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MINI REVIEW

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Live to fight another day: The bacterial nucleoid under stress

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Abstract

The bacterial chromosome is both highly supercoiled and bound by an ensemble of proteins and RNA, causing the DNA to form a compact structure termed the nucleoid. The nucleoid serves to condense, protect, and control access to the bacterial chromosome through a variety of mechanisms that remain incompletely understood. The nucleoid is also a dynamic structure, able to change both in size and composition. The dynamic nature of the bacterial nucleoid is particularly apparent when studying the effects of various stresses on bacteria, which require cells to protect their DNA and alter patterns of transcription. Stresses can lead to large changes in the organization and composition of the nucleoid on timescales as short as a few minutes. Here, we summarize some of the recent advances in our understanding of how stress can alter the organization of bacterial chromosomes.

KEYWORDS
Dps, nucleoid, prokaryote, stationary phase, stress

1 | THE NUCLEOID IN THE EXPONENTIAL PHASE

The bacterial nucleoid is typically the largest identifiable structure in the bacterial cytoplasm and is comprised of thousands of proteins and RNA strands bound to the chromosome. While this review encompasses multiple bacterial species, much of our knowledge of the nucleoid comes from studies of *Escherichia coli*, and the results cited herein refer to *E. coli* unless specifically noted. Nucleoids adopt a hierarchical organization (Lioy et al., 2018). At the smallest scales, we observe wrapping and bending of DNA by nucleoid associated proteins (NAPs). Transcription by RNA polymerase along with the binding of NAPs serves to separate the DNA into distinct regions of continuous supercoiling density termed supercoil domains (SDs) with an average size of 10–20 kilobases (kb) (Shen & Landick, 2019). These SDs are further organized into 30–400 kb chromosome interaction domains (CIDs), which are groups of SDs identified in Hi-C studies that contact each other much more frequently than SDs in neighboring CIDs (Shen & Landick, 2019). On a broader scale, CIDs are then organized into macrodomains that span 500–1000 kb and adopt largely fixed positions within the cell (Valens et al., 2004) Contact maps show that regions of DNA from different macrodomains rarely interact with each other (Lioy et al., 2018; Valens et al., 2004). At the largest scale, the entire nucleoid adopts a helical ellipsoid shape with density decreasing radially from the center of the cell (Fisher et al., 2013).

During exponential phase, the nucleoid of *E. coli* is primarily organized by several major NAPs. The most abundant of these are factor for inversion stimulation (Fis), Histone-like protein from strain U93 (HU), histone-like nucleoid structuring protein (H-NS), and suppressor of td mutant phenotype A (StpA) (Talukder & Ishihama, 2015). Each of these proteins contribute to the nucleoid structure via distinct DNA-binding mechanisms. Fis is the most abundant NAP during exponential phase, binding to sequence-specific targets throughout the genome. Fis bends DNA as it binds and has been proposed to stabilize plectonemes (Dame et al., 2020). HU binds nonspecifically to DNA, with a preference for naturally bent regions of DNA. HU creates a bend in the DNA where it binds and can form larger complexes that inhibit transcription (Amemiya et al., 2021). H-NS is an NAP that preferentially binds to AT-rich regions and can nucleate and spread along DNA at higher-affinity binding sites, contributing to modulating protein transcription as well as constraining supercoils (Cristofalo et al., 2020). H-NS can form either linear filaments or more dense bridged complexes with DNA depending on temperature and osmolarity (Dame

et al., 2020). StpA binds DNA in a similar manner, although with much tighter affinity than H-NS and without a preference for bent DNA (Keatch, 2005). StpA acts to constrain DNA supercoiling and can protect DNA from double strand DNAses and has also been shown to bind RNA and affect splicing, potentially coordinating transcription and translation (Lucchini et al., 2009).

Several additional NAPs are present in the nucleoid at lower levels during exponential phase, including DNA-binding protein from starved cells (Dps) and integration host factor (IHF). Dps forms homooligomeric dodecamers that bind to DNA nonspecifically (Azam & Ishihama, 1999; Martinez & Kolter, 1997). IHF shares homology to HU but binds to specific sequences and creates a much larger U-turn bend in the DNA (Dillon & Dorman, 2010). With this brief overview in mind, this review will focus on recent new data highlighting how the nucleoid changes in response to different forms of stress.

2 | NUTRIENT DEPRIVATION

Nutrient deprivation in bacteria leads to the onset of stationary phase, characterized by slower cell division rates accompanied by a suite of transcriptional and physiological changes. In stationary phase the nucleoid is reorganized on a global level, becoming more compact (Janissen et al., 2018), increasing the number of long range contacts (Lioy et al., 2018), and altering patterns of supercoiling (Lal et al., 2018). In *E. coli*, the general stress response regulator sigma(S) is upregulated during stationary phase, altering the expression of hundreds of genes including several that are central to nucleoid maintenance. Several NAPs that are highly abundant in the nucleoid in exponential phase decrease in abundance in stationary phase, including Fis, Hfq, and HU (Azam & Ishihama, 1999). In the case of Fis this change is particularly marked, with the concentration of Fis decreasing by 500–1000-fold. Concurrently, several other NAPs increase in abundance, with Dps exhibiting the largest increase in concentration during stationary phase.

In *E. coli*, accumulation of polyphosphates can slow growth through limiting cytoplasmic magnesium availability (Rudat et al., 2018). In *Pseudomonas aeruginosa*, cell cycle exit and concomitant entrance into stationary phase have been shown to be associated with the development of polyphosphate granules throughout the nucleoid (Racki et al., 2017). These granules, which rely on transcriptional regulatory protein AlgP for spatial positioning in the cell (Chawla et al., 2022), form three-component, phase-separated droplets with DNA and Hfq as a potential method for modulating transcription and translation of specific genomic elements (Beaufay et al., 2021). Polyphosphate granules attenuate Hfq binding to DNA, causing it to target AT-rich sites, further modulating transcription and specifically targeting mobile genetic elements and prophages. The widespread transcriptional repression caused by polyphosphate granules has resulted in the proposal that polyphosphate can act as a form of "bacterial heterochromatin" (Beaufay et al., 2021).

By mid-to-late stationary phase, Dps concentration has increased so dramatically that it becomes the third most abundant protein in the cell, dominating the architecture of the stationary-phase nucleoid (Figure 1). Dps performs two primary biochemical functions within bacterial cells (Calhoun & Kwon, 2011). Dps binds and condenses the nucleoid, associating with DNA via unstructured N-terminal DNA binding domains that line the periphery of the dodecamer and protrude into solution (Antipov et al., 2017; Grant et al., 1998; Roy et al., 2007). Additionally, Dps dodecamers have ferroxidase active sites that are capable of mineralizing and sequestering iron atoms within the core of the dodecamer (Orban & Finkel, 2022). These two functions are biochemically separable and both independently contribute to preserving cell viability and protecting the DNA from damage during stationary phase (Karas et al., 2015).

Early studies of the condensates formed by Dps and DNA showed highly regular, dense arrays termed biocrystals, both in vivo and in vitro (Dadinova et al., 2019; Frenkiel-Krispin, 2001; Wolf et al., 1999). These crystalline structures have primarily been studied under conditions of Dps overexpression, which can produce large and regular repeating patterns. Dps from wild-type cells in stationary phase can form smaller biocrystalline regions within their nucleoids as well. Recent results indicate that Dps:DNA condensates can adopt a variety of crystal packing states as well as other non-crystalline morphologies in stationary phase with different levels of Dps expression (Chesnokov et al., 2023; Loiko et al., 2020). Therefore, the packing of Dps and DNA can vary considerably within the broad umbrella of stationary phase conditions.

2.1 | Diverse morphologies of Dps in stationary-phase condensates

Our knowledge of the molecular-scale details of packing of Dps and DNA within condensates comes primarily from in vitro studies using small angle X-ray scattering (SAXS) and cryo-electron tomography (CET) (Dadinova et al., 2019; Dubrovin et al., 2021). In some condensates, SAXS measurements are consistent with liquid-crystalline phases (Dadinova et al., 2019). SAXS measurements also provide evidence that the N-terminal regions of the Dps subunits can extend away from the dodecamer and are highly flexible, potentially allowing them to bind in multiple configurations. Early studies of Dps:DNA condensates demonstrated a pseudohexagonal crystal packing of Dps dodecamers (Grant et al., 1998). In these structures, the path of the DNA could not be resolved. More recent CET studies have resolved structures that have crystalline packing, with unit cells that include both DNA and Dps densities (Chesnokov et al., 2023). This increased resolution has allowed the identification of multiple crystal packing geometries in different condensates: a densely packed triclinic unit cell that allows four parallel DNA strands to contact each dodecamer, a more loosely packed cubic unit cell that allows each dodecamer to bind six DNA strands arranged in three

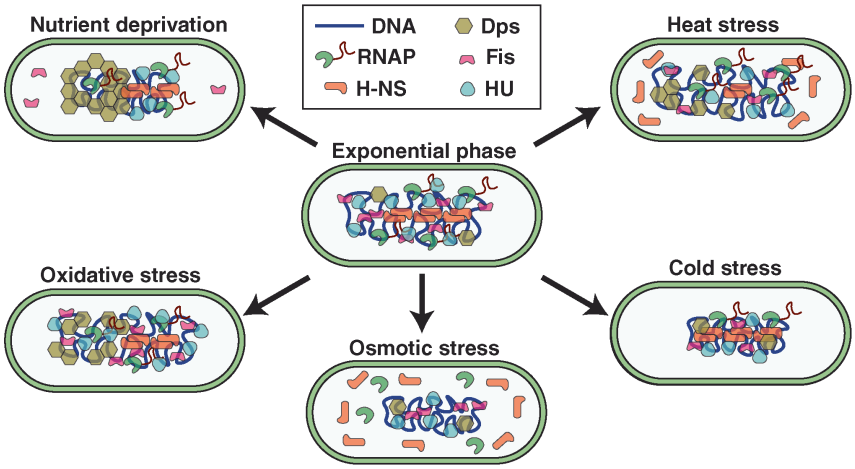

FIGURE 1 The nucleoid dynamically responds to environmental stresses with different responses. The exponential phase nucleoid (center) is condensed by NAPs such as Fis, HU, and H-NS. After nutrient deprivation (upper left), *E. coli* DNA shows further condensation. The amount of bound Dps increases dramatically while the amount of bound Fis is greatly reduced. Under oxidative stress (lower left) Dps bound to the DNA prevents damage, Fis remains bound, and compaction has not been detected to change. In *S. aureus*, which lacks a Fis paralog, Dps homolog MrgA has been observed to further condense DNA similar to what is observed under nutrient deprivation. Under hyperosmotic shocks (lower center), DNA transiently becomes condensed while RNAP and H-NS are relocalized to the cell periphery. Cold shocks (lower right) also condense the nucleoid, possibly due to an increase in plectonemic supercoiling. Heat shock (upper right) causes H-NS bridging complexes to deoligomerize while bound Dps protects the DNA.

orthogonal pairs, and a hexagonal packing that allows each dodecamer to bind six strands of parallel DNA. A recent study also identified cylindrical assemblies with pseudo-crystalline packing of Dps dodecamers around curved DNA strands (Chesnokov et al., 2023). Taken together, the range of packing geometries observed speaks to the structural flexibility of interactions between Dps and DNA.

These diverse packing geometries observed on the molecular scale can give rise to a variety of higher-order structures in the nucleoid as well. In late-stationary phase cells overexpressing Dps, Dps and DNA have been shown to form large biocrystals (Wolf et al., 1999). In early stationary phase, the same strain of cells produced toroidal nucleoid assemblies (Frenkiel-Krispin et al., 2004). More recently, evidence has emerged suggesting that Dps and DNA can form liquid-like condensates under appropriate conditions (Gupta et al., 2022, 2023). Gupta et al. reported spherical droplets formed by genomic salmon sperm ssDNA and Dps in vitro. These droplets showed a high degree of internal mobility based on fluorescence recovery after photobleaching (FRAP) analysis. These data raise the possibility that Dps could organize the nucleoid into phase-separated liquid droplets in vivo. This organization would be consistent with transcriptional studies showing that RNAP can transcribe DNA condensed by Dps without any noticeable decrease in activity (Janissen et al., 2018).

Diverse morphologies of Dps:DNA condensates have recently been reported in heavily aged *E. coli* cells starved for 7 months (Loiko et al., 2020). These morphologies have been described by the authors as "nanocrystalline", "liquid-crystalline", and "folded nucleosome-like". Nanocrystalline condensates were frequently observed in conditions of Dps overexpression, consistent with previous studies. Liquid-crystalline condensates were observed in all types of cells, including Dps-overexpressing cells, wild-type cells, and *dps*- cells. In cells expressing Dps, energy-dispersive X-ray (EDX) analysis indicated that Dps was present in these liquid-crystalline condensates. This morphology resembles the cholesteric phase previously observed in *dps*- cells in late stationary phase (Frenkiel-Krispin, 2001). The third morphology was characterized by numerous spherical condensates of roughly uniform size (~30nm) that resembled DNA wrapped around a protein core, which the authors compared to nucleosome-DNA complexes. Elemental analysis indicated that Dps was associated with these spherical structures. Given the wide range of observed structures formed by Dps and DNA, further research is needed to identify which conditions promote the distinct structural morphologies and how each of these structures can affect cell physiology.

The next steps for studying nucleoid condensation during stationary phase should focus on the causative factors underlying the creation of the different structures formed by Dps and DNA, and whether the specific morphologies formed depend solely upon the concentrations of Dps and DNA, or other cellular or extracellular factors. Additionally, the influence that these different DNA structures have on protein mobility and access to genomic DNA within the cell should also be investigated. The varying crystallinity of nucleoid morphologies in starved cells could have a differential impact on transcriptional patterns and the activities of other DNA-binding proteins, as well as affecting the degree of protection of DNA integrity against external stressors.

3 | OXIDATIVE STRESS

Reactive oxygen species (ROS) can act as a damaging stressor for bacteria, resulting in direct damage to DNA, RNA, membranes, and

proteins. ROS can arise from many sources, including cellular respiration, exposure to metal ions, and radiation. DNA is particularly susceptible to damage from ROS produced by the reaction of H_2O_2 and iron(II) through Fenton chemistry (Imlay et al., 1988). Bacteria respond to oxidative stress in several ways, including enzymatic removal of ROS from the cell and repair of damage caused by the ROS (Farr & Kogoma, 1991).

Bacteria can modify their genomes in response to the ROS. In *E. coli*, Dps plays a major role in the oxidative stress response. Cells lacking the *dps* gene or containing mutations that reduce its affinity for DNA or its ferroxidase activity were more susceptible to damage from both iron(II) and H_2O_2 (Karas et al., 2015). In *E. coli*, Dps expression is upregulated by the transcriptional regulator OxyR, which senses H_2O_2 and disulfide stress, and the sRNA DsrA, both of which are key regulators of the oxidative stress response (Zhu et al., 2023). In addition, Fis and H-NS have both been shown to bind to the Dps promoter and prevent initiation by RNAP containing σ^{70}, the primary exponential phase sigma factor. However, neither NAP prevents initiation mediated by σ^{38}, which is upregulated in response to multiple stresses including oxidative stress (Grainger et al., 2008). In *Bacillus subtilis* and *Staphylococcus aureus*, Dps homolog MrgA also plays a significant role in the oxidative stress response. MrgA is activated by PerR, which responds to both H_2O_2 and Fe(II), and MrgA has both DNA binding and iron binding capability (Chen et al., 1995; Chen & Helmann, 1995; Horsburgh et al., 2001). The Fe(II) scavenging ability of MrgA prevents the occurrence of H_2O_2-related DNA damage, as H_2O_2 mediated killing occurs through interacting with Fe(II) via the Fenton reaction (Chen & Helmann, 1995; Imlay & Linn, 1986). *S. aureus* MrgA also displays similar regulation to *B. subtilis* MrgA (Morikawa et al., 2006).

Moreover, single cells exposed to H_2O_2 showed activation of the *dps* promoter proportional to the concentration of H_2O_2 (Martino et al., 2016). In *S. aureus*, the nucleoid is compacted in response to oxidative stress in a manner dependent on the Dps homolog MrgA (Morikawa et al., 2006) (Figure 1). MrgA protects cell viability during oxidative stress, and this protection is dependent on both the ferroxidase activity of the enzyme as well as its DNA-binding ability (Ushijima et al., 2014). Interestingly, Dps has also been shown to be able to compact DNA in response to oxidative stress, but only if Fis were deleted (Ohniwa et al., 2006).

The next steps for studying the nucleoid response to oxidative stress should include understanding the protection offered through DNA binding by Dps and Dps-like proteins such as MrgA in response to oxidative stressors. Given that the DNA-binding activity of Dps is necessary to achieve wild-type levels of oxidative stress resistance (Karas et al., 2015), it is somewhat unexpected that Dps does not appear to condense DNA during oxidative stress to the same extent observed in stationary phase cells (Ohniwa et al., 2006). Given the recent evidence that Dps can produce diverse binding morphologies cited above, it is possible that Dps binds DNA in a different manner or extent during oxidative stress compared to stationary phase. Several species, including *B. subtilis*, *B. anthracis*, *and D. radiodurans*, have two or more Dps paralogs, which raises the question of how these paralogs are differentially expressed and what distinct biochemical roles these different paralogs may play in the cells (Antelmann et al., 1997; Kim et al., 2006; Orban & Finkel, 2022; Papinutto et al., 2002). Additionally, any effect on the ability of DNA repair machinery to repair DNA while it is condensed should also be identified.

4 | OSMOTIC STRESS

Osmotic shock describes the response of bacteria to sudden changes in solute concentrations surrounding the cell. Plunging bacteria into a high salt environment causes water to leave the cells via osmosis (Bremer & Krämer, 2019). Conversely, a sudden decrease in solute concentrations in the environment can force cells to quickly take in water. Both these forms of stress negatively impact cell physiology. Bacteria have therefore developed a range of countermeasures to adapt to osmotic stress, including the accumulation of osmoprotectants to rebalance the pressure on the cell (Csonka & Hanson, 1991).

Until osmotic adaptation takes place, the nucleoid undergoes major changes in response to osmotic stress. Rapid increases in cytoplasmic potassium associated with osmotic shock have been observed to cause RNA polymerase (RNAP) to quickly dissociate from the nucleoid (Cagliero & Jin, 2012). As the RNAP dissociates, the nucleoid abruptly condenses, causing much of the nucleoid to concentrate in the center of the cell while RNAP diffuses throughout the cytoplasm (Cagliero & Jin, 2012) (Figure 1). It is unclear whether the compaction of the nucleoid is a cause or consequence of the dissociation of RNAP, but neither Dps nor IHF were shown to affect the osmotically-induced compaction (Cagliero & Jin, 2012).

A similar pattern was observed in a different study tracking the distribution of the H-NS in stationary phase cells exposed to hyperosmotic shock. The nucleoid became more compact and H-NS moved to the periphery, a behavior that was not observed in exponential-phase cells exposed to hyperosmotic shock (Rafiei et al., 2019). In this case, the osmotic-shock-induced redistribution of H-NS was shown to depend on the overall level of supercoiling in the nucleoid, as the stationary-phase behavior could be reconstituted in exponential phase when gyrase was inhibited. This response to hyperosmotic shock is likely due to a conformational change in H-NS from an open state to a closed state, which modulates its ability to form bridging interactions (van der Valk et al., 2017). Recently it was shown that this change in ability to form bridging interactions allows H-NS to modulate the local three-dimensional chromatin structure near the osmoresponsive *proVWX* operon in response to hyperosmotic shock. This change in the local architecture of the nucleoid was observed to modulate transcriptional activity of the operon, analogous to eukaryotic chromosome remodeling (Rashid et al., 2023).

The differential responses of the bacterial nucleoid to osmotic stress highlight areas for further study. Future work should focus on uncovering the factors driving DNA condensation during osmotic stress response, as well as the mechanism underlying its sensitivity

to DNA supercoiling, and whether the changes in H-NS DNA binding are sufficient to induce the osmoprotective response.

5 | THERMAL STRESS

Sudden changes in temperature will alter the viscosity of the bacterial cytoplasm and membranes as well as the kinetics of transcription and translation (Di Bari et al., 2023; Oliveira et al., 2016). Over long periods of time, the cell responds by altering its growth rate, but on short time scales many transient transcriptional changes are observed. In *E. coli*, half of the genes repressed in the short-term response to cold shocks were also sensitive to gyrase inhibition (Dash et al., 2022). In addition, the nucleoid density was shown to increase (Figure 1), and gyrase became more colocalized with the nucleoid. These results support the hypothesis that a transient decrease in negative supercoiling plays a major role in the cold shock response (Dash et al., 2022).

During periods of heat shock, two NAPs have been shown to aid the cell. Dps confers no measurable advantage in fitness during cold shock, but greatly aids in survival during heat shock (Karas et al., 2015; Nair & Finkel, 2004). In addition to protecting the DNA from damage, Dps has been hypothesized to play a role as a molecular chaperone, preventing proteins from undergoing thermally-induced aggregation (Park et al., 2023) (Figure 1). In addition, elevated temperatures reduce the size of large H-NS oligomers. Oligomerization is necessary for strong DNA binding by H-NS, so reduced oligomerization of H-NS at higher temperatures provides a mechanism to alter the transcription of temperature-sensitive genes (Lukose et al., 2024; Ono et al., 2005).

While several changes to the nucleoid in response to thermal stress have recently been identified, the next steps should be to further elucidate the mechanical rationales driving these changes. The portfolio of potential cellular clients of Dps molecular chaperone activity have yet to be identified, as well as the structural effects of reduced H-NS oligomerization on its interaction with DNA-binding motifs. In addition, the cause of the transient change in DNA supercoiling during cold shock is yet undetermined, as well as the global impact of DNA supercoiling on nucleoid architecture and access to transcriptional factors.

6 | CONCLUSIONS

Although the bacterial nucleoid was first observed nearly seventy years ago (Mason & Powelson, 1956), the small size of bacteria made it difficult to study nucleoid organization for many decades. Recent advances in chromosome conformation capture, high resolution fluorescence microscopy, electron microscopy, and atomic force microscopy have greatly advanced our ability to study the nucleoid at scales ranging from microns to nanometers (Dame & Tark-Dame, 2016). Our knowledge of nucleoid organization has therefore progressed considerably in the past decade. However, much of our knowledge is still based on studies of *E. coli* in exponential phase. Here we have summarized some of the recent results from studies of bacterial nucleoids under stress. These results serve to highlight the dynamic nature of the nucleoid and the importance of nucleoid organization in preserving cell viability. More research is needed to explore the full scope of changes the nucleoid undergoes in response to different stresses in a variety of bacterial species, as well as to link how these changes affect cell viability and physiology in harsh conditions. Greater insight into nucleoid dynamics during bacterial stress responses could provide useful tools for regulating nucleoid structure, leading to the creation of novel bacterial strains with controllable viability and tunable gene expression under a range of environmental conditions.

AUTHOR CONTRIBUTIONS

Azra M. Walker: Writing – original draft; investigation; writing – review and editing; conceptualization. **Elio A. Abbondanzieri:** Writing – review and editing; investigation; supervision; visualization; conceptualization. **Anne S. Meyer:** Writing – review and editing; supervision; project administration; funding acquisition; conceptualization.

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DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ETHICS STATEMENT

No ethical considerations apply to this work.

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