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# Control of Ribosome Synthesis During the Cell Division Cycles of *E. coli* and *Synechococcus*

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**Abstract**
The regulation of ribosome synthesis has been investigated for nearly five decades. In earlier studies, the control of rRNA synthesis in bacteria was found to be dependent on nutrient composition of the growth media or cell growth rates, and these observations led to the growth rate-dependent regulation model. Also developed were stringent control, feedback ribosome synthesis, passive regulation, and antitermination models. Current evidence indicates that upstream (UP) element, molecular effectors, ppGpp and iNTP (initiating nucleoside triphosphate), and trans-acting proteins, Fis and H-NS, play important roles in the control of rRNA synthesis in response to changing nutritional environments. The mechanisms for the ribosome feedback regulation, and growth rate-dependent controls of rRNA synthesis remain to be determined despite numerous investigations. r-protein synthesis can be controlled by translational coupling, translation repression, or premature transcription termination. In *Synechococcus,* a photoautotroph*,* ribosome synthesis occurs early in the cell cycle as programmed events under conditions that support balanced growth. Periods of r-protein synthesis occur before rRNA synthesis periods, and rRNA synthesis is stimulated by a light-activated gene regulatory protein. These observations suggest that gene regulatory proteins are involved in the coordinate regulation of ribosome assembly in *Synechococcu*s.

**Introduction**
*E. coli*, a chemoheterotroph, and *Synechococcus*, a photoautotroph, are on opposite ends of the microbial nutritional categories. Basic differences in the nutritional process of chemoheterotrophs and photoautotrophs lie in their methods of generating energy and in the nature of the carbon sources needed for cell replication. In chemoheterotrophs organic compounds serve as sources of both energy and carbon. The energy source for photoautotrophs is radiant energy, and the carbon source for biosynthesis of cellular molecules is mainly $CO_2$. Chemoheterotrophs can take in organic substrates from the environment to support cell growth, while photoautotrophs must produce all cellular molecules and cellular structures from inorganic compounds to support cell growth. Chemoheterotrophs can grow in light or dark conditions, while photoautotrophs grow in light conditions only. Nevertheless, there are similarities in biosynthetic paths of building block molecules, the synthesis of macromolecules, and the assembly of macromolecules to form basic cell structures in both bacteria. The cell cycle events that occur sequentially in the cell division cycle of *E.coli* and *Synechococcus* are genome replication, genome segregation and cell septum formation, suggesting that these cell events are coordinately regulated events (Vinella and D'Ari, 1995; Donachie, 2001; Asato, 2003). However, ribosome synthesis in *E. coli* is stimulated by the quality of external nutritional environments, or by growth phases, or growth rates (see reviews by Condon et al., 1995; Keener and Nomura, 1996; Gourse et al., 1996; Nomura, 1999). In *Synechococcus,* ribosome production occurs early in the cell cycle followed by genome replication, genome segregation and cell septum formation (Asato, 1979). It is obvious that the regulation of ribosome synthesis in *E. coli* and *Synechococcus* as described above is not controlled by similar mechanisms. What is less obvious is the complexity of the regulatory mechanisms involved in ribosome synthesis in *E. coli* and *Synechococcus*. Comprehensive and authoritative reviews on the regulation of ribosome synthesis in *E. coli* have been published by authors indicated above. The aim of this article, however, is to focus on current issues regarding the control of rRNA and r-protein syntheses in ribosome formation in *E. coli* and *Synechococcus.*

**Control of r-protein synthesis in *E. coli***
Ribosome synthesis involves a coordinated, sequential appearance of r-protein and rRNA. Some forms of direct genetic regulation must be involved in coordinating r-protein expression and rRNA synthesis (Kjeldgaard and Gausing, 1974; Nomura, 1999). However, the elucidation of genetic mechanisms that coordinate the syntheses of r-protein and rRNA presents a formidable problem (Nomura, 1999). Additionally, the regulation of the entire r-protein operons by gene regulatory proteins remains to be elucidated. A more amenable problem is the determination of the mechanisms that regulate r-protein and rRNA concentrations. Two mechanisms have been proposed for controlling r-protein concentration, and these control mechanisms are referred to as feedback regulation (Keener and Nomura, 1996) or autogenous control (Zengel and Lindahl, 1994). The first represents the translational coupling that increases the rate of r-protein translation, while the second represents the repression of translation by repressor r-protein (Keener and Nomura, 1996). The translational coupling can increase r-protein translation rate when r-protein concentration is low as explained in the following way. Genes on the upstream positions on r-protein mRNAs could melt the secondary mRNA structure and thereby facilitate the translation

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of genes located downstream. Alternatively, ribosomes engaged in translation might be transferred directly from the upstream genes to the initiation codons of downstream genes. In the repression of translation, one of the r-proteins, produced from r-protein mRNA, acts as an inhibitor of r-protein mRNA translation. Accordingly, when excessive amounts of r-proteins are produced a specific r-protein can bind to the mRNA transcript that codes for the r-proteins and prevent mRNA translation. The binding of r-protein to mRNA is possible since, the r-protein binding sequences on rRNA are also present in r-protein mRNA. The repression of translation by r-protein could occur when r-protein binding sites on rRNA are fully occupied or when rRNA transcription rates are decreased during ribosome assembly.

The regulation of r-protein operons by translational coupling and translation repression are manifested in a variety of ways (Lindhal and Zengel, 1986; Keener and Nomura, 1996). One example, the translational coupling of L10 operon presents an interesting albeit a complex scheme. The L10 operon codes for L10, L7/L12 r-proteins, and the $\beta$ and $\beta'$ subunits of RNA polymerase (RNAP). L7 and L12 occur as acetylated and unacetylated forms, respectively. Translation of the L10 operon is regulated by the binding of L10 or by L10, L7/L12 complex to a region located at the 5‘ end of *rplJ* cistron (Petersen, 1989). However, the protein copies of L10:L7/L12:$\beta$:$\beta'$ are synthesized in a ratio of 1:>4:0.2:0.2 (Post and Nomura, 1980). A mechanism for the translational coupling of L10 operon was proposed to explain the synthesis of L10 and L7/L12 in a ratio of four to one (Yates et al., 1981). It was suggested that the translation of L10 opens up the region between L10 and L7/L12 which consists of 66 base pairs. This event allows other ribosomes to initiate translation at the region such that synthesis of L7/L12 r-proteins are translated about four times as fast. It should also be noted that overproduced free L10 is rapidly degraded, and this activity could contribute to the unusual stoichiometry of L10 and L7/L12 (Petersen, 1990).

Translation repression is proposed as the major mechanism that controls r-protein concentration in relation to rRNA concentration in steady state growth or growth in rapidly changing environments. An alternative mechanism for controlling r-protein synthesis is premature transcription termination. The premature terminations of transcription of r-protein mRNA can occur at terminator sites located upstream of the first gene of the operon (Zengel and Lindahl, 1994; Lindahl, Archer, and Zengel, 1983; Stelzl, et al., 2003). For example, the transcription termination of the S10 r-protein operon is regulated by L4 r-protein which is a product of the S10 operon. L4 can also serve as a translation repressor of S10 mRNA. Further investigations of transcription termination in other r-protein operons are needed in order to determine their prevalence and the possible relationships with other mechanisms that coordinate r-protein and rRNA syntheses.

**Control of rRNA synthesis in *E.coli* during steady state growth**

Investigations into the control of rRNA synthesis in bacteria have been ongoing for about 46 years. Described below are some of the major developments in the control of rRNA synthesis that indicate the enormously difficult problem of trying to determine the mechanisms involved in ribosome synthesis. The arduous quest in determining the fundamental mechanisms in the control of rRNA synthesis began with the publication of a classic paper on RNA content of *Salmonella typhimurium* during balanced growth conditions (Schaecter et al., 1958). A major finding was that RNA contents increased exponentially when plotted against linear increase of growth rates. The data indicated that the composition of the growth media controls growth rates, and growth rates, in turn, control ribosome synthesis (Maaloe and Kjeldgaard, 1966). Subsequent experiments indicated that the number of ribosomes per unit cellular protein is equal to the growth rate ($\mu$) and the rate of ribosome accumulation is roughly proportional to $\mu^2$ (Gausing, 1980). These observations gave rise to a growth rate-dependent regulation model of ribosome synthesis during steady state growth and served to guide a great number of studies on this subject.

In line with the principle of growth rate-dependent regulation is ribosome feedback model which was developed to explain the control of rRNA synthesis during balanced or steady state growth (Jinks-Robertson et al., 1983). Experiments to test this model were based on rRNA activities in relation to gene dosage of rRNA operons. The results of gene dosage experiments indicated that rRNA expression was regulated by the amount of functional rRNAs assembled in ribosomes, and the appropriate concentration of ribosomes was controlled by some form of feedback mechanisms. Originally, it was thought that non-translating ribosomes acted as feedback regulators. Upon further experiments (Cole et al., 1987), translating ribosomes were proposed to be the feedback regulators. Unfortunately, the mechanism of feedback regulation by translating ribosome turned out to be very elusive and signal molecules of ribosome feedback regulation remained to be identified. Nevertheless, the notion that feedback mechanism plays a role in ribosome synthesis was found to be intriguing. That is, the expression of rRNA is controlled by feedback mechanisms and the feedback loops balanced rRNA synthesis rates with the demands for protein syntheses (Jinks-Robertson et al. 1983; Yanagishi et al., 1987).

A number of experiments were done to determine the relationship between feedback mechanism and control of rRNA transcription. In these studies, rRNA promoter activities were monitored where gene dosages of rRNA operons were increased or decreased (Jinks-Robertson et al., 1983; Cole et al., 1987; Condon et al., 1993; Voulgaris et al., 2000), or in conditional expression of rRNA genes (Gourse et al., 1985), or when mutant forms of *fis*, (Ross et al., 1990), *rpoA* (Ross et al., 1993), and *nus*B (Sharrock et al., 1985) were introduced into the cells. The results of these experiments showed that rRNA core promoter activities were decreased. When the corresponding changes in the rRNA core promoter activities were made rRNA synthesis rates were

restored indicating that the feedback mechanisms were responsible in restoring rRNA synthesis rates in response to the demand for protein synthesis.

It is now known, however, that ppGpp and iNTP serve as the feedback regulators in controlling rRNA expression, and these molecular effectors play major roles in controlling rRNA synthesis during rapidly changing environments (Schneider and Gourse, 2003). The participation of ppGpp and iNTP in rRNA synthesis will be described in the next section dealing with the control of rRNA in rapidly changing environments. Nevertheless, ppGpp was once considered to be involved in the growth-rate dependent regulation of rRNA synthesis ( see Condon et al*.,* 1995). It turned out, however, that ppGpp was not required for the growth rate-dependent regulation of rRNA synthesis (Gaal and Gourse, 1990). The role of iNTP in the growth rate-dependent regulation of rRNA has been a controversial issue as indicated below. In an earlier report ATP and GTP pools were found to increase substantially with increases in growth rates (Gaal et al, 1997). On the other hand, Peterson and Moller (2000) found that RNA precursor nucleotide concentrations were increased in a narrow range while total RNA accumulation increased as growth rates were increased. The discrepancies in the results reported above were resolved in a recent paper (Schneider and Gourse, 2004). A new luciferase bioassay for ATP was developed and used to measure NTP concentration at increasing growth rates. These results are now in agreement; *i.e*., nucleotide triphosphate pools are not involved in the growth rate-dependent regulation of rRNA synthesis

Two other control mechanisms in the regulation of rRNA synthesis have been proposed: the rRNA transcription antitermination model (Aksoy *et. al*., 1984), and the passive model (Maaloe. 1969). The rRNA antitermination model was proposed as an independent mechanism that coordinates the r-protein and rRNA syntheses. In this model, the Rho dependent transcription terminators (nut sites) located downstream of *rrn* P2 can prevent RNAP from transcribing *rrn* operons. The antitermination complexes, composed of r-proteins S10 (=NusE), S4, L3, L4 L13 and Nus factors (A, B, G) are able to permit read-through of nut sites (Torres et al., 2001; Luttgen et al., 2002;Torres et al., 2004). In addition, r-proteins, S4 and L4, can act as regulatory elements in repressing mRNA translation by means of translational feedback, and they play key roles in restoring the stoichiometry of r-protein and rRNA. The passive model of ribosome production is based on the relative strength of mRNA promoters over rRNA promoters, and on the premise that the concentration of RNAP in cells is limited. Serious arguments against the passive model have come from the observations that promoters of *rrn* are the strongest promoters in cells, and that RNAP concentration is not a limiting factor (see reviews by Condon et al., 1995; Gourse et al., 1996; Keener and Nomura, 1996).

**Control of rRNA synthesis in rapidly changing environments**

It is ironic that one of the first indications of the control of rRNA synthesis was the discovery of ppGpp as a possible effector molecule that represses rRNA synthesis in non-steady state growth conditions (Cashel and Gallant, 1969). In cells subjected to amino acid starvation or cells that are entering or are in a stationary phase of growth, ppGpp concentration increased significantly above basal level while rRNA transcription is decreased. The role of ppGpp as an effector molecule that controls the rRNA synthesis is a complex one. In any case, the control of the rRNA synthesis has been found to be associated with the *rrn* promoter strength, the regulatory molecules, Fis, H-NS, and feedback regulators, ppGpp and iNTP (Gourse et al., 1996; Gaal et al., 1997; Barker et al., 2001; Schneider et al., 2002). The promoter strength of *rrn* operons is increased by the UP element (Ross et al., 1993). Fis proteins enhance the binding of RNAP to promoters by direct interaction with the C-terminal domain of the alpha subunit of RNAP (Bokal et al., 1997; Alyar et al., 1998). The UP elements and trans-acting Fis protein (Ross et al., 1990) are able to increase transcription at least 300-fold (Rao et al., 1994). On the other hand, H-NS proteins inhibit the promoter activity at P1 (Afferbach et al., 1998). However, how does ppGpp decrease *rrn* expression and how does iNTP increase *rrn* expression? Experimental results show that ppGpp binds to $\beta$, $\beta'$ of RNAP and $s^{70}$ (Chatterji et al., 1998; Toulokhonov et al., 2001; Hernandez and Cashel, 1995; Schneider et al., 2003). The effector-RNAP complex binds to the *rrn* promoters and decreases the half-life of the open complex at P1 promoter sites (Barker et al., 2001) resulting in decreased transcription from *rrn* P1. In translation inhibited cells or when cells emerge from the stationary phase or when the growth media is enriched, ppGpp is decreased and iNTP is increased. iNTP binds to RNAP and the iNTP-RNAP complex increases the half-lives of the transcription complex thereby enhancing transcription rate of rRNA (Schneider et al., 2002).

Is it possible that ppGpp and iNTP act also as feedback regulators linking promoter activities and the need for protein synthesis in conditions where the growth media are enriched (Schneider and Gourse, 2003; Murray et al., 2003)? Experiments were done to compare promoter activities in WT and *nus*B5, *rpo*A, and $\Delta$*fis* strains (transcription-compromised mutants) as the media were nutritionally enriched (Schneider and Gourse, 2003). The results showed that an increase in promoter activity correlated with observed increased concentrations of ATP (=iNTP) and decreased ppGpp concentration in mutant strains. It was concluded that ppGpp and iNTP serve as effectors in controlling rRNA synthesis and play significant roles in feedback regulation in linking *rrn* P1 activities to the demands for protein synthesis upon enriching the nutrition of the media. However, in cells transformed with multiple copies of *rrn* operons (*i.e*., an *rrn* dosage compensation experiment), results showed that decreased promoter activities were associated with a small decrease in ATP concentration; surprisingly ppGpp did not increase as expected. This result reinforced the notion that other signal molecules are involved in the ribosome feedback regulation model during balanced growth conditions. It should also be noted that ppGpp, Fis and H-NS proteins are not involved in the growth rate-dependent control of rRNA expression (Ross et al., 1990; Afferbach et al., 1998; Murray et al., 2003).

It is interesting to note that ppGpp has taken an additional function. In this case, ppGpp, a product of *spoT* gene, is involved in adaptive responses to detrimental nutritional and physical conditions. For example, during depletion of amino acid, carbon source, fatty acid, and exposure to weak emission of UV light, *spoT*-dependent ppGpp accumulation occurs and wasteful rRNA synthesis is inhibited (Cashel, 2000; Seyfzadeh et al., 1993; Gong et al., 2002). In addition, *spoT*-dependent ppGpp accumulation activates the synthesis of RpoS which in turn stimulates several stress-resistance genes (see Loewen et al., 1998).

**Coordination of rRNA and r-protein syntheses in ribosome formation, an enigmatic problem**

In the assembly of ribosome, r-proteins bind to rRNA as rRNA is being transcribed (Cowgill de Narvaez and Schaup, 1979). In this transcriptionally coupled ribosome assembly process, r-proteins added early onto the nascent rRNA were found to be present in greater quantities. The order of specific r-proteins added to rRNA in the *in vivo* assembly process is similar to the order of r-protein added in *in vitro* reconstitution of 30S ribosome (Nomura and Held, 1974). The transcription of rRNA operons occurs in the order of 16S, 23S and 5S. The orderly transcription of rRNA genes, and the orderly binding of r-proteins to the nascent rRNA, suggests that transcriptions of rRNA and r-proteins are coordinately regulated by gene regulatory proteins.

It is becoming clear that the crucial processes of ribosome assembly are the mechanisms that coordinate the regulation of rRNA and r-protein syntheses. Experiments performed several decades ago indicated that pools of rRNA and r-protein are not found in any appreciable size (Kjeldgaard and Gausing, 1974). However, the synthesis of both rRNA and r-protein occurs immediately after the nutritional shift-up, while total protein production occurs several minutes later (Maaloe and Kjeldgaard, 1966; Schleif, 1967; Kjeldgaard and Gausing, 1974). These observations suggest a logical progression of ribosome synthesis and total protein production. Results from subsequent investigations indicated that r-protein genes are regulated at the level of initiation of transcription during both steady state growth and nutritional shift-up (Dennis and Nomura, 1975; Gausing, 1976). However, as indicated earlier, the transcriptional control of r-protein and rRNA syntheses by gene regulatory proteins has not yet been determined in *E. c*oli.

A rapid change in the nutritional environment brought about by the nutritional shift-up would result in an increased demand for ribosomes. Several responses are now known to occur following nutritional shift-up. Increase synthesis of both rRNA and r-protein synthesis occurs immediately. The increased rRNA synthesis is stimulated by an increase in iNTP and decrease in ppGpp concentrations. However, the regulatory mechanisms that increase r-protein synthesis during the nutritional shift-up have not been determined. In any case, molecular effectors, ppGpp and iNTP, act as feedback regulators in controlling the synthesis of rRNA. It should be noted, however, that feedback mechanisms by definition, operate after the r-protein, rRNA and ribosmes are produced. It appears, therefore, that feedback mechanisms by operational definition are not involved in coordinating the initiations of r-protein and rRNA syntheses

There could be other models of ribosome synthesis that are not discussed above. Nevertheless, a major obstacle in constructing ribosome biosynthesis models is determining the coordinate regulation of r-protein and rRNA syntheses. The task of finding the regulatory molecules that coordinate the synthesis of both r-protein and rRNA in *E. coli* prior to and during ribosome assembly is not an easy undertaking.

**Cell division cycle and ribosome synthesis of a photoautotroph, *Synechococcus sp* PCC 6301**

The sequential order of macromolecular synthesis that occurs during the cell cycle in *Synechococcus* permits orderly production of major cellular structures in the reproduction of daughter cells in light growth conditions (Asato, 2003). The initiation of r-protein and rRNA transcriptions, DNA replication and cell growth do not occur in the dark (Marino and Asato, 1986). Periodic macromolecular synthesis patterns occur at medium to fast growth rates, and the cell cycle displays distinct $D_1$, S, and $D_2$ phases (Asato, 1984). A working model has been constructed (Asato, 1983) which indicates that timely appearances of macromolecular synthesis are controlled by timely coordinated light-activated gene regulatory proteins. The production of ribosomes in *Synechococcus* is one of the major cell events and displays unique features that differ in some ways from ribosome production in *E. coli*. The synthesis of r-protein and rRNA, and the assembly of ribosomes occur early in the cell division cycle as regularly programmed events. Two *rrn* operons were identified in *Synechococcus,* rather than the seven *rrn* operons found in *E. coli*. *In vitro* experiments (Kumano et al., 1986) indicate the synthesis of three rRNA transcripts that differ in molecular size, suggesting the existence of three promoters in the *rrn*A operon. Base sequence analyses have, indeed, located three promoters in *rrn*A (Asato, 1998). A $G_6$ tract was found upstream of *rrn*A P1, although the function of the $G_6$ tract in stimulating rRNA transcription is not known. In *E. coli* A-tracts upstream of the UP element of *rrn*B P1were found to strengthen the promoter activity in stimulating rRNA transcription (Aiyar et al., 1998). There were, however, sites found in *rrn* operons of *E.coli* that are similar to those present in *Synechococcus*. A base sequence resembling the UP element were found upstream of *rrn*A P1, and sequences resembling the antitermination sequences found in *E.coli* had been found in the leader region. In addition, sequences similar to box B of *E.coli* were identified within the first stem and loop structure in the leader region of *Synechococcus* (Kumano et al., 1986).

The rRNA synthesis period is about three hours in synchronized cultures growing at a doubling time of 8 h. Any scheme in ribosome production in S*ynechococcus* must be consistent with the following observations. (1) rRNA synthesis occurs early in the cell cycle as a genetically programmed cell event. (2) rRNA synthesis must be coordinated with r-protein synthesis such that r-

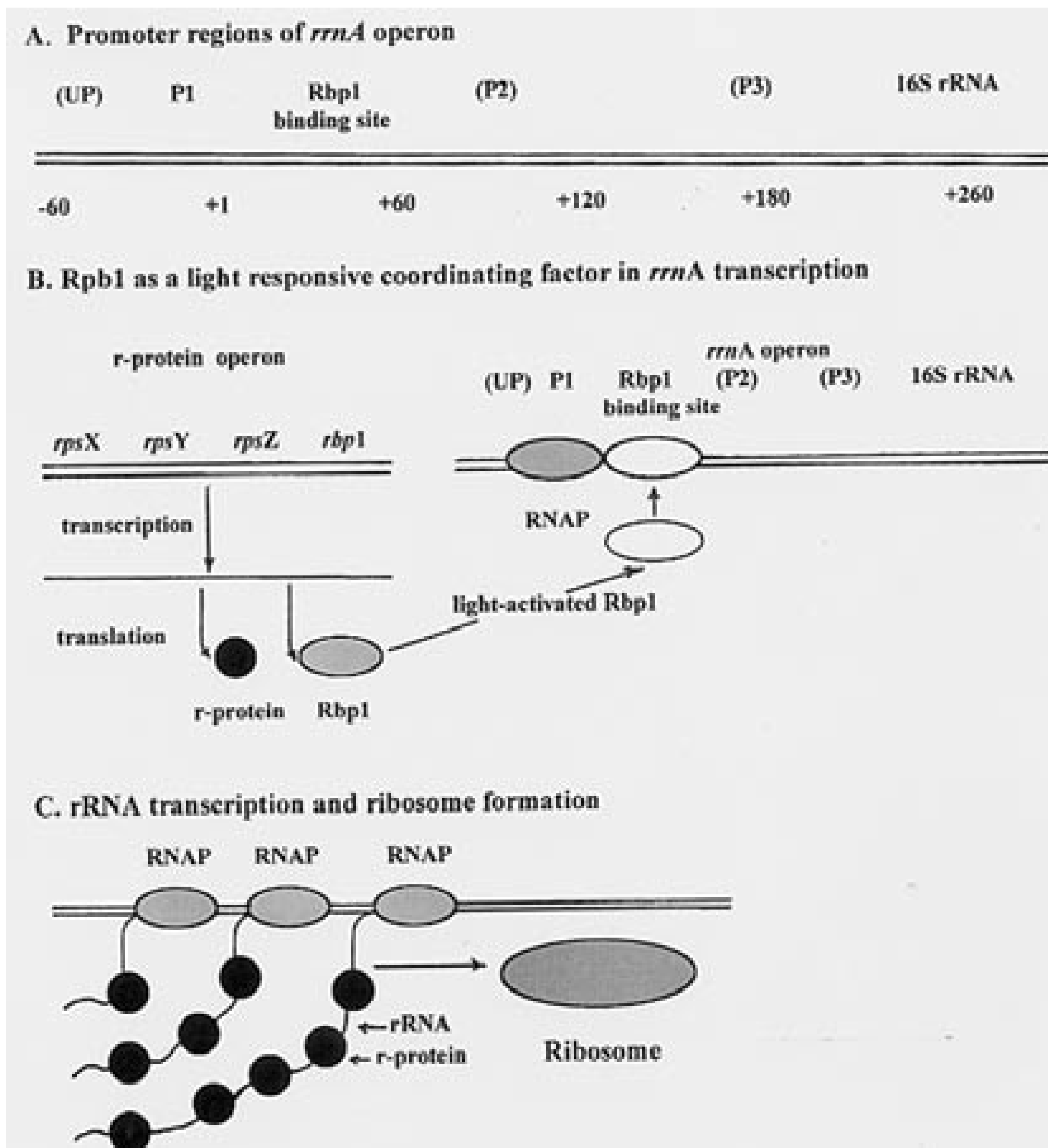


Figure 1. Coordinate Regulation of Ribosome Synthesis: A. Physical map of the promoter region of *rrn*A operon B. Rbp1 serves as a light responsive coordinating factor. r-protein operon displayed in Fig 1B is an idealized operon harboring the hypothetical rbp1 locus. As the r-protein operon is transcribed and translated r-proteins and Rbp1 are produced. Rbp1 is light-activated and binds to the Rbp1 binding site. C. 16S rRNA transcription and assembly of 30S ribosomal subunit . Ribosome assembly is modeled according to Cowgill de Narvaez and Schaup (1979). The implication of the ribosome assembly is described in the text. Although not described in Fig 1, excess concentrations of r-proteins will repress mRNA translation, and the production of r-proteins, including Rbp1, will be curtailed. As a result, the production of r-protein and rRNA syntheses will decline.

protein synthesis occurs before rRNA synthesis, and not in the reverse order. (3) rRNA transcription is stimulated by a light-responsive gene regulatory protein that activates rRNA synthesis in light but not in darkness (Asato, 1998). (4) Down regulation of rRNA and r-protein syntheses must also be coordinated so that ribosome synthesis does not occur in excess, or within the S and $D_2$ periods. Possible models of coordinate regulation of r-protein and rRNA synthesis were described elsewhere (Asato, 1983). Fig. 1 depicts a revised model in coordinate regulation of r-protein and rRNA syntheses. In this model, *rpb*1 is part of a hypothetical r-protein operon (Fig 1B). As r-protein is transcribed and translated Rpb1 is also produced. Rpb1 is activated by light and binds to the *rpb*1 binding site located downstream of *rrn*A P1 on rDNA (Asato, 1998). RNAP binding to *rrn* P1 promoter is enhanced by Rpb1 and the proposed UP element. The binding of both RNAP and Rpb1 transforms the closed complex into open complex and rRNA transcription proceeds. As rRNA is being transcribed, r-proteins bind to r-protein binding

sites on the 5'-end of the16S rRNA as the sites become exposed during the transcription process (Fig 1C).

The ribosome assembly process as depicted in Fig.1indicates that r-proteins are synthesized before the transcription of rRNA. The involvement of a light-activated gene regulatory protein and the coordinated transcriptions of r-protein and rRNA in the figure are consistent with the observations made in previous reports (Asato, 2003; Asato, 1983; Asato, 1998; Marino and Asato, 1986). The curtailment of r-protein and rRNA syntheses can be accomplished by negative feedback control (Keener and Nomura, 1996). Rpb1 synthesis is also curtailed and, as a consequence, the initiation of rRNA transcription stops as well. In support of the feedback control mechanism, a r-protein exhibiting the characteristics of a repressor r-protein has been reported in *Synechococcus (*Meng et al*.,* 1989). S7 is a product of *rps*7 in a r-protein operon structurally similar to *str* operon of *E. coli.* Binding sites of S7 are found on 16S rRNA and r-protein mRNA, indicating that S7 can bind to rRNA transcripts in the formation of ribosomes or bind to mRNA of *str* operon to hinder r-protein translation.

Model 1 assumes the involvement of anti-termination and sigma factors in the regulation of rRNA synthesis in *Synechococcus.* The involvement of other ancillary molecules in stimulating rRNA transcription is not ruled out. The regulatory roles of ppGpp (Mann et al., 1975) and iNTP in the control of rRNA synthesis in *Synechococcus* have not been demonstrated. These molecular effectors do regulate promoter activities in rRNA transcriptions in *E. coli* in conditions of amino acid starvation, or changes in nutrient composition and growth phases. It appears that these molecular effectors are not involved in coordinating r-protein and rRNA synthesis in *Synechococcus* in conditions that support balanced growth (Asato, 2003). Locating *rpb*1 on the r-protein operon as indicated in model I is not a radical proposal. Genes coding for proteins involved in transcription ($\beta$, $\beta_2$ $\sigma$ factor), translation (elongation and initiation factors Ef-G, Ef-Tu, TufA, EF-Ts) and DNA replication (DnaG) are found in r-protein operons.

**Summary, conclusions, and perspectives**

*E. coli* evolved multi-functional control systems of r-protein and rRNA syntheses that respond to changes in the nutritional environments. The control of r-protein synthesis at the translational level involves translational coupling and regulation of translation by repressor r-protein. r-protein transcription can be terminated prematurely by specific r-proteins as in the transcription termination of S10 operon by L4. Significant progress has been made recently in describing the participation of molecular effectors (ppGpp and iNTP) and trans-acting proteins (Fis and H-NS) in controlling the transcription of rRNA synthesis in changing nutritional environments or in different growth phases. It would be of interest to know if there are other molecular effectors that could stimulate r-protein synthesis, and whether these molecular effectors could coordinate r-protein synthesis with rRNA synthesis in ribosome formation. In any case, ppGpp, iNTP, Fis and H-NS proteins are not involved in the growth-rate dependent control of rRNA transcription or feedback ribosome synthesis models; regulators and regulatory mechanisms of these models remain to be determined. The transcription antitermination models of r-RNA operons involve antiterminators and r-protein molecules that permit the read-through of strategically located termination sites within *rrn* operons. There are, however, several problems that must be overcome in order to fully explain the coordinate regulation of r-protein and rRNA in ribosome synthesis. Gene regulatory proteins that stimulate r-protein operons and rrn operons, and the genetic factors that coordinate the r-protein and rRNA syntheses during the progress of the cell cycle need to be determined. The resolution of these problems is will not be easy.

In *Synechococcus,* sequential r-protein and rRNA synthesis periods occur early in the cell cycle during balanced growth. A light responsive DNA binding protein, Rbp1, stimulates rRNA synthesis in light but not in darkness. These observations led to the construction of a plausible model involving Rbp1 as a gene regulatory protein that coordinates r-protein production and rRNA synthesis. The location of the *rbp1*gene locus within a r-protein operon will be crucial in verifying the existence of the model. The value of a genetic scheme is in presenting a heuristic model of coordinate regulation of rRNA and r-protein syntheses where none exits at present. There are several important questions that remain. How is transcription of the entire set of r-protein operons initiated? What will trigger the cell to engage the feedback loop in terminating r-protein and rRNA syntheses?

A similar conundrum exists in elucidating the coordinate regulation of cell cycle events in bacteria. In *E coli* and *Synechococcus*, the common characteristics of cell cycle events are the sequential occurrences of DNA replication, genome segregation and cell division septum formation. In *Synechococcus,* ribosome synthesis appears first in the cell cycle. The coordinate regulation of these major cell cycle events cannot be based solely on physical and physiological parameters or by cell age. The well-ordered sequence of cell cycle events must involve coordinated transcriptions by gene regulatory proteins. A model that describes the coordinate regulation of macromolecular synthesis by employing sequentially expressed regulatory molecules in *Synechococcus* has been reported. It would be of great interest to determine the coordinate regulation of cell cycle in *E. coli*. As indicated by Donachie (2001) attempts must be made to clear the “cloud of unknowing” in the coordinate regulation of cell cycle events in *E. coli*. Recent advances in genetics and molecular biology techniques, and the availability of genomic sequences of increasing numbers of bacteria, may eventually reveal the gene regulatory proteins in coordinating cell cycle events.

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