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Minireview

Coupling the distribution of RNA polymerase to global gene regulation and the dynamic structure of the bacterial nucleoid in *Escherichia coli*

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Abstract

Prokaryotic genomes are contained in a cellular structure termed the nucleoid. However, despite a complete genome sequence and years of intensive study of *Escherichia coli*, our knowledge of nucleoid structure remains quite rudimentary. Moreover, little is known about the *in vivo* relationship between nucleoid structure and global gene regulation. Recent studies have shown that the structure of the nucleoid responds dynamically to changing environmental conditions and that this metastable nature of the nucleoid is mediated to a large extent by the distribution and activity of RNA polymerase (RNAP). For example, during rapid growth, the nucleoid is highly condensed with RNAP concentrated into transcription foci or factories, structures analogous to the eukaryotic nucleolus, where active transcription of rRNA genes occurs. However, during nutrient starvation and/or limitation, RNAP is redistributed throughout the genome and this is accompanied by a decondensation of the nucleoid. Thus, the distribution of RNAP, global gene regulation and the dynamic structure of the nucleoid are coupled in the bacterial cell.

Keywords: RNA polymerase; RNA polymerase distribution; Nucleoid; rRNA synthesis; Stable RNA synthesis; Global gene regulation; Transcription foci; Transcription factories; *Escherichia coli*

1. The *Escherichia coli* cell, genome and RNA polymerase

Escherichia coli is a small rod-shaped bacterium about 2–4 μm long with a diameter of about 1 μm resulting in a cell volume of approximately 2–3 fl. In general, the more optimal the growth conditions, the larger the cell size at division. Each cell harbors at least one genome (chromosome or nucleoid) equivalent with faster growing cells containing multiple genome equivalents, particularly of sequences near the origin of replication (*ori*). The *E. coli* genome consists of about 4.6 million base pair (bp) of DNA which stretches approximately 1.5 mm in length when fully extended. Thus, the genome must be compacted about 1000 fold to fit in the cell.

Updated annotation indicates there are 4453 genes shared between the two sequenced *E. coli* K12 strains, MG1655 and W3110 (Blattner et al., 1997; Riley et al., 2006). Current estimates are that these genes are organized into approximately 2390 operons in MG1655, each of which is controlled by one or more promoters. Among them, the genes/operons encoding for rRNA and tRNA (collectively known as stable RNA) represent only about 1% of the genome with the vast majority of the remaining 99% of the genome essentially encoding mRNA.

Unlike eukaryotes, which have three different RNAPs (Pol I, II, and III), each of which synthesizes a different RNA species (rRNA, mRNA, and tRNA/5S rRNA, respectively), *E. coli* contains a single RNAP that transcribes all genes. The number of RNAP molecules is estimated to be approximately 2000–5000 per genome equivalent (Grigorova et al., 2006; Ishihama, 2000), and thus is likely present in sub-stoichiometric amounts relative to the number of promoters in the genome. This constraint on global

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transcription is further exacerbated by the tremendous demand for stable RNA synthesis during rapid growth (see below). Together, these considerations imply that RNAP is limiting for transcription *in vivo*, and therefore, its distribution on the nucleoid will be determined in large part by the expression potential of the most actively transcribed genes.

Recently, the effects of cellular processes including replication, transcription, translation and transertion, as well as nucleoid-associated proteins and DNA supercoiling on nucleoid structure have been reviewed (Cook, 2002; Dame, 2005; Deng et al., 2005; Travers and Muskhelishvili, 2005; Woldringh, 2002). In this special issue, several minireviews consider different aspects of the bacterial nucleoid. Here, we focus on how transcription and RNAP distribution under different growth conditions affect the dynamic structure of the nucleoid in *E. coli*. The sophisticated genetics together with the advanced knowledge of physiology and biochemistry associated with *E. coli* make it an ideal model system to probe this issue.

2. Global gene regulation by cell growth

rRNAs and tRNAs, or stable RNA collectively, stand out for their unique role in cell growth. Transcription of rRNA, in particular, is intimately coordinated with the cellular growth rate in responding to nutrient availability (Gralla, 2005; Paul et al., 2004). This is because the rate of ribosome synthesis is proportional to the square of the growth rate and rRNA synthesis is rate limiting for the production of ribosomes (Gausing, 1980). There are seven rRNA operons, each of which also contains one or more tRNA genes, and 37 additional operons harboring the remaining tRNA genes in the K12 genome. Among them, the majority of rRNA operons is located near the *ori* and is oriented in the same direction as the corresponding replichore.

The synthesis of the stable RNA operons is potentially so robust and tightly correlated with nutrient availability and growth rate that growth conditions could have profound effects on RNAP distribution and global gene expression. During optimal growth, stable RNA promoters are the most actively transcribed in the cell and the expression of these genes can account for >80% of total RNA synthesis (Bremer and Dennis, 1996) despite representing only about 1% of the genome. It is estimated that during rapid growth there are 58 initiations per rRNA operon per min, or about one initiation per second. This leads to initiating and elongating RNAP molecules being densely packed at each rRNA operon with one RNAP molecule every 85 bp, whereas elsewhere in the genome there is only one RNAP per 10 000–20 000 bp, as visualized by electron microscopy (French and Miller, 1989). In contrast, slow growth on nutrient-poor media results in a significant reduction of stable RNA expression with only four initiations per rRNA operon per min. A more dramatic example of this effect occurs when cells are abruptly starved for a key nutrient(s), such as an amino acid, leading to what is termed the stringent response (Cashel et al., 1996). During a stringent response, expression of stable RNA is totally inhibited while other operons, such as those for amino acid biosynthetic pathways, are activated. Relative synthesis of stable RNA and mRNA for amino acid biosynthetic operons under different growth conditions is summarized in Table 1. Together, this argues that stable RNA synthesis "drains" RNAP pools during optimal growth, thus reducing the availability of free RNAP for transcription of other genes in the genome. This notion is consistent with the recent observations that most RNAP molecules are located in the genes encoding for stable RNA and ribosomal proteins during rapid growth (Grainger et al., 2005), and that there are fewer expressed genes during optimal growth than in nutrient-limiting conditions (Liu et al., 2005; Traxler et al., 2006).

Two classes of mutations affecting cell growth and the stringent response have been characterized. One class has a "relaxed phenotype" during the stringent response: mutations in the *relA* gene render cells insensitive to nutrient down-shifts as evidenced by continued stable RNA synthesis even during amino acid starvation (Metzger et al., 1989). Conversely, the second class of mutants has a "constitutive stringent phenotype", i.e., mutants exhibit the stringent phenotype even in optimal growth conditions: certain RNAP mutations cause a slow growth phenotype and are defective in stable RNA synthesis even in rich media (Zhou and Jin, 1997, 1998). Relative synthesis of stable RNA and mRNA for amino acid biosynthetic operons in these two mutants compared to that of wild type under different growth conditions is also summarized in Table 1. Both

Table 1
Relative synthesis of stable RNA and mRNA for amino acid biosynthetic operons in wild type and mutant strains under different growth conditions

	Rich media		Minimal media		Amino acid starvation	
	Stable RNA	mRNA for amino acid biosynthetic operons	Stable RNA	mRNA for amino acid biosynthetic operons	Stable RNA	mRNA for amino acid biosynthetic operons
Wild type	Maximum	Minimal	Low	High	Minimal	Maximum
relA "relaxed" mutant	~	~	↑	↓	↑	↓
rpoB "stringent" mutant	↓	↑	↓	↑	~	~

The entries in the table are intended qualitatively only to show the trend of relative expression of stable RNA compared to that of mRNA for amino acid biosynthetic operons under different growth conditions. For wild type, values in other growth conditions are compared to that in rich media. For the two *E. coli* mutants which have altered the stringent response phenotypes, ~, ↑, and ↓ mean similar, increased or decreased RNA synthesis rate compared to that of the wild type strain in the same growth condition.