CellPress

Spatial organization of transcription in bacterial cells

Xiaoli Weng and Jie Xiao

Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD, USA

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

Corresponding author: Xiao, J. (xiao@jhmi.edu).

Keywords: prokaryotic transcription; spatial organization; single molecule; chromosome conformation capture; transcription factors; RNA polymerase.

0168-9525/

 http://dx.doi.org/10.1016/j.tig.2014.04.008

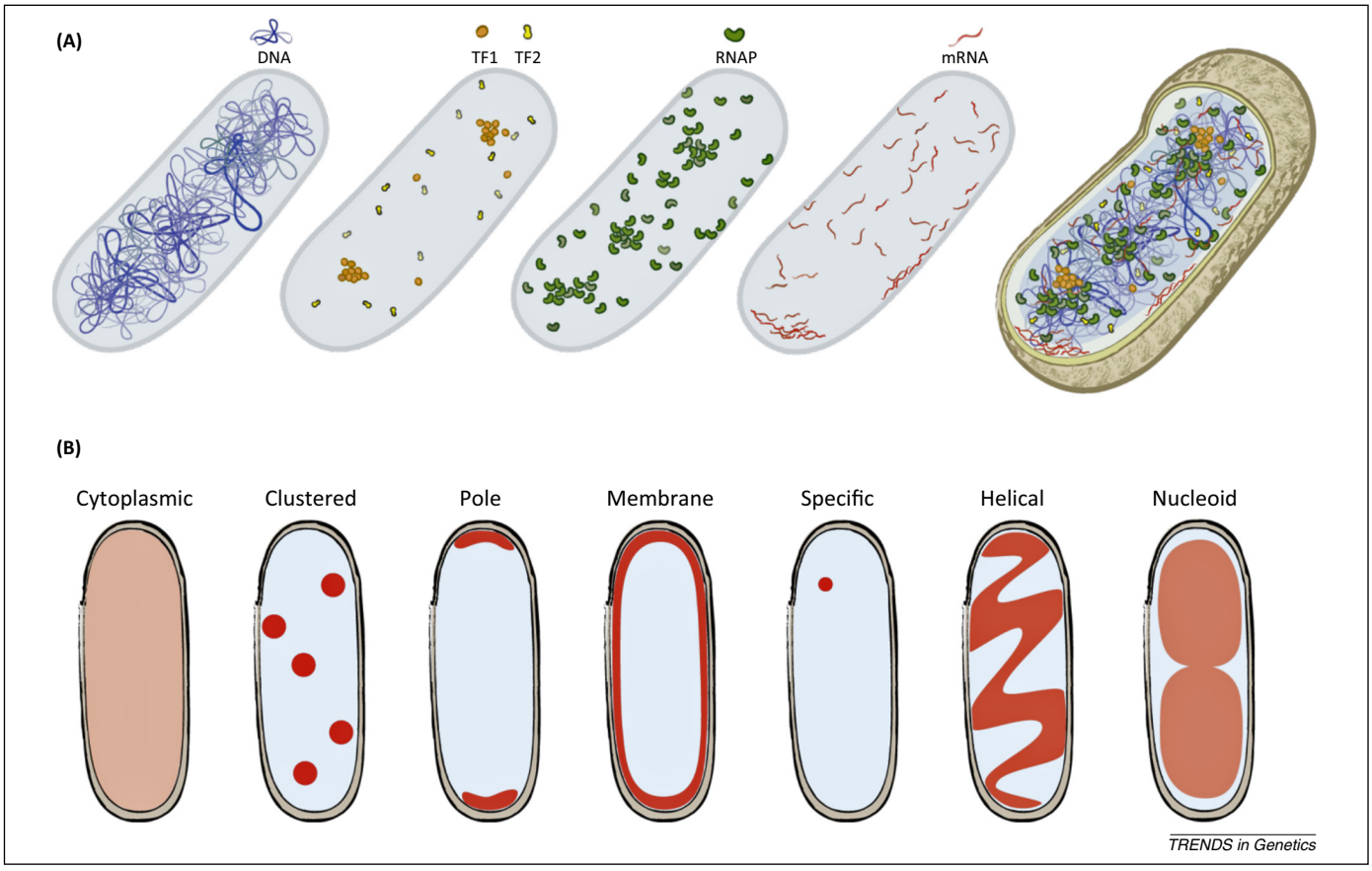

Figure 1. Spatial organization of transcription in an *Escherichia coli* cell. **(A)** Genes (DNA), transcription factors (TF1 and TF2), RNA polymerase (RNAP), and mRNAs may be organized differentially in space instead of being homogenously distributed. **(B)** Overview of experimentally observed molecular 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