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Cellular organization of the transfer of genetic information

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Abstract

Each step involved in the transfer of genetic information is spatially regulated in eukaryotic cells, as transcription, translation and mRNA degradation mostly occur in distinct functional compartments (e.g., nucleus, cytoplasm and P-bodies). At first glance in bacteria, these processes seem to take place in the same compartment—the cytoplasm—because of the conspicuous absence of membrane-enclosed organelles. However, it is becoming increasingly evident that mRNA-related processes are also spatially organized inside bacterial cells, and that this organization affects cellular function. The aims of this review are to summarize the current knowledge about this organization and to consider the mechanisms and forces shaping the cell interior. The field stands at an exciting point where new technologies are making long-standing questions amenable to experimentation.

Two types of cellular organization

The first hint of spatial organization in the bacterial cell came as early as 1937 when Piekarski described a central DNA-rich region, which he named nucleoid for “nucleus-like” [1]. Microscopy studies have since revealed that ribosomes preferentially accumulate outside this nucleoid. Sucrose gradient centrifugation experiments show that 50–85% of the ribosomal material in *E. coli* extracts elutes with the polyribosome fraction [2,3], suggesting that the majority of ribosomes are bound to mRNA and engaged in translation. Most RNA polymerase (RNAP) molecules bind to the nucleoid, with only 7–14 % of them remaining diffuse in the cytoplasm [4–8]. Thus, distinct zones of enrichment of DNA (RNAP) and ribosomes indicate that the bulk of transcription and translation is largely segregated in space. The partitioning of ribosomes and DNA has been observed in a variety of bacterial species and has become a textbook picture of bacterial cellular organization (Figure 1A). However, bacteria such as *Caulobacter crescentus*, *Agrobacterium tumefaciens*, *Sinorhizobium meliloti*, and some mollicutes exemplify another type of organization that has been underappreciated [9–14]. In these organisms, the chromosomal DNA spreads throughout the cytoplasmic space, and the ribosomes are evenly dispersed in the cytoplasm (Figure 1B). This variation in the cellular architecture demonstrates that ribosomes and

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chromosomal DNA can share the same space. It should be noted that, even in this case, there is evidence showing that the transfer of genetic information remains under spatial constraints, as discussed below.

The reason for the strikingly different cellular organization between these two groups of bacteria remains mystery, and it cannot simply be explained by a difference in genome or cell size. For example, *A. tumefaciens* and *Escherichia coli* O157:H7 have comparable cell and chromosome sizes, but their chromosome and ribosome distributions are different (Figure 1). Furthermore, the tiny bacterium *Bdellovibrio bacteriovorus* displays a confined nucleoid surrounded by a ribosome-rich region [15] despite its very small cell size compared to *C. crescentus*.

Entropy and the nucleoid organizers

What influences the spatial distribution of the chromosome and ribosomes? The mass density of the bacterial cytoplasm is very high, with concentrations of macromolecules reaching 400 mg/ml in *E. coli* [16]. Hence, the supercoiled chromosome is subject to crowding forces that condense the DNA [17]. Recently, Mondal et al. deployed a Monte Carlo simulation based on experimentally-derived assumptions to show that separation between ribosomes and DNA in *E. coli* could be explained by the maximization of the conformational entropy of the chromosome and the translational entropy of ribosomes [18]. While this appealing model identifies important entropic forces to consider, other factors are likely at play. For instance, this physical model does not easily explain how some bacteria display an alternate cellular organization in which ribosomes and DNA are mixed (Figure 1B). In addition, it has been shown that the chromosome architecture is dominated by intra-chromosomal interactions [19,20]. Nucleoid-associated proteins (NAPs) bind, bend, coat and bridge DNA segments, thereby participating in chromosomal organization [21], as recently demonstrated by atomic force microscopy, super-resolution fluorescence microscopy and chromosome conformation capture analysis [22,23]. Thus, the spatial distribution of the chromosome and ribosomes likely depends on a balance between physical constraints and biological interactions that alter the physical properties of the DNA. A different balance may result in a different cellular organization. The distinct structures and orientations of *E. coli* and *C. crescentus* chromosomes hint at this possibility [24–26].

Spatially separated, yet functionally coupled

The segregation of ribosomes and DNA (and RNAP) in *E. coli* argues that transcription and translation are separated in space. Yet, a functional coupling between translation and transcription (i.e., translation is initiated on nascent mRNAs as they are being transcribed) has been extensively documented in the same bacterium. The notion of coupling was proposed as early as 1964 [27], and began receiving experimental support in the early days of molecular biology [28–30]. The physical interaction between a transcribing RNAP and a trailing ribosome was recently described in atomic detail [31,32]. How can elements of this apparent paradox be reconciled? We envision at least three non-mutually exclusive scenarios (Figure 2).

A) The first scenario (Figure 2A) questions the generality of the coupling between transcription and translation. What if the coupling was a marginal mechanism applicable only to a small subset of genes? If true, then in most cases, transcription would occur independently of translation, resulting in the release of naked (ribosome-free) transcripts in the nucleoid region. The mRNAs would then move away from their site of transcription toward the ribosome-rich regions for translation. However, the instability of naked transcripts and the abundant evidence that translation is initiated during transcription make

this first scenario unlikely to be common, although this is not to say that it does not happen for some mRNAs (see below).

B) The second scenario (Figure 2B) rests on the premise that genes are spatially distributed based on their promoter activity. In this scenario, actively transcribed genes would reside at the interface between the nucleoid and the ribosome-rich regions, whereas silent genes would be buried inside the nucleoid. This, in turn, would imply that if a gene is located inside the nucleoid, induction of its expression would result in gene relocation to the nucleoid periphery prior to initiation of translation. How such directed gene relocation would occur independently of translation is unclear. Gene relocation has, however, been shown to occur in a translation-dependent manner for genes encoding membrane proteins, presumably through a process known as transertion (coupling between transcription, translation and translocation of the nascent protein into the membrane) [33]. The local mobility of gene loci (within a ~300 nm domain [34,35]) could facilitate the relocation process.

C) The third scenario (Figure 2C) takes into consideration that ribosomes are less abundant but still present (10–15%) inside the nucleoid [5]. It also posits that transcription can take place anywhere in the nucleoid region and that ribosomes bind the nascent mRNA shortly after transcription is initiated. Relocation of the ribosome-bound transcript to the ribosome-rich phase would then simply be driven by entropic forces.

O mRNA, where art thou?

Key to our understanding is the determination of where mRNAs localize in the cell. The task of localizing mRNAs has proven to be challenging for several reasons. Bacterial mRNAs are present in very low copy numbers [36,37], their half-life is very short, on the time scale of a few minutes only, and they represent only a few percent of the RNA cellular content. Punctate patterns of mRNA localization have been reported from fluorescence in situ hybridization (FISH) and live-cell fluorescence microscopy experiments in a bacteria [11,37–41]. Dual labeling of specific mRNAs and corresponding gene loci in *C. crescentus* and *E. coli* revealed that the mRNA signal largely [11] colocalizes with the corresponding gene. Given the resolution of light microscopy, these results suggest that these mRNAs tend to accumulate within ~ 200–300 nm of their site of transcription, implying that the synthesis of the proteins produced from these mRNAs is spatially regulated by the location of the encoding gene. Thus, even in *C. crescentus*, where there is no evidence of cytoplasmic segregation of DNA and ribosome, translation can be under spatial constraints. Remarkably, Kuhlman and Cox provided evidence that this localized source of protein synthesis can affect the localization and activity of transcriptional regulators [42]. They showed that the location of the *lacI* gene and mRNA in *E. coli* affects the location and activity of the LacI repressor. These exciting results were surprising considering that proteins, which are long-lived compared to transcripts, are expected to display homogeneous distribution at steady-state due to rapid diffusion.

A fundamentally different picture of localization has been reported for other mRNAs. For example, in *B. subtilis*, the *comE* mRNA was shown to accumulate at the septum where its post-transcriptional regulator ComN localizes [43]. Amster-Choder and colleagues showed that the *bglF* mRNA, which codes for a membrane-bound permease in *E. coli*, localizes as a halo around the cell membrane where the BglF protein is inserted [44]. In contrast, a truncated version of the *bglF* mRNA coding for the soluble N-terminal domain exhibits a patchy pattern throughout the cytoplasm. Surprisingly, it was found that the apparent membrane localization of the *bglF* mRNA is independent of translation, leading to the

proposal that the sequences of mRNA can contain "zipcodes" that target mRNAs to the location where their protein product is needed [44].

So far, the localization of only a handful of mRNA species has been examined. A comprehensive analysis of mRNA localization, which is central to our understanding of mRNA-dependent processes, will likely require some technical advances. The FISH technique is based on fixed, dead cells and thus intrinsically lacks a temporal dimension. The live-cell imaging methods typically rely on tagging an mRNA of interest with an array of aptamers that are recognized by a fluorescently-labeled RNA-binding protein such as the bacteriophage coat protein MS2 (used here for discussion). These live-cell methods currently have major drawbacks. Because of the low dissociation rate for the MS2-GFP/aptamer complex [45], MS2 binding increases the lifetime of the RNA [38,46], presumably by blocking RNase access. It is unclear how the stabilization of the mRNA may affect its localization, but it must, at the very least, increase the time of mRNA dispersion from the site of synthesis. Given the difference of stability between most bacterial mRNAs and the MS2-GFP/aptamer complex, we can even question whether the mRNA of interest is still attached to the array of aptamers that is visualized. In addition, MS2-GFP has been shown to aggregate upon mRNA binding [11], affecting the localization pattern, as aggregates accumulate at the poles through nucleoid exclusion [47].

The background fluorescence from the unbound MS2-GFP molecules is also problematic. An exciting prospect to reduce background fluorescence is bimolecular fluorescence complementation. In the bacterial system pioneered by the Broude lab [48,49], an RNA-binding protein (eIF4A) is split in two, and each fragment is fused to a complementary fragment of GFP. Reconstitution of GFP occurs only when each split protein fusion is brought into close proximity by binding to aptamers fused to an RNA of interest. Hence, fluorescence production occurs upon RNA binding, resulting in a very low background signal. However, reconstitution of the GFP complex is essentially irreversible *in vitro* [50]. The fluorescent signal from the reconstituted GFP is thus expected to remain long after the mRNA has been degraded.

Ribosome mobility reveals mRNA dynamics

While mRNA imaging methods aim to examine one particular mRNA at a time, examining the dynamics of fluorescently labeled ribosomes can provide information about the average behavior of mRNAs in live cells since most ribosomes are active (i.e., in a translational complex with a mRNA). Ribosome dynamics have been examined in single-molecule tracking experiments in *E. coli* [5,51,52] and by FRAP microscopy in *C. crescentus* [53]. These two techniques offer a complimentary view as they sample ribosome dynamics at different temporal and spatial scales (Figure 3). Single-molecule super-resolution approaches are capable of tracking motion of single ribosomes beyond the diffraction limit of light microscopy, and are most effective for tracking short-range (submicrometer) motion during short (<1 sec) periods of time. Conversely, FRAP methods monitor the motion of the ribosome ensemble over long (micrometer scale) distances and can do so at longer (>1 sec) timescales.

By analyzing thousands of short single-ribosome trajectories in *E. coli*, the Elf and Weisshaar groups showed that ribosomes display considerable motion within microdomains, with submicrometer-scale diffusion coefficients of 0.02–0.14 $\mu\text{m}^2/\text{sec}$ [5,51,52]. Since most ribosomes are active, the majority of ribosome traces must represent the dynamics of translating ribosomes. Interestingly, the mean square displacement (MSD) curves plateaued over time [51,52], consistent with the observation that while ribosomes move significantly, this motion remains confined within microdomains [5]. This spatial confinement was also

observed in *C. crescentus* by FRAP microscopy [53]. In these FRAP experiments, the active ribosomes, which constitute 70% of the ribosome population, displayed very little long-range motion, with a low micrometer-scale diffusion coefficient value ($<0.001 \mu\text{m}^2/\text{sec}$).

This spatial confinement of ribosomes would be understandable if the ribosomes were in complex with nascent mRNAs—still tethered to their respective genes or operons—as opposed to released, full-length mRNAs. However, there are several observations suggesting that mRNAs, in general, spend most of their lifetime as full-length. First, northern blots usually show one RNA species corresponding to the full-length transcript. Second, transcriptome analysis of *Bacillus anthracis* by RNA-Seq showed a similar representation between 5' and 3' ends of transcripts [36]. Third, the lifetime of mRNAs is typically long in comparison to the time of their synthesis [37,54,55]. Hence, the reason for the observed spatial confinement of ribosomes on the micrometer scale is not obvious.

RNA degradosome localization

While transcription and translation tend to receive most of the attention, another important process in the flow of genetic information is mRNA degradation as it affects the levels (and possibly the localization) of mRNAs. In most models of gene expression, mRNA decay is assumed to occur uniformly throughout the cytoplasm. However, recent studies of various components of the RNA degradosome suggest otherwise. RNase E, a major component of the mRNA degradation machinery, has been shown to associate with the membrane in *E. coli* [56–58]. RNase Y (YmdA), the functional equivalent of RNase E in *B. subtilis* [59], was also found to be membrane-associated [60]. Furthermore, other RNA degradosome components (e.g., RNA helicase B, polynucleotide phosphorylase, enolase) show non-homogeneous distributions inside cells [58]. Interestingly, a fluorescent fusion of RNase E does not display any membrane accumulation in *C. crescentus*, instead it exhibits a patchy pattern throughout the cell [11]. More strikingly, this fusion segregates with the chromosome in filamentous cells containing DNA-free regions [53]. This suggests that the *C. crescentus* RNase E (directly or indirectly) associates with the chromosomal DNA. Alternatively, its localization may reflect the location of the (slowly diffusing) mRNAs. Either way, the difference in RNase E localization patterns between *C. crescentus* and *E. coli* raises the question of whether it is a cause or a consequence of the two types of cellular organization in bacteria (Figure 1).

The next frontier

Bacteria have shown us that intracellular organelles are clearly dispensable for cellular organization and functional compartmentalization to take place. Even core processes such as transcription, translation and mRNA degradation are subject to spatial constraints. The field has made great strides in recent years, but so much remains to be understood. How is the spatial organization achieved, and how does it impact the physiology and behavior of the cell? Addressing these fundamental questions will require multidisciplinary approaches spanning from microscopy to genomics to modeling. The repertoire of species examined should be expanded beyond the traditional bacterial models in order to extract the general organizing principles. One thing is for sure, the journey is bound to lead to exciting discoveries.

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Highlights

- The cytoplasm organization into separated ribosome-rich and nucleoid regions is not universal.
- The segregation of chromosome and ribosomes is compatible with a transcription-translation coupling.
- The life of mRNA is spatially organized, from transcription to translation and degradation.

Figure 1.

Two distinct patterns of spatial organization of chromosomes and ribosomes in bacteria.

Figure 2.

Models to reconcile the transcription-translation coupling with the spatial separation of DNA and ribosomes in bacteria that have the cellular organization illustrated in Figure 1A. See text for details about these three non-exclusive models.

Figure 3.

FRAP and super-resolution single-molecule microscopy probe ribosome dynamics at different spatial and temporal scales. FRAP-based experiments examine the mobility of the ribosome ensemble (shown in yellowgreen) whereas super-resolution single-molecule experiments track individual ribosomes (depicted as red spheres). The green line shows the trajectory and rapid escape of a free ribosome whereas the blue line represents the trajectory of a ribosome bound to an mRNA, displaying motion in a spatially confined microdomain.