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# Transcription-induced domains form the elementary constraining building blocks of bacterial chromosomes

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Transcription generates local topological and mechanical constraints on the DNA fiber, leading to the generation of supercoiled chromosome domains in bacteria. However, the global impact of transcription on chromosome organization remains elusive, as the scale of genes and operons in bacteria remains well below the resolution of chromosomal contact maps generated using Hi-C (~5–10 kb). Here we combined sub-kb Hi-C contact maps and chromosome engineering to visualize individual transcriptional units. We show that transcriptional units form discrete three-dimensional transcription-induced domains that impose mechanical and topological constraints on their neighboring sequences at larger scales, modifying their localization and dynamics. These results show that transcriptional domains constitute primary building blocks of bacterial chromosome folding and locally impose structural and dynamic constraints.

Bacterial genomes are organized into the nucleoid, a well-defined membrane-less compartment where DNA, RNA and proteins interact to shape the conformation of chromosome(s)<sup>1–3</sup>. DNA opening, associated with replication and transcription, modulates transiently the supercoiling level of the DNA fiber<sup>4</sup> by creating twin domains spanning 25 kb in each direction<sup>5</sup>. Topoisomerases, mainly Topo I and DNA gyrase, maintain supercoiling homeostasis, to keep the negatively supercoiled state necessary for DNA compaction and strand opening operations<sup>6</sup>. Radial plectoneme loops are proposed to decorate the bacterial chromosomes, either in association with protein complexes of the structural maintenance of chromosome (SMC) family<sup>7,8</sup> or with supercoil-induced processes<sup>9,10</sup>. Hi-C contact maps have also revealed higher-order levels of organization in bacterial chromosomes<sup>11–15</sup>, with directionality index (DI) analysis (a statistical parameter that assesses the degree of upstream or downstream contact bias for a genomic region) pointing at ~30 chromosome self-interacting domains (or CIDs) ranging in size

from ~30 to 300 kb (ref. 11). A careful analysis further unveiled a correlation between highly expressed (and long) genes (HEGs) and CID boundaries, although it was not systematic<sup>12,14</sup>. A short-range correlation was also described between the transcription level and the contact frequencies between pairs of adjacent, 5-kb DNA segments (bins)<sup>13</sup>. Furthermore, inhibition of transcription initiation by rifampicin abrogates domains and decondense nucleoids within minutes, suggesting a direct role for transcription in folding the chromosome<sup>16,17</sup>. On top of supercoiling generation, other transcription-related effects can influence the chromosome conformation. Recent experiments and biophysical models revealed that RNA production reduces the effective solvent quality of the cytoplasm and consequently impacts the local conformation of the DNA fiber<sup>18</sup>. However, with respect to the scale of gene and operon (<10 kb)<sup>9</sup> of bacterial genomes, these analyses remain relatively coarse. In addition, gene density, concomitant transcription and cell-to-cell variability of hundreds of genes could

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lead to intermingled patterns, leaving the possibility that fundamental underlying structural features have been overlooked.

In this Article, we combine a high-resolution Hi-C protocol recently adapted for bacteria<sup>19</sup> with chromosome engineering and cellular imaging to address the link between chromosome architecture and transcription at a higher level. We show that all active transcription units (TUs) form discrete individual, insulated three-dimensional (3D) domains that form the primary building blocks for larger chromosome folding.

## Results

### High-resolution Hi-C reveals transcription-associated contacts

High-resolution (0.5 or 1 kb) Hi-C contact maps of exponentially growing *Escherichia coli* cells reveals strong heterogeneity in the short-range contact signal (Fig. 1a and Methods), with ~200 short regions exhibiting strong and dense short-range signal, subsequently referred to as bundled domains (for calling of these regions, see Methods). These patterns, which cover approximately 1,300 kb, are strongly correlated with transcriptional activity and disappear upon addition of rifampicin (Fig. 1b and Extended Data Fig. 1a). They range in size from 1 to 20 kb and are distributed over the entire genome map (Extended Data Fig. 1b). The potential to make protein–DNA crosslinks will influence local Hi-C contacts<sup>20</sup>. Therefore, the local protein concentration on the DNA (protein occupancy) may contribute to the local Hi-C bundled signal. We took advantage of recent high-resolution maps of protein occupancy on the *E. coli* genome<sup>19</sup> to test whether silent regions nevertheless strongly enriched in proteins (EPODs) would appear as bundled domains in Hi-C maps. As shown on Fig. 1c, only ~10% of EPODs regions appear to be involved in a bundled domain, suggesting that protein occupancy per se is not sufficient to promote their formation. Overall, the positioning of the bundled domains are not correlated with Hi-C coverage, nor protein occupancy as quantified in ref. 21, suggesting that they do not correspond to DNA regions that are more visible or captured by the Hi-C protocol (Extended Data Fig. 1b,c and Methods)<sup>22</sup>.

In addition, a plaid-like pattern was often observed, corresponding to enrichment in contacts between successive transcribed DNA regions, alternating with nontranscribed regions with which they make fewer contacts (Fig. 1b and Extended Data Fig. 1a). The pattern was even more pronounced in the origin region that contains four ribosomal operons (Extended Data Fig. 1d). These contacts involved both ribosomal operons and highly expressed protein-coding genes. These observations suggest that neighboring transcribed regions tend to contact each other locally, either because they may relocate to the nucleoid external periphery, as suggested by super-resolution imaging<sup>17,23,24</sup>, or through an unknown transcription-dependent clustering mechanism.

To further quantify the correlation between local Hi-C contacts and gene expression, a pileup analysis of the averaged contacts centered on the start codon of the 5% and 10% most transcribed genes was performed (Fig. 1d). A bundled signal centered on the start codon appeared, strongly correlated with the corresponding averaged transcription signal (Pearson correlation 0.81). Because bacteria genes are often organized into operons and cotranscribed, we then plotted the pileup contact windows centered on the start codon of the first gene of the most transcribed operons (transcription start site, TSS) (Fig. 1e and Methods). The pileup displays an enrichment in local Hi-C contacts that increases abruptly precisely at TSS positions, and extends over the area spanned by the transcription track, further reinforcing the notion that short-range (0–5 kb) Hi-C contacts are correlated with transcription levels (Pearson correlation 0.62). Faint stripes crossing the map highlight slight enrichment of contacts between the TU and upstream and downstream regions is also observed, a signal that corroborates the plaid-like pattern observed on the sub-kb contact map (Fig. 1d,e, pointed at by green triangles).

Taken together, these observations suggest that the primary blocks organizing the *E. coli* chromosome consist of a succession of bundled domains, that make short-range contacts in Hi-C maps, and which we

term transcription-induced domains (TIDs). TIDs are separated by non-transcribed regions depleted in local Hi-C contacts but can interact together as long as the genomic distance between them remains relatively small (with the present experimental approach: <25 kb).

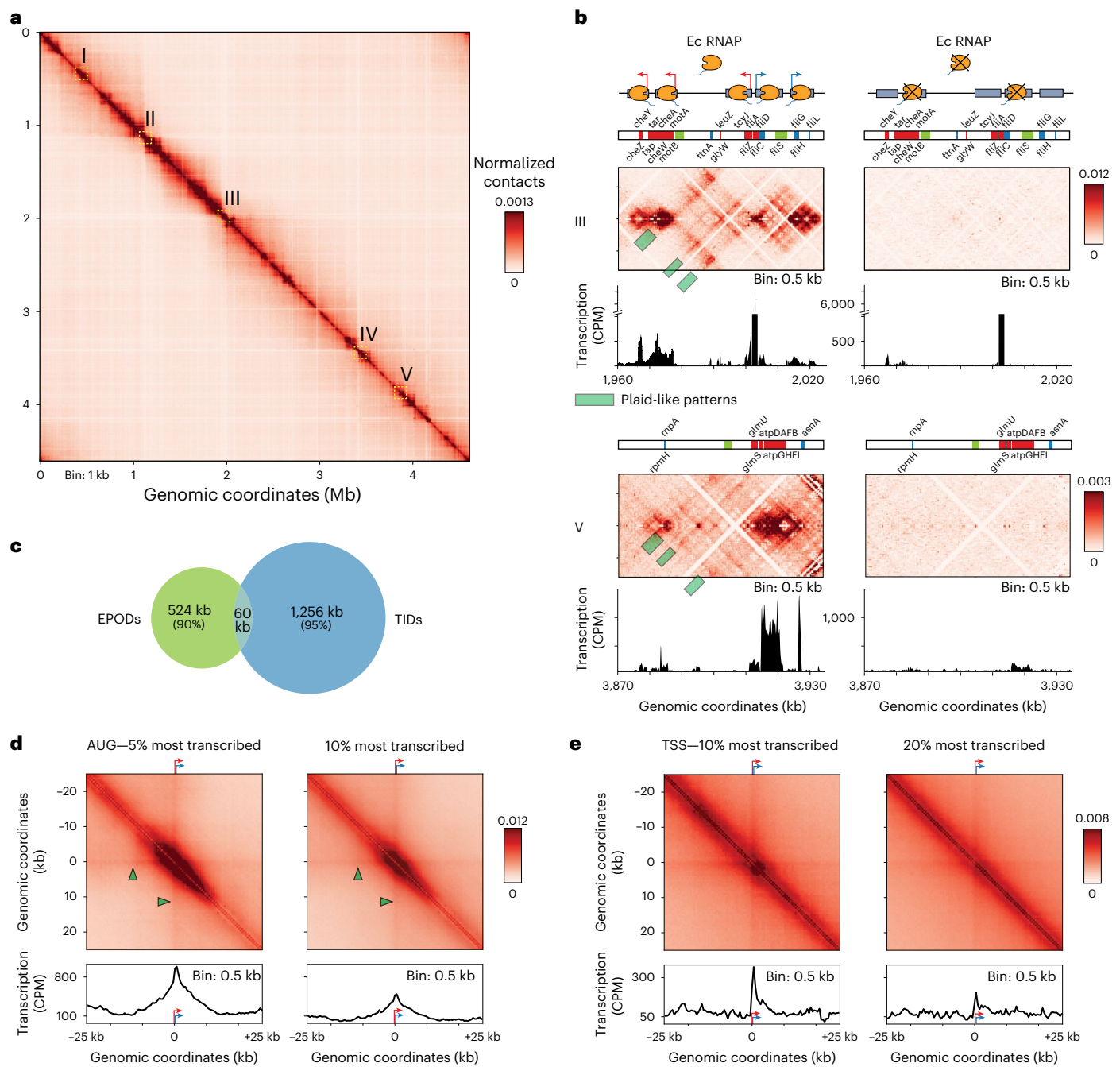
### TIDs explain CIDs detection in low-resolution maps

We next compared the positions of TIDs with the boundaries of CIDs previously identified along the *E. coli* genome<sup>13</sup>. First, we called CIDs in the 5-kb contact map using DI analysis, revealing 27 domains (Methods). Twenty-two of these domains' boundaries overlapped those previously identified using the same approach<sup>13</sup>, while the others lie at the edge of the detection threshold (Extended Data Fig. 2a–b and Extended Data Table 1). As shown before, these boundaries are enriched with HEGs (Extended Data Fig. 2c–e). The same DI analysis performed over a 2-kb binned contact map yielded 30 new boundaries (green signal, Extended Data Fig. 2b and Extended Data Table 1). Finally, DI analysis proved too noisy when applied on a 1-kb contact map (Extended Data Fig. 2b and Extended Data Table 1). To call CID-like signals in 1-kb contact maps, we adapted HiC-DB, another insulation score approach<sup>25</sup> (Methods). We detected 135 boundaries, delineating 135 CID-like regions ranging in size from 5 to 125 kb (magenta signal, Extended Data Fig. 2c and Extended Data Table 1). Among those, 22 overlap with the boundaries called with the DI analysis of the 5 kb binned contact map and enriched HEG annotations (blue signal, Extended Data Fig. 2). The remaining 113 positions correspond to less expressed genes (Extended Data Fig. 2d,e). Altogether, these results suggest that the chromosome, rather than being structured into large self-interacting regions, is organized by a succession of short, transcription-induced, compact domains alternating with unstructured regions. This structuring is reminiscent of those observed in budding yeast using the microC technique<sup>26</sup>.

### A single TU is sufficient to imprint a Hi-C domain

To further understand the nature of the transcription-dependent, short-range contacts increase observed in the high-resolution contact maps, we designed an artificial inducible system. A T7 promoter was inserted at the *lacZ* locus, facing towards the *ter*. The T7 RNA polymerase (Pol) is specific to its own promoters and was put under the control of the inducible arabinose promoter (Fig. 2). Upon arabinose addition, a bundled domain appears on the Hi-C map, originating at the pT7 position and propagating towards the *ter* over ~70 kb when it abruptly stops at the level of the bundled generated by the highly expressed *cyoABCD* operon (Fig. 2a,b). In addition, the Hi-C signal at pT7 is shaped roughly like an arrowhead, while an enrichment in local contacts is also observed upstream the activated promoter. Chromatin immunoprecipitation of the T7 RNA Pol showed a strong enrichment at the pT7 (Extended Data Fig. 3a), whereas RNA sequencing (RNA-seq) analysis further confirmed the strong induction of this artificial TU (Fig. 2b). Since the T7 RNA Pol is insensitive to the bacterial RNA Pol inhibitor rifampicin, we reasoned that treating the cells with the drug should highlight a single transcriptional unit induced by the T7 promoter as genome-wide transcription is turned off<sup>27</sup>. In presence of rifampicin, the chromosome indeed displayed a single TU starting at the pT7 promoter, as determined by RNA-seq and T7 RNA pol chromatin immunoprecipitation followed by sequencing (ChIP-seq; Fig. 2a,b). The absence of neighboring transcription leads to a longer T7 transcription track covering ~110–120 kb, as compared to 70 kb in absence of rifampicin (Fig. 2a,b). This observation further supports the hypothesis that the endogenous *E. coli* RNA Pol of the closest native transcription peak of the *cyoABCD* operon was indeed responsible for blocking T7-induced transcription.

The corresponding normalized contact map displays a clear, discrete bundled domain overlapping the active T7 TU, further magnified when plotting the ratio between the maps of cells treated with rifampicin but with or without T7 induction (Fig. 2c, bottom). Magnification of the induced T7 region from the normalized wild type (WT) with rifampicin map reveals two types of contact pattern at



**Fig. 1 | The bacterial chromosome is structured by tens of small transcriptionally active 3D units. a**, Hi-C normalized contact map of WT *E. coli* cells (bin: 1 kb). The five yellow squares I–V underline representative 64-kb regions magnified in either **b** or in Extended Data Fig. 1. **b**, Magnifications of regions III and V in absence (left) and presence (right) of rifampicin. Ec RNAP: *E. coli* RNA Pol II. For each window and condition: Top: a schematic representation of the region's genetic content, with the names of genes within the 10% most transcribed indicated in blue and red for forwards and reverse orientation, respectively, and silent EPODs regions in green<sup>21</sup>. Middle: normalized contact map (bin: 0.5 kb). Bottom: RNA-seq profile in CPM. Plaid-like pattern positions

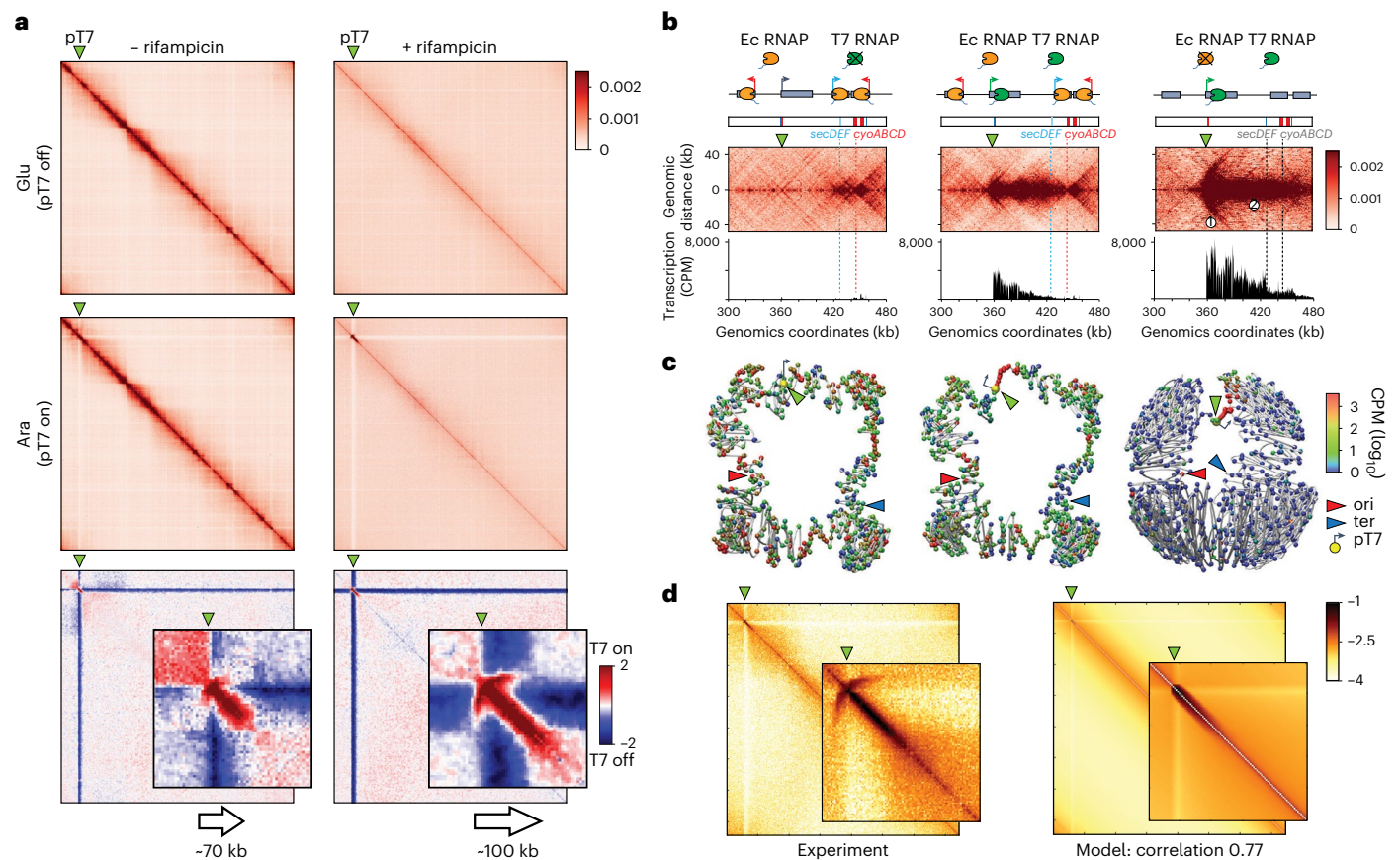
are pointed with greenish rectangles on the maps. **c**, Venn diagram of EPODs labeled regions<sup>21</sup> and of regions labeled as bundle domain. The metric used corresponds to the total size of the corresponding regions, in kb. **d**, Top: pileup of 50 kb contact map windows (bin: 0.5 kb) centered on the start codons (AUG) of the 5% (left) and 10% (right) most transcribed genes of the genome. Bottom: corresponding pileup of transcription (RNA-seq) tracks. Green arrowheads indicate a faint stripe signal extending from the TSS. **e**, Pileup of 50-kb contact map windows centered on the TSS of the 10% and 20% most transcribed TUs (that is operons). Bottom: corresponding pileup of transcription (RNA-seq) tracks.

the induced promoter: an 'arched stripe', which supplants the rough arrowhead observed earlier and extends from the TSS (Fig. 2b, label (1)) over -25 kb, and the thick bundled signal that extends across the transcription and T7 RNA Pol deposition tracks (Fig. 2b, label (2); Extended Data Fig. 3a). Consequently, this system allows to magnify a

bundled signal emanating from a single TU and decreasing smoothly along a -110-kb track.

Both signals were observed upon inversion of the gene (Extended Data Fig. 3a). The bundled pattern, but not the arched stripe, is strongly reminiscent of that observed from the pileup plots of highly





**Fig. 2 | Contact profile of single, active transcription unit within an entire genome. a**, Hi-C contact maps (bin: 1 kb) of *E. coli* chromosome carrying a single T7 promoter (green triangle), in absence (left) or presence (right) of rifampicin. Ec RNAP: *E. coli* RNA Pol II. Top: cells grown in glucose media, when the T7 RNA Pol is not expressed. Middle: cells grown in presence of arabinose, with expression of the T7 RNA Pol. Bottom:  $\log_2$  ratio contact maps with and without induction of the T7 promoter. Magnification of the T7 promoter region, represented using Serpentine flexible binning (Methods)<sup>48</sup>. **b**, Magnification of the T7 promoter in the normalized contact maps, with and without induction and in presence and absence of rifampicin. From left to right: T7 promoter off, no rif; T7 on, no rif; T7 on, + rif. For each window and condition, a schematic representation of the region's genetic content is presented on the top, with the operons within the 10%

most transcribed indicated in blue and red for forwards and reverse orientation, respectively (that is, *secDEF* and *cyoABCD*). The corresponding RNA-seq tracks (CPM) are plotted under the maps. In presence of T7 RNA Pol and rifampicin, the numbered labels on the map highlight the features discussed in the text: (1) arched stripe pattern; (2) bundle region. **c**, Average genome structures using Shrek of the corresponding 2D contact maps of the *E. coli* bacterial chromosome in the different conditions. The green, red and blue arrows represent the pT7, ori and ter positions, respectively. The 3D representations are not the physical structure of the genome, but the average structure of the population of cells that we observed. **d**, Modeling of the Hi-C contact maps using the RNA Pol distribution on the genome and using the second model (Methods).

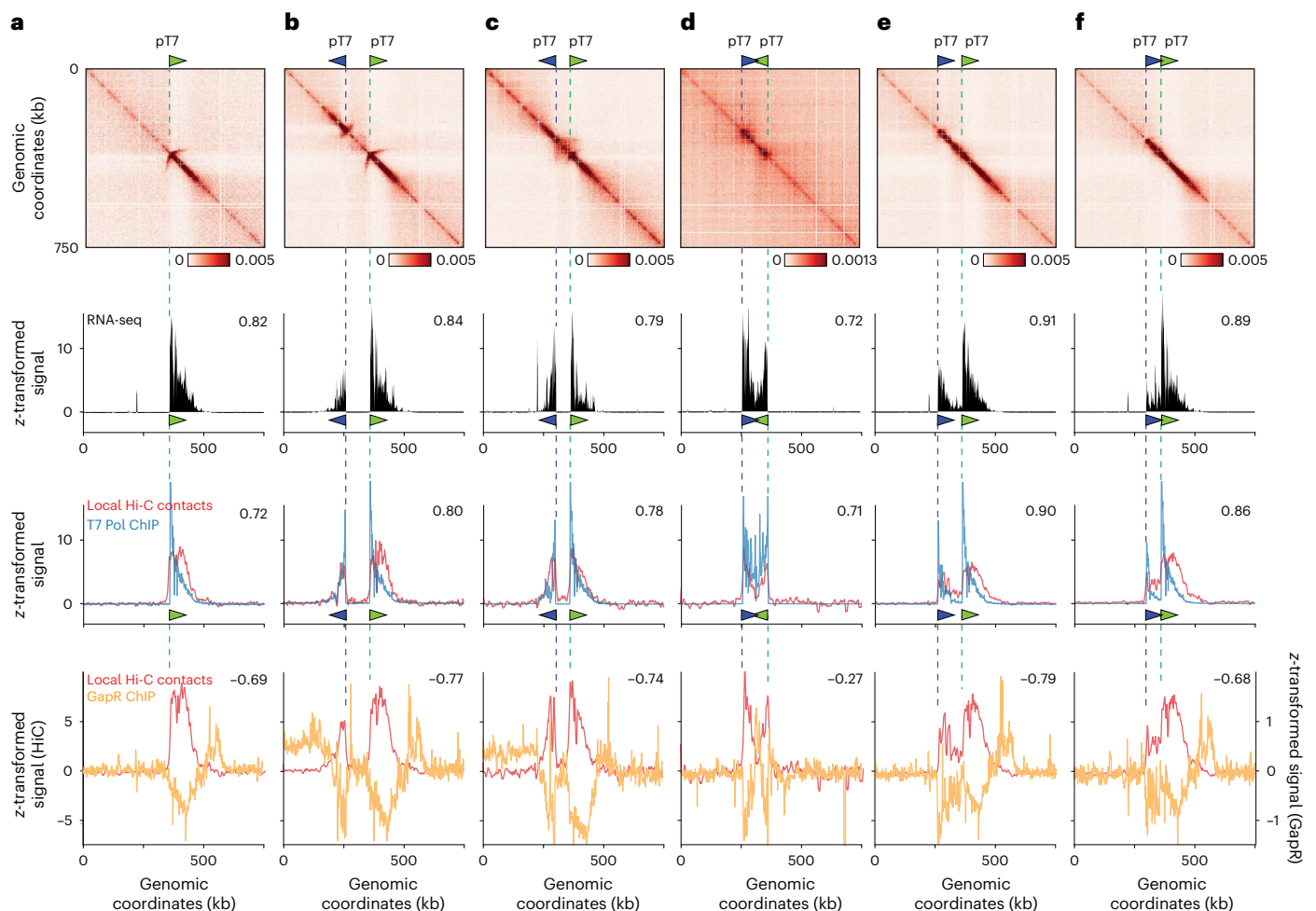
expressed TSS of the native genome (Fig. 1d). Using less potent endogenous promoters (*PompA* and *PrpsM*) introduced at the *lacZ* locus, we observed a few kilobases bundles and no obvious arched stripe (Extended Data Fig. 4a). Finally, the transcribed region is covered with polysomes, and thus most likely translated (Extended Data Fig. 5). However, the contact signal is unchanged when (1) two stop codons are introduced downstream the T7 promoter (*pT7lacZ<sup>2xstop</sup>*), preventing the synthesis of the first genes of the T7 TU *lacZYA*, and (2) translation elongation is inhibited by chloramphenicol, a drug that inhibits ribosome translocation and stabilizes messenger RNAs<sup>28,29</sup> (Extended Data Fig. 5). The former result suggests that the signal could be independent of translation although we cannot exclude a role of ribosomes in TIDs maintenance.

### Modeling TIDs

The data contained in the 2D contact maps can also be visualized in 3D using the shortest-path reconstruction algorithm ShRec3D (Methods). These structures are an alternative, lower-dimension representation of the 2D maps in the 3D space and are based on any physical model. They nevertheless illustrate how the highly transcribed T7 TU contact

map forms a discrete structure within the chromosome that appears to insulate flanking regions (Fig. 2d)<sup>30</sup>.

To gain further quantitative insight into the link between transcription and increased short range contacts, we developed two probabilistic modeling approaches to emulate the observed contact map under two different assumptions. In the first hypothesis, the increase in short-range contacts is due to the existence of preferential contacts between T7 RNA Pols that cover the TU. In the second model, we added an insulation effect of the polymerases such that the contact probability between two polymerases decreases if another polymerase is present between them. The models take as inputs the experimentally measured decay in contact frequency with increasing genomic distance and the ChIP deposition profile of T7 RNA Pol. The only fitting parameter, the maximum T7 RNA Pol occupancy along the TU (between 0% and 100%), was set to get the highest correlation between the experimental Hi-C and the model contact maps. The best result (correlation of 0.77) was obtained for the second model and a maximum occupancy of 15% (Extended Data Fig. 3c; compared to a maximum correlation of 0.67 for model 1 in Extended Data Fig. 3c). The extended bundled pattern correlates nicely with the experimental T7 RNA Pol occupancy,



**Fig. 3 | Supercoiling and contacts resulting from combination of pairs of transcription units. a–f**, Genomic characterization of chromosomal regions carrying pairs of pT7 promoters in different orientations. The orientations are as follows: a single promoter (a), two divergent promoters at 100 kb (b), 60 kb (c), two convergent promoters at 100 kb (d), two unidirectional (collinear) promoters at 100 kb (e) and 60 kb (f). From top to the bottom: Hi-C contact map

(first row; bin: 1 kb), RNA-seq track (second row; in CPM), T7 RNA Pol ChIP-seq track (third row, blue curve) and short-range Hi-C contacts (third row, red curve), and GapR ChIP-seq revealing positive supercoiling (fourth row, yellow curve) and short-range Hi-C contacts (fourth row, red curve). Values on the top right corner of each panel are the Spearman correlation coefficients of the track with the short-range Hi-C contacts. All tracks are z-transformed.

suggesting that crosslinking of trains of consecutive RNA Pol along the transcribed track could account for the contact pattern observed (Fig. 2e). These results therefore suggest that the bundled motifs corresponding to TIDs in Hi-C contact maps correspond to trains of RNA Pools that each have a cumulative local insulating effect.

### Interactions between adjacent T7 RNA Pol-induced domains

We next combined pairs of transcribed T7 units (*pT7lacZ* and *pT7mCherry*) to further characterize the potential structural interplay between two neighboring genes. The second pT7 was introduced at either 60 or 100 kb upstream of *pT7lacZ*, either in collinear, convergent or divergent orientation (Fig. 3, first row). Exponentially growing cells were induced for T7 RNA Pol using arabinose, treated with rifampicin and processed with Hi-C and RNA-seq. In all cases, we observed an excellent correlation between the short-range contacts, transcription tracts and T7 RNA Pol as quantified using ChIP-seq (Spearman correlation between 0.62 and 0.91) (Fig. 3, first to third rows). First, and in contrast to the native TUs for which interactions between neighboring TUs are observed (Fig. 1b), no interactions between the pairs of T7 TU were observed (Fig. 3b–f). Second, the arched stripe pattern appears affected by the orientation of the promoters with respect to each other. Upon induction, the two promoters positioned in divergent orientations and separated by

100 kb displayed similar contact patterns, that is, an arched stripe and the bundle signal (Fig. 3b, first and third rows). The arched stripe pattern nevertheless vanished when the distance separating the divergent promoters was shortened (60 kb) (Fig. 3c, first row). Concomitantly, a self-interacting domain of enriched contact emerged in-between the two genes, which strengthens when the distance between the divergent promoters decreases (Fig. 3b,c). These upstream contacts are consistent with the observation made using a single promoter (Fig. 2b). In contrast, the two genes in convergent orientation resulted in the two transcription tracks abruptly ending at mid-distance, resulting in a sharp boundary right in-between the two promoters (Fig. 3d, first to third rows). When positioned in collinear orientation, the well-defined and visible arched stripe of the *pT7lacZ* promoter is strongly reduced, if not entirely suppressed, by the incoming transcription tract of the upstream pT7mCherry (Fig. 3e,f, first to third rows). Transcription induces positive and negative supercoils in front of and behind the RNA Pol<sup>4,5</sup>. These supercoils may indirectly influence the arched stripe by decreasing initiation or elongation by the T7 RNA Pol<sup>31–33</sup>. Because we did not observe dramatic changes of T7 expression besides the abrupt termination of convergent tracks that probably reflect the documented effect(s) of supercoiling on elongation<sup>33</sup>, we favor the hypothesis that an adjacent TU will directly affect the arched stripe because it consists of negative supercoils.



### The arched stripe of the T7 RNA Pol-induced domains

To further explore the nature of the arched stripe, we tested the effects of *topA* overexpression, which encodes for TopoI that actively relaxes negative supercoils into DNA<sup>34</sup>. TopoI overexpression modestly affected the bundle but resulted in 50% reduction of the intensity of the arched stripe signal, suggesting that the latter may result from an accumulation of negative supercoiling upstream of the promoter that overcomes the capacity of the topoisomerase to remove them. By contrast, the inhibition of gyrase, which removes positive supercoil ahead of the RNA Pol, with novobiocin, shortens the transcription bundle signal while concomitantly strongly reducing the arched stripe (Extended Data Fig. 4c). A possibility is that in absence of gyrase the accumulation of positive supercoiling downstream of the track triggers earlier termination of the polymerase, and thus diminishes negative supercoiling upstream of the promoter. To measure the supercoiled nature of the DNA template at the level of the T7 TU(s), we quantified using ChIP-seq the deposition of GapR, a protein of *Caulobacter crescentus* recently introduced as a marker of positive supercoiling along bacterial and yeast chromosomes<sup>35</sup>. In *W. coli*, GapR is enriched downstream endogenous active genes<sup>35</sup>. Enrichment of GapR was observed at the 3' end of the single T7 TU (Fig. 3a, fourth row). This enrichment corresponds to positive supercoils that diffuse over a 50–100 kb region after the T7 TU. No enrichment was observed in-between genes in divergent orientations, but the GapR signal was enriched downstream these transcription tracks (Fig. 3b,c, fourth row), and also in-between genes positioned in convergent orientations (Fig. 3d, fourth row). In colinear orientation, no enrichment was seen after the first gene, in agreement with the suppression of the positive supercoils by the neighboring negative one (Fig. 3e,f, fourth row). However, a strong enrichment was observed after the second gene. Altogether, these results strongly suggest that the arched stripe pattern is the Hi-C signature of a negative supercoiled structure positioned in the upstream 5' region of the TU. These observations agree with simulated and experimental data pointing at a preferred positioning of RNA Pol at the apical positions of supercoiling loops<sup>36,37</sup>. A fine observation of the signal suggests that, indeed, the T7 promoter is positioned in the middle of the arched stripe. On endogenous genes, this pattern would be either two small to be visualized at the present resolution, erased by neighboring supercoiling (similarly to the collinear T7 units), or most likely both (see Discussion).

### TIDs impose mechanical constraints on adjacent regions

To assess the importance of TIDs in living cells, we used fluorescence imaging to monitor two chromosomal regions flanking the T7

promoter. We positioned two markers, separated by 230 kb, one in a 'silent' region (*parS<sup>PI</sup>*; about 200 normalized RNA-seq reads in the 20-kb flanking region) and the other in a moderately expressed region (*parS<sup>MTI</sup>*; about 700 normalized RNA-seq reads in the 20-kb flanking region; Fig. 4a and Extended Data Fig. 4a–c). The positioning of these regions relative to cell length was similar (Fig. 4b and Extended Data Fig. 6d). However, the lateral positioning of the expressed *parS<sup>MTI</sup>* region is closer to the nucleoid periphery than the neighboring silent *parS<sup>PI</sup>* region (Fig. 4c) suggesting that endogenous transcription moderately influences gene localization. Rifampicin-induced inactivation of transcription relocates the *parS<sup>MTI</sup>* towards the center of the nucleoid (Fig. 4d). This observation is in agreement with previous findings showing using super-resolution imaging that clusters of RNA Pol tend to (though not systematically) localize near nucleoid periphery, in rich<sup>17</sup> and minimal<sup>17,24</sup> medium. Upon transcription activation of the neighboring T7 TU, both loci (silent *parS<sup>PI</sup>* and expressed *parS<sup>MTI</sup>*) localize at the nucleoid periphery (Fig. 4e,f).

In the presence of rifampicin, the longitudinal (Fig. 4g and Extended Data Fig. 6e) and lateral (Fig. 4d) localization of the expressed and silent regions were affected, moving closer to the center. Activation of T7 expression counteracted the effect of rifampicin by moving foci away from the medial position of the cell (Fig. 4h and Extended Data Fig. 6f).

In addition, we studied the impact of transcription on the organization of flanking chromosomal regions. Under natural conditions, approximately 80% of the cell population exhibited colocalization of the two regions separated by 230 kb. However, upon T7 expression, this frequency increased to 90%, regardless of the presence of rifampicin. This suggests that T7-mediated folding influences the convergence of neighboring regions (Fig. 4i).

To monitor the influence of T7 transcription on the mobility of chromosome loci, we used strains carrying fluorescently labeled *lacO<sup>20</sup>* arrays inserted at two positions downstream of the T7 promoter (Fig. 4j). We compared individual foci dynamics with or without T7 induction, in the absence or presence of rifampicin, by recording their position every second for 120 s (Methods). For each trajectory, we computed the mean-squared displacement (MSD), a technique that describes the mode of displacement of particles followed over time. We plotted the slope ( $\alpha$ ) of the MSD versus time interval.  $\alpha$  is indicative of the nature of the locus movement.  $\alpha = 1$  describes normal diffusion, whereas  $\alpha < 1$  is subdiffusive. For the two loci close to the T7 TU (*betT* and *ecpR*), T7 activation correlated with a reduction of the  $\alpha$  median value, suggesting that T7 transcription constrains the movement of the flanking region

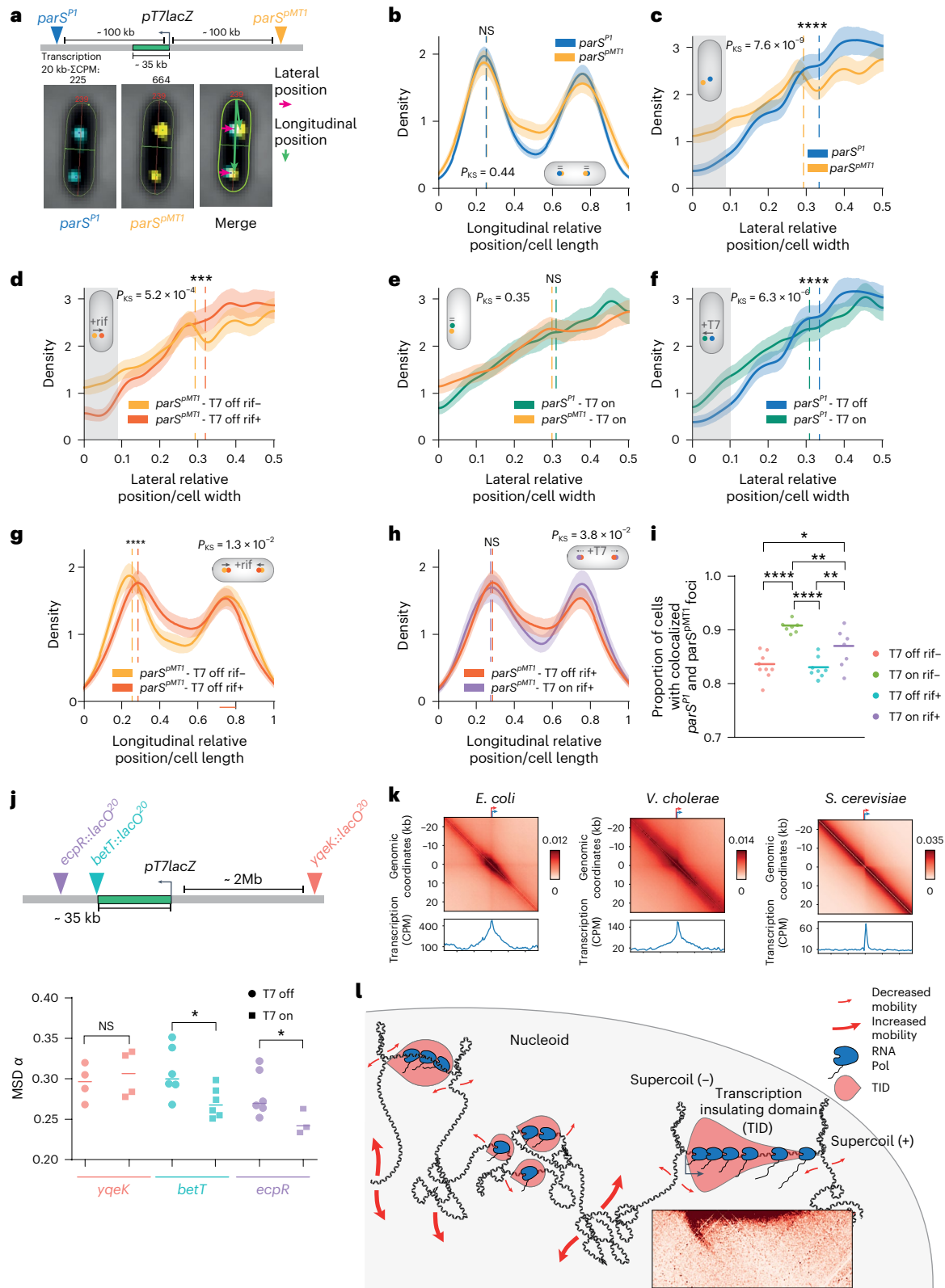
**Fig. 4 | Dynamic influence of the T7 transcription unit. a**, Positions of the *parS* tags inserted in a TID enriched region (*parS<sup>MTI</sup>* near *yajQ* gene) and a poorly expressed region (*parS<sup>PI</sup>* near *crI* gene). The T7 promoter was inserted at the *lacZ* promoter position in-between the two *parS* tags. The arrows on the right indicate how we measure the lateral (pink) and longitudinal (green) positions in **b–h** and in Extended Data Fig. 6d–f. **In b–h**, the positions of one or two *parS* tags in one or two conditions are compared. The *x* axis represents either the relative longitudinal or lateral relative position. 0 (and 1) corresponds to the cell periphery, whereas 0.5 corresponds to the middle of the cell. For each panel, a cell cartoon illustrates the position that the two monitored loci tend to occupy. Statistical differences between the distributions are analyzed with a two-sample Kolmogorov–Smirnov test. On each plot, the dotted lines indicate the median of the tags positions, and the significance of the one-sided *t*-test between average position of both conditions is indicated by NS (not significant) or stars ( $*P < 5 \times 10^{-2}$ ;  $**P < 1 \times 10^{-3}$ ;  $***P < 1 \times 10^{-4}$ ;  $****P < 1 \times 10^{-5}$ ). The error bars are defined as the 95% confidence interval of 1,000 bootstraps. Finally, a gray area can highlight the peripheral localization where and if foci redistribution occurs. **b,c**, The longitudinal (**b**) and lateral (**c**) foci positions of *parS<sup>MTI</sup>* and *parS<sup>PI</sup>* (as shown in **a**), in absence of T7 transcription, are plotted (*t*-test *P* value: 0.62,  $8.7 \times 10^{-8}$ , respectively). The lateral position of the transcribed *parS<sup>MTI</sup>* tag region is shifted toward the periphery of the nucleoid compared to the inactive *parS<sup>PI</sup>* region. **d**, Lateral position of the *parS<sup>MTI</sup>* tag in absence or presence of rifampicin (*t*-test *P* value:  $8.3 \times 10^{-4}$ ). **e**, Lateral position of the *parS<sup>MTI</sup>* and *parS<sup>PI</sup>*

tags in the presence of T7 transcription (*t*-test *P* value: 0.12). **f**, Lateral position of the *parS<sup>PI</sup>* tag with or without T7 transcription (*t*-test *P* value:  $3.5 \times 10^{-5}$ ). **g**, Longitudinal position of the *parS<sup>MTI</sup>* tag in absence or presence of rifampicin (*t*-test *P* value:  $1.5 \times 10^{-5}$ ). **h**, Same as **g** but in presence of T7 transcription (*t*-test *P* value:  $6.8 \times 10^{-2}$ ). **i**, Colocalization of pairs of *parS* tags from **a** flanking the T7 unit. The proportion of cells presenting at least one couple of foci closer than 200 nm or less was plotted. Each replicate is an average of 400 cells. Statistical differences are measured by an analysis of variance (ANOVA) Kruskal–Wallis test with Bonferroni correction,  $*P < 0.033$ ,  $**P < 0.0021$ ,  $***P < 0.0002$ ,  $****P < 0.0001$ . Replicates:  $N = 7–9$ . **j**, Positions of the three *lacO* arrays inserted in the vicinity of the T7 promoter. **Lacl**–YFP foci dynamics was analyzed for 100 time intervals of 1 s, for each replicate ( $N = 3–6$ ) the median MSD $\alpha$  measurement for -1,000 trajectories of the fluorescently labeled loci was computed. Experiments were performed in the absence of rifampicin upon induction of the T7 TU. Statistical differences are measured by an ANOVA Kruskal–Wallis test with Bonferroni correction,  $*P < 0.033$ ,  $**P < 0.0021$ ,  $***P < 0.0002$ ,  $****P < 0.0001$ . **k**, *E. coli*, *V. cholerae* and *S. cerevisiae* genes pileups. Left: pileup for each species of 50-kb windows contact maps centered on TSS of the 10% most transcribed genes. Bottom: corresponding RNA-seq pileup profiles. **l**, Schematic representation of the proposed nucleoid structuring into a mosaic of small 3D transcriptional units or TIDs. We propose that TIDs tend to cluster together, and/or relocate to the nucleoid periphery, resulting in enriched contacts between adjacent units separated by -20–40 kb.

(Fig. 4j). In contrast, a focus positioned 2 Mb away at the *yqeK* locus did not show notable changes upon T7 activation (Fig. 4j). Note that rifampicin appears to have heterogeneous impact on DNA mobility according to the reporter region monitored, perhaps because of the combination of indirect perturbations (Extended Data Fig. 6g).

Overall, live imaging analysis revealed that the T7 transcription track exhibits less mobility and appears to promote colocalization

of its flanking regions. In addition, the regions flanking native active genes and transcribed T7 TU tend to (re)localize along the lateral edges of the nucleoid. These experiments suggest that, in *E. coli*, local transcription modulates DNA localization<sup>17,18,24</sup>, while imposing a mechanical constraint on neighboring loci by bringing them closer together<sup>38,39</sup>, and possibly also affecting mobility.





## Discussion

The thinner grain scale made available by resolution improvements, combined with the analysis of native and artificial single transcription unit(s), suggests that transcription shapes bacteria chromosomes by imposing local constraints with multilevel consequences. First, we demonstrate that the large CIDs identified from the long HEG are the tip of a more general phenomena, also visible in the high-resolution Hi-C contact maps of another bacterium (for example, *Vibrio cholerae*, Fig. 4k)<sup>19</sup>. Transcription locally stimulates the formation of bundled domains (TIDs) and promotes contacts between adjacent active genes or operons separated by a few tens of kb (Fig. 4l). Transcription induced by highly expressed artificial T7 promoters also display bundled domains in Hi-C maps and striking arched stripe patterns in 5' position, which most likely correspond to negative supercoils constrained in-between the promoter and the first quarter of the T7 TU. Furthermore, by combining two divergent T7 TUs, a self-interacting domain appears in the untranscribed region between them. The differences observed between native genes and T7 UT may result from inherent differences between the polymerases, but could also reflect an amplification effect of T7 activity on native processes that are either indistinguishable with current technologies, or overridden by other activities.

We propose that this is the case for the 5' arched stripe of T7 TU that appears linked to supercoiling. This pattern is manifest at the T7 TSS in absence of neighboring transcription (Fig. 2b), but not so much in the presence of transcription or at the level of active endogenous *E. coli* genes. This suggests that this signal probably reflects dramatic DNA underwinding following strong T7 RNA Pol transcription, which topoisomerase I fails to counteract. In agreement, the divergent or collinear orientation of pairs of T7 promoters can strongly affect the arched stripe patterns, by blurring or alleviating them, respectively. This could explain why this pattern is not observed along native *E. coli* TIDs, since the density, expression level and orientation of these regions may result in similar effects. Therefore, we propose that the constraints imposed by transcription along the fiber balance each other to modulate chromosome organization and dynamics. Note that this arched pattern is therefore different from the 'stripes', 'flames' or 'lines' probably generated through SMC-mediated loop extrusion mechanisms in other species<sup>40–42</sup>. However, transiently these constraints could have multiple consequences for DNA transactions, including transcription, DNA repair and segregation, and contribute as well to the regulation of the extrusion of large DNA loops by bacteria condensins as they travel along the chromosome<sup>7,43,44</sup>.

Sub-kb resolution Hi-C further reveals plaid-like patterns corresponding to contacts between neighboring active endogenous TUs separated by inactive regions (Fig. 1b and Extended Data Fig. 4a), a new feature of bacterial chromosome folding. Furthermore, T7 TUs facing towards *OriC* (that are slightly less strong than TU facing the terminus) also display long-range *trans* contacts with neighboring endogenous active transcription unit upstream the promoter (Extended Data Fig. 3a), suggesting that this pattern is not specific to the *E. coli* polymerase. Since these distant contacts (~20–40 kb) can involve protein-coding genes (membrane and cytoplasmic proteins) and transfer RNA regulated by different transcription factors and different sigma factors (Fig. 1b,d), they may only rely on transcription. Several hypotheses may explain this phenomenon. Firstly, the decreased mobility of transcribed units (Fig. 4j), in association with either relocalization to the nucleoid periphery (Fig. 4c,e,f and ref. 17), and/or into cluster of active genes (as suggested in ref. 24), could explain such inter-TU contacts (as schematized on Fig. 4l). Secondly, the proximity of transcribing RNA Pols may favor protein–protein interactions as biomolecular condensates<sup>45</sup>. In addition, RNA production may locally reduce effective solvent quality of the cytoplasm and drive local chromosome deformation as proposed by the group of Christine Jacobs-Wagner<sup>18</sup>. Alternatively, or in combination, contacts between adjacent TUs may also be modulated by loop extrusion by the *E. coli* condensin MukB<sup>43</sup>.

Loops would extend until they encounter actively transcribed regions that would act as permissive roadblocks or extrusion slowing zones, resulting in enriched contacts between them. Future experiments will be required to assess the contribution(s) of these elements to the folding of transcribed units.

TIDs structural features depend on both transcription level and genomic context, demonstrating that all loci along the chromosome are not subject to transcription-induced mechanical stresses in the same way. For instance, TIDs should form the center of twin-supercoiled domains<sup>4</sup> recently described using psoralen crosslinking detectable around HEGs (that is, ribosomal operons)<sup>5</sup> that will span  $\pm 25$  kb. The relationship between the basic structuring elements that are TIDs and higher-level features of chromosome organization (for example, plectonemic loops<sup>9,10</sup>, macrodomains<sup>46</sup> and supercoiling domains<sup>5</sup>) is not deciphered in *E. coli*. However, it has been shown that HEG (that results in strong TIDs) represent permissive barriers to cohesin loop extrusions in other species such as *Bacillus subtilis*<sup>43</sup>. A potential influence of TIDs on the *E. coli* SMC MukB could emerge in the future. Also, HEG (for example, ribosomal operons) are frequent in the *oriC* proximal part of the genome, and the resulting TIDs and associated pronounced 'plaid-like' patterns may influence *oriC* region folding revealed by Hi-C<sup>13,14</sup>, recombination<sup>46</sup> and imaging<sup>14</sup> or supercoiling of this region<sup>5</sup>. This pattern falls within the more general propensity of all adjacent expressed sequences to contact each other more frequently, and thus this behavior would not be specific but only magnified at ribosomal DNA operons.

In eukaryotes, transcription shapes chromosome architecture but the contact patterns differ, with active genes delineating clear boundaries in contact maps for instance in *Saccharomyces cerevisiae* (Fig. 4k), as shown by past and recent work<sup>26,47</sup>. In this species, the average gene size is ~1.4 kb, and few genes are larger than 5 kb. As a consequence, it is also possible that the bundle is much shorter and less visible than in bacteria where operons are on average ~3–5 kb in size, and sometimes larger. This pattern appears modulated by SMC complex DNA translocase activity<sup>37,47</sup>. The presence of nucleosomes in eukaryotes and in some archaea is also expected to thicken the contact pattern at short distance, therefore blurring the crisper signal observed in bacteria. Nevertheless, the underlying constraints unveiled in this work imposed by transcription on the DNA sequence stand to be a fundamental aspect of chromosome biology.

## Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41594-023-01178-2>.

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## Methods

### Media culture conditions and strains

Strains used in this study are derived from MG1655 and BW25113 *E. coli* strains. They are listed in Extended Data Table 2. All strains were grown in minimal media A (0.26 M  $\text{KH}_2\text{PO}_4$ , 0.06 M  $\text{K}_2\text{HPO}_4$ , 0.01 M tri sodium citrate, 2 mM  $\text{MgSO}_4$ , 0.04 M  $(\text{NH}_4)_2\text{SO}_4$ ) supplemented with 0.2% of casamino acids and 0.5% of glucose at 37 °C. BW25113 strains were grown with 0.2% arabinose for 2 h to induce T7 RNA Pol expression under the control of the PBAD promoter. TopA overexpression was also controlled by arabinose for 2 h.

### Drugs and antibiotics

Rifampicin was used for 10 min at a 100  $\mu\text{g ml}^{-1}$  working concentration to inhibit transcription. Novobiocin was used for 10 min at a 50  $\mu\text{g ml}^{-1}$  working concentration to inhibit gyrase. Chloramphenicol was used for 10 min at a 30  $\mu\text{g ml}^{-1}$  working concentration to inhibit translation. When required, ampicillin was used at 100  $\mu\text{g ml}^{-1}$ .

### Western blot

Bacteria were resuspended in Laemmli buffer at  $2.00 \times 10^6$  cells  $\mu\text{l}^{-1}$ . Protein extracts were ran on 7.5% gel and then transferred to a membrane that was saturated with 10% TBS-T and then labeled with anti-TopA antibodies (mouse antibodies, gift from Yuk-Ching Tse-Dinh) and finally revealed with horseradish peroxidase-coupled anti-mouse antibodies. Revealing was carried out on the femto with the FX fusion device. Membranes were stripped, saturated and labeled with horseradish peroxidase-coupled anti-RpoB antibodies (a loading indicator of protein quantity). Revelation was performed at pico using the FX fusion device. For quantification, the gray level was calculated for each protein, the background was subtracted and the amount of TopA was normalized to the amount of protein (loading control, RpoB).

### Hi-C procedure and sequencing

Cell fixation with 3% formaldehyde (Sigma-Aldrich, cat. no. F8775) was performed as described in ref. 49. Quenching of formaldehyde with 300 mM glycine was performed at 4 °C for 20 min. Hi-C experiments were performed as described in ref. 49. Samples were sonicated using Covaris (DNA 300 bp).

### ChIP-seq and RNA-seq experiments

Chromatin immunoprecipitation was performed as described<sup>50</sup>. Briefly, overnight cultures were diluted to  $\text{OD}_{600\text{nm}}$  of 0.01, grown until  $\text{OD}_{600\text{nm}}$  of 0.2–0.25, diluted and crosslinked using formaldehyde (Sigma-Aldrich; final concentration of 1%) for 10 min at 22.5 °C. Formaldehyde was then quenched by adding 2.5 M glycine (final concentration 0.5 M), for 10 min at room temperature (for example, 19–22 °C). Cells were collected by centrifugation at 1,500g for 10 min and washed three times in ice-cold 1× phosphate-buffered saline. The pellets can be stored at –80 °C or used straight away. A pellet was then resuspended into 500  $\mu\text{l}$  of 1× TE buffer, supplemented with 5  $\mu\text{l}$  of ready-lyse lysozyme, and incubated with shaking at 37 °C for 30 min. Then 500  $\mu\text{l}$  of 2× ChIP buffer (50 mM HEPES–KOH pH 7.5, 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate and 1× Roche Complete EDTA-free protease inhibitor cocktail) was added, and the sample was transferred to ice. The sample was transferred to a prechilled 1-ml Covaris tube (Covaris), and sonicated using Covaris S220 for 7 min (settings as followed: target size, 200–700; Peak Incident Power 140; Duty Factor 5%; Cycle Per Burst 200). One-hundred microliters of the sample was removed as input and stored at 20 °C. Immunoprecipitation was performed overnight under rotation at 4 using 1/100 T7RNA antibody (Biolabs CB MAB-0296MC) and antitag (Sigma F1804 and F3165). Immunoprecipitated samples were incubated with Protein G Dynabeads (Invitrogen) with rotation for 2 min at room temperature. The tube was washed three times with 1× phosphate-buffered saline

with 0.02% Tween-20 using the Dynamag magnet setup. The beads were resuspended in 200  $\mu\text{l}$  TE buffer with 1% sodium dodecyl sulfate and 1  $\mu\text{l}$  RNaseA (10 mg  $\text{ml}^{-1}$ ) and 1  $\mu\text{l}$  proteinase K (20 mg  $\text{ml}^{-1}$ ). Samples were incubated at 65 °C for 10 h to reverse the formaldehyde crosslinking. The beads were removed using the Dynamag magnet and DNA of the supernatant purified using Qiagen Minelute polymerase chain reaction (PCR) purification kit using two elution steps. DNA was eluted into a 50  $\mu\text{l}$  TE buffer and stored at –20 °C until further processing.

### RNA-seq

Total RNA was extracted from *E. coli* using the Nucleospin RNA Extraction Kit (Macherey-Nagel) according to the manufacturers' instructions. DNase was depleted using an additional DNase treatment with Turbo DNase (Thermo Fisher). The DNase was inactivated and RNA was purified by a phenol–chloroform extraction (pH 4.5, Amresco) and ethanol precipitation. The RNA was then resuspended in diethyl pyrocarbonate-treated water. Ribosomal RNA depletion was done using Ribo-Zero magnetic beads according to the manufacturer's protocol (Illumina). Complementary DNA library preparation was performed following standard protocols. Briefly, RNA was fragmented using the NEBnext mRNA first and second strand synthesis kits (NEB). One to three biological replicates were generated for each condition, and on average ~10 million reads were generated per sample.

### DNA libraries preparation

For Hi-C, RNA-seq and ChIP-seq libraries, preparation of the samples for paired-end sequencing was performed using Invitrogen Colibri PS DNA Library Prep Kit for Illumina according to the manufacturer's instructions. The detailed protocol is available in ref. 49. All libraries used or generated during the course of this study are listed in Extended Data Table 3.

### Gradient preparation of *E. coli* polysomes

To preserve the polysomes, cultures of *E. coli* are incubated with 100  $\mu\text{g ml}^{-1}$  of chloramphenicol before centrifugation. Fresh cell paste (0.7 g) was homogenized in the buffer (150 mM  $\text{NH}_4\text{Cl}$ , 10 mM  $\text{MgCl}_2$ , 2 mM Tris–Cl pH 7.5, 10  $\mu\text{M}$  phenylmethylsulfonyl fluoride and 0.2  $\mu\text{g ml}^{-1}$  chloramphenicol, Complete EDTA-free, RNAsin) at a 1:2 (w:v) ratio and set aside for 20 min at 4 °C. Disrupt cells using FastPrep sample preparation system and lysing matrix B tubes (2 ml) containing 0.1 mm silica beads. Add sodium deoxycholate (1% final), DNase I to a final concentration of 2  $\mu\text{g ml}^{-1}$  (20 U  $\text{ml}^{-1}$ ) and let 30 min on ice, then clear the lysate of cell debris by centrifugation at 4 °C using benchtop centrifuge for 20 min and a second centrifuge for 5 min. Divide the supernatant equally, and treat one part by adding EDTA (70 mM) and incubate on ice for 30 min. Layer the fractions (600  $\mu\text{l}$ ) on top of 10 ml sucrose gradient (10–40%) and centrifuge for 2.5 h at 4 °C in SW41Ti rotor at 35,000 rpm (151,000g). Gradients are next fractionated by collecting 500- $\mu\text{l}$  fractions. To analyze RNA, 170  $\mu\text{l}$  of each fractions is mixed to 400  $\mu\text{l}$  of RNase-free water and 570  $\mu\text{l}$  of phenol, vortexed and centrifuged to extract RNA from proteins, then aqueous supernatant is precipitated with  $\text{CH}_3\text{COONa}$ , glycogen and isopropanol. Collected RNA present in each fraction is next analyzed in agarose gel.

### Processing of reads and Hi-C data analysis

Reads were aligned with bowtie2 v2.4.4 and Hi-C contact maps were generated using hicstuff v3.0.3 (ref. 51) with default parameters and using HpaII enzyme to digest. Contacts were filtered as described in ref. 22, and PCR duplicates (defined as paired reads mapping at exactly the same position) were discarded. Matrices were binned at 0.5, 1, 2 or 5 kb. Balanced normalizations were performed using ICE algorithm<sup>52</sup>. Reads with ambiguous mapping were removed such as reads mapping on the rDNA operons, resulting in missing values into the Hi-C contact map (white lines). Contact maps are stored in cool file format using cooler (v0.8.11)<sup>53</sup>. For all comparative analyses, matrices were downsampled to the same number of contacts. Comparison between



matrices was done using  $\log_2$  ratio and serpentine v0.1.3 (ref. 48) for flexible binning. Serpentine was used with 5-kb binned matrices, with 25 iterations and a threshold of 100. The Hi-C signal was computed as the contacts between adjacent 5 kb bins as described in Liroy et al.<sup>13</sup>. To compare this signal with other genomics tracks, we binned it at the desired resolution and z-transformed it.

### Border detection

To detect the borders we first used the directional method as described in ref. 13. The directional index is a statistical parameter that quantifies the degree of upstream or downstream contact bias for a genomic region<sup>54</sup>. For each bin, we extracted the vector of contacts from the correlation matrix between that bin and bins up to a window size in both left and right directions. To assess if the strength of interactions is stronger with one direction relative to the other we used a paired *t*-test between the two vectors. A *P* value of 0.05 was used as a threshold to assess a statistical significant difference. The directional preferences for the bin along the chromosome are represented as a bar plot with positive and negative *t* values shown as red and green bars, respectively. We trimmed the bars of the bins with *t* values below  $-2$  or above  $2$  (corresponding to a *P* value of 0.05). At the borders identified in the contact matrices, the directional index changes from negative to positive *t* values. The implementation of the code is available at ref. 55, v1.0.1, and it is based on the one used for Liroy et al.<sup>13</sup>. The DI method depends on the binning resolution and on the window size. At small window size, it misses the larger domains visible at larger scale, and at large window size it finds only the larger domains. Moreover, the resolution impacts on the performance of the DI: at low resolution it cannot find the smallest domains that are merged in few bins, and at high resolutions it starts to be noisy as the resolution directly impacts the width of the vectors used to compute the DI. In our study, we decided to use an insulation score method to improve the borders detection at higher resolution. For our analysis, we developed a python implementation<sup>55</sup> of the HiCDB algorithm<sup>25</sup>. This method allows multiple window sizes, which reduces the dependence between the window size and the size of the detected domains. Furthermore, it does not depend on the resolution of the matrix, which allows for efficient detection of boundaries even at high resolution. We used the 1 kb resolution contact map with 10, 15, 20, 25 and 30 kb windows (Extended Data Table 1).

### Pileup analysis

The Hi-C contacts were built and normalized as explained before at a resolution of 500 bp. For each gene we extract a 100 kb matrix centered on the start codon of the gene. For reverse genes, we flip the matrix to have the centered genes pointing always in the same direction. The pileup plot is the average of all the extracted windows, without taking into account the white lines (that is, bins with less than the median minus three times the median absolute deviation are considered as white lines). To select active genes, we select a fraction of the most transcribed genes (values in reads per kilobase per million) as the active genes. For the transcription units analysis, to center our windows on the first transcribed genes, we selected active genes only if there are no other active genes in the 3 kb upstream of the start codon of the gene. To compare the pileups of the first transcribed genes with the noncoding or nontranscribed regions, we calculated the ratio between the pileup of the first transcribed genes and the pileup of random windows taken from the same region (center on a random position within 100 kb around the gene). We chose to use random regions instead of the pileup of noncoding genes or the expected matrix (matrix corresponding to the contacts of the genomic distance law) to avoid having a bias of the region where we extract the active genes.

### Detection of contact bundles (that is, TIDs) along the main diagonal

To detect contact bundles on the main diagonal, we used a convolution kernel on the balanced matrix. The method is implemented in

ref. 55. We used a computer vision approach similar to the program Chromosight<sup>56</sup>, which uses a convolution kernel describing a given pattern as a template to detect the local similarity with it. Here we aim at detecting the bundles on the main diagonal of the matrix. To detect them, we build a gaussian kernel of size *n* as follows (*n* = 5 in our study):

$$M_{ij} = \frac{1}{\sqrt{2}} e^{-\frac{1}{2} \left[ \frac{|i-j| + |n-1-i-j|}{2(n-1)} \right]^2}$$

By computing the convolution product between each local image centered on each bin of the main diagonal and the kernel, we obtain a convolution score. The higher the score is, the closer the local image is to the kernel and the more likely it is to be a bundle. To remove the effect of local regions, we remove the second envelope of the signal as it's described in the HiCDB insulation score algorithm<sup>25</sup>. Finally, the borders of the bundles are detected by taking each peak of the local convolution score superior to the median of the local convolution score. The bundle region is then extended until the value gets inferior to one-third of the peak.

### RNA-seq processing

Processing is done using tinymapper v0.10.0 (ref. 57) with default RNA parameters. The reads are mapped with bowtie2 v2.4.4, PCR duplicates are filtered using samtools v1.14 and count per million (CPM) is made with bamCoverage v3.5.1. We used only the unstranded signal, and binned it depending on the displaying resolution. For the comparisons with other signals, a z-transformation is done.

### ChIP-seq processing

Processing of the ChIP-seq of T7 RNA Pol and GapR is done using tinymapper v0.10.0 (ref. 57) with default ChIP-seq parameters without input. The reads are mapped with bowtie2 v2.4.5, PCR duplicates are filtered using samtools v1.15 and CPM is made with bamCoverage function from deeptools (v2.29.1). For the GapR-seq, we do a gaussian blur of the signal with the gaussian\_filter1d function from scipy v1.7.3 with 'wrap' mode and sigma value of 2,500, as described in Guo et al.<sup>35</sup>. The data are then binned at the displaying resolution and z-transformed to compare it to other signals.

### Imaging and analysis

Cells were grown similarly to Hi-C samples (above). One hour after arabinose induction of T7 RNA Pol, 2 ml of cells were pelleted and resuspended in 50  $\mu$ l of fresh medium. Three drops of 2  $\mu$ l are deposited on a freshly made agarose pad (1 $\times$  supplemented medium A, 1% agarose) incubated 30 min in the microscope incubation chamber at 30 °C and imaged. For foci mobility analysis, imaging was performed on a Nikon Eclipse Ti inverted microscope equipped with a Spinning-Disk CSU-X1 (Yokogawa), an EM-CCD Evolve 512 $\times$ 512, magnification lens 1.2, pixel size: 13.3  $\mu$ m  $\times$  13.3  $\mu$ m camera at 600-fold magnification. Focal plane was maintained during acquisition using Nikon Hardware autofocus. Illumination and acquisition was controlled by Metamorph. Imaging was performed for 120 s every second with a 100 ms acquisition time. Time series images were registered using Stackreg<sup>58</sup> and analyzed with the MOSAIC suite<sup>59</sup> as Fiji plugins. Median MSD ( $\alpha$ ) distribution was analyzed and plotted with Graphpad-PRISM. An average of 1,000 trajectories were analyzed for each replicate. For interfocal distances and nucleoid organization measurements, cells were observed live on agarose pad on a thermo-controlled stage with an epifluorescence-LED system mounted on a Zeiss inverted confocal microscope and a C-MOS Hamamatsu 2,048  $\times$  2,048/pixel size: 6.45  $\times$  6.45  $\mu$ m camera at 630-fold magnification. The position of foci in the cell in each condition was analyzed with the ObjectJ plugins of ImageJ<sup>60</sup>. Two-color localization was performed with *cr::parS<sup>P1</sup>* and *yajQ::parS<sup>pMT1</sup>* tags<sup>61</sup>. An average of 1,200 cells were analyzed per strain and condition. Distributions were analyzed and plotted with MATLAB. Confidence intervals on the plot



were made using a bootstrap of sampling of the original values. The  $P$  values were computed using a Kolmogorov–Smirnov test.

### Modeling approach

We devised a simple model to reproduce the contact maps obtained experimentally under a few hypotheses. We start our approach by computing the contact probability decline with increasing genomic distance from experimental data  $p(s)$ . We then make the hypothesis that two different types of contacts are found in the experiments: contacts mediated by polymerases and contacts mediated by other proteins. We assume that the proportion of contacts mediated by polymerases at bin  $i$  is  $C_i$ , where  $C_i$  is the normalized experimental Pol- $\chi$ IP signal. To normalize the signal, we define its maximum value as  $\varepsilon$ , which is between 0 and 1. The proportion of contacts mediated by other proteins at bin  $i$  is then simply  $1 - C_i$ . We then compute the contact probability between any couple of bins  $i$  and  $j$  using two different models:

- In model 1, there is a preferential interaction between polymerases so that the contact frequency is proportional to:  $p(s_{ij}) \times (C_i C_j + (1 - C_i)(1 - C_j))$
- In model 2, there is a preferential interaction only between consecutive polymerases. The idea behind this model is that polymerases also act as contact insulators. The contact frequency is then modified from model 1:  $p(s_{ij}) \times (m \times C_i C_j + (1 - C_i)(1 - C_j))$  with  $m = \sum_{i+1}^{j-1} C_n$ .  $m$  represents the insulation factor, which is proportional to the total amount of polymerase that is found between bins  $i$  and  $j$ .

After all contact probabilities have been computed for each model, the contact matrix is normalized so that the sum of each line and each column is equal to 1 so that it corresponds to contact probabilities. The Spearman rank correlation is then computed between the experimental map, and the model map is then computed to find the best value for epsilon and to compare the relevance of each of the two models.

### Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

### Data availability

The accession number for the sequencing reads reported in this study is [PRJNA844206](https://www.ncbi.nlm.nih.gov/assembly/GCF_000005845.2). The reference genome for *E. coli* K12 MG1655 strain, GCF\_000005845.2, is provided at [https://www.ncbi.nlm.nih.gov/assembly/GCF\\_000005845.2](https://www.ncbi.nlm.nih.gov/assembly/GCF_000005845.2), and for *V. cholerae* O1 El Tor N16961, GCF\_003063785.1, at [https://www.ncbi.nlm.nih.gov/assembly/GCF\\_003063785.1](https://www.ncbi.nlm.nih.gov/assembly/GCF_003063785.1). For *S. cerevisiae*, the reference genome of the W303 strain used is available at <https://www.ncbi.nlm.nih.gov/assembly/GCA800216351.1>. Microscopy data are available at Mendeley Data, V1, at <https://data.mendeley.com/datasets/fzrmgjfyg7/1>. Source data are provided with this paper. Strains of this study are available from the corresponding authors.

### Code availability

The custom-made code of the analysis is available at [https://github.com/koszullab/transcription\\_T7\\_analysis](https://github.com/koszullab/transcription_T7_analysis). Open-access versions of the programs and pipeline used are available online.

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### Author contributions

Conceptualization: A.B., C.C., O.E. and R.K. Methodology: C.C., A.B., J.M., M.M., O.E. and R.K. Investigation: C.C., J.G., C.B. and A.T. with contributions from E.A. Formal analysis: A.B., J.M., C.C., J.G., C.B. and O.E. Data curation: A.B., O.E. and R.K. Visualization: A.B. and R.K. Writing—original draft: R.K. Writing—other drafts and editing: R.K., O.E. and A.B. Supervision: O.E. and R.K. Funding acquisition: O.E., J.M. and R.K.

### Competing interests

The authors declare no competing interests. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

### Additional information

**Extended data** is available for this paper at <https://doi.org/10.1038/s41594-023-01178-2>.

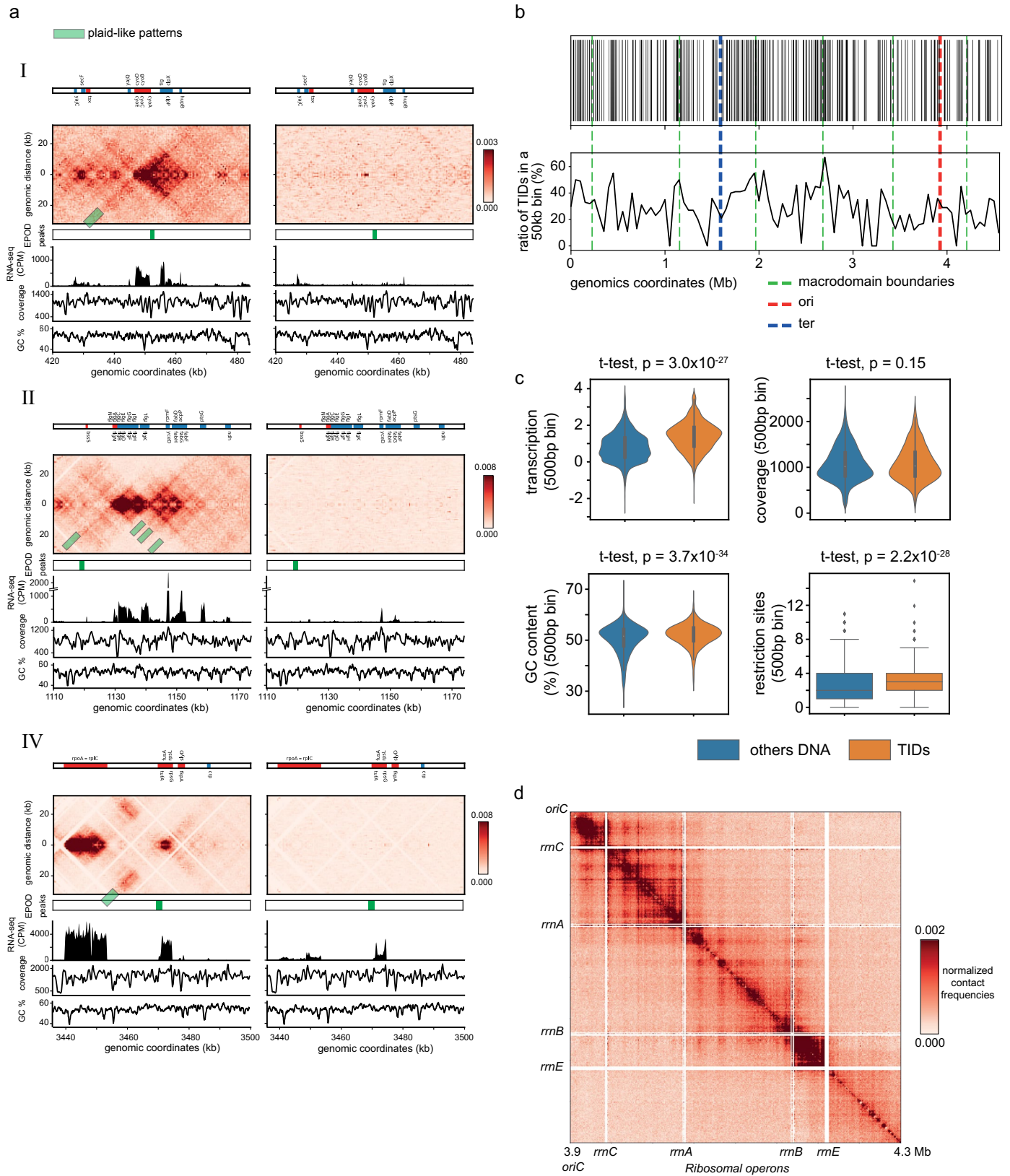
**Supplementary information** The online version contains supplementary material available at <https://doi.org/10.1038/s41594-023-01178-2>.

**Correspondence and requests for materials** should be addressed to Olivier Espéli or Romain Koszul.

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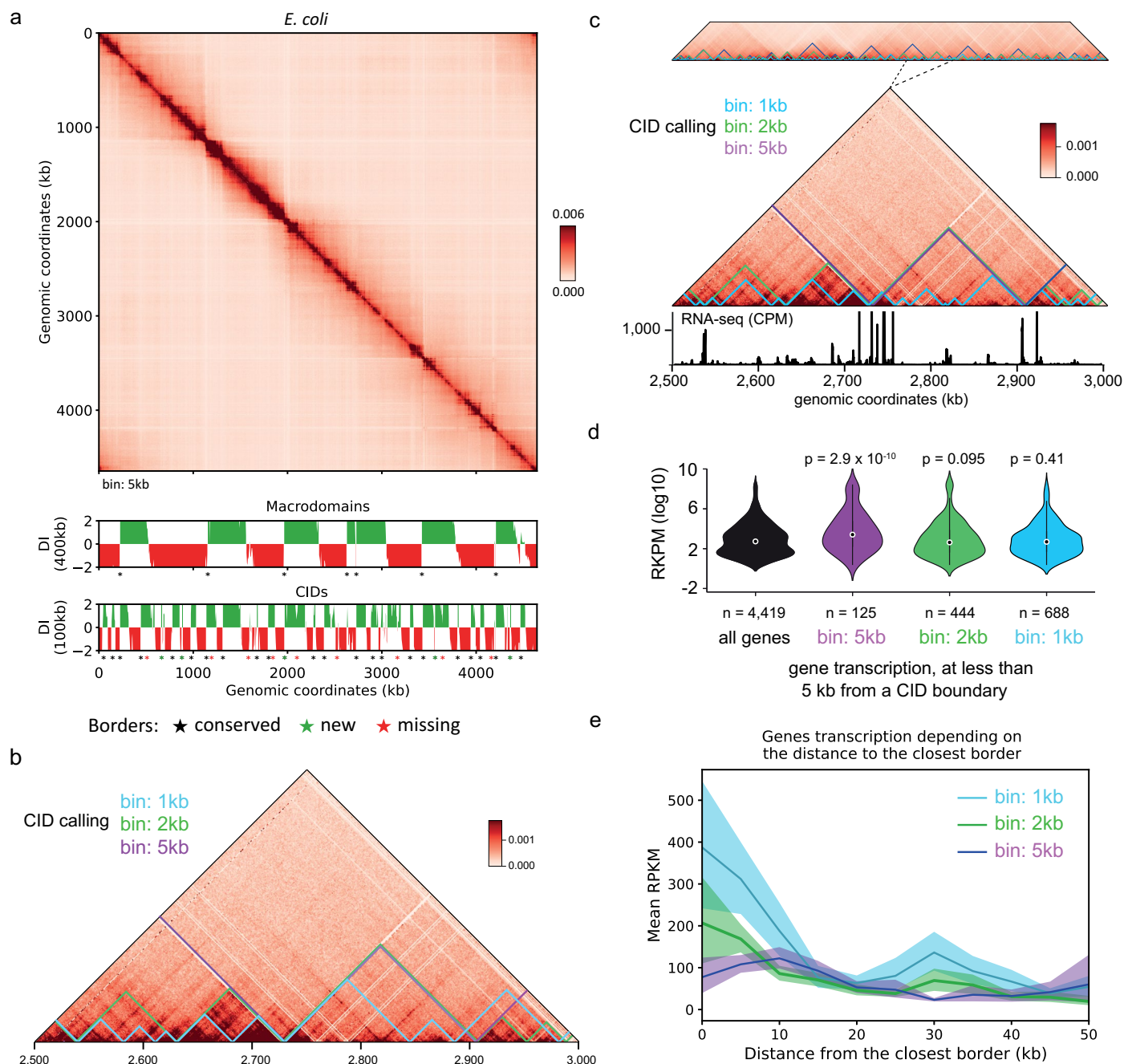


Extended Data Fig. 1 | See next page for caption.

**Extended Data Fig. 1 | Transcription impact on WT bacterial chromosome folding.** **a**, Magnifications of regions I, II and IV. The names of the genes within the 10% most transcribed are indicated (blue and red correspond to forwards and reverse genes, respectively). Left panels: normalized contact map (bin: 0.5 kb) over the corresponding EPODs<sup>12</sup> peaks and RNA-seq profile (in count per million or CPM), Hi-C coverage, GC content (%), in absence of rifampicin. Right panel: same region and analysis but in presence of rifampicin. **b**, Distributions of the bundle domains across the whole genome (x axis). Top: each strip represents a 500 bp bin called within a bundle domains (that is TID; Methods). Bottom: same data as above but binned into 50 kb bins. The positions of the macrodomains as defined in Liroy et al.<sup>8</sup> are indicated by green dotted lines. Ori and ter are indicated

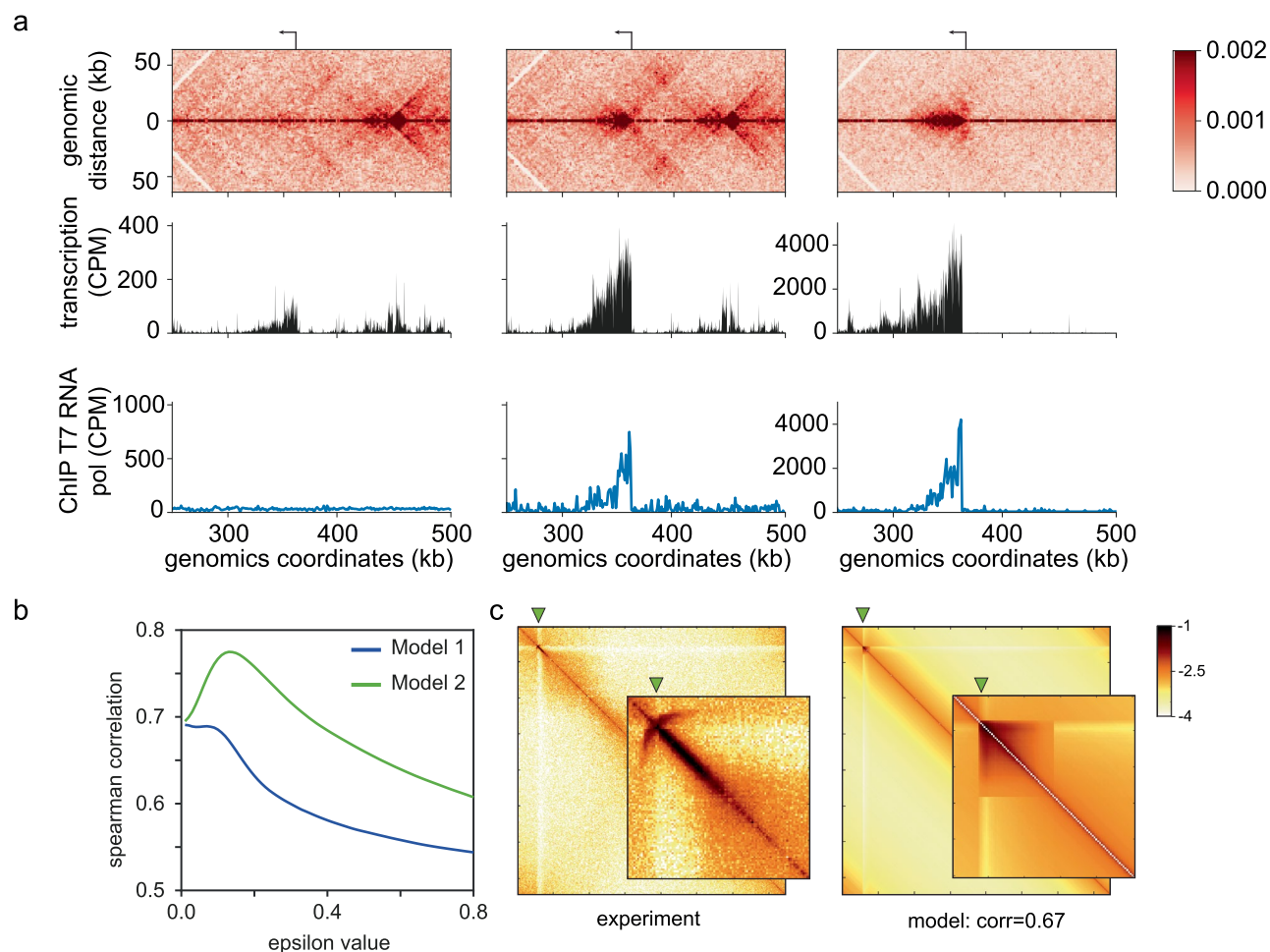
by red and blue lines, respectively. **c**, Distributions of transcription (CPM, in log 10), coverage, GC content and numbers of restrictions sites in pairs of bins with either low (blue) and high (that is in TIDs; orange) contact frequency at short range (Methods). Boxplots represent the first quantile, the median and the third quantile and the bar is between the first and ninth decile. The p-values are from independent one-sided t-tests (Others DNA: n = 6772, TIDS: n = 2512). **d**, Magnification of the WT *E. coli* contact map binned at 1kb on the rDNA loci. rDNA positions are indicated with their names. As rDNA operons are repeated sequences, reads cannot be mapped unambiguously, resulting in no signal in these loci (white lines in the contact map).





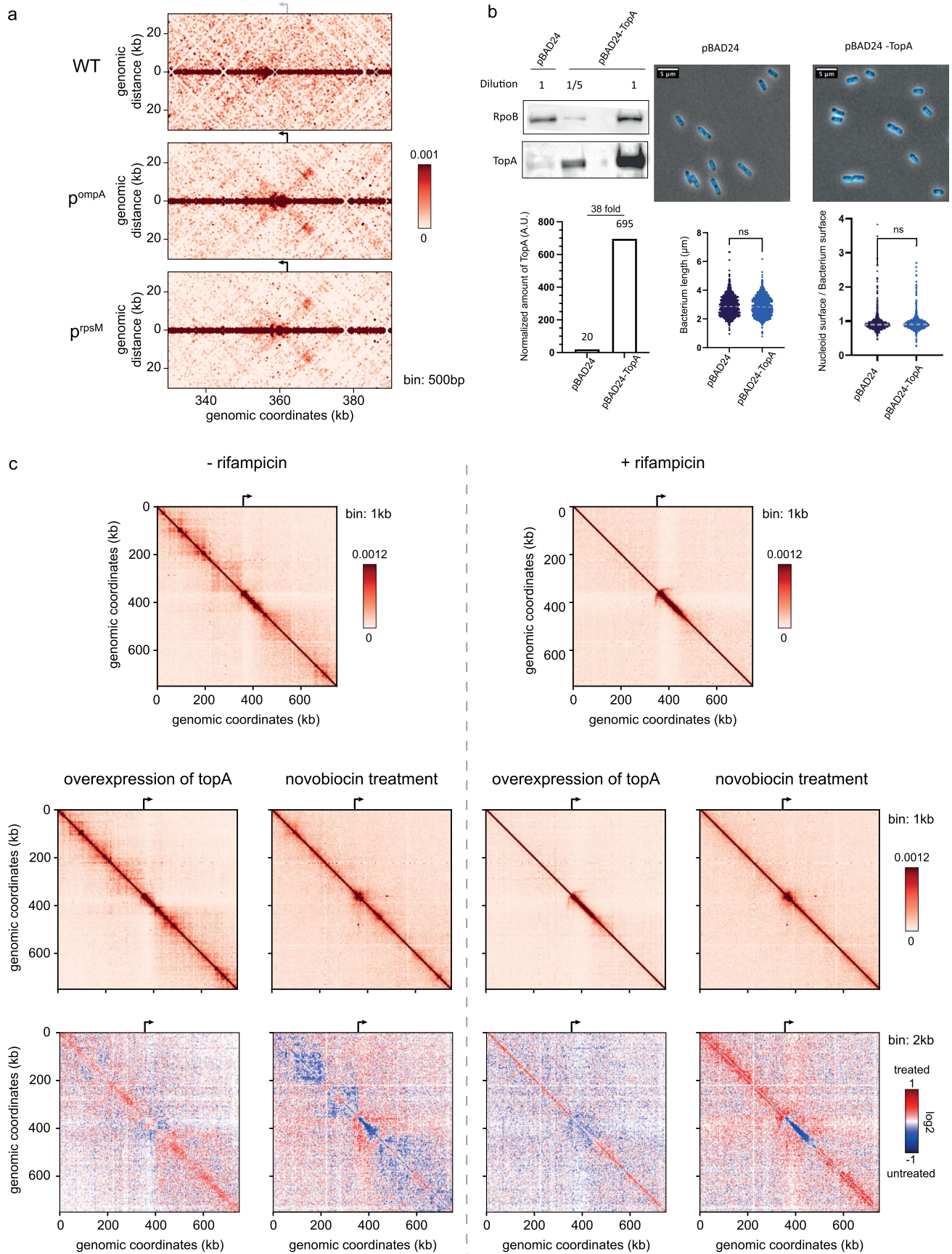
**Extended Data Fig. 2 | Relationships between Transcription Induced Domains and CIDs.** **a**, *E. coli* contact map binned at 5 kb at the top. Below the corresponding detected macrodomains and CIDs based on directional index method<sup>9</sup>. Stars show the significant borders detected in both Lioy et al. data<sup>8</sup> and the present one (black), only in Lioy et al. data (red) and only with our data (green). **b**, Domains detected based on DI analysis only at different resolutions; 1 kb (cyan), 2 kb (green) and 5 kb (blue). **c**, Bundle domains called using insulation score detection at 1 kb (cyan), or DI analysis on contact maps binned either 2 kb (green) and 5 kb (blue). **c**, Top, visualization of domains over the entire genome. Middle, magnification of a 500 kb region. Below, Corresponding RNA-seq

track in CPM. **d**, Violin plot distributions of transcript levels for all genes in the genome (black), and for all genes in 10 kb windows centered on the domain boundaries called on the 5 kb (blue), 2 kb (green) and 1 kb (cyan) binned maps. The bars represent the first and ninth deciles, and the dots is the mean of each distribution. The p-values of non-parametric one-sided Mann-Whitney test of whether the later distributions follow a genomewide distribution are indicated. **e**, Gene transcription in RPKM depending on the distance from the closest borders detected at different resolutions. The errors bars are defined as the 95% confidence interval of 1,000 bootstraps.



**Extended Data Fig. 3 | Activation of a single transcription unit within the *E. coli* chromosome. **a**, Magnifications of the Hi-C contact maps (bin: 1kb) of *E. coli* chromosome carrying a single T7 promoter facing toward the ori, with below the corresponding RNA-seq and the signal from ChIP of the T7 RNA polymerase. From**

left to right: the T7 promoter off, the T7 promoter on and the T7 promoter on with rifampicin. **b**, Correlation between the maps recovered from each of the two models and the experimental map, depending on the epsilon values (Methods). **c**, Best correlation map of Model I (right), aside the experimental map (left).

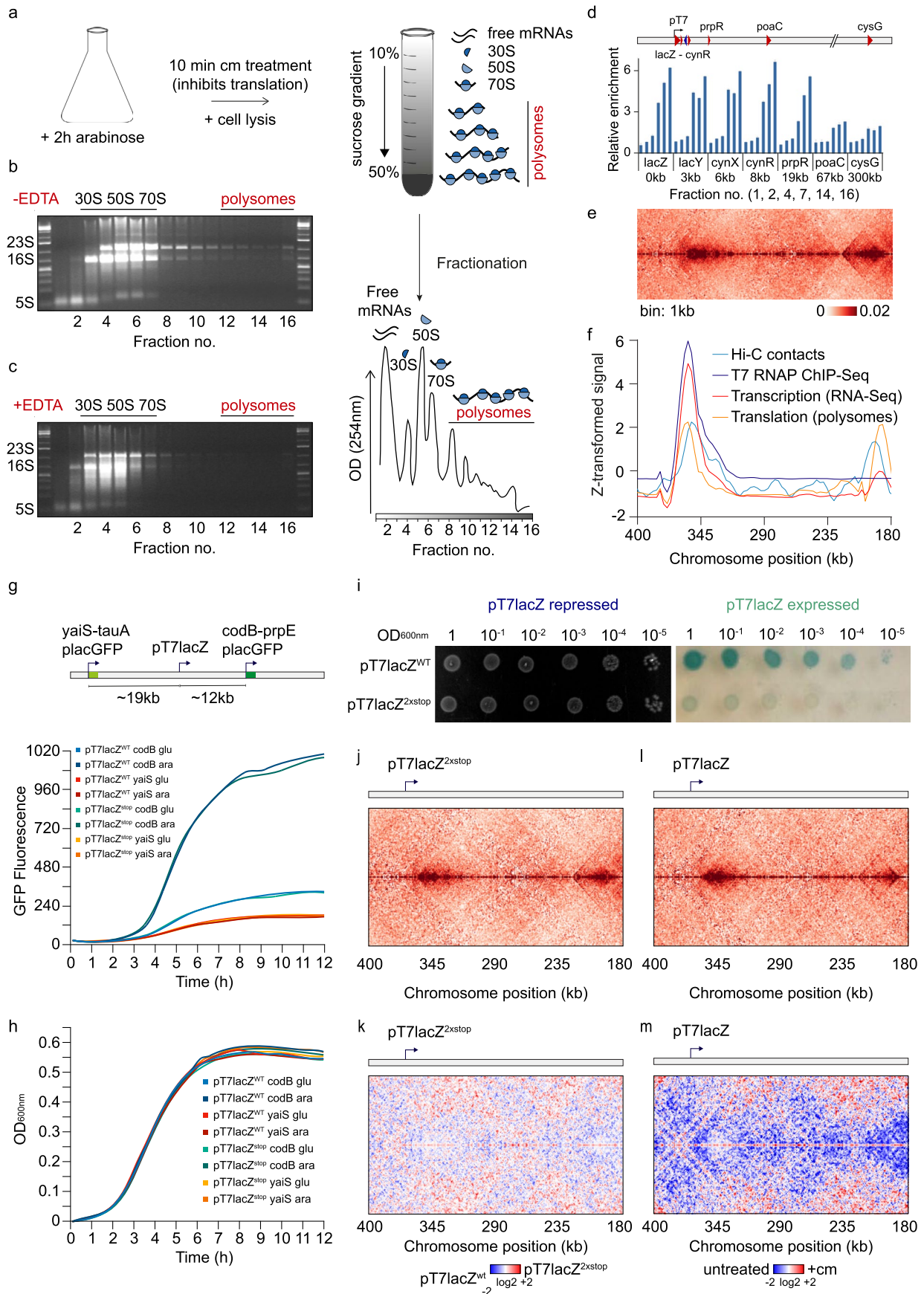


Extended Data Fig. 4 | See next page for caption.

**Extended Data Fig. 4 | Topoisomerase impact on the transcription unit folding.** **a**, Hi-C contact map magnifications (bin: 500 bp) of an *E. coli* strain carrying endogenous promoters facing the origin of replication. From top to bottom: without any additional promoter; with two  $p^{\text{ompA}}$  promoters; with two  $p^{\text{rpsM}}$  promoters. **b**, Analysis of TopA overexpression. Left panel, measurement of TopA amount by western blot in RSGB834 pBAD24 and RSGB834 pBAD24-TopA with an anti TopA antibody (gift from Dr. Yuk-Ching Tse-Dinh). Quantification of the western-blot showed a 38 fold overexpression of TopA after 2h of arabinose induction. This experiment was representative of 3 replicates. Right panel, microscopy imaging of the arabinose treated cell. The cells were fixed 2h after

arabinose induction and stained with DAPI. Bacteria length and DAPI amount per cell surface was measured with a custom macro of the Omnipose software. The significance of the two-tailed Mann-Whitney test between average of both conditions is indicated by *ns* (not significant) or stars (\*: <0.032; \*\*: <0.0021; \*\*\*: <0.0002; \*\*\*\*: <0.0001). **c**, Hi-C contact map magnifications of the T7 system while interfering with the topoisomerases activity; contact map binned at 1kb. top; wt system with 2h arabinose treatment. Middle left; overexpression of the topA, right; gyrase inhibition using a 10 min novobiocin treatment. Bottom; log2 ratio of the interfered over the wild type; 2 kb binned. On the left same with 10 min rifampicin treatment.

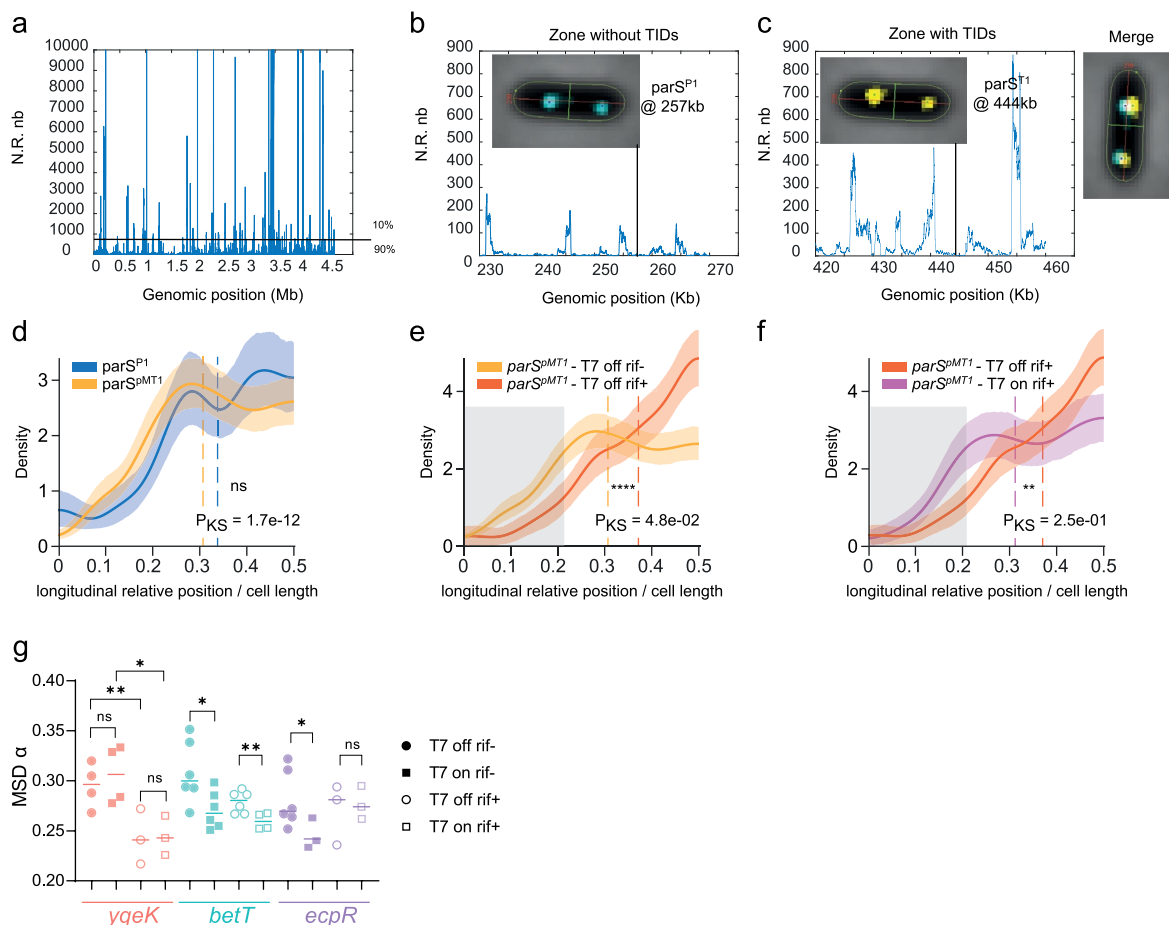




Extended Data Fig. 5 | See next page for caption.

**Extended Data Fig. 5 | Translation impact on bacterial chromosome folding.** **a**, Schematic view of the polysome extraction experiment. **b, c**, Gel migration of the different fractions for polysome extraction without EDTA (**b**), and with EDTA (**c**) (ladder: GeneRuler 1 kb Plus DNA Ladder). **d**, Relative enrichment along the chromosome as a function of polysome extraction fraction number. **e**, Magnification of the Hi-C contact map of the *E. coli* carrying T7 promoter facing the origin (oriented from left to right). **f**, Corresponding z-transformed signals of the short range Hi-C signal, T7 RNA polymerase ChIP-seq, transcription and translation. **g, h**, Gene expression upstream (*yaiS*) and downstream (*codB*)

of the T7 promoter lacZ system with or without STOP codons based on GFP fluorescence (**g**) and growth of the corresponding strains (**h**). **i**, Bacterial colony dilution with pT7lacZ repressed on the left and expressed on the right. **j**, Contact map of the bacteria carrying a T7 promoter lacZ system with two stop codons into the lacZ gene. **k**, Log<sub>2</sub> ratio between the contact map with the lacZ2xSTOP system over the contact map with the WT lacZ. **l**, Contact map of the bacteria carrying a T7 promoter lacZ system treated with chloramphenicol. **m**, Log<sub>2</sub> ratio between the contact map treated over the untreated.



**Extended Data Fig. 6 | Dynamic influence of the T7 transcription unit. a,** RNaseq signal over the whole genome. Values are the normalized number of reads at a given position. **b,** Magnification of the RNaseq signal over the *parS<sup>P1</sup>* locus. **c,** Magnification of the RNaseq signal over the *parS<sup>PMT1</sup>* locus. **d-f,** The dotted lines on the plot indicate the median of the loci positions, and the significance of the one-sided t-test between average position of both conditions is indicated by *ns* (not significant) or stars (\*:  $<5.10^{-2}$ ; \*\*:  $<1.10^{-3}$ ; \*\*\*:  $<1.10^{-4}$ ; \*\*\*\*:  $<1.10^{-5}$ ). The errors bars are defined as the 95% confidence interval of 1,000 bootstraps. The gray area highlights the shift of distributions across conditions. **d,** Longitudinal position of the tags with one focus (t-test p-value:

0.12). **e,** Longitudinal position of the *parS<sup>PMT1</sup>* locus with one focus with or without rifampicin treatment (t-test p-value:  $9.8 \times 10^{-3}$ ). **f,** Longitudinal position of the *parS<sup>PMT1</sup>* locus with one focus with rifampicin treatment is rescued upon T7 activation (t-test p-value:  $1.4 \times 10^{-3}$ ). **g,** Positions of the three *lacO* arrays inserted in the vicinity of the T7 promoter. LacI-YFP foci dynamics was analyzed for 100 time intervals of 1 sec, for each replicate (N=3-6) the median MSD $\alpha$  measurement for -1,000 trajectories of the fluorescently labeled loci was computed. Experiment were performed in the presence of rifampicin upon induction of the T7 TU. Statistical differences are measured by an Anova Kruskal-Wallis test with Bonferroni correction, \*  $<0.033$ , \*\*  $<0.0021$ , \*\*\*\*  $<0.0002$ , \*\*\*\*\*  $<0.0001$ .

Extended Data Table 1 | Positions of the detected borders

Liroy <i>et al.</i> 2018	DI 5kb - window 100kb	DI 2kb - window 50kb	DI 1kb - window 20kb	HiCBD 1kb - window: 10, 15, 20, 25, 30kb
40	50	36	36;89;112	36;89;112
145	145	130	144;189	128;156;189;204
220	225	230	231;290;381	226;269;305;315;326; 343;364;379;437
440	445	446	446;465	445
510	665	526;590;662	527;658;753	552;581;608;619;629; 658;689;708;753
760	780;880	782;848;876	840;913;948	779;808;821;836;911;946
980	980	976;1016	986;1051	999;1013.1086;1106
1145	1140	1140	1140	1145;1167
1195			1245	1196;1268;1273;1300
1300	1315	1316;1402;1418	1317;1409;1471;1566	1318;1342;1366;1422;1437 ;1455;1497;1544;1549
1585		1598	1605	1601;1616;1621;1645
1670	1675	1702;1722	1674	1673;1702;1752;1757;1774
1795	1800	1800	1793	1789
1845	1970	1910;1970;2002	1843;1911;2970; 2002;2024	1844;1865;1870;1906;1942; 1963;2000;2024;2046;2084
2100		2108;2184	2139;2175;2228	2107;2130;2168
2255	2275	2270;2330	2304;2330;2369	2263
2380	2390	2390	2389;2425;2452	2389;2410;2425;2248;2495
2525		2538;2630	2540;2582;2611; 2631;2682	2527;2537;2555;2614;2631;2691
2720	2730	2728	2730;2845;2886	2725;2756;2773;2811;2816;2833
2895	2905	2906;2952		2906;2942;2958
2990	3000	2988;3046;3062;3140	2982;3022;3066;3140	2973;3030;3156
3165		3194	3232	3222
3295	3300	3304;3360	3376;3392;3424	3304;3393;3425
3430	3440;3565	3442;3566	3446;3511	3440;3469;3511;3535;3566;3598
3645		3670	3701;3778	3635;3654;3696;3748
3800	3805	3808	3865;3910	3836;3876;3896;3910
3935	3945	3946	3948;3983	3943;3982;4004
4030	4045	4042	4041;4072;4146	4038;4062;4115;4136
4160		4170	4171;4190	4169;4190
4205	4210;4360	4212;4308;4362;4412	4214;4324;4386;4417	4210;4282;4385;4409
4465	4475	4474;4584	4475;4571	4473;4538;4458;4605

Borders from the different methods and Liroy *et al.* 2018<sup>8</sup>.



## Extended Data Table 2 | Strains used in the study

<i>E. coli</i> train name	Genetic background	Genotype	Reference
K-12 MG1655	K-12 MG1655	WT	lab collection
RSG_B012	BW27784	<i>lacZ::PT7 lacO ascFG::ParaBADT7</i> -RNApol (PT7 at position 362063 facing towards <i>oriC</i> )	This work
RSG_B212	BW27784	RSG_B012 <i>lacZ::PT7 2xStop</i>	This work
RSG_B229	BW27784	RSG_B012 <i>proA-thrW::FRT-cm-FRT::PT7mCherry A</i> (PT7mCherryA towards terminus)	This work
RSG_B834	BW27784	<i>lacZ::PT7</i> (PT7lacZ facing towards terminus) <i>ascFG::ParaBADT7</i> -RNApol (PT7 at position 358927)	This work
RSG_B835	BW27784	RSG_B384 <i>proA-thrW::FRT-cm-FRT::PT7mCherry A</i> (towards terminus)	This work
RSG_B836	BW27784	RSG_B384 <i>proA-thrW::FRT-cm-FRT::PT7mCherry B</i> (towards origin) (PT7lacZ TU facing towards terminus)	This work
RSG_B837	BW27784	RSG_B384 <i>paoA-yagU::FRT-cm-FRT::PT7mCherry A</i> (towards terminus) (PT7lacZ TU facing towards terminus)	This work
RSG_B838	BW27784	RSG_B384 <i>paoA-yagU::FRT-cm-FRT::PT7mCherry B</i> (towards origin) (PT7lacZ TU facing towards terminus)	This work
RSG_B791	BW27784	RSG_B834+ pKvS45-GapR3xFLAG	This work
RSG_B792	BW27784	RSG_B835+ pKvS45-GapR3xFLAG	This work
RSG_B793	BW27784	RSG_B836+ pKvS45-GapR3xFLAG	This work
RSG_B794	BW27784	RSG_B837+ pKvS45-GapR3xFLAG	This work
RSG_B795	BW27784	RSG_B838+ pKvS45-GapR3xFLAG	This work
RSG_B789	BW27784	RSG_B229+ pKvS45-GapR3xFLAG	This work
OE3420	DH5 $\alpha$	pKvS45-GapR3xFLAG	<sup>35</sup>
OE3421	BW27784	RSG_B834 + pBAD24	This work
OE3422	BW27784	RSG_B834 + pBAD24-TopA	This work
OE3435	K-12 MG1655	<i>PlacZ::FRT-kan-FRT::PrpsM</i>	This work
OE3436	K-12 MG1655	<i>PlacZ::FRT-kan-FRT::PompA</i>	This work
OE1305	BW27784	RSG_B012 <i>parS<sup>PMT1</sup> ::yajQ parS<sup>P1</sup> ::crl +pFH2973</i>	This work
OE3313	BW27784	RSG_B012 <i>lacO20::ecpR</i>	This work
OE3310	BW27784	RSG_B012 <i>lacO20::betT</i>	This work
OE3311	BW27784	RSG_B012 <i>lacO20::yqeK</i>	This work

Description of the different genotype used.

## Extended Data Table 3 | Libraries used in this study

Figure	Condition	Strains	Associated fastq raw files	Total genome-wide contacts or aligned reads for ChIP-seq or RNA-seq	
Figure 1, 4, Extended Data Figure 1, 2, 4	Hi-C (HpalI) of <i>Escherichia coli</i> : K12-MG1655 rep1	K-12 MG1655	SRR10394904 (CC328)	30,079,548	
	Hi-C (HpalI + MluCI) of <i>Escherichia coli</i> : K12-MG1655 rep1	K-12 MG1655	CC330	8,041,129	
	Hi-C (HpalI) of <i>Escherichia coli</i> : K12-MG1655 rep2	K-12 MG1655	SRR10394903 (CC334)	13,736,815	
	Hi-C (HpalI + MluCI) of <i>Escherichia coli</i> : K12-MG1655 rep2	K-12 MG1655	SRR10394901 (CC336)	10,949,945	
	RNA-seq of <i>Escherichia coli</i> : K12-MG1655	K-12 MG1655	CC_Ec15	1,409,288	
Figure 1, Extended Data Figure 1	Hi-C (HpalI) of <i>Escherichia coli</i> : K12-MG1655 + 10 min rifampicin	K-12 MG1655	CC419	19,977,855	
	RNA-seq of <i>Escherichia coli</i> : K12-MG1655 + 10 min rifampicin	K-12 MG1655	CC_Ec16	222,09	
Figure 2	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B834 - 2h glucose	RSG_B834	CC408	23,131,267	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B834 - 2h glucose + 10 min rifampicin	RSG_B834	CC409	22,378,178	
Figure 2, Extended Data figure 4	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose	RSG_B834	CC410	17,238,493	
Figure 2, 3, 4, Extended Data	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose + 10 min rifampicin	RSG_B834	CC307	2,634,760	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose + 10 min rifampicin	RSG_B834	CC411	10,537,600	
Figure 2	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h glucose - Rep1	RSG_B834	CC_Ec01a	4,848,538	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h glucose - Rep1 - resequenced	RSG_B834	C_Ec01b	299,8	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h glucose - Rep2	RSG_B834	C_Ec02	4,016,249	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h glucose - Rep3	RSG_B834	C_Ec03	4,334,775	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h glucose + 10 min rifampicin	RSG_B834	CC_Ec13	122,275	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose - Rep1	RSG_B834	CC_Ec04a	3,222,697	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose - Rep1 - resequenced	RSG_B834	CC_Ec04b	7,227,247	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose - Rep2	RSG_B834	C_Ec05	3,290,101	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose - Rep3	RSG_B834	C_Ec06	3,380,846	
	RNA-seq of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose + 10 min rifampicin	RSG_B834	CC_Ec14	1,594,533	
Figure 2-3	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B835 - 2h arabinose + 10 min rifampicin	RSG_B835	CC308	10,238,592	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B836 - 2h arabinose + 10 min rifampicin	RSG_B836	CC309	8,762,672	
Figure 3	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B837 - 2h arabinose + 10 min rifampicin	RSG_B837	CC310	8,867,936	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B838 - 2h arabinose + 10 min rifampicin	RSG_B838	CC311	9,914,456	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B229 - 2h arabinose + 10 min rifampicin	RSG_B229	CC366	22,601,813	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B229 - 2h arabinose + 10 min rifampicin	RSG_B229	CC417	26,706,874	
	RNA-seq of <i>Escherichia coli</i> : RSG_B835 - 2h arabinose + 10 min rifampicin	RSG_B835	CCR55	2,747,926	
	RNA-seq of <i>Escherichia coli</i> : RSG_B836 - 2h arabinose + 10 min rifampicin	RSG_B836	CCR56	1,815,746	
	RNA-seq of <i>Escherichia coli</i> : RSG_B837 - 2h arabinose + 10 min rifampicin	RSG_B837	CCR57	2,404,906	
	RNA-seq of <i>Escherichia coli</i> : RSG_B838 - 2h arabinose + 10 min rifampicin	RSG_B838	CCR58	802,962	
	RNA-seq of <i>Escherichia coli</i> : RSG_B229 - 2h arabinose + 10 min rifampicin	RSG_B229	CCR08	207,68	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose + 10 min rifampicin	RSG_B834	CC_Chip08	2,822,568	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B835 - 2h arabinose + 10 min rifampicin	RSG_B835	CC_Chip09	2,023,450	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B836 - 2h arabinose + 10 min rifampicin	RSG_B836	CC_Chip10	462,016	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B837 - 2h arabinose + 10 min rifampicin	RSG_B837	CC_Chip11	1,985,554	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B838 - 2h arabinose + 10 min rifampicin	RSG_B838	CC_Chip12	871,262	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B229 - 2h arabinose + 10 min rifampicin	RSG_B229	CC_Chip06	1,424,510	
	ChIP-seq GapR of <i>Escherichia coli</i> : RSG_B791 - 2h arabinose + 10 min rifampicin	RSG_B791	CC_Chip16	33,709,944	
	ChIP-seq GapR of <i>Escherichia coli</i> : RSG_B792 - 2h arabinose + 10 min rifampicin	RSG_B792	CC_Chip18	33,416,300	
	ChIP-seq GapR of <i>Escherichia coli</i> : RSG_B793 - 2h arabinose + 10 min rifampicin	RSG_B793	CC_Chip19	35,992,914	
	ChIP-seq GapR of <i>Escherichia coli</i> : RSG_B794 - 2h arabinose + 10 min rifampicin	RSG_B794	CC_Chip20	37,961,852	
	ChIP-seq GapR of <i>Escherichia coli</i> : RSG_B795 - 2h arabinose + 10 min rifampicin	RSG_B795	CC_Chip21	36,086,046	
	ChIP-seq GapR of <i>Escherichia coli</i> : RSG_B789 - 2h arabinose + 10 min rifampicin	RSG_B789	CC_Chip17	35,006,280	
	Figure 4	HiC (HpalI) of <i>Vibrio cholerae</i> : N16961	N16961	SRR10394900	27,094,804
		RNA-seq of <i>Vibrio cholerae</i> : N16961	N16961	SRR18009750	15,192,244
		RNA-seq of <i>Vibrio cholerae</i> : N16961	N16961	SRR18009751	13,621,052
		RNA-seq of <i>Vibrio cholerae</i> : N16961	N16961	SRR18009754	15,186,376
		HiC (DpnII) of <i>Saccharomyces cerevisiae</i> : W303	W303	SRR12284724	21,297,734
		HiC (DpnII, HinfI) of <i>Saccharomyces cerevisiae</i> : W303	W303	SRR12284725	21,802,154
		HiC (DpnII) of <i>Saccharomyces cerevisiae</i> : W303	W303	SRR12284732	15,516,227
RNA-seq of <i>Saccharomyces cerevisiae</i> : W303		W303	SRR11684203	20,881,992	
RNA-seq of <i>Saccharomyces cerevisiae</i> : W303		W303	SRR11684204	44,943,506	
Extended Data figure 3, 5		Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose	RSG_B012	CC440	6,999,634
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B012 - 2h glucose	RSG_B012	CC439	6,300,015	
Extended Data figure 3	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose + 10 min rifampicin	RSG_B012	CC441	9,743,558	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B012 - 2h glucose + 10 min rifampicin	RSG_B012	CC102	2,113,567	
Extended Data figure 3	RNA-seq of <i>Escherichia coli</i> : RSG_B012 - 2h glucose	RSG_B012	CCR01	7,698,850	
	RNA-seq of <i>Escherichia coli</i> : RSG_B012 - 2h glucose + 10 min rifampicin	RSG_B012	CCR02	1,267,321	
	RNA-seq of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose	RSG_B012	CCR03	6,594,578	
	RNA-seq of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose + 10 min rifampicin	RSG_B012	CCR06	231,918	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B012 - 2h glucose	RSG_B012	CC_C01	428,26	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B012 - 2h glucose	RSG_B012	CC_C02	234,548	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose	RSG_B012	CC_C03	5,111	
	ChIP-seq T7 RNAP of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose + 10 min rifampicin	RSG_B012	CC_Chip05	396,112	
Extended Data Figure 4	Hi-C (HpalI) of <i>Escherichia coli</i> : OE3436	OE3436	AT692	5,526,950	
	Hi-C (HpalI) of <i>Escherichia coli</i> : OE3435	OE3435	AT693	4,729,955	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose + 10 min novobiocin	RSG_B834	CC352	9,942,198	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B834 - 2h arabinose + 10 min rifampicin/novobiocin	RSG_B834	CC353	11,134,014	
	Hi-C (HpalI) of <i>Escherichia coli</i> : OE3422 - 2h arabinose	OE3422	AT691	4,404,342	
Extended Data figure 5	Hi-C (HpalI) of <i>Escherichia coli</i> : OE3422 - 2h arabinose + 10 min rifampicin	OE3422	AT708	1,878,078	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B212 - 2h arabinose	RSG_B212	CC444	5,061,732	
	Hi-C (HpalI) of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose + 10 min chloramphenicol	RSG_B012	CC443	10,287,171	
	Polysome sequencing of <i>Escherichia coli</i> : RSG_B012 - 2h arabinose	RSG_B012	CCR49	572,637	

Data are available at [PRJNA844206](https://www.ncbi.nlm.nih.gov/sra/PRJNA844206).

## Reporting Summary

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### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | n/a                                 | Confirmed  |
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| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated   |

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

### Software and code

Policy information about [availability of computer code](#)

Data collection	No software have been used.
Data analysis	<p>All the scripts used in this study are available in the following github repository: <a href="https://github.com/koszullab/T7_promoter_analysis">https://github.com/koszullab/T7_promoter_analysis</a>            The programs involved in this study. The following programs are the one cutsom made or open sources programs from Koszul laboratory:            hicstuff v3.1.4 (available at <a href="https://github.com/koszullab/hicstuff">https://github.com/koszullab/hicstuff</a>)            bacchus v1.0.1 (available at <a href="https://github.com/ABignaud/bacchus">https://github.com/ABignaud/bacchus</a>)            TinyMapper v0.10.0 (available at <a href="https://github.com/js2264/tinyMapper">https://github.com/js2264/tinyMapper</a>)            serpentine v0.1.3 (<a href="https://github.com/koszullab/serpentine/tree/master">https://github.com/koszullab/serpentine/tree/master</a>)</p> <p>As well as programs developed and published by others:            bowtie2 v2.4.5 (available at <a href="https://github.com/BenLangmead/bowtie2">https://github.com/BenLangmead/bowtie2</a>)            samtools v1.15 (available at <a href="http://www.htslib.org/">http://www.htslib.org/</a>)            deepTools v2.29.1 (available at <a href="https://github.com/deeptools/deepTools">https://github.com/deeptools/deepTools</a>)            cooler v0.8.11 (available at <a href="https://github.com/open2c/cooler">https://github.com/open2c/cooler</a>)            scipy v1.7.3 (available at <a href="https://scipy.org/">https://scipy.org/</a>)</p>

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Sequencing datasets for all figures have been deposited in SRA under the accession code of PRJNA844206

Reference genomes:

- E. coli K12 MG1655: [https://www.ncbi.nlm.nih.gov/nuccore/NC\\_000913.3/](https://www.ncbi.nlm.nih.gov/nuccore/NC_000913.3/)
- V. cholerae O1 El Tor N16961: [https://www.ncbi.nlm.nih.gov/data-hub/genome/GCF\\_000006745.1/](https://www.ncbi.nlm.nih.gov/data-hub/genome/GCF_000006745.1/)
- S. cerevisiae W303: <https://www.ncbi.nlm.nih.gov/assembly/GCA800216351.1/>

Microscopy data are available at Mendeley Data, V1, doi: 10.17632/fzrmgjfyg7.

Source data are provided with this paper.

Strains of this study are available from the corresponding authors.

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

Reporting on sex and gender

NA

Population characteristics

NA

Recruitment

NA

Ethics oversight

NA

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences       Behavioural & social sciences       Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No samples size calculation were performed: experiments are bulk and each measurement reflects millions of cells. The experimental design follows the standard guidelines and procedures in the field (i.e. similar samples sizes, incubation time, etc.). The sample size (n) of independent experiments is provided in the methods of the manuscript. Sample sizes were chosen to support meaningful conclusions. See following publications for similar guidelines: Lioy et al., Cell, 2018 ; Cockram et al., Mol Cell, 2021.

Data exclusions

No data were excluded from the analysis.

Replication

4 biological HiC replicates have been used for the WT HiC on E. coli. For the RNA-seq experiments with one T7 promoter in the forward strand 3 replicates have been done for the library without rifampicine and only one for the library with rifampicine as we just want to control that transcription is shot down. For other genomics libraries we did only one replicate. the microscopy analysis of the positioning of the two parS sites have been made based on between 796 and 1823 cells in each strain and each condition. Between 7 to 9 biological replicates of each strain and conditions (different cultures at different time) have been pooled. To compute the trajectories of the locus, between 3 and 6 replicates have been made for each strain and conditions to have at the end the trajectories of approximately 1000 cells for each. Different numbers of replicates have been made to have the similar number of cells once it's pooled. All attempts at replication were successful.

Randomization

No randomization of samples was relevant nor performed: randomization is not relevant because we did not use experiment groups in our



Randomization 
  
Blinding

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

- n/a  Involved in the study
- Antibodies
  - Eukaryotic cell lines
  - Palaeontology and archaeology
  - Animals and other organisms
  - Clinical data
  - Dual use research of concern

### Methods

- n/a  Involved in the study
- ChIP-seq
  - Flow cytometry
  - MRI-based neuroimaging

## Antibodies

### Antibodies used

ChIP-seq antibodies:

- T7 RNA pol antibody (Biolabs CB MAB-0296MC) for the T7 RNA pol ChIP-seq diluted 1/1000.
- Mouse anti-flag (Sigma F1804 clone M2 and F3165) for the GapR ChIP-seq
- Mouse monoclonal antibody against E.coli TopA antibody (gift from Tse-dinh) used as described in Zhou, et al 2017 NAR for the western-blot diluted 1/1000
- HRP:
- Primary antibody : anti-E. coli RNA Polymerase  $\beta$  Antibody (Biolegend 663907) for the western blot diluted 1/2000
- Secondary antibody : HRP anti-mouse antibody for the western-blot diluted 1/10000

### Validation

- Mouse anti-FLAG (Sigma Aldrich, F1804 clone M2) is validated on Sigma Aldrich website.  
<http://www.sigmaaldrich.com/FR/fr/product/sigma/f1804>
- GapR ChIP-seq profiles yield expected values as the ones previously published (see Freddolino et al., PLOS Biol., 2021).
- T7 RNA pol antibody was test by Western blot.  
[https://www.creativebiolabs.net/Anti-T7-RNA-Polymerase-Antibody-78687.htm?gclid=CjwKCAjwsfuYBhAZEiwA5a6CDIznSVjE0dkEZ6ZQFHli3oH1D\\_oTvWoOWe1uvWJ8H5QfHvC7VkYeBoC9oIQAvD\\_BwE](https://www.creativebiolabs.net/Anti-T7-RNA-Polymerase-Antibody-78687.htm?gclid=CjwKCAjwsfuYBhAZEiwA5a6CDIznSVjE0dkEZ6ZQFHli3oH1D_oTvWoOWe1uvWJ8H5QfHvC7VkYeBoC9oIQAvD_BwE)
- The anti-TopA monoclonal antibodies were generated in the 1990's by Rolf Menzel at Bristol-Myers Squibb for a project on topA transcription. TopA antibodies were used previously in the study: Direct interaction between Escherichia coli RNA polymerase and the zinc ribbon domains of DNA topoisomerase I.  
Cheng B, Zhu CX, Ji C, Ahumada A, Tse-Dinh YC. J Biol Chem. 2003 Aug 15;278(33):30705-10. doi: 10.1074/jbc.M303403200. Epub 2003 Jun 4. PMID: 12788950
- HRP : ([https://urldefense.com/v3/\\_\\_https://www.biolegend.com/en-gb/search-results/direct-blot-hrp-anti-e-coli-rna-polymerase-beta-antibody-13499?GroupID=GROUP26\\_\\_;!!JFdNOqOXpB6UZW0!qTAVjUvvrh3s1B3zQHMIHLTERap\\_IDp2ggew3F0GuWiCoXzaBAJl4cJZ\\_wb8dS-xUnYyIkGdESvVg-c1rm\\_dBvb-q7jKs8mENCv96RO\\$](https://urldefense.com/v3/__https://www.biolegend.com/en-gb/search-results/direct-blot-hrp-anti-e-coli-rna-polymerase-beta-antibody-13499?GroupID=GROUP26__;!!JFdNOqOXpB6UZW0!qTAVjUvvrh3s1B3zQHMIHLTERap_IDp2ggew3F0GuWiCoXzaBAJl4cJZ_wb8dS-xUnYyIkGdESvVg-c1rm_dBvb-q7jKs8mENCv96RO$))

## ChIP-seq

### Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

### Data access links

*May remain private before publication.*

<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA844206>  
<https://www.ncbi.nlm.nih.gov/geo/query/qcc.cgi?acc=GSE213028>

### Files in database submission

Filenames included in the ChIP-seq and HiC database submission are provided in the supplementary table 3: "Libraries used in this study available at PRJNA844206."

### Genome browser session (e.g. [UCSC](#))

No longer applicable.

## Methodology

### Replicates

The coherence between the different constructions carrying similar features were used as replicates. GapR ChIP-seq profiles yield expected values as the ones previously published (see Freddolino et al., PLOS Biol., 2021).

Sequencing depth	<p>Numbers of reads uniquely mapped and paired on <i>E. coli</i> are reported in the supplementary table 3 (Libraries used in this study available at PRJNA844206.) provided in the manuscript.</p> <p>CC_ChIP08; 5,062,356 reads          CC_ChIP09; 5,014,893 reads          CC_ChIP10; 5,850,694 reads          CC_ChIP11; 5,134,958 reads          CC_ChIP12; 5,260,568 reads          CC_ChIP06; 3,518,621 reads          CC_ChIP16; 21,701,615 reads          CC_ChIP18; 21,541,473 reads          CC_ChIP19; 23,914,039 reads          CC_ChIP20; 25,858,578 reads          CC_ChIP21; 24,347,473 reads          CC_ChIP17; 22,673,532 reads          CC_C01; 24,563,583 reads          CC_C03; 5,282,265 reads ; reads aligned          CC_ChIP05; 7,200,704 reads ; reads aligned</p>
Antibodies	<p>ChIP-seq antibodies:</p> <ul style="list-style-type: none"> <li>- T7 RNA pol antibody (Biolabs CB MAB-0296MC) for the T7 RNA pol ChIP-seq</li> <li>- anti-flag (Sigma F1804 and F3165) for the GapR ChIP-seq</li> </ul> <p>The validation has been described upper in the antibodies section.</p>
Peak calling parameters	No peak calling have been performed: GapR and T7 RNAPol yield broad signals and we were not interested on the peak detection.
Data quality	Reads quality was assessed using FastQC. T7 RNA-pol ChIP-seq were concordant with the transcriptomic profiles. GapR ChIP-seq profiles yield expected values as the ones previously published (see Freddolino et al., PLOS Biol., 2021).
Software	Bowtie2 was used for the alignments on the <i>E. coli</i> genome, allowing the generation of an alignment of IP and WCE that exclusively mapped on the genome. The obtained SAM files were converted into a BAM files, sorted, filtered and indexed using samtools. ChIP-seq profiles were then normalised by the number of million sequences and converted into BigWig using bamCoverage.