Bacterial promoter opening underpins ubiquitous transcriptional regulation by DNA supercoiling

Raphaël Forquet, Maïwenn Pineau, William Nasser, Sylvie Reverchon and Sam Meyer

October 1, 2020

Abstract

DNA supercoiling acts as a basal transcriptional regulator, which contributes to the quick and global transcriptional response of bacteria to many environmental changes. In spite of this importance, mechanistic models explaining the differential response of promoters to global topological variations of the chromosome remain essentially lacking. Here, we present the first quantitative transcriptional regulatory model by DNA supercoiling, focusing on the specific step of promoter opening during transcription initiation. Based on the known physico-chemical properties of DNA denaturation, it involves only one global adjustable parameter and is yet able to predict the global supercoiling response of promoters in a wide range of bacteria, based on the sequence content of their "discriminator" element. We first show that it quantitatively predicts both *in vitro* and *in vivo* data from transcription assays focusing on individual model promoters. We then assess the universality of the mechanism by analyzing transcriptomes of phylogenetically distant bacteria under conditions of supercoiling variation: (1) by gyrase-inhibiting antibiotics, (2) by biologically relevant environmental stresses, (3) naturally acquired and inherited in the longest-running evolution experiment. The model robustly predicts a significant contribution of the entire transcriptomic response to transient or inherited supercoiling variations in various species. This study strongly suggests that the proposed physical mechanism is used as an ubiquitous regulatory mechanism in the whole prokaryotic kingdom, based on the fundamental mechanical properties of the double-helix.

Importance

DNA supercoiling acts as a global yet underestimated transcriptional regulator in bacteria. We propose the first quantitative model of this regulation mode, based on the specific step of promoter opening during transcription initiation, explaining the differential response of promoters to global topological variations of the chromosome. In contrast to classical mechanisms requiring dedicated regulatory molecules to bind target promoters, we show that global deformations of the DNA template itself underpin a selective response of each particular promoter, according to its "discriminator" sequence, by modulating the ability of RNA Polymerase to initiate transcription. This study defines the first systematic rule underpinning the ubiquitous regulatory action of DNA supercoiling on the core transcriptional machinery, in particular in response to quick environmental changes.

DNA supercoiling | transcriptional regulation | discriminator | quantitative modeling | stress response

Introduction

Bacteria encounter rapid changes of environmental conditions (availability of nutrients, physical or chemical stresses) to which they respond by quick and global modifications of their transcriptional program. Inspired by early studies [1], current mechanistic models of this regulation are mostly based on transcription factors (TFs) which bind at specific promoters and interact with RNA Polymerase (RNAP). Yet more than half of *E. coli* promoters are not targeted by any known TF [2], and entire organisms are even almost devoid of them [3, 4] but exhibit nonetheless a complex regulation. Global transcriptional control has been further explained by variations in RNAP composition (sigma factors, [5]) or abundance [6] depending on growth conditions, as well as RNAP-binding regulatory molecules such as ppGpp [7].

Besides this variability of the transcription machinery, the physical state of the DNA template itself is subject to cellular control through DNA supercoiling (SC), *i.e.*, the over- or under-winding of the double-helix by the action of topoisomerase enzymes and architectural proteins [8, 9, 10]. The SC level of the chromosome has long been

shown to change in response to environmental conditions [10], and was proposed to act as a global transcriptional regulator involved in growth control [8]. However, in contrast to aforementioned mechanisms relying on regulatory proteins, quantitative models of this physical regulatory factor are lacking. Even the features defining SC-sensitive promoters remain obscure, and the direction of their response to SC variations unpredictable. A possible reason for this shortcoming is that SC and RNAP interact at multiple steps of the transcription process, both directly and via regulatory proteins during initiation, as well as elongation [10]; as a result, virtually every investigated promoter exhibits a distinct SC response, making it difficult to analyze the underlying mechanisms and propose a general and predictive model.

In this paper, we address the latter objective and propose a first quantitative model of transcriptional regulation by global SC, by focusing specifically on the step of open-complex formation during transcription initiation. This step is strongly facilitated by negative SC and depends primarily on the "discriminator" sequence located downstream of the -10 box [11, 12]. Based on existing physico-chemical descriptions of DNA denaturation [13, 14], we propose a simple thermodynamic model of this regulatory mechanism involving only one global parameter, and yet able to predict its contribution in the transcriptional response to global SC variations affecting the entire chromosome in a wide range of bacterial species.

The model is first applied on specific model promoters analyzed *in vitro* or *in vivo*, showing that this intuitive and basal physical effect explains most of their activation by negative SC. Since open-complex formation is required for the expression of all promoters, independently from additional regulatory mechanisms, we then analyze its contribution to the global transcriptional response of various organisms to SC variations, either transiently induced by gyrase-inhibiting antibiotics or by biologically relevant environmental stresses, or inherited over generations due to naturally selected mutations in the longest-running evolution experiment [15]. This analysis robustly and consistently suggests that the proposed physical mechanism, imposed by the structure of the double-helix itself, is used as an ubiquitous regulatory mechanism in the prokaryotic kingdom.

Results

Thermodynamic regulatory model of open-complex formation

Transcription requires RNAP to destabilize the double-helix and gain access to the bases (Fig. 1A), which is thermodynamically highly unfavorable in torsionally relaxed DNA. In contrast to eukaryotes relying on ATP hydrolysis by TFIIH for this task [16], transcription initiation is a purely thermal process in prokaryotes, possible only because the DNA gyrase maintains the chromosome at an out-of-equilibrium negative SC level in which the double-helical state is strongly destabilized. This dependence is shown on Fig. 1B for the *tyrT* promoter, based on an existing physical model of DNA thermodynamics [13, 17] relying on knowledge-based enthalpic and entropic parameters of all base sequences. *In vivo*, the SC level is subject to cellular control, especially through ATP-dependent gyrase activity [18, 10], and its variations should then directly affect the opening facility of promoters and thus their expression. Such a dependence was indeed observed for the *tyrT* promoter (blue) in both *in vitro* (Fig. 1C) or *in vivo* (Fig. 1D) transcription assays [19].

The observed correlation between promoter opening thermodynamics and expression strength is far from trivial, since transcription initiation is a complex process involving successive kinetic steps before and after promoter opening [20, 21], all of which might be influenced by the SC level, leading for example to DNA scrunching [22] and abortive rather than processive transcription if the open-complex is too stable [8]. Such effects were dissected in specific promoters like those of ribosomal RNAs (which likely evolved particular mechanisms for an optimized expression), and might explain the decreased expression of *tyrT* at high SC levels (Figs. 1 C and D, rightmost datapoints). Yet interestingly, mostly monotonous activation curves following DNA opening thermodynamics, as we see in *tyrT*, were observed with many bacterial promoters analyzed *in vitro* in the physiological SC range [10]. Our working hypothesis is therefore that thermodynamics of open-complex formation alone (Fig. 1B) may explain a significant part of the SC regulation of most bacterial promoters expressed at a moderate level in the cell. The key advantage of this approximation is the simplicity of the resulting regulatory model ([23]):

$$k(\sigma, s) = k_0 \exp\left(\min\left(\frac{\Delta G_{op}(\sigma, s) + \Delta G_P^0}{k_B T}, 0\right)\right)$$

where k is the transcription rate, k_0 the basal (maximal)

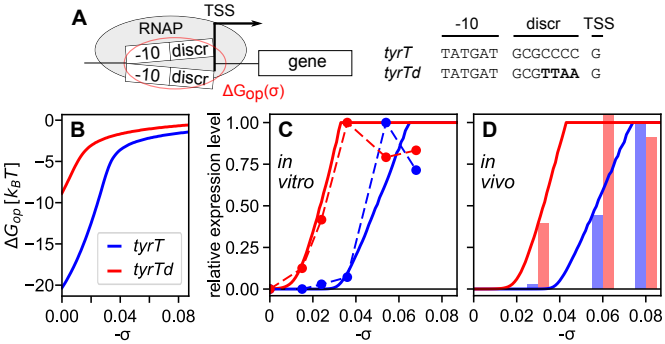


Figure 1: Discriminator sequence determines transcription bubble opening free energy and resulting regulation of promoter expression by DNA supercoiling. **(A)** Diagram and sequences from wild-type *tyrT* and the mutant *tyrTd* promoter with A/T-rich discriminator [19]. **(B)** Transcription bubble opening free energies of *tyrT* and *tyrTd* promoters. **(C)** Transcription model predictions (solid lines) compared to the *in vitro* (dots) and **(D)** *in vivo* (bars) expression data from [19]. Data and computed values of the *tyrT* promoter are shown in blue, and those of *tyrTd* in red.

rate, s is the precise 14-nt sequence of the denaturated region in the open-complex [11] of estimated negative opening penalty ΔG_{op} (Fig. 1B), and k_BT is the Boltzmann factor. $\Delta G_P^0 = 3.5\,k_BT \simeq 2$ kcal/mol is a global energy parameter representing an opening assistance by RNAP during open-complex formation (typically due to conformational changes in the complex), adjusted on the data of Fig. 1 and kept constant henceforth for all promoters and species. At high negative SC levels, the opening penalty becomes negligible ($\Delta G_{op}(\sigma, s) + \Delta G_P^0 > 0$, Fig. 1B) and the maximal rate k_0 is achieved, whereas the promoter is mostly closed when DNA is strongly relaxed.

Without any detailed description of the complex RNAP-DNA interactions, this minimalistic model reproduces both *in vitro* and *in vivo* expression data of Fig. 1. We note that in the underlying unidimensional description of DNA, SC is entirely present in the form of twist, neglecting the writhe contribution present in the SC levels measured *in vivo* with plasmids. As visible on Fig. 1, this limitation does not prevent quantitative estimates, consistent with the notion that twist is the main contributor to DNA denaturation. The balance between these two contributions might however differ depending on the species, growth conditions and location on the chromosome. Since this distinction is equally inaccessible by usual chloroquine-agarose SC assays, the approximation used in the model matches the level of knowledge currently available for most expression data. Crucially though, due to the monotonous nature of the underlying activation curves (Fig. 1), almost all model predictions and all results presented henceforth are robust when the SC values used in the computations are slightly varied, and should thus not be affected by this limitation.

Discriminator sequence underpins promoter selectivity by DNA relaxation

The denaturation energy of the transcription bubble is known to be strongly dependent on the proportion of G/C bases, in particular in the discriminator region (Fig. 1A) lying between the -10 element and the transcription start site (TSS) [24, 8]. Replacing four C/G by A/T nucleotides at that location (*tyrTd* mutant) indeed shifts the opening curve to the left (Fig. 1B, red curve), *i.e.*, favors DNA opening already at weaker SC levels. Strikingly, the resulting transcriptional activation curves (Figs. 1 C and D) closely follow the thermodynamic predictions, showing that the discriminator sequence is the primary contributor in the supercoiling sensitivity of these promoters in the investigated conditions. The latter notion has long been proposed based on model promoters [25], but is here translated into a quantitative model. Interestingly, while G/C-rich discriminators were sometimes described as "more supercoiling-sensitive" than A/T-rich ones [8], Fig. 1 rather suggests that both types are equally sensitive, but at different SC levels, with the former indeed more relevant to the physiological range. As an example, if the chromosome is relaxed from $\sigma = -0.06$ to -0.04 (*e.g.*, during a switch from exponential to stationary phase), we then expect the A/T-rich promoter (red) to be hardly affected, whereas the G/C-rich one should be repressed. Experimentally, such a relaxation may result from a change in medium composition, or be induced by various biologically relevant environmental stress conditions (see below). However, many of such shocks also trigger specific stress-response or additional metabolic regulatory pathways, which contribute to the transcriptional response and may hide the generic effect of chromosomal relaxation on many promoters [10].

To avoid this issue and specifically confirm the predictions above, we analyzed the response of different promoters to DNA relaxation induced by a shock of the antibiotic novobiocin at sublethal concentration, which inhibits the ATPase activity of DNA gyrase. Two families of synthetic promoters with mutated discriminators were constructed (Fig. 2A and Supplementary Tab. S1), based on (1) the *pheP* promoter of *E. coli*, which is SC-sensitive [26, 27] and is not regulated by any identified TF [2], and (2) the *pelD-pelE* genes of the enterobacterial phytopathogen *Dickeya dadantii*, which are paralogous viru-

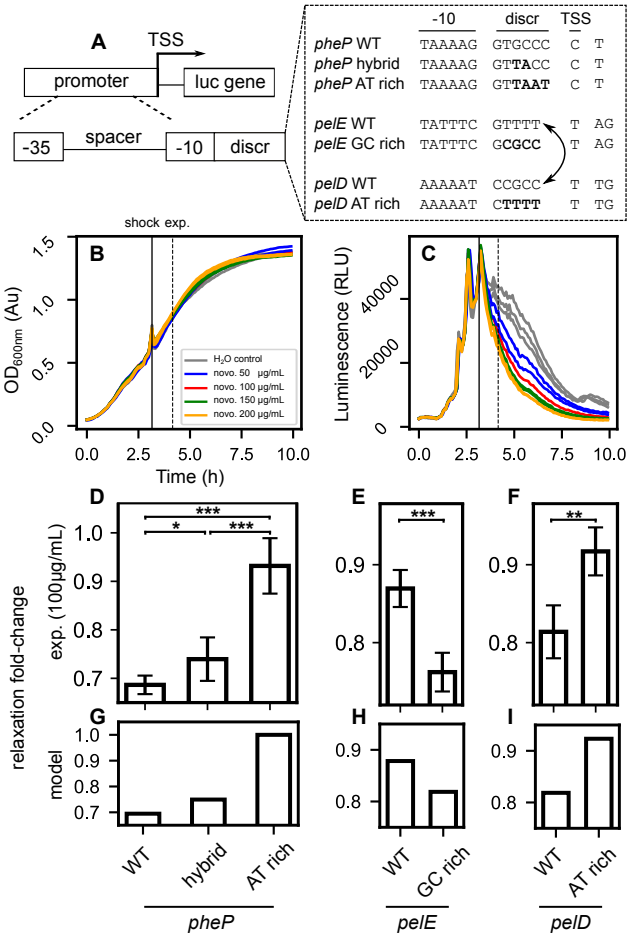


Figure 2: The DNA relaxation response of promoters with mutated discriminators match the predictions of the regulatory model. **(A)** Promoter sequences were derived from *pheP* (*E. coli*) and *pelD*/*pelE* (*D. dadantii*), with mutated discriminators of various G/C content. **(B)** Bacterial growth monitored in a microplate reader (*pheP* hybrid strain in rich medium). A novobiocin shock was applied in mid-exponential phase (different sublethal concentrations shown). The slight increase at shock time is an optical artifact due to the opening of the recorder. **(C)** Expression of the *pheP* hybrid promoter monitored by luminescence (see all raw datapoints in Supplementary Fig. S1). **(D)** Relaxation fold-changes computed 60' after novobiocin shock (100 μg/mL) in *pheP*-derived promoters. As expected, the repression factor reduces with increasing A/T%. **(E)** The DNA relaxation response of *pelE* and **(F)** *pelD* are reversed when a tetranucleotide is swapped between their discriminators, with low and high G/C content respectively. **(G)** Relaxation fold-changes predicted by the model reproduce the experimental observations on *pheP*-derived promoters, as well as **(H)** *pelE*- and **(I)** *pelD*-derived promoters, assuming a weak relaxation compatible with the observed repression levels (see Supplementary Information).

lence genes encoding similar pectinolytic enzymes, are directly regulated by more than ten TFs and are both supercoiling-sensitive [28] but harbor different discriminators. Promoters were fused on plasmids in front of a luciferase reporter gene (Fig. 2A), and their expression was analyzed in *E. coli* cells in a microplate reader (see Fig. 2, Supplementary Fig. S1 and Supplementary Information).

For the *pheP*-derived promoters (Fig. 2D), cells were grown in LB medium. As expected, we found that the expression fold-change (treated vs non-treated wells) was strongest for the native G/C-rich promoter, significantly reduced for the hybrid promoters (with two mutated nucleotides in the discriminator), whereas the A/T-rich discriminator (with four mutated nucleotides) was almost insensitive to DNA relaxation. Similarly, swapping four nucleotides between the discriminators of *pelE* and *pelD* (Fig. 2 E and F) completely reversed their response to DNA relaxation in minimal medium. The levels of repression observed in these data are highly significant but quantitatively weaker than those obtained in batch cultures [28], which we attribute to a milder effect of novobiocin in these growth conditions in microplates, as well as an additional buffering effect of the reporter system. Accordingly, all results are reproduced with the model assuming a weak relaxation magnitude (Fig. 2 G-I and Supplementary Information), where the direction of the promoters' predicted response is inscribed in their sequences and is therefore robust when this magnitude is varied. The predictive power of the model thus extends to very different promoters in terms of biological function and regulation complexity, as well as differences in growth conditions (rich vs minimal medium). This result supports the notion that the investigated regulation mode affects bacterial promoters independently from additional regulatory mechanisms involving promoter-specific actors, leading us to enlarge the scale of the analysis to entire genomes.

A global regulation of bacterial promoters

The transcriptomic response to DNA relaxation induced by gyrase inhibitors has been recorded in various species, providing lists of "supercoiling-sensitive genes" [27, 26, 29, 4, 30], although no sequence or structural signature was ever clearly identified in support of this notion. The results above yet suggest that the discriminator sequence could be a strong determinant of such a property, which can be tested at the scale of entire genomes by comparing these sequences for promoters activated or repressed by DNA relaxation. We developed such an analysis based on published transcriptomes and genome-wide TSS maps (Tab. 1 and Supplementary Tab. S1). Inevitably, the precision of the analysis is affected by the biological and

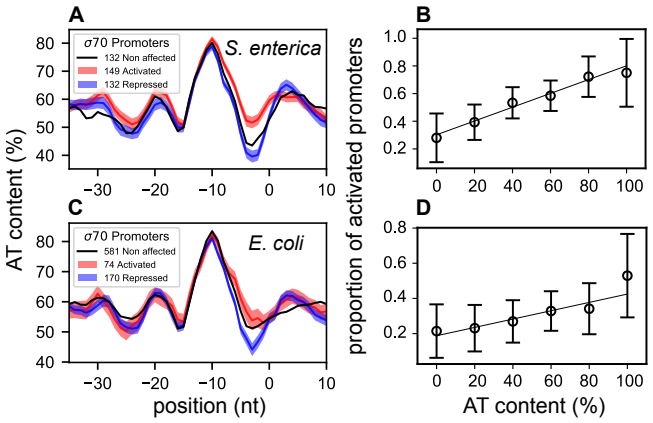


Figure 3: Discriminator sequence controls the global selectivity of promoters to a relaxation response. **(A)** Average A/T% profiles of *S. enterica* $\sigma 70$ promoters along 5-nt-centered windows, depending on their response to novobiocin-induced chromosomal relaxation [30]. The profiles are very similar (shaded areas represent $\pm$ one standard error) except in the discriminator region (between -10 and +1 positions). **(B)** Proportion of activated promoters among those responsive to DNA relaxation, depending on their A/T% in a 5-nt window around position -2. The resulting linear regression is highly significant ($P < 10^{-4}$). **(C)** Average A/T% profiles of *E. coli* $\sigma 70$ promoters depending on their response to norfloxacin-induced DNA relaxation (LZ54 vs LZ41 strains, [26]). The resulting pattern is very similar to that observed in *S. enterica* in spite of strong differences in protocols. **(D)** Same as B, for the *E. coli* data ($P = 0.011$).

technical limitations of these data: (1) the relaxation response differs quite strongly depending on the drugs and protocols used; (2) the response of each gene is regulated at different transcription steps besides open-complex formation [10]; (3) the local SC level experienced by each promoter differs from the global SC level of the chromosome [31]; (4) bacterial promoters are often annotated with a resolution below one single basepair, which is however required for an accurate definition of the discriminator sequence. All these sources of noise contribute to blur out the investigated effect, which may thus only emerge as a statistical feature, whose contribution in global regulation might however be quantified.

Fig. 3A shows the average A/T% profile of the $\sigma 70$ promoters of *Salmonella enterica*, depending on their response 20 minutes after a novobiocin shock [30]. Strikingly, although this content exhibits a characteristic non-uniform pattern along the promoter (with an expected peak at the -10 element), the signals of the two groups of promoters are indistinguishable everywhere except in the region between -10 and +1, precisely where we expected the observed difference ($P < 10^{-5}$ around position -2, Tab. 1 and Fig. 4), demonstrating that this region is the primary location of selectivity for the relaxation response. As a comparison, no significant difference is detected at the -10 site, suggesting that this selectivity is not related to a difference in sigma factor usage. Further, classifying the promoters based on their discriminator sequence composition (Fig. 3B) exhibits a clear and highly significant (approximately linear) effect on the proportion of activated promoters (correlation $P < 10^{-4}$).

To assess the universality of the observation, we repeated the analysis with transcriptomic data from *E. coli*, obtained with DNA microarrays after norfloxacin shock in two alternate mutant strains synthetizing drug-resistant gyrase and topoisomerase IV enzymes respectively, resulting in a strong magnitude of DNA relaxation [26]. In spite of strong differences in the experimental protocol compared to the *S. enterica* dataset, the obtained pattern is remarkably similar (Fig. 3C and D), confirming its robustness. Importantly, whereas in the first experiment (treated vs non-treated cells), this pattern might include contributions from SC-independent drug-response pathways, here the two compared samples received exactly the same treatment, and any such unwanted contribution should thus not be apparent. The slightly weaker observed effect might also be due to the lower sensitivity of the employed transcriptomic technology.

Can we explain the observed dependence (Figs. 3 B and D) based on the simple model developed above? From the curves of Fig. 1, the expression rate of all promoters is predicted to decrease upon DNA relaxation, which might seem contradictory with the existence of a large subset of activated promoters in the transcriptomic data. However, (1) the total transcription level in the cell is probably limited by the availability of RNAP holoenzymes, for which promoters are in competition [6]; (2) even if the total amount of cellular mRNA changes after the shock, it is then normalized during the transcriptomics experiment protocol (*e.g.,* predefined sequencing depth) and analysis. To mimic the latter protocol, we therefore included a comparable normalization step in the modeling (see Supplementary Information), which was sufficient to reproduce the competition mechanism: while G/C-rich promoters still experience repression, A/T-rich ones consequently represent a higher fraction of the total mRNAs after relaxation, and therefore appear as activated (Supplementary Fig. S2), in line with the observed trend (Fig. 3B and D) and keeping in mind that the latter is affected by the various factors of noise mentioned above. In spite of these latter, the predictive power of the model can be quantified by computing the proportion of accurately predicted promoters (sensitivity) and comparing it to a null (random) prediction, yielding

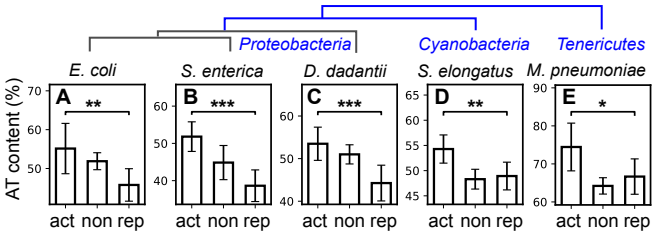


Figure 4: A robust statistical relation between discriminator A/T% and promoter's response to DNA relaxation by novobiocin (act: activated, non: no significant variation, rep: repressed) is observed in distant bacterial species: **(A)** *E. coli* ($P = 0.010$, relaxation by norfloxacin in LZ54 vs LZ41 mutant strains) [26]; **(B)** *S. typhimurium* ($P < 10^{-5}$); **(C)** *D. dadantii* ($P < 10^{-3}$); **(D)** *S. elongatus* ($P = 0.004$); **(E)** *M. pneumoniae* ($P = 0.029$). In enterobacteria, only $\sigma 70$ promoters were considered, and aligned at the -10 site. In the two other species, all promoters were aligned at their annotated TSS. A/T% are computed in a 5-nt window in the discriminator (see Supplementary Information). A schematic phylogeny is depicted above.

a gain in accurate prediction of as much as 14% of the differentially expressed genes in *S. enterica* ($P < 10^{-6}$, Tab. 1). Since this rate is only marginally affected by the precise SC levels considered in the calculation, our essentially parameter-free model of a single transcription step is able to predict a remarkably large contribution of the entire genomic response to DNA relaxation.

An ancestral regulation principle used in distant bacterial phyla

Since the investigated mechanism relies on highly conserved molecular actors, RNAP and topoisomerases, it might affect a particularly broad range of bacterial species. We therefore tested if our observations can be reproduced in various organisms of increasing evolutionary distance to *E. coli*, where comparable experiments were conducted (Fig. 4). In the enterobacterium *D. dadantii* (C), the response to relaxation by novobiocin was monitored in minimal medium based on identified gene promoters ([32]), in both exponential and stationary phase, exhibiting the same pattern (10% predictive power, $P < 10^{-3}$) as in *E. coli* (A) and *S. enterica* (C) (Tab. 1). At a drastically larger evolutionary distance, in the cyanobacterium *Synechococcus elongatus*, SC was shown to be a major determinant of the circadian oscillatory genomic expression [33]. The transcriptomic response to DNA relaxation was not monitored directly, but the phasing of gene expression in this oscillation can be used as an indirect proxy of this response [33], although many other metabolic signals may be equally correlated and could contribute to this signal. The promoter analysis yields a similar difference of discriminator sequence (Fig. 4D) and partial predictability (5%, $P = 0.010$, Tab. 1), this lower magnitude being possibly due to these additional regulatory mechanisms and a poorer definition of promoters (and associated sigma-factors). Finally, the response to novobiocin was also monitored in the small tenericute *Mycoplasma pneumoniae* [4], in which transcriptional regulation is poorly understood due to the quasi-absence of TFs [34]. Although the signal is here possibly also weakened by the lower number of promoters, it still goes in the expected direction (Fig. 4E) and yields a significant proportion of accurately predicted promoters (8%, $P = 0.020$, Tab. 1). Altogether, although these two latter species differ widely from the others in terms of phylogeny, lifestyle, and G/C content (high and low, respectively), these robust results consistently suggest that the ancestral infrastructural constraint of DNA opening, coupled with the conserved activity of topoisomerases, indeed underpins a global regulatory mechanism throughout the prokaryotic kingdom.

Global response to stress conditions and inheritable supercoiling variations

While sublethal antibiotic shocks provide a convenient method to specifically induce rapid DNA relaxation, in natural conditions the latter is rather triggered by sudden changes of environmental conditions, especially by physico-chemical stress factors like temperature, acidity, oxidative agents etc. In the case of pathogenic species, such signals are responsible for triggering the expression of many virulence factors required during infection [10]. Interestingly, the resulting rapid SC variations were found to be conserved even in distant species (e.g., increase of negative SC by cold shock, DNA relaxation by heat shock or oxidative stress) [10]. We therefore expect these variations to contribute to the subsequent global transcriptional response of the bacteria, besides other described global or more specific stress-related regulatory pathways (*e.g.*, alternative sigma factors, ppGpp, metabolic switches, two-component systems, etc). Since our model allows predicting the former contribution based on discriminator sequences, we now look for this signature in genome-wide stress-response data.

Temperature shocks provide a useful example, since heat and cold shocks both put the bacteria under stress, while affecting the SC level in opposite directions (relaxation [37] and overtwisting [38], respectively, Tab. 1 and Supplementary Tab. S1). The analysis of the correspond-

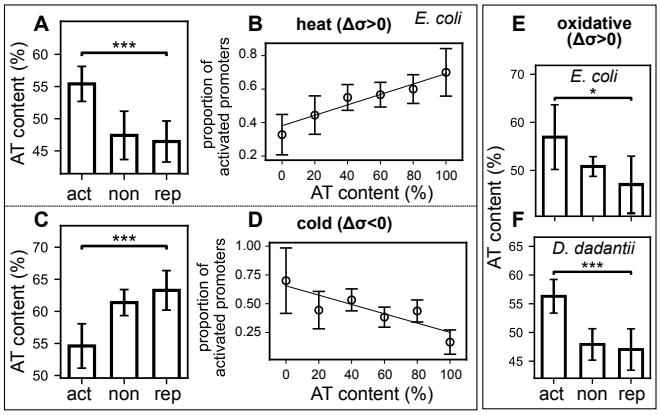


Figure 5: Relation between discriminator sequence and response to SC variations induced by environmental stress conditions. **(A)** During heat shock in *E. coli* [35] triggering a transient DNA relaxation ($\Delta\sigma$>0), activated promoters have discriminators with higher A/T% than repressed ones ($P < 10{-}5$) as expected from the presented model. **(B)** The proportion of promoters activated by heat shock increases linearly with A/T content (correlation $P = 0.007$, same data as A). **(C)** In a cold shock in *E. coli* [36] inducing an opposite SC variation (increase in negative SC, $\Delta\sigma$<0), the relation is reversed, with a preference of G/C-rich discriminators among activated promoters ($P < 10^{-4}$), as we expected. **(D)** The promoters' response is linear and significant (correlation $P = 0.026$, same data as C). **(E)** Same as A, during an oxidative shock in *E. coli* [36] inducing DNA relaxation (σ>0, $P = 0.017$). **(F)** Same in *D. dadantii* ($P < 10{-}4$) [29] where the shock was shown to induce the same SC response [28], showing the conservation of the mechanism.

ing transcriptomic datasets obtained in independent studies clearly match the expected response, with G/C-rich discriminators being respectively repressed and activated with a linear dependence in the sequence content (Figs. 5 A to D). The predictive power of the model is comparable to that observed with antibiotics (7.5%, $P < 0.001$ for heat shock; 4%, $P = 0.038$ for cold shock, Tab. 1). In the case of oxidative stress (induced by H_2O_2), the response was analyzed in different species, allowing to assess the robustness and universality of the signal. In the enterobacteria *E. coli* and *D. dadantii* where oxidative stress induces relaxation [39, 28], the pattern is indeed very similar and consistent with our predictions (9%, $P = 0.013$ for *E. coli*; 7.6%, $P < 0.001$ for *D. dadantii*, Tab. 1). Altogether, a compilation of several investigated species and conditions (Tab. 1) consistently confirms that, besides stress-specific regulatory pathways affecting a defined subset of the genome through dedicated regulatory molecules, chromosomal SC variations induced by these shocks may directly induce rapid and global changes of the transcriptional program, based on a predictable promoter selectivity.

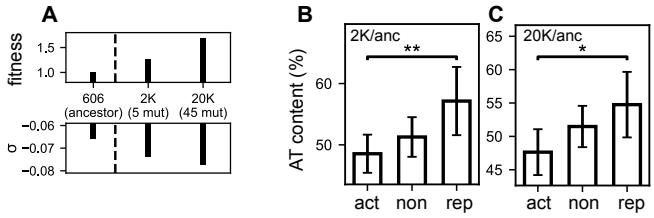


Figure 6: Discriminator sequence selectivity during inheritable increase in SC. **(A)** In the longest-running evolution experiment [40], two point mutations naturally acquired by *E. coli* induce successive increases of negative SC [15], associated to fitness gains through modifications of global gene expression [31]. **(B)** As expected from our modeling, G/C-rich discriminators are more activated in the 2K evolved strain, compared to A/T-rich ones ($P = 0.005$). **(C)** In the 20K evolved strain, the same difference is observed ($P = 0.011$) although less significant, possibly due to many other mutations affecting the regulatory network.

Finally, we address the question, if the investigated mechanism could be involved not only in transient responses, but also in inheritable modifications of the expression program. Point mutations inducing variations of the SC level were indeed quickly and naturally selected in the longest-running evolution experiment [15, 40], providing substantial fitness gains attributed to the associated global transcriptional change. In the investigated conditions of growth in nutrient-poor medium, a first mutation (in *topA*) before 2000 generations, and a second mutation (in *fis*) before 20,000 generations both lead to an inheritable increase of negative SC (Fig. 6A). We therefore expected promoters with a G/C-rich discriminator to experience enhanced expression in the evolved strains compared to the ancestor. Such a relation is indeed observed, both after 2000 generations where the signal is strongest (Fig. 6B, $P = 0.005$) and after 20,000 generations (Fig. 6C, $P = 0.011$, Tab. 1), where 43 accumulated mutations besides these two affecting SC probably contribute in rewiring the regulatory network and blurring the signal. Altogether, this generic regulatory system embedded in the double-helical structure of DNA may thus be used as a driving force in the evolution of genomes.

Discussion

The pervasive effect of SC on gene expression has been demonstrated for decades, but mostly regarded as a mere disturbance in the transcription process. In this study, we show that a significant contribution of this effect can be predicted from and depends on the promoter sequence, and thus, constitutes a *bona fide* regulation mechanism.

The basic nature of the mechanism involved, *i.e.*, the physical constraints imposed by the core infrastructure of the transcription process, suggests that it might constitute an ancestral and basal mode of regulation, a notion confirmed by the robustness of our analyses even in organisms like *Mycoplasma* which lack many classical regulation pathways and where SC is believed to play a pivotal role [41].

The present model focuses on a single step of transcription initiation, based on a simplified thermodynamic description of promoter opening, and neglects in particular any additional effect of positive SC accumulation in the elongation stage, which has been demonstrated in several studies and is likely critical for a few highly expressed genes such as those of ribosomal RNAs [10]. Similarly, while we focused on the propensity of DNA denaturation at the initiation site, the same as well as other DNA structural transitions (cruciform exclusion, G-quadruplex, Z-DNA) may occur in nearby regions depending on the SC level [13] and compete with the former, strongly enhancing the complexity of the SC regulation of individual promoters beyond the basal behavior analyzed here [18]. Finally, while our calculations were based on the hypothesis of a uniform SC level along the chromosome, the regulatory contribution of SC will probably appear even stronger when the heterogeneity of SC levels affecting different topological domains will be measured, allowing more precise predictions.

Simultaneous regulation by SC and ppGpp at the discriminator

Among various further regulatory mechanisms possibly involved in the conditions investigated in this study, there is one deserving particular attention: the alarmone ppGpp, classically associated to the stringent response (or amino-acid starvation), triggering a sudden growth arrest [7]. In contrast to many TFs, ppGpp affects the expression of a large subset of the genome by binding RNAP in combination with the transcription factor DksA [42]. Interestingly, transcriptional repression by ppGpp is not dependent on a strict sequence motif, but rather on the presence of a C nucleotide at position -1, allowing ppGpp to bind and destabilize the open complex. This mechanism and the associated sequence signal thus present remarkable similarities with the one investigated here [8, 42]. Since the regulation by ppGpp has been also associated to some of the stress conditions that we considered in this analysis, the question arises of a possible relationship between these two pathways. Before the advent of high-throughput data, it was proposed that ppGpp and SC were responsible for two partially entangled yet distinct pathways, respectively termed stringent and growth control, with the latter being still active in ppGpp-depleted cells [8]. In the datasets analyzed here, gyrase inhibitors do not trigger any growth arrest (Fig. 2B) nor signature of stringent response [26], and accordingly, an analysis of the expression levels of genes involved in ppGpp synthesis (*gppA*, *spoT*, *relA*) does not exhibit any significant response (slight repression in two datasets in *E. coli* [27, 26], slight activation by novobiocin in *D. dadantii* [29] and *S. enterica* [30], no effect in *M. pneumoniae* [4]. Thus, even if the regulation by ppGpp is associated to a similar discriminator sequence pattern as we have found, the observations made with gyrase inhibitors are indeed due to a ppGpp-independent effect of SC. Further, we analyzed the promoters of genes directly regulated by ppGpp through its binding to RNAP, as identified in a recent study [42]. As expected, a strong difference in G/C% between the many promoters activated and repressed by ppGpp induction (representing 70% of $\sigma 70$ promoters in total) is detected in the discriminator (Supplementary Fig. S3A). But interestingly, in mutant cells where RNAP is unable to bind ppGpp, almost half as many genes respond as in the wild-type cells (representing 35% of $\sigma 70$ promoters), although with weaker magnitudes and slightly slower response times (10 min instead of 5). Remarkably, these promoters exhibit a weaker but similar sequence signature at the same location (Supplementary Fig. S3B). An alternate mechanism must therefore be responsible for this broad but quantitatively milder transcriptional response to ppGpp induction in the control dataset obtained with mutant cells. We propose SC relaxation as a plausible candidate, since *gyrA/B* genes are indeed repressed and *sbmC* (encoding a gyrase inhibitor) is activated (by an unknown mechanism) in both WT and mutant cells [42]; based on our modeling, this relaxation should generate the selective repression of G/C-rich promoters observed in both datasets, with an additional strong contribution of direct ppGpp binding in WT cells (Supplementary Fig. S3A). Altogether, this combined analysis of transcriptomic data fully confirms the notion that the regulation by SC relaxation and ppGpp are distinct but partially redundant in their transcriptional effect, since they share the same criterion of promoter selectivity. More precisely, SC relaxation may be considered as a milder but more fundamental form of regulation relying on the basic infrastructure of transcription, whereas ppGpp synthesis has a stronger effect on many promoters and may itself trigger DNA relaxation (but not conversely).

In the datasets obtained with environmental stress con-

Species	Condition	Reference	SC change	A/T% difference p-value (act vs rep)	Model sensitivity gain (p-value)
Salmonella typhimurium	novobiocin	[30]	-	$< 10^{-5}$	13.8% ($< 10^{-6}$)
Dickeya dadantii	novobiocin	[31]	-	< 0.001	9.8% (< 0.001)
Escherichia coli	norfloxacin	[26]	-	0.010	5.1% (0.039)
Synechococcus elongatus	correlation*	[33]	-	0.004	4.7% (0.099)
Mycoplasma pneumoniae	novobiocin	[4]	-	0.029	7.8% (0.020)
Escherichia coli	heat shock	[35]	-	$< 10^{-5}$	7.5% ($< 10^{-4}$)
	cold shock	[36]	+	$< 10^{-4}$	4.1% (0.038)
	oxidative shock	[36]	-	0.017	9.0% (0.013)
Dickeya dadantii	oxidative shock	[29]	-	$< 10^{-4}$	7.6% (< 0.001)
Escherichia coli	experimental evolution (2K mutant)	[31]	+	0.005	3.7% ((0.011)
	experimental evolution (20K mutant)	[31]	+	0.011	1.8% (0.18)

Table 1: Compilation of investigated species, conditions and results. The SC change refers to the direction of SC variation induced by the condition (-: DNA relaxation, +: increase in negative SC). The correlation* condition from *S. elongatus* corresponds to the phasing of gene expression in the SC circadian oscillation and provides an indirect proxy of gene response to SC relaxation [33]. A/T% contents are compared with Student tests, in 5-nt windows centered at position -2. The sensitivity gain is computed as the increase in accurate prediction rate (among significantly activated or repressed genes) compared to a null (random) model. Additional detail in Supplementary Information and Supplementary Tab. S2.

ditions that we have analyzed, the genes associated to ppGpp synthesis are partly responsive, but rather in an opposite direction to the discriminator sequence signature observed (repression in heat and oxidative stress, slight activation in cold stress) and this pathway does probably not contribute significantly to the observed signal. Remarkably, in the evolution experiment, the two genes most quickly and robustly affected by mutations are *topA* and *spoT* [43, 15], involved precisely in DNA relaxation and ppGpp synthesis/degradation [7] respectively. Interestingly, the *spoT* mutation alone explains only a part of the observed transcriptional change [44], while similarly, the *topA* mutation alone generates only a fraction of the observed signal at the discriminator (Supplementary Fig. S4), suggesting a synergistic action of these two mutations [43, 15]. The additive selection of promoters based on the same sequence signal at the discriminator provides a plausible and natural mechanistic explanation for this feature.

Concluding remarks

The proposed regulation mode likely constitutes an important piece of the complex puzzle relating the structure and expression of bacterial chromatin. An original and striking feature of this specific mechanism, however, is the lack of any dedicated regulatory molecule (either protein or effector) required to bind in vicinity of the affected genes: although the local SC level experienced by a given promoter might be modified by the local binding of architectural or regulatory proteins and by nearby transcription, the regulatory principle highlighted in this study derives primarily from the global activity of topoisomerases at the scale of the entire chromosome, which then affects promoters differently depending on their distinct DNA thermodynamic properties, deviating from the usual notions of transcriptional regulation.

Materials and Methods

All details of experimental procedures, modeling computations, procedures and datasets used in the analyses can be found in Supplementary Information. All A/T%

shown are computed in a 5-nt window in the discriminator (centered at position -2). All error bars shown are 95% confidence intervals.

Acknowledgements

We thank the whole CRP team for helpful discussions, as well as Georgi Muskhelishvili and Ivan Junier for their critical reading of the manuscript.

References

[1] F. Jacob and J. Monod. Genetic regulatory mechanisms in the synthesis of proteins. *Journal of Molecular Biology*, 3:318–356, June 1961.

[2] Ingrid M. Keseler, Amanda Mackie, Alberto Santos-Zavaleta, Richard Billington, César Bonavides-Martínez, Ron Caspi, Carol Fulcher, Socorro Gama-Castro, Anamika Kothari, Markus Krummenacker, Mario Latendresse, Luis Muñiz-Rascado, Quang Ong, Suzanne Paley, Martin Peralta-Gil, Pallavi Subhraveti, David A. Velázquez-Ramírez, Daniel Weaver, Julio Collado-Vides, Ian Paulsen, and Peter D. Karp. The EcoCyc database: reflecting new knowledge about Escherichia coli K-12. *Nucleic Acids Research*, 45(Database issue):D543–D550, January 2017.

[3] Lilia Brinza, Federica Calevro, and Hubert Charles. Genomic analysis of the regulatory elements and links with intrinsic DNA structural properties in the shrunken genome of Buchnera. *BMC Genomics*, 14(1):73, February 2013.

[4] Ivan Junier, E. Besray Unal, Eva Yus, Verónica Lloréns-Rico, and Luis Serrano. Insights into the Mechanisms of Basal Coordination of Transcription Using a Genome-Reduced Bacterium. *Cell Systems*, 2(6):391–401, 2016.

[5] Andrey Feklístov, Brian D. Sharon, Seth A. Darst, and Carol A. Gross. Bacterial sigma factors: a historical, structural, and genomic perspective. *Annual Review of Microbiology*, 68:357–376, 2014.

[6] Stefan Klumpp and Terence Hwa. Growth-rate-dependent partitioning of RNA polymerases in bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 105(51):20245–20250, December 2008.

[7] Melanie M. Barker, Tamas Gaal, Cathleen A. Josaitis, and Richard L. Gourse. Mechanism of regulation of transcription initiation by ppGpp. I. Effects of ppGpp on transcription initiation in vivo and in vitro1 1Edited by R. Ebright. *Journal of Molecular Biology*, 305(4):673–688, January 2001.

[8] Andrew Travers and Georgi Muskhelishvili. DNA supercoiling - a global transcriptional regulator for enterobacterial growth? *Nat Rev Microbiol*, 3(2):157–169, 2005.

[9] Charles J. Dorman and Matthew J. Dorman. DNA supercoiling is a fundamental regulatory principle in the control of bacterial gene expression. *Biophysical Reviews*, 8(Suppl 1):89–100, November 2016.

[10] Shiny Martis B., Raphaël Forquet, Sylvie Reverchon, William Nasser, and Sam Meyer. DNA Supercoiling: an Ancestral Regulator of Gene Expression in Pathogenic Bacteria? *Computational and Structural Biotechnology Journal*, 17:1047–1055, January 2019.

[11] Jookyung Lee and Sergei Borukhov. Bacterial RNA Polymerase-DNA Interaction-The Driving Force of Gene Expression and the Target for Drug Action. *Frontiers in Molecular Biosciences*, 3:73, 2016.

[12] Emily F. Ruff, M. Thomas Record, and Irina Artsimovitch. Initial events in bacterial transcription initiation. *Biomolecules*, 5(2):1035–1062, May 2015.

[13] Dina Zhabinskaya and Craig J. Benham. Theoretical Analysis of Competing Conformational Transitions in Superhelical DNA. *PLoS Computational Biology*, 8(4), April 2012.

[14] Daniel Jost, Asif Zubair, and Ralf Everaers. Bubble statistics and positioning in superhelically stressed DNA. *Physical Review. E, Statistical, Nonlinear, and Soft Matter Physics*, 84(3 Pt 1):031912, September 2011.

[15] Estelle Crozat, Nadège Philippe, Richard E. Lenski, Johannes Geiselmann, and Dominique Schneider. Long-term experimental evolution in Escherichia coli. XII. DNA topology as a key target of selection. *Genetics*, 169(2):523–532, February 2005.

[16] B. P. Leblanc, C. J. Benham, and D. J. Clark. An initiation element in the yeast CUP1 promoter is recognized by RNA polymerase II in the absence

of TATA box-binding protein if the DNA is negatively supercoiled. *Proceedings of the National Academy of Sciences of the United States of America*, 97(20):10745–10750, September 2000.

[17] Daniel Jost. Twist-DNA: computing base-pair and bubble opening probabilities in genomic superhelical DNA. *Bioinformatics*, 29(19):2479–2481, 2013.

[18] G. Wesley Hatfield and Craig J. Benham. DNA topology-mediated control of global gene expression in Escherichia coli. *Annual Review of Genetics*, 36:175–203, 2002.

[19] Helge Auner, Malcolm Buckle, Annette Deufel, Tamara Kutateladze, Linda Lazarus, Ramesh Mavathur, Georgi Muskhelishvili, Iain Pemberton, Robert Schneider, and Andrew Travers. Mechanism of Transcriptional Activation by FIS: Role of Core Promoter Structure and {DNA} Topology. *Journal of Molecular Biology*, 331(2):331 – 344, 2003.

[20] Kate L. Henderson, Lindsey C. Felth, Cristen M. Molzahn, Irina Shkel, Si Wang, Munish Chhabra, Emily F. Ruff, Lauren Bieter, Joseph E. Kraft, and M. Thomas Record. Mechanism of transcription initiation and promoter escape by E. coli RNA polymerase. *Proceedings of the National Academy of Sciences of the United States of America*, 114(15):E3032–E3040, 2017.

[21] James Chen, Courtney Chiu, Saumya Gopalkrishnan, Albert Y. Chen, Paul Dominic B. Olinares, Ruth M. Saecker, Jared T. Winkelman, Michael F. Maloney, Brian T. Chait, Wilma Ross, Richard L. Gourse, Elizabeth A. Campbell, and Seth A. Darst. Stepwise Promoter Melting by Bacterial RNA Polymerase. *Molecular Cell*, 78(2):275–288.e6, 2020.

[22] Jared T. Winkelman and Richard L. Gourse. Open complex DNA scrunching: A key to transcription start site selection and promoter escape. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 39(2), 2017.

[23] Lacramioara Bintu, Nicolas E. Buchler, Hernan G. Garcia, Ulrich Gerland, Terence Hwa, Jané Kondev, Thomas Kuhlman, and Rob Phillips. Transcriptional regulation by the numbers: applications. *Current Opinion in Genetics & Development*, 15(2):125–135, April 2005.

[24] A. A. Travers. Promoter sequence for stringent control of bacterial ribonucleic acid synthesis. *Journal of Bacteriology*, 141(2):973–976, February 1980.

[25] I. K. Pemberton, G. Muskhelishvili, A. A. Travers, and M. Buckle. The G+C-rich discriminator region of the tyrT promoter antagonises the formation of stable preinitiation complexes. *Journal of Molecular Biology*, 299(4):859–864, June 2000.

[26] Nicolas Blot, Ramesh Mavathur, Marcel Geertz, Andrew Travers, and Georgi Muskhelishvili. Homeostatic regulation of supercoiling sensitivity coordinates transcription of the bacterial genome. *EMBO Reports*, 7(7):710–715, July 2006.

[27] Brian J Peter, Javier Arsuaga, Adam M Breier, Arkady B Khodursky, Patrick O Brown, and Nicholas R Cozzarelli. Genomic transcriptional response to loss of chromosomal supercoiling in Escherichia coli. *Genome Biology*, 5(11):R87, 2004.

[28] Zghidi-Abouzid Ouafa, Sylvie Reverchon, Thomas Lautier, Georgi Muskhelishvili, and William Nasser. The nucleoid-associated proteins H-NS and FIS modulate the DNA supercoiling response of the pel genes, the major virulence factors in the plant pathogen bacterium Dickeya dadantii. *Nucleic Acids Research*, 40(10):4306–4319, May 2012.

[29] Xuejiao Jiang, Patrick Sobetzko, William Nasser, Sylvie Reverchon, and Georgi Muskhelishvili. Chromosomal "Stress-Response" Domains Govern the Spatiotemporal Expression of the Bacterial Virulence Program. *mBio*, 6(3), April 2015.

[30] Natalia E. Gogoleva, Tatiana A. Konnova, Alexander S. Balkin, Andrey O. Plotnikov, and Yuri V. Gogolev. Transcriptomic data of Salmonella enterica subsp. enterica serovar Typhimurium str. 14028S treated with novobiocin. *Data in Brief*, 29, February 2020.

[31] Bilal El Houdaigui, Raphaël Forquet, Thomas Hindré, Dominique Schneider, William Nasser, Sylvie Reverchon, and Sam Meyer. Bacterial genome architecture shapes global transcriptional regulation by DNA supercoiling. *Nucleic Acids Research*, 47(11):5648–5657, June 2019.

[32] Raphael Forquet, Xuejiao Jiang, William Nasser, Florence Hommais, Sylvie Reverchon, and Sam

Meyer. Mapping the complex transcriptional landscape of the phytopathogenic bacterium Dickeya dadantii. *bioRxiv*, page 2020.09.30.320440, September 2020. Publisher: Cold Spring Harbor Laboratory Section: New Results.

[33] Vikram Vijayan, Rick Zuzow, and Erin K. O'Shea. Oscillations in supercoiling drive circadian gene expression in cyanobacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 106(52):22564–22568, December 2009.

[34] Eva Yus, Verónica Lloréns-Rico, Sira Martínez, Carolina Gallo, Hinnerk Eilers, Cedric Blötz, Jörg Stülke, Maria Lluch-Senar, and Luis Serrano. Determination of the Gene Regulatory Network of a Genome-Reduced Bacterium Highlights Alternative Regulation Independent of Transcription Factors. *Cell Systems*, 9(2):143–158.e13, 2019.

[35] Alexander Bartholomäus, Ivan Fedyunin, Peter Feist, Celine Sin, Gong Zhang, Angelo Valleriani, and Zoya Ignatova. Bacteria differently regulate mRNA abundance to specifically respond to various stresses. *Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences*, 374(2063), 2016.

[36] Szymon Jozefczuk, Sebastian Klie, Gareth Catchpole, Jedrzej Szymanski, Alvaro Cuadros-Inostroza, Dirk Steinhauser, Joachim Selbig, and Lothar Willmitzer. Metabolomic and transcriptomic stress response of Escherichia coli. *Molecular Systems Biology*, 6:364, May 2010.

[37] Y. Ogata, T. Mizushima, K. Kataoka, T. Miki, and K. Sekimizu. Identification of DNA topoisomerases involved in immediate and transient DNA relaxation induced by heat shock in Escherichia coli. *Molecular & general genetics: MGG*, 244(5):451–455, September 1994.

[38] Tohru Mizushima, Kazuhiro Kataoka, Yasuyuki Ogata, Ryu-ichi Inoue, and Kazuhisa Sekimizu. Increase in negative supercoiling of plasmid DNA in Escherichia coli exposed to cold shock. *Molecular Microbiology*, 23(2):381–386, 1997.

[39] Dalit Weinstein-Fischer, Maya Elgrably-weiss, and Shoshy Altuvia. Escherichia coli response to hydrogen peroxide: a role for DNA supercoiling, Topoisomerase I and Fis. *Molecular Microbiology*, 35(6):1413–1420, 2000.

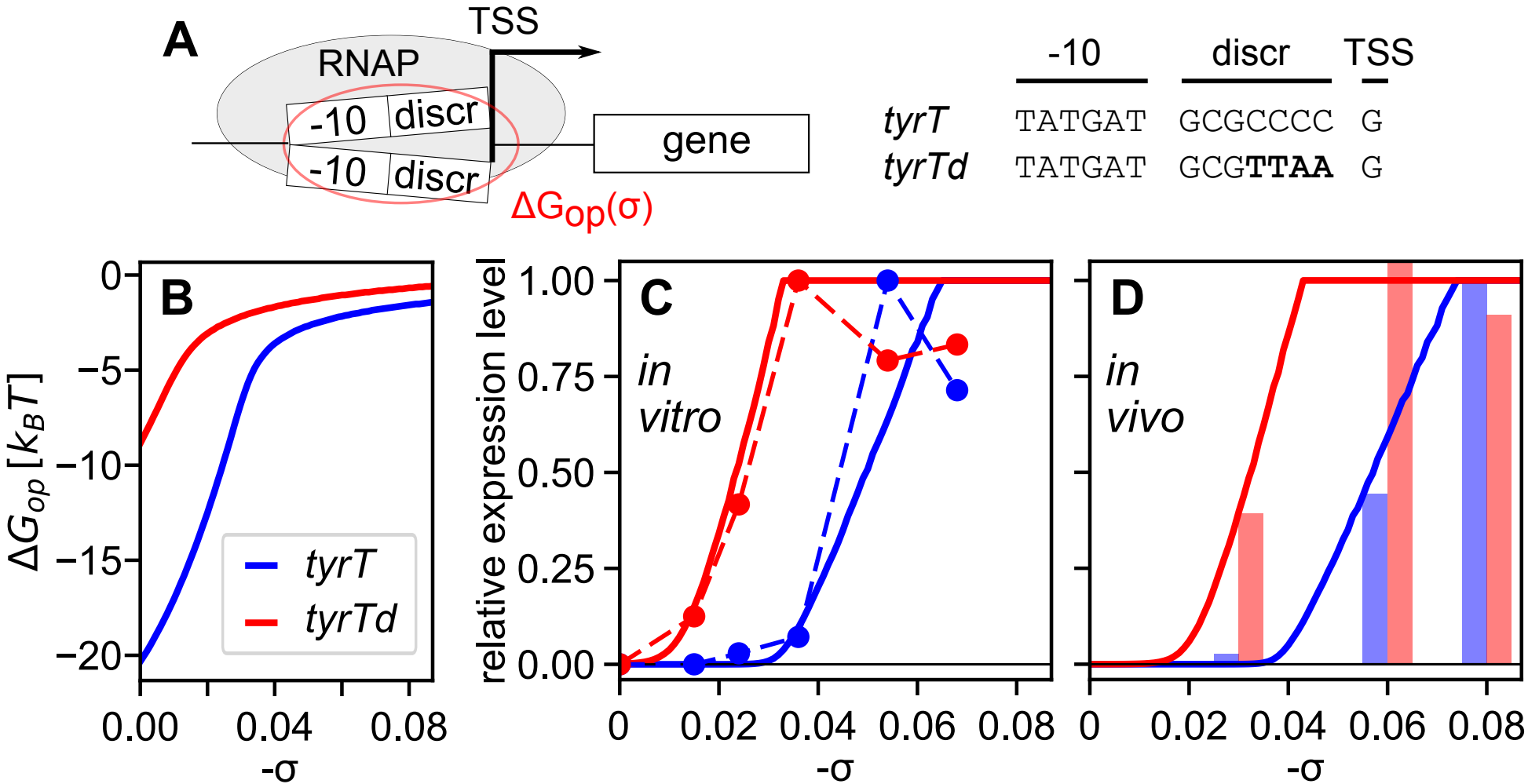
[40] Olivier Tenaillon, Jeffrey E. Barrick, Noah Ribeck, Daniel E. Deatherage, Jeffrey L. Blanchard, Aurko Dasgupta, Gabriel C. Wu, Sébastien Wielgoss, Stéphane Cruveiller, Claudine Médigue, Dominique Schneider, and Richard E. Lenski. Tempo and mode of genome evolution in a 50,000-generation experiment. *Nature*, 536(7615):165–170, August 2016.

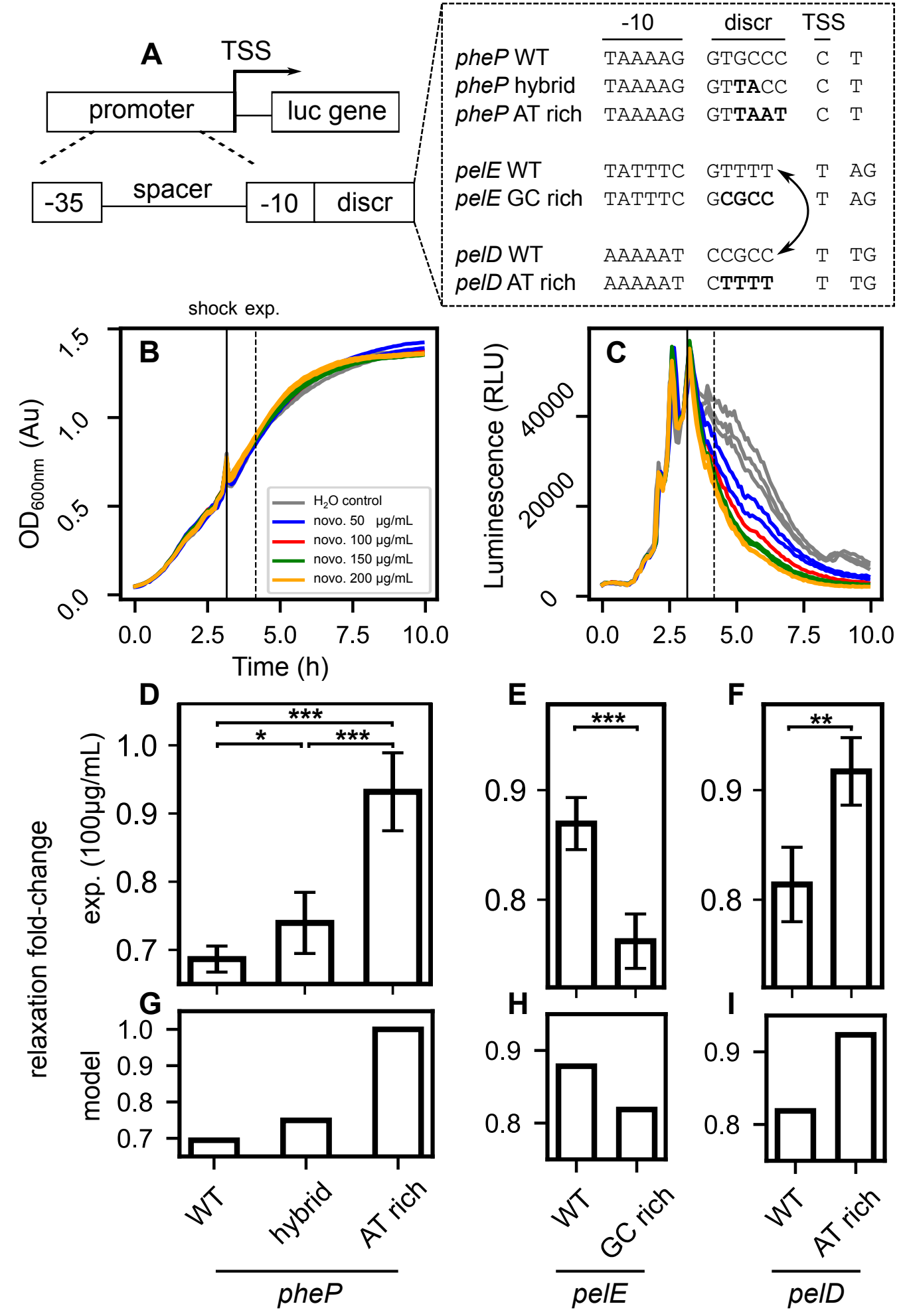
[41] Charles J. Dorman. Regulation of transcription by DNA supercoiling in Mycoplasma genitalium: global control in the smallest known self-replicating genome. *Molecular Microbiology*, 81(2):302–304, July 2011.

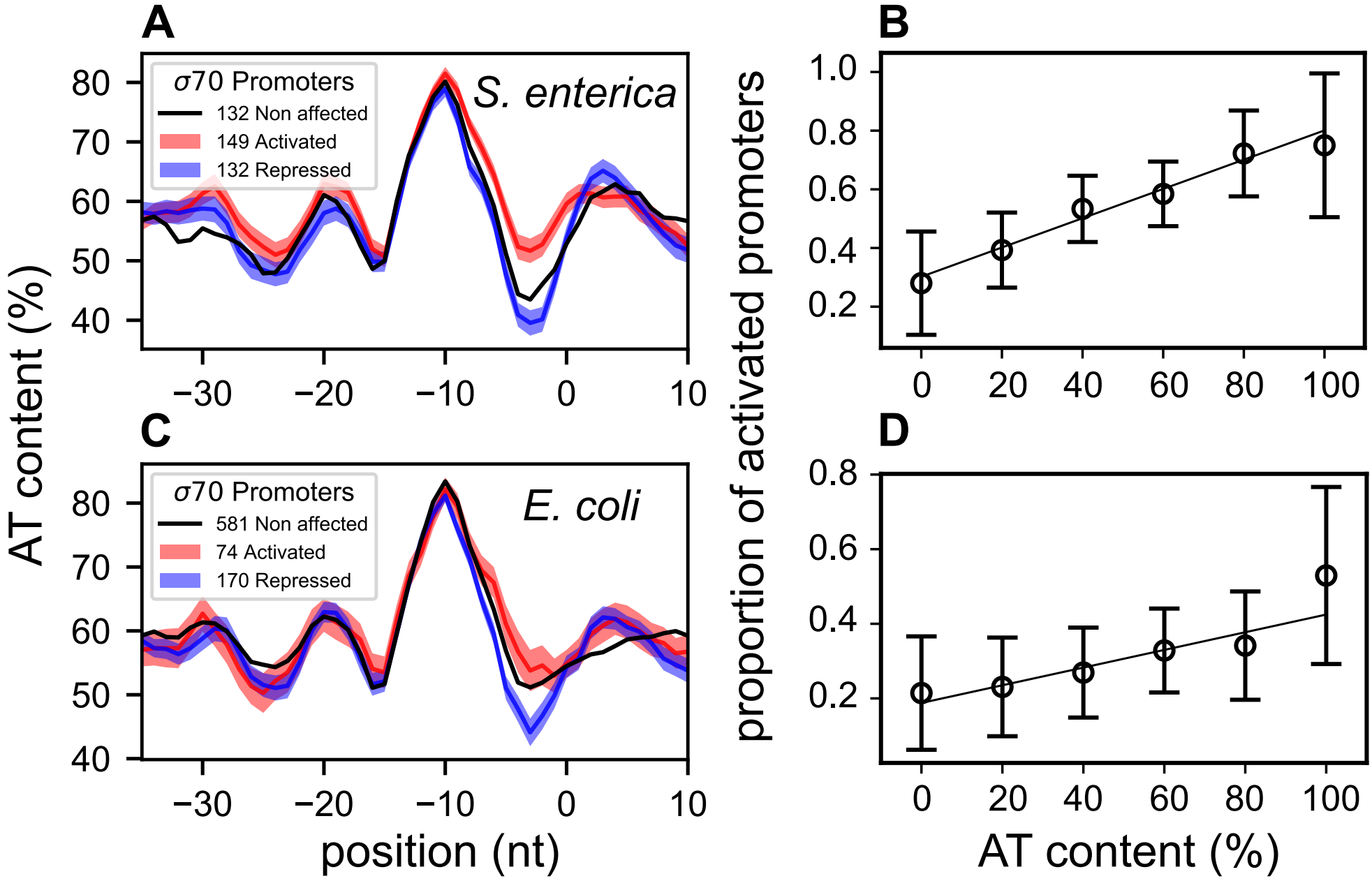
[42] Patricia Sanchez-Vazquez, Colin N. Dewey, Nicole Kitten, Wilma Ross, and Richard L. Gourse. Genome-wide effects on Escherichia coli transcription from ppGpp binding to its two sites on RNA polymerase. *Proceedings of the National Academy of Sciences of the United States of America*, 116(17):8310–8319, 2019.

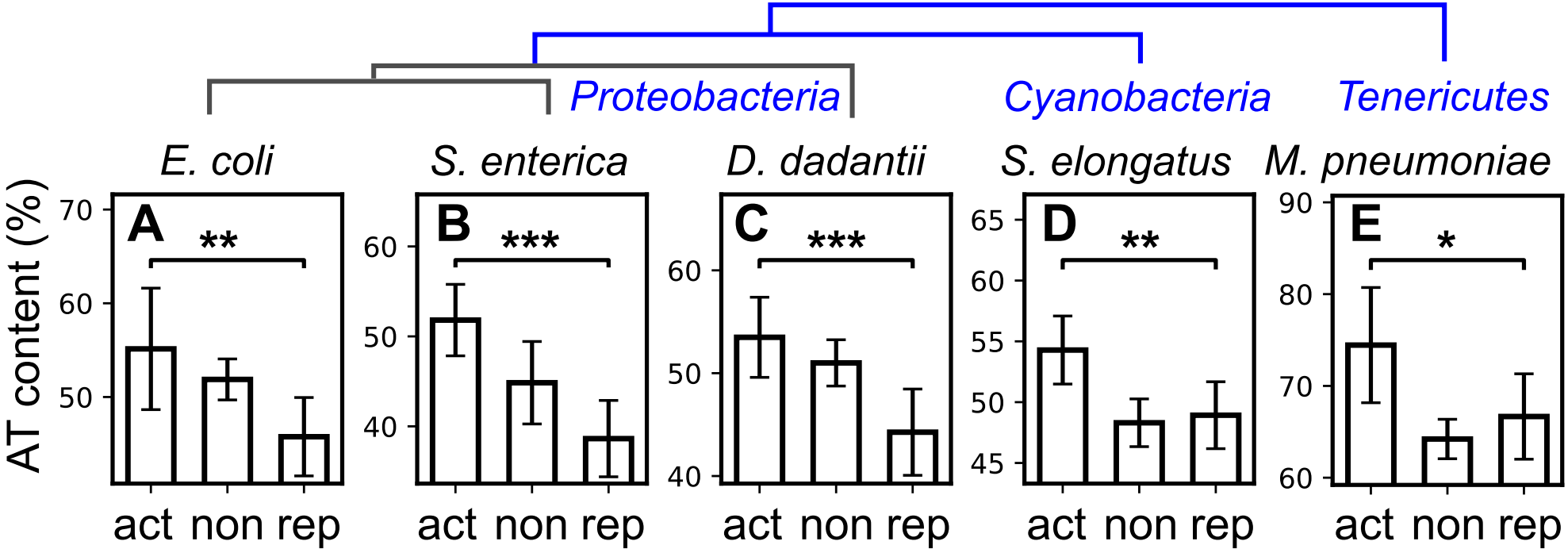
[43] Nadège Philippe, Estelle Crozat, Richard E. Lenski, and Dominique Schneider. Evolution of global regulatory networks during a long-term experiment with Escherichia coli. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 29(9):846–860, September 2007.

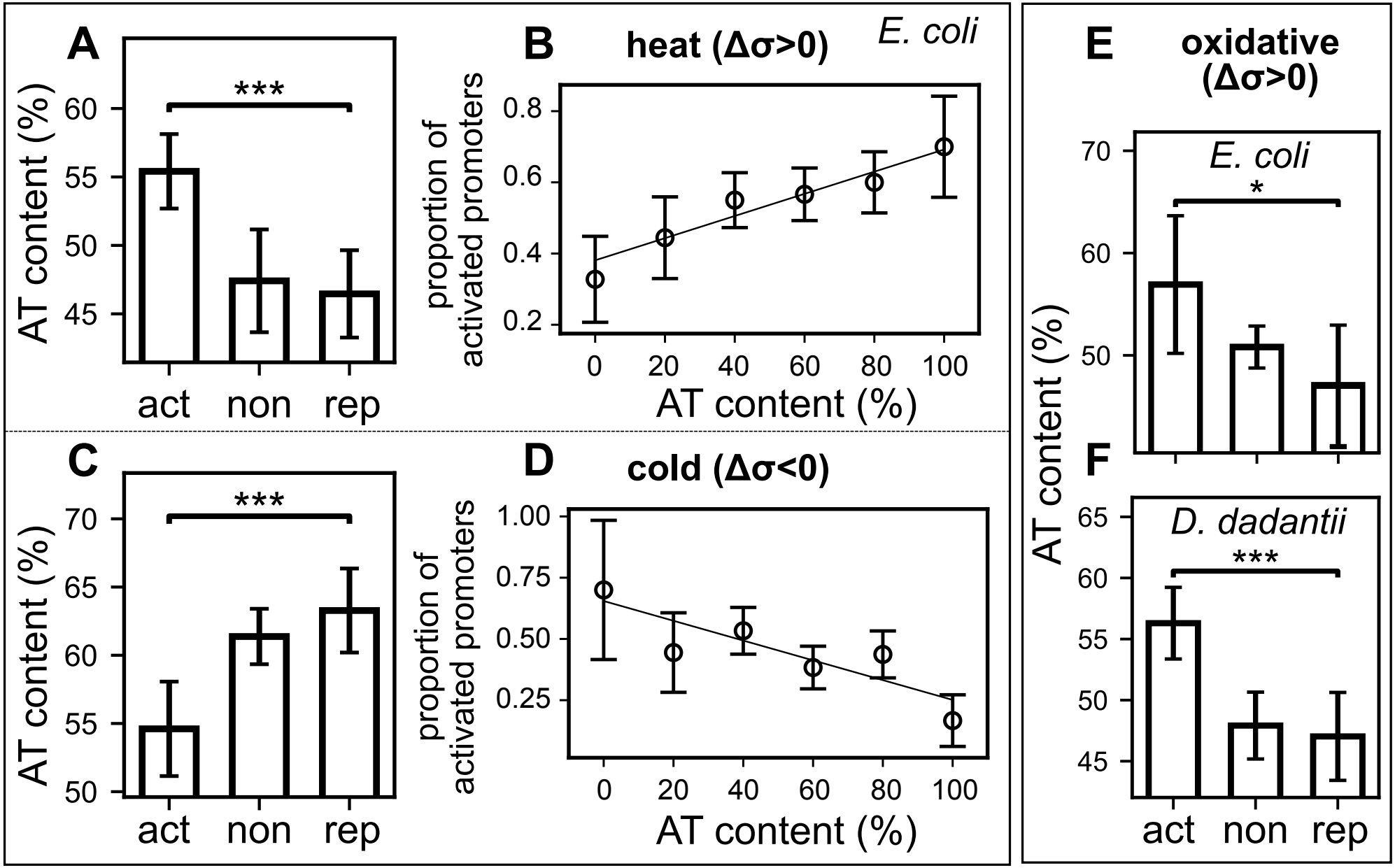
[44] Tim F. Cooper, Daniel E. Rozen, and Richard E. Lenski. Parallel changes in gene expression after 20,000 generations of evolution in Escherichiacoli. *Proceedings of the National Academy of Sciences of the United States of America*, 100(3):1072–1077, February 2003.

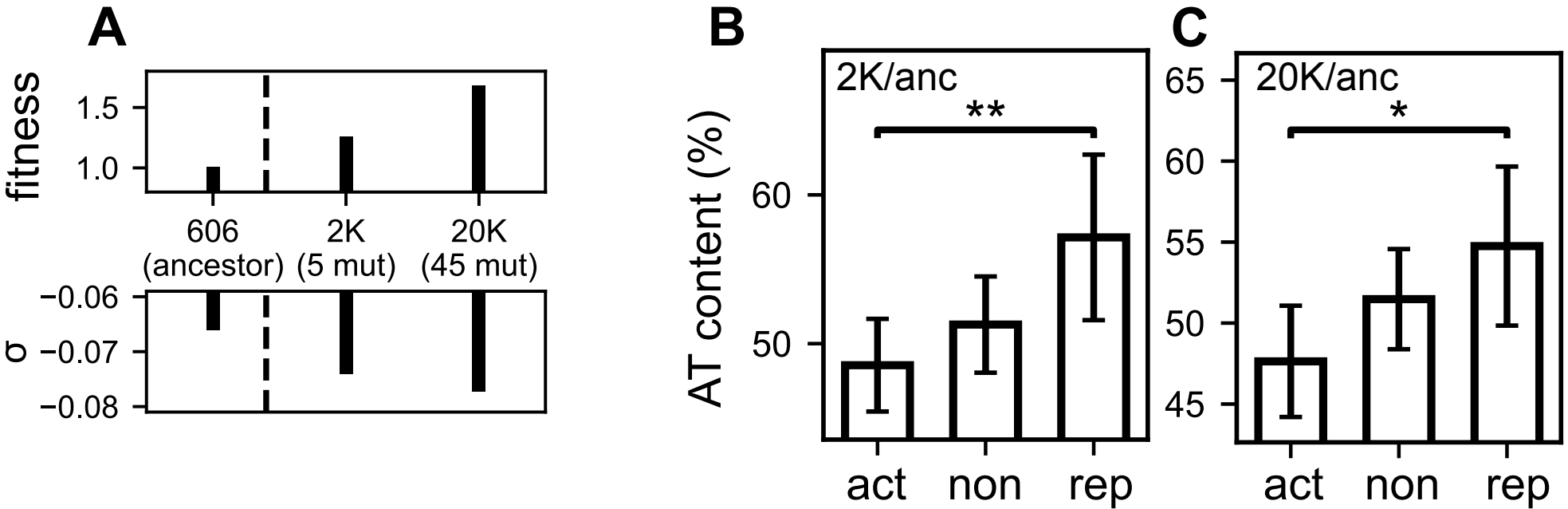

A
TSS
RNAP
-10
discr
-10
discr
gene
ΔG_op(σ)
-10
discr
TSS
tyrT TATGAT GCGCCCC G
tyrTd TATGAT GCGTTAA G
B
ΔG_op [k_B T]
tyrT
tyrTd
-σ
C
in vitro
relative expression level
-σ
D
in vivo
-σ


A
TSS
promoter
luc gene
-35
spacer
-10
discr
-10
discr
TSS
pheP WT TAAAAG GTGCCC C T
pheP hybrid TAAAAG GTTACC C T
pheP AT rich TAAAAG GTTAAT C T
pelE WT TATTTC GTTTT T AG
pelE GC rich TATTTC GCGCC T AG
pelD WT AAAAAT CCGCC T TG
pelD AT rich AAAAAT CTTTT T TG
shock exp.
B
OD600nm (Au)
Time (h)
H2O control
novo. 50 µg/mL
novo. 100 µg/mL
novo. 150 µg/mL
novo. 200 µg/mL
C
Luminescence (RLU)
D
E
F
G
H
I
relaxation fold-change
exp. (100µg/mL)
model
WT
hybrid
AT rich
GC rich
pheP
pelE
pelD


A
σ70 Promoters
132 Non affected
149 Activated
132 Repressed
S. enterica
B
C
σ70 Promoters
581 Non affected
74 Activated
170 Repressed
E. coli
D
AT content (%)
position (nt)
proportion of activated promoters
AT content (%)


Proteobacteria
Cyanobacteria
Tenericutes
E. coli
S. enterica
D. dadantii
S. elongatus
M. pneumoniae
AT content (%)
A
B
C
D
E
**
***
***
**
*
act non rep
act non rep
act non rep
act non rep
act non rep


A
***
AT content (%)
60
55
50
45
act
non
rep
B
heat (Δσ>0)
E. coli
proportion of activated promoters
0.8
0.6
0.4
0.2
0
20
40
60
80
100
AT content (%)
C
***
AT content (%)
70
65
60
55
50
act
non
rep
D
cold (Δσ<0)
proportion of activated promoters
1.00
0.75
0.50
0.25
0
20
40
60
80
100
AT content (%)
E
oxidative (Δσ>0)
E. coli
*
AT content (%)
70
60
50
F
D. dadantii
***
65
60
55
50
45
act
non
rep


A
fitness
1.5
1.0
606
(ancestor)
2K
(5 mut)
20K
(45 mut)
σ
−0.06
−0.07
−0.08
B
2K/anc
**
AT content (%)
60
50
act
non
rep
C
20K/anc
*
65
60
55
50
45
act
non
rep