Published in final edited form as:
FEMS Microbiol Rev. 2012 March ; 36(2): 269–287. doi:10.1111/j.1574-6976.2011.00279.x.

Growth rate regulation in *Escherichia coli*

Ding Jun Jin, **Cedric Cagliero**, and **Yan Ning Zhou**
Transcription Control Section, Gene Regulation and Chromosome Biology Laboratory, National Cancer Institute-Frederick, National Institutes of Health, Frederick, MD, USA

Abstract

Growth rate regulation in bacteria has been an important issue in bacterial physiology for the past 50 years. This review, using *Escherichia coli* as a paradigm, summarizes the mechanisms for the regulation of rRNA synthesis in the context of systems biology, particularly, in the context of genome-wide competition for limited RNA polymerase (RNAP) in the cell under different growth conditions including nutrient starvation. The specific location of the seven *rrn* operons in the chromosome and the unique properties of the *rrn* promoters contribute to growth rate regulation. The length of the *rrn* transcripts, coupled with gene dosage effects, influence the distribution of RNAP on the chromosome in response to growth rate. Regulation of rRNA synthesis depends on multiple factors that affect the structure of the nucleoid and the allocation of RNAP for global gene expression. The magic spot ppGpp, which acts with DksA synergistically, is a key effector in both the growth rate regulation and the stringent response induced by nutrient starvation, mainly because the ppGpp level changes in response to environmental cues. It regulates rRNA synthesis via a cascade of events including both transcription initiation and elongation, and can be explained by an RNAP redistribution (allocation) model.

Keywords

growth rate regulation; rRNA synthesis; RNA polymerase distribution; transcription factories; nucleolus-like structure; ppGpp

Introduction

More than half a century ago two accompanying papers by Maaloe and his colleagues were the first to describe the growth rate regulation in bacteria, an important issue in bacterial physiology (Kjeldgaard *et al*., 1958; Schaechter *et al*., 1958). They reported that during balanced growth, cell mass and RNA level can be described as an exponential function of the growth rate afforded by the various media. Further, during transitions between different growth rates, either by nutrient up-shift from a minimal medium to rich broth or, conversely, by nutrient down-shift, RNA synthesis is the first to respond to the change in medium, either accelerating or decreasing rapidly to the rate characteristic of the new condition. Changes in the relative rates of synthesis of other macromolecules, including DNA and protein, are delayed relative to that of RNA synthesis during periods of changing growth rates (Neidhardt & Fraenkel, 1961; Maaloe & Kjeldgaard, 1966). rRNA, along with tRNA (collectively they are called stable RNA), comprises the majority of total RNA in bacterial cells. Because most of the stable RNA is rRNA, for simplicity, total RNA reflects rRNA in

Correspondence: Ding Jun Jin, Transcription Control Section, Gene Regulation and Chromosome Biology Laboratory, National Cancer Institute-Frederick, National Institutes of Health, 1050 Boyles St. Frederick, MD 21702, USA. Tel.: +1 301 846 7684; fax: +1 301 846 1489; djjin@helix.nih.gov.

the cell. In *Escherichia coli*, the number of ribosomes in the cell is proportional to the growth rate in order to meet the demand for protein synthesis (Nomura *et al.*, 1984; Bremer & Dennis, 1996; Keener & Nomura, 1996). However, it is the synthesis of rRNA, but not of ribosomal protein (r-protein), that is sensitive to the growth rate (Gausing, 1977).

In the past 50 years, many researchers have studied growth rate regulation in bacteria, with a focus on the regulation of rRNA synthesis. The main challenge has been to understand how rRNA synthesis is regulated during two extreme growth conditions (analogous to two opposite ends of the spectrum): in rapidly growing cells, rRNA is actively synthesized and commandeers the majority of RNA polymerase (RNAP) in the cell, whereas rRNA synthesis is suppressed upon nutrient starvation, which induces the stringent response (Cashel *et al.*, 1996). In the latter case, because Cashel & Gallant (1969) discovered >40 years ago that two alarmone molecules or magic spots, pppGpp and ppGpp (hereafter collectively named ppGpp) are rapidly accumulated immediately following an amino acid starvation, accompanied by concomitant inhibition of almost total RNA synthesis in the cell, the question has also become how rRNA synthesis is stringently controlled by ppGpp. Subsequent works by many groups showed that both the stringent control and the growth rate regulation are mediated by ppGpp, indicating an interconnection of the two regulatory mechanisms for rRNA synthesis. Relatively recently, Gourse and colleagues discovered that DksA acts with ppGpp synergistically in the regulation of rRNA synthesis (Paul *et al.*, 2004a).

The extensive literature on the subject, although at times controversial and contentious, demonstrates the significance of the research and reflects a long intellectual journey in seeking to solve the puzzle. Many reviews on various aspects of the subject have been written (Nomura *et al.*, 1984; Gourse *et al.*, 1996; Wagner, 2002; Dennis *et al.*, 2004; Paul *et al.*, 2004b; Gralla, 2005). Recognizing that different mechanisms of growth rate regulation have evolved in different bacteria, however, this review intends to summarize mechanisms for the regulation of rRNA in *E. coli* with systems biology perspectives. The authors hope this review will facilitate discussions in the continuation of the journey toward understanding this important issue.

Organization of rRNA operon and bacterial growth

There are seven almost identical rRNA operons in the *E. coli* chromosome. The genome contains about 4.6 million base pairs (bp) of DNA. Each of the rRNA operons is named *rrn* with a capital letter (Fig. 1). Four of them, *rrn*CABE, are clustered near the origin of DNA replication (*ori*) and the other three are located in other regions within the *ori* half of the chromosome. Together all seven *rrn* operons encompass about 38 kbp, <1% of the genome size. The synthesis of rRNA has to compete with the remaining 99% of the genome for RNAP in response to environmental cues. Several features associated with the special location and organization of rRNA operons are important for cell growth and global gene regulation.

Clustering with the genes for r-proteins

Most of the genes/operons encoding the 55 r-proteins are clustered near the *rrn* operons, which facilitate the coordination of the production of the components of the ribosome (Nomura *et al.*, 1984). Certain r-proteins act as translational repressors by binding to their own mRNAs if they are in excess of the capacity for ribosome assembly, for example, under conditions when rRNA synthesis is reduced. Thus, the synthesis of rRNA coordinates the production of r-proteins by a translational feedback regulation mechanism (Nomura *et al.*, 1980). Conceivably, the clustering of the *rrn* operons and the genes for r-proteins would also

facilitate ribosome assembly, as those gene products are expressed in close proximity in the cell.

Codirection of *rrn* transcription and DNA replication

In *E. coli* DNA replication starts bidirectionally at the *ori*, from which the resulting replication forks propagate. The transcription direction of all seven *rrn* operons is the same as the direction of DNA replication (Ellwood & Nomura, 1982). This feature is critical as it minimizes the collision between the machineries of transcription and DNA replication (Brewer, 1988; Liu & Alberts, 1995; Pomerantz & O'Donnell, 2010a, b), particularly during rapid growth, because each of actively transcribing RNAP molecules along the *rrn* operons is a powerful biological motor that can exert considerable force (Yin *et al.*, 1995). Transcription factor DksA, which is involved in regulation of rRNA synthesis, has an important role in preventing the collisions (Tehranchi *et al.*, 2010). Collisions between DNA and RNAP can lead to DNA breakage and chromosomal instability (Vilette *et al.*, 1996; Srivatsan *et al.*, 2010); thus, the growth advantage of codirectionality of *rrn* transcription and DNA replication is evolutionarily selected. Note also that expression of essential genes tends to be codirectional with rRNA synthesis and DNA replication in bacteria (Rocha & Danchin, 2003).

Gene dosage effect

In *E. coli*, the time needed for one round of DNA replication is fixed at 40 min while the time needed for cell division varies depending on growth rate; consequently, multiple replication forks are located at different locations in chromosome before cell division in a fast-growing cell (Bremer & Dennis, 1996). Because the four *rrn*CABE operons are close to the *ori*, the number of *rrn* operons is significantly larger in a fast-growing cell than in a slow-growing cell. For example, it is estimated that a wild-type *E. coli* cell, with a doubling time of about 23 min, will have an equivalent of 38 *rrn* operons (Bremer & Dennis, 1996), whereas, a cell with a doubling time of 36 min will have an equivalent of 22 *rrn* operons. The rate of rRNA synthesis thus will be higher in a fast-growing cell than in a slow-growing cell due to their special locations and increased gene dosage. The gene dosage effect of the *rrn* operons, coupled with the long *rrn* transcripts, explains why synthesis of rRNA prominently influences RNAP distribution in the cell.

Nucleolus-like structures

Results from cell biology of RNAP (Cabrera & Jin, 2003; Jin & Cabrera, 2006) demonstrate that in rapidly growing cells cultured in nutrient rich Luria–Bertani (LB), concentrated RNAP form a few (two to three on average per nucleoid) dominant transcription foci. These transcription foci are proposed to be special transcription factories (Cook, 1999) and structures analogous to the eukaryotic nucleolus, where most of the cellular RNAP molecules are engaged in the synthesis of rRNA (Fig. 2a). It is further inferred that under optimal growth conditions, multiple *rrn* operons are folded together to be in proximity three dimensionally forming the putative nucleolus-like structure, and the nucleoid structure is relatively compact (Fig. 2b). In fast-growing cells, the nucleolus-like structures would considerably facilitate RNAP recycling and recruitment for active rRNA synthesis locally, which in turn would facilitate rRNA processing and ribosome assembly in the proximity. These hyperstructures (Norris *et al.*, 2007), however, are dynamic and sensitive to the environment. For example, the transcription foci and, possibly, nucleolus-like structures disappear leading to an expanded nucleoid when growth is arrested, such as by amino acid starvation, which induces the stringent response, or with rifampicin treatment, which inhibits all transcription initiation (Cabrera & Jin, 2003). Studying the formation and degeneration of the transcription foci and understanding the nature of the nucleolus-like structures for rRNA synthesis will provide another dimension for growth rate regulation in *E. coli*.

Regulatory determinants of rRNA operons

Two elements of *rrn* operons are important for the growth regulation of rRNA synthesis (Fig. 3). The first is the long transcripts of *rrn* operons, which are among the longest transcripts in the cell. The other is the unique properties of the *rrn* promoters.

Structure of *rrn* operons

All seven *rrn* operons have a similar structure as illustrated in Fig. 3. In addition to two tandem promoters, they all have the *boxB-boxA-boxC* elements of the *rrn* antitermination system (Theissen *et al.*, 1990; Condon *et al.*, 1995b; Torres *et al.*, 2001; Greive *et al.*, 2005; Quan *et al.*, 2005), which are located downstream from the second promoter, and two strong Rho-independent terminators at the end of the operons. The *rrn* antitermination system is required for maintaining a higher elongation rate compared with rates of elongation of mRNAs, and for preventing premature termination, particularly because untranslated *rrn* transcripts tend to form secondary and/or tertiary structures, which are termination-prone. The almost identical structure of different *rrn* operons explains why all *rrn* operons are subject to the same or similar regulatory mechanisms, although there are some fine-tuning differences (Condon *et al.*, 1992). Only five of the seven *rrn* operons are necessary to support near-optimal growth; however, all seven *rrn* operons are required for rapid adaptation to nutrient changes (Condon *et al.*, 1995a), suggesting an evolutionary advantage of having extra *rrn* operons in changing environments during growth for bacteria (Klappenbach *et al.*, 2000). For rRNA synthesis, regulation at processing/maturation and elongation/antitermination are important; however, the primary regulatory mechanism is focused at the transcription initiation step (Gourse *et al.*, 1986).

Each *rrn* operon encodes a long transcript with an average length of about 5500 nt, which is subsequently processed into three mature rRNA species, 16S, 23S and 5S RNA genes (Dunn & Studier, 1973; Srivastava & Schlessinger, 1990; Schaferkordt & Wagner, 2001), as well as some tRNA species, which are located in the spacer region between 16S and 23S RNA genes and/or at the distal ends of some *rrn* operons (Condon *et al.*, 1995b). The long length of the *rrn* transcripts allows visible identification of rRNA synthesis in the chromosome and the estimation of the number of RNAP engaged in rRNA synthesis. From electron micrographs of chromatin spreads of cells grown in rich medium LB, active synthesis of the nascent *rrn* transcript can be readily identified as a double Christmas tree morphology (Miller *et al.*, 1970), and the gap between the two Christmas trees reflects the RNase III processed 16S and 23S RNA genes (Hofmann & Miller, 1977). In contrast, under suboptimal conditions leading to slower growth rates, the nascent *rrn* transcript is difficult to identify in electron micrographs, as rRNA synthesis is reduced (Hamkalo & Miller, 1973a, b). Under the optimal growth conditions used where active rRNA synthesis occurs, as manifested by the double Christmas tree patterns, RNAP is found to be densely packed. It is estimated that on average there are about 65 RNAP molecules per *rrn* operon, which corresponds to one RNAP molecule for every 85 bp (French & Miller, 1989). Similar density of RNAP is also found in genes encoding some tRNA and r-proteins. In contrast, there is only one RNAP for each 1 kbp in the *rpoBC* region and only one RNAP molecule for every 10–20 kbp in many regions of the chromosome in fast-growing cells (French & Miller, 1989). The high density of RNAP in transcribing *rrn* in a fast-growing cell demonstrates that the *rrn* promoters have extremely high strength and efficiency compared with other *E. coli* promoters under the same condition.

Promoter regions

All seven *rrn* operons have extended promoter regions with similar sequences. Each *rrn* operon has tandem promoters, P1 and P2, which are separated by about 120 bp and

recognized by RNAP holoenzyme containing σ^{70} (de Boer *et al.*, 1979; Gilbert *et al.*, 1979; Glaser & Cashel, 1979). P1 and P2 have similar activities *in vitro* (Gilbert *et al.*, 1979; Glaser & Cashel, 1979). However, the two promoters behave differently *in vivo*. It is the activity of the upstream promoter P1, rather than that of P2, that predominates at fast growth rates and is subject to growth rate regulation and the stringent response (Lund & Dahlberg, 1979; Sarmientos & Cashel, 1983; Sarmientos *et al.*, 1983; Zhang & Bremer, 1995). The differential expression of P1 and P2 in fast-growing cells is likely due to the interference at P2 from the transcriptions of RNAP originating from P1 across P2 (Gafny *et al.*, 1994), a phenomenon known as 'promoter occlusion' (Adhya & Gottesman, 1982). At low growth rate and during the stringent response, however, P2 is mainly used (Sarubbi *et al.*, 1988). One of the advantages of having the two promoters is that RNAP is always poised in the extended promoter regions, ready for transcription in response to environmental cues. During rapid growth, multiple *rrn* promoters are likely to be in proximity in the putative nucleolus-like structures to facilitate the capture and reuse of concentrated RNAP for active synthesis of rRNA.

A typical *rrn* P1 promoter, as illustrated in Fig. 3, has the following features: (1) There is an UP element (Ross *et al.*, 1993; Aiyar *et al.*, 1998) located at about ' − 60 to − 40' upstream from the transcription initiation site (+1). (2) Nucleoid-associated proteins including Fis (Johnson *et al.*, 1988) and H-NS (Falconi *et al.*, 1988), as well as transcription factor Lrp (Platko *et al.*, 1990), bind upstream of the UP element and/or overlap the element (Nilsson *et al.*, 1990; Ross *et al.*, 1990; Tippner *et al.*, 1994; Bokal *et al.*, 1995; Aiyar *et al.*, 2002; Pul *et al.*, 2005). The binding sites for these proteins are nonidentical, but overlap in the extended promoter region. (3) The DNA sequences of the '− 35' regions as well as the spacing between the '− 35' and '− 10' regions of *rrn* P1 do not match the consensus of other σ^{70} promoters in *E. coli*. In addition, the *rrn* P1 promoter does not have an extended '− 10' region (Burr *et al.*, 2000). (4) There is a G/C-rich 'discriminator' region (Travers, 1980a) located immediately downstream the '− 10' region, but before the initiation site '+1.' It has been reported that the actual sequence of the discriminator' region, in addition to its being G/C rich, is critical for the regulation (Haugen *et al.*, 2006). The first two initially transcribed nucleotides of *rrn* transcripts are either AC or AU with the exception of *rrnD* where it is GU. It is important to emphasize that these several features, together as an integral system, determine the unique properties of the *rrn* P1 promoter and its regulation.

There are two key properties of the *rrn* P1 promoter that distinguish it from other typical *E. coli* promoters. The first is its exceptional strength potential, which means that the promoter has the potential to be the strongest promoter only in the cell grown in nutrient-rich media; the promoter strength weakens in the cell grown in nutrient-poor media. The first property is mainly attributed to the presence of the UP element, which enhances RNAP recruitment by providing additional interaction sites (Gourse *et al.*, 1986; Ross *et al.*, 1993, 1998), similar to the upstream region of the stringent promoter of *tyrT*, encoding a major $tRNA^{Tyr}$ species (Travers *et al.*, 1983). The UP element enhances the activity of the P1 core promoter (defined as '− 41 to +1') >30-fold, giving *rrn* P1 the potential to be the strongest promoter in fast-growing cells. The superb strength may explain why rRNA synthesis is the driving force for the distribution of RNAP in the cell (Cabrera & Jin, 2006).

The second property of *rrn* P1 is its rapid responsiveness to growth conditions in the cell. This property is its opposition to the robust promoter strength potential and is caused by the initiation complexes (or open complex) of *rrn* P1 being intrinsically unstable before the formation of the first few initial phosphodiester bonds (Gourse, 1988; Borukhov *et al.*, 1993). This second property is the primary reason that the *rrn* P1 promoter can be regulated rapidly in response to growth conditions. For example, the activity of *rrn* P1 changes during the stringent response (Cashel *et al.*, 1996), i.e. from being the strongest and most efficient

in fast-growing cells grown in nutrient-rich medium to being almost nonexpressive during growth arrest in nutrient-starved cells. Thus, the promoter is termed growth rate regulated and/or stringently controlled.

In addition to *rrn* P1, there are a few other stringently controlled promoters (hereafter called stringent promoters) with similar features. In general, *rrn* P1 and the stringent promoters are regulated similarly (Zhou & Jin, 1998). Other stringent promoters include those of abundant tRNAs (Lamond & Travers, 1985; Emilsson & Kurland, 1990; Emilsson *et al.*, 1993) and the *rpoD* operon including *rpsU* and *dnaG* (Taylor *et al.*, 1984; Grossman *et al.*, 1985), as well as *rnpB* (Jung & Lee, 1997), a component of RNase P, important for tRNA processing, *pyrBI* operon encoding pyrimidine biosynthetic enzymes (Donahue & Turnbough, 1990) and flagella genes (Durfee *et al.*, 2008; Lemke *et al.*, 2009). Note that these stringent genes are involved in the translational machinery, synthesis of nucleotides, which are the substrates for RNAP, and the cell motility machinery, the latter having a high demand for energy resources of the cell.

Several features of *rrn* P1 contribute to its intrinsic instability of the open complexes and the stringent response. The G/C-rich 'discriminator' region (Travers, 1980a, b; Zacharias *et al.*, 1991) is critical for the instability of the open complexes. It has been suggested that the DNA duplex of the G/C-rich 'discriminator' sequences is extremely stable, thus requiring extra energy to form and/or maintain the open complex (Opel *et al.*, 2004). Mutations at the 'discriminator' region of *rrn* P1 and other stringent promoters are no longer sensitive to the stringent response and growth-rate regulation, and enhance the stability of the open complexes, leading to increased efficiency of initiation (Travers, 1980b; Travers *et al.*, 1986; Josaitis *et al.*, 1995; Pemberton *et al.*, 2000; Barker *et al.*, 2001b; Zhi *et al.*, 2003). Moreover, the nonoptimal nature of the ' − 35' region, as well as the spacing between the '− 35' and '− 10' regions, also contribute to the intrinsic instability of the *rrn* P1 and other stringently controlled promoters (Lamond & Travers, 1985; Josaitis *et al.*, 1995; Park *et al.*, 2002).

Regulation of *rrn* transcription *in vitro*

Biochemical analyses of the *rrn* P1 promoter and other stringent promoters *in vitro* have provided many insights into the regulation of rRNA synthesis and the stringent response in the cell (Fig. 4). For nonstringent promoters, formation of open complexes from closed complexes is an irreversible step in general (McClure, 1980; von Hippel *et al.*, 1984). By contrast, the open complexes (σRP_o) of stringent promoters are conspicuously unstable kinetically, as if RNAP is in a rapid ON/OFF equilibrium before formation of the first few phosphodiester bonds (Gourse, 1988; Ohlsen & Gralla, 1992a; Borukhov *et al.*, 1993; Zhou & Jin, 1998; Barker *et al.*, 2001b; Paul *et al.*, 2004a) (Fig. 4). Thus, the formation and/or maintenance of the open complexes of *rrn* P1 and other stringent promoters is a rate-limiting step, and is proposed to be the target for regulation (Zhou & Jin, 1998). This shared regulatory mechanism enables the coordinated expression of these genes in response to the nutrient quality of the environment. There are multiple factors that act at this regulatory step by either increasing or decreasing the efficiency of open complex formation and, thus, controlling transcription activity of *rrn* P1.

Positive regulators in transcription initiation

Two factors, negative supercoiling and the Fis protein, act as positive regulators by stabilizing the open complexes of *rrn* P1 and other stringent promoters. Both factors are likely to be relevant *in vivo*. A third factor, the initiating NTPs, is also found to be important *in vitro*, likely by promoting the rate of formation of the stable initially transcribing complex (σRP_i) of *rrn* P1.

Supercoiling—A positive effect of supercoiling on rRNA synthesis is implied from the reports that the antibiotic novobiocin, which is a gyrase inhibitor, reduces the synthesis of 16S and 23S RNA genes both *in vitro* and *in vivo* (Yang *et al.*, 1979; Oostra & Gruber, 1980). Using typical *in vitro* transcription assays, high efficiency of transcription of the *rrn* promoters occurs when supercoiled DNA is used; however, only minimal transcription activity of the promoters is detected when linear DNA is used (Glaser *et al.*, 1983). The open complex of *rrn* P1 on a linear DNA template is more unstable and more sensitive to salt (Gourse, 1988; Ohlsen & Gralla, 1992b, c). The inhibitory effect of salt on transcription of *rrn* P1 is discussed below. Even with supercoiled DNA, the half-life (seconds) of the open complexes of *rrn* P1 is limiting in the presence of 40 mM KCl (Zhi *et al.*, 2003), allowing for regulation. Supercoiling can be influenced locally by the transcription process itself because an elongating RNAP generates negative supercoiling behind it and positive supercoiling ahead of it when constrained DNA is used (Liu & Wang, 1987). It is reported that the highest transcription rate associated with rRNA synthesis from bacterial *rrn* operons produces a very high level of constrained supercoiling density locally (Booker *et al.*, 2010). As proposed (Zhou & Jin, 1998), such a supercoiling feedback mechanism by transcription activity *per se* at *rrn* is likely to be an important integral element for the growth regulation of *rrn* P1 by stabilizing the open complexes.

Fis—Fis is a nucleoid-associated protein with multiple functions (Finkel & Johnson, 1992). As described above, there are multiple Fis-binding sites upstream of the *rrn* P1 promoter region (Fig. 3). Binding of Fis to these sites activates transcription from the promoter (Ross *et al.*, 1990), by interaction with RNAP (Gosink *et al.*, 1993, 1996; Bokal *et al.*, 1995). It has been suggested that Fis also acts at a step subsequent to closed complex formation (Bartlett *et al.*, 2000), and experiments have demonstrated that Fis stabilizes the open complex of *rrn* P1, leading to the activation of the promoter (Zhi *et al.*, 2003). How Fis stabilizes the open complex of *rrn* P1 is still unknown; it is possible that Fis binding results in a translocation of superhelical energy required for the formation and/or maintenance of the open complexes, similar to the action of Fis binding on the *leuV* promoter, which is a stringent promoter (Opel *et al.*, 2004). The Fis protein thus is a positive modulator important for the growth regulation of rRNA synthesis.

Initiating NTPs—Formation of the first few phosphodiester bonds bypasses the intrinsically unstable open complex step (Gourse, 1988; Ohlsen & Gralla, 1992a, b; Borukhov *et al.*, 1993), indicating the importance of the rate of the initially transcribing complex formation in the equilibrium as proposed (Zhou & Jin, 1998). Similarly, the initiation of *rrn* P1 is activated *in vitro* with a high concentration of the first nucleotide when other NTPs are constant (Gaal *et al.*, 1997), likely due to promoting the formation of the initially transcribing complex. Potentially, any element affecting the rate of formation of the initially transcribing complex containing the first few phosphodiester bonds should influence the transcription of *rrn* P1. The role of NTPs on the rRNA synthesis *in vivo* has been reported and proposed (Gaal *et al.*, 1997; Schneider *et al.*, 2002). Subsequent work showed that the concentration of ATP, which is the first nucleotide for most of the *rrn* transcripts, changes little, if at all, with growth rate; thus, NTP sensing is most likely not responsible for growth rate regulation of rRNA synthesis in *E. coli* (Petersen & Moller, 2000; Schneider & Gourse, 2004). However, ATP concentration increases significantly during cells' outgrowth from stationary phases, which correlates with the expression of *rrn* P1 (Murray *et al.*, 2003), indicating that NTP sensing works under some growth conditions.

Negative regulators in transcription initiation

In contrast to the actions of supercoiling and Fis, there are also several negative modulators, which act in the opposite direction of the equilibrium; collectively, they reduce the efficiency of the open complex formation at *rrn* P1 and other stringent promoters.

Salt and competitive DNA—The interaction between RNAP and promoter in general is sensitive to salt concentration (Record *et al.*, 1978); however, the interaction between RNAP and *rrn* P1 is particularly weakened by salt due to the intrinsic instability of the open complex of the promoter (Gourse, 1988; Zhi *et al.*, 2003; Gralla & Vargas, 2006).

A high concentration of salt decreases transcription of *rrn* P1 (Kajitani & Ishihama, 1984; Zhi *et al.*, 2003) by reducing the efficiency of open complex formation at *rrn* P1 *in vitro* (Ohlsen & Gralla, 1992c). Regardless of the template topology, the open complexes of *rrn* P1 are very sensitive to salt; however, as discussed above, the salt effect is much more severe on the open complex of *rrn* P1 on a linear DNA template than that on a supercoiled DNA. Although it is not known whether there are changes in salt concentration in cells with different growth rates, it is established that cellular salts change when cells are exposed to high salt in the environment (Dinnbier *et al.*, 1988; Cayley *et al.*, 1991; Cayley & Record, 2003). The effect of salt on initiation of *rrn* P1 is biologically significant because rRNA synthesis is significantly inhibited during hyperosmotic response (Gralla & Vargas, 2006).

Heparin competes for RNAP and decreases the efficiency of open complex formation at the *rrn* P1 and other stringent promoters (Ohlsen & Gralla, 1992b). This is the expected result as the intrinsic instability of the open complex of *rrn* P1 is only manifested in the presence of a DNA competitor. Analogous to the action of heparin, nonspecific DNA also competes effectively for RNAP on preformed initiation complexes of *rrn* P1, which leads to inhibition of transcription from the promoter (Zhou & Jin, 1998). Because there is an overwhelming presence of non-*rrn* DNA (>99%) in the genome, this negative effect of nonspecific competitive DNA is likely relevant to the regulation of RNA synthesis in cells during the stringent response or under nutrient-poor conditions. Under these stress conditions, RNAP would be easily titrated out from the *rrn* P1 promoter by the competitive genomic DNA.

ppGpp—Immediately following its discovery >40 years ago, ppGpp was thought to be a negative regulator of rRNA synthesis (Cashel & Gallant, 1969). Since then, many studies have analyzed the effect of ppGpp on transcription from the *rrn* promoters, but with inconsistent results (for comprehensive references, see Cashel *et al.*, 1996). The literature, however, reveals that some of those contradictory results could be reconciled if the different experimental conditions used are considered. Notably, inhibitory effects of ppGpp on the initiation of *rrn* P1 are consistently found under conditions in which the efficiency of open complex formation at *rrn* P1 is reduced, such as with a linear DNA template (Gilbert *et al.*, 1979; Kingston *et al.*, 1981a), in the presence of nonspecific competitor DNA (Kajitani & Ishihama, 1984), or in the presence of a relatively moderate concentration of salt (50 mM KCl) (Glaser *et al.*, 1983). These results are also consistent with reports that ppGpp is ineffective on open complexes of *rrn* P1 with supercoiled DNA and low salt (Ohlsen & Gralla, 1992c), conditions known to stabilize the open complexes of *rrn* P1. When ppGpp is effective, it significantly decreases further the half-life of the open complex of *rrn* P1 (Barker *et al.*, 2001b). Inhibition of initiation of *rrn* P1 *in vitro* requires a relatively high concentration of ppGpp. It appears that a key role of ppGpp is to effectively affect the lifetime of initiation complexes and shift the equilibrium toward the closed complex conformation during initiation of *rrn* P1 under conditions in which formation and/or maintenance of the open complex or *rrn* P1 are constrained. Importantly, the effect of ppGpp is significantly enhanced by DksA.

Several derivatives of ppGpp bind to RNAP (Owens *et al.*, 1987; Reddy *et al.*, 1995; Toulokhonov *et al.*, 2001), indicating that the target for ppGpp is RNAP. Although structures of RNAP•ppGpp complexes have been determined (Artsimovitch *et al.*, 2004), mutational analysis of RNAP based on these structures does not support the structural basis, and the true interaction site(s) between RNAP and ppGpp remains unresolved (Vrentas *et al.*, 2008).

DksA—DksA (Kang & Craig, 1990), an RNAP-associated protein, is also discovered to play a key role in the regulation of rRNA synthesis by Gourse and colleagues in 2004 (Paul *et al.*, 2004a). DksA decreases the half-life of the open complex of *rrn* P1; moreover, DksA acts synergistically with ppGpp to further reduce the half-life of the open complex of *rrn* P1. The DksA structure is similar to the transcript cleavage factor GreA (Perederina *et al.*, 2004); thus, it is likely to regulate transcription through the secondary channel of RNAP where GreA binds.

H-NS and Lrp—H-NS, a nucleoid-associated protein, binds upstream of the *rrn* P1 promoter and inhibits transcription of *rrn* P1 (Tippner *et al.*, 1994; Afflerbach *et al.*, 1998). H-NS antagonizes the activation function of Fis in a concentration-dependent manner. In the absence of Fis, H-NS traps the bound RNAP at the *rrn* P1 promoter by bridging or looping the upstream and downstream sequences of the promoter (Schroder & Wagner, 2000; Dame *et al.*, 2002). The binding of H-NS to *rrn* P1 is enhanced by the leucine-responsive regulatory protein Lrp (Pul *et al.*, 2005). Lrp alone inhibits transcription initiation from *rrn* P1 by binding to the extended promoter region, similar to H-NS. However, in the presence of leucine, Lrp loses its ability to bind to the promoter, leading to the derepression of the transcription of *rrn* P1.

Inhibition of transcription elongation by ppGpp

In addition to its inhibitory effect in initiation, ppGpp also enhances pausing of RNAP at several sites during transcription of *rrn* operons on either linear or supercoiled DNA template (Kingston & Chamberlin, 1981; Krohn *et al.*, 1992). NusA, which binds to core RNAP after the release of σ70 (Greenblatt & Li, 1981), further enhances pausing and/or termination at some sites in the leader regions of the 16S RNA gene (Kingston & Chamberlin, 1981). Although ppGpp also enhances pausing of RNAP during transcription of other non-*rrn* DNA templates (Kingston *et al.*, 1981b), there is evidence that ppGpp shows the strongest pausing enhancement during RNA chain elongation from *rrn* P1 and other stringent promoters with the G/C-rich ‘discriminator’ sequences (Krohn & Wagner, 1996). It has been shown that ppGpp inhibits mRNA chain elongation *in vivo*; however, elongation of a truncated *rrn* operon, in which about 1 kb DNA encompassing the *rrn* P1 promoter, pausing sites and about half of 16S RNA gene are deleted, appears to be unaffected by ppGpp in the cell (Vogel & Jensen, 1994). The effect of ppGpp on rRNA chain elongation in the native context *in vivo* needs to be further investigated.

Regulation of rRNA synthesis in the cell

From what is described in the above sections (Fig. 4), it is understood that regulation of rRNA synthesis involves a concerted action of multiple factors in response to growth conditions. The key regulatory step is at the efficiency of the open complex formation, which is positively controlled by a proposed transcription-coupled supercoiling mechanism and Fis, as well as being negatively controlled by salt, noncompetitive DNA and most importantly, by a concert action of ppGpp/DksA. The role of ppGpp is special because it affects both initiation and elongation; the collective effects of ppGpp are interconnected and amplified. The nucleoid-associated protein H-NS and transcriptional regulator Lrp also

negatively control initiation of *rrn* P1. In this section, we discuss mechanisms that have been proposed for the regulation of rRNA synthesis. Although controversies still remain, some of which could be explained by variables used in the studies, overwhelming evidence underscores the interconnections of different mechanisms.

Establishing active rRNA synthesis by Fis during nutrient upshift

The role of Fis in rRNA synthesis was first identified in 1990 (Nilsson *et al.*, 1990; Ross *et al.*, 1990). Many nucleoid-associated proteins are growth phase-dependent; however, Fis is unique in that it is the first one to accumulate dramatically upon nutrient upshift (Nilsson *et al.*, 1992b; Ali Azam *et al.*, 1999). For example, immediately after stationary cells are subcultured into fresh nutrient-rich LB, Fis increases rapidly and approaches its peak level (about 50 000–100 000 molecules per cell) within the first cell division; shortly afterward, however, the level of Fis decreases rapidly and is undetectable in stationary phase cells (Ball *et al.*, 1992). The burst of the Fis level during the initial outgrowth corresponds to an immediate rapid increase in rRNA synthesis from a level undetectable in stationary phase cells, and leads immediately to exponential growth (Zhi *et al.*, 2003). In contrast to wild type (MG1655), in the isogenic *fis* mutant there is a significant lag time (>30 min) before exponential growth accompanied by a continued twofold reduction of rRNA synthesis. After the prolonged lag time, however, the *fis* mutant has a growth rate and rate of rRNA synthesis comparable to those of the wild type during exponential growth. These results demonstrate that Fis is a key effector for the establishment of active rRNA synthesis during initial outgrowth from stationary phase and/or nutrient upshift.

How does Fis promote the establishment of active rRNA synthesis from a basal level expression of *rrn* during nutrient upshift? It is likely attributed to the role of Fis in both the recruitment of RNAP and stabilization of open complexes of *rrn* P1, as described in the above section. This positive effect is particularly critical, as DNA supercoiling is reduced in nutrient-starved stationary cells, leading to decreased efficiency of the open complex formation at *rrn* P1 (Ohlsen & Gralla, 1992c). During the outgrowth, the ATP level also increases, which activates rRNA synthesis (Murray *et al.*, 2003). By working together, transcription from the *rrn* P1 promoter increases from the basal level. Afterward, elongation of rRNA chain by RNAP would generate ‘waves’ of negative supercoiling at the upstream promoter region, which in turn would further propagate the formation and maintenance of the open complexes of *rrn* P1 by the proposed supercoiling feedback mechanism described above. Thus, an initial activation of rRNA synthesis promoted by Fis and ATP would reinforce and amplify subsequent initiations of *rrn* P1, further activating rRNA synthesis. This hypothesized supercoiling feedback mechanism by the *rrn* transcription *per se* could explain why active rRNA synthesis would sustain its momentum after the establishment of the *rrn* transcription. Consequently, Fis would no longer be needed shortly after the establishment of rRNA synthesis. The effects of Fis on rRNA synthesis and growth rate underscore the importance of kinetic assays in assessing the function of Fis in the cell. Different types of assays used could therefore account for variable results in the magnitude of the effects of Fis reported in the literature (Ross *et al.*, 1990; Condon *et al.*, 1992; Nilsson *et al.*, 1992a; Zhang & Bremer, 1996; Afflerbach *et al.*, 1998; Bartlett *et al.*, 2000; Zhi *et al.*, 2003).

Fis antagonizes the H-NS negative effect on *rrn* P1 (Afflerbach *et al.*, 1998) as the binding sites for Fis and H-NS overlap with each other and the UP element of the *rrn* P1 promoter. Changes in the binding pattern of these nucleoid-associated proteins at the regulatory region of *rrn* would affect the local architectural structure of the nucleoid, thus affecting transcription of *rrn* P1. The level of H-NS reaches a peak value of about 20 000 molecules in the mid-exponential phase when Fis declines rapidly, and gradually decreases to only about half of the peak value in stationary phase when Fis level becomes minimal (Ali Azam *et al.*,

1999; Hansen *et al.*, 2005). The differential levels of Fis and H-NS at different growth phase provide a basis for competition of the two regulators binding to the *rrn* P1 promoter. Because H-NS is a global repressor for gene expression in the cell (Atlung & Ingmer, 1997; Dorman, 2007; Dillon & Dorman, 2010), a genome-wide silencing of gene expression by H-NS would also facilitate allocation of RNAP to the rRNA synthesis in exponential cells grown in a nutrient-rich medium, assuming that RNAP is limiting in the cell.

The stringent control and growth rate regulation by ppGpp/DksA

In *E. coli*, ppGpp is produced by two ppGpp synthetases, RelA and SpoT; SpoT also has an additional ppGpp hydrolase activity (for a review, see Cashel *et al.*, 1996). RelA and SpoT, however, sense different signals for nutrient richness or quality of growth media. RelA, a ribosome-associated protein, is the major ppGpp synthetase; the ppGpp synthetase activity of RelA is activated when an increased ratio of uncharged to charged tRNA is present due to starvation for amino acid(s). Accumulation of ppGpp by SpoT can be due to either increasing ppGpp synthetases activity or decreasing ppGpp hydrolase activity. Both activities of SpoT are stimulated by other starvations, such as carbon source, fatty acid, phosphate, iron and energy depletion (Xiao *et al.*, 1991; Spira *et al.*, 1995; Murray & Bremer, 1996; Vinella *et al.*, 2005; Battesti & Bouveret, 2006); however, exactly how the two SpoT activities are regulated in the cell remains unclear. Collectively, RelA and SpoT are responsible for any accumulation of ppGpp in the cell in response to growth conditions, as no ppGpp is produced (ppGpp^0) in a double null *relA spoT* mutant (Hernandez & Bremer, 1991; Xiao *et al.*, 1991).

The accumulation of ppGpp inversely corresponds to the nutrient richness or quality of a growth medium, indicating an intimate link between the steady-state growth rate regulation in bacteria afforded by growth medium and the basal levels of ppGpp in the cell (Zacharias *et al.*, 1989; Bremer & Dennis, 1996). Even with the same growth medium, there is an inverse linear relationship between cells' steady-state growth rates and the amounts of ppGpp accumulated in different *spoT* mutants; in parallel, there is an inverse linear relationship between the transcription of *rrn* P1 and the ppGpp level in those cells (Sarubbi *et al.*, 1988). If ppGpp is the major source of growth rate control in *E. coli*, it would be expected that the regulation is abolished in a ppGpp^0 mutant. Indeed, in contrast to wild type, in the ppGpp^0 mutant RNA/DNA and RNA/protein ratios remain constantly high, independent of the steady-state growth rates (Potrykus *et al.*, 2010); however, contradictory results have been reported when β-galactosidase activities are measured from different *rrn* P1-*lacZ* fusions (Gaal & Gourse, 1990; Hernandez & Bremer, 1993). The cause for the differences in the results from different groups is not clear; however, it is known that the double null *relA spoT* mutation easily accumulates suppressors that conferred growth advantage over the ppGpp^0 mutant (Xiao *et al.*, 1991; Potrykus *et al.*, 2010).

The effect of the stringent response or ppGpp on the rRNA synthesis is universally accepted. When a fast-growing cell is subject to amino acid starvation, which induces the stringent response (Cashel *et al.*, 1996), a rapid accumulation of ppGpp (over 100-fold increase from the basal level) is accompanied by an immediate shutoff of rRNA synthesis and growth arrest (Cashel, 1969). Such an inhibitory effect of the stringent response/ppGpp on rRNA synthesis during amino acid starvation can be visualized by a rapid disintegration of the special transcription factories, where concentrated RNAP engages in active rRNA synthesis during optimal growth (Fig. 2). It should be noted that complex effects of ppGpp have been reported (for reviews, see Chatterji & Ojha, 2001; Nystrom, 2004; Potrykus & Cashel, 2008; Srivatsan & Wang, 2008), particularly because the stringent response invokes reprogramming of global gene expression patterns in the cell (Durfee *et al.*, 2008; Traxler *et al.*, 2008, 2011). However, the consensus is that ppGpp directly inhibits rRNA synthesis and transcription of the stringent genes in the cell.

The inhibitory effect of ppGpp on rRNA synthesis is potentiated by the DksA protein, as the inhibition is significantly reduced upon nutrient starvation in the Δ*dksA* mutant (Paul *et al.*, 2004a). The structure of DksA suggests that it interacts with RNAP at the secondary channel of RNAP (Perederina *et al.*, 2004). Mutational analyses of DksA and RNAP have identified the regions important for the interaction (Blankschien *et al.*, 2009; Rutherford *et al.*, 2009). Similar to the ppGpp^0 mutant, the growth rate regulation is abolished in the Δ*dksA* mutant as RNA/protein ratios remain constantly high, independent of the steady-state growth rates (Paul *et al.*, 2004a). Unlike ppGpp, however, the level of DksA remains unchanged with different growth rates and growth phases (Brown *et al.*, 2002; Paul *et al.*, 2004a). Further, only high nonphysiological levels of DksA can substitute for ppGpp in the growth rate control in the ppGpp^0 mutant (Potrykus *et al.*, 2010). Together, these results are consistent with the conclusion that ppGpp is the primary effector in the regulation of rRNA synthesis.

How does ppGpp control rRNA synthesis in the cell? From the *in vitro* effects of ppGpp on the transcription of *rrn* described above, it is proposed that the regulation of rRNA synthesis by ppGpp involves a cascade of events during the stringent response (Fig. 4). The target for ppGpp is at both initiation and elongation of *rrn*, probably simultaneously; such synergistic inhibitory effect of ppGpp on transcription of *rrn* would effectively turn down rRNA synthesis immediately after nutrient starvation. On the one hand, it is likely that ppGpp increases pausing of elongating RNAP on *rrn* operons, which would promptly decrease the propagation of supercoiling at the *rrn* promoters region due to the proposed supercoiling feedback mechanism described above, reducing the efficiency of the open complex formation at the *rrn* P1 promoter. The pausing effect would also cause an immediate blockage of escape from the *rrn* P1 promoter by the jamming of RNAP downstream, particularly when RNAP molecules are densely packed. On the other hand, ppGpp and DksA act in concert synergistically to destabilize the weakened initiation complexes of *rrn* P1. This promotes RNAP dissociation from *rrn* P1, resulting in inhibition of initiation of the promoter. The synergistic effect of ppGpp and DksA could be augmented further by the challenge of vast amounts of non-*rrn* genomic DNA, which would effectively titrate RNAP out from the *rrn* P1 promoter into other parts of the nucleoid in the cell. Decreased rRNA synthesis would facilitate the binding of the H-NS proteins at the *rrn* P1 regulatory region, preventing further RNAP initiation from the *rrn* P1 promoter. Lrp (in the absence of leucine) binding in the region can further stimulate the interaction of H-HS in the *rrn* P1 promoter region. Lrp is a global feast/famine regulator of metabolism in the cell (Newman *et al.*, 1992; Calvo & Matthews, 1994; Yokoyama *et al.*, 2006). Expression of Lrp is stimulated by ppGpp (Landgraf *et al.*, 1996). Note also that H-NS, Lrp and DksA are modulators of chromosomal supercoiling (Hardy & Cozzarelli, 2005). Together, this mode of action of ppGpp *in vivo* is consistent with the known effects of various factors on the *rrn* transcription *in vitro* described in the above section. However, the *in vivo* effect of ppGpp in the scheme needs to be further studied.

The inhibitory effect of ppGpp on rRNA synthesis and bacterial growth is likely a continuum depending on the level of the inhibitor. As described above, bacterial growth rate is negatively correlated with the basal levels of ppGpp (Sarubbi *et al.*, 1988; Zacharias *et al.*, 1989; Bremer & Dennis, 1996). Similarly, stable RNA synthesis is inversely related to the concentration of ppGpp, ranging >100-fold from basal level to high level during the stringent response (Baracchini & Bremer, 1988). Moreover, mutations in the G/C-rich discriminator regions of the *rrn* and *tyrT* promoters affect both the stringent response and growth-rate regulation simultaneously (Travers *et al.*, 1986; Zacharias *et al.*, 1989). Altogether, these results strongly support the argument that both the stringent and growth-rate control of rRNA synthesis are mediated by ppGpp and DksA. Conceivably, basal levels of ppGpp work similarly for the growth rate regulation, but to lower extents compared with the effect caused by high levels of ppGpp during the stringent response.

Promoting growth by polyphosphate/ppGpp during nutrient downshift

After *E. coli* cells grown in a nutrient-rich medium are subcultured into a fresh nutrient-poor minimal medium, bacteria assume exponential growth after a lag time. In contrast to wild type, however, the lag time is extended significantly to several hours in a strain carrying a null mutation in the *ppk* gene, which encodes the inorganic polyphosphate kinase (PPK), demonstrating that inorganic polyphosphate is important for bacterial to adapt to growth during nutrient downshift (Kuroda *et al.*, 1999). The rapid accumulation of polyphosphate in starved cells depends on the inhibition of exopolyphosphatase (PPX) by ppGpp (Kuroda *et al.*, 1997), indicating another role of ppGpp in growth regulation. Polyphosphate binds the Lon protease and stimulates the protease activity to degrade r-proteins (Kuroda *et al.*, 2001), providing amino acids to support growth of starved cells during the nutrient limitation. Connection of ppGpp and polyphosphate demonstrates an important role of the two molecules in the nutrient starvation response. Recently, it has been proposed that polyphosphate could act as a second messenger by binding to the major sigma factor of RNAP in *Helicobacter pylori* and possibly, in other human pathogens during nutrient starvation (Yang *et al.*, 2010).

The distribution of RNAP and the regulation of rRNA synthesis

Regulation of rRNA synthesis, in essence, reflects the competition of RNAP between transcription of *rrn* and that of other non-*rrn* (or nonstringent) genes under different growth conditions. This competition indicates the fact that RNAP is limiting in the cell. In *E. coli*, it is estimated that there are about 2000 RNAP molecules per genome equivalent (Ishihama, 2000), or about 2600 RNAP molecules per cell in cells grown in LB (Piper *et al.*, 2009), although a higher number of about 5000 RNAP molecules per genome equivalent has also been reported (Grigorova *et al.*, 2006). The *E. coli* genome encodes about 4500 genes, which are organized into about 2390 operons, many of which have more than one promoter (Blattner *et al.*, 1997; Riley *et al.*, 2006). Moreover, it is likely that during cell growth more RNAP molecules are engaged in elongation than initiation in the cell. These estimates also argue for the notion that RNAP is limiting for genome-wide transcription.

The limitation of RNAP for genome-wide transcription is exacerbated in a fast-growing cell. It is estimated that the rate of rRNA synthesis approaches one initiation per second per *rrn* operon in a cell under optimal growth conditions (Bremer & Dennis, 1996; Voulgaris *et al.*, 1999). The long length of the *rrn* transcripts coupled with the gene dosage effect described above has clear implications for the distribution of RNAP in the genome in response to cell growth. For example, rRNA synthesis of an equivalent of 38 *rrn* operons, which is calculated to be the copy number for these operons in a mid-log growing cell with a doubling time of 23 min, would capture about 2500 RNAP molecules. This amounts to almost all of the RNAP in the cell if using the lowest estimate. Under a suboptimal growth condition with minimal medium, however, the rate of rRNA synthesis decreases to about one initiation every 15 s per *rrn* operon, and during nutrient starvation such as amino acid starvation leading to the stringent response, the rate of synthesis of rRNA is even more reduced. Growth rate regulation in bacteria is largely about how RNAP is allocated for rRNA synthesis in response to environmental cues.

The rate of rRNA synthesis will determine the differential allocation of RNAP between *rrn* and non-*rrn* (and non-stringent) genes in the genome, which in turn will have profound consequences on global gene regulation and the structure of the nucleoid in response to growth conditions (Jin & Cabrera, 2006). During optimal growth, active rRNA synthesis consumes the majority of RNAP molecules; thus, RNAP available for transcription of genome-wide non-*rrn* genes is limited. Conversely, during the stringent response, more RNAP molecules become free and available for genome-wide transcription due to the

amounts of RNAP released from rRNA synthesis. Such an RNAP redistribution (or allocation) concept during the stringent response is originally suggested by the analysis of stringent RNAP mutants that are defective in transcription of *rrn* P1 and other stringent promoters both *in vitro* and *in vivo* (Zhou & Jin, 1998). These RNAP mutants exhibit the stringent response phenotype (thus they are named the stringent RNAP) even when they are grown in nutrient-rich LB. They have slower growth rates compared with wild type; and as expected, the rates of RNA synthesis are reduced and the dominant transcription foci are not evident in these stringent RNAP mutants when grown in LB (Zhou & Jin, 1997; Cabrera & Jin, 2003). Results from biochemical studies, transcriptional profiling and cell biology analysis are consistent with the model underlying the redistribution of RNAP by ppGpp during the stringent response and carbon source limitation (Barker *et al.*, 2001a; Cabrera & Jin, 2003; Liu *et al.*, 2005; Durfee *et al.*, 2008). The direction for the newly available RNAP engaging in genome-wide transcription is provided by ppGpp/DksA, transcription factors or the concentration of free RNAP *per se*, which is a topic beyond the scope of this review.

Two other models addressing the effect of ppGpp on RNAP have been proposed. One model (Baracchini & Bremer, 1988) proposes that RNAP is partitioned into two forms: one ppGpp-bound, which is unable to participate in initiation from *rrn* P1, but is able to for other non-*rrn* promoters, and the other ppGpp free, which is competent for initiation at all promoters. The other model (Jensen & Pedersen, 1990) proposes that RNAP is sequestered by ppGpp-mediated pausing during elongation, leading to reduced RNAP available for global gene expression during the stringent response. Both models were attractive at the time they were proposed and helped us understand the regulation of rRNA synthesis by ppGpp; however, each model mainly emphasizes either the effect of ppGpp on initiation or elongation. As discussed above, there are multiple effects of ppGpp on transcription that can be explained by the RNAP redistribution (allocation) model, which takes into account the effects of ppGpp on both initiation and elongation.

The RNAP redistribution (allocation) model could also explain and/or reconcile some results related to the regulation of rRNA synthesis. For example, a ribosome feedback model has been proposed to account for reduced rRNA synthesis by the presence of a plasmid-borne *rrn* operon in the cell (Jinks-Robertson *et al.*, 1983); the effect of the extrachromosomal copies of *rrn*B on rRNA synthesis has been attributed to components involved in translation initiation machinery (Cole *et al.*, 1987), an increased level of ppGpp (Baracchini, 1991) or about a 20% reduction of ATP and no change in ppGpp level (Schneider, 2003). Studies from cell biology of RNAP have shown that RNAP is exclusively colocalized with the nucleoid under normal conditions, whereas, in the presence of a plasmid-borne *rrn* operon RNAP is located both in the nucleoid and in the cytoplasmic space. Under this condition when RNAP is driven into the cytoplasmic space by the plasmid-borne *rrn* operon, the dominant transcription foci are diminished in the nucleoid (Cabrera & Jin, 2006). Thus, reduced RNAP in the nucleoid by the plasmid-borne *rrn* operon could account for the decreased rRNA synthesis in the chromosome (Voulgaris *et al.*, 1999). Also, it has been reported that *E. coli* mutants, which deleted one to two *rrn* operons in the chromosome, have a growth rate comparable to the wild type (Condon *et al.*, 1993, 1995a; Asai *et al.*, 1999). The total RNA synthesis in these *rrn* deletion mutants is unchanged; however, the rate of rRNA synthesis for the remaining intact *rrn* operons is increased compared with the wild type, as demonstrated by electron micrographs (Condon *et al.*, 1993). These results can be explained by the RNAP redistribution model as the same amount of RNAP in these mutants will be redistributed to the remaining intact *rrn* operons, leading to an increased frequency of RNAP occupying each of the remaining *rrn* operons without the need for a reduced ppGpp level in the cells.

Summary and future prospects

Regulation of rRNA synthesis in response to growth conditions involves multiple factors and affects the allocation of RNAP for global gene expression and the structure of the nucleoid in *E. coli*. The magic spot ppGpp, which acts in concert with DksA, is the major source of growth rate regulation and the stringent response in the bacterium. In the future, identifying the sites in RNAP that are involved in the regulation of rRNA synthesis, particularly defining the ppGpp-binding sites in RNAP, will shed light on the mechanisms of ppGpp in transcription. In addition, studying the dynamics of the dominant transcription foci and the putative nucleolus-like structures will be important to help our understanding of the link between rRNA synthesis, global gene expression, and chromosome structure in this model system.

Acknowledgments

We thank our colleagues for comments on the manuscript. This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

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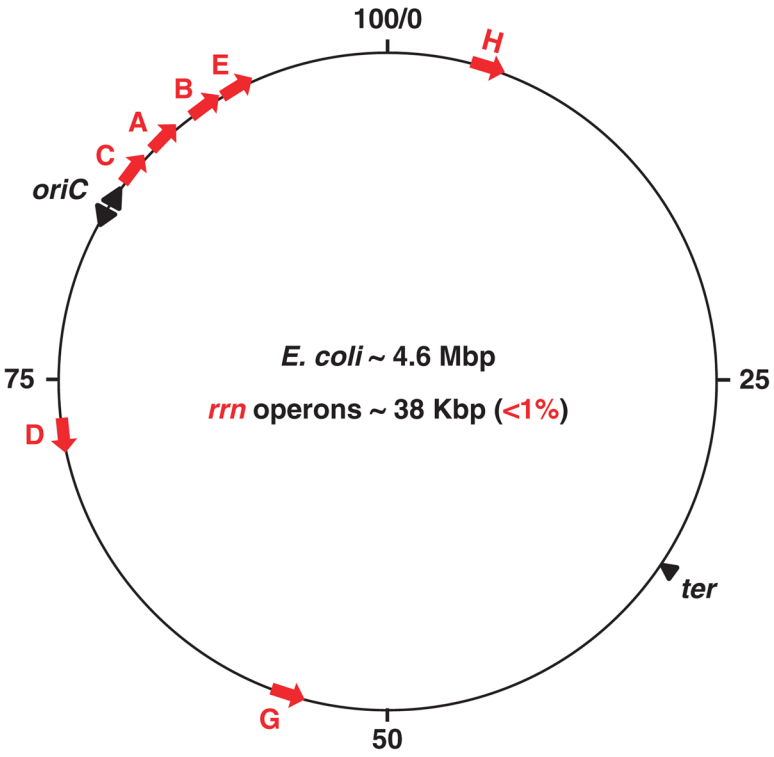

Fig. 1.

Location of seven *rrn* operons as indicated by capital letters (red) in *Escherichia coli* chromosome map. The numbers indicate the minute in the map, *oriC* for the origin of chromosome replication and *ter* for the terminus of replication. The direction of *rrn* transcription is indicated by arrows (red) and the bidirectional replication by arrowheads. Only a tiny fraction of *E. coli* genome encodes for the seven *rrn* operons.

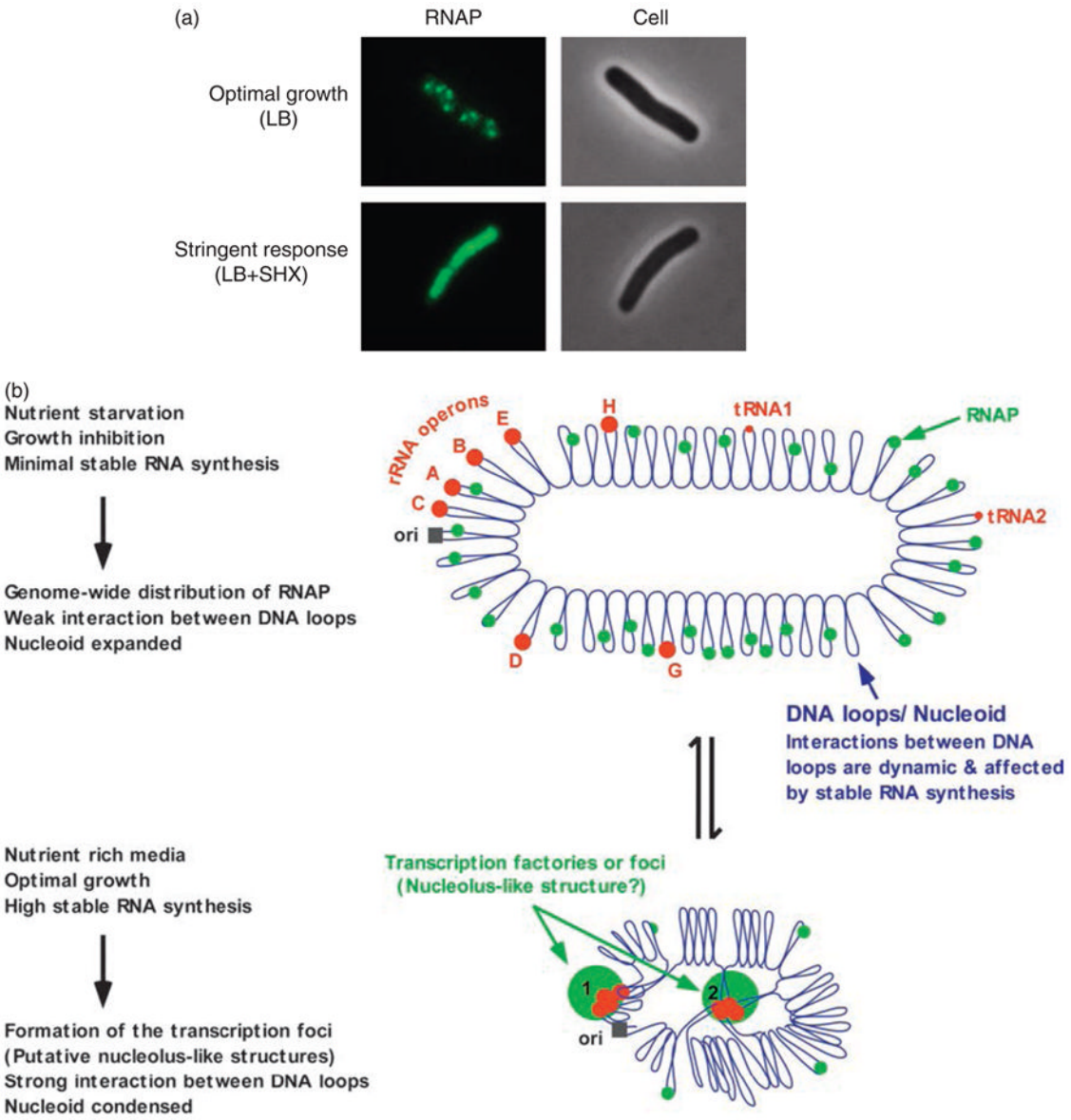

Fig. 2.

(a) The distribution of RNAP is sensitive to environmental cues. During optimal growth, concentrated RNAP (pseudo-colored green) forms dominant transcription foci, which are proposed to be special transcription factories and the nucleolus-like structures, where rRNA is being actively synthesized, in a mid-log cell grown in nutrient rich LB. With the addition of serine hydroxamate (SHX), which caused cell starvation for amino acid and induced the stringent response, RNAP is redistributed relatively homogeneously in the nucleoids. The RNAP is fused with a fluorescent protein and imaged with a fluorescent microscope with the corresponding cells in phase contrast as described (Cabrera & Jin, 2003). (b) Model illustrating the dynamics of the transcription factories or foci and the putative nucleolus-like structures under the two extreme growth conditions. The *Escherichia coli* chromosome is represented as blue lines folded in loops, the *ori* of replication as a black square, the seven rRNA operons as large red circles with letters, and two representative tRNA operons as small red circles. The RNAP molecules are represented as small green circles. For simplicity, during optimal growth only two transcription factories/foci and putative

nucleolus-like structures, which make the nucleoid more compact by pulling different stable RNA operons into proximity, are indicated (bottom part of the diagram, large green circles encompassing multiple large red cycles labeled 1 and 2) (Adapted from the study by Cabrera & Jin, 2003).

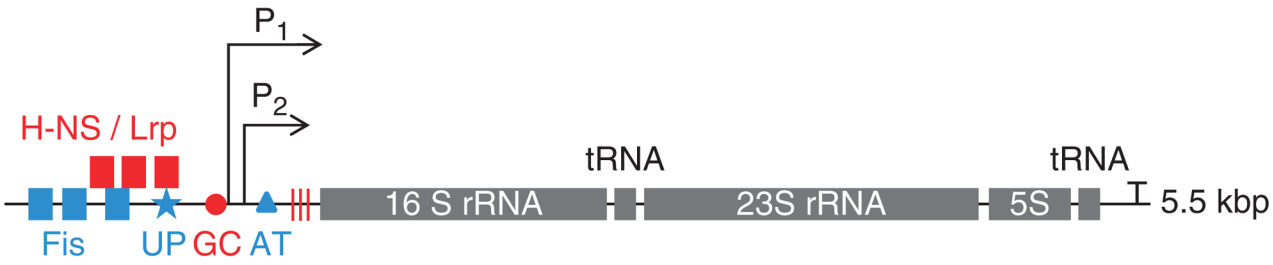

Fig. 3.

A typical *rrn* operon is schematically illustrated. Two promoters (P1 and P2) and terminators (T) for the long *rrn* transcript are indicated. Blue symbols indicate positive elements for the regulation of *rrn*, including Fis-binding sites (box) and UP element (UP, star) at the extended P1 promoter region, and antitermination system (AT, triangle) after the P2 promoter. Red symbols indicate negative elements, including H-NS and/or Lrp binding site (box), G/C-rich 'discrimination sequence' (GC, circle) at the extended promoter region, and multiple pausing sites (vertical line) before the 16S RNA gene. The illustration is not drawn to scale.

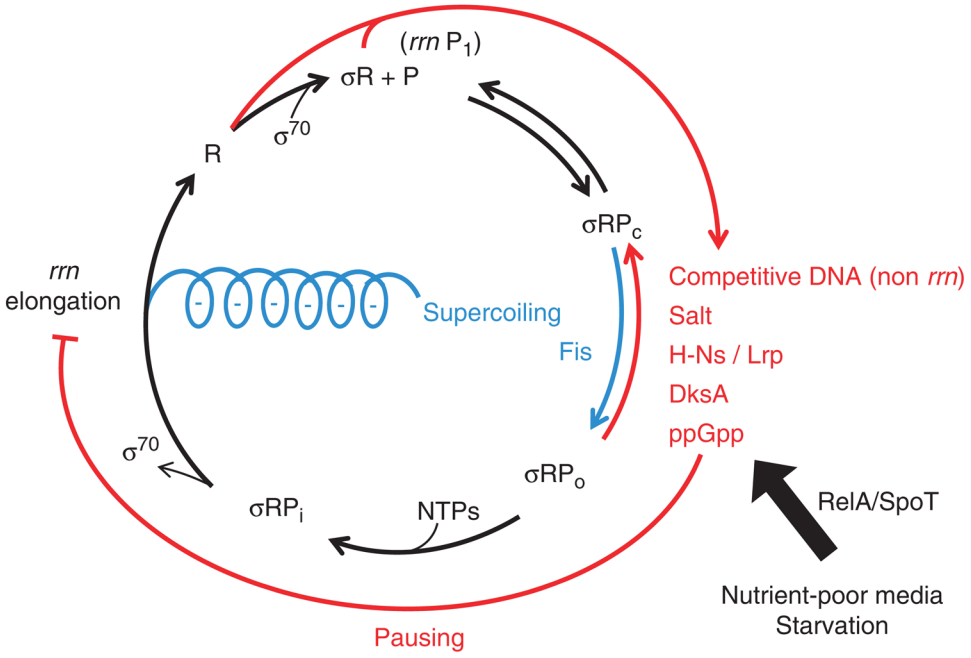

Fig. 4.

Model illustrating regulation of rRNA synthesis at system-level in *Escherichia coli*. RNAP holoenzyme (σR) binds to the *rrn* P1 promoter (P), forming multiple initiation complexes, including close complex (σRP_c) and open complex (σRP_o). Uniquely, the initiation complexes, particularly open complex, are intrinsically unstable and in rapid equilibrium with each other, before the formation of the stable initially transcribing complex σRP_i in the presence of NTPs. Many positive (blue) and negative (red) elements as indicated control the expression of *rrn* P1. The activities of RelA and/or SpoT are responsible for the basal level of ppGpp, which are inversely proportional to the nutrient quality of the media and rapid accumulation of high level of ppGpp during starvation. The effects of ppGpp on rRNA synthesis are twofold, including inhibition of initiation, and enhancing pausing of elongation RNAPs, which in turn decreases elongation-induced supercoiling and causes jamming of RNAP at the *rrn* P1 promoter. Depending on cell growth conditions, released RNAP (R) from *rrn* operons either rebinds to *rrn* P1 for reinitiation or redistributes to other non-*rrn* genome-wide DNA. See text for details.