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Nucleolus-like compartmentalization of the transcription machinery in fast-growing bacterial cells

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Abstract

Overview. We have learned a great deal about RNA polymerase (RNA Pol), transcription factors, and the transcriptional regulation mechanisms in prokaryotes for specific genes, operons, or transcriptomes. However, we have only begun to understand how the transcription machinery is 3-dimensionally (3D) organized into bacterial chromosome territories to orchestrate the transcription process and to maintain harmony with the replication machinery in the cell. Much progress has been made recently in our understanding of the spatial organization of the transcription machinery in fast-growing *Escherichia coli* cells using state-of-the-art superresolution imaging techniques. Co-imaging of RNA polymerase (RNA Pol) with DNA and transcription elongation factors involved in ribosomal RNA (rRNA) synthesis and ribosome biogenesis has revealed similarities between bacteria and eukaryotes in the spatial organization of the transcription machinery for growth genes, most of which are rRNA genes. Evidence supports the notion that RNA Pol molecules are concentrated, forming foci at the clustering of rRNA operons resembling the eukaryotic nucleolus. RNA Pol foci are proposed to be active transcription factories for both rRNA genes expression and ribosome biogenesis to support maximal growth in optimal growing conditions. Thus, in fast-growing bacterial cells, RNA Pol foci mimic eukaryotic Pol I activity, and transcription factories resemble nucleolus-like compartmentation. In addition, the transcription and replication machineries are mostly segregated in space to avoid the conflict between the two major cellular functions in fast-growing cells.

Keywords

RNA polymerase; bacterial nucleolus; 3D organization of transcription machinery; chromosome territories; transcription factories

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Differences in genome organization, transcription machinery for rRNA synthesis and ribosome biogenesis between eukaryotes and prokaryotes

An emerging theme for the transcriptional regulation of gene expression is that the 3D organization of chromosome and chromatin architecture orchestrate the transcription process in biological systems. The concepts of transcription factories (Cook, 1999; Papantonis and Cook, 2013; Zhao et al., 2014) and chromosome territories (Cremer et al., 2000; Fritz et al., 2016; Meaburn and Misteli, 2007) are active research areas almost exclusively in eukaryotes. A eukaryotic cell has a membrane-enclosed nucleus in which the chromosome(s) and nucleolus are located in distinct spaces and with specialized functions. The nucleolus is the most dominant nuclear compartment in eukaryotes and plays a key role in controlling cellular growth and proliferation (Olson and Dundr, 2015). It is an organization of multiple-component complexes of nucleic acid and proteins, including tandemly repeated copies of rDNA for rRNA genes, RNA polymerase I (Pol I, one of the three RNA polymerases) that exclusively transcribes rDNA (Goodfellow and Zomerdijs, 2013) and factors involved in ribosome biogenesis, which is coupled to the transcription of rRNA genes. Malfunction in Pol I transcription of rRNA genes and abnormality in rDNA clusters are common features in cancer cells and human diseases (Hariharan and Sussman, 2014; Peltonen et al., 2014; Quin et al., 2014; van Sluis and McStay, 2014).

In a prokaryotic cell such as the bacterium *E. coli*, there is no nucleus organelle. The bacterial chromosome is named the nucleoid, which has long been shown as a blob shape with little structural details in literatures and textbooks. There is only one RNA polymerase (RNA Pol) species for transcription of all transcript species including rRNA, tRNA, mRNA and noncoding RNA, in contrast to three RNA Pol species (Pol I, Pol II and Pol III) in eukaryotes. In *E. coli*, the growth rate is determined by growth medium (Kjeldgaard et al., 1958; Schaechter et al., 1958), which in turn controls rRNA synthesis and ribosome biogenesis. The rRNA operons are the major growth genes, which also include those for tRNA and other components of the translation machinery. Most RNA Pol engage in rRNA synthesis in nutrient-rich broth such as LB as visualized by electron microscopy (French and Miller, 1989) and rRNA measurements (Bremer and Dennis, 2008). In contrast, rRNA synthesis is minimal in nutrient-poor minimal media, but the expression of genome-wide genes increases (Liu et al., 2005). Therefore, growth-rate regulation reflects the competition of RNA Pol distribution between rRNA synthesis and other vast regions of the genome for limiting RNAP in the cell (Jin et al., 2012).

In *E. coli* there are seven rRNA operons (~1% of the genome), four of which are near the origin of chromosome replication *oriC*. The copy number of rRNA operons will amplify rapidly in a fast-growing cell containing multiple *oriC* copies. When cells grow rapidly with a doubling time (τ) far shorter than the time needed to complete one round of chromosome (nucleoid) replication and segregation (> 74 min) (Stokke et al., 2012), maximum expression of growth genes and multiple genome replications are concurrently achieved. For example, cells grown in LB at 37°C have a τ = 20 min. Under these optimal growth conditions, it is estimated that there are ~five nucleoid equivalents and ~50 rRNA operons on average per cell prior to cell division (Jin et al., 2015; Nielsen et al., 2007). Although electron

chromatin-spreading micrographs reveal densely packed RNA Pol molecules in each of the rRNA operons individually (French and Miller, 1989), it was unknown whether RNA Pol molecules actively synthesizing rRNA are concentrated at the clustering of rRNA operons in those fast-growing cells. Several transcription elongation factors including NusA and NusB participate in rRNA synthesis and ribosome biogenesis in *E. coli*. NusA but not NusB binds to RNA Pol *in vitro* (Greenblatt and Li, 1981). NusB interacts with the BoxA sequence of the ribosomal RNA precursor (pre-rRNA) (Greive et al., 2005; Nodwell and Greenblatt, 1993). However, it was also unknown whether both NusA and NusB form complexes with RNA Pol in actively transcribing rRNA operons. Moreover, whether the transcription machinery for rRNA synthesis and ribosome biogenesis is 3D organized in the bacterial nucleoid remained an unexplored realm in prokaryotes until very recently.

Developments of imaging tools and procedures in studying the spatial organization of the transcription machinery in *E. coli*

Several advances in imaging techniques have contributed to our understanding of the 3D organization of transcription machinery in fast-growing bacterial cells. (i) RNA Pol-GFP fusion: Following its use in eukaryotes, green fluorescent protein (GFP) technology was introduced into bacteria (Gordon et al., 1997; Lewis et al., 2000; Margolin, 2000). An *E. coli* chromosomal *rhoC-gfp* fusion was constructed to image RNAP in fast-growing cells and in cells undergoing an amino acid starvation-induced stress response using widefield fluorescent microscopy (Cabrera and Jin, 2003). Since then, many derivatives of the RNAP-GFP reporter have been constructed in *E. coli* to study different aspects of RNA Pol in the cell (Bakshi et al., 2012; Cabrera et al., 2009; Cabrera and Jin, 2006; Cagliero and Jin, 2013; Endesfelder et al., 2013; Stracy et al., 2015). (ii) Superresolution imaging: Because *E. coli* cells are small ($\sim 1 \mu\text{m}$ [short axis] $\times \sim 1$ to $5 \mu\text{m}$ [long axis] depending on growth conditions) and the nucleoid is even smaller, it is difficult to study the sub-nucleoid organization using conventional widefield microscopy (WFM), which has a diffraction limit of $\sim 250 \text{ nm}$ in the lateral dimension and $\sim 500 \text{ nm}$ in the axial dimension. Photoactivated localization microscopy (PALM), which has a resolution beyond the diffraction limit and is able to detect single molecules, has been used for one-color imaging of RNA Pol in *E. coli* and provided much useful information about the distribution and mobility of RNA Pol in living cells (Bakshi et al., 2012; Endesfelder et al., 2013). However, to understand the spatial organization of the transcription machinery in the bacterial chromosome, two-color co-imaging of RNA Pol and DNA is required. Moreover, to understand the spatial relationships among RNA Pol, a transcription factor (or other interested factor) and DNA, three-color co-imaging is necessary. For this purpose, superresolution Structured Illumination Microscopy (SIM) (Fiolka et al., 2012; Gustafsson, 2000) is currently the ideal choice. Using imaging tools such as NIH ImageJ and Matlab, SIM Images can be analyzed to provide insight into the spatial relationship of the transcription machinery with DNA and other factor(s) in the cell. (iii) A fixed-cell procedure to capture snapshots of the dynamic organization of transcription machinery: The distribution of *E. coli* RNA Pol is extremely sensitive to perturbations in the environment, which poses a challenge to be able to image the spatial organization of transcription machinery in living cells, particularly in fast-growing cells (Cabrera and Jin, 2003). Procedures involved in sampling of fast-growing cells from a

shaking flask in water bath to mounting the cells onto a microscopy slide followed by imaging, even within a short time frame of 5–10 min, will result in changes in growth environments, and will cause perturbation in bacterial physiology. Consequently, the resulted live-cell images will be an adapted state, rather than images of the fast-growing cells as intended (Jin et al., 2015). To overcome this problem, a procedure using formaldehyde to fix cells immediately after sampling has revolutionized the imaging of RNA Pol in fast-growing cells, because the procedure is able to “freeze” or immobilize RNAP and other subcellular structures before being imaged (Cabrera and Jin, 2003). Comparison of fixed-cell images with images from live-cell procedures, including the use of microfluidic device, not only validates the fixed-cell technique, but also highlights the advantage and necessity in using the fixed-cell procedure to probe the organizations of the transcription machinery and the nucleoid in fast-growing cells (Jin et al., 2015). Note that the formaldehyde-fixed cells procedure has also been widely used in ChIP-chip assays to study the distribution of *E. coli* RNAP genome wide under different growth conditions (Davis et al., 2011; Grainger et al., 2005; Herring et al., 2005). Importantly, the fixed-cell images are consistent with extensive studies of *E. coli* genetics and physiology (Jin et al., 2013). It is mandatory to use fixed cells for SIM imaging of fast-growing cells (LB, 37°C), because immobility of cells is essential for the procedure. Currently, the time needed for three-color co-imaging of bacterial cells using SIM is about 10 min, not including the extra time for sampling and mounting of cells, during which the organizations of RNA Pol and the nucleoid would be completely altered if live cells were used (Jin et al., 2015).

Compartmentalization of RNA Pol foci at the clustering of rRNA operons in fast-growing cells

The spatial organization of transcription machinery in the bacterial chromosome of fast-growing cells under optimal growth conditions (LB, 37°C, $\tau = 20$ min) was revealed by SIM two-color co-imaging of RNA Pol and DNA (Cagliero et al., 2014). Figure 1 shows 2D SIM images of RNA Pol and DNA, and an overlay of the two images in a typical fast-growing cell. DNA image from Hoechst 33342 staining reflects the nucleoid. There are four distinct nascent nucleoids in the cell, and the nucleoids are compact and have textural variations and apparent void areas. RNA Pol image from the RNA Pol-Venus fusion shows a non-evenly distribution pattern; most RNA Pol forms foci or clusters with different sizes and intensities. An overlay of RNA Pol (green) and DNA (red) reveals the spatial relationship of the two; RNA Pol foci are at the surface (likely in DNA loops) of the compact nucleoids. Such a feature can be quantified by the ratio of the intensity of RNA Pol over the intensity of DNA at each pixel of the images. The heat map shows that RNA Pol foci are enriched at the periphery of the compact nucleoids (red regions) but DNA intensities are enriched (or RNA Pol depleted) in the core interior region of the nucleoids (blue regions).

Some parameters are helpful to understand the nature of RNA Pol foci in the fast-growing cells. As mentioned above, based on the growth rate ($\tau = 20$ min), there are ~50 rRNA operons in a typical fast-growing cell. Assuming that there are about 65 RNA Pol molecules actively transcribing one rRNA operon (French and Miller, 1989), a total number of 3,250 RNA Pol molecules are allocated for the synthesis of 50 rRNA operons. The number of

RNA Pol molecules in cells grown in LB is estimated to be ~4,000 to 5,000 per cell (Bakshi et al., 2012; Endesfelder et al., 2013); therefore, the vast majority (>65%) of RNA Pol molecules are allocated for rRNA synthesis in fast-growing cells, a value that is consistent with the measurements of rRNA synthesis in nutrient-rich media (Bremer and Dennis, 2008). From SIM imaging analysis, the median number of RNA Pol foci from a population of cells ($n > 500$) is eight (Cagliero et al., 2014). One can then deduce that for each RNA Pol foci there are on average clustering of ~six rRNA operons, resembling the eukaryotic nucleolus structure. This proposition is supported by the finding that RNA Pol in fast-growing cells forms clusters ranging from 70 to >500 RNA Pol molecules using PALM (Endesfelder et al., 2013), which are likely to be RNA Pol foci at single or clustering of rRNA operons, respectively. Because even small clusters (foci) of 70 RNA Pol molecules form a sphere of ~160nm in diameter (Endesfelder et al., 2013), SIM, which has a diffraction limit of ~140nm in the lateral dimension for RNA Pol-Venus, is as effective as PALM in detecting RNA Pol foci at single and clustering of rRNA operons in fast-growing cells. Compartmentalization of the transcription machinery under optimal growth conditions has revealed for the first time an important spatial-organization element for maximal expression of rRNA operons in fast-growing cells.

SIM and WFM images of living cells under non-optimal or moderate growth conditions such as EZ Rich Defined Media (Neidhardt et al., 1974) supplemented with 0.2% glucose at 30°C also reveal a similar feature of compartmentalization of RNA Pol in clusters or foci (Bakshi et al., 2012; Stracy et al., 2015), but those images are less sharp and lack more details compared with the fixed cells under the optimal growth conditions (LB, 37°C) (Cagliero et al., 2014). These results are consistent with the report that the organization of RNA Pol and the nucleoid are sensitive to growth medium and temperatures (Jin et al., 2013). It is also possible that the quality of those live-cell images is compromised by the mobility of cells and/or perturbations associated with cell sampling and imaging processes (Cabrera and Jin, 2003; Jin et al., 2015), as mentioned above.

Compartmentalization of RNA Pol foci requires active rRNA synthesis from multiple rRNA operons located either in the genome or on an extrachromosomal plasmid

Several lines of evidence have demonstrated that the formation and compartmentalization of RNA Pol foci at the surface of compact nucleoid require active rRNA synthesis in the cell. Changes either in growth conditions or mutations in RNA Pol which decreased rRNA synthesis will result in disappearance of RNA Pol foci and changes in nucleoid organization (Jin et al., 2013). Figure 2 shows some examples. When serine hydroxamate (SHX), a structural analogue of serine, was added into fast-growing cells (LB, 37°C) for 30 min, cells underwent amino acid starvation leading to a stringent response (Cashel et al., 1996) during which rRNA synthesis is down and expressions of genome-wide stress genes are up (Durfee et al., 2008; Traxler et al., 2008). In these stressed cells (LB+SHX 30'), RNA Pol foci dissipated and the nucleoid expanded compared to fast-growing cells. Similarly, when fast-growing cells (LB, 37°C) were subject to nutrient down-shift by transferring to a minimal medium (M63 + 0.2% glucose), rRNA synthesis decreased immediately; RNA Pol foci

rapidly disappeared within 5 min after the down shift (LB>M63 5') and the nucleoids become less structured. In these two cases, most RNA Pol distribute at the peripheries of either expanded or less structured nucleoids. When fast-growing cells (LB, 37°C) were shocked with 0.5 M NaCl (LB+NaCl), however, RNA Pol initially dissociated from the chromosome at 10 min (LB+NaCl 10'), which was accompanied by hyper-condensation of the nucleoid, followed by reassociation with the genomic DNA forming a ring of RNA Pol surrounding the hyper-condensed nucleoids at 20 min (LB+NaCl 20') in the cell (Cagliero and Jin, 2013). It was also reported that RNA Pol redistributes to the cytoplasm in wild-type cells that contain the promoter(s) and fractions of the rRNA operon in a plasmid; however, there is no RNA Pol foci in those cells. This is likely due to the titration of RNA Pol from the nucleoid by the plasmid leading to slow growth and reduced rate of rRNA synthesis (Cabrera and Jin, 2006). Therefore, although there are variations in terms of RNA Pol distribution and the organization of bacterial nucleoid during different stress responses, one theme is common: RNAP Pol foci either dissipate or are absent in stressed cells. Clearly, RNA Pol foci at the surface of each of multiple compact-nascent nucleoids are hallmarks of active expression of rRNA operons in the cell.

The formation and compartmentalization of RNA Pol foci at the surface of the compact nucleoid require multiple copies of chromosomal rRNA operons. The organization of RNA Pol and the nucleoid were examined (Jin et al., 2016) in *E. coli* strains that have different numbers of rRNA operons deleted (Quan et al., 2015). Apparently, cells (*2rrn*) that have five rRNA operons (two of the seven rRNA operons in the genome were deleted) have similar growth rate (Quan et al., 2015) and organization of RNA Pol and the nucleoid as wild type under optimal growth conditions. With more deletions of rRNA operons in the genome, such features gradually disappear. In a strain (*6rrn*) that has only one remaining rRNA operon (deleted six rRNA operons in the genome), RNA Pol is relatively evenly distributed without apparent foci, but with textual variations of RNA Pol distribution, and an expanded nucleoid (Figure 3) (Cabrera et al., 2009). This strain has a reduced growth rate with $\tau = 37$ min, and it is estimated there are four copies of rRNA operons in the cell under the optimal growth conditions (LB, 37°C). After the treatment of SHX, the textual variations of RNA Pol reduce in the expanded nucleoids (LB+SHX). Therefore, formation of RNA Pol foci and nucleoid compaction requires active rRNA synthesis from the genome with more than four rRNA operons.

Intriguingly, the defect of the *6rrn* strain in the formation of RNA foci and nucleoid structure can be complemented by a plasmid-borne rRNA operon (*prnB*) in the cell. When the *6rrn* strain was transformed with *prnB*, the resulting transformant strain *6rrn/prnB* has an apparent $\tau = 43$ min slower than the parent strain, which can be explained in part by an unbalanced growth caused by the plasmid borne rRNA operon in these cells. The plasmid *prnB* is a pBR322 derivative and has multiple copies in the cell. One or two large RNA Pol foci associated with compact nucleoid are apparent in the transformant cells under optimal growth conditions (LB, 37°C), and they are usually located at the poles of the cell (Figure 3). As expected, RNA Pol foci dissipated after the treatment with SHX. Similar features are also observed in the *7rrn/prnB* strain which has no chromosomal rRNA operon remaining. Thus, the presence of the extrachromosomal copies of rRNA operons from the plasmid effectively substitutes the missed chromosomal copies in the *6rrn* and *7rrn* strains for two

functions: the spatial organization of RNA Pol foci and nucleoid compaction. These results further support the notion that active rRNA synthesis from the clustering of rRNA operons is the driving force for the nucleoid compaction and compartmentalization of RNA Pol foci. These findings provided a framework for future mechanistic analysis on how RNA Pol allocation on rRNA synthesis and 3D organizations of transcription machinery and bacterial chromosome are interconnected.

RNA Pol foci are transcription factories for rRNA synthesis and other growth genes and ribosome biogenesis: a model

The functional nature of RNA Pol foci has been determined by three-color co-imaging of RNA Pol, DNA and each of the two transcription factors, NusA or NusB fused to mCherry fluorescent protein (Cagliero et al., 2014). Both NusA and NusB are important for rRNA synthesis and ribosome biogenesis (Bubunencko et al., 2013). Only NusA binds to RNA Pol *in vitro*. NusB binds to BoxA sequence of the nascent pre-rRNA. Because both factors behaved similarly, only images with NusB are shown (Figure 4). NusB forms foci, and most of NusB foci (87%) and RNA Pol foci are co-localized in space. These results are consistent with the idea that RNA Pol foci are transcription factories comprising of factors for active rRNA synthesis and ribosome biogenesis. Therefore, compartmentalization of RNA Pol foci at the clustering of rRNA operons are functionally transcription factories for rRNA synthesis and ribosome biogenesis in fast-growing cells.

Evidence from *E. coli* chromosome conformation capture (GCC) assays supports the idea that the transcription factories and functional compartmentalization of growth genes are intrinsically interconnected. Comparative GCC analyses were performed in cells during optimal growth conditions (LB, 37°C) and in cells undergoing amino acid starvation after the SHX treatment (LB+SHX) for 30 min (Cagliero et al., 2013). A key finding from these statistical analyses in the population of cells is that growth genes, which are down regulated in SHX-treated cells, are in a highly interactive environment and in large clusters, demonstrating functional compartmentalization of growth genes. Connecting the finding from GCC assays with the spatial compartmentalization of the transcription machinery under optimal growth conditions described above, it is proposed that RNA Pol foci are transcription factories for rRNA synthesis and other growth genes located in the distance in the genome (Jin et al., 2015). The physical interaction between rRNA synthesis and the expression of other growth genes is also indicated by the large RNA Pol foci at the poles of the compacted nucleoid in *6rrn/prmB* cells (Figure 3). In this case, the connection of RNA Pol foci at clustering of plasmid-borne rRNA operons with other growth genes in the genome is also manifested by the effect on the nucleoid compaction.

Nucleolus-like compartmentalization of the transcription factories for rRNA synthesis and ribosome biogenesis in fast-growing bacterial cells suggests a new concept that the 3D organization of genome and chromatin architecture also orchestrate the transcription process in prokaryotes too. We can only speculate at this time on what forces (factors) help the formation of such spatial organization. Supercoiling likely plays a role because active rRNA synthesis by RNA Pol would impose the supercoiled domains in the genome (Booker et al.,

2010; Jin et al., 2012; Liu and Wang, 1987). It is suggested that the entropic depletion-attraction forces facilitate the formation of RNAP Pol foci (Marenduzzo et al., 2006). The transcription factories are macromolecule complexes in addition to RNA Pol and NusA/NusB. They likely also include other transcription factors and factors involved in the formation of RNA Pol foci and compaction of genomic DNA, as well as components of DNA and RNA of growth genes. Further studies on the identification and characterization of the composition of transcription factories, for example, using the *6rrn/prmB* cells (LB, 37°C) (Figure 3), will shed new light on the structure-function of the transcription factories.

The transcription and the replication machineries are spatially segregated: an explanation for why the two major cellular functions avoid the conflicts in fast-growing cells

It has been a longstanding question regarding how to avoid the conflicts between replication and transcription in *E. coli* (Bedinger et al., 1983; Merrikh et al., 2012), particularly in fast-growing cells in which both processes are robust and intertwined. There are multiple ways to accomplish this. Co-directional replication and transcription of growth genes including rRNA and other components of translational machinery in the *E. coli* genetic map indicates the importance of genome organization in avoiding head-on collisions (Boubakri et al., 2010; French, 1992; Ivanova et al., 2015). Recently, the spatial relationship between the transcription and the replisome machineries was examined by three-color co-imaging of RNA Pol with DNA and each of the two protein factors (fused to mCherry) that have been used to track replication forks/replisomes, single-stranded DNA binding protein (SSB) and SeqA (Cagliero et al., 2014). Because both SSB and SeqA behave similarly, only co-images of the former are shown here (Figure 5). SSB forms foci by coating the single-stranded DNA at replication forks and interacts with DNA polymerase III holoenzyme (DNA Pol) (Marceau et al., 2011; Reyes-Lamothe et al., 2008). Like RNA Pol foci, SSB foci are also compartmentalized at the surface of compact nascent nucleoids (overlay of SSB and DNA). However, foci of RNA Pol and SSB are mostly segregated in space (overlay and RNA Pol and SSB). This finding can explain why the two major cellular functions maintain harmony in fast-growing cells. As expected, the spatial segregation of the two machineries is not absolute and there is about 22% co-localization between SSB foci and RNA Pol foci. How replication forks propagate and segregate at clustering of rRNA operons which are actively expressed in fast-growing cells remains to be determined.

Summary

Recent advances using cutting edge imaging tools have shown the landscape of bacterial chromosome in fast-growing cells (Figure 6). An important finding is the compartmentalization of RNA Pol foci at the clustering of rRNA operons in fast-growing cells under optimal growth conditions. RNA Pol foci colocalize with elongation factors NusA and NusB, both of which participate in rRNA synthesis and ribosome biogenesis. Thus, RNA Pol foci are proposed to be transcription factories that mimic the functions of eukaryotic nucleolus. Such a spatial organization has logistical advantages, including coupling of rRNA synthesis with ribosome biogenesis. Nucleolus-like compartmentalization

of the transcription factories in fast-growing bacterial cells indicates similarities between prokaryotes and eukaryotes in terms of chromosome organization and function. Although there is only one RNA Pol species in bacteria, the *E. coli* RNA Pol foci mainly function as eukaryotic RNA Pol I for rRNA synthesis to support fast growth. In the future, structure-function analysis of the transcription factories will provide mechanistic insight into the formation of RNA Pol foci and DNA compaction. Another new finding is the spatial segregation of the chromosome territories for the two major cellular functions of transcription and replication, which not only explains why transcription and replication largely remain in harmony in fast-growing cells, but also raises the question regarding how cells accomplish the segregation. These findings are the first evidences revealing the spatial organization of transcription machinery and its relationship with replisomes in fast-growing cells and they have provided frameworks for future mechanistic studies using the simple *E. coli* model system.

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Figure 1. SIM co-imaging of RNAP and DNA reveals spatial compartmentalization of RNA Pol foci at the surface of compact nucleoid in fast-growing cells

Shown are images of DNA, RNA Pol (RNAP) and an overlay of RNAP (green) and DNA (red) from a representative fast-growing *E. coli* cell (LB, 37°C). The cell shape is outlined by a dotted line. The scale bar represents 1 μ m. The $\log_2$ (RNAP/DNA) plot (heat map) is a quantitative representation of the ratio between intensities of RNAP and DNA, represented by a color scale bar ranging from -3 to 3 . Note that regions enriched in RNAP up to eightfold over DNA are at the periphery of the compact nucleoid (red foci), and regions enriched in DNA up to eightfold over RNAP are in the center of the nucleoid (blue regions). The SIM images from this and other figures were acquired using a Nikon N-SIM system and reconstituted using Nikon NIS-Elements AR/NIS-Elements C.

Figure 2. Perturbations in growth conditions and/or stress responses that decrease rRNA synthesis result in the disappearance of RNA Pol foci and changes in nucleoid organization
 Shown are SIM images of DNA, RNA Pol (RNAP) and an overlay of RNAP (green) and DNA (red) from a fast-growing cell (LB, 37°C) as a control, and after different perturbations induced by SHX treatment for 30 min (+SHX 30'), nutrient down shift from LB to M63+Glu for 5 min (>M63 5') and after high salt shock (0.5 M NaCl) for 10 min (+NaCl 10') and 20 min (+NaCl 20'). The cell shape is outlined by a dotted line. The scale bar represents 1 μ m.

Figure 3. Formation and compartmentalization of RNA Pol foci at the surface of the compact nucleoid require multiple copies of rRNA operons located either in the genome or on an extrachromosomal plasmid

Shown are SIM images of DNA, RNA Pol (RNAP) and an overlay of RNAP (green) and DNA (red) in $\Delta 6rrn$ and $\Delta 6rrn/prrnB$ cells either under optimal growth conditions (LB, 37°C) (first and third panels) or after SHX treatment for 30 min (+SHX) (second and fourth panels). Note that the defect of the $\Delta 6rrn$ strain in the formation of RNA foci and nucleoid structure can be complemented by a plasmid-borne rRNA operon ($prrnB$) in the cell (compare the first and third panels). The cell shape is outlined by a dotted line. The scale bar represents 1 μ m.

Figure 4. Nascent rRNA-binding protein NusB forms foci and colocalizes with RNA Pol foci in fast-growing cells

Shown are SIM images of DNA, RNA Pol (RNAP), NusB, two-color overlays of RNAP (green) and NusB (red), NusB(red) and DNA(green), and three-color overlays of RNAP(green), NusB (red) and DNA (blue) from a representative fast-growing *E. coli* cell (LB, 37°C). Note that NusB foci are at the periphery of the nucleoid (separate red and green colors on the RNAP/NusB overlay) and the NusB signals perfectly colocalize with RNAP signals (overall yellow color on the RNAP/NusB overlay). The cell shape is outlined by a dotted line. The scale bar represents 1 μ m.

Figure 5. Spatial segregation of transcription foci and replisomes tracked by SSB in fast-growing cells

Shown are SIM images of DNA, RNA Pol (RNAP), SSB, two-color overlays of RNAP (green) and SSB (red), SSB(red) and DNA(green), and three-color overlays of RNAP(green), SSB (red) and DNA (blue) from a representative fast-growing *E. coli* cell (LB, 37°C). Note that SSB foci and RNA Pol foci are largely located at different positions (red and green colors on the overlay of SSB and RNAP). Note also that most of the SSB foci appear to be separated from high intensities of DNA signals in the nucleoids (red and green colors on the overlay of SSB and DNA). The cell shape is outlined by a dotted line. The scale bar represents 1 μm .

Figure 6. Model illustrating the chromosome territories of the major transcription machinery and replisome in a fast-growing cell

The *E. coli* chromosome is represented by the blue lines folded in loops, the *oriC* by a black square, and the RNA Pol molecules by green circles. For simplicity, only one of the prominent RNA Pol foci and the replisome are shown. RNA Pol foci at the clustering of *rrn* (red), resembling a bacterial nucleolus, are spatially organized at the periphery of the nucleoid for compartmentalization. Foci of NusA and NusB co-localize with RNA Pol foci, indicating that RNA Pol foci are transcription factories for the coupling of rRNA synthesis and ribosome biogenesis including processing of the pre-rRNA. Long-distance interactions of DNA loops are indicated. Compartmentalization of the replisome is also shown, but the two major cellular functions of transcription and replication are largely spatially segregated. Modified from (Jin et al., 2015).