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GUEST COMMENTARY

Regulation of Ribosome Biosynthesis in *Escherichia coli* and *Saccharomyces cerevisiae*: Diversity and Common Principles

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In celebrating the centennial of the American Society for Microbiology, many people will surely recall the central importance that research using microbial systems played in the birth and the subsequent development of molecular biology in the latter half of the 100-year history. Starting from the demonstration of DNA as the genetic material, a series of key experiments, such as the proof of semiconservative replication of DNA, the discovery of mRNA as the information carrier between DNA and protein, and the eventual elucidation of the genetic code, were done mostly with microbial systems, the enteric bacterium *Escherichia coli* and its bacteriophages in particular. These basic principles in molecular genetics discovered with bacterial systems soon proved to be true for almost all organisms. Consequently, early research activities in molecular biology were concentrated on *E. coli* and related bacterial and phage systems, generating the initial attitude of many molecular biologists reflected in the well-publicized phrase, "What is true for *E. coli* is true for elephants." (The acceptance of such an attitude at that time was not very surprising. Prior to the successful development of molecular biology, research in the field of intermediary metabolism from the 1920s through 1940s had demonstrated abundant evidence for the unity of biochemistry from microorganisms to humans, e.g., the mechanism of energy [ATP] production and its use for anabolic reactions [see also reference 42)]. Starting my first research as a student of fermentation biochemistry in 1950, I was certainly influenced by the prevalent belief, the unity of biochemistry, at that time.) Of course, in view of the bewildering diversity known in biology, especially some fundamental differences between prokaryotes and eukaryotes or single-cell versus multicellular organisms, such a view was expected to be too simple and naïve. Thus, it was soon realized that the actual mechanisms and principles underlying certain biological functions, including diverse modes found in regulation of gene expression, are the consequences of evolutionary tinkering and may not necessarily be universal among diverse organisms (for a detailed discussion on evolution and tinkering, see reference 27). Nevertheless, attempts to extend factual observations or concepts obtained in one system (e.g., prokaryotes) to another (e.g., eukaryotes) have been made repeatedly and often turned out to be stimulating if not successful. As a person who was engaged in studies of synthesis of ribosomes and ribosomal components first in *E. coli* and later in *Saccharomyces cerevisiae*, I will recount some of the research activities on this subject which I have touched upon in this context.

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REGULATION OF SYNTHESIS OF RIBOSOMES AND RIBOSOMAL COMPONENTS IN *E. COLI*

In the 1960s, regulation of ribosome synthesis became one of the central questions in bacterial physiology, mostly triggered by the discovery of a simple linear relationship between growth rates and cellular concentrations of ribosomes in exponentially growing cultures of enterobacteria (47, 65). For a long time, microbiologists had been interested in various factors and conditions that influence growth. In contrast to the complex patterns of development and growth of multicellular organisms, growth of bacteria (e.g., *E. coli*) meant an increase of cell numbers, which followed exactly the equation of an exponential increase, and specific growth rate constants could be measured precisely under carefully set up experimental conditions. Bacterial physiologists were interested in the question of what really determines growth rates. Identification of the ribosome as the essential machinery of protein synthesis in the mid-1950s followed by the discovery of the relationship between ribosome concentrations and growth rates led to the notion that the rate of protein synthesis per unit amount of cellular ribosomes is constant and the rate of bacterial growth is in fact determined by the number of ribosomes in the cell. This notion, the constant efficiency of ribosomes, was especially advocated by the Copenhagen group led by Ole Maaløe and stimulated research on the synthesis of ribosomes and its control (41). However, it should be noted that the initial experiments carried out by the Copenhagen group did not include cultures in conditions in which the bacteria grew slowly (slow growth conditions) and the constant efficiency of ribosomes was only approximate and could be applicable only for medium- to fast-growing cultures. Later studies, especially those done by Arthur Koch and coworkers (36, 37), demonstrated that functional ribosomes are clearly present in excess under slow growth conditions; that is, under such conditions, bacterial growth is not limited by the number of ribosomes. (It should be noted that the rate of peptide elongation for individual ribosomes is constant regardless of growth rates and that the level of free ribosomes not engaged in protein synthesis is elevated in slow growth conditions. Koch argued that the presence of excess functional ribosomes, especially in very slow growth conditions, is advantageous to *E. coli* cells in their natural environment, the human gut, where famine and feast alternate, and prompt adaptation to a nutritional upshift is of great advantage [36].) Consequently, the major question of regulation of ribosome synthesis, growth rate-dependent control, became defined as the question of how bacterial cells adjust ribosome synthesis in relation to synthesis of other cellular components so that the optimum growth rate is attained under medium to fast growth conditions.

Another initial event related to the study of regulation of ribosome synthesis is the discovery of stringent control. Al-

though the cessation of accumulation of stable RNA (rRNAs and tRNAs) in auxotrophic bacteria starved for a required amino acid had been known for some time, it was the discovery of the *relA* gene by Gunther Stent and Sydney Brenner, which clearly defined the phenomenon of stringent control of stable RNA synthesis (70). This discovery stimulated many people to study the mechanism involved in this regulatory phenomenon, leading to the identification of guanosine tetraphosphate (ppGpp) as the key effector molecule in this regulation (5).

Although I was mostly concerned with in vitro studies of ribosome structure, function, and assembly in the 1960s, I started to work seriously in the mid-1970s on the question of ribosome synthesis and its regulation in vivo. With the belief that one has to know and isolate genes for ribosomal components for regulation studies, our initial efforts were aimed at this goal, and by the end of the 1970s, we succeeded in isolating more than half of the ribosomal protein (r-protein) genes and all of the seven rRNA operons and completed mapping and characterization of these genes (reviewed in references 53 and 54). Measurements of synthesis rates of rRNAs, r-protein mRNAs, and r-proteins under various nutritional conditions were performed by using these isolated genes and more improved techniques by several groups, the Copenhagen group, Hans Bremer's group, and my research group in particular. As a result, it became clear that under medium to fast growth conditions, the synthesis rates of all r-proteins reflect their accumulation rates, which in turn reflect the stoichiometric relationship within the ribosome. In addition, the synthesis rates of rRNAs also approximately reflect their accumulation rates under these conditions. Thus, two specific questions were asked: first, what mechanisms ensure the coordination and balancing of synthesis of all the r-proteins as well as those of synthesis of r-proteins and rRNA; second, what mechanism is responsible for adjusting the overall synthesis rates of ribosomes so that most of the ribosomes synthesized are those required for growth, that is, to explain the apparent constant ribosome efficiency?

Regarding the apparent coordination of rRNA and r-protein synthesis, three possibilities were considered. The first was that rRNA synthesis was the primary target of regulatory mechanisms, and the regulation of r-protein synthesis was a consequence of the regulation of rRNA synthesis. The second possibility was opposite to the first, namely, that r-protein synthesis was regulated and regulation of rRNA synthesis was a secondary consequence of this regulation. The third possibility was that both rRNA and r-protein syntheses were regulated directly, and exact coordination was achieved either by a balance of transcriptional and translational efficiencies inherent in the DNA and mRNA structures themselves or by degradation of products synthesized in excess or by both. Maaløe favored the second possibility by proposing the passive regulation model, suggesting that passive regulation acts on transcription of r-protein genes, and r-protein products somehow regulate rRNA synthesis, perhaps by acting as an inducer (40). I thought about the first possibility and considered a negative-feedback inhibition of r-protein synthesis by free unassembled r-proteins to explain coordination and balancing of synthesis of r-proteins and rRNA. Having worked on the in vitro ribosome assembly reaction, the idea of coupling ribosome assembly with gene expression was appealing. The first test of this idea was gene dosage experiments. By increasing the dosages of r-protein operons, it was observed that the rate of transcription increases in proportion to gene dosage increases but the rate of r-protein synthesis does not increase, indicating negative-feedback inhibition at the level of r-protein mRNA translation (14). Direct proof of the model and actual identification of repressor r-proteins were done by in vitro as well as in vivo experiments (84; for detailed historical accounts as well as independent experiments done by others, supporting the translational feedback regulation, see reference 50). Briefly, most, if not all, r-protein operons encode a protein which functions as an autogenous translational repressor acting at a target on the polycistronic mRNA, leading to inhibition of synthesis of all proteins encoded by the mRNA. As long as rRNA synthesis continues, repressor r-proteins are incorporated into ribosomes and the operons continue to express. When rRNA synthesis declines, repressor concentrations increase and the operons are repressed. Coregulation of all the genes within a single operon is achieved because of translational coupling of these r-protein genes combined with some other mechanisms, such as stimulation of mRNA degradation (for reviews, see references 31, 53, and 85). Coregulation of unlinked r-protein operons is achieved not by using a common regulatory protein(s) and target sites with a shared structure but by coupling the translation of all of these unlinked r-protein mRNAs with a single major reaction, ribosome assembly. Although the actual mechanisms of repression are different, depending on the specific operons, and are complex, I thought that the general principle of regulation is simple and beautiful and, therefore, must (or may) be true for other organisms including eukaryotic cells. As will be mentioned below, this supposition turned out to be incorrect and eukaryotic cells were found to use the third possibility mentioned above, i.e., separate and direct regulation of both rRNA and r-protein syntheses.

After the discovery of the occurrence of translational feedback regulation, it was demonstrated that this regulation is in fact responsible for apparent growth rate-dependent and stringent control of r-protein synthesis at least for some r-protein genes; that is, these two control systems act primarily on rRNA synthesis, and their apparent effects on r-protein synthesis are almost certainly consequences of their primary effects on rRNA synthesis (8). The question was then how rRNA synthesis is regulated. Regarding growth rate-dependent control, I thought again about a model using negative feedback to prevent production of excess ribosomes. Gene dosage experiments were performed to test this idea, and it was demonstrated that increases in gene dosage did not increase rRNA synthesis rate but that increasing the dosage of rRNA genes in a mutant form that would not lead to functional ribosomes led to an increased rate of transcription of all rRNA genes combined (29). Although we initially thought about the possibility of free ribosomes in the pool themselves acting as a repressor, later experiments led to the conclusion that an excess translation caused by the excess ribosomes will give a signal, leading to feedback inhibition of transcription of rRNA (and tRNA) genes (7). Since each of the seven rRNA operons has tandem promoters, P1 and P2, and growth rate-dependent control acts on the major P1 promoter and not on the minor P2 promoter (19), deviation from the constant ribosome efficiency model, the deviation in slow growth conditions in particular, appears to be explained based on the ribosome feedback model. Recent work from the Rick Gourse laboratory showed that the basis of the negative feedback is the special property of the P1 promoters requiring high initiating nucleoside triphosphate (NTP) (ATP or GTP) concentrations and thus sensitive to a reduction of NTP concentrations caused by excess translation (16). Other models proposed to explain growth rate-dependent control will be commented upon below.

Studies on stringent control of rRNA synthesis were easier to explain. The mechanism of production of ppGpp upon amino acid starvation was well clarified by in vitro studies (22), and evidence has accumulated indicating that this compound

must be the key effector molecule, leading to various stringent responses (for a review, see reference 6). Cessation of rRNA synthesis may well be a direct inhibition of rRNA transcription by ppGpp produced in a very high concentration upon amino acid starvation, although attempts by many people to test the direct inhibitory effects of ppGpp in vitro yielded conflicting results, and definitive proof for the direct action must still await further studies (6).

It is always appealing to find a unitary model that could be used to explain several related phenomena. Because of the discovery of ppGpp as the primary effector to mediate stringent response reactions, it was natural to consider ppGpp to explain growth rate-dependent control of rRNA synthesis. Although the basal levels of ppGpp in growing cells are not high relative to the level found upon amino acid starvation, there is an excellent inverse correlation between basal ppGpp levels and growth rates (64). The unitary model advocated by Hans Bremer et al. (64) and another model proposed by Jensen and Pedersen (28) have been extensively discussed previously (6, 31) and are beyond the scope of this essay. My only comment here is that the proportionality between ribosome content and growth rates (as originally used to define the growth rate-dependent control) was observed in the mutant (Δ*relA* Δ*spoT* [82]) which does not produce any ppGpp (17, 24); that is, the growth rate-dependent control can take place without ppGpp. Nevertheless, ppGpp might still be involved in the regulation of rRNA synthesis in normal *E. coli* cells. Thus, a compromise we thought about seriously in the past was to postulate ppGpp as an effector of ribosome feedback, i.e., to hypothesize that an excess production of ribosomes leads to excess translation, exceeding the capacity of the cell to maintain charged tRNA levels, resulting in increased ppGpp production that would prevent transcription of rRNA genes from the P1 promoter. However, as mentioned above, it now appears that negative feedback may be achieved simply by a decrease in substrate NTP concentrations. As discussed below in connection with regulation in *S. cerevisiae*, finding two (and perhaps more) different mechanisms in two different contexts may not be surprising at all. In addition, there are several mechanisms involving *cis* elements and *trans* factors known to participate in rDNA transcription, such as Fis-dependent activation and antitermination (for reviews, see references 9 and 20). Although these mechanisms were shown not to be directly responsible for growth rate-dependent control of rRNA synthesis (19, 63, 67), they might play important regulatory roles under conditions that have not been carefully studied.

SYNTHESIS OF RIBOSOMES AND RIBOSOMAL COMPONENTS IN EUKARYOTES

In the mid-1980s, I started to shift research subjects from *E. coli* to yeast, *S. cerevisiae* in particular. I was following research on ribosome synthesis in eukaryotes and knew that there are considerable similarities in regulatory features between prokaryotes and eukaryotes. A series of major discoveries in the 1970s made cloning and manipulation from any organism and the yeast systems *S. cerevisiae* and *Schizosaccharomyces pombe* were especially amenable to genetic and molecular analyses. Genes for r-proteins and rRNA were being cloned and characterized. My first interest was to test whether some of the regulatory mechanisms discovered in the *E. coli* system were applicable to eukaryotes.

Regarding regulation of the synthesis of ribosomes and ribosomal components, there had been considerable research; in particular, many papers had been published by Jon Warner's group and Rudy Planta's group using *S. cerevisiae*. Like *E. coli*, ribosome content increases with increasing growth rate and synthesis of all ribosomal components appeared to be coordinately regulated (e.g., reference 32). With several r-protein genes cloned, it soon became clear that, as already suspected, there was no indication of gene clustering, i.e., no operon structures as seen in *E. coli*. By analyzing the effects of increased r-protein gene dosages on r-protein synthesis, as was done for *E. coli*, people were just beginning to examine the question of whether there is any feedback mechanism to prevent wasteful production of r-proteins. Although it was initially thought that a translational feedback regulation similar to that found in *E. coli* might exist in *S. cerevisiae*, the results were later explained by instability of free r-proteins which are not assembled into ribosomes. In these gene dosage experiments, feedback regulation was not observed in most cases and r-proteins were overproduced, followed by rapid degradation of excess r-proteins (e.g., references 13, 43, 73, and 79). We took a complementary approach, devising genetic systems in which the rate of rRNA synthesis is specifically reduced. For the *S. cerevisiae* system, because of the rapidity of free r-protein degradation, convincing overproduction followed by degradation, i.e., the absence of feedback regulation, was demonstrated only for several r-proteins (80). However, for the *S. pombe* system, the experimental results were convincing because of relatively higher stability of free r-proteins; the synthesis of all the 19 r-proteins analyzed was found not to be significantly affected by cessation of rRNA synthesis and the r-proteins synthesized in excess were slowly degraded (83). From all these experiments, combined with some earlier experiments using anucleolate mutant embryos of *Xenopus laevis* (58) and mammalian cells during inhibition of rRNA synthesis (77), it became clear that a feedback system similar to that found in *E. coli* does not exist in these and perhaps in most eukaryotes. It was a disappointment for me not to find the universality of the feedback mechanism discovered for *E. coli*. However, it should be noted that these attempts to test the *E. coli* regulation model led to the discovery of feedback inhibition of r-protein gene expression at the level of mRNA splicing for two r-protein genes, rpL32 and rpS14 (11, 15), and at the level of mRNA degradation for another r-protein, rpL4 (formerly L2) (61). Dabeva et al. (11) suggested that since the assembly of ribosomes in eukaryotes takes place inside the nucleus (i.e., at the nucleolus) which is separated from the site of mRNA translation, feedback at the level of splicing may be analogous to (and more reasonable than) feedback at the level of translation as observed in *E. coli*. Similarly, degradation of rpL4 mRNA induced by excess rpL4 was concluded to take place in the nucleus and analogy to the feedback regulation of *E. coli* r-protein synthesis was discussed (62). Since many r-protein genes have not been critically analyzed yet, it still may be possible that such feedback systems are not limited to just a few exceptional r-protein genes and may play roles in fine regulation, minimizing wasteful production of r-proteins. (Interestingly, homologs of two of these three r-proteins are also feedback regulated in higher eukaryotes. The *X. laevis* homolog of rpL4 was shown to be feedback regulated at the level of splicing and turnover of precursor mRNA [4], and the human homolog of rpS14 was shown to be feedback regulated at the level of transcription [71]).

Regardless of the extent of operation of the feedback regulation of r-protein synthesis in *S. cerevisiae* and other eukaryotes, it is now abundantly clear that regulation of r-proteins and that of rRNA in eukaryotes takes place mostly independently and mechanisms involved are also likely to be different from those used in prokaryotes. Regarding coordination of synthesis of many (nominally 78) r-proteins in *S. cer-*

evisiae in response to nutritional changes, coregulation appears to be achieved mostly at the level of transcription by the use of target sites with shared sequence features upstream of promoters for r-protein genes where regulatory signals may act (see below). This is a striking contrast to the coregulation of unlinked r-protein operons discussed above. (Interestingly, in mammalian cells, regulation of r-protein synthesis in response to nutritional conditions appears to take place at the level of mRNA translation, although the mechanisms are different from that used for *E. coli* [reviewed in reference 44].)

Another informative case is stringent control. As in the case of *E. coli*, amino acid starvation causes inhibition of the synthesis of not only rRNA but also r-proteins in *S. cerevisiae*. In addition, derepression of many genes involved in biosynthesis of amino acids takes place in response to amino acid starvation. In *E. coli*, all these three (and many other) responses are caused (directly or indirectly) through the initial production of ppGpp by the use of uncharged tRNA and RelA protein on the ribosome. Extensive efforts to look for ppGpp in eukaryotic cells soon after the discovery of ppGpp in *E. coli* all failed, and it is therefore clear that the mechanisms involved must be different between *E. coli* and *S. cerevisiae*. Regarding derepression of amino acid biosynthetic genes in *S. cerevisiae*, extensive work by Hinnebusch and coworkers identified specific genes, such as *GCN1*, *-2*, *-3*, and *-4*, required for this response reaction (caused by histidine starvation) and clarified many steps involved in this regulatory response. According to their model, uncharged tRNA stimulates an eIF2 (translation initiation factor 2) protein kinase encoded by *GCN2* and initiates a signal transduction leading to eventual activation of amino acid biosynthetic genes by transcription factor Gcn4 (25). Remarkably, the activation of the Gcn2 kinase by uncharged tRNA appears to take place on the ribosome and the mechanism of sensing amino acid depletion resembles that used in *E. coli*, namely, the activation of the RelA protein by uncharged tRNA on the ribosome (25). Regarding the stringent control of r-protein synthesis in *S. cerevisiae*, repression was demonstrated not to be affected by mutation in any of the genes *GCN1* to *GCN4* (46). Instead of the pathway involving the *GCN* genes, involvement of protein kinase A (PKA) was suggested, because mutants that express PKA constitutively did not show repression of r-protein gene transcription during amino acid starvation (33). Therefore, the signal transduction pathway(s) including the initial sensor(s) of amino acid starvation for repression of r-protein gene is distinct from that used for derepression of amino acid biosynthetic genes. Thus, even though many of the physiological responses to amino acid starvation are shared by both *E. coli* and *S. cerevisiae* and some particular features of mechanisms might also be shared, the actual mechanisms used are clearly different between the two organisms.

COOPERATIVITY OF RIBOSOME ASSEMBLY AND COORDINATE REGULATION OF rRNA AND r-PROTEIN SYNTHESIS

At the time of the discovery of translational feedback regulation of r-protein synthesis in *E. coli*, I (and other investigators) thought that synthesis of protein is energetically very expensive (much more expensive than RNA synthesis) and thus the feedback mechanism may have evolved to avoid a wasteful overproduction of r-proteins, which all together comprise a significant fraction of total protein in *E. coli*. Therefore, after finding the absence of general feedback regulation of r-protein synthesis in eukaryotes, I wondered why an efficient feedback system was evolved in *E. coli*, but not in eukaryotes. On the other hand, *S. cerevisiae* and other eukaryotes have very efficient regulatory systems to repress rRNA synthesis in response to a decrease in protein synthesis, even though rRNA synthesis is not as energetically expensive as protein synthesis, namely, stringent control of rRNA synthesis in response to amino acid starvation (e.g., references 69 and 78) and repression of rRNA synthesis in response to inhibition of protein synthesis by specific inhibitors such as cycloheximide (69). While thinking about this question, I remembered our own earlier experiments examining the degree of cooperativity of ribosome assembly published in 1969 (55) and thought about the possible importance of the conclusion obtained in relation to this question.

Soon after the successful reconstitution of functional 30S ribosomal subunits from 16S rRNA and a mixture of all 30S r-proteins (TP30) in 1968 (72), we performed simple experiments in which reconstitution reactions were performed with a constant amount of 16S rRNA and various amounts of TP30. If in vitro assembly were completely cooperative, one would expect that the formation of 30S subunits is proportional to the amount of TP30 added in the range of rRNA excess. For example, if the (molar) ratio of r-protein to rRNA were 0.4 to 1, the expectation is that 40% of 16S rRNA would form 30S subunits and 60% of 16S rRNA would be left as free 16S rRNA. The results showed that when the ratio was 0.6 or lower, cooperativity was clearly not complete; e.g., the efficiency of 30S formation was only 14% when the ratio of TP30 to 16S rRNA was reduced to 0.4; i.e., the 2.5-fold decrease of TP30 without decreasing rRNA caused a sevenfold decrease in the efficiency of ribosome assembly. The data suggested the presence of two or three independent nucleation sites (55). I wanted to confirm this earlier conclusion, the absence of complete cooperativity. So, more than 20 years later, we repeated the same in vitro reconstitution experiments, and in addition, we examined the question of cooperativity of ribosome assembly in *E. coli* in vivo (12).

First, we were able to confirm the earlier results regarding cooperativity of in vitro ribosome reconstitution. Second, we measured syntheses of rRNAs, r-proteins, and ribosomes in *E. coli* cells treated with various concentrations of chloramphenicol. It was known that under these conditions rRNA synthesis rates are stimulated, presumably through the operation of the ribosome feedback regulation system that senses "deficiency" of ribosomes through ribosome activity, as described above, and this stimulation was observed. Synthesis rates of all individual r-proteins analyzed relative to total protein synthesis rates were also found to increase greatly, almost certainly through the operation of another feedback system regulating r-protein synthesis as a result of increased rRNA synthesis, as discussed above. However, because of increased inhibition of total protein synthesis, ratios of synthesis rates of r-proteins to those of rRNA were, as expected, found to decrease with increasing concentrations of chloramphenicol. By analyzing synthesis of new intact ribosomes simultaneously, we found that synthesis of completely assembled ribosomes is much more sensitive to chloramphenicol than is r-protein synthesis; analysis of the data indicated that the cooperativity of ribosome assembly in vivo is also not complete as in the case of in vitro ribosome reconstitution (12). Therefore, avoiding conditions of high rRNA/r-protein ratios by regulatory mechanisms such as stringent control must be important to prevent breakdown of cooperative assembly of ribosomes, as evidenced by adverse effects of relaxed mutations under several conditions such as during the recovery from amino acid starvation (1, 6).

As for *S. cerevisiae* and other eukaryotes, no in vitro ribosome reconstitution system is available and the question of cooperativity of ribosome assembly in vivo has never been

specifically asked and studied. In view of the presence of many nonribosomal components in the nucleolus, including many snoRNPs which contain snoRNA (small nucleolar RNA) that appear to interact with nascent rRNAs, helping rRNA modification and presumably rRNA processing and ribosome assembly, we would expect that in vivo the ribosome assembly reaction in eukaryotes must be highly efficient and may be largely cooperative. Nevertheless, eukaryotes have a very efficient regulatory system to repress rRNA synthesis in response to a decrease in r-protein synthesis (as a result of a general decrease in total protein synthesis or of specific amino acid starvation), but apparently not a reverse regulatory system (to repress r-protein synthesis in response to a decrease in rRNA synthesis). Therefore, avoiding conditions of high rRNA/r-protein ratios (but not the reverse ratios) appears to be important for *S. cerevisiae* (and other eukaryotic) cells. Thus, it is quite possible that as in the case of *E. coli*, ribosome assembly in vivo may not be completely cooperative.

From these considerations, I would like to suggest that stringent control seen in both *E. coli* and *S. cerevisiae* may have evolved to prevent overproduction of rRNA relative to r-protein, thus avoiding possible breakdown of cooperative assembly of ribosomes under conditions of high rRNA/r-protein ratios. Similarly, in achieving growth rate-dependent control, the mechanisms that evolved appear to be ones that will ensure preventing high rRNA/r-protein ratios. In *S. cerevisiae* (and other eukaryotic organisms), the mechanism that evolved is independent control of both rRNA and r-protein synthesis, but with some tolerance of wasteful overproduction of r-proteins. In *E. coli*, the mechanism that evolved is direct control of rRNA synthesis with apparently "unregulated" overproduction of r-protein mRNA with efficient feedback at the translation level that adjusts r-protein production to rRNA synthesis and simultaneously prevents wasteful r-protein synthesis.

It should also be noted that stringent control induced by amino acid starvation acts on both rRNA and tRNA syntheses in *E. coli* (26), whereas it acts only on rRNA synthesis and not on tRNA synthesis in *S. cerevisiae* (69). The significance of this difference in stringent control between *E. coli* and *S. cerevisiae* was difficult to understand in the past, but the difference can be easily explained by the hypothesis described above; stringent control may have evolved to prevent states of high rRNA/r-protein ratios, and excess production of tRNA is basically harmless to cell growth. In the case of *E. coli*, the operon structure, which ensures cotranscription of rRNA genes and several tRNA genes, might have evolved first, and the stringent-control system might have evolved subsequently, and hence, have included genes for tRNAs in addition to rRNA genes as its target. A prediction of the hypothesis, the absence of complete cooperativity in ribosome assembly in *S. cerevisiae* (and other eukaryotes), might be difficult to test experimentally, but the answer would be very informative in connection with the questions discussed above.

COMPLEXITY OF CONTROL OF r-PROTEIN AND rRNA GENE EXPRESSION RELATED TO NUTRITIONAL AVAILABILITY IN *S. CEREVISIAE*

S. cerevisiae cells (and most other eukaryotic cells) contain more ribosomes under conditions of fast growth than under slow growth conditions. Even though the relationship between ribosome synthesis rates and growth rates is not established as satisfactorily as for *E. coli* (see, e.g., the article reporting the absence of constant ribosome efficiency [76]), *S. cerevisiae* cells certainly regulate both rRNA and r-protein synthesis rates coordinately, but independently as mentioned above, in response to nutritional availability (32). Recent studies, mostly performed to examine regulation of r-protein gene transcription, have demonstrated participation of several different signal transduction pathways in the regulation. For example, addition of glucose to *S. cerevisiae* cells growing slowly on media containing a nonfermentable carbon source causes an increase in transcription of r-protein genes. It was shown that this up-regulation consists of two phases: an immediate temporary response reaction followed by a second response reaction that reflects regulation during the steady-state growth (21). The first response reaction involves PKA (see also 33 and 48), but the second response reaction is apparently independent of the PKA system (21). The rapamycin-sensitive TOR signaling pathway has been shown to be involved in both phases (60). As mentioned above, stringent control observed during amino acid starvation is apparently achieved through the PKA system (33). Thus, stringent control and growth rate-dependent control are partly overlapped but are separable.

The complexity of regulation of r-protein gene expression is even more bewildering. Warner and coworkers discovered that transcription of both r-protein and rRNA genes is repressed under conditions of inhibition of the secretion pathway (45) and that this down-regulation requires PKC, but not PKA (49). It was proposed that defects in the secretion pathway cause defects in plasma membrane synthesis and that this defect is monitored by a signal transduction system involving PKC, leading to repression of synthesis of r-proteins (and rRNA). The relationship between these various signal transduction pathways and the question of whether they all act on the same target are currently unknown. Even though upstream activation sequences (UASs) for most r-protein genes are similar in their sequence features, containing Rap1 or Abf1 protein binding sites and T-rich elements (59, 81), and those UASs may be the *cis* elements where *trans* factors such as Rap1p or Abf1p may act for regulation, how regulatory signals modulate the rate of transcription is unknown for any of the transduction pathways mentioned above. In addition, the targets for rRNA gene transcription and for r-protein gene transcription are certainly different.

For *S. cerevisiae* rRNA gene transcription, four transcription factors in addition to RNA polymerase I (Pol I) are shown to be required both in vivo and in vitro (30; reviewed in reference 51); therefore, any of these components could be the target for regulation in response to external and/or internal regulatory signals. Although mechanisms of regulation of rDNA transcription are just beginning to be studied for the *S. cerevisiae* system, studies on mammalian Pol I regulation have suggested that a variety of mechanisms may be involved. For example, repression caused by nutritional depletion may be due to inactivation of the transcription factor called TIF-IA (or a similar factor), a factor which is not completely characterized but is distinct from TIF-IB (e.g., reference 66), whereas repression during mitosis may involve inactivation of both SL1 (TIF-IB) and UBF by phosphorylation (23, 34). Thus, independent regulation of transcription of rRNA and r-proteins in *S. cerevisiae* (and other eukaryotes) and its complexity are in a great contrast to the apparent simplicity (and beauty) of the feedback systems involved in growth rate-dependent control of rRNA and r-protein syntheses in *E. coli*. Again, it is evident that there is no necessity for trying to explain known regulation of rRNA synthesis in *E. coli* by a unitary model. As mentioned earlier, it would not be surprising if new regulatory mechanisms were discovered under conditions not well studied so far and if additional complexity were also recognized in *E. coli*.

COMPARISON OF rRNA TRANSCRIPTION SYSTEMS IN PROKARYOTES AND EUKARYOTES

There are three features of ribosomal DNA (rDNA) transcription in most eukaryotes that distinguish it clearly from rRNA synthesis in prokaryotes: (i) the use of a specific Pol I, (ii) the presence of tandemly repeated rRNA genes, and (iii) the presence of the nucleolus. Regarding the number of rRNA genes, *E. coli* has seven, four of which are located fairly close to the origin of replication but are not tandemly connected, whereas the yeast *S. cerevisiae* carries about 150 in tandem repeats. It is not clear why these numbers have been selected during evolution. As mentioned above, a two- to threefold increase in the number of rRNA genes (29) or deletion of four of the seven rRNA genes (10) did not significantly affect the rate of total rRNA synthesis in *E. coli*. Similarly, an *S. cerevisiae* strain which has only about 40 tandem copies, i.e., only one-fourth of that of the wild type, was constructed, and its growth rate and rRNA synthesis rate were the same as those of the wild type (reference 35 and our unpublished data). Thus, both *E. coli* and *S. cerevisiae* cells appear to carry rRNA genes in excess over the number required for maximum growth. Similarly, it is known that the repeat number of rRNA genes in different organisms varies greatly, ranging from less than 100 to over 10,000 per haploid genome, and there does not appear to be a correlation between gene numbers and a cellular demand for high rates of rRNA synthesis in these organisms (for a review, see reference 38).

While studying mutants of Pol I transcription factor UAF, we have recently discovered a phenomenon we call polymerase switch (55a, 75). UAF is a multiprotein transcription factor, which is required for a high level of transcription, but not for basal transcription, of rDNA by Pol I in vitro. It was found that strains defective in one of the specific subunits of this factor give rise to variants able to grow by transcribing endogenous rRNA by Pol II. It was demonstrated that the switch to growth using the Pol II system consists of two steps: a mutational alteration in UAF and an expansion of chromosomal rDNA repeats to the level of about 400. The switch is also accompanied by a striking alteration in the localization and morphology of the nucleolus. We think that rDNA expansion and the alteration of nucleolar structures in these polymerase-switched strains are an extreme example of a general plasticity of rDNA repeat numbers and nucleolar structures. From these and other studies on the relationship between rDNA repeat numbers and components of the Pol I machinery in *S. cerevisiae* (35), we have hypothesized that extra rDNA repeats might be present simply to form suitable nucleolar structures rather than for the necessity to function as templates for rRNA synthesis.

It has been gradually recognized recently that the nucleolus in eukaryotes may have functions other than synthesizing ribosomes (for reviews, see references 56 and 57). A most recent, exciting development is the discovery of nucleolar proteins participating in regulation of cell cycle progression in *S. cerevisiae* (68, 74; reviewed in reference 18). Perhaps the number of rDNA repeats unique to each organism reflects the presence of particular nucleolar structures unique to these organisms (and environmental or developmental conditions), reflecting not only structures required for ribosome synthesis but also other important functions. The plasticity of rDNA repeat numbers and nucleolar structures may also be advantageous to organisms in this respect.

The presence of the nucleolus as the specific site of rDNA transcription and ribosome assembly in eukaryotes raises the question of whether such a structure exists in prokaryotes. More specifically, one can ask whether each of the seven rRNA operons of *E. coli* is located in a different site or whether all of the seven operons are located in close proximity, forming a single factory corresponding to the nucleolus, coordinating rRNA transcription, rRNA processing or modification, and ribosome assembly. If the latter is the case, we could then ask the significance of the chromosomal locations of the seven rRNA operons. As commented previously (52) with respect to the recent work by Asai and coworkers on rRNA gene deletion strains (3), such an analysis should now be feasible using the advanced technology of fluorescence microscopy (see reference 39). In connection with the plasticity of rDNA copy numbers in eukaryotes mentioned above, it should also be noted that *E. coli* growing fast in rich medium has multiple chromosomal replication forks, increasing the copy number of rRNA genes proximal to the replication origin. Perhaps because of this or perhaps because of a selective advantage, tandem genetic duplication by unequal recombination between different rRNA operons takes place at a high frequency, especially under conditions of rapid growth, increasing the number of rRNA gene copies further (3% of population was reported [for *Salmonella typhimurium*] to have such a duplication [2]). Thus, bacteria like *E. coli* have a plasticity in rRNA operon numbers, even though, as mentioned above, the rRNA synthesis rate in *E. coli* is not limited by the number of rRNA operons. If the increase in the number of rRNA genes really has a selective advantage for bacterial cells, the basis for the advantage may have to be something other than increasing rRNA synthesis rate.

By concentrating on a few model organisms, initially on *E. coli* and then on *S. cerevisiae* and a few other model eukaryotic organisms, molecular biologists have been successful in elucidating mechanisms of regulation of gene expression. Comparison of prokaryotes exemplified by *E. coli* with eukaryotes exemplified by these model eukaryotic organisms has revealed very often or almost always some significant differences in underlying molecular mechanisms, even though they often share apparently similar regulatory features, as discussed with respect to stringent control and growth rate-dependent control of ribosome biosynthesis. The diversity of regulatory mechanisms among different organisms confirms the notion of evolutionary tinkering mentioned at the beginning of this article. Careful analysis of differences and diversity may sometimes reveal the significance of the mechanisms and some general biological principles that may have been left unnoticed, if such comparative analyses were not done. Even comparisons among different bacterial species, e.g., comparison of regulatory systems between *E. coli* and *Bacillus subtilis*, may be rewarding in this respect. Because of the rapid increase in the number of diverse microorganisms whose genome sequences are completely determined, combined with remarkable technological progress (such as DNA chips) that is making comprehensive analysis of gene expression pattern easier, comparative analysis of gene expression must soon face enormous amounts of information revealing similarities and differences in regulatory patterns of gene expression among diverse organisms. We expect and hope that those abundant data to be generated in the coming genomics era will lead to new and deeper insights into general and specific features of regulation of gene expression and their evolutionary significance.

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