in growth and physiology, when cells are sampled from a rapidly shaking flask and agarose embedded on coverslips. To overcome the complications owing to rapid bacterial adaptation, a procedure was developed in which cells are rapidly fixed with formaldehyde to “freeze” RNAP and subcellular structures before imaging, yielding reproducible results that are consistent with the genetics and physiology of *E. coli* cells. Microfluidic technology will be useful for live-cell imaging of RNAP and nucleoid to examine whether results from fixed-cells represent the distribution of RNAP and structure of nucleoid found in living cells.

With a new generation of fluorescent protein Venus²³⁸, *E. coli* strains with the *rpoC-venus* fusion exhibits wild type growth phenotype and growth rate at different temperatures and media¹³¹; thus, experiments can be performed at optimal conditions at 37°C in LB. Compared to the RNAP-GFP protein, the RNAP-Venus protein is brighter, thus significantly enhancing the signal-to-noise ratio. Coupled with a more sensitive imaging system, new detailed structure and texture information for both RNAP and the genomic DNA are revealed (in sharp contrast, previous studies with the *rpoC-gfp* fusion showed that the nucleoid has blob morphology with no structural and textural details). As described below with the new images of the strains containing the *rpoC-gfp* fusion, these improvements have significantly advanced our study allowing some detailed analysis of the intensities of RNAP and the DNA in images for further understanding the role of RNAP in the organization of the nucleoid during growth and stress.

Recently, superresolution imaging of RNAP using γ -GFP 59 and photoactivated localization microscopy (PALM) imaging of RNAP are reported²³⁹. These new superresolution techniques provide new details on the distribution of RNAP in the cell.

3.2. Nucleoid Structures and Growth Conditions

As shown in figure 3, the sizes of *E. coli* cells vary significantly under different growth media. Fast growing cells in optimal growth conditions (LB 37°C, doubling time (DT) 20 min) are the largest with dimensions of 0.7–1.7 μm (short axis) $\times$ 3.5–10.7 μm (long axis), while slow growing cells (minimal MOPS with poor carbon source glycerol) are the smallest (among growing cells) with dimensions of 0.5–1.2 μm (short axis) $\times$ 2.0–3.3 μm (long axis). Temperature also affects the growth rates and, in the same medium, cells grow slower at 30°C than at 37°C. However, stationary phase cells from LB overnight cultures are much smaller (0.7–1.1 $\times$ 1.0–2.1 μm) than those of mid-log cultures. Regulation of cell size in response to fatty acid availability has been reported²⁴⁰. Note that the growth rate of *E. coli*

in the intestine of mice can reach the same fast growth rate during optimal laboratory growth condition with LB and vary with similar ranges in animals as in the test tubes ²⁴¹. Thus the growth rate regulation by nutrient status likely reflects *E. coli* physiology in the hosts.

The relative nucleoid compaction in the cell can be measured empirically as the relative nucleoid size (RNS), which is the ratio of the nucleoid(s) length to that of the cell ^{235,242}. In fast growing cells (LB, 37°C) the four apparent nascent nucleoids are relatively compact as indicated by the ample cytoplasmic space and a smaller relative nucleoid size (RNS) value (0.55). Additionally, the nucleoids reveal structural details with textural variations and apparent voids. In rich defined-minimal medium (MOPS+RD), there are only two well separated nascent nucleoids, each of which has two undefined nascent nucleoids; the RNS value (0.64) is significantly higher than that of LB cells. The single nucleoid in the slowest growing cells is expanded with a RNS value (0.74) and there is little texture variation in the nucleoid. In the stationary phase cells which are the smallest, the smooth nucleoid has an average RNS value of 0.7 within a cell not much bigger than the nucleoid.

Media apparently have far more influence on nucleoid compaction than growth rate *per se*. For example, nucleoids are significantly more compact (RNS = 0.56) in cells grown in LB at 30°C with a doubling time of 45 min compared with those (RNS = 0.64) in cells grown in MOPS-RD at 37°C with a doubling time of 25 min. It is probable that there are some undefined nutrients in LB which are missing in the rich defined (RD) MOPS medium.

3.3. Dominant Transcription Foci for Active rRNA Synthesis

During rapid growth in LB, the majority of RNAP molecules is concentrated at transcription foci ^{131,234} (Figure 4). These dominant foci presumably locate at the putative nucleolus structure for active rRNA synthesis; thus they are named TF(*rnm*). Recent PALM imaging of RNAP in rapidly growing cells shows large clusters of >500 RNAP that are likely engaging in rRNA synthesis from multiple *rnm* operons ²³⁹. In addition small clusters of 70 RNAP that are attributed to the transcription of a single *rnm* operon are also revealed. Formation of transcription foci is sensitive to growth media as the transcription foci diminish in rich defined-minimal medium (MOPS + RD, DT 25 min) and there are no apparent foci in very slow growing cells in minimal medium with poor carbon source (MOPS + glycerol, DT 93 min). The lack of transcription foci is consistent with the low transcription of *rnm* from cells grown in nutrient-poor media ²⁴³.

Evidently, both the nucleoid structure and the formation of transcription foci are sensitive to growth media; further, the RNS value, which is an index for nucleoid compaction/expansion, is inversely correlated to the richness of the growth media. To recognize the role of RNAP in the organization of nucleoid it is essential to understand growth rate regulation including the stringent response and its effect on the distribution of RNAP in the cell.

3.4. Growth Rate Regulation and rRNA Synthesis

A landmark in bacterial physiology is the finding more than 50 years ago that bacterial growth rate as measured by cell mass and RNA level is regulated in response to growth media ^{244–245}. Subsequently, it was determined that growth rate is a function of the rate of rRNA synthesis (including tRNA), from which ribosomes are assembled to meet the demand for protein synthesis ^{243,246–248}. Since then, regulation of rRNA synthesis has been extensively studied ^{249–251}. The effect of the differential distribution of RNAP between rRNA and mRNA genes on growth rate regulation has been reviewed elsewhere ¹⁸⁶.

There are seven *rnm* operons in *E. coli* and their positions in the *E. coli* map are shown in figure 5A. The direction of transcription of the seven *rnm* operons is the same as that of replication. This co-directionality likely evolved to avoid replication–transcription conflicts

in bacteria ²⁵², as transcription of *rnm* is extremely active in fast growing cells. In addition, 93% of ribosomal protein genes and about 66% of the essential genes are transcribed in the same direction as that for *rnm* ^{253–255}. Having multiple *rnm* operons is likely advantageous for the cells to rapidly respond to changing environmental conditions ^{256–257}.

The seven *rnm* operons encompass less than 1% of the genome; however, the status of rRNA synthesis in response to changes in growth conditions has the most profound effect on the distribution of RNAP in the nucleoid. Three features of *rnm* contribute to the unique role of rRNA synthesis in driving the distribution of RNAP. First, all seven *rnm* operons are located in the origin half of the chromosome and, particularly, four of them are clustered near *oriC*. Their proximity to *oriC* makes the copy number (or gene dosage) of *rnm* especially sensitive to growth rate regulation.

The copy numbers of *rnm* genes and genome equivalents can be calculated on average per cell based on the growth rate of the cell ^{243,258}. In fast growing cells during optimal growth conditions (LB, 37°C, doubling time 20 min), it is calculated that there are ~50 copies of *rnm*, of which ~33 copies of *rnm* are located near the multiple *oriC* loci (8–16 copies), and five genome-equivalents (23Mb) per cell. Before cell division, each cell has four nascent nucleoids; each nucleoid could have up to four copies of *oriC* (Figure 5B). Thus, mature cells have up to 16 copies of *oriC*, whereas newly divided daughter cells have up to eight copies of *oriC* ¹⁰⁰.

Two additional features are related to the length of the *rnm* transcripts and their regulation. The seven *rnm* operons have essentially the same organization and regulation ²⁵⁹. The coding sequence for the *rnm* operons is long; the ~5.5 kb *rnm* transcript is processed to mature rRNA including 16S, 23S and 5S and tRNAs ²⁶⁰. Regulation of rRNA synthesis is mainly at the initiation ^{250,261}, although it is also regulated to a lesser extent by elongation and the antitermination system ^{262–264}. There are two very strong promoters for each *rnm* ²⁶⁵, of which P1 is the major promoter responsible for growth rate regulation ²⁶⁶. The P1 promoter has extensive regulatory elements including a G/C rich “discriminator sequence” ²⁶⁷ and binding sites for small NAPs including Fis, H-NS and Lrp (leucine-responsive protein) ^{268–270}. In nutrient-rich media, P1 is the strongest promoter in the cell approaching one initiation per second ²⁷¹ and rRNA synthesis comprises the most active transcription activity in the cell ²⁴³. Multiple factors contribute to the superior strength of the P1 promoter including the “UP” element ^{264,272} and multiple Fis binding sites that recruit and stabilize the initiation complex ^{268,273}. However, in nutrient-poor media the P1 promoter activity decreases significantly ^{264,274–275}, and during nutrient starvation the P1 activity is minimal ²⁷⁶.

3.5. Stringent Response vs. Growth Rate Regulation

When cells are starved for amino acids, rRNA synthesis ceases almost immediately, accompanied by a rapid accumulation of the alarmones, pppGpp and ppGpp, collectively called (p)ppGpp ²⁷⁷. This starvation-induced response is named the stringent response ²⁷⁸. (p)ppGpp is synthesized by both RelA and SpoT ²⁷⁹; however, RelA is mainly responsible for its production during amino acid starvation. Thus, in contrast to wild type having a “stringent” phenotype, the *relA* mutant cells exhibit a “relaxed” phenotype as rRNA synthesis remains active during the amino acid starvation ^{280–281}.

The alarmone (p)ppGpp binds to RNAP ^{282–284} and inhibits initiation from the P1 promoter by destabilization of the initiation complex ²⁸⁵. The DksA protein potentiates the regulation of P1 by (p)ppGpp ²⁸⁶. In addition, (p)ppGpp could affect rRNA synthesis by enhancing the pausing of elongating RNAP on *rnm* ²⁸⁷. Thus, rRNA synthesis is negatively controlled by (p)ppGpp and P1 is a stringent promoter.

In addition to *rrn*, there are a few other genes with promoters that are regulated similarly to *rrn* P1 both *in vivo* and *in vitro*; thus they are classified as genes that are regulated by stringent promoters²⁸⁸. Significantly, these promoters are extremely sensitive to negative supercoiling; the initiation complexes are stabilized by supercoiled DNA but RNAP dissociates rapidly from relaxed DNA²⁸⁸. Active transcription from these strong promoters will likely generate “waves” of negative supercoiling behind towards the promoters in fast growing cells, whilst inhibition of transcription will abolish supercoiling during starvation, constituting a supercoiling feedback regulation¹⁸⁶. This class of genes includes those involved in the translational machinery (tRNA and tRNA processing enzymes)^{289–293}, synthesis of nucleotides²⁹⁴, cell motility machinery^{276,295}, and replication and transcription machinery²⁹⁶. The *rrn* operons and these “*rrn*-like” genes are regulated similarly (for simplicity hereafter they are represented by *rrn*) which are distinguished from the vast majority of the “non-*rrn*” genes in the genome.

Because both bacterial growth rate and rRNA synthesis are inversely correlated to the concentration of (p)ppGpp, ranging over 100-fold from basal level to maximum level during the stringent response^{297–299}, growth rate regulation and the stringent response are clearly interrelated. At one end of the spectrum during optimal growth in nutrient-rich media, P1 has maximum strength, while at the other end of spectrum during nutrient starvation the promoter has minimum strength owing to the intrinsic instability of the initiation complexes²⁶¹. The growth rate regulation of *rrn* is thus likely a continuum depending on nutrient availability in the media.

Studies of a special class of Rif^R RNAP mutants have suggested that the dual aspects of the stringent response are mechanistically related. These RNAP mutants are named “stringent” mutants because they exhibit constitutive stringent phenotypes affecting the dual aspects, i.e. decreasing rRNA synthesis even in nutrient-rich LB and activation of amino acid biosynthetic operons in the absence of (p)ppGpp³⁰⁰. Biochemical characterizations of these “stringent” mutant RNAPs have shown that the initiation complexes of *rrn* P1 and several other stringent promoters with these mutant RNAPs are extremely unstable and prone to dissociation compared to wild type RNAP²⁸⁸, thus providing an apparent link between dissociation of RNAP from *rrn* P1 and more available RNAP for transcription of other non-*rrn* genes in the genome.

Based on the characterizations of these stringent RNAP mutants both *in vivo* and *in vitro*, it is hypothesized that the redistribution of RNAP between *rrn* and “non-*rrn*” genes is a key mechanism for the stringent response in the cell. Specifically, during rapid growth, most RNAP molecules are used up by active rRNA synthesis (and other stringent genes), thus RNAP is limiting for transcription of other “non-*rrn*” genes such as amino acids biosynthetic operons. In contrast, during a starvation stress that induces the stringent response, RNAP molecules released from the rRNA synthesis become available and can be directed by transcription factors for transcription of non-*rrn* genes, such as amino acids biosynthetic operons. Between the two ends of the spectrum is the continuum of growth rate regulation. This model predicts that the distribution of RNAP is skewed (or biased) to rRNA synthesis on 1% of the genome at the expense of global genome-wide transcription in fast growing cells; conversely, RNAP redistribution enables “balanced” transcription across the genome in nutrient-poor and starved cells.

Transcription profiling from wild type and the “relaxed” *relA* mutant cells before and after amino acid starvation has been analyzed^{276,301–302}. An important finding from these studies is that, compared to non-starved cells, the number of genes with increased expression (or activation) is about 3-fold higher than that of genes with reduced expression during starvation in wild type cells. Similarly, an apparent inverse correlation between the number

of genes which are activated and rRNA synthesis is also observed during carbon sources starvation³⁰³. Amino acids biosynthetic operons are typical examples of the genes which are positively controlled (or activated) by (p)ppGpp during the stringent response, as (p)ppGpp-less double *relA spoT* null mutant cells are defective in growth on minimal media without supplementation of amino acids²⁷⁹. Promoters for amino acid biosynthetic operons are stimulated by (p)ppGpp³⁰⁴ and further potentiated by DksA³⁰⁵. Thus, decreasing rRNA synthesis is linked to activating genome-wide transcription, consistent with the RNAP redistribution model during the stringent response. The principle is likely to be true in cells with other stresses.

3.6. RNAP Distribution dictated by rRNA Synthesis

3.6.1. Imaging RNAP Distribution by Chromatin Spreading—The distribution of RNAP in the nucleoids has been examined by chromatin spreading from *E. coli* cells grown in different media. The distinctive double Christmas tree morphology is the signature of active rRNA synthesis on *rnm* and is easily identified on an electron micrograph from fast growing cells⁴⁶. This unique morphology is the consequence of nascent pre-rRNA cleavage by RNase III resulting in two gradients of increasing length of 16S and 23S rRNA transcripts^{306–307}. As expected, the double Christmas tree morphology is absent in the chromatin spread from slow growing cells^{308–309} because rRNA synthesis is minimal in nutrient-poor media. This is also consistent with the lack of transcription foci in slow growing cells with the MOPS+glycerol medium mentioned above.

Figure 6 illustrates a skewed genome-wide distribution of RNAP in fast growing cells with LB⁴⁷. The high density of nascent rRNA transcripts makes the two *rnm* operons (*rnmB* and *rnmE*), which are separated ~40kb, the most easily identified in the electron micrographs. Each of the *rnm* operons has almost the same densities of *rnm* transcripts; RNAP is densely packed having one RNAP every 85 bp with little space between them. Thus, there are 65 RNAP per *rnm* operon on average. A higher number of ~70 RNAP per *rnm* operon (or one RNAP per 79 bp) has also been reported^{258,271}. In contrast, RNAP densities transcribing other genes are significantly reduced, ranging from one RNAP per 1 kb in the *rpoBC* genes to unidentified lone transcripts with one RNAP separated by 10 to 20 kb.

Significantly, the chromosome is mostly transcriptionally inactive and there are regions as long as 30 to 40 kb totally devoid of transcript and RNAP in the chromatin spread images from cells grown in nutrient-rich LB. Larger regions of nucleoid thus appear to be “naked” with a smooth appearance morphology and no other bound NAPs are apparent. These data support the results that (i) *E. coli* nucleoid has a low mass ratio of tightly-bound protein and DNA and (ii) RNAP is the predominant and stably-bound NAP in contrast to the “histone-like” proteins and other NAPs.

The chromatin spread images are also consistent with the results that nascent rRNA molecules are the predominant RNA species in the isolated nucleoids from cells grown in a nutrient-rich medium^{101–102}. However, these analyses have provided no information on whether RNAP engaging in active rRNA synthesis is concentrated together with multiple *rnm* operons or separated with each individual *rnm* operon in the nucleoids of rapidly growing cells. Nor do they suggest any role of RNAP associated with rRNA synthesis in the organization of the nucleoid in the cell. However, as described below, microscopy images with RNAP-Fluorophore Fusion and with nucleoid (DNA) from co-imaging suggest that rRNA synthesis is concentrated in the transcription foci consisting of multiple *rnm* operons and that the transcription foci are critical for the nucleoid compaction in rapidly growing cells.

3.6.2. Active *rrn* Transcription Is the Driving Force for RNAP Distribution in the Cell—The bias of RNAP distribution toward the *rrn* operons in fast growing cells has also been observed *in vivo*²³⁷. Normally, RNAP is located in the nucleoid and its surroundings in the cell²³⁴. However, in the cell that harbors a plasmid containing an entire *rrn* operon, the RNAP is found largely in the cytoplasmic space suggesting that the transcription of rRNA is critical in governing the distribution/localization of the RNAP in the cell. Both the extremely strong *rrn* promoters and the length of the *rrn* operon in the plasmids are essential in driving RNAP from the nucleoid into the cytoplasm, as either the replacement of the *rrn* promoters with a strong *ptac* promoter or the shortening of the *rrn* operon abolishes the cytoplasmic re-localization of RNAP (Table 1). These results indicate that the two features of the *rrn* operons (promoter strength and transcript length) are critical in governing the distribution of RNAP in living cells.

3.6.3. The Majority of RNAP Is Concentrated in the Transcription Foci at the Nucleolus-like Structure in Fast Growing Cells—The numbers of RNAP in *E. coli* cells with different growth media are estimated by different methods to range from 2000 to 5000^{59,109,239,310–311}. Assuming 5000 RNAP molecules/cell and ~80 bp of DNA bound by RNAP, RNAP occupies 4×10^5 bp or 8.7% of the *E. coli* genome. In rapidly-growing cells with five genome equivalents, the total coverage is reduced to 1.7%. These estimates highlight the fact that at a given time only a small fraction of the nucleoids is occupied by RNAP in fast growing cells.

The combined effects of gene dosage, long *rrn* transcripts and growth regulation drive RNAP allocation in the genome. In the fast growing cells (LB 37°C, DT 20 min) where rRNA is most active, assuming there are 70 RNAP per *rrn*, it is estimated that there are 3500 RNAP molecules transcribing a total of 50 copies of *rrn* (Figure 5B). Thus, about 70% of RNAP molecules are highly concentrated in the transcription foci for *rrn* at the putative nucleolus structure using a high estimate number of RNAP. Obviously, there are not many RNAP molecules left for the transcription of non-*rrn* genes in the remaining vast regions of the nucleoid during optimal growth.

The number of transcription foci on average appears to be approximating the number of nascent nucleoids in the cell (Figure 4). For example, during optimal growth (LB 37°C, DT 20 min) there are 4–5 transcription foci (Figure 4B) which correspond to 5 nucleoid equivalents per cell (Figure 5B). Imaging reveals four distinctive nascent nucleoids in the cell (Figure 4A). With a total number of 50 *rrn* operons in the cell, it is calculated that there are more than 12 *rrn* operons in each of the foci in each nascent nucleoid. Presumably, each of these dominant transcription foci is a hub of transcription activity encompassing as yet undefined transcription factories^{312–313}, each of which is likely actively transcribing one or more *rrn* operons and possibly with other highly transcribed non-*rrn* genes. At present, it is not clear whether non-*rrn* genes are also transcribed in factories. The concept of transcription hub is based on recent observations from Genome-wide Chromosome Conformation Capture analysis that in fast growing cells, highly transcribed genes are clustered and in a highly interactive environment²³². Whether all 7 *rrn* operons are clustered in the transcription foci is unknown. A recent study of the distribution of RNAP in superresolution suggests the presence of loner *rrn* that are not associated with the large clusters of RNAP²³⁹.

3.7. Nucleoid Compaction Modulated by Transcription Foci

Several experiments described below have further demonstrated a clear correlation between the formation of transcription foci and nucleoid compaction. Together, they strongly suggest that transcription foci modulate nucleoid organization in the cell.

3.7.1. Transcription Foci Disappearance Accompanies Nucleoid Expansion during Amino-Acid-Starvation Induced Stringent Response in Wild Type Cells

—The addition of serine hydroxamate (SHX) into a rapidly growing LB culture, causes starvation for serine³¹⁴ (and eventually inhibition of protein synthesis), thus inducing the stringent response²⁷⁸. As expected, transcription foci disperse during the stringent response accompanied by nucleoid expansion (Figure 7). This process is reversible as transcription foci reappeared and the nucleoids were re-compacted when the culture was supplemented with the starved amino acid²³⁴.

3.7.2. Absence of Transcription Foci and Nucleoid Expansion in RNAP

Mutants Defective in rRNA Synthesis in Nutrient-rich LB—The stringent RNAP mutants are defective in rRNA synthesis even in nutrient-rich media as described above. As expected, transcription foci are not apparent and RNAP distributes evenly across the expanded nucleoid in one of the mutant cells grown in LB (Figure 8). Consequently, the *rpoB3443* (L533P) mutant cells grow slower with a doubling time of ~40 min (LB, 37°C) which is only one half of the growth rate of wild type cells and are smaller in size compared to wild type (Figure 3).

3.7.3. Lacking Transcription Foci and Expanded Nucleoid in $\Delta \Delta 6rrn$ Mutant Cells

—When six of the seven *rrn* operons in the chromosome are deleted, the resulting *6rrn* mutant has a slow growth rate with a doubling of ~34 min in LB at 37°C. There are no apparent transcription foci and the nucleoid is expanded with an RNS value of 0.67 (Figure 9). Based on the growth rate, it is calculated that there are four *rrn* on average per cell. These results suggest that formation of transcription foci with multiple *rrn* is required for nucleoid compaction²³⁵.

3.7.4. Apparent Transcription Foci and Nucleoid Compaction in the “Relaxed” *relA* Mutant Cells during Amino-Acid Starvation

—Unlike wild type, the *relA* mutant is defective in the production of (p)ppGpp during amino acid starvation^{280,315}; thus it is proficient in rRNA synthesis with the addition of SHX in LB culture. Transcription foci are apparent during amino acid starvation and the nucleoids are further compacted (Figure 10). Apparently the two nascent nucleoids in each half of the cell are fused together and DNA appears to be highly condensed with an average RNA value of 0.31, a phenotype that resembles that of the nucleoids in cells treated with the protein inhibitor chloramphenicol (see below). Note that the cells cease to grow after the addition of SHX owing to inhibition of protein synthesis. Inhibition of translation could reduce the coupling of transcription-translation and transertion, which in turn could cause nascent nucleoid fusion and further condensation compared to untreated cells. Under this condition active rRNA synthesis decoupled from cell growth still has an impact on the nucleoid.

3.7.5. Evident Transcription Foci and Nucleoid Compaction in Cells Treated with Protein Synthesis Inhibitors

—When rapidly growing cells in LB are treated with chloramphenicol or spectinomycin, cell growth stops immediately. Both antibiotics have the same effect on transcription foci and nucleoid compaction. As shown in figure 11, the transcription foci remain evident and nucleoids become further compacted. Similar to the morphology of the nucleoids in the *relA* mutant cells treated with SHX (Figure 10), the fused nascent nucleoid in each half of the cell is highly condensed with the same average value of RNS (0.31) in chloramphenicol-treated cells.

Chloramphenicol treatment enhances nucleoid compaction as indicated by increased sedimentation of the isolated nucleoid⁷⁶. Nucleoid compaction in chloramphenicol-treated cells has been used as evidence for the hypothesis that transertion is an expansion force for

the nucleoid, thus inhibition of protein synthesis leading to the inhibition of transertion results in nucleoid compaction⁵⁵. In wild type cells treated with SHX, protein synthesis and transertion are inhibited; however, the nucleoids are fully expanded (Figure 7). These results are not consistent with the hypothesis that transertion is a nucleoid expansion force. Furthermore, with the additional treatment of rifampicin that inhibits transcription, the nucleoids compacted by chloramphenicol convert to expanded ones accompanied with the disappearance of transcription foci²³⁵ (Figure 11). Together, these data are consistent with the hypothesis that transcription foci modulate nucleoid compaction.

3.8. Transcribing RNAP Locates at the Surface of the Nucleoid

As described below, co-imaging RNAP and the nucleoid in cells containing *rpoC-venus* under different growth conditions reveals new features and details. Analysis of the overlays (RNAP/Nucleoid) has provided new insights on how RNAP remodels nucleoid organization.

3.8.1. Transcription Foci Appear to Position at the Voids of the Nucleoid—

Overlay of the images of RNAP and the nucleoid from cells during optimal growth shows that transcription foci tend to locate at the apparent voids of the nucleoid where DNA densities are low (Figures 4 & 7). This feature suggests that highly active transcription foci for *rnm* and possibly other genes are in a distinct compartment separated from the main body of the nucleoid. As most of the RNAP molecules are concentrated in the transcription foci, larger areas of the nucleoid are devoid of RNAP as indicated by red color in the overlay of the two images, consistent with the hypothesis that RNAP is limited and mainly engaged in rRNA synthesis during fast growth.

3.8.2. DNA Loops Enriched with RNAP locate at the Periphery of the Nucleoid

—One common theme from the overlays of the RNAP and DNA images is that the area covered by RNAP signal is usually larger than that of the nucleoid signal. This is particularly evident for the nucleoids without transcription foci such as *6rrn* (Figure 9) and wild type cells treated with SHX for 30 min (Figure 7). Because RNAP is a strong DNA binding protein and is always associated with DNA in the cell¹³¹, we speculate that the enlarged RNAP areas suggest the presence of DNA loops at the periphery of the observed nucleoid. It seems very likely that DNA loops are the main locations for transcription in the nucleoid. The DNA densities of the loops are likely too low to be detected by conventional fluorescence microscopy. Super-resolution imaging is necessary to reveal more details on these features.

The inverse relationship between RNAP and nucleoid can be better observed when the nucleoids isolated from the lysed cells are analyzed (Figure 12). In the isolated nucleoids from fast growing cells (LB), the transcription foci are at the periphery of the nucleoid where DNA density is low. The overlay image shows a distinct pattern of green (RNAP) or red (DNA) but not much of orange that would be indicative of co-localization (RNAP/DNA). As described in the previous section (2.3.3.2), there are temporal changes in nucleoid structure in cells during a hyper-osmotic stress response. The nucleoids become hyper-condensed shortly (10 to 20 min) after initial high salt shock followed by gradually remodeling and returning to the pre-stress state during the later adaptation stage. Accompanying the changes of the nucleoid structure, RNAP initially dissociates from the nucleoid releasing into the cytoplasmic space followed by re-associates with the nucleoid¹³¹. The nucleoids isolated from the cells 20 min after the salt shock is hyper-condensed (NaCl 20 min) similarly to that in the intact cells (Figure 2). At this time, RNAP associates (being crosslinked) with the unseen DNA loops and forms a distinct ring at the periphery of the isolated nucleoid. As this is a 2-D image, it indicates a sphere of RNAP

associated with DNA loops surrounding the nucleoid. These observations are consistent with the preferential binding of RNAP to DNA loops at the periphery of the nucleoid.

3.8.3. Non-Transcribing RNAP Scatters across the Nucleoid

In the presence of rifampicin, which inhibits transcription initiation³¹⁶ but not DNA binding³¹⁷, the nucleoid expands fully and RNAP redistributes relatively homogeneously across the nucleoid to promoters and non-specific sites^{318–319}, as indicated by minimum texture variation of the RNAP image (Fig. 13). The number of sites occupied increases more than 5-fold compared to untreated cells³²⁰; similar morphology is observed in cells treated with SHX for 2 hrs (Figure 7). These results are in agreement with the early observations that isolated nucleoids from cells treated with rifampicin or amino acid starvation exhibit reduced sedimentation compared to untreated cells^{21,76} as mentioned in the last section (2.3.1.2).

4. Model: Transcription Foci Condense the Nucleoid

How do RNAP and transcription influence nucleoid organization? A model is proposed to explain how transcription and particularly transcription foci are intimately linked to the structure of the nucleoid (Figure 14). The presence of transcription foci and the structure of the nucleoid are both highly dynamic and are largely affected by growth conditions and stress. This model is based on the premise that RNAP is limited and cannot sustain both active *rnn* synthesis and genome-wide transcription at the same time²⁸⁸ and modified from the ones described previously^{14,186,234}.

In fast growing cells, the nucleoid compaction is mediated by the formation of the transcription foci. The extremely active *rnn* transcription acts as a driving force for RNAP distribution in the cell²³⁷, thus, titrating RNAP away from transcription of the majority non-*rnn* genes in the genome. Extremely high expression of *rnn* is also likely to impose the supercoiled domains in the region. Formation of transcription foci with multiple highly transcribing *rnn* operons could be facilitated by depletion-attraction⁷³ or other unknown mechanisms. TF(*rnn*) are centered at nucleolus-like organization and located at the periphery of the nucleoid in low density DNA regions, supporting the idea of a subcellular compartmentalization. Like other hyperstructures³²¹, subcellular compartmentalization of the foci would have logistic advantages, including maximizing efficiency of transcription, coupling rRNA synthesis with processing and ribosome assembly and reducing traffic jams. Also importantly, the “imprinted” transcription foci at each of the nascent nucleoids would retain active rRNA synthesis apparatuses upon cell division to sustain fast growth.

Additionally, as suggested by the recent *E. coli* Genome-wide Chromosome Conformation Capture analysis²³², the transcription foci could represent a hub of transcription factories encompassing multiple *rnn* and non-*rnn* genes, including essential and growth-related genes located in distant DNA loops of the nucleoid. An average size of DNA loops being 10 kb¹⁷¹ could easily reach a few μm distances for networking with the transcription hub. Bridging of intra- and/or inter-loops by small NAPs and transcription factors^{26,322} could enhance the networking. The hub and networking could maximize the use of the limited RNAP by sharing as a pool, as RNAP released from “run off” in the hub could hop around three-dimensionally in search of promoters¹⁵⁸. The transcription foci networking of distant DNA loops could further create tension promoting a higher order of the nucleoid compaction and organization.

Finally, the basic nucleoid organization is in a dynamic equilibrium between the “closed” and “open” states. Transcription activities promote the “open” state generating supercoiling loops at the surface of the nucleoid, resembling eukaryotic re-modeled nucleosomes active

for transcription. RNAP dissociation or minimal transcription favors the “closed” state with a high density of DNA in the core interior of the nucleoid, analogous to the un-remodeled nucleosomes. In fast growing cells, only a relatively small fraction of the genome is highly transcribed, the vast majority of regions of the nucleoid are devoid of transcription and RNAP, leading to global nucleoid compaction. During nutrient limitation or stress, the transcription from the *rnn* operons is reduced to a minimum, thus destabilizing the transcription foci and reducing the supercoiling in the region. The loops that were associated with the hubs will disperse and the tension is lost, leading to nucleoid relaxation or expansion. In addition the re-distribution of RNAP enables transcription of larger fractions of the genome, thus encompassing more of the “open” state. Inhibition of transcription by rifampicin causes the nucleoid to fully expand maximizing the “open” state because the “run off” RNAP scatters across the nucleoid homogeneously.

This model could explain the effects of growth conditions and antibiotics treatments on nucleoid structure reported in the literature. It also provides the framework for further studies on the mechanism whereby RNAP and transcription remodels the nucleoid.

5. Conclusion and Perspectives

In the nucleoid, which appears to be largely devoid of tightly-bound proteins, RNAP is the predominant protein and binds to DNA most strongly among all NAPs. Formation of transcription foci accompanies nucleoid compaction, which is the central feature of the nucleoid structure in fast growing cells. It is postulated that the transcription foci acts as hubs for networking gene expression in distant loops, which in turn generates tension for a higher order of nucleoid compaction. Transcription and networking are likely influenced by small NAPs and transcription factors. Thus, the presence or absence of transcription foci will influence the organization of the nucleoid, including compaction and expansion in response to changes in growth and cell cycle. Furthermore, the compartmentation and “imprint” of transcription foci in the nucleoid have important implications for growth regulation, cell cycles and other chromosome functions in *E. coli*.

Future research needs interdisciplinary approaches to understand the dynamic changes of transcription foci and nucleoid structures under different physiologies in real time in single living cells. Imaging RNAP, DNA and other transcription factors with high resolution will shed lights on the structure of the transcription foci or other potential transcription factories and further our understanding on the role of RNAP in the organization of the bacterial nucleoid. Studying the formation and composition of the transcription foci is critical for our understanding of the networking in transcription and long-distant interactions leading to nucleoid compaction. Determining the RNAP allocation profiling in changing environments will provide physical genomic maps of RNAP in transition. Genome-wide Chromosome Conformation Capture analysis will reveal the interaction network of *E. coli* in different growth conditions. With more data available, it is possible to perform modeling on how transcription foci and RNAP distribution influence networking and nucleoid organization. Studying the relationship between transcription foci and the replication machinery during cell cycle will further our understanding on how cells resolve conflicts of the two processes. Advanced information and tools of genetics and physiology combined with the extensive history and diversity of previous work make the simple *E. coli* model system attractive for studying the structure and function of the chromosome.

Acknowledgments

The authors thank Dr. Julio E. Cabrera who initiated the cell biology of RNAP in the laboratory and Mr. Bo Xiao who introduced the MatLab tools. Also, thank our colleagues and reviewers for helpful comments on the

manuscript. This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

References

1. Fuerst JA. Annual review of microbiology. 2005; 59:299.
2. Piekarski G. Arch Mikrobiol. 1937; 8:428.
3. Robinow C, Kellenberger E. Microbiol Rev. 1994; 58:211. [PubMed: 7521510]
4. Pettijohn, DE. Escherichia coli and Salmonella Typhimurium. Neidhardt, FC.; Curtiss, R., III; Ingraham, JL.; Lin, ECC.; Low, KB.; Magasanik, B.; Reznikoff, WS.; Riley, M.; Schaechter, M.; Umberger, HE., editors. American Society for Microbiology; Washington, D. C: 1996.
5. Clapier CR, Cairns BR. Annu Rev Biochem. 2009; 78:273. [PubMed: 19355820]
6. Grunstein M. Nature. 1997; 389:349. [PubMed: 9311776]
7. Guetg C, Santoro R. Epigenetics. 2012; 7:811. [PubMed: 22735386]
8. Ito T. J Biochem. 2007; 141:609. [PubMed: 17405795]
9. Strahl BD, Allis CD. Nature. 2000; 403:41. [PubMed: 10638745]
10. Higgins, TNP.; Booker, BM.; Manna, D.; Dame, RT.; Dorman, CJ. Springer; 2012.
11. Holmes VF, Cozzarelli NR. Proc Natl Acad Sci U S A. 2000; 97:1322. [PubMed: 10677457]
12. Sherratt DJ. Science. 2003; 301:780. [PubMed: 12907786]
13. Toro E, Shapiro L. Cold Spring Harb Perspect Biol. 2010; 2:a000349. [PubMed: 20182613]
14. Jin DJ, Cabrera JE. J Struct Biol. 2006; 156:284. [PubMed: 16934488]
15. Neidhardt, FC.; Curtiss, R., III; Ingraham, JL.; Lin, ECC.; Low, KB.; Magasanik, B.; Reznikoff, WS.; Riley, M.; Schaechter, M.; Umberger, HE. Escherichia coli and Salmonella. ASM Press; Washington, D.C: 1996.
16. Hecht RM, Taggart RT, Pettijohn DE. Nature. 1975; 253:60. [PubMed: 1089200]
17. Zimmerman SB. J Struct Biol. 2006; 156:255. [PubMed: 16697220]
18. Margolin, W. In Bacterial Chromatin. Dame, RT.; Dorman, CJ., editors. Springer; 2012. p. 13-30.
19. Pettijohn DE. CRC Crit Rev Biochem. 1976; 4:175. [PubMed: 827418]
20. Stonington OG, Pettijohn DE. Proc Natl Acad Sci U S A. 1971; 68:6. [PubMed: 4924971]
21. Worcel A, Burgi E. J Mol Biol. 1972; 71:127. [PubMed: 4564477]
22. Worcel A, Burgi E. J Mol Biol. 1974; 82:91. [PubMed: 4594427]
23. Robinow CF. Bacteriol Rev. 1956; 20:207. [PubMed: 13403844]
24. Pettijohn DE. Cell. 1982; 30:667. [PubMed: 6291779]
25. Skoko D, Yan J, Johnson RC, Marko JF. Physical review letters. 2005; 95:208101. [PubMed: 16384101]
26. Skoko D, Yoo D, Bai H, Schnurr B, Yan J, McLeod SM, Marko JF, Johnson RC. J Mol Biol. 2006; 364:777. [PubMed: 17045294]
27. Luijsterburg MS, Noom MC, Wuite GJ, Dame RT. J Struct Biol. 2006; 156:262. [PubMed: 16879983]
28. Yamazaki K, Nagata A, Kano Y, Imamoto F. Mol Gen Genet. 1984; 196:217. [PubMed: 6387397]
29. Maurer S, Fritz J, Muskhelishvili G. J Mol Biol. 2009; 387:1261. [PubMed: 19254726]
30. McLeod SM, Johnson RC. Curr Opin Microbiol. 2001; 4:152. [PubMed: 11282470]
31. Grainger DC, Hurd D, Goldberg MD, Busby SJ. Nucleic Acids Res. 2006; 34:4642. [PubMed: 16963779]
32. Dillon SC, Dorman CJ. Nat Rev Microbiol. 2010; 8:185. [PubMed: 20140026]
33. Ali Azam T, Iwata A, Nishimura A, Ueda S, Ishihama A. J Bacteriol. 1999; 181:6361. [PubMed: 10515926]
34. Browning DF, Grainger DC, Busby SJ. Curr Opin Microbiol. 2010; 13:773. [PubMed: 20951079]
35. Rimsky S, Travers A. Curr Opin Microbiol. 2011; 14:136. [PubMed: 21288763]
36. Liu Y, Chen H, Kenney LJ, Yan J. Genes Dev. 2010; 24:339. [PubMed: 20159954]
37. Dame, RT.; Dorman, CJ.; Dame, RT.; Dorman, CJ., editors. Springer; 2012.

38. Dame RT, Espeli O, Grainger DC, Wiggins PA. *Mol Microbiol.* 2012; 86:1023. [PubMed: 23066904]
39. Macvanin, M.; Adhya, S. *Biochim Biophys Acta.* 2012.
40. Espeli O, Boccard F. *J Struct Biol.* 2006; 156:304. [PubMed: 16979349]
41. Espeli O, Mercier R, Boccard F. *Mol Microbiol.* 2008; 68:1418. [PubMed: 18410497]
42. Lesterlin C, Mercier R, Boccard F, Barre FX, Cornet F. *EMBO Rep.* 2005; 6:557. [PubMed: 15891766]
43. Boccard F, Esnault E, Valens M. *Mol Microbiol.* 2005; 57:9. [PubMed: 15948945]
44. Eltsov M, Zuber B. *J Struct Biol.* 2006; 156:246. [PubMed: 16978880]
45. Kellenberger E, Arnoldschulzgahmen B. *Fems Microbiology Letters.* 1992; 100:361. [PubMed: 1478471]
46. Miller OL Jr, Hamkalo BA, Thomas CA Jr. *Science.* 1970; 169:392. [PubMed: 4915822]
47. French SL, Miller OL Jr. *J Bacteriol.* 1989; 171:4207. [PubMed: 2666391]
48. Olins AL, Olins DE. *Science.* 1974; 183:330. [PubMed: 4128918]
49. Olins DE, Olins AL. *Nat Rev Mol Cell Biol.* 2003; 4:809. [PubMed: 14570061]
50. Woodcock CL, Safer JP, Stanchfield JE. *Exp Cell Res.* 1976; 97:101. [PubMed: 812708]
51. Kellenberger E, Ryter A. *J Biophys Biochem Cytol.* 1958; 4:323. [PubMed: 13549505]
52. Kellenberger E, Ryter A, Sechaud J. *J Biophys Biochem Cytol.* 1958; 4:671. [PubMed: 13610928]
53. Woldringh, C.; Nanninga, N.; Nanninga, Ne, editors. Academic Press; London: 1985.
54. Ryter A, Chang A. *J Mol Biol.* 1975; 98:797. [PubMed: 811810]
55. Woldringh CL, Jensen PR, Westerhoff HV. *FEMS Microbiol Lett.* 1995; 131:235. [PubMed: 7557335]
56. Binenbaum Z, Parola AH, Zaritsky A, Fishov I. *Mol Microbiol.* 1999; 32:1173. [PubMed: 10383759]
57. Norris V, Madsen MS. *J Mol Biol.* 1995; 253:739. [PubMed: 7473748]
58. Woldringh CL, Nanninga N. *J Struct Biol.* 2006; 156:273. [PubMed: 16828313]
59. Bakshi S, Siryaporn A, Goulian M, Weisshaar JC. *Mol Microbiol.* 2012; 85:21. [PubMed: 22624875]
60. McGuffee SR, Elcock AH. *PLoS Comput Biol.* 2010; 6:e1000694. [PubMed: 20221255]
61. Zimmerman SB, Trach SO. *J Mol Biol.* 1991; 222:599. [PubMed: 1748995]
62. Ellis RJ. *Curr Opin Struct Biol.* 2001; 11:114. [PubMed: 11179900]
63. Minton AP. *J Biol Chem.* 2001; 276:10577. [PubMed: 11279227]
64. Zimmerman SB, Murphy LD. *FEBS Lett.* 1996; 390:245. [PubMed: 8706869]
65. Murphy LD, Zimmerman SB. *Biophys Chem.* 1995; 57:71. [PubMed: 8534838]
66. Ando T, Skolnick J. *Proc Natl Acad Sci U S A.* 2010; 107:18457. [PubMed: 20937902]
67. de Vries R. *Biochimie.* 2010; 92:1715. [PubMed: 20615449]
68. Han J, Herzfeld J. *Biophys J.* 1993; 65:1155. [PubMed: 8241395]
69. Wieczorek G, Zielenkiewicz P. *Biophys J.* 2008; 95:5030. [PubMed: 18757562]
70. Zimmerman SB, Minton AP. *Annu Rev Biophys Biomol Struct.* 1993; 22:27. [PubMed: 7688609]
71. Odijk T. *Biophys Chem.* 1998; 73:23. [PubMed: 9697298]
72. Marenduzzo D, Finan K, Cook PR. *J Cell Biol.* 2006; 175:681. [PubMed: 17145959]
73. Marenduzzo D, Micheletti C, Cook PR. *Biophys J.* 2006; 90:3712. [PubMed: 16500976]
74. Yazdi NH, Guet CC, Johnson RC, Marko JF. *Mol Microbiol.* 2012; 86:1318. [PubMed: 23078205]
75. Pelletier J, Halvorsen K, Ha BY, Paparcone R, Sandler SJ, Woldringh CL, Wong WP, Jun S. *Proc Natl Acad Sci U S A.* 2012; 109:E2649. [PubMed: 22984156]
76. Dworsky P, Schaechter M. *J Bacteriol.* 1973; 116:1364. [PubMed: 4584813]
77. Pettijohn DE, Hecht R. *Cold Spring Harb Symp Quant Biol.* 1974; 38:31. [PubMed: 4598638]
78. Blattner FR, Plunkett G 3rd, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y. *Science.* 1997; 277:1453. [PubMed: 9278503]

79. Kornberg, A.; Baker, TA. DNA Replication. Freeman; New York: 1992.
80. Lemon KP, Grossman AD. Science. 1998; 282:1516. [PubMed: 9822387]
81. Hiraga S, Ichinose C, Niki H, Yamazoe M. Mol Cell. 1998; 1:381. [PubMed: 9660922]
82. Lemon KP, Grossman AD. Mol Cell. 2000; 6:1321. [PubMed: 11163206]
83. Dingman CW. J Theor Biol. 1974; 43:187. [PubMed: 4813535]
84. Morigen, Odsbu I, Skarstad K. Genes Cells. 2009; 14:643. [PubMed: 19371375]
85. Waldminghaus T, Weigel C, Skarstad K. Nucleic Acids Res. 2012; 40:5465. [PubMed: 22373925]
86. Wang X, Liu X, Possoz C, Sherratt DJ. Genes Dev. 2006; 20:1727. [PubMed: 16818605]
87. Wang X, Possoz C, Sherratt DJ. Genes Dev. 2005; 19:2367. [PubMed: 16204186]
88. Nielsen HJ, Ottesen JR, Youngren B, Austin SJ, Hansen FG. Mol Microbiol. 2006; 62:331. [PubMed: 17020576]
89. Bates D, Kleckner N. Cell. 2005; 121:899. [PubMed: 15960977]
90. Lesterlin C, Gigant E, Boccard F, Espeli O. Embo J. 2012; 31:3468. [PubMed: 22820946]
91. Nielsen HJ, Li Y, Youngren B, Hansen FG, Austin S. Mol Microbiol. 2006; 61:383. [PubMed: 16771843]
92. Possoz C, Junier I, Espeli O. Front Biosci. 2012; 17:1020.
93. Helmstetter, CE. Escherichia coli and Salmonella. Neidhardt, FC., editor. Vol. 2. ASM Press; Washington, D.C: 1996.
94. Boye E, Stokke T, Kleckner N, Skarstad K. Proc Natl Acad Sci U S A. 1996; 93:12206. [PubMed: 8901558]
95. Cooper S, Helmstetter CE. J Mol Biol. 1968; 31:519. [PubMed: 4866337]
96. Akerlund T, Nordstrom K, Bernander R. J Bacteriol. 1995; 177:6791. [PubMed: 7592469]
97. Skarstad K, Boye E, Steen HB. Embo J. 1986; 5:1711. [PubMed: 3527695]
98. Fossum S, Crooke E, Skarstad K. Embo J. 2007; 26:4514. [PubMed: 17914458]
99. Lau IF, Filipe SR, Soballe B, Okstad OA, Barre FX, Sherratt DJ. Mol Microbiol. 2003; 49:731. [PubMed: 12864855]
100. Nielsen HJ, Youngren B, Hansen FG, Austin S. J Bacteriol. 2007; 189:8660. [PubMed: 17905986]
101. Pettijohn DE, Stonington OG, Kossman CR. Nature. 1970; 228:235. [PubMed: 4920919]
102. Pettijohn DE, Clarkson K, Kossman CR, Stonington OG. J Mol Biol. 1970; 52:281. [PubMed: 4991980]
103. Murphy LD, Zimmerman SB. J Struct Biol. 1997; 119:321. [PubMed: 9245770]
104. Varshavsky AJ, Nedospasov SA, Bakayev VV, Bakayeva TG, Georgiev GP. Nucleic Acids Res. 1977; 4:2725. [PubMed: 333393]
105. Drlica K, Rouviere-Yaniv J. Microbiol Rev. 1987; 51:301. [PubMed: 3118156]
106. Ball CA, Osuna R, Ferguson KC, Johnson RC. J Bacteriol. 1992; 174:8043. [PubMed: 1459953]
107. Azam TA, Ishihama A. J Biol Chem. 1999; 274:33105. [PubMed: 10551881]
108. Murphy LD, Rosner JL, Zimmerman SB, Esposito D. J Bacteriol. 1999; 181:3842. [PubMed: 10368163]
109. Ishihama A. Annu Rev Microbiol. 2000; 54:499. [PubMed: 11018136]
110. Johnson RC, Bruist MF, Simon MI. Cell. 1986; 46:531. [PubMed: 3524854]
111. Cukier-Kahn R, Jacquet M, Gros F. Proc Natl Acad Sci U S A. 1972; 69:3643. [PubMed: 4566454]
112. Rouviere-Yaniv J, Gros F. Proc Natl Acad Sci U S A. 1975; 72:3428. [PubMed: 1103148]
113. Nash HA, Robertson CA. J Biol Chem. 1981; 256:9246. [PubMed: 6267068]
114. Dorman CJ. Adv Appl Microbiol. 2009; 67:47. [PubMed: 19245936]
115. Dorman CJ, Kane KA. FEMS Microbiol Rev. 2009; 33:587. [PubMed: 19207739]
116. Fang FC, Rimsky S. Curr Opin Microbiol. 2008; 11:113. [PubMed: 18387844]
117. Ball CA, Johnson RC. J Bacteriol. 1991; 173:4032. [PubMed: 1829454]
118. Esposito D, Gerard GF. J Bacteriol. 2003; 185:3076. [PubMed: 12730167]

119. Shiga Y, Sekine Y, Kano Y, Ohtsubo E. *J Bacteriol.* 2001; 183:2476. [PubMed: 11274106]
120. Kleckner N, Chalmers RM, Kwon D, Sakai J, Bolland S. *Curr Top Microbiol Immunol.* 1996; 204:49. [PubMed: 8556869]
121. Lavoie BD, Chaconas G. *Curr Top Microbiol Immunol.* 1996; 204:83. [PubMed: 8556870]
122. Pul, U.; Wagner, R. In *Bacterial Chromatin*. Dame, RT.; Dorman, CJ., editors. Springer; 2012. p. 149-173.
123. Luijsterburg MS, White MF, van Driel R, Dame RT. *Crit Rev Biochem Mol Biol.* 2008; 43:393. [PubMed: 19037758]
124. Muskhelishvili, G.; Travers, A. In *Bacterial Chromatin*. Dame, RT.; Dorman, CJ., editors. Springer; 2012. p. 323-351.
125. Murphy LD, Zimmerman SB. *J Struct Biol.* 1997; 119:336. [PubMed: 9245771]
126. Schmid MB. *Cell.* 1990; 63:451. [PubMed: 2121364]
127. Englander SW, Kallenbach NR, Heeger AJ, Krumhansl JA, Litwin S. *Proc Natl Acad Sci U S A.* 1980; 77:7222. [PubMed: 6938969]
128. Nowak-Lovato K, Alexandrov LB, Banisadr A, Bauer AL, Bishop AR, Usheva A, Mu F, Hong-Geller E, Rasmussen KO, Hlavacek WS, Alexandrov BS. *PLoS Comput Biol.* 2013; 9:e1002881. [PubMed: 23341768]
129. Hancock SP, Ghane T, Cascio D, Rohs R, Di Felice R, Johnson RC. *Nucleic Acids Res.* 2013
130. Schmiedeberg L, Skene P, Deaton A, Bird A. *PLoS One.* 2009; 4:e4636. [PubMed: 19247482]
131. Cagliero C, Jin DJ. *Nucleic Acids Res.* 2013; 41:315. [PubMed: 23093594]
132. Prieto AI, Kahramanoglou C, Ali RM, Fraser GM, Seshasayee AS, Luscombe NM. *Nucleic Acids Res.* 2012; 40:3524. [PubMed: 22180530]
133. Kahramanoglou C, Seshasayee AS, Prieto AI, Ibberson D, Schmidt S, Zimmermann J, Benes V, Fraser GM, Luscombe NM. *Nucleic Acids Res.* 2011; 39:2073. [PubMed: 21097887]
134. Wang W, Li GW, Chen C, Xie XS, Zhuang X. *Science.* 2011; 333:1445. [PubMed: 21903814]
135. Afflerbach H, Schroder O, Wagner R. *Mol Microbiol.* 1998; 28:641. [PubMed: 9632265]
136. Afflerbach H, Schroder O, Wagner R. *J Mol Biol.* 1999; 286:339. [PubMed: 9973555]
137. Falconi M, Brandi A, La Teana A, Gualerzi CO, Pon CL. *Mol Microbiol.* 1996; 19:965. [PubMed: 8830277]
138. Grainger DC, Goldberg MD, Lee DJ, Busby SJ. *Mol Microbiol.* 2008; 68:1366. [PubMed: 18452510]
139. Dame RT, Wyman C, Wurm R, Wagner R, Goosen N. *J Biol Chem.* 2002; 277:2146. [PubMed: 11714691]
140. Dorman CJ, Deighan P. *Curr Opin Genet Dev.* 2003; 13:179. [PubMed: 12672495]
141. Cramer P, Armache KJ, Baumli S, Benkert S, Brueckner F, Buchen C, Damsma GE, Dengl S, Geiger SR, Jasiak AJ, Jawhari A, Jennebach S, Kaminski T, Kettenberger H, Kuhn CD, Lehmann E, Leike K, Sydow JF, Vannini A. *Annu Rev Biophys.* 2008; 37:337. [PubMed: 18573085]
142. Vassilyev DG, Vassilyeva MN, Perederina A, Tahirov TH, Artsimovitch I. *Nature.* 2007; 448:157. [PubMed: 17581590]
143. Vassilyev DG, Vassilyeva MN, Zhang JW, Palangat M, Artsimovitch I, Landick R. *Nature.* 2007; 448:163. [PubMed: 17581591]
144. Murakami KS. *J Biol Chem.* 2013
145. Burgess, RR.; Erickson, B.; Gentry, D.; Gribskov, M.; Hager, D.; Lesley, S.; Strickland, M.; Thompson, N. *RNA Polymerase and the Regulation of Transcription*. Reznikoff, WS., editor. Elsevier Science Publishing; New York, NY: 1987.
146. Darst SA, Kubalek EW, Kornberg RD. *Nature.* 1989; 340:730. [PubMed: 2671751]
147. Tichelaar W, Schutter WG, Arnberg AC, van Bruggen EF, Stender W. *Eur J Biochem.* 1983; 135:263. [PubMed: 6350000]
148. Polyakov A, Severinova E, Darst SA. *Cell.* 1995; 83:365. [PubMed: 8521466]
149. Jin DJ, Zhou YN, Shaw G, Ji X. *Biochim Biophys Acta.* 2011; 1809:470. [PubMed: 21419241]
150. Krummel B, Chamberlin MJ. *Biochemistry.* 1989; 28:7829. [PubMed: 2482070]

151. Metzger W, Schickor P, Heumann H, Embo J. 1989; 8:2745. [PubMed: 2555184]
152. Wang MD, Schnitzer MJ, Yin H, Landick R, Gelles J, Block SM. Science. 1998; 282:902. [PubMed: 9794753]
153. Wang HY, Elston T, Mogilner A, Oster G. Biophys J. 1998; 74:1186. [PubMed: 9512018]
154. Herbert KM, Greenleaf WJ, Block SM. Annu Rev Biochem. 2008; 77:149. [PubMed: 18410247]
155. deHaseth PL, Lohman TM, Burgess RR, Record MT Jr. Biochemistry. 1978; 17:1612. [PubMed: 350271]
156. Kabata H, Kurosawa O, Arai I, Washizu M, Margaron SA, Glass RE, Shimamoto N. Science. 1993; 262:1561. [PubMed: 8248804]
157. Harada Y, Funatsu T, Murakami K, Nonoyama Y, Ishihama A, Yanagida T. Biophys J. 1999; 76:709. [PubMed: 9929475]
158. Wang F, Redding S, Finkelstein IJ, Gorman J, Reichman DR, Greene EC. Nat Struct Mol Biol. 2012
159. Delius H, Worcel A. J Mol Biol. 1974; 82:107. [PubMed: 4206502]
160. Kavenoff R, Bowen BC. Chromosoma. 1976; 59:89. [PubMed: 795620]
161. Kavenoff R, Ryder OA. Chromosoma. 1976; 55:13. [PubMed: 767075]
162. Portalier R, Worcel A. Cell. 1976; 8:245. [PubMed: 786463]
163. Drlica K, Burgi E, Worcel A. J Bacteriol. 1978; 134:1108. [PubMed: 350839]
164. Wolf-Watz H. J Bacteriol. 1984; 157:968. [PubMed: 6321451]
165. Jacob F, Brenner S, Cuzin F. Cold Spring Harb Symp Quant Biol. 1963; 28:329.
166. Dubarry N, Possoz C, Barre FX. Mol Microbiol. 2010; 78:1088. [PubMed: 21091498]
167. Deghorain M, Pages C, Meile JC, Stouf M, Capiaux H, Mercier R, Lesterlin C, Hallet B, Cornet F. PLoS One. 2011; 6:e22164. [PubMed: 21799784]
168. Sherratt DJ, Arciszewska LK, Crozat E, Graham JE, Grainge I. Biochem Soc Trans. 2010; 38:395. [PubMed: 20298190]
169. Grainge I, Lesterlin C, Sherratt DJ. Nucleic Acids Res. 2011; 39:5140. [PubMed: 21371996]
170. Woldringh CL. Mol Microbiol. 2002; 45:17. [PubMed: 12100545]
171. Postow L, Hardy CD, Arsuaga J, Cozzarelli NR. Genes Dev. 2004; 18:1766. [PubMed: 15256503]
172. Deng S, Stein RA, Higgins NP. Mol Microbiol. 2005; 57:1511. [PubMed: 16135220]
173. Ohniwa RL, Morikawa K, Kim J, Ohta T, Ishihama A, Wada C, Takeyasu K. Embo J. 2006; 25:5591. [PubMed: 17093499]
174. Sinden RR, Pettijohn DE. Proc Natl Acad Sci U S A. 1981; 78:224. [PubMed: 6165987]
175. Wegner AS, Alexeeva S, Odijk T, Woldringh CL. J Struct Biol. 2012
176. Kuehner DE, Heyer C, Ramsch C, Fornefeld UM, Blanch HW, Prausnitz JM. Biophys J. 1997; 73:3211. [PubMed: 9414232]
177. Bates, AD.; Maxwell, A. DNA Topology. Oxford University Press; USA: 2005.
178. Zechiedrich EL, Khodursky AB, Bachellier S, Schneider R, Chen D, Lilley DM, Cozzarelli NR. J Biol Chem. 2000; 275:8103. [PubMed: 10713132]
179. Hsu YH, Chung MW, Li TK. Nucleic Acids Res. 2006; 34:3128. [PubMed: 16757578]
180. Higgins, NP.; Booker, BM.; Manna, D. In Bacterial Chromatin. Dame, RT.; Dorman, CJ., editors. Springer; 2012. p. 117-148.
181. Gellert M, Mizuuchi K, O'Dea MH, Nash HA. Proc Natl Acad Sci U S A. 1976; 73:3872. [PubMed: 186775]
182. Gamper HB, Hearst JE. Cell. 1982; 29:81. [PubMed: 6286146]
183. Liu LF, Wang JC. Proc Natl Acad Sci U S A. 1987; 84:7024. [PubMed: 2823250]
184. Wu HY, Shyy SH, Wang JC, Liu LF. Cell. 1988; 53:433. [PubMed: 2835168]
185. Jeong KS, Ahn J, Khodursky AB. Genome Biol. 2004; 5:R86. Epub 2004 Oct 27. [PubMed: 15535862]
186. Jin DJ, Cagliero C, Zhou YN. FEMS Microbiol Rev. 2012; 36:269. [PubMed: 21569058]

187. Rovinskiy N, Agbleke AA, Chesnokova O, Pang Z, Higgins NP. PLoS Genet. 2012; 8:e1002845. [PubMed: 22916023]
188. Balke VL, Gralla JD. J Bacteriol. 1987; 169:4499. [PubMed: 3308843]
189. Schneider R, Travers A, Muskhelishvili G. Mol Microbiol. 1997; 26:519. [PubMed: 9402022]
190. Hardy CD, Cozzarelli NR. Mol Microbiol. 2005; 57:1636. [PubMed: 16135230]
191. Mojica FJ, Higgins CF. J Bacteriol. 1997; 179:3528. [PubMed: 9171396]
192. Dorman CJ. Sci Prog. 2006; 89:151. [PubMed: 17338437]
193. Pruss GJ, Drlica K. J Bacteriol. 1985; 164:947. [PubMed: 2997140]
194. Richardson SM, Higgins CF, Lilley DM. Embo J. 1988; 7:1863. [PubMed: 2844526]
195. Wu HY, Tan J, Fang M. Cell. 1995; 82:445. [PubMed: 7634334]
196. Scheirer KE, Higgins NP. Biochimie. 2001; 83:155. [PubMed: 11278064]
197. Deng S, Stein RA, Higgins NP. Proc Natl Acad Sci U S A. 2004; 101:3398. [PubMed: 14993611]
198. Booker BM, Deng S, Higgins NP. Mol Microbiol. 2010; 78:1348. [PubMed: 21143310]
199. Broccoli S, Rallu F, Sanscartier P, Cerritelli SM, Crouch RJ, Drolet M. Mol Microbiol. 2004; 52:1769. [PubMed: 15186424]
200. Lynch AS, Wang JC. J Bacteriol. 1993; 175:1645. [PubMed: 8383663]
201. Leng F, Chen B, Dunlap DD. Proc Natl Acad Sci U S A. 2011
202. Chintakayala K, Singh SS, Rossiter AE, Shahapure R, Dame RT, Grainger DC. PLoS Genet. 2013; 9:e1003152. [PubMed: 23341772]
203. Niki H, Yamaichi Y, Hiraga S. Genes Dev. 2000; 14:212. [PubMed: 10652275]
204. Azaro, MA.; Landy, A. Mobile DNA II. Craig, NL.; Gellert, M.; Lambowitz, AM., editors. ASM Press; Washington, DC: 2002.
205. Valens M, Penaud S, Rossignol M, Cornet F, Boccard F. Embo J. 2004; 23:4330. [PubMed: 15470498]
206. Adachi S, Fukushima T, Hiraga S. Genes Cells. 2008; 13:181. [PubMed: 18233960]
207. Cortes-Ledesma F, de Piccoli G, Haber JE, Aragon L, Aguilera A. Cell Cycle. 2007; 6:914. [PubMed: 17404505]
208. Niki H, Imamura R, Kitaoka M, Yamanaka K, Ogura T, Hiraga S. Embo J. 1992; 11:5101. [PubMed: 1464330]
209. Melby TE, Ciampaglio CN, Briscoe G, Erickson HP. J Cell Biol. 1998; 142:1595. [PubMed: 9744887]
210. Niki H, Jaffe A, Imamura R, Ogura T, Hiraga S. Embo J. 1991; 10:183. [PubMed: 1989883]
211. Sawitzke JA, Austin S. Proc Natl Acad Sci U S A. 2000; 97:1671. [PubMed: 10660686]
212. Weitao T, Nordstrom K, Dasgupta S. EMBO Rep. 2000; 1:494. [PubMed: 11263493]
213. Weitao T, Nordstrom K, Dasgupta S. Mol Microbiol. 1999; 34:157. [PubMed: 10540294]
214. Dame RT, Kalmykova OJ, Grainger DC. PLoS Genet. 2011; 7:e1002123. [PubMed: 21698131]
215. Sobetzko P, Travers A, Muskhelishvili G. Proc Natl Acad Sci U S A. 2012; 109:E42. [PubMed: 22184251]
216. Zeth K. Biochem J. 2012; 445:297. [PubMed: 22788214]
217. Nair S, Finkel SE. J Bacteriol. 2004; 186:4192. [PubMed: 15205421]
218. Almiron M, Link AJ, Furlong D, Kolter R. Genes Dev. 1992; 6:2646. [PubMed: 1340475]
219. Wolf SG, Frenkiel D, Arad T, Finkel SE, Kolter R, Minsky A. Nature. 1999; 400:83. [PubMed: 10403254]
220. Levin-Zaidman S, Frenkiel-Krispin D, Shimoni E, Sabanay I, Wolf SG, Minsky A. Proc Natl Acad Sci U S A. 2000; 97:6791. [PubMed: 10829063]
221. Minsky A, Shimoni E, Frenkiel-Krispin D. Nat Rev Mol Cell Biol. 2002; 3:50. [PubMed: 11823798]
222. Kempf B, Bremer E. Arch Microbiol. 1998; 170:319. [PubMed: 9818351]
223. Dinnbier U, Limpinsel E, Schmid R, Bakker EP. Arch Microbiol. 1988; 150:348. [PubMed: 3060036]
224. Csonka LN. Microbiol Rev. 1989; 53:121. [PubMed: 2651863]

225. Wood JM. *Annu Rev Microbiol.* 2011; 65:215. [PubMed: 21663439]
226. Cheung KJ, Badarinarayana V, Selinger DW, Janse D, Church GM. *Genome Res.* 2003; 13:206. [PubMed: 12566398]
227. Shaner SL, Melancon P, Lee KS, Burgess RR, Record MT Jr. *Cold Spring Harb Symp Quant Biol.* 1983; 47(Pt 1):463. [PubMed: 6345063]
228. Roe JH, Record MT Jr. *Biochemistry.* 1985; 24:4721. [PubMed: 2934084]
229. Thanbichler M, Wang SC, Shapiro L. *J Cell Biochem.* 2005; 96:506. [PubMed: 15988757]
230. Joshi MC, Bourniquel A, Fisher J, Ho BT, Magnan D, Kleckner N, Bates D. *Proc Natl Acad Sci U S A.* 2011; 108:2765. [PubMed: 21282646]
231. Umbarger MA, Toro E, Wright MA, Porreca GJ, Bau D, Hong SH, Fero MJ, Zhu LJ, Marti-Renom MA, McAdams HH, Shapiro L, Dekker J, Church GM. *Mol Cell.* 2011; 44:252. [PubMed: 22017872]
232. Cagliero C, Grand RS, Jones MB, Jin DJ, O'Sullivan JM. *Nucleic Acids Res.* :2013.
233. Bradley MD, Beach MB, de Koning AP, Pratt TS, Osuna R. *Microbiology.* 2007; 153:2922. [PubMed: 17768236]
234. Cabrera JE, Jin DJ. *Mol Microbiol.* 2003; 50:1493. [PubMed: 14651633]
235. Cabrera JE, Cagliero C, Quan S, Squires CL, Jin DJ. *J Bacteriol.* 2009; 191:4180. [PubMed: 19395497]
236. Cabrera JE, Jin DJ. *Methods Enzymol.* 2003; 370:3. [PubMed: 14712629]
237. Cabrera JE, Jin DJ. *J Bacteriol.* 2006; 188:4007. [PubMed: 16707692]
238. Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A. *Nat Biotechnol.* 2002; 20:87. [PubMed: 11753368]
239. Endesfelder U, Finan K, Holden SJ, Cook PR, Kapanidis AN, Heilemann M. *Biophys J.* 2013; 105:172. [PubMed: 23823236]
240. Yao Z, Davis RM, Kishony R, Kahne D, Ruiz N. *Proc Natl Acad Sci U S A.* 2012; 109:E2561. [PubMed: 22908292]
241. Poulsen LK, Licht TR, Rang C, Krogfelt KA, Molin S. *J Bacteriol.* 1995; 177:5840. [PubMed: 7592332]
242. Manasherob R, Zaritsky A, Metzler Y, Ben-Dov E, Itsko M, Fishov I. *Microbiology.* 2003; 149:3553. [PubMed: 14663087]
243. Bremer, H.; Dennis, PP. *Escherichia coli and Salmonella*. Neidhardt, FC.; Curtiss, R., III; Ingraham, JL.; Lin, ECC.; Low, KB.; Magasanik, B.; Reznikoff, WS.; Riley, M.; Schaechter, M.; Umbarger, HE., editors. Vol. 2. ASM Press; Washington, D.C: 1996. p. 1553-1560.
244. Kjeldgaard NO, Maaloe O, Schaechter M. *J Gen Microbiol.* 1958; 19:607. [PubMed: 13611203]
245. Schaechter M, Maaloe O, Kjeldgaard NO. *J Gen Microbiol.* 1958; 19:592. [PubMed: 13611202]
246. Nomura M, Gourse R, Baughman G. *Annu Rev Biochem.* 1984; 53:75. [PubMed: 6206783]
247. Gausing K. *J Mol Biol.* 1977; 115:335. [PubMed: 338910]
248. Keener, J.; Nomura, M. *Escherichia coli and Salmonella*. Neidhardt, FC., editor. Vol. 1. ASM Press; Washington, D.C: 1996.
249. Condon C, Squires C, Squires CL. *Microbiol Rev.* 1995; 59:623. [PubMed: 8531889]
250. Paul BJ, Ross W, Gaal T, Gourse RL. *Annu Rev Genet.* 2004; 38:749. [PubMed: 15568992]
251. Gourse RL, Gaal T, Bartlett MS, Appleman JA, Ross W. *Annu Rev Microbiol.* 1996; 50:645. [PubMed: 8905094]
252. Merrikh H, Zhang Y, Grossman AD, Wang JD. *Nat Rev Microbiol.* 2012; 10:449. [PubMed: 22669220]
253. McLean MJ, Wolfe KH, Devine KM. *J Mol Evol.* 1998; 47:691. [PubMed: 9847411]
254. Lin Y, Gao F, Zhang CT. *Biochem Biophys Res Commun.* 2010; 396:472. [PubMed: 20417622]
255. Rocha EP, Danchin A. *Nucleic Acids Res.* 2003; 31:6570. [PubMed: 14602916]
256. Condon C, Liveris D, Squires C, Schwartz I, Squires CL. *J Bacteriol.* 1995; 177:4152. [PubMed: 7608093]
257. Stevenson BS, Schmidt TM. *Appl Environ Microbiol.* 2004; 70:6670. [PubMed: 15528533]

258. Condon C, French S, Squires C, Squires CL. *Embo J*. 1993; 12:4305. [PubMed: 8223440]
259. Condon C, Philips J, Fu ZY, Squires C, Squires CL. *Embo J*. 1992; 11:4175. [PubMed: 1396599]
260. Srivastava AK, Schlessinger D. *Annu Rev Microbiol*. 1990; 44:105. [PubMed: 1701293]
261. Gourse RL. *Nucleic Acids Res*. 1988; 16:9789. [PubMed: 3054811]
262. Torres M, Condon C, Balada JM, Squires C, Squires CL. *Embo J*. 2001; 20:3811. [PubMed: 11447122]
263. Quan S, Zhang N, French S, Squires CL. *J Bacteriol*. 2005; 187:1632. [PubMed: 15716433]
264. Gourse RL, de Boer HA, Nomura M. *Cell*. 1986; 44:197. [PubMed: 2416474]
265. Lund E, Dahlberg JE. *Proc Natl Acad Sci U S A*. 1979; 76:5480. [PubMed: 392503]
266. Sarmientos P, Sylvester JE, Contente S, Cashel M. *Cell*. 1983; 32:1337. [PubMed: 6188537]
267. Travers AA. *J Bacteriol*. 1980; 141:973. [PubMed: 6154042]
268. Ross W, Thompson JF, Newlands JT, Gourse RL. *Embo J*. 1990; 9:3733. [PubMed: 2209559]
269. Tippner D, Afflerbach H, Bradaczek C, Wagner R. *Mol Microbiol*. 1994; 11:589. [PubMed: 7512187]
270. Pul U, Wurm R, Lux B, Meltzer M, Menzel A, Wagner R. *Mol Microbiol*. 2005; 58:864. [PubMed: 16238633]
271. Voulgaris J, French S, Gourse RL, Squires C, Squires CL. *J Bacteriol*. 1999; 181:4170. [PubMed: 10400572]
272. Ross W, Gosink KK, Salomon J, Igarashi K, Zou C, Ishihama A, Severinov K, Gourse RL. *Science*. 1993; 262:1407. [PubMed: 8248780]
273. Zhi H, Wang X, Cabrera JE, Johnson RC, Jin DJ. *J Biol Chem*. 2003; 278:47340. [PubMed: 13679374]
274. Hernandez VJ, Bremer H. *J Biol Chem*. 1990; 265:11605. [PubMed: 2114400]
275. Bartlett MS, Gourse RL. *J Bacteriol*. 1994; 176:5560. [PubMed: 8071240]
276. Durfee T, Hansen AM, Zhi H, Blattner FR, Jin DJ. *J Bacteriol*. 2008; 190:1084. [PubMed: 18039766]
277. Cashel M, Gallant J. *Nature*. 1969; 221:838. [PubMed: 4885263]
278. Cashel, M.; Gentry, DR.; Hernandez, VJ.; Vinella, D. *Escherichia coli* and *Salmonella*. Neidhardt, FC.; Curtiss, R., III; Ingraham, JL.; Lin, ECC.; Low, KB.; Magasanik, B.; Reznikoff, WS.; Riley, M.; Schaechter, M.; Umberger, HE., editors. Vol. 1. ASM Press; Washington, DC: 1996. p. 1458-1496.
279. Xiao H, Kalman M, Ikehara K, Zemel S, Glaser G, Cashel M. *J Biol Chem*. 1991; 266:5980. [PubMed: 2005134]
280. Fiil N, Friesen JD. *J Bacteriol*. 1968; 95:729. [PubMed: 4867755]
281. Metzger S, Schreiber G, Aizenman E, Cashel M, Glaser G. *J Biol Chem*. 1989; 264:21146. [PubMed: 2556396]
282. Ross W, Vrentas CE, Sanchez-Vazquez P, Gaal T, Gourse RL. *Mol Cell*. 2013; 50:420. [PubMed: 23623682]
283. Zuo Y, Wang Y, Steitz TA. *Mol Cell*. 2013; 50:430. [PubMed: 23623685]
284. Mechold U, Potrykus K, Murphy H, Murakami KS, Cashel M. *Nucleic Acids Res*. 2013
285. Barker MM, Gaal T, Josaitis CA, Gourse RL. *J Mol Biol*. 2001; 305:673. [PubMed: 11162084]
286. Paul BJ, Barker MM, Ross W, Schneider DA, Webb C, Foster JW, Gourse RL. *Cell*. 2004; 118:311. [PubMed: 15294157]
287. Kingston RE, Chamberlin MJ. *Cell*. 1981; 27:523. [PubMed: 6086107]
288. Zhou YN, Jin DJ. *Proc Natl Acad Sci U S A*. 1998; 95:2908. [PubMed: 9501189]
289. Emilsson V, Kurland CG. *Embo J*. 1990; 9:4359. [PubMed: 2265611]
290. Lamond AI, Travers AA. *Cell*. 1985; 41:6. [PubMed: 2581696]
291. Lamond AI, Travers AA. *Nature*. 1983; 305:248. [PubMed: 6350894]
292. Jung YH, Lee Y. *Biochem Biophys Res Commun*. 1997; 230:582. [PubMed: 9015366]
293. Choi HS, Kim KS, Park JW, Jung YH, Lee Y. *Mol Cells*. 2005; 19:239. [PubMed: 15879709]
294. Donahue JP, Turnbough CL Jr. *J Biol Chem*. 1990; 265:19091. [PubMed: 1699940]

295. Lemke JJ, Durfee T, Gourse RL. *Mol Microbiol.* 2009; 74:1368. [PubMed: 19889089]
296. Grossman AD, Taylor WE, Burton ZF, Burgess RR, Gross CA. *J Mol Biol.* 1985; 186:357. [PubMed: 3910841]
297. Baracchini E, Bremer H. *J Biol Chem.* 1988; 263:2597. [PubMed: 2449428]
298. Sarubbi E, Rudd KE, Cashel M. *Mol Gen Genet.* 1988; 213:214. [PubMed: 2460731]
299. Zacharias M, Goringe HU, Wagner R, Embo J. 1989; 8:3357. [PubMed: 2479545]
300. Zhou YN, Jin DJ. *J Bacteriol.* 1997; 179:4292. [PubMed: 9209046]
301. Traxler MF, Summers SM, Nguyen HT, Zacharia VM, Hightower GA, Smith JT, Conway T. *Mol Microbiol.* 2008; 68:1128. [PubMed: 18430135]
302. Traxler MF, Zacharia VM, Marquardt S, Summers SM, Nguyen HT, Stark SE, Conway T. *Mol Microbiol.* 2011; 79:830. [PubMed: 21299642]
303. Liu M, Durfee T, Cabrera JE, Zhao K, Jin DJ, Blattner FR. *J Biol Chem.* 2005; 280:15921. [PubMed: 15705577]
304. Barker MM, Gaal T, Gourse RL. *J Mol Biol.* 2001; 305:689. [PubMed: 11162085]
305. Paul BJ, Berkmen MB, Gourse RL. *Proc Natl Acad Sci U S A.* 2005; 102:7823. [PubMed: 15899978]
306. Hofmann S, Miller OL Jr. *J Bacteriol.* 1977; 132:718. [PubMed: 334750]
307. Bubunenko M, Court DL, Al Refaii A, Saxena S, Korepanov A, Friedman DI, Gottesman ME, Alix JH. *Mol Microbiol.* 2013; 87:382. [PubMed: 23190053]
308. Hamkalo BA, Miller OL Jr. *Basic Life Sci.* 1973; 1:63. [PubMed: 4204123]
309. Hamkalo BA, Miller OL Jr. *Annu Rev Biochem.* 1973; 42:379. [PubMed: 4581229]
310. Piper SE, Mitchell JE, Lee DJ, Busby SJ. *Mol Biosyst.* 2009; 5:1943. [PubMed: 19763331]
311. Grigorova IL, Phleger NJ, Mutalik VK, Gross CA. *Proc Natl Acad Sci U S A.* 2006; 103:5332. [PubMed: 16567622]
312. Cook PR. *Science.* 1999; 284:1790. [PubMed: 10364545]
313. Papantonis A, Cook PR. *Chemical Reviews.* 2013
314. Tosa T, Pizer LI. *J Bacteriol.* 1971; 106:972. [PubMed: 4934072]
315. Metzger S, Dror IB, Aizenman E, Schreiber G, Toone M, Friesen JD, Cashel M, Glaser G. *J Biol Chem.* 1988; 263:15699. [PubMed: 2844820]
316. Sippel A, Hartmann G. *Biochim Biophys Acta.* 1968; 157:218. [PubMed: 4868249]
317. Kassavetis GA, Kaya KM, Chamberlin MJ. *Biochemistry.* 1978; 17:5798. [PubMed: 365234]
318. Herring CD, Raffaele M, Allen TE, Kanin EI, Landick R, Ansari AZ, Palsson BO. *J Bacteriol.* 2005; 187:6166. [PubMed: 16109958]
319. Sasse-Dwight S, Gralla JD. *J Biol Chem.* 1989; 264:8074. [PubMed: 2722774]
320. Grainger DC, Hurd D, Harrison M, Holdstock J, Busby SJ. *Proc Natl Acad Sci U S A.* 2005; 102:17693. [PubMed: 16301522]
321. Norris V, Blaauwen TD, Doi RH, Harshey RM, Janniere L, Jimenez-Sanchez A, Jin DJ, Levin PA, Mileykovskaya E, Minsky A, Misevic G, Ripoll C, Saier M, Skarstad JK, Thellier M. *Annu Rev Microbiol.* 2007; 61:309. [PubMed: 17896876]
322. Lyubchenko YL, Shlyakhtenko LS, Aki T, Adhya S. *Nucleic Acids Res.* 1997; 25:873. [PubMed: 9016640]

Biographies

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Figure 1. Macromolecular crowding in *E. coli* cytoplasm. (A) List of the 50 major proteins found in *E. coli* cytoplasm (~85% in mass of the total cytoplasm). (B) Modeling of *E. coli* cytoplasm using the content in (A). Reproduction from McGuffee & Elcock⁶⁰.

Figure 2.

The nucleoid is hyper-condensed shortly after high salt shock. Nucleoid and nucleoid/cell overlay of *E. coli* cells before (LB), 20 min and 45 min after induction of an osmotic shock by 0.5M NaCl. The nucleoid initially becomes hyper-condensed and then gradually re-expanded to a pre-stress state. The nucleoid is stained with Hoescht 33342. The scale bar represents 2 μm . Adapted from Cagliero & Jin¹³¹.

		Nucleoid	Nucleoid / Cell	Doubling time	Avg. RNS	Cell size (W x L)
37°C	LB			20 min.	0.55	0.7-1.7 3.5-10.7
	MOPS RD			25 min.	0.64	0.4-1.3 3.2-7.5
	MOPS glycerol			93 min.	0.74	0.5-1.2 2.0-3.3
	LB O/N			-	0.70	0.7-1.1 1.0-2.1
30°C	LB			45 min.	0.56	0.6-1.4 3.0-6.8
	MOPS RD			53 min.	0.62	0.5-1.5 2.8-7.6
	MOPS glycerol			168 min.	0.63	0.5-1.2 1.2-3.6

Figure 3.

Cell size and nucleoid structure are sensitive to growth rate and media. Nucleoid and nucleoid/cell overlay of exponential phase cells (*rpoC-venus*) growing in different media (LB, MOPS rich defined (RD), MOPS glycerol) at different temperatures (37°C or 30°C) or in stationary phase cells growing in LB at 37°C overnight (LB, O/N). The nucleoid is stained with Hoescht 33342. Cell size represents the range of cell width (W) and cell length (L) in a population of unsynchronized cells. The scale bar represents 2µm.

Figure 4.

Growth-rate dependence of transcription foci in the *rpoC-venus* cells grown at 37°C. (A) RNAP, nucleoid, RNAP/nucleoid overlay and nucleoid/cell overlay (RNS value is indicated) of cells grown in different media (LB, MOPS rich defined (RD) and MOPS glycerol; the growth rates are indicated). In the RNAP/nucleoid overlay, the RNAP is false colored in green and the nucleoid is false colored in red. The scale bar represents 2 μ m. (B) The number of transcription foci per cell in a population (about 100 cells) of asynchronous cells grown in different media. The number of transcription foci decreases as the nucleoid expands and the available nutrients in the media decrease leading to reduced growth rates.

Figure 5.

(A) Position of the *rrn* operons, *oriC* and *ter* regions in the *E. coli* chromosome map. The red arrows represent each of the seven *rrn* operons, all of which are localized in the *oriC* proximal half of the chromosome and four of them are near the *oriC*. Transcription of *rrn* occurs in the same direction as replication. Adapted with modification from Jin *et al.*¹⁸⁶. (B) Typical cell cycle of *E. coli* growing in rich media (LB). The red dots represent the multiple copies of *oriC* in the cell. The C period (period of replication) or the D period (termination and post replication period) is also represented. Multiple rounds of replication initiation increase the gene dosage of *oriC* (8 to 16 *ori*) and the number of nascent nucleoids (4 nucleoids). Adapted from Nielsen *et al.*¹⁰⁰. The calculated numbers of replication forks, *oriC* and *rrn* as well as the DNA content of the genomes in a fast growing cell with a doubling time of 20 min are listed.

Figure 6.

Chromatin spread of *rrnB-rrnE* region from cells grown in LB. (A) Large view of the *rrnB-rrnE* region. The *rrnB* and *rrnE* operons are easily distinguished due to their heavy transcription. The other highly transcribed genes (*tufB*, *rplKALJL*, *rpoBC*) contain on average only 1 RNAP per Kb. The effect of the transcription attenuator between the *rplKALJL* operon and *rpoBC* operon is visible by a drop in the number of RNAP. The rest of the DNA (between *rpoBC* and *rrnE*) contains much less RNAP with only one per 10 to 20 Kb. (B) Higher magnification of the *rrnB* operon and *tufB* gene. The double Christmas tree is visible. There is on average one RNAP per 70 bp in each *rrn* operon. Reproduction with permission from French & Miller ⁴⁷.

Figure 7.

The transcription foci disperse accompanied by nucleoid expansion during the amino-acid-starvation induced stringent response in wild type (*rpoC-venus*). RNAP, nucleoid, RNAP/nucleoid overlay and nucleoid/cell overlay of cells growing in LB or in LB + serine hydroxamate (SHX, 30 min and 120 min) at 37°C after the induction of the stringent response. In the RNAP/nucleoid overlay, the RNAP is false colored in green and the nucleoid is false colored in red. The scale bar represents 2 μm .

Figure 8.

The stringent RNAP mutant cells grown in LB behave like wild type during the amino-acid-starvation induced stringent response. RNAP, nucleoid, RNAP (green)/nucleoid (red) overlay and nucleoid/cell overlay of the *rpoB3443* (L533P) mutant cell containing *rpoC-venus* grown in LB at 37°C. The cell has no visible transcription foci and an expanded nucleoid. Cell size represents the range of cell width (W) and cell length (L) in a population (about 100 cells) of unsynchronized cells. The doubling time of this mutant strain is 40 min. The scale bar represents 2 μ m.

Figure 9.

The presence of multiple *rrn* operons is critical for the formation of transcription foci and nucleoid compaction. RNAP, nucleoid, RNAP (green)/nucleoid (red) overlay and nucleoid/cell overlay of the $\Delta 6rrn$ mutant cell containing *rpoC-venus* that has only one *rrn* operon (*rrnC*) remaining on the chromosome grown in LB at 37°C. There are no apparent transcription foci and the nucleoids are expanded in the mutant cells. Cell size represents the range of cell width (W) and cell length (L) in a population (about 100 cells) of unsynchronized cells. The doubling time of this mutant strain is 34 min. The scale bar represents 2 μm .

Figure 10.

The transcription foci are apparent and the nucleoids are hyper-condensed in the “relaxed” *reIA* mutant cells during amino acid starvation induced by SHX. RNAP, nucleoid, RNAP/nucleoid overlay and nucleoid/cell overlay of the mutant cells containing *rpoC-venus* grown in LB or in LB + SHX for 30 min at 37°C. In the RNAP/nucleoid overlay, the RNAP is false colored in green and the nucleoid is false colored in red. The scale bar represents 2 μ m.

Figure 11.

The hyper-condensed nucleoids in chloramphenicol-treated cells depend on active transcription. RNAP, nucleoid, RNAP/nucleoid overlay and nucleoid/cell overlay of wild type cells containing *rpoC-venus* after chloramphenicol treatment for 30 min or sequential treatment of chloramphenicol for 30min followed by an additional rifampicin treatment for 60 min. The hyper-condensed nucleoids and the transcription foci are apparent in chloramphenicol-treated cells. With the additional rifampicin treatment, however, the foci disappear and the nucleoids expand. In the RNAP/nucleoid overlay, the RNAP is false colored in green and the nucleoid is false colored in red. The scale bar represents 2 μm .

Figure 12.

The nucleoids released from lysed cells highlight the presence of RNAP on the peripheral DNA loops. The nucleoids of exponential phase cells grown in LB or after an osmotic stress (0.5M NaCl) for 20 min and 60 min are isolated and imaged. RNAP, nucleoid and overlay of RNAP (green) and nucleoid (red) are shown. In LB, the transcription foci appear to be located in the voids or low density regions of the compact nucleoids. After induction of the osmotic response, the initially released RNAP re-associates to DNA loops at the periphery of the hyper-condensed nucleoid, forming a sphere of RNAP surrounding the nucleoid. After 60 min, the nucleoids approximate the pre-stress state and RNAP is enriched at the periphery of the nucleoids. The scale bar represents 2 μ m. Reproduced from Cagliero & Jin¹³¹.

Figure 13.

Inhibition of transcription by rifampicin causes RNAP to evenly scatter across the nucleoid and the nucleoid to fully expand. RNAP, nucleoid, RNAP/nucleoid overlay and nucleoid/cell overlay of wild type cells after rifampicin treatment for 30 min. In the RNAP/nucleoid overlay, the RNAP is false colored in green and the nucleoid is false colored in red. The scale bar represents 2 μm .

Nutrient poor, starvation or transcription inhibition

- Minimal or no *rrn* synthesis
- Genome-wide distribution of RNAP
- Nucleoid expanded

11

Nutrient rich media

- High *rrn* synthesis
- Formation of the putative nucleolus structure(s)
- Nucleoid moderately condensed

11

Optimal growth in LB

- Highest *rrn* synthesis
- Formation of the transcription hub networking highly transcribed genes from distant loops
- Nucleoid further condensed

Figure 14.

A model illustrating how transcription foci compact the nucleoid in fast growing cells in LB. The nucleoid organization is dynamic and sensitive to the pattern of RNAP distribution which responds to changes in growing environments. The *E. coli* chromosome is represented as blue lines folded in loops, the *ori* of replication as a black square, the seven rRNA operons as large red circles with letters, and two representative tRNA operons as small red circles. The RNAP molecules are represented as small green circles. In very slow growing or nutrient starved cells (top), RNAP distributes relatively evenly across the nucleoid for broad global gene expression leading to the full “open” state of the nucleoid or nucleoid expansion. Inhibition of transcription by rifampicin has a similar effect on the nucleoid because RNAP scatters across the nucleoid homogeneously. During rapid growth (middle), RNAP is concentrated at the putative nucleolus structures (~1% of the genomic DNA) and biased for rRNA synthesis, leaving only a small fraction of RNAP in the vast remaining regions of the nucleoid, leading to nucleoid compaction. For simplicity, only two nucleolus-like structures which make the nucleoid more compact by pulling different *rrn* operons (red cluster) into proximity in a nascent nucleoid are shown. During optimal growth (bottom), the transcription foci represent transcription hubs networking with the transcription of other genes located in distant loops, further condensing the nucleoid. The transcription hub is indicated. See text for details.

Table 1RNAP distribution in cells harboring plasmid-borne derivatives of *rrn* Adapted from Cabrera & Jin ²³⁷

Plasmid ^a	<i>rrnB</i> (transcript length [kb])	RNAP distribution ¹¹
pBR322	None
pNO1301	<i>rrnB</i> P1-P2-16S-tRNAs-23S-5S (5.4)
pNO1302	<i>rrnB</i> P1-P2-16S - 23S-5S (2.7)
pDJ2791A	<i>rrnB</i> P1-P2-16S (1.8)
pDJ2791B	<i>rrnB</i> P1-P2-16S (1.0)
pDJ2754-11	<i>rrnB</i> P1-P2 -16S-tRNAs-23S-5S (0)
pDJ2754-17	<i>rrnB</i> P1-16S-tRNAs-23S-5S (5.4)
pDJ2754-15	<i>rrnB</i> P2-16S-tRNAs-23S-5S (5.4)
pDJ2845	<i>Ptac</i> -16S (1.8)

^a All plasmids are pBR322 derivatives.^b The cell is represented by a rounded rectangle, the nucleoid by an oval. The green represents the RNAP distribution.