11
Regulation of Ribosome Biosynthesis in *Escherichia coli*

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Overview of Ribosome Biosynthesis Regulation

In all living organisms, protein biosynthesis, a central process important for cellular growth and development, is catalyzed by the ribosome. The ribosome represents a cellular paradigm for a macromolecular machine and, essentially, translates genetically encoded information into functionally active proteins. Structurally, the ribosome is composed of both RNA and proteins interwoven in a complex three-dimensional-fold (see Chap. 2). This complex structure necessitates an ordered assembly process (see Chap. 3), as well as coordinated production of the constituent RNA and protein molecules (discussed below). Specifically, the regulated synthesis of the ribosomal components and the cooperativity of the assembly process ensure that each ribosome contains a full compliment of it constitutive parts.

Additionally, the ribosome is physically large (2.5 MDa in *Escherichia coli*), its 3 RNA chains comprise 4566 nucleotides [1] and the 54 proteins, 7343 amino acids [2]. In bacteria, ribosomes can account for as much as 50% of the cell dry mass [3–5], whereas in eukaryotes the corresponding value is less than 5% [6]. Taking into account that the translational apparatus comprises, in addition to ribosomes, elongation factors (e.g., EF-Tu is the most common protein in the bacterial cell contributing ~10% of the total protein mass), synthetases and tRNAs, one can estimate that up to 60% of the total cell energy is expended on the synthesis of the translational apparatus. This enormous energetic commitment by the bacterial cell requires a coordinated synthesis of rRNA and ribosomal proteins. Accordingly, an intricate network of regulations exists in bacteria (i) to ensure a balanced synthesis of rRNAs and ribosomal proteins and (ii) to adapt ribosome synthesis to the cells nutritional environment. Only under unfavorable conditions and during the stationary phase, are ribosomes present in excess. During stationary phase, 70S ribosomes are present as inactive 100S dimers due to a ribosome-associated protein (ribosome modulation factor, [7]). Consistently, null mutations of this factor affect viability of cells at stationary, but not log phase [8].

The tight coupling of ribosome synthesis to growth rate of the cell is termed *growth rate control* and is defined as "the number of ribosomes per unit amount of cellular protein in *E. coli* is proportional to the growth rate (μ), and the rate of ribosome biosynthesis is proportional to μ^2 [9]". One important control mechanism not

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observed in eukaryotes is termed the stringent control, which results in an immediate stop of RNA synthesis upon a shortage of amino acids. Further bacterial control mechanisms for ribosome biosynthesis are present at the level of ribosomal RNA (rRNA) transcription (see Ref. [9] for a review of rRNA regulation) as well as translation of ribosomal proteins. We will see that the latter, called "translational control", is regulated in response to the rRNA levels (see Ref. [10] for a review of r-protein regulation), whereas rRNA synthesis is directly regulated (growth rate control).

In this review, we will outline the fundamental ideas governing the regulation of ribosome biosynthesis and where established and proven models exist we will delve into specific examples. In the case where more detailed knowledge is required we would direct the reader to the comprehensive reviews given by Refs. [9–11].

11.1 Regulation of rRNA Synthesis

11.1.1 Organization of rRNA Operons and Elements of rRNA Promoters

In *E. coli* there are seven copies of the rRNA operons arranged within the first half of the chromosome relative to the origin of replication (Fig. 11.1A). As is characteristic for highly expressed genes the rRNA operons are transcribed in the direction of DNA synthesis (Fig. 11.1A) to avoid clashes between replicase and transcriptase. The reason is that only under these conditions can transcriptase transcribe the operon continuously from initiation to termination in harmony with the replicase synthesizing the leading DNA strand, rather than being interrupted by gaps between the Okazaki pieces of the lagging strand of the replication fork. Furthermore, the clustering of the rRNA operons around the origin of replication (Fig. 11.1A) ensures that their relative gene dosage is much greater then the absolute gene dosage, since replication eyes around the replication origin can lead to partial di- and tetra-diploidy (Fig. 11.2). This may be important in rapidly growing cells where initiation of DNA synthesis is faster than cell division, ensuring that multiple copies of the genome around the origin of replication exist in the cell.

In *E. coli* the rRNA operons are designated *rrnA*, *rrnB*, *rrnC*, *rrnD*, *rrnE*, *rrnG*, and *rrnH*. In the rRNA operons, the 16S, 23S, and 5S rRNA, as well as several tRNA genes are co-transcribed as a single transcript (Fig. 11.1B), which is subsequently processed to generate the individual rRNA (see Chap. 3) and tRNA molecules. Transcription of the 5S, 16S, and 23S molecules as a single transcript ensures stoichiometric production of the rRNAs; however, it should be noted that in a few prokaryotic species the rRNA operons are fragmented, e.g., in the bacteria *Thermoplasma* 23S, 16S, and 5S rRNA are all transcribed from separate promoters, whereas the bacterium *Thermus thermophilus* and archaeon *Desulfococcus*, the 16S and (23S+5S) have individual promoters (Fig. 11.3)

The rRNA genes are transcribed from tandem promoters called the *rrn* P1 and *rrn* P2 promoters (Fig. 11.1B). Both promoters have similar core sequences consisting of –10 and –35 regions that are recognized by the σ^{70} subunit of the RNA polymerase

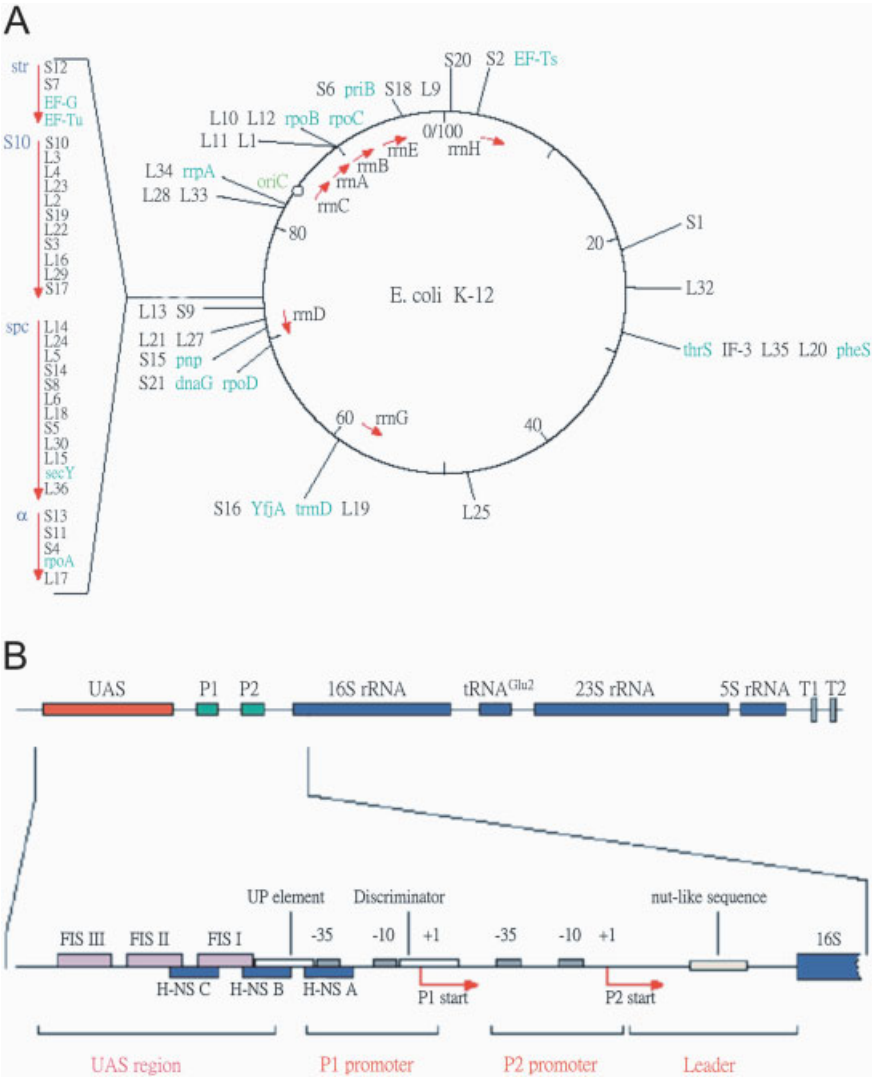

Figure 11.1 (A) Genetic organization of the rRNA and r-protein operon within the *E. coli* chromosome. The cluster of the four large protein operons is shown on the left (str, S10, spc and α operon). Non-ribosomal components are colored. The red arrows indicate the direction of transcription. OriC, origin of replication. (B) The architecture of the rRNA operons, where promoter and regulatory regions are enlarged. See text for details. (A) and (B) have been reproduced from Refs. [50] and [24] respectively.

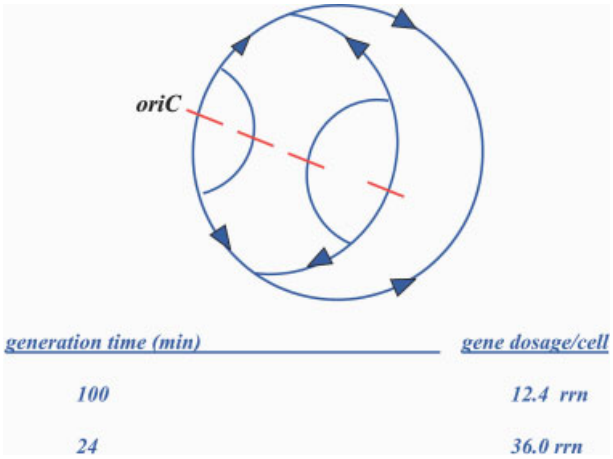

Figure 11.2 Relative gene dosage: during DNA synthesis, transcription units closer to oriC exist in a higher relative copy numbers compared with more distal operons. The relative copy numbers of rrnC, A, B, and E will exceed those of rrnH, D, or G, when cells grow in a rich medium.

(RNAP), although they do not have perfect σ^{70} consensus sequences. Additionally, both have a G+C-rich sequence downstream of the –10 region called a discriminator sequence, which plays a role in regulation during the stringent response (see Sect. 11.1.3). Until recently, it was believed that the *rrn* P1 promoter was regulated whereas the *rrn* P2 was constitutively active at low levels. However, recent work suggests that the P2 promoter is regulated similar to the P1 but not nearly to the same extent [12]. Table 11.1 compares structural features and the extent of regulation with that of the *lac* promoter.

The fact that in fast growing *E. coli* cells the majority of the RNAP initiates transcription at the *rrn* P1 is due to recruitment by the upstream activating sequences (UAS; Fig. 11.1B; Ref. [13]). The UAS includes the UP element which is a A+T-rich sequence upstream of the –35 element as well as 3–5 binding sites for a *trans*-acting

Table 11.1 Features of the rrn P1 and P2 promoters and comparison of their relative transcription intensities with that of the *lac* promoter

	rrn		
	P1	**P2**	***lac* promoter**
–35 region	TTGTC	TTGACT	TTGACA
–10 (TATA box)	TATAATG	TATATA	TATAAT
Discriminator	CGCC(T/A)CC	C(G/A)C(C)ACC	–
Start transcription	A/G	C/T	A
Regulation	+++	+	++ (*lacI*)
Transcription	3-10	1	0.25-1

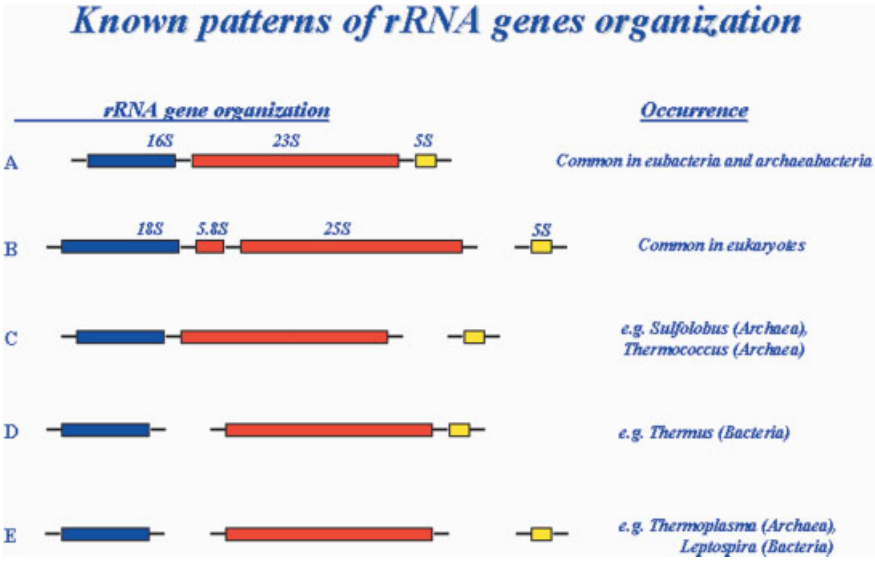

Figure 11.3 Various patterns of organization of rRNA genes in different organisms. In most prokaryotes, rRNA genes are expressed as one operon, and in eukaryotes, the 5S rRNA genes are expressed independently. Some exceptional organizations of rRNA genes in a few prokaryotes are presented in this figure. Similar to *Sulfolobus* [85] and *Thermococcus* [86], 16S and 23S rRNA genes are linked, whereas the 5S rRNA gene is not. In *Thermus thermophilus* [87], the 16S rRNA gene is separated from and transcribed independently of the 23S and 5S genes. *Thermoplasma* contains one copy of each of the rRNA genes, dispersed in the genome and separated by at least 52 kbp [88]. Also in *Leptospira* the three rRNAs are transcribed separately [89].

protein called Fis. In the UAS of the *rrn* P1 promoter, the UP element is the most important factor for activating transcription (20–50-fold activation; Ref. [13]). This activation is promoted through an interaction with the C-terminal domain of the α subunit of RNAP (αCTD) and the minor groove of the DNA representing the UP element [14, 15]. Similar to the UP element, Fis also interacts with the αCTD but instead uses protein–protein interactions [16, 17] to stimulate transcription 3–4-fold [13]. The binding sites of Fis on the DNA are spaced in a way that on the DNA helix the Fis proteins bind on the same side of the helix and induce a DNA bending, which facilitates the downstream TATA box to be melted [16, 18]. Fis is not essential for growth, but plays an important role in the feedback mechanism of growth rate control [11]. The binding of Fis proteins is counteracted by the H-NS proteins that bind downstream, partially overlapping the Fis-binding sites (Fig. 11.1B; Ref. [19]).

11.1.2 Models for rRNA Regulation

Regulation of rRNA synthesis is believed to be primarily due to a feedback mechanism that relates the excess translational ability of the cell to the production of rRNA. This is logical because the translation ability of the cell is synonymous with the amount of ribosomes present, which in turn is influenced by the production of RNA. Although this feedback mechanism was demonstrated in several ways, it can be nicely elucidated from experiments that show overproduction of the rRNA inhibits its transcription from the *rrn* promoters, but only if the overproduced rRNA was competent to form active ribosomes that could engage in protein synthesis [20].

Although the Fis and UP elements are responsible for the strength of the *rrn* promoters [13], they are not probably involved in growth rate regulation, since promoters with deletions in the UAS are still actively regulated [21]. Although there is still disagreement over the source and mechanism of growth rate regulation (see Ref. [22] for a discussion), the most probable molecules to be involved in regulating rRNA synthesis are ppGpp and iNTPs [23, 24]. The ability of ppGpp to regulate rRNA synthesis during extreme amino acid starvation or stringent response has been well studied (see Sect. 11.1.3). ppGpp is proposed to regulate transcription by decreasing the half-life of the open complex formed by RNAP during initiation of transcription. The half-life of the open complex at rRNA promoters is normally much shorter than that of other cellular promoters, e.g., promoters of genes involved in amino acid biosynthesis [25]. This marked difference in half-life can then explain the extreme sensitivity of rRNA promoters to the destabilizing effects of ppGpp [25]. The intercellular levels of ppGpp vary with growth rate in agreement with the idea that they couple rRNA synthesis with the growth rate [26].

In addition to (p)ppGpp, iNTPs (the NTPs that represent the initiating nucleotide in rRNA synthesis: GTP in *rrn*D P1 and ATP in the other *rrn* P1 promoters; Fig. 11.1B and Table 11.1) are presumed to confer growth rate control, because initiation of transcription from the RNA promoters is strongly dependent on their concentration. Gaal *et al.* [24] propose that the intercellular levels of GTP and ATP would vary depending on (i) the nutritional environment and (ii) on the translational capacity of the cell, since the process of translation would consume ATP and GTP. In the model presented in Fig. 11.4 by Gaal *et al.* [24], these properties are combined to explain both feedback regulation and growth rate control of rRNA synthesis. It should be noted however that this model is largely contested, as it is not known if the intercellular concentrations of NTPs vary significantly [27].

Schneider *et al.* [26, 28] observe that the roles of ppGpp and iNTPs in rRNA regulation may be complementary to "increasing the regulatory robustness of the system" and function during different growth phases.

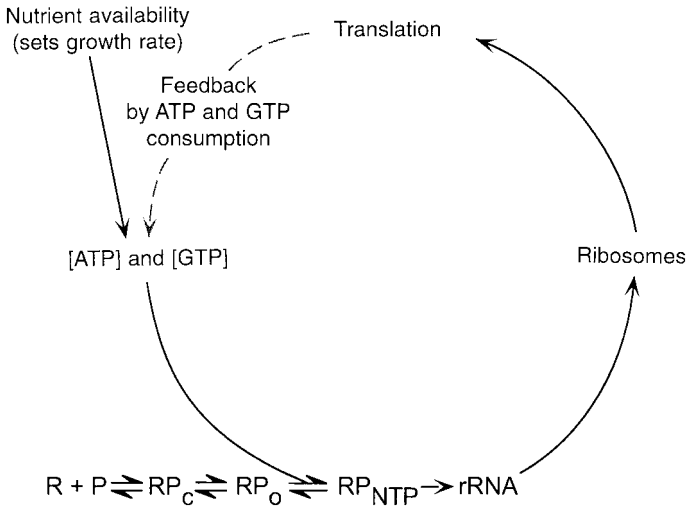

Figure 11.4 NTP-sensing model for RNA regulation. The intercellular ATP and GTP concentrations regulate the stability of the complex of the RNAP at the *rrn* promoters. When the concentrations are sufficient, initiation of rRNA transcription ensues leading to the production of ribosomes. Upon formation, the ribosome will engage in protein synthesis (translation) and in doing so consume GTP and ATP. If the translational activity of the cell is too high with respect to nutrient availability, the GTP and ATP levels will fall, which will in turn limit the initiation of rRNA transcription and therefore couple ribosome production to the growth rate of the cell. This figure has been reproduced from Ref. [24].

11.1.3
Stringent Response

Curtailment of nutrient supply results in an adaptation response in many bacteria termed the *stringent* response. The stringent response is a unique case of rRNA regulation that is triggered by amino acid starvation. It stringently couples protein synthesis with that of RNA and is characterized by (i) a rapid shut down of stable RNA (rRNA, tRNA) synthesis, and (ii) a sudden and significant increase in the levels of ppGpp and pppGpp. The basic level of (p)ppGpp in the cell is about 60 μM and can increase to mM values, i.e., virtually the whole cell content of GDP and GTP is converted to (p)ppGpp [23].

The stringent response provides the cell with a prominent regulatory means to control gene expression. The effect is two-fold: (i) transcriptional repression of genes associated with the translational apparatus, e.g., genes encoding tRNAs, rRNAs [29], ribosomal proteins, translational factors and synthetases [30]. (ii) Up-regulation of genes encoding metabolic enzymes, especially those involved in amino acid biosynthesis [31].

Activation of the stringent response initially stems from the shortage of one (or more) amino acid(s), which in turn produces a significant increase in uncharged-tRNA (deacylated tRNA) for the corresponding amino acid(s). In log-phase bacterial

cells, deacylated tRNA constitutes approximately 15% of the total tRNA, the majority of which is present in a bound state, namely, bound either to ribosomes or synthetases. Under conditions of amino acid starvation, the deacylated tRNA fraction can increase to over 80% of the total tRNA [32]. The scarcity of the aminoacylated tRNA, compounded by the large pools of free deacylated tRNA, enables deacylated tRNA to bind an empty ribosomal A site, conditional to the presence of a cognate codon. The presence of a deacylated tRNA at the A site triggers RelA-dependent synthesis of guanosine 5′-triphosphate 3′ diphosphate (pppGpp) and guanosine 3′, 5′ bisphosphate (ppGpp), collectively referred to as (p)ppGpp. The reaction catalyzed by RelA utilizes ATP and GTP, or GDP, to produce AMP and pppGpp, or ppGpp, respectively (Fig. 11.5, Refs. [33–35]). The products, (p)ppGpp, most probably exert a regulatory effect on transcription via an interaction with the β-subunit of the transcriptase [36–38]. (p)ppGpp is again converted to GTP and GDP, respectively, by the enzyme SpoT (Fig. 11.5; Ref. [39]).

The inhibition of transcription, resulting from the starvation-induced inhibition of translation, is almost immediate (within a minute) and is termed stringent control [40]. This stringent coupling between translation and transcription can be relieved by mutations in either *relA*, the gene encoding the stringent factor RelA [40, 41], or in *relC*, the gene for ribosomal protein L11 ([42, 43]; *relC* ≡ *rplK*). Uncoupling the activities establishes a relaxed phenotype, where RNA synthesis can continue for a period of more than 1 h following translation inhibition [44].

Early studies demonstrated that RelA binding to 70S ribosomes is essential for the production of (p)ppGpp synthesis [45–47] and that RelA binding is enhanced by the presence of a poly(U)-mRNA [48]. The synthesis of (p)ppGpp has been shown to be dependent on a deacylated tRNA at the A site [33] and inhibited in the absence of L11 *in vivo* [42].

Recently, the mechanism of RelA-mediated (p)ppGpp synthesis has been dissected *in vitro* [49]. It was shown that binding of RelA to the ribosome is predominantly influenced by mRNA and not by deacylated tRNA or L11. In contrast, RelA-catalyzed (p)ppGpp synthesis is strictly dependent on L11. Furthermore, it has been clearly demonstrated that it is the release of RelA from the ribosome, not release of the deacylated tRNA, that is concomitant with (p)ppGpp synthesis. Figure 11.6 illustrates

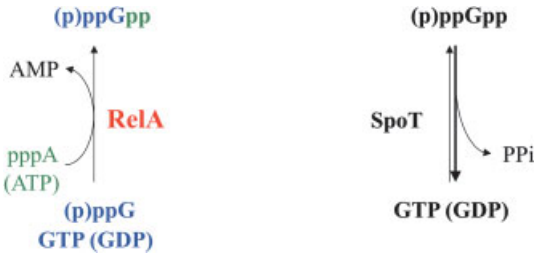

Figure 11.5 Synthesis of (p)ppGpp by RelA and its degradation by SpoT.

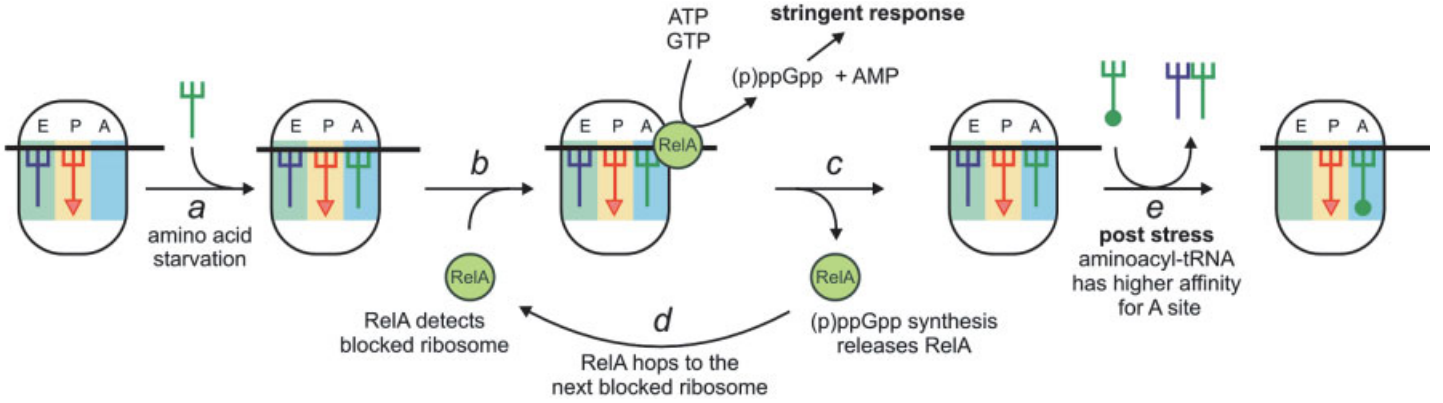

Figure 11.6 Model for mechanism of RelA-mediated (p)ppGpp synthesis: (a) amino acid starvation generates large pools of deacylated tRNAs, which binds to the ribosomal A site with low affinity and blocks the ribosome, (b) RelA detects a blocked ribosome with a 3′-extension of the mRNA, (c) RelA mediates the conversion of ATP and GTP(GDP) to AMP and (p)ppGpp in the presence of a deacylated tRNA at the A site. Release of RelA but not the A-site-bound deacylated tRNA occurs simultaneously with RelA-mediated (p)ppGpp synthesis. (d) RelA 'hops' to the next blocked ribosome and the synthesis of (p)ppGpp is repeated. High levels of (p)ppGpp activate the stringent response. (e) Aminoacylated tRNAs are replenished following post-stress conditions. The higher affinity of an aminoacylated tRNA over deacylated tRNAs for the A site enables displacement of the deacylated tRNAs, which rescues blocked ribosomes and reactivates translation. Note that binding of an aminoacylated tRNA at the A site also results in concomitant release of the E-site-bound tRNA (reviewed in Ref. [90]), thus two deacylated tRNAs are released. Reproduced from Wendrich *et al.* [49].

the present understanding of the mode of action of RelA during the stringent response.

11.2 Regulation of r-protein Synthesis

11.2.1 Some General Remarks

In *E. coli* there are 54 proteins comprising the ribosome. Unlike the rRNA genes, which generally exist in multiple copies within the ribosome, the r-protein genes are present in only a single copy [50]. About half the r-proteins are arranged in the *spc*, S10, *str*, and α operons, whereas the remaining r-proteins are scattered throughout the *E. coli* chromosome in operons containing 1–4 genes (Fig. 11.1A; Ref. [10, 50]). The r-protein operons are often named based on the fact that they harbor genes whose mutations confer resistance to an antibiotic [10]. The r-proteins operons are depicted in Fig. 11.7; however, it should be noted that the division of the operons is not so clear, as often an upstream operon will transcribe into a downstream operon [10]. For example, the α operon can be transcribed from both its own promoter and by RNAP initiating on the *spc* operon promoter [51, 52].

As mentioned above, regulated production of the r-proteins with respect to the nutritional state of the cell is believed to be mediated indirectly by the cellular rRNA levels (reviewed in Ref. [10]). Namely, r-proteins are produced to a level which matches the production of rRNA and when r-protein production exceeds this level, then the accumulation of free ribosomal proteins *feedbacks* and negatively regulates r-protein expression. This is termed *autogenous control* and dictates that, after synthesis from a polycistronic mRNA, a single protein acts both as a r-protein and as a regulatory protein such that its accumulation in an rRNA-free form leads to inhibition of expression of the entire mRNA (Fig. 11.8).

As seen in Fig. 11.7, the regulatory proteins (red boxes) are very often primary rRNA-binding proteins, i.e., that they are capable of binding to the free ribosomal RNA. In contrast with a widespread assumption [10, 53, 54], this does not mean that they associate with the ribosome very early in its synthesis – in fact, only two proteins, L24 and L3, are capable of initiating the assembly of prokaryotic ribosomes (see Chap. 3.2), whereas all other proteins can bind to the rRNAs with high affinity only via the help of other proteins. Nevertheless, if a ribosomal protein can bind already to the naked rRNA, its affinity to a partially assembled subunit is even higher.

Inherent in the *autogenous control* model is the idea that the rRNA and the mRNA targets compete for binding to the regulatory protein. Direct binding to the mRNA targets has been shown for many of the regulatory proteins including L10–L12 [55], S4 [56], S8 [57], S15 [58] and most recently L4 [59]. Additionally, it has been proposed that a *molecular mimicry* exists between the mRNA and rRNA targets and, in some cases, e.g., L1 [60], S8 [57], S15 [61], L20 [62] and L4 [59], this has been established.

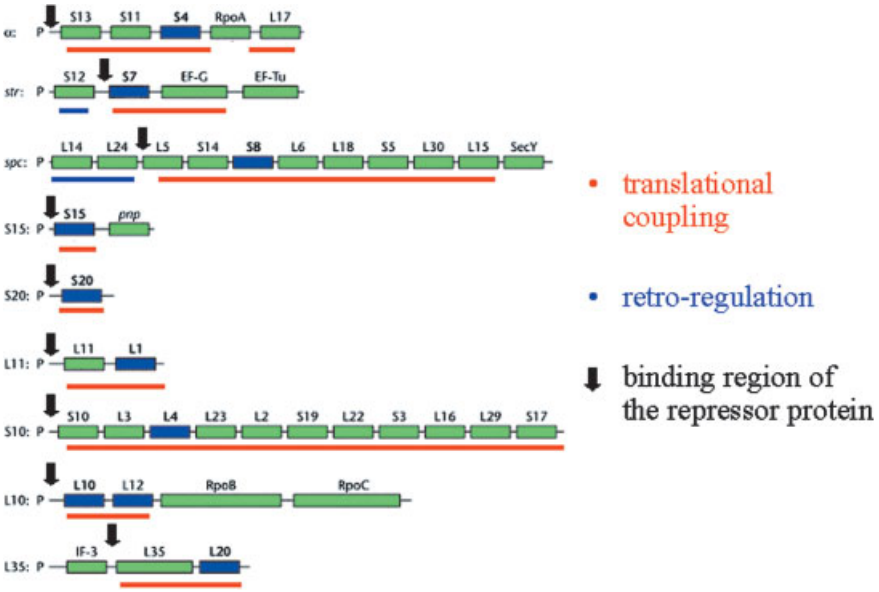

Figure 11.7 The operon structure of the r-proteins. Ribosomal protein operons regulated by translational feedback. The name of each operon is given. P denotes the transcription start site. Individual genes of the operon are shown as green boxes and labeled according to the gene product. The regulatory product is indicated by a blue box and the respective mRNA binding site is shown by an arrow. Genes underlined red are under translational feedback regulation; genes underlined blue are not. In case of the L10 operon the regulator is a complex of L10(L7/L12)$_4$. This figure has been modified from Ref. [50].

The *autogenous control* of r-protein operons is believed to be regulated at various levels:

1. *Transcription*: The binding of the regulatory r-protein effects the elongation of the mRNA. This control mechanism is seen in the S10 operon, where L4 functions in translational attenuation (Sect. 11.2.2.2).
2. *Translation*: The simplest assumption is that the regulatory ribosomal protein binds to the ribosomal binding site of the first cistron and thus interferes with the 30S *de novo* initiation. However, this is not what is observed; instead quite often two alternative secondary structures can form including the ribosomal binding site; one conformation preventing the formation of a productive initiation complex, the other allowing initiation. The regulatory protein is stabilizing the non-productive conformer. In particular, the binding of the regulatory r-protein can (i) entrap the ribosome–mRNA complex in a state that is not competent to initiate translation as seen with the α-operon (called entrapment; see Sect. 11.2.2.3), and (ii) trigger a conformational change in the

mRNA that obscures the RBS in secondary structure so that initiation is inhibited.

3. *mRNA stability*: The binding of the regulatory r-protein destabilizes the mRNA such that it is more readily degraded and therefore cannot be translated (see Sects. 11.2.2.1 and 11.2.2.4).

In many cases, the regulatory protein binds to the 5′ leader sequence directly inhibiting translation of the first gene of the operon, whereas the translation of the following genes is inhibited indirectly by disrupting *translational coupling*. Translational coupling is the phenomenon, where the ribosomal binding sites (RBS) are buried in secondary structures, which are unfolded by ribosomes translating the preceding cistron. When translation of the preceding cistron is finished, the empty 70S ribosome does not necessarily fall off the mRNA but can scan until it reaches the ribosomal binding site of the downstream cistron. fMet-tRNA is bound to the P site with the help of IF2, and translation of the downstream cistron commences. The initiation type is called "70S type initiation" in contrast with the canonical 30S *de novo* initiation (see Chap. 7.1). This "70S type initiation" is a speciality of polycistronic mRNAs encoding ribosomal proteins, and it is this type of initiation that requires N-blocked methionyl-$tRNA_i$ rather than the 30S *de novo* initiation [63, 64]. Since eukaryotic mRNAs are monocistronic and therefore require 40S *de novo* initiation, the initiator Met-$tRNA_i$ does not need to be formylated.

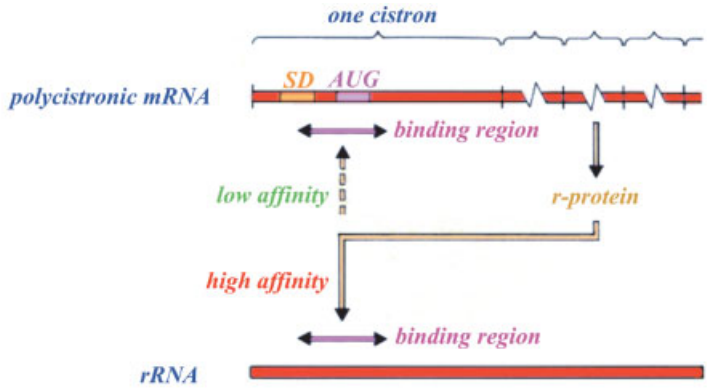

Figure 11.8 Scheme of the translational control of ribosomal proteins. Under fast growth conditions ribosomal proteins do not have a free pool, therefore synthesized ribosomal proteins flow directly into ribosome assembly. However, under some unfavorable conditions, a pool of ribosomal proteins does exist, so that the regulatory proteins indicated below (see also Fig. 11.7) can now bind to their respective mRNA and block the translation of the whole polycistronic mRNA. If the synthesis of ribosomal proteins and rRNA reaches again a molar balance, the ribosomal proteins will dissociate from the mRNA and bind to the higher-affinity binding site on the partially assembled ribosome.

11.2.2 Various Models for r-protein Regulation

11.2.2.1 *spc* operon

The regulatory protein S8 binds between the second and third cistrons, just in front of the L5 cistron of the *spc* mRNA (see Fig. 11.7). Therefore, the downstream genes are regulated via translational coupling. What happens with the genes L14 and L24?

They are repressed by S8 indirectly: when the binding of S8 blocks the translation of the L5 cistron and the downstream cistrons, the mRNA is freed of ribosomes and is thus a target for endonucleolytic RNases. Afterwards, 3′ to 5′ exonucleases will digest the L24 and L14 cistrons ("retroregulation", Ref. [65]).

S8 binds to an mRNA structure similar to the rRNA-binding site with a 5-fold lower affinity [57]. The L5 initiation codon AUG is bulged out but not the Shine–Dalgarno sequence [66, 67]. The ribosomes might be trapped on the SD in a non-productive complex, if S8 is bound in the front of the L5 cistron.

11.2.2.2 **S10 operon**

The S10 operon encodes 11 proteins (Fig. 11.7) and expression of the r-proteins from this operon is regulated by L4 [54, 68]. The regulation by L4 is unique among the r-proteins because it acts at both the transcriptional and translational levels, where both contribute about equally to attain a maximal repression of 25-fold [69]. The determinants for the transcriptional and translational controls are both located within the 172 nucleotide 5′ untranslated region seen in Fig. 11.9. Within this region helices HD and HE are required for transcriptional control, whereas helix HE and the unstructured downstream sequence are required for translational control ([69–72]; Fig. 11.9). Within this region, L4 is proposed to form specific interactions with the loop of helix HD and non-sequence specific interactions with helix HE (Fig. 11.9A; Ref. [59]).

L4-mediated transcription termination occurs on the descending side of helix HE at a string of U's [73] that resemble a rho-independent terminator. It is believed that as the RNAP bound by the transcription factor NusA – a protein that helps RNAP correctly recognize termination signals – transcribes the S10 operon it briefly pauses at a NusA-dependent site before resuming transcription ([74, 75]; Fig. 11.9B). However, under conditions where there is an excess of free cellular L4, this r-protein would bind to its target on the nascent mRNA transcript resulting in structural changes that are propagated to the RNAP–NusA complex [59]. This would prolong the NusA-dependent pause and seemingly stimulate transcription termination (Refs. [74, 75]; Fig. 11.9B), thereby down-regulating r-protein expression from the S10 operon.

The mechanism of L4-mediated translational control is not so well studied; however, it may also operate using an entrapment-based mechanism. This is because the mRNA target for L4 does not include the RBS [59] and, therefore, is not probably

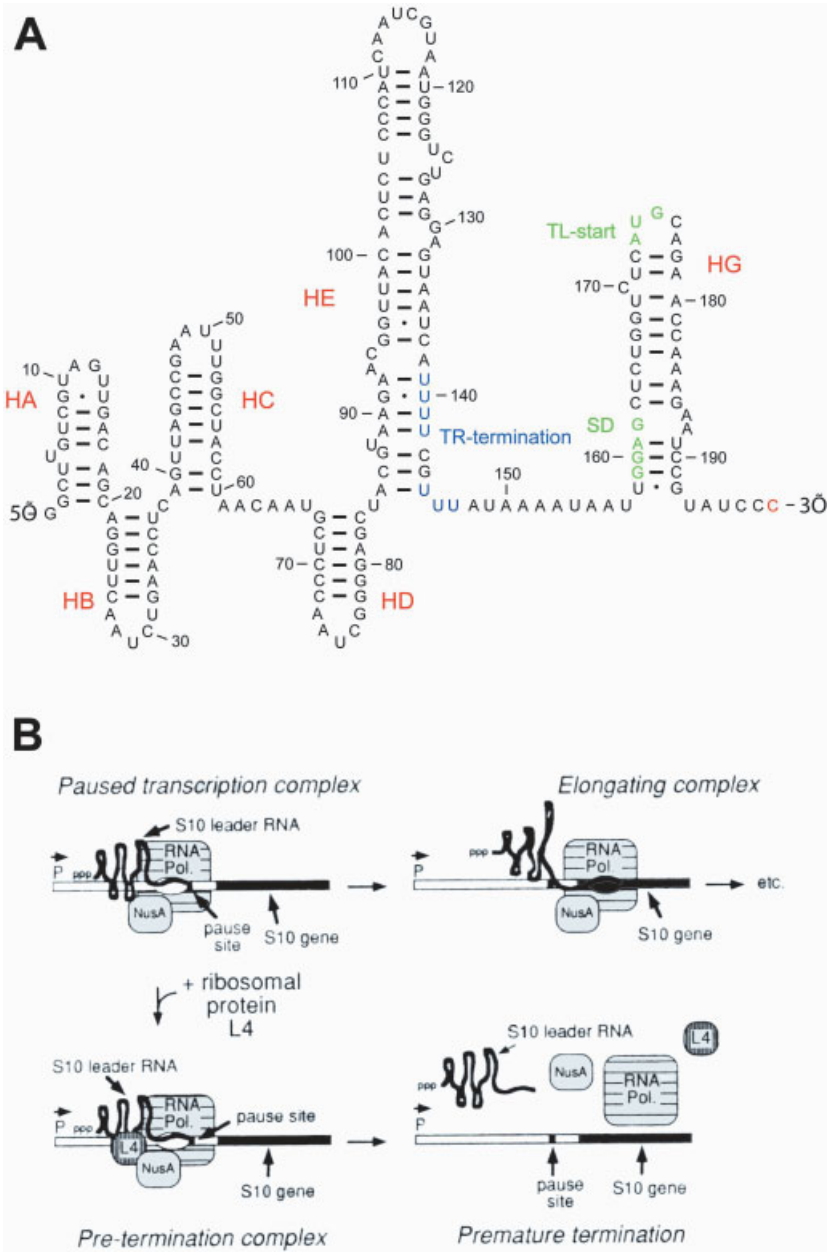

Figure 11.9 Transcriptional regulation of the S10 operon. (A) The secondary structure of leader sequence is illustrated with the L4-dependent transcriptional termination site colored blue, and the translational start site and SD sequence for the S10 gene colored green. (B) A model for L4-dependent transcriptional attenuation is illustrated. Panel A and B have been reproduced from Refs. [59] and [10], respectively.

competing with the ribosome for binding to the mRNA. Additionally, chemical probing experiments show no structural rearrangements in the mRNA leader sequence in the vicinity of the RBS [59] and therefore it is unlikely that L4 binding results in the RBS becoming sequestered in secondary structure. In this case, it is probable that L4 binding stabilizes the ribosome–mRNA complex in a pre-initiation state that is not competent to continue along the initiation pathways as described below for the α operon. The inhibition of translation initiation would block translation of the first gene in this operon, S10, whereas expression of the other downstream genes would be inhibited by disrupting translational coupling [76].

11.2.2.3 α operon

The α operon comprises four r-proteins (S13, S11, S4, and L17), as well as the gene encoding the α-subunit of the RNAP (Fig. 11.7). Expression of the r-proteins is regulated by S4 [77]. S4 binds to a nested pseudoknot structure (Fig. 11.10A) in the 5′ leader sequence upstream of S13 [56, 78]. This pseudoknot structure is believed to exist in two folded states, one that is active and one that is inactive for formation of a ternary initiation complex (Fig. 11.10B; Refs. [79, 80]). In the scheme presented in Fig. 11.10B, the 30S subunit is capable of binding both forms with equal affinity, whereas S4 binds and stabilizes the inactive form driving the equilibrium towards the inactive state [81]. In the absence of free S4, the 30S is capable of binding the mRNA in either state and eventually form an initiation complex, since the active and inactive forms are inter-convertible (Fig. 11.10B). The S4-bound inactive mRNA is free to interact with the 30S subunit (i.e., it does not prevent 30S binding), but the 30S-mRNA–S4 complex cannot be converted to an active state that is capable of progressing through the initiation pathway and bind fMet-tRNA^{fMet} (Fig. 11.10B; Ref. [81]). Therefore, translation of S13 is blocked by 'entrapping' the 30S subunit on the RBS in a non-productive state and thereby prevents subsequent initiation attempts by a new incoming 30S subunit [81]. Spedding and Draper [79] state that "an advantage of an entrapment mechanism is that it does not demand that the repressor bind tightly enough to displace the ribosome, which has substantial affinity for the mRNA".

11.2.2.4 *str* operon

This operon is named *str* operon, since a mutation in the first gene encoding S12 (*rpsL*) can confer resistance against the antibiotic streptomycin (see Chap. 12). The *str* operon comprises genes for, in the order, S12, S7, elongation factor G, and one of the two genes coding for elongation factor Tu (*tufA*). The repressor protein is S7, which binds in front of its own cistron after the S12 cistron. The expression of S12 is also regulated by S7, obviously by "retroregulation" similar to the regulation of L14 and L24 syntheses (see Sect. 11.2.2.1), whereas overproduction of S7 partially represses EF-G synthesis and that of EF-Tu only weakly. Expression of S7 is translationally coupled to the synthesis of the preceding S12, but interestingly S12 is also expressed independent of S7, which is depressing exclusively the coupled translation of both

proteins [82]. The fact that coupled and independent translation follows distinct mechanisms is convincing evidence that for translational coupling the ribosome translating the preceding cistron is continuing the synthesis of the downstream cistron without mixing with the pool of free ribosomal subunits [82, 83].

11.2.2.5 **IF3 operon**

IF3 operon contains three genes coding for IF3, and ribosomal proteins L35 and L20. L20 can act as a repressor of the translation of both the cistron encoding L35 and its own cistron by translational coupling. L20-mediated repression requires a long base-pairing interaction of its polycistronic mRNA, namely between nucleotide residues within the IF3 cistron and residues just upstream of the L35 cistron. This interaction results in the formation of a pseudoknot. Springer and co-workers [62, 84]

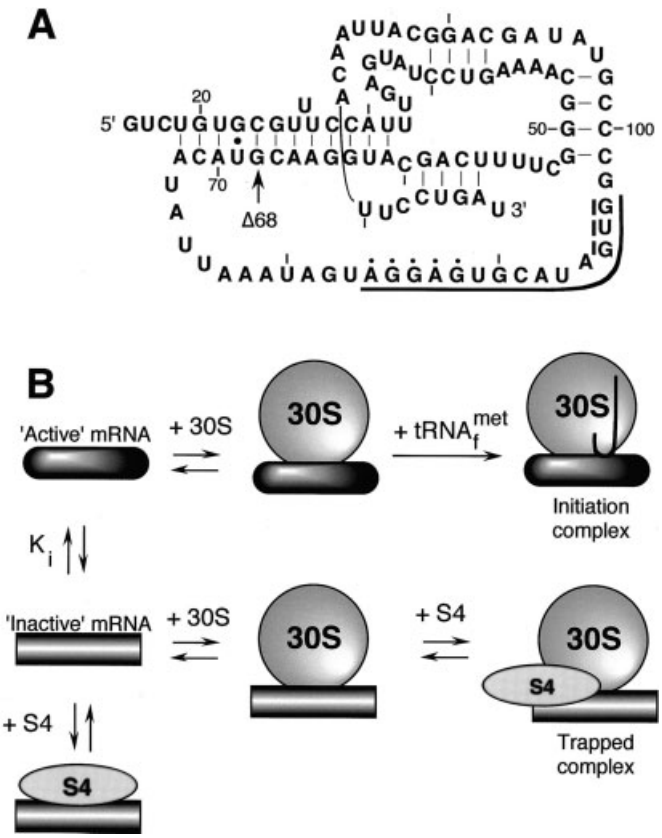

Figure 11.10 Regulation of the α-operon by S4. (A) The secondary structure of the nested pseudoknot structure in the 5′ leader sequence of the α-operon is illustrated. The RBS is underlined with the SD indicated with *dots* and the GUG initiation codon marked with *dashes*. (B) A schematic showing an 'entrapment' model for regulation of the α-operon. This figure has been reproduced from Ref. [81].

showed that L20 causes protection of nucleotide residues in two regions *in vitro*: the first region is the pseudoknot itself and the second lies in an irregular stem located upstream of the L35 cistron (Fig. 11.11). Both regions bind independently to L20 *in vitro*, and mutation and deletion studies demonstrated that they are essential for repression *in vivo*. Both sites are similar to the L20-binding site on 23S rRNA. This observation suggests that L20 recognizes its mRNA and its binding site on the 23S rRNA in a similar way.

11.3 Conclusion

These few examples demonstrate that the regulation of ribosomal proteins (1) depends on the synthesis of rRNA, (2) follows a general scheme whereby the repressor protein binds to its own mRNA and stabilizes one of two conformers, which prevents the formation of a productive 30S *de novo* initiation, and (3) that the recognition mechanism that the repressor protein uses to bind to its own mRNA is unique in every case, as are the protein–protein interactions. Furthermore, the polycistronic mRNAs coding for ribosomal proteins are distinct in that the cistron blocked by the repressor protein initiates via the canonical 30S *de novo* initiation, whereas the downstream cistrons are translationally coupled, as characterized by a 70S initiation type, where the ribosome translating the preceding cistron melts a secondary structure that sequesters the ribosomal binding site of the downstream cistron (see Sect. 3.1).

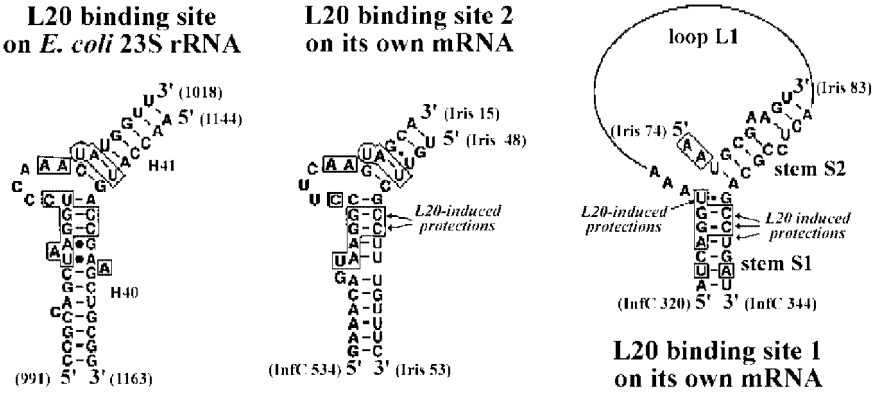

Figure 11.11 Secondary-structure similarities between the L20-binding site on the *E. coli* 23S rRNA and on the *rpm*I mRNA. The putative L20 binding site on *E. coli* 23S rRNA was deduced from the L20-binding site on 23S rRNA in the large ribosomal subunit of *D. radiodurans*. Thin lines and small dots indicate canonical and G+U base pairings, respectively; non-canonical base-pairings are indicated with large dots. Numbering of the terminal nucleotide residues on each strand of L20-binding site on 23S rRNA is that of *E. coli* 23S rRNA. Regions of sequence similarity between the L20-binding site on *E. coli* 23S rRNA and its own mRNA are boxed. Nucleotide residues that contain phosphate groups protected by L20 in iodine footprinting experiments are indicated by black arrows. The relevant features of the pseudoknot structure of L20-binding site 1 (stems 1 and 2 and loop L1) are also indicated [62].

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