Species-specific supercoil dynamics of the bacterial nucleoid

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Abstract Bacteria organize DNA into self-adherent conglomerates called nucleoids that are replicated, transcribed, and partitioned within the cytoplasm during growth and cell division. Three classes of proteins help condense nucleoids: (1) DNA gyrase generates diffusible negative supercoils that help compact DNA into a dynamic interwound and multiply branched structure; (2) RNA polymerase and abundant small basic nucleoid-associated proteins (NAPs) create constrained supercoils by binding, bending, and forming cooperative protein–DNA complexes; (3) a multi-protein DNA condensin organizes chromosome structure to assist sister chromosome segregation after replication. Most bacteria have four topoisomerases that participate in DNA dynamics during replication and transcription. Gyrase and topoisomerase I (Topo I) are intimately involved in transcription; Topo III and Topo IV play critical roles in decatenating and unknotting DNA during and immediately after replication. RNA polymerase generates positive (+) supercoils downstream and negative (–) supercoils upstream of highly transcribed operons. Supercoil levels vary under fast versus slow growth conditions, but what surprises many investigators is that it also varies significantly between different bacterial species. The MukFEB condensin is dispensable in the high supercoil density (σ) organism *Escherichia coli* but is essential in *Salmonella* spp. which has 15 % fewer supercoils. These

observations raise two questions: (1) How do different species regulate supercoil density? (2) Why do closely related species evolve different optimal supercoil levels? Control of supercoil density in *E. coli* and *Salmonella* is largely determined by differences encoded within the gyrase subunits. Supercoil differences may arise to minimize toxicity of mobile DNA elements in the genome.

Keywords Gyrase · Topoisomerase · Nucleoid-associated proteins · Supercoil density · MukFEB condensin

Introduction

Progress in understanding the structure/function mechanics of the bacterial chromosome greatly improved with development of techniques that allow real time visualization of bacterial nucleoids inside living cells and the ability to follow specific chromosomal loci using fluorescent labels (Kleckner et al. 2014; Wang et al. 2008). Nucleoids are self-adherent filaments stochastically organized locally into approximately 10-kb domains in growing cells (Cunha et al. 2005; Espeli et al. 2008; Hadizadeh Yazdi et al. 2012; Postow et al. 2004; Stein et al. 2005; Wiggins et al. 2010). The 4-Mb circular *Escherichia coli* chromosome is the best studied nucleoid in prokaryotic biology. In this review I focus on DNA condensation that exploits negative supercoiling to produce a highly interwound plectonemic network of DNA. This network exhibits two forms of movement that will juxtapose two points along the network over distances of 10 to 100 kb, depending on cell growth conditions. These movements are slithering, which is a reptilian-like sliding of the interwound network, and branching, which is a rotational movement that causes segments to extrude and be resorbed along a fiber axis (Higgins et al. 1996) (Fig. 1). Supercoil diffusion is critical for many

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Fig. 1 Structure and sequence alignment of DNA gyrase A and B genes (*GyrA*, *GyrB*) from *Salmonella* and *Escherichia coli*. **a** For *GyrA* the catalytic tyrosine (Y122) is shown in purple. There are 77 positions with amino acid substitutions between the species (black lines). The reference sequence is *Salmonella* *GyrA*, which is 3 amino acids longer than *E. coli* *GyrA* and terminates at 875 amino acids. **b** *GyrB* is more highly conserved than *GyrA*, with the two proteins differing at only 28 positions in this 804-amino acid protein. Domains for *GyrA* include the DNA Gate, the C Gate, and the CTD Chiral DNA Binding elements. For *GyrB* domains include the ATP Gate, Metal Binding site,

and *GyrA* Gate interaction region. **c**. Long-range diffusion mechanisms of negative supercoiling is illustrated for a segment of DNA that includes two directly repeated recombination sites for the $\gamma\delta$ resolvase (*Res*). One *Res* dimer binds to three DNA sites, numbered I (blue), II (red), and III (yellow). Branch extrusion (**c**, left) or reptilian slithering (**c**, center) move the two *Res* sites into a three-node tangle. (**c**, far right). The products of recombination are two catenated circles (**c** right). In vivo-catenated links are separated by topoisomerase IV (Topo IV)

biochemical reactions of the chromosome, including site-specific recombination, transcription, transposition of mobile elements, and initiation of DNA replication (Higgins and Vologodskii 2015). Yet this DNA movement is not discernable in real time, even with the highest resolution techniques currently available. I discuss the control of supercoil density in two closely related species, *E. coli* and *Salmonella* Typhimurium. These organisms have essentially identical tool kits of proteins that generate diffusible and constrained supercoiling, but they maintain significantly different values of supercoil density ($\sigma = -0.070$ and -0.060 , respectively). Multiple studies show that negative supercoils present in *E. coli* are partitioned between a diffusible supercoiled state that moves rapidly throughout thousands of base pairs of chromosomal DNA and constrained supercoils that behave locally in a manner reminiscent of nucleosomes.

Constrained supercoils

The *E. coli* chromosome contains about 30,000 supercoils ($\sigma = -0.07$). Approximately half of these supercoils can

diffuse freely (σ_D) and the other half are constrained (σ_C) so that DNA writhe persists when a chromosomal region is relaxed, resulting in the relationship $\sigma = \sigma_D + \sigma_C$ (Higgins and Vologodskii 2015). RNA polymerase (RNAP) is one protein that constrains supercoils. The enzyme has five proteins ($\alpha_2\beta\beta'\omega$) with a molecular mass of about 400 kDa. About 3,000 molecules of RNAP are present in *E. coli* cells grown exponentially in rich medium, of which two-thirds are actively engaged in transcription. One consequence of transcription is that each polymerase unwinds a short segment of the DNA template that moves with the enzyme. This creates a linking number change (ΔLk) of -1.7 supercoils per RNA polymerase (Gamper and Hearst 1982) so that RNAP accounts for about 3400 supercoils, or roughly 10 % of σ_C . A group of abundant small basic proteins that are often called histone-like or nucleic-associated proteins (NAPs) bind and bend DNA; these proteins are thought to account for the rest of constrained supercoiling. The leading member is HU protein, a hetero-dimer composed of HupA and HupB protomers in *E. coli* (Dillon and Dorman 2010). HU can produce supercoiled DNA when incubated with a relaxed substrate and an enzyme such as calf thymus topoisomerase I (Topo

I), and it accounts for 6000 supercoils or roughly 40 % of the constrained DNA supercoils. About 50 % of σ_C is presumed to associate with the NAPs IHF, H-NS, STPA, FIS, and DPS (Johnson et al. 2005; Pul and Wagner 2010).

Diffusible supercoils

DNA gyrase is primarily responsible for maintaining approximately 15,000 diffusible supercoils in *E. coli*. A tetramer of two proteins, GyrA and GyrB (Fig. 1), gyrase was the first type II topoisomerase discovered. The enzyme introduces two (-) supercoils in each enzymatic cycle (Fig. 2). By first breaking both strands of DNA and then forming a transient covalent phospho-tyrosine (Y122) complex with both of the DNA strands, the enzyme passes one double-strand segment through the gap and reseals the bond before releasing the DNA product. Gyrase is a factor in many DNA transactions and a fundamental force in compacting chromosomal DNA; consequently, it has an impact on much of the chemistry of DNA metabolism (Higgins et al. 2005). The structure and sequence of the gyrase subunits are highly conserved. After millions of years of evolutionary divergence separating *E. coli* and *Salmonella* there are only 28 amino acid changes in GyrB and 77 amino acid differences in GyrA (Fig. 1). All four subunits have been purified and cross-complement each other in in vitro supercoiling reactions (Pang et al. 2005). Nonetheless, *Salmonella* has a supercoil density that is 15 % lower than that of *E. coli* (Champion and Higgins 2007; Higgins et al. 2005), and *E. coli* is not viable at the wild-type (WT) *Salmonella*

supercoil density. The mystery of how these differences arose and why they have been stably maintained is discussed in a following section.

Gyrase mutations with different phenotypes in two species

In biochemistry and molecular genetics it is often assumed that metabolic pathways and enzymes that catalyze the critical reactions all work the same way. If you understand the mechanism of an essential *E. coli* enzyme (DNA gyrase or RNA polymerase for example), you expect the phenotype of specific mutations to apply in most types of bacteria. This is the assumption in the emerging fields of “metabolomics” and systems biology. However, the assumption is often wrong. Zhenhua Pang studied a temperature-sensitive (TS) mutation of *gyrB* in *Salmonella* (Pang et al. 2005). The *gyrB*-652 mutation changes R436-S in the TOWPRIM domain of GyrB (Fig. 1), and it makes cells TS for growth at 42 °C and resistant to low (5 µg/ml) levels of nalidixic acid. Normally, nalidixic acid resistance mutations map near the catalytic tyrosine present in the *gyrA* gene. To study the catalytic mechanism of the mutant, Pang cloned, expressed, and purified GyrA, GyrB, and GyrB652 proteins to assemble and study *Salmonella* WT gyrase and GyrB652 enzyme activity in vitro (Pang et al. 2005). WT gyrase and GyrB652 were both proficient in supercoiling relaxed circular DNA substrates at 30 °C. Surprisingly, the GyrB652 enzyme supercoiled DNA faster at 42 °C than at 30 °C. GyrB652 supercoiling reactions carried

Fig. 2 **a** Model for supercoil density control based a branch point leading to DNA dissociation (*D*) or processive reactions (*P*) where the enzyme remains bound to the same site. ΔLk Linking number change, *Pi* inorganic phosphorus, *Mu SGS* strong DNA gyrase cleavage site at the center of the *Mu* genome, *CTD* C-terminal domain. **b** Comparison of the C-terminal acidic tails of *E. coli* (*E.c.*) and *Salmonella* Typhimurium (*S.T.*) Acidic tail residues are shown in red

E. c. 841-875 EPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE
S.T. 841-878 EPVDDEELDAIDGSVAEGDEDIAPEAESDDDDVADDADE

out at 42 °C worked twice as fast as those at 30 °C. However, GyrB652 gyrase had a slower catalytic rate compared to the WT gyrase at all temperatures tested, and it is this slow enzyme mechanism in *Salmonella* that causes problems at high temperature.

GyrB652 was the first topoisomerase exhibiting growth rate toxicity (Champion and Higgins 2007). To determine whether the R436-S GyrB protein would also cause TS growth in *E. coli*, Champion and Higgins (2007) used the lambda RED “recombineering” (recombination-mediated genetic engineering) method (Datta et al. 2006) to introduce this mutation into the *E. coli gyrB* chromosomal gene and found that this single base pair change was lethal in *E. coli* at all growth temperatures. This experiment provided the first example of a gyrase mutation that caused strikingly different phenotypes in two closely related species of bacteria. A subsequent study carried out by the Higgins’ group showed that many TS mutations in GyrA and Topo IV decrease catalytic efficiency rather than causing protein dysfunction at the high temperature (Rovinskiy et al. 2012). In all of these cases, chromosomal supercoiling losses are observed even at the permissive growth temperatures.

Species-specific phenotypes of other homologous genes

To better understand the GyrB phenotype, Keith Champion studied other phenotypes linked to negative supercoiling (Champion and Higgins 2007). In this study, he first measured the supercoil density of pUC18 plasmid DNA isolated from *Salmonella* and found to be 15 % lower than that in the same plasmid isolated from *E. coli*. Experiments with supercoil-dependent Z-DNA-forming plasmids confirmed that *E. coli* creates a significantly higher DNA torsional strain than does *Salmonella*. Second, he introduced either a *Salmonella* GyrB652 protein or a WT *Salmonella* GyrB protein into WT *E. coli* and observed that both proteins were toxic, even at very low expression levels, suggesting that WT and mutant gyrase subunits from *Salmonella* cause problems in vivo for *E. coli*. Third, species differences in supercoil density changed many phenotypes linked to replication and chromosome segregation. For example, a null mutation in the *mukB* subunit of condensin is viable in *E. coli*, but lethal in *Salmonella*. The explanation is that at a low supercoil set point, the *Salmonella* chromosome relies more heavily on condensin to compact and organize the chromosome than does *E. coli* with higher supercoil compaction forces (Holmes and Cozzarelli 2000; Sawitzke and Austin 2000). Fourth, strains with null mutations in *seqA* were healthy in *Salmonella*, whereas *E. coli seqA* deletions were sick and exhibited RecA-induced SOS phenotypes. The high supercoil density of *E. coli* promotes premature initiation at

the origin of replication (OriC) because a replisome can auto-assemble at unpaired regions near the OriC. SeqA suppresses this reaction by binding to hemi-methylated sites within the OriC and sequestering it from interacting with the replication initiation protein DnaA for many minutes. At the lower supercoil density of *Salmonella*, there is no over-initiation and SeqA sequestration is not needed.

Life on a flat chromosome

Many reports dealing with gyrase and supercoiling start with a litany of the cellular functions reported to be intimately connected to gyrase-driven negative supercoiling. The list includes initiation of replication, recombination, transcription, transposition, homologous and site-specific recombination, among others. However, bacterial cells can survive, grow, and carry out well-organized cell divisions even while their chromosomes have lost all detectable diffusible negative supercoils (Higgins 2014; Rovinskiy et al. 2012). Such growth is not possible for *E. coli*, which is the paradigm case, so this result in *Salmonella* is not widely understood or appreciated, although it may be more characteristic of most bacterial species.

Discovery of viable flat chromosomes started with experiments by Booker, who set out to study DNA topology of the highly transcribed *rrnG* operon of *Salmonella* (Booker et al. 2010). Booker introduced supercoil sensors upstream and downstream of the 5-kb *rrnG* operon and measured supercoil-dependent resolution efficiency. The results revealed that in *Salmonella* with a WT complement of genes for DNA topology and transcription, there was a significant excess of negative supercoiling upstream from the promoter, and a supercoil deficit downstream from the transcription terminator. These findings confirmed the Liu and Wang hypothesis of twin domains of opposite handed supercoiling up- and downstream of a transcribing RNA polymerase (Liu and Wang 1987) and proved that twin domain supercoiling exists even in WT cells. Booker extended the experiment to test how mutations in TopA (*E. coli* strains with a Topo I enzyme of low activity) and TS mutations in gyrase altered the result. TopA mutants significantly increased (–) supercoiling upstream of the promoter, thereby confirming its role in preventing hyper-supercoiling on the chromosome (Drolet et al. 2003). A TS GyrB mutant growing at permissive temperature (30 °C) caused a significant loss of (–) supercoiling downstream of the terminator. However, the GyrB652 mutation caused a complete loss of negative supercoiling in a 9-kb domain downstream from the *rrnG* terminator, even though this strain grows at the permissive temperature (30 °C).

The complete loss of supercoiling downstream of *rrnG* in strains with GyrB652 raised an intriguing possibility. A flat chromosome lacking nearly all negative supercoiling might be

produced in cells with a sluggish GyrB subunit. This hypothesis was tested and confirmed in several ways (Rovinskiy et al. 2012). First, supercoil sensors at eight positions were evaluated for chromosome supercoiling. Analysis of these positions revealed that 95 % of all diffusible chromosomal supercoiling disappeared in GyrB652 cells growing exponentially at 30 °C. Second, if transcription can run the chromosome flat, can supercoiling be restored by blocking transcription? Rifampicin was added for 10 min after cells had been induced for resolvase expression, and this treatment restored supercoil density to 70 % of the WT level. Third, the possibility of a slow GyrB652 influencing the normal rate of transcription elongation was tested by measuring the rates of RNA polymerase elongation in the Lac operon at the same positions used to analyze recombination. The average RNA polymerase elongation rate fell from 50 nucleotides/s in WT cells growing at 30 °C to roughly half that in the GyrB652 mutants. This result reaffirms the close linkage between transcription and gyrase catalytic rates. Fourth, if rates of transcription elongation and gyrase supercoiling are tuned to match in WT cells, what happens to supercoiling if one slows down RNAP? When an RNAP mutant with a slow elongation rate was introduced into *Salmonella* cells, the global supercoil density of the chromosome increased, confirming again the intimate connection between gyrase catalytic activity and the transcription elongation properties of RNAP. This result confirms that RNAP is critical for establishing the mean supercoil density of chromosomes in collaboration with gyrase and Topo I.

What controls supercoil density?

To examine critical elements that participate in regulating supercoil levels, Nikolay Rovinskiy made transgenic swaps of GyrA between *E. coli* and *Salmonella*. As Champion had found earlier for GyrB, GyrA proteins caused supercoiling disruptions as single-copy transgenes. For his thesis Rovinskiy constructed chimeras to see if one GyrA region might control proper supercoiling. He found that in both cases when a transgenic GyrA from the wrong host was fused to the correct species GyrA sequence containing the last two pinwheel elements and acidic tail, proper supercoiling was restored. One model proposed for species-specific supercoiling involves the pinwheel elements of the C-terminal domain (CTD) and the C-terminal 35–38 acidic tail (Fig. 1a red tail on GyrA in Fig. 2b). Purified *E. coli* and *Salmonella* GyrA dimers do not bind to DNA, but GyrA dimers lacking the tail do bind to DNA because the positively charged residues of the CTD inhibit DNA binding. As shown in Fig. 2a (Stage 0), gyrase forms two chambers, with the top chamber open and the lower C chamber closed. When DNA

enters the upper chamber (Stage 1), it is drawn to the floor, which bends DNA into a V shape caused by two 75° bends on opposing sides of the DNA cleavage site (Wendorff et al. 2012). DNA bending orients the DNA arms so that they compete with GyrA CTD for DNA binding and formation of a chiral (+) loop on one pinwheel element. Looping of the DNA over the pinwheels places the DNA (green) transfer segment over the DNA cleavage site. In solution, gyrase–DNA complexes are stable with a half-life of >> 24 h. (Higgins and Cozzarelli 1982). DNase footprinting (Oram et al. 2006) and topology mapping (Kirkegaard and Wang 1981) indicate that in solution the equilibrium strongly favors a (+) wrapped conformation. However, stretching tension, which is applied in both single molecule rotorbead studies (Basu et al. 2012) and magnetic tweezers experiments (Fernandez-Sierra et al. 2015), exerts tension that appears to shift the equilibrium to favor the unwrapped condition. In Stage 2, binding of ATP to both GyrB subunits stabilizes the + chiral loop (Basu et al. 2012) and the enzyme closes the upper chamber by dimerization of ATP–GyrB subunits (Stage 3). At Stage 3, cleaved complexes are efficiently formed with both GyrA subunits attached to opposing DNA strands via a phospho-tyrosine linkage to Y122. In the cleaved state, solid body rotation of the GyrA Tower and GyrB TOPRIM domains opens both the DNA gate for passage of the transfer DNA segment (green) down to the lower chamber and the C gate at the bottom. ATP hydrolysis promotes re-ligation of the covalent intermediate complex and opening of the ATP gate, which places the enzyme in position to follow one of two tracks—release of DNA and return to Stage 0, or retain the bend and proceed to Stage 2 for another rapid reaction. In rotor bead experiments, processive supercoil cycles rapidly introduce >100 supercoils in continuous bursts (Basu et al. 2012).

We have proposed that the *Salmonella/E. coli* supercoil density difference is controlled by the energetics of the branch point of distributive versus processive reaction. The *E. coli* tail has 35 residues with 19 acidic side chains, whereas *Salmonella's* tail has 38 residues and 21 acidic amino acids. This difference means that the *Salmonella* tail is a better DNA competitor than *E. coli*, and consequently, a less processive enzyme. When bound to the identical sites, *E. coli* gyrase is predicted to be more processive and to achieve high supercoiled levels faster than *Salmonella* gyrase.

Critical partners with gyrase in chromosome supercoiling reactions are high-affinity binding sites that promote processive bursts and contribute to the processive/distributive equilibrium (Chong et al. 2014). The strongest known gyrase DNA binding site is the strong DNA gyrase cleavage site (SGS) at the center of the Mu genome, which

is stronger than all other gyrase binding sites in *E. coli* and *Salmonella* (Pato and Banerjee 2000). Because of its stability and processive properties, the Mu SGS has been used in both rotorbead and molecular tweezers experiment to study supercoiling cycles. Unfortunately, very little is known about processivity and gyrase dynamics when gyrase is bound to sites like the BIMES of *E. coli* and *Salmonella* (Espeli and Boccard 1997; Stern et al. 1984; Yang and Ames 1988). We note that when a site is too strong, it can interfere with competing DNA processes. In dilute solution the off-rate of a DNA with the SGS from gyrase is longer than 1–2 days (Scheirer and Higgins 1997), and collisions between gyrase and RNA polymerase, DNA polymerase, and other helicases can lead to traffic jams during transcription, replication, and DNA repair in vivo. Therefore, gyrase and its optimum binding sites are most likely under strong evolutionary pressure for optimal genetic fitness. Recent results also show that even synonymous codons can have remarkable selective phenotypes in *E. coli* (Brandis and Hughes 2016).

Why do bacteria evolve significantly different supercoil set points?

Work on gyrase from species like *Borrelia burgdorferi* and *Mycobacterium tuberculosis* (*M. tb*) show that gyrase from different bacterial species varies significantly in terms of mechanism and the ability to supercoil DNA in vitro. The DNA binding and wrapping mechanism of *M. tb* gyrase differs dramatically from that of *E. coli*, resulting in the in vitro set point of the former being lower than that of the *E. coli* gyrase (Tretter and Berger 2012a, b). The GyrA of *Borrelia burgdorferi* and *E. coli* differs in terms of C-terminal β -pin-wheel structure, and the *Borrelia* GyrA DNA binding domain is actually expressed from an independent promoter inside the GyrA gene such that the C-terminal region functions as an independent DNA-bending protein. This protein will substitute for the *E. coli* HU protein in biochemical assays for in vitro Mu transposition (Knight and Samuels 1999; Ruthenburg et al. 2005). These differences between gyrases from very distantly related bacteria are not too surprising. However, why do supercoil differences arise between closely related species like *E. coli* and *Salmonella* that have nearly identical genetic maps and enzymes?

One possible answer to this question is the presence of multiple mobile DNA elements in the two organisms (Fig. 3a). The transcriptional landscape and location of highly expressed genes is remarkably conserved between *E. coli* and *Salmonella* (Fig. 3b). The sequential order of the most highly transcribed genes is preserved along with the relative transcript abundance measured with microarray technology. However, the types and number of mobile elements are dramatically different between the species (Fig. 3a) *Escherichia*

coli K12 encodes a large cast of insertion sequence (IS) elements, which includes multiple copies of IS1, IS3, IS4, IS5, IS30, IS150, IS186, and IS911. These IS sequences are active and move frequently (Manna et al. 2001). *Escherichia coli* K12 has no inducible plaque-forming prophage.

In contrast, *Salmonella* has none of the IS elements present in *E. coli*. It has six copies of the IS200 element that appear to be inactive since they have not been seen to move in almost 50 years of study in *Salmonella*. More significant are the four plaque-forming prophage, Gifsy-1, Gifsy-2, Fels-1, and Fels-2. These viruses are under selection because they include multiple genes and operons that increase the ability of *Salmonella* to infect and reside in mammalian hosts (Bacciu et al. 2004; Coombes et al. 2005; Figueroa-Bossi and Bossi 1999; Ho et al. 2002). These elements are also potentially lethal if and when spontaneous induction leads to viral replication and cell lysis. A low supercoil density could provide insulation from spontaneous induction and lytic phage development.

Comparing the phage lytic/lysogenic equilibriums in *E. coli* and *Salmonella*

To prove that a lower supercoil density in *Salmonella* can reduce the lytic burden of four lysogenic viruses, we needed to test the lytic induction potential of both species. An efficient induction method to study *Salmonella* prophages was not available; therefore, Champion carried out experiments that compared the lethality of the bacteriophage Mu (Champion and Higgins 2007) in *E. coli* and *Salmonella* (Fig. 4). Lysogenic WT strains of *E. coli* and *Salmonella* were isolated by infecting cells with the thermal-inducible plaque-forming MupA1 phage. Multiple cultures of both species were grown at 30 °C, then shifted to a 42 °C shaking water bath, and the OD₆₀₀ as well as the number of viable cells that plated on LB agar at 30 °C were sampled at 10-min intervals for the next 80 min. *Escherichia coli* cultures began to lyse at 30 min after the temperature shift, while *Salmonella* cultures began lysis 10 min later. Thus, Mu phage development took longer in *Salmonella* than it did in *E. coli*. More dramatically, the number of cells surviving the shift to 42 °C were vastly different. In *E. coli*, viable cell numbers fell immediately after the shift and rapidly declined; by 50 min, viability had fallen by 8 log units and no survivors were detected. In *Salmonella*, viable cell numbers also dropped immediately after the temperature shift, but they then leveled off at 20 min and the viable cell count remained at 10⁷/ml for the next hour. Whereas both species support the Mu lytic cycle, *Salmonella* survived the strong thermo-induction 10,000,000-fold better than *E. coli*. The results of this experiment strongly support the hypothesis that the low supercoil density in *Salmonella* is an important factor in tolerating four prophage genomes in one bacterial chromosome.

Fig. 3 **a** Map of *E. coli* mobile DNA elements (inside) and *S. Typhimurium* mobile elements (outside). *E. coli* includes multiple copies of insertion sequence 1 (IS1; 1A–H), IS2 (2A–H), IS3 (3A–F), IS5 (5A–R), IS30 (30A–C), IS150 (150), IS186 (186A–C), IS600 (600), and IS911 (911A–B). In *Salmonella* Typhimurium LT2 there are six copies of IS200 (200A–F) in addition to four lysogenic plaque-forming

prophages, namely, Fels-1, Gifsy-2, Gyfysy-1, and Fels-2, shown in purple in clockwise order. **b** The positions of the 27 most highly transcribed protein operons are shown in black and those of the seven ribosomal RNA operons are shown in blue. Steady-state RNA/DNA level for the protein-encoding genes from microarray experiments in exponential phase on LB medium are listed following the gene name

In the final analysis, the critical factor for gene expression systems for all organisms is that they must be highly efficient at matching gene expression output with growth rate demands of the most common niches occupied by the species. In bacteria, transcription and translation are intimately connected by

Fig. 4 Lytic response of a Mu lysogen in *E. coli* and *Salmonella*. Fresh overnight cultures of lysogens of *E. coli* (open squares) and *Salmonella* Typhimurium (filled squares) were diluted 1:100 in fresh LB and grown at 30 °C to the log phase (OD₆₀₀ = 0.4). Part of each culture was shifted to a shaking water bath maintained at 42 °C, and the OD₆₀₀ and colony-forming units (CFU) were measured at 10-min intervals through the lysis period. *E. coli* cultures began to lyse at 30 min, whereas lysis in *Salmonella* cultures was delayed to 40 min. The number of viable *Salmonella* cells (filled circles) dropped by 50-fold, while the number of viable *E. coli* cells (open circles) dropped by seven orders of magnitude — i.e., no survivors after 40 min at 42 °C

temporal synchrony. Ribosomes follow the emerging transcript in most genes, and there is a large collection of proteins dedicated to delivering specific proteins to their proper cell location. The genetic investment is huge, and examples of proteins, RNAs, and small molecules that collaborate include gyrase, DksA, NusA, NusG, MFD, Rho, RfhA, GreA, GreB, RNAP, ppGpp, tmRNA, Topo I, cAMP, and cyclic GMP. Other results indicate that transcription is coupled with DNA repair and replication machinery so that conflicts between RNA and DNA polymerases are quickly and efficiently resolved (Cagliero et al. 2014; Hanawalt and Spivak 2008; Tehranchi et al. 2010).

In the post-genomic era, there has been a shift in focus from funding research designed to uncover biochemical mechanisms and test genetic relationships to funding correlation science. New fields of proteomics, metabolomics, and translational science are based on the notion that the current level of understanding of biological systems is sufficient to enable epidemics to be predicted, fundamental problems to be solved with massive computational power and cures can be developed from designer drugs that target almost any protein. Daily media predictions hint that new work in mouse genetics will lead to cures for diabetes, cancer, and Parkinson's disease. However, there has been little interest in testing the limits of our knowledge. Work described above shows that unexpected responses of *E. coli* and *Salmonella* to highly homologous proteins from the other species proves that we lack information about important connections between gyrase, condensins, and the transcription machinery. In addition, the Ventner group in La

Jolla published a recent paper in *Science* in which they describe the smallest free-living organism, which they isolated by whittling down a larger bacterial genome to only 531,560 bp and 473 genes (Hutchinson et al. 2016). Shockingly, 147 essential genes in this bacterium have no known structure or function. Correlations have little meaning without an understanding of cause and effect. Shakespeare said it well. “There are more things under heaven and earth, Horatio, than are dreamt of in your philosophy” (Hamlet I.5:159–167).

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Compliance with ethical standards

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