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Spatial organization of transcription in bacterial cells

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Prokaryotic transcription has been extensively studied over the past half a century. However, there often exists a gap between the structural, mechanistic description of transcription obtained from *in vitro* biochemical studies, and the cellular, phenomenological observations from *in vivo* genetic studies. It is now accepted that a living bacterial cell is a complex entity; the heterogeneous cellular environment is drastically different from the homogenous, well-mixed situation *in vitro*. Where molecules are inside a cell may be important for their function; hence, the spatial organization of different molecular components may provide a new means of transcription regulation *in vivo*, possibly bridging this gap. In this review, we survey current evidence for the spatial organization of four major components of transcription [genes, transcription factors, RNA polymerase (RNAP) and RNAs] and critically analyze their biological significance.

Location matters

Traditionally, bacterial cells have been viewed as bags of enzymes. Subcellular localization was thought unimportant because enzymes could reach their substrates fast enough through simple diffusion. For example, a protein molecule with a typical cytoplasmic diffusion constant of 8 $\mu m^2/s$ [1] will travel on average approximately 700 nm within 10 ms, which is comparable to the dimensions of a typical bacterial cell. However, over the past two decades, studies in bacterial cells have emerged to suggest the opposite: subcellular locations of biomolecules may matter for their function [2,3].

In this review, we focus on the spatial organization of transcription in bacterial cells, defined as the intracellular localization of various transcription components and their dynamic response to transcriptional activity (Figure 1A). Such spatial organization suggests possible functional compartmentalization, and may provide a new means of regulation of enzymatic activities by spatial colocalization or segregation. We review recent work documenting the subcellular localizations (Figure 1B) of four major components of transcription: genes, transcription factors, RNAP, and RNAs. Most of these studies have been made possible by recent developments in innovative single-molecule, single-cell imaging techniques and high-throughput, large-scale biochemical methods (Table 1). However, we note that the field is currently still at the level of observations, and most evidence come from a relatively small number of specific case studies. To move beyond these observations, we critically analyze the evidence in the context of previous genetic and biochemical studies and consider whether the observed spatial localization pattern is pertinent to a specific biological function.

Organization of genes: spatial clustering versus dynamic relocation

A typical bacterial chromosome forms a compact DNA mass called the nucleoid in the center of the cell (Box 1). Although there is no nuclear envelope separating the nucleoid from the cytoplasm, it is well documented that the chromosome is not a random bowl of spaghetti but is instead structurally organized [4–9]. One of the many factors involved in organizing the nucleoid is transcription [10–12]. For example, nucleoids of cells treated with rifampicin, an antibiotic that traps RNAP on promoters by binding to the β subunit of RNAP, showed clear expansion [13–15]. Recent studies using chromosome conformation capture (3C)-based methods (Table 1) also found that specific chromosomal domains are established and maintained by highly expressed genes [7,9].

The coupling of nucleoid structure and transcriptional activity suggests that genes are spatially organized according to their transcriptional activities irrespective of their linear orders on the chromosome. Intuitively, there could be two ways to organize genes spatially. The first is that genes sharing similar regulatory controls could spatially cluster together (Figure 2A); the second is that the cellular location of a gene could dynamically correlate with its transcriptional activity (Figure 2B).

Spatial clustering of genes is supported by computational analyses that showed pairs of distant genes (>100 kb) with correlated expression levels, suggesting that these genes share a similar environment [16–20]. Spatially clustered genes may also have the advantage of confining transcription to local areas where high concentrations of RNAP and transcription factors allow rapid response and efficient transcription [21]. This scenario is similar to the transcription factory theory proposed for eukaryotic cells [22] (Box 2).

The hypothesis of spatially clustered genes initially stemmed from the observation that a functional RNAP-GFP fusion (green fluorescent protein, labeled on the β′ subunit, RpoC-GFP) forms one or two dense foci per

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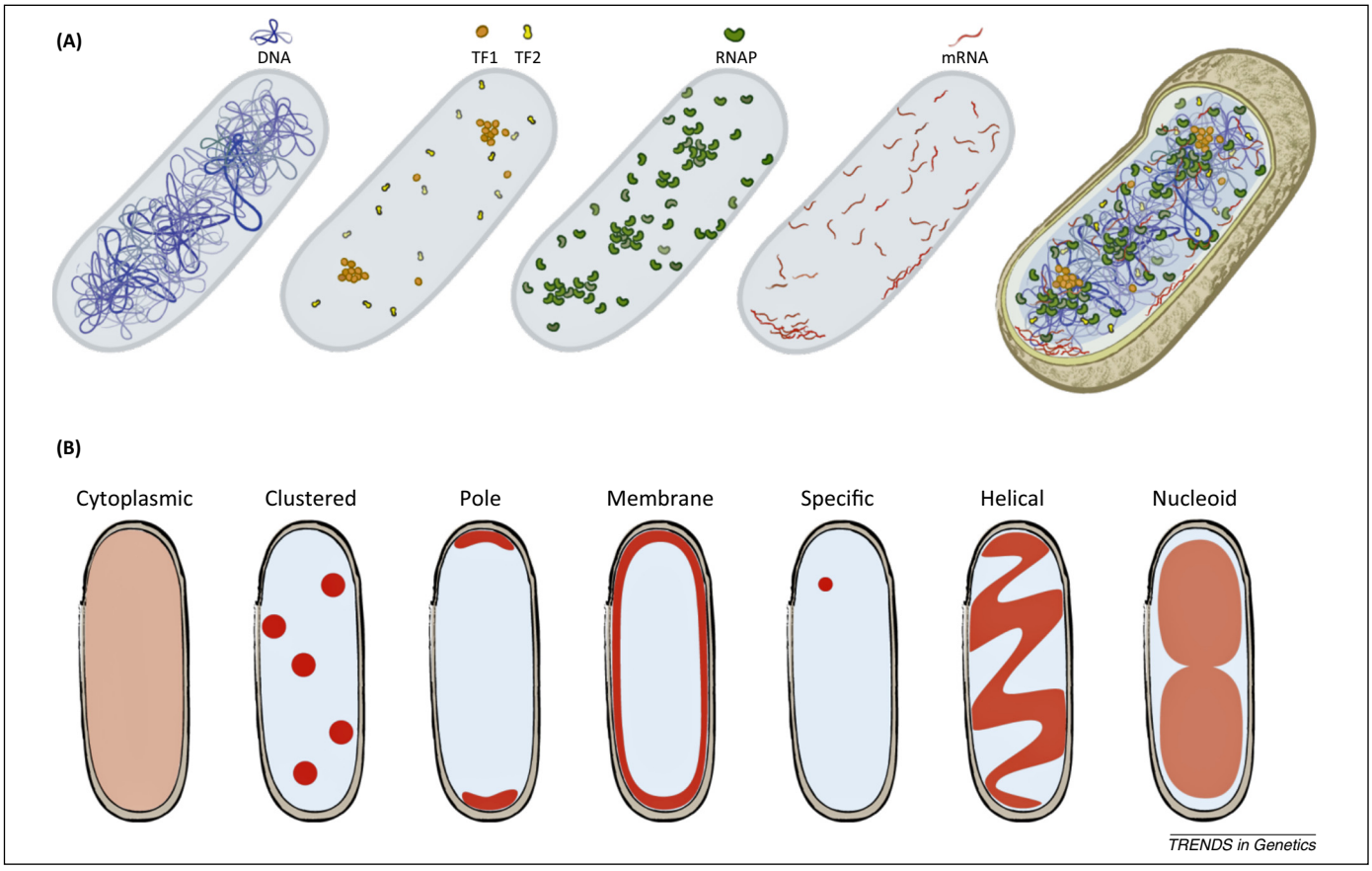

Figure 1. Spatial organization of transcription in an *Escherichia coli* cell. **(A)** Genes (DNA), transcription factors (TF1 and TF2), RNA polymerase (RNAP), and mRNAs may be organized differentially in space instead of being homogenously distributed. **(B)** Overview of experimentally observed molecular spatial distribution patterns of transcription components in a model bacterial cell. From left to right are cytoplasmic, clustered, pole, membrane, specific, helical, and nucleoid distributions.

chromosome in *Escherichia coli* cells growing in rich media (see more discussions in the RNAP section) [23–25]. Given that ribosomal RNA (rRNA) synthesis is the major transcription activity in cells with high growth rates [26–28], it is assumed that these RNAP foci are active RNAP molecules engaged in rRNA synthesis. In addition, because multiple rRNA operons are spaced far away from each other on the chromosome (seven in *E. coli* and ten in *Bacillus subtilis* [29,30]), and the copy number of *rrn* operons in a fast-growing *E. coli* cell can reach up to 50, the observation of far fewer RNAP foci suggests that multiple rRNA operons are transcribed while clustered together [23–25].

A recent study that investigated the spatial distribution of H-NS, one of the nucleoid-associated proteins (NAPs), in *E. coli* showed additional evidence for clustering of genes. Using single-molecule based super-resolution imaging (Table 1), it was found that H-NS forms on average two clusters per chromosome in cells (Figure 3A) [31]. More interestingly, two-color colocalization showed that these clusters colocalized with genes that H-NS regulates (Figure 3A) [31]. In another study, the subcellular localizations of multiple *gal* operons (all regulated by the Gal repressor GalR) were investigated using both fluorescence microscopy and 3C in *E. coli* [21]. It was found that yellow fluorescent protein (YFP) labeled GalR molecules formed one to three punctate foci in cells at stationary phase, and subsequent 3C experiments detected interactions between multiple *gal* operons that were hundreds of kilobases away on the chromosome. However, in cells lacking GalR, such interactions were abolished, suggesting that the clustering of these operons is related to the binding of GalR.

It is important to note that the studies described above used the spatial clustering of RNAP or transcription factors to infer the spatial distribution of the genes they bind to. The spatial proximity of these genes was then investigated using 3C and its derivatives, or inferred by correlating with known transcription activities under the same growth conditions (see more discussions in the section on transcription factors). The actual cellular localizations or spatial clustering of these genes were not directly visualized and compared to the protein clusters. As such, the results are still controversial. For example, it has not been experimentally proven that multiple rRNA operons colocalize with each other in RNAP foci. In fact, interactions between rRNA operons were not detected in a recent 3C study, which was attributed to technical limitations in the 3C analysis of repetitive gene loci [7]. The same 3C study also failed to detect interactions between H-NS regulated genes. This discrepancy was attributed to the improved resolution in the new 3C study. However, other experimental differences, such as cell growth conditions, may also contribute to the discrepancies. In addition, because 3C and its derivatives detect the juxtaposition of DNA sites by evaluating their cross-linking frequencies with averaging across an ensemble of cells, transient interactions between

Table 1. Summary of microscopy and biochemical methods used in detecting the spatial organization of transcription[a]

Name	Target	Detection principle	Strength	Limitation	Refs
3C and derivatives	DNA	Fixation and cross-linking of genome interactions; detection is by PCR, immunoprecipitation, and/or sequencing	Capturing native interactions in one reaction High-throughput, large-scale detection of long-range genome interactions	Interaction frequencies inferred from cross-linking efficiency; low resolution (>10 kb)	[33]
FROS	DNA	Tandem arrays of DNA-binding sites are inserted chromosomally; detection is by binding of fluorescent protein fused to DNA-binding proteins	Live cell compatible Strong signal:noise ratio if hundreds of binding sites are used Enables tracking of chromosome positions in real time Orthogonal systems available: *lacO*-LacI; *tetO*-TetR; *parS*-ParB	Tight binding of fusion proteins on DNA may be detrimental to cell physiology	[41]
RNA stem-loop motif-phage protein system	RNA	Tandem arrays of RNA stem-loops are inserted into genes of interest. Detection is by the binding of fluorescent protein fused to phage proteins that bind to the stem-loops	Live cell compatible Strong signal:noise ratio if multiple binding sites are used Enables the tracking of real-time RNA production and diffusion Orthogonal systems available: MS2-MCP; PP7-PCP; boxB-lambdaN	Tight binding of phage proteins on RNA stem-loops may alter RNA stability or inhibit translation	[94,104–106]
smFISH	RNA	Multiple complementary short DNA oligonucleotides labeled with organic fluorophores hybridize to RNA of interest	Detecting native RNAs Able to achieve single RNA molecule detection	Requires fixation; hybridization efficiency varies depending on sequence	[107,108]
PALM	Protein	Photoactivatable fluorescent protein fused to protein of interest. Stochastic photoactivation of single molecules allows for sub-diffraction limited precision in determining the position of molecules	Live cell compatible 15–30 nm spatial resolution achievable	Functionality of fluorescent protein fusions need to be verified	[71]
STORM	Protein and nucleic acids	Antibodies and/or oligonucleotides labeled with photoswitchable fluorophores to detect protein or nucleic acids; blinking of dye molecules allows for subdiffraction-limited precision in determining position of molecules	15–30 nm spatial resolution achievable	Usually requires fixation	[72]
SMT	Protein and nucleic acids	Spatial positions of fluorescently tagged single molecules are tracked at various time points	Enables measurement of diffusion behavior and binding and/or unbinding kinetics	Fluorophore bleaching and/or photoblinking limits length of tracking trajectories	[109]

[a]*Abbreviations*: 3C, Chromosome Conformation Capture; FROS, fluorescent repressor-operator system; smFISH, single-molecule fluorescence *in situ* hybridization; PALM, photo-activated localization microscopy; STORM, stochastic optical reconstruction microscopy; SMT, single-molecule-tracking.

genes may not be easily detectable [32–34]. As we will describe at the end of this section, new methods that enable the direct, sensitive detection of individual gene locus in single cells are key to resolving these issues.

The second way to organize genes spatially is through dynamically changing the cellular location of a gene depending on its transcription activity (Figure 2B). The regulation of transcription activity may involve moving genes in or out of particular subcellular locations, in addition to the binding and unbinding of transcription factors in the traditional view of gene regulation. This notion may have stemmed from early electron microscopy (EM) studies, where it was shown that transcriptionally active genes and RNAP were primarily located on the nucleoid surface instead of the interior [35,36]. Intuitively, it is easier for RNAP and transcription factors to access genes on the surface rather than in the interior of a packed nucleoid.

The dynamic relocation of genes in response to their transcription activities has been demonstrated in eukaryotic cells [37–40]. However, in bacteria, direct evidence is scarce. In one *E. coli* imaging study, a plasmid DNA was labeled using the fluorescent repressor-operator system (FROS; Table 1 [41]). It was found that, when the plasmid carried a constitutive promoter, it formed a clear focus at the edge of the nucleoid toward the cell pole [42]. However, in the absence of the promoter, the plasmid diffused randomly

Box 1. Bacterial nucleoid structure

The typical size of a bacterial genome is a few million base pairs. This chromosomal DNA must adopt a highly compact but orderly configuration to accommodate the spatial constraints of a bacterial cell while coordinating with DNA-centric activities, such as replication and transcription.

Taking the most well-studied bacterial model system *Escherichia coli* as an example, the chromosome is organized on multiple levels (Figure I). At the first level, the negatively super-coiled chromosomal DNA naturally generates plectonemic loops, which are actively maintained by the opposing actions of gyrase and topoisomerases [12,110,111]. Protein factors, such as the abundant NAPs, can influence DNA structure both locally by bending and wrapping DNA segments and globally by bridging and providing boundaries for DNA topological domains [112]. These independent topological microdomains domains are on the scale of 10–100 kb and are positioned stochastically along the chromosome [66]. The next level of organization, as assayed by both the accessibility of DNA to homologous recombination and chromosome dynamics as observed through fluorescence imaging, comprises four macrodomains of approximately 1 Mbp each (*ori*, *ter*, *left* and *right*), along with two unstructured regions flanking the *ori* domain [4,5]. These macrodomains are likely further folded into a compact rod shaped nucleoid, as suggested in a recent genome conformation capture study [7]. Finally, the chromosome has a defined orientation within the cell. For *E. coli*, the replication origin and terminus are situated close to mid cell, whereas the left and right chromosome arms are stretched toward the two poles [48,113].

Chromosome organization in other bacterial model systems, such as *Bacillus subtilis* and *Caulobacter crescentus* show that the basic level of organization by DNA supercoiling and NAPs is similar to that of *E. coli*. Furthermore, fluorescent-labeling experiments showed a helical arrangement of the chromosome in both *B. subtilis* and *C. crescentus* [8,114]. Higher resolution chromosome interaction maps obtained in recent 3C-based experiments showed that the *C. crescentus* chromosome may adopt a bottle-brush configuration [9]. The orientation of the chromosome in the cell is different in *B. subtilis* and *C. crescentus* compared with *E. coli*. In *B. subtilis*, the replication origin and terminus have a preference for opposite poles early in the cell cycle [45,114]. In *C. crescentus*, the chromosome is positioned with the origin always at the old pole and the terminus at the new pole, with the left and right chromosome arms spanning the length of the cell [8,115]. Notably, both *B. subtilis* and *C. crescentus* (but not *E. coli*) contain an origin proximal centromere-like region *parS* (Partition system), which is used for positioning of the chromosomal origin of replication, and assists in the proper orientation of the chromosome and regulation of the cell cycle [116,117].

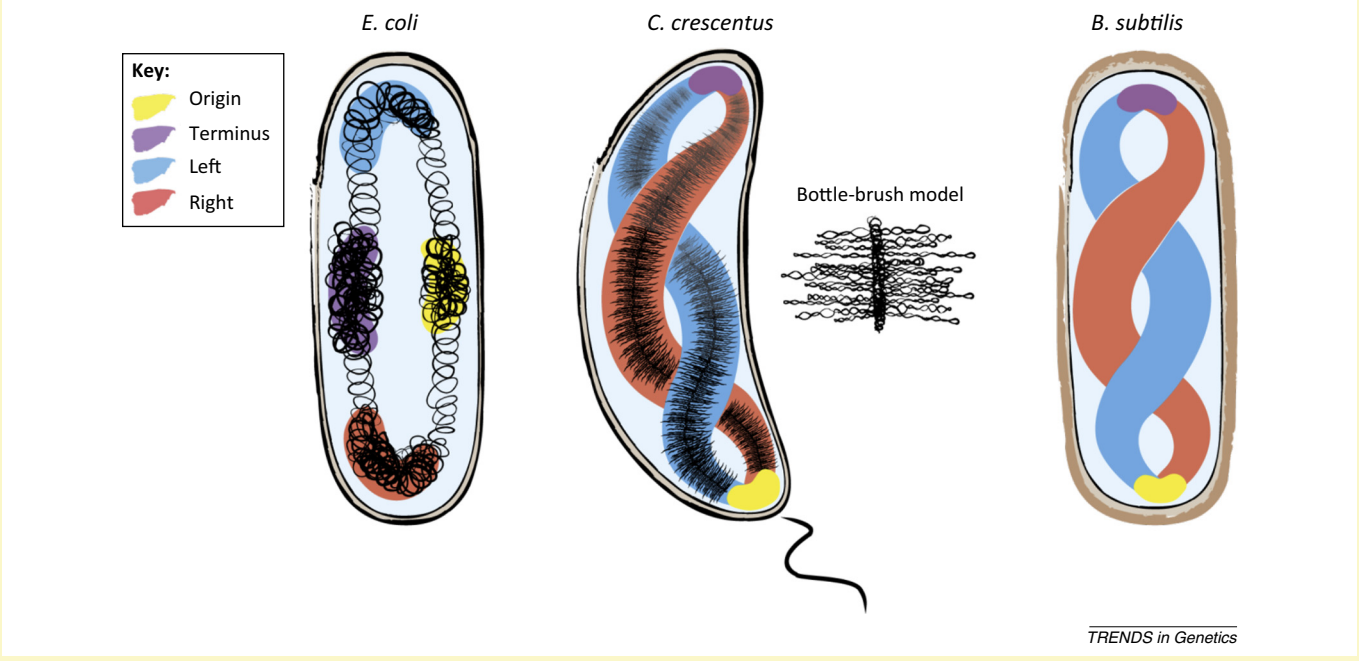

Figure I. Models for nucleoid organization in *Escherichia coli*, *Caulobacter crescentus*, and *Bacillus subtilis*. Chromosomal domains are colored as indicated by legend.

inside the cytoplasm. Although the plasmid DNA is not integrated onto the chromosome, the study did show that transcription activity can influence the subcellular location of a gene. In another *E. coli* study, two genes encoding different membrane proteins were found to move toward the membrane upon induction. However, this movement is most likely due to co-transcriptional translation and insertion of proteins into the membrane (transertion), so may not be a direct effect of transcription activity [43].

Other studies also used the FROS method to label specific DNA segments on the chromosome and imaged their cellular localizations in live bacteria cells. Although not specifically looking for the correlation with transcription activities, these studies found that the intracellular localization of a DNA segment is largely dependent on where it is on the chromosome and which stage of replication it is in, in contrast to the view of a dynamically relocating gene [44–52]. Furthermore, a clever experiment in *Caulobacter crescentus* found that, when the chromosome is rotated by moving the chromosome-anchoring sequence *parS* to a new genomic location, the global gene expression profile was not significantly altered [8]. This study suggests that gene expression activity is not related to specific subcellular positions.

How can these seemingly contradictory results be reconciled? It is certainly possible that the dynamic relocation of genes in response to transcription activity is not necessary; the interior of the nucleoid may be equally accessible to transcription as the surface. An imaging study in mouse cells found that active transcription could also occur deep

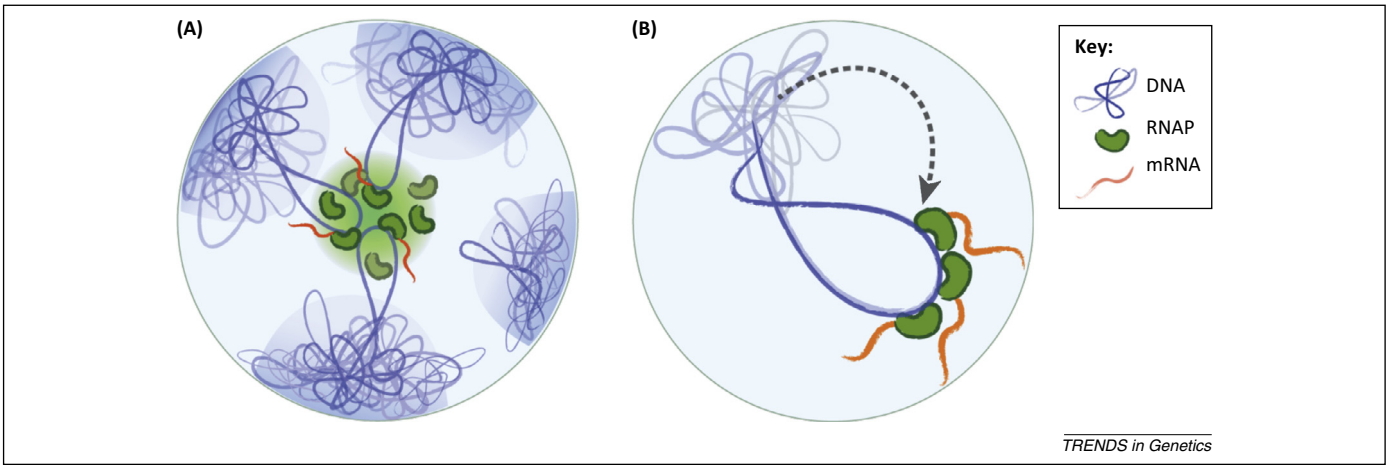

Figure 2. Spatial organization of genes. **(A)** Multiple genes spatially cluster together to be coregulated. **(B)** A gene may move out of its ordinary boundary to be activated. Adapted from [118] (A).

inside chromosomal territories [53]. In this case, it could be that the intracellular location of a gene does not change, but the local environment of the gene changes to promote active transcription.

Alternatively, it is also possible that transcription activity-induced change in the subcellular location of a gene may not be significant enough to be detected by FROS-based methods (Table 1). FROS traditionally uses FPs fused to DNA-binding proteins bound to tandem arrays of hundreds of binding sites spanning several kilobases; thus, the accuracy and precision in determining the intracellular position of a gene similar in length are compromised. Furthermore, it is especially challenging when probing small changes in positions with respect to the short axis of the cell in rod-shaped bacteria, which is on the scale of 1 μm.

To resolve these issues, a method allowing for direct visualization of subcellular positions of genes with high resolution should be used. A recent single-molecule imaging study used a modified FROS strategy to track the position and movement of a 2.3-kb DNA segment in live *E. coli* cells with an accuracy of approximately 40 nm [54]. Instead of hundreds of tandem arrays, the use of only three DNA-binding sites spanning less than 100 bp results in a diffraction-limited fluorescent spot. Thus, the position of the binding sites can be determined with subdiffraction-limited precision by fitting its fluorescence profile to a Gaussian function [55]. This method, if combined with other methods that directly visualize the transcription activity of individual genes in live cells (see below), allows for in-depth examination of whether and how genes are spatially organized in response to their transcription activities.

Spatial distributions of transcription factors: clustering or randomly diffusing?

In the conventional view, a transcription factor (TF) molecule randomly diffuses inside a cell until it encounters a specific binding site, at which it associates tightly to regulate the corresponding gene. This notion is supported by the measured diffusion constant of approximately 0.4 μm^2/s on a YFP-labeled Lac repressor, LacI [56]. As such, if the number of TF molecules is significantly greater than its binding sites, TF distribution should be fairly homogenous over the nucleoid.

However, a recent single-molecule study suggests the opposite. In this work, the spatial distribution of a Venus-labeled LacI fusion protein in an *E. coli* strain lacking a LacI-binding site was imaged. It was found that LacI-Venus preferentially localized to regions close to where its coding gene resides [57]. This biased spatial distribution may

Box 2. The transcription factory theory

The transcription factory theory was initially proposed for RNAPII in eukaryotic cells [22]. In this model, multiple active RNAPII molecules form foci or clusters to reel genes through for transcription. One of the features of this model is that genes far away from each other could be brought into the same factory and, thus, can be coordinately regulated by the same factors despite their physical distance (reviewed in [118]). Past studies using a combination of conventional fluorescence microscopy, EM, and 3C techniques in eukaryotic cells have well established that some active genes indeed colocalize with active RNAPII foci [37,38,40]. Nonetheless, many aspects of the model, such as whether RNAPII factories are stable or dynamic cellular structures, and whether it is a general mode of transcription, remain unclear.

Recently, two single-molecule-based super-resolution imaging studies investigated the spatial distribution of RNAPII in the same type of human cell [119,120]. One study found that RNAPII indeed forms large clusters in live cells; these clusters are dynamic, transitory, and respond to changes in the transcriptional state of the cell [119]. However, due to the live cell nature of the study, it was difficult to quantify on average how many RNAPII molecules are in a cluster, and how many of these clusters are in a cell. The other study, conducted in fixed cells where the number of detected molecules could be accurately counted, found that most RNAPII foci (>70%) only contained a single RNAPII molecule, and <10% of foci contained more than four RNAPII molecules [120]. Regardless of differences in the experimental and quantification methods used in the two studies (live versus fixed cells, wide-field versus light-sheet illumination, FP fusion versus affinity tag labeling, etc.), both studies indicate that, although transcription factories may exist, they are not static cellular structures as previously thought, and may not be a prevalent mode of transcription in eukaryotic cells.

The observation of dense RNAP foci in bacterial cells inspired a similar theory in prokaryotes. However, there are large differences in eukaryotic and prokaryotic transcription. For example, RNAPI in eukaryotes is responsible for synthesizing rRNA, and localizes to the nucleolus, whereas a single RNAP is responsible for all transcription in bacteria. Furthermore, transcription foci in bacterial cells are sensitive to growth conditions and cell physiology: punctate RNAP clusters are only observed in *Escherichia coli* cells under fast growth [23]. To date, the transcription factory theory remains controversial in both eukaryotes and prokaryotes.

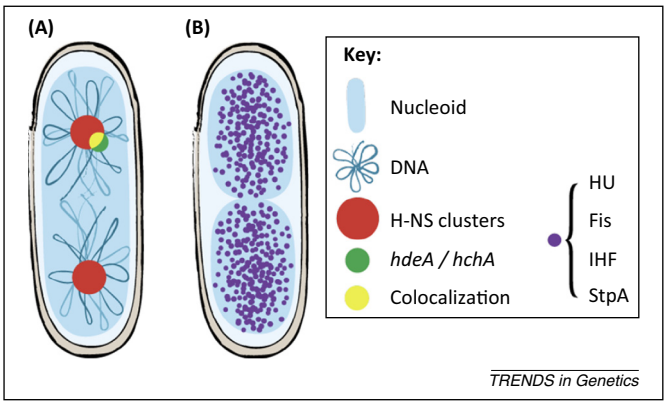

Figure 3. Different spatial distributions of transcription factors. In *Escherichia coli*, global nucleoid-associated proteins (NAPs) are differentially distributed, with H-NS forming on average two clusters per chromosome **(A)**, HU, Fis, IHF, and StpA all distributing relatively homogonously on the nucleoid **(B)**. H-NS clusters were shown to colocalize with H-NS regulated genes *hdeA* and *hchA*; H-NS binding sites across the chromosome are inferred to be colocalized with H-NS clusters (A) [31].

explain previous observations that the closer the target gene is to the TF-encoding gene, the stronger the regulation by the TF is [58,59]. It is interesting to note that, to explain this spatial distribution, a model in which the *lacI-venus* gene is transcribed and translated inside the nucleoid is needed. This is in contrast to the hypothesis that a gene moves to the surface of the nucleoid for transcription.

When TF molecules are bound to multiple binding sites across the chromosome, the spatial distribution of the TF should reflect that of the bound DNA sites. As we described in the first section, GalR and H-NS both form clusters [21,31], suggesting that their multiple DNA-binding sites are also spatially clustered. Another TF in *B. subtilis*, Rok, binds to AT-rich DNA and is likewise distributed nonuniformly on the nucleoid when visualized by fluorescence microscopy [60]. By contrast, other global TFs such as HU, IHF, Fis, and StpA, are largely uniformly distributed on the nucleoid in *E. coli* (Figure 3B) [31,61].

What determines the spatial clustering of a TF? Oligomerization of a TF has been suggested as a driving force: it was found that dimerization mutants GalR^{T322R} and H-NS^{L30P} abolished the clustered appearance of the TFs and reduced the regulation of corresponding genes [21,31]. It was verified for GalR^{T322R} that the mutation did not affect the DNA-binding properties of the protein [62]. However, H-NS^{L30P} seems to have altered DNA-binding properties [63,64], hence the abolishment of the clustering in the mutant cannot be solely attributed to the loss of oligomerization. In addition, other TFs, including HU, IHF, Fis, and StpA, form dimers or higher-ordered oligomers [65], but do not form spatial clusters, arguing that the oligomerization of TFs may not be sufficient to bring DNA sites together. Indeed, other factors, such as cell growth conditions, expression levels of NAPs, and the supercoiling state of the chromosome [65–67], may contribute to the formation of spatial clusters. For example, it was shown previously that repression by GalR is likely mediated by a higher-order molecular complex comprising GalR, HU, and supercoiled DNA [68]. It is possible that the local chromosome structure, along with the binding of other protein factors, contributes significantly to the stabilization of the spatial distribution pattern of a TF.

What could be the functional significance of spatially clustered TFs? As discussed previously, clusters may increase the local concentration of a TF and, consequently, enhance transcription regulation. In other cases, clustered TFs may bring DNA regulatory elements that are far away close to a promoter in a way that is similar to eukaryotic enhancers and, hence, strengthen transcription regulation [69]. Finally, the formation of DNA loops, long and short, by the spatial colocalization of TFs and their target genes, may organize chromosomal domains, as suggested by a recent computational study [70]. To examine these possibilities, it would be desirable to analyze systematically the spatial distributions of TFs with high resolution in small bacteria cells. In this regard, recent advances in single-molecule-based super-resolution imaging methods, such as photo-activated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) (Table 1) [71,72], offer great opportunities for bacteriologists [31,54,61].

Spatial distribution of RNA polymerase: are RNAP clusters active transcription centers?

Bacterial RNAP is a multisubunit enzyme. The core enzyme comprises subunits $\alpha_2\beta\beta'\omega$, and is responsible for transcription elongation. The holoenzyme contains an additional σ factor, which is necessary for promoter recognition and initiation of transcription [73]. By using alternative σ factors, RNAP can be directed to transcribe different subsets of genes [74]. Currently, all imaging studies probing the spatial distribution of RNAP in *E. coli* and *B. subtilis* have used FP fusions of the largest subunit, β′ (RpoC-FP). These FP fusions have been shown to be able to replace the endogenous β′, and >90% is incorporated into the RNAP core [75].

Using these fusion proteins, large and distinct RNAP foci have been observed in *E. coli* (Figure 4A) [23–25]. These foci were attributed to active transcription sites, reminiscent of eukaryotic transcription factories (Box 2). Under super-resolution imaging using RpoC-PAmCherry or RpoC-yGFP, these dense foci resolved into clusters ranging from 50 to 300 nm in diameter and often containing more than 100 localizations of RNAP [76,77]. Given that these clusters were largely diminished in cells growing in minimal media or treated with transcription inhibitors, they were suggested to be active transcription centers (transcription factories) transcribing *rrn* operons (Figure 4B) [23–25,76]. Chromatin immunoprecipitation experiments supported this notion by showing that, in rapidly growing cells, RNAP predominately associates with approximately 90 transcription units that are involved in rRNA and ribosomal protein synthesis, and such associations are diminished in rifampicin-treated cells [78,79]. Such a spatial arrangement, if proven true, would be reminiscent of the eukaryotic nucleolus, a specific subnuclear region where rRNA synthesis takes place.

One critical factor remaining unresolved for suggesting these foci as active transcription sites is that there is no direct evidence showing that RNAP molecules in observed foci are indeed actively engaged in transcription. Theoretically, these foci could be formed by RNAP molecules that are bound to DNA in nonproductive transcription

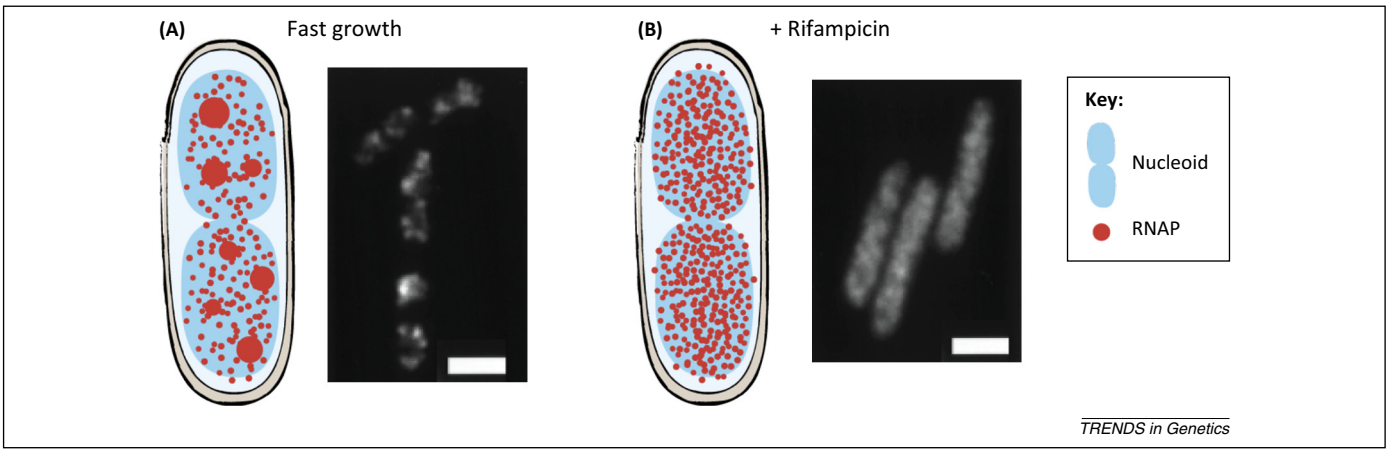

Figure 4. Spatial distribution of RNA polymerase (RNAP) in *Escherichia coli*. **(A)** Under fast growth, distinct foci of GFP-labeled RNAP can be seen. **(B)** Upon rifampicin treatment, RNAP distribution becomes more homogenous. Fluorescent images adapted from [23]. Scale bars = 1 μm.

complexes or on strong promoters poised for transcription [79]. In the latter cases, different growth conditions or drug treatment may alter the nucleoid structure and, hence, the spatial distribution of RNAP bound on the nucleoid. Theoretical modeling and experimental quantifications have suggested that most RNAP is associated with the nucleoid, but only approximately 20–30% of RNAP molecules are actively engaged in transcription, including both rRNA and mRNA synthesis [26,28,75,80,81]. Moreover, fluorescence recovery after photobleaching (FRAP) and single-molecule-tracking experiments (Table 1) showed that, in both rich and minimal media, most RNAP (50–80%) was stably associated with DNA [76,82,83]. Given that the expression level and transcription activity of RNAP differ several fold under different growth conditions [26,28], these studies suggest that the decreased mobility of RNAP and the formation of foci are not necessarily good indicators for transcription activity.

It is difficult to examine the spatial distribution of RNAP activity due to lack of specific drugs and antibodies targeting bacterial RNAP at different stages of transcription as is possible in eukaryotic cells. However, co-labeling RNAP with nascent RNAs using uridine analogs [84], transcription elongation factors, such as NusA, using antibodies [85], or genes using the super-resolution FROS system as described above, may identify RNAP molecules that are engaged in active transcription. This will provide more concrete evidence to examine the hypothesis that these foci are active transcription factories.

The same critical analysis also applies to recent observations using fluorescence microscopy that RNAP mainly colocalizes with the nucleoid, whereas ribosomes localize outside the nucleoid in *E. coli* and *B. subtilis* [25,77]. The latter is consistent with earlier EM evidence that ribosomes reside mostly outside of the nucleoid in *E. coli* [86]. However, the nucleoid-localized RNAP suggests that transcription and translation is spatially separated, or decoupled, in these bacterial cells (note that, in *C. crescentus*, ribosomes are distributed throughout the nucleoid, but not at the periphery [87,88]). The spatial segregation between translation and transcription is in contrast to the long-held view supported by the classical Miller 'Christmas Tree' spreads and many other biochemical studies that transcription and translation are coupled [89,90]. One way to reconcile these observations is that this large spatial separation between RNAP and ribosome does not necessarily represent a functional separation; as we have described above, not all RNAP molecules localized to the nucleoid are actively engaged in transcription. Given that transcription of mRNA is only a small portion of total RNAP activity in fast growth conditions, it is possible that a small population of RNAP transcribing mRNAs is at the periphery of the nucleoid, where transcription and translation are coupled at the interface of RNAP and ribosome distributions. In addition, recently it was shown that the nucleoid has apparent voids in the middle of the cell and, hence, it is possible that ribosome and RNAP are still coupled in these voids [91]. Alternatively, transcription-coupled translation could initiate inside the nucleoid and, when transcription is finished, the mRNA detaches from the gene locus and moves outside of the nucleoid to finish translation.

Spatial distributions of RNAs: a combined result of translation and degradation

Where does an RNA molecule go when it is detached from its gene locus after transcription? Does it randomly diffuse inside the cell, localize to a specific cellular position, or just simply remain where it was transcribed? Moreover, is a particular localization pattern biologically important, or is it merely the result of physical constraints, without any biological consequences? To answer these questions, two important factors, translation and degradation of RNA, should be discussed. These two factors may significantly influence the spatial localization of RNAs.

Translation can influence the localization of mRNAs in two different ways. First, it is conceivable that, with ongoing translation, the presence of multiple ribosomes and nascent peptide chains could significantly slow down the diffusion of mRNA purely due to the increased molecular size. Hence, translating mRNA molecules may exhibit limited mobility and largely remain where they are transcribed. Single-molecule-tracking experiments using a fluorescently labeled ribosome subunit [92] or FP-fused bacteriophage MS2 coat protein (MCP)-MS2 labeling scheme (Table 1) [93,94] found that 70S ribosomes and mRNAs displayed locally confined motion, although the diffusion constants (0.02–0.2 $\mu m^2/s$) were still large enough to enable random distributions over long timescales. In a few cases examined

in *C. crescentus* and *E. coli*, both fluorescent *in situ* hybridization (FISH) (Table 1) on native mRNAs and the MS2 labeling method (Table 1) showed that six different mRNAs encoding diverse protein products colocalized with their coding genes [88]. Such localization, as suggested by the authors, may be biologically significant: it could facilitate rapid interactions between proteins and their partners encoded in close gene clusters.

Note here that, although these observations are consistent with the idea that translating ribosomes limit the mobility of mRNAs, other mechanisms could still be at play to localize (or delocalize) mRNAs. In *E. coli*, for example, a *lacZ* mRNA transcribed from a BAC plasmid carrying a disabled ribosome binding site still localized to cell poles and centers at which the plasmid resides [93]. In another study in *E. coli*, it was found that an untranslated RNA formed localized spots at cell poles, whereas a *lacZ* mRNA highly induced from the same multicopy plasmid displayed largely homogenous distribution throughout the cells [95]. Whether these localization patterns are related to specific biological functions is unknown, but they demonstrate that the localization patterns may be dependent on the individual identity of RNA.

Another way for translation to influence the localization of mRNA is that co-translational insertion of nascent peptides into the membrane could target an mRNA to the membrane [96]. This has been demonstrated for three *E. coli* membrane proteins, lactose permease LacY, tetracycline efflux pump TetA, and a glucose transporter $IICB^{Glc}$ [43,97] using fluorescence microscopy and membrane fractionation. In all three cases, the localization of mRNAs to the membrane is dependent on translation: frame-shifting or drug inhibition of translation abolished the localization. Interestingly, using the MS2 labeling scheme, another study reported similar membrane localization of the *E. coli* sugar permease BglF mRNA, but in a translation-independent way (Figure 5A) [98]. Most strikingly, the mRNAs encoding two additional proteins (a cytoplasmic enzyme BglB and a pole-localizing TF BglG) in the same polycistronic messenger, once decoupled from the *bglF*

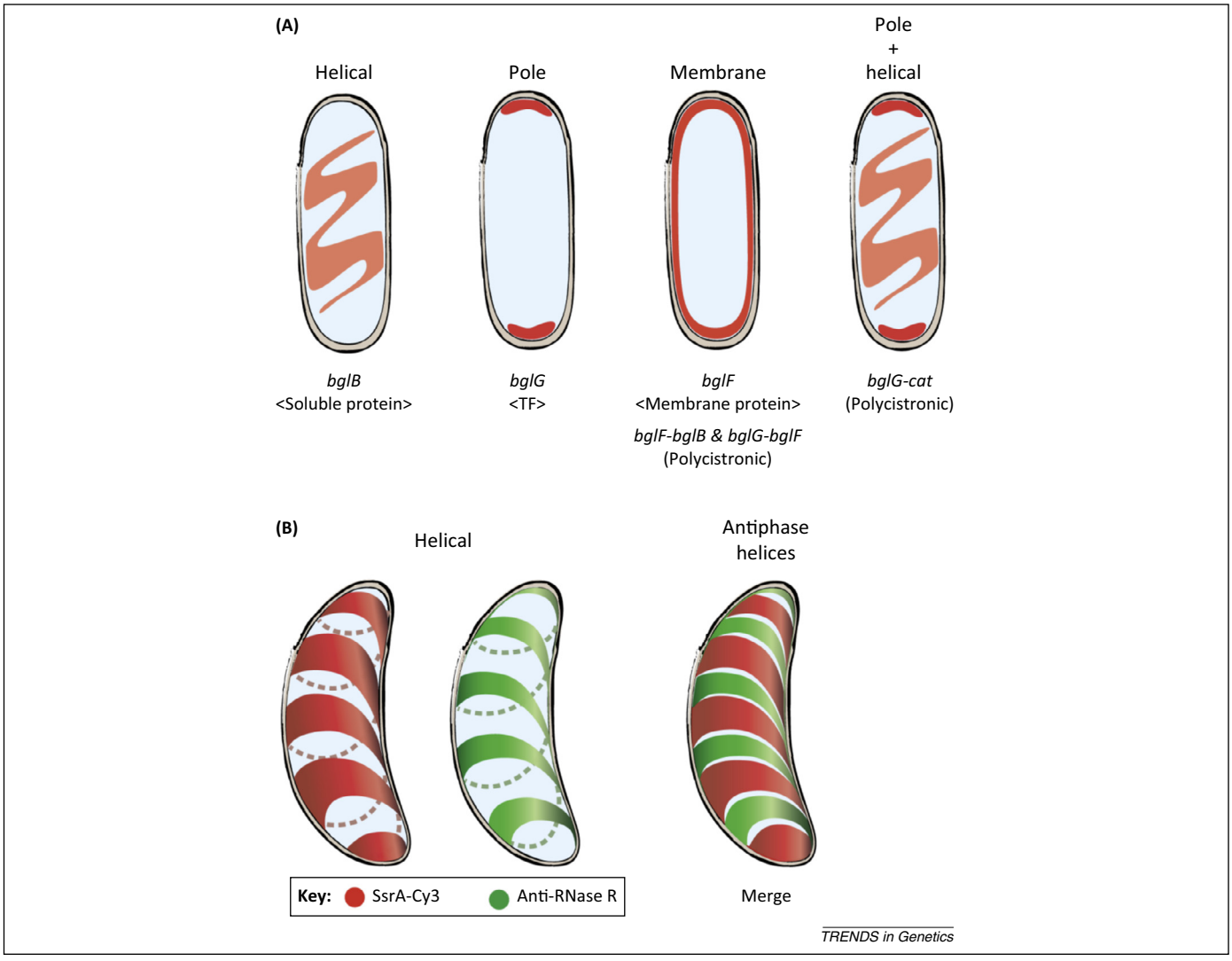

Figure 5. Distinct localization patterns of various bacterial mRNAs. **(A)** Localization of mRNAs (labeled with the MS2 system) encoding membrane protein BglF, cytoplasmic protein BglB, and pole-localizing transcription factor (TF) BglG show distinct cellular patterns. When in one polycistronic mRNA, *bglF* mRNA dominates the localization pattern of the cytoplasmic *bglB* and pole-localizing *bglF* to become membrane localized (cell 3 from left). When the pole-localizing *bglG* mRNA and a cytoplasm-localizing *cat* mRNA are placed on the same polycistronic mRNA, the resulting localization pattern is mixed (cell 4 from left) [98]. **(B)** A small *Caulobacter crescentus* regulatory RNA, tmRNA forms a helix-like localization pattern in the cytoplasm (in red), and that of RNase R (which targets tmRNA for degradation) is localized in a similar anti-phase helical pattern (in green). tmRNA and RNase R are spatially segregated (merged) [102].

mRNA, exhibited drastically different localization patterns: *bglB* mRNA showed a helix-like pattern in the cytoplasm, whereas *bglG* showed polar localization. The helix-like pattern was also observed for the cytoplasmic chloramphenicol acetyltransferase CAT mRNA [98]. Notably, these localization patterns were also detected in unlabeled mRNAs using FISH, arguing that they were not caused by the MS2 labeling scheme. The authors suggested that the localization of mRNAs to where their protein products are required serves as a way to deliver proteins more efficiently to the desired locations; however, the translation-independent mechanism to transport these mRNAs to the cell pole or membrane remains unknown.

The degradation of RNA may also influence localization patterns. In *E. coli*, RNase E, the major enzyme that degrades RNAs [99], was reported to localize to the inner membrane or associate with cytoskeleton structures close to the membrane [100,101]. This spatial distribution suggests that RNAs are targeted to membranes for degradation in *E. coli*. In fact, in the case described above for the *ptsG* mRNA encoding the glucose transporter $IICB^{Glc}$, it was found that membrane targeting enabled efficient degradation of the mRNA with the help of a small RNA, which binds to the RNA chaperone, Hfq [97]. In *C. crescentus*, a small regulatory RNA, called tmRNA, was found to localize in a helix-like pattern in the cytoplasm when probed using FISH (Figure 5B) [102]. The tmRNA encodes the protein degradation tag ssrA in a process called '*trans*-translation', in which the ssrA peptide is added on to a translating protein molecule stalled on the ribosome. Subsequently, the ssrA-tagged protein is targeted for degradation and the stalled ribosome released [103]. Interestingly, the degradation enzyme for tmRNA in *C. crescentus*, RNase R, also forms a helix-like structure in the cytoplasm, but out of phase. The spatial segregation of RnaseR and tmRNA suggests that tmRNA is protected from Rnase R degradation when its activity is needed [102].

Concluding remarks

As of today, the intracellular spatial distributions of genes, TFs, RNAP, and RNAs have been documented with unprecedented sensitivity and resolution. However, knowing precisely where molecules are inside a cell is only the first step toward a better understanding of the connection between spatial organization and functionality. In analogy to functional magnetic resonance imaging, where brain activity can be visualized spatially, identifying the functional aspect of a particular spatial distribution pattern is critical. New imaging and biochemical methods focusing on correlating transcription activity with spatial localizations of various molecular species will no doubt shed light on the emerging question of the field: whether and how the spatial information is converted to regulatory signals of transcription.

This question is particularly important for bacterial cells, because they lack membrane compartmentalization for dedicated enzymatic tasks. Highly controlled but dynamic spatial organization of molecular components in a compact cell space may represent a new paradigm of regulation (Figure 6). In the canonical scheme for TF-mediated gene regulation, the response is often mediated by signal-induced

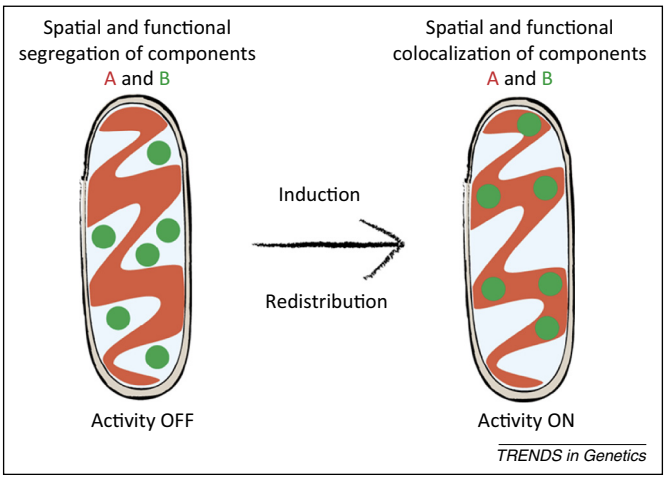

Figure 6. Model of transcription regulation by spatial organization. Molecular components of transcription can be spatially and functionally segregated. Upon induction, spatial reorganization of one or more of the components brings about a change in enzymatic activity.

protein concentration changes through induction of protein expression at the transcription level, which occurs on a relatively long time scale. Reorganizing local environments by redistributing molecular components of transcription could be a potentially faster and more efficient regulatory mechanism to respond to a signal. Furthermore, spatial segregation of enzymes and substrates, such as the case for tmRNA degradation, could serve as a new way to regulate enzymatic activity without altering enzyme structure. New knowledge obtained from investigations into the spatial organization in bacteria will tie together previous known *in vitro* mechanisms of molecular interactions to produce a better understanding of this new type of regulation at the cellular level.

References

1 Elowitz, M.B. *et al.* (1999) Protein mobility in the cytoplasm of *Escherichia coli*. *J. Bacteriol.* 181, 197–203
2 Losick, R. and Shapiro, L. (1999) Changing views on the nature of the bacterial cell: from biochemistry to cytology. *J. Bacteriol.* 181, 4143–4145
3 Bi, E.F. and Lutkenhaus, J. (1991) FtsZ ring structure associated with division in *Escherichia coli*. *Nature* 354, 161–164
4 Valens, M. *et al.* (2004) Macrodomain organization of the *Escherichia coli* chromosome. *EMBO J.* 23, 4330–4341
5 Espeli, O. *et al.* (2008) DNA dynamics vary according to macrodomain topography in the *E. coli* chromosome. *Mol. Microbiol.* 68, 1418–1427
6 Thiel, A. *et al.* (2012) Long-range chromosome organization in *E. coli*: a site-specific system isolates the Ter macrodomain. *PLoS Genet.* 8, e1002672
7 Cagliero, C. *et al.* (2013) Genome conformation capture reveals that the *Escherichia coli* chromosome is organized by replication and transcription. *Nucleic Acids Res.* 41, 6058–6071
8 Umbarger, M.A. *et al.* (2011) The three-dimensional architecture of a bacterial genome and its alteration by genetic perturbation. *Mol. Cell* 44, 252–264
9 Le, T.B. *et al.* (2013) High-resolution mapping of the spatial organization of a bacterial chromosome. *Science* 342, 731–734
10 Browning, D.F. *et al.* (2010) Effects of nucleoid-associated proteins on bacterial chromosome structure and gene expression. *Curr. Opin. Microbiol.* 13, 773–780
11 Dorman, C.J. (2013) Genome architecture and global gene regulation in bacteria: making progress towards a unified model? *Nat. Rev. Microbiol.* 11, 349–355
12 Liu, L.F. and Wang, J.C. (1987) Supercoiling of the DNA template during transcription. *Proc. Natl. Acad. Sci. U.S.A.* 84, 7024–7027

13 Harrington, E.W. and Trun, N.J. (1997) Unfolding of the bacterial nucleoid both in vivo and in vitro as a result of exposure to camphor. *J. Bacteriol.* 179, 2435–2439
14 Cabrera, J.E. *et al.* (2009) Active transcription of rRNA operons condenses the nucleoid in *Escherichia coli*: examining the effect of transcription on nucleoid structure in the absence of transertion. *J. Bacteriol.* 191, 4180–4185
15 Jin, D.J. and Cabrera, J.E. (2006) Coupling the distribution of RNA polymerase to global gene regulation and the dynamic structure of the bacterial nucleoid in *Escherichia coli*. *J. Struct. Biol.* 156, 284–291
16 Carpentier, A.S. *et al.* (2005) Decoding the nucleoid organisation of Bacillus subtilis and *Escherichia coli* through gene expression data. *BMC Genomics* 6, 84
17 Riva, A. *et al.* (2008) Analyzing stochastic transcription to elucidate the nucleoid's organization. *BMC Genomics* 9, 125
18 Junier, I. *et al.* (2012) Genomic organization of evolutionarily correlated genes in bacteria: limits and strategies. *J. Mol. Biol.* 419, 369–386
19 Audit, B. and Ouzounis, C.A. (2003) From genes to genomes: universal scale-invariant properties of microbial chromosome organisation. *J. Mol. Biol.* 332, 617–633
20 Kepes, F. (2004) Periodic transcriptional organization of the *E.coli* genome. *J. Mol. Biol.* 340, 957–964
21 Qian, Z. *et al.* (2012) Galactose repressor mediated intersegmental chromosomal connections in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.A.* 109, 11336–11341
22 Iborra, F.J. *et al.* (1996) Active RNA polymerases are localized within discrete transcription 'factories' in human nuclei. *J. Cell Sci.* 109, 1427–1436
23 Cabrera, J.E. and Jin, D.J. (2003) The distribution of RNA polymerase in *Escherichia coli* is dynamic and sensitive to environmental cues. *Mol. Microbiol.* 50, 1493–1505
24 Cabrera, J.E. and Jin, D.J. (2006) Active transcription of rRNA operons is a driving force for the distribution of RNA polymerase in bacteria: effect of extrachromosomal copies of rrnB on the in vivo localization of RNA polymerase. *J. Bacteriol.* 188, 4007–4014
25 Lewis, P.J. *et al.* (2000) Compartmentalization of transcription and translation in *Bacillus subtilis*. *EMBO J.* 19, 710–718
26 Bremer, H. *et al.* (1973) Regulation of ribonucleic acid synthesis in *Escherichia coli* B-r: an analysis of a shift-up. II. Fraction of RNA polymerase engaged in the synthesis of stable RNA at different steady-state growth rates. *J. Mol. Biol.* 75, 161–179
27 Bremer, H. and Dennis, P.P. (1996) Modulation of chemical composition and other parameters of the cell by growth rate. In *Cellular and Moleuclar of Escherichia coli and Salmonella* (2nd edn) (Neidhardt, F.C., ed.), pp. 1553–1569, American Scoiety for Microbiology
28 Klumpp, S. and Hwa, T. (2008) Growth-rate-dependent partitioning of RNA polymerases in bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 105, 20245–20250
29 Berlyn, M.K. (1998) Linkage map of *Escherichia coli* K-12, edition 10: the traditional map. *Microbiol. Mol. Biol. Rev.* 62, 814–984
30 Piggot, P.J. and Hoch, J.A. (1985) Revised genetic linkage map of *Bacillus subtilis*. *Microbiol. Rev.* 49, 158–179
31 Wang, W. *et al.* (2011) Chromosome organization by a nucleoid-associated protein in live bacteria. *Science* 333, 1445–1449
32 de Wit, E. and de Laat, W. (2012) A decade of 3C technologies: insights into nuclear organization. *Genes Dev.* 26, 11–24
33 Dekker, J. *et al.* (2002) Capturing chromosome conformation. *Science* 295, 1306–1311
34 Umbarger, M.A. (2012) Chromosome conformation capture assays in bacteria. *Methods* 58, 212–220
35 Ryter, A. and Chang, A. (1975) Localization of transcribing genes in the bacterial cell by means of high resolution autoradiography. *J. Mol. Biol.* 98, 797–810
36 Kellenberger, E. (1990) Intracellular organization of the bacterial genome. In *The Bacterial Chromosome* (Drlica, K. and Riley, M., eds), pp. 173–186, American Society for Microbiology
37 Osborne, C.S. *et al.* (2004) Active genes dynamically colocalize to shared sites of ongoing transcription. *Nat. Genet.* 36, 1065–1071
38 Osborne, C.S. *et al.* (2007) Myc dynamically and preferentially relocates to a transcription factory occupied by Igh. *PLoS Biol.* 5, e192
39 Papantonis, A. *et al.* (2010) Active RNA polymerases: mobile or immobile molecular machines? *PLoS Biol.* 8, e1000419
40 Ragoczy, T. *et al.* (2006) The locus control region is required for association of the murine beta-globin locus with engaged transcription factories during erythroid maturation. *Genes Dev.* 20, 1447–1457
41 Straight, A.F. *et al.* (1996) GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Curr. Biol.* 6, 1599–1608
42 Sanchez-Romero, M.A. *et al.* (2012) Location and dynamics of an active promoter in *Escherichia coli* K-12. *Biochem. J.* 441, 481–485
43 Libby, E.A. *et al.* (2012) Membrane protein expression triggers chromosomal locus repositioning in bacteria. *Proc. Natl. Acad. Sci. U.S.A.* 109, 7445–7450
44 Mohl, D.A. and Gober, J.W. (1997) Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus*. *Cell* 88, 675–684
45 Webb, C.D. *et al.* (1997) Bipolar localization of the replication origin regions of chromosomes in vegetative and sporulating cells of *B. subtilis*. *Cell* 88, 667–674
46 Bates, D. and Kleckner, N. (2005) Chromosome and replisome dynamics in *E. coli*: loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation. *Cell* 121, 899–911
47 Lau, I.F. *et al.* (2003) Spatial and temporal organization of replicating *Escherichia coli* chromosomes. *Mol. Microbiol.* 49, 731–743
48 Wang, X. *et al.* (2006) The two *Escherichia coli* chromosome arms locate to separate cell halves. *Genes Dev.* 20, 1727–1731
49 Wang, X. *et al.* (2005) Dancing around the divisome: asymmetric chromosome segregation in *Escherichia coli*. *Genes Dev.* 19, 2367–2377
50 Viollier, P.H. *et al.* (2004) Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. *Proc. Natl. Acad. Sci. U.S.A.* 101, 9257–9262
51 Wiggins, P.A. *et al.* (2010) Strong intranucleoid interactions organize the *Escherichia coli* chromosome into a nucleoid filament. *Proc. Natl. Acad. Sci. U.S.A.* 107, 4991–4995
52 Hong, S.H. *et al.* (2013) Caulobacter chromosome in vivo configuration matches model predictions for a supercoiled polymer in a cell-like confinement. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1674–1679
53 Mahy, N.L. *et al.* (2002) Spatial organization of active and inactive genes and noncoding DNA within chromosome territories. *J. Cell Biol.* 157, 579–589
54 Hensel, Z. *et al.* (2013) Transcription-factor-mediated DNA looping probed by high-resolution, single-molecule imaging in live *E. coli* cells. *PLoS Biol.* 11, e1001591
55 Thompson, R.E. *et al.* (2002) Precise nanometer localization analysis for individual fluorescent probes. *Biophys. J.* 82, 2775–2783
56 Elf, J. *et al.* (2007) Probing transcription factor dynamics at the single-molecule level in a living cell. *Science* 316, 1191–1194
57 Kuhlman, T.E. and Cox, E.C. (2012) Gene location and DNA density determine transcription factor distributions in *Escherichia coli*. *Mol. Syst. Biol.* 8, 610
58 Bintu, L. *et al.* (2005) Transcriptional regulation by the numbers: models. *Curr. Opin. Genet. Dev.* 15, 116–124
59 Janga, S.C. *et al.* (2009) Transcriptional regulation shapes the organization of genes on bacterial chromosomes. *Nucleic Acids Res.* 37, 3680–3688
60 Smits, W.K. and Grossman, A.D. (2010) The transcriptional regulator Rok binds A+T-rich DNA and is involved in repression of a mobile genetic element in *Bacillus subtilis*. *PLoS Genet.* 6, e1001207
61 Lee, S.F. *et al.* (2011) Super-resolution imaging of the nucleoid-associated protein HU in *Caulobacter crescentus*. *Biophys. J.* 100, L31–L33
62 Geanacopoulos, M. and Adhya, S. (2002) Genetic analysis of GalR tetramerization in DNA looping during repressosome assembly. *J. Biol. Chem.* 277, 33148–33152
63 Ueguchi, C. *et al.* (1997) Clarification of the dimerization domain and its functional significance for the *Escherichia coli* nucleoid protein H-NS. *J. Mol. Biol.* 274, 145–151
64 Lim, C.J. *et al.* (2012) Nucleoprotein filament formation is the structural basis for bacterial protein H-NS gene silencing. *Sci. Rep.* 2, 509
65 Azam, T.A. and Ishihama, A. (1999) Twelve species of the nucleoid-associated protein from *Escherichia coli*. Sequence recognition specificity and DNA binding affinity. *J. Biol. Chem.* 274, 33105–33113

66 Dillon, S.C. and Dorman, C.J. (2010) Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nat. Rev. Microbiol.* 8, 185–195
67 Sobetzko, P. *et al.* (2012) Gene order and chromosome dynamics coordinate spatiotemporal gene expression during the bacterial growth cycle. *Proc. Natl. Acad. Sci. U.S.A.* 109, E42–E50
68 Lewis, D.E. *et al.* (1999) Role of HU and DNA supercoiling in transcription repression: specialized nucleoprotein repression complex at gal promoters in *Escherichia coli*. *Mol. Microbiol.* 31, 451–461
69 Cui, L. *et al.* (2013) Enhancer-like long-range transcriptional activation by lambda CI-mediated DNA looping. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2922–2927
70 Fritsche, M. *et al.* (2012) A model for *Escherichia coli* chromosome packaging supports transcription factor-induced DNA domain formation. *Nucleic Acids Res.* 40, 972–980
71 Betzig, E. *et al.* (2006) Imaging intracellular fluorescent proteins at nanometer resolution. *Science* 313, 1642–1645
72 Rust, M.J. *et al.* (2006) Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat. Methods* 3, 793–795
73 Ebright, R.H. (2000) RNA polymerase: structural similarities between bacterial RNA polymerase and eukaryotic RNA polymerase II. *J. Mol. Biol.* 304, 687–698
74 Gruber, T.M. and Gross, C.A. (2003) Assay of *Escherichia coli* RNA polymerase: sigma-core interactions. *Methods Enzymol.* 370, 206–212
75 Shepherd, N. *et al.* (2001) Cytoplasmic RNA polymerase in *Escherichia coli*. *J. Bacteriol.* 183, 2527–2534
76 Endesfelder, U. *et al.* (2013) Multiscale spatial organization of RNA polymerase in *Escherichia coli*. *Biophys. J.* 105, 172–181
77 Bakshi, S. *et al.* (2012) Superresolution imaging of ribosomes and RNA polymerase in live *Escherichia coli* cells. *Mol. Microbiol.* 85, 21–38
78 Grainger, D.C. *et al.* (2005) Studies of the distribution of *Escherichia coli* cAMP-receptor protein and RNA polymerase along the *E. coli* chromosome. *Proc. Natl. Acad. Sci. U.S.A.* 102, 17693–17698
79 Herring, C.D. *et al.* (2005) Immobilization of *Escherichia coli* RNA polymerase and location of binding sites by use of chromatin immunoprecipitation and microarrays. *J. Bacteriol.* 187, 6166–6174
80 Bremer, H. *et al.* (2003) Free RNA polymerase and modeling global transcription in *Escherichia coli*. *Biochimie* 85, 597–609
81 Shepherd, N.S. *et al.* (1980) Synthesis and activity of ribonucleic acid polymerase in *Escherichia coli*. *J. Bacteriol.* 141, 1098–1108
82 Bratton, B.P. *et al.* (2011) Spatial distribution and diffusive motion of RNA polymerase in live *Escherichia coli*. *J. Bacteriol.* 193, 5138–5146
83 Bakshi, S. *et al.* (2013) Partitioning of RNA polymerase activity in live *Escherichia coli* from analysis of single-molecule diffusive trajectories. *Biophys. J.* 105, 2676–2686
84 Jao, C.Y. and Salic, A. (2008) Exploring RNA transcription and turnover in vivo by using click chemistry. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15779–15784
85 Burmann, B.M. and Rosch, P. (2011) The role of *E. coli* Nus-factors in transcription regulation and transcription:translation coupling: From structure to mechanism. *Transcription* 2, 130–134
86 Robinow, C. and Kellenberger, E. (1994) The bacterial nucleoid revisited. *Microbiol. Rev.* 58, 211–232
87 Briegel, A. *et al.* (2006) Multiple large filament bundles observed in *Caulobacter crescentus* by electron cryotomography. *Mol. Microbiol.* 62, 5–14
88 Llopis, P. *et al.* (2010) Spatial organization of the flow of genetic information in bacteria. *Nature* 466, 77–81
89 Miller, O.L., Jr *et al.* (1970) Visualization of bacterial genes in action. *Science* 169, 392–395
90 French, S.L. and Miller, O.L., Jr (1989) Transcription mapping of the *Escherichia coli* chromosome by electron microscopy. *J. Bacteriol.* 171, 4207–4216
91 Cagliero, C. and Jin, D.J. (2013) Dissociation and re-association of RNA polymerase with DNA during osmotic stress response in *Escherichia coli*. *Nucleic Acids Res.* 41, 315–326
92 English, B.P. *et al.* (2011) Single-molecule investigations of the stringent response machinery in living bacterial cells. *Proc. Natl. Acad. Sci. U.S.A.* 108, E365–E373
93 Golding, I. and Cox, E.C. (2004) RNA dynamics in live *Escherichia coli* cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11310–11315
94 Bertrand, E. *et al.* (1998) Localization of ASH1 mRNA particles in living yeast. *Mol. Cell* 2, 437–445
95 Valencia-Burton, M. *et al.* (2007) RNA visualization in live bacterial cells using fluorescent protein complementation. *Nat. Methods* 4, 421–427
96 Driessen, A.J. and Nouwen, N. (2008) Protein translocation across the bacterial cytoplasmic membrane. *Annu. Rev. Biochem.* 77, 643–667
97 Kawamoto, H. *et al.* (2005) Implication of membrane localization of target mRNA in the action of a small RNA: mechanism of post-transcriptional regulation of glucose transporter in *Escherichia coli*. *Genes Dev.* 19, 328–338
98 Nevo-Dinur, K. *et al.* (2011) Translation-independent localization of mRNA in *E. coli*. *Science* 331, 1081–1084
99 Mackie, G.A. (2013) RNase E: at the interface of bacterial RNA processing and decay. *Nat. Rev. Microbiol.* 11, 45–57
100 Liou, G.G. *et al.* (2001) RNA degradosomes exist in vivo in *Escherichia coli* as multicomponent complexes associated with the cytoplasmic membrane via the N-terminal region of ribonuclease E. *Proc. Natl. Acad. Sci. U.S.A.* 98, 63–68
101 Taghbalout, A. and Rothfield, L. (2007) RNaseE and the other constituents of the RNA degradosome are components of the bacterial cytoskeleton. *Proc. Natl. Acad. Sci. U.S.A.* 104, 1667–1672
102 Russell, J.H. and Keiler, K.C. (2009) Subcellular localization of a bacterial regulatory RNA. *Proc. Natl. Acad. Sci. U.S.A.* 106, 16405–16409
103 Keiler, K.C. (2008) Biology of *trans*-translation. *Annu. Rev. Microbiol.* 62, 133–151
104 Golding, I. *et al.* (2005) Real-time kinetics of gene activity in individual bacteria. *Cell* 123, 1025–1036
105 Hocine, S. *et al.* (2013) Single-molecule analysis of gene expression using two-color RNA labeling in live yeast. *Nat. Methods* 10, 119–121
106 Baron-Benhamou, J. *et al.* (2004) Using the lambdaN peptide to tether proteins to RNAs. *Methods Mol. Biol.* 257, 135–154
107 Lawrence, J.B. *et al.* (1988) Sensitive, high-resolution chromatin and chromosome mapping in situ: presence and orientation of two closely integrated copies of EBV in a lymphoma line. *Cell* 52, 51–61
108 Raj, A. and Tyagi, S. (2010) Detection of individual endogenous RNA transcripts in situ using multiple singly labeled probes. *Methods Enzymol.* 472, 365–386
109 Schmidt, T. *et al.* (2009) Single-molecule analysis of biomembranes. In *Handbook of Single-Molecule Biophysics* (Hinterdorfer, P. and Oijen, A., eds), pp. 19–42, Springer
110 Hsu, Y.H. *et al.* (2006) Distribution of gyrase and topoisomerase IV on bacterial nucleoid: implications for nucleoid organization. *Nucleic Acids Res.* 34, 3128–3138
111 Tadesse, S. and Graumann, P.L. (2006) Differential and dynamic localization of topoisomerases in *Bacillus subtilis*. *J. Bacteriol.* 188, 3002–3011
112 Luijsterburg, M.S. *et al.* (2008) The major architects of chromatin: architectural proteins in bacteria, archaea and eukaryotes. *Crit. Rev. Biochem. Mol. Biol.* 43, 393–418
113 Nielsen, H.J. *et al.* (2006) The *Escherichia coli* chromosome is organized with the left and right chromosome arms in separate cell halves. *Mol. Microbiol.* 62, 331–338
114 Berlatzky, I.A. *et al.* (2008) Spatial organization of a replicating bacterial chromosome. *Proc. Natl. Acad. Sci. U.S.A.* 105, 14136–14140
115 Violin, J.D. *et al.* (2003) A genetically encoded fluorescent reporter reveals oscillatory phosphorylation by protein kinase C. *J. Cell Biol.* 161, 899–909
116 Toro, E. *et al.* (2008) *Caulobacter* requires a dedicated mechanism to initiate chromosome segregation. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15435–15440
117 Lee, P.S. *et al.* (2003) Effects of the chromosome partitioning protein Spo0J (ParB) on oriC positioning and replication initiation in *Bacillus subtilis*. *J. Bacteriol.* 185, 1326–1337
118 Sutherland, H. and Bickmore, W.A. (2009) Transcription factories: gene expression in unions? *Nat. Rev. Genet.* 10, 457–466
119 Cisse, I.I. *et al.* (2013) Real-time dynamics of RNA polymerase II clustering in live human cells. *Science* 341, 664–667
120 Zhao, Z.W. *et al.* (2014) Spatial organization of RNA polymerase II inside a mammalian cell nucleus revealed by reflected light-sheet superresolution microscopy. *Proc. Natl. Acad. Sci. U.S.A.* 111, 681–686