The Molecular Biology of the Bacilli

Volume II

Edited by

DAVID A. DUBNAU

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Department of Microbiology
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Preface

Volume II of *The Molecular Biology of the Bacilli* combines, as does Volume I, material of interest to molecular biologists concerned with acquiring basic knowledge and to investigators attempting to develop the bacillus system for industrial applications. These concerns are, or should be, inextricably linked. Our progress in developing applications will benefit from our understanding of fundamental problems, and attempts at the former can provide an expanded data base and an important impetus for the latter. The clear relationships between the development of bacilli as insecticides and the biology of sporulation, or between the production of useful cloned products and the biochemistry and genetics of protein secretion, dramatically exemplify this reciprocal and dynamic interaction.

In this volume we have stressed those areas of bacillus research that have recently received attention either because they are unique to bacilli (Chapters 1, 4, 5, 8) or because they present interesting comparisons with other bacteria, primarily *Escherichia coli* (Chapters 2, 3, 6).

I would like to thank Issar Smith and Eugenie Dubnau for advice and encouragement during the preparation of this volume and Annabel Howard for much of the secretarial assistance.

David A. Dubnau

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1

Translational Specificity in *Bacillus subtilis*

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I. Introduction

Elucidation of the process and components involved in protein synthesis, like so many other biological problems, has depended on the availability of an active cell-free system. Such a system was first described in bacteria for *Escherichia coli* (Lamborg and Zamecnik, 1960). It was composed of ribonucleoprotein particles, later recognized as ribosomes, a high-speed supernatant fraction, an ATP-generating system, GTP, and Mg^{2+}. Results related to translation in heterologous systems in which ribosomes and enzymatic factors derived from *E.*

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coli were used to translate mRNA derived from either bacteria or phages related to bacteria other than *E. coli* suggested that the mRNA was efficiently translated by the *E. coli* system (Bassel *et al.*, 1974) and led to the general assumption that components of the protein translational system, including the mRNA, were interchangeable. This point of view was generally accepted because it was found that the cellular components of the protein synthesis machinery were very similar chemically among the bacterial species examined.

However, a limited number of observations were reported suggesting that the components of the protein translational apparatus of prokaryotes were not altogether interchangeable. Ribosomes from different bacterial species differed in their ability to translate the same mRNA (Lodish, 1969, 1970a). The ribosomes from *E. coli* and *Bacillus stearothermophilus* were found to translate the *E. coli* phage f2 RNA quite differently. *Escherichia coli* ribosomes produced unequal levels of three protein products *in vitro*. (More recently it has been recognized that there are four products.) The most abundant product was the coat protein, followed by the replicase, with the A (maturation) protein made in the least amount. In contrast, the overall incorporation by *B. stearothermophilus* ribosomes in response to f2 RNA was only ~5% of that of *E. coli*, although the amount of A protein made by the two systems was equal. This difference in selection of translation initiation sites was due to the source of the ribosomes and not to the source of the supernatant fraction or tRNAs used (Lodish, 1969). Lodish (1970a) showed that this selectivity in initiation was dependent on the source of the 30S subunit of the ribosome. The origin of the 50S subunit or initiation factors (present in a salt wash of 70S ribosomes) had no effect.

Species-specific translation has also been observed with ribosomes from *Clostridium pasteurianum* (Himes *et al.*, 1972). As with *B. stearothermophilus*, the *C. pasteurianum* ribosomes are active on poly(U), but not on f2 RNA. This work showed that *C. pasteurianum* polyribosomes were translationally active, and that crude mRNA from *C. pasteurianum* was active *in vitro* with both *E. coli* and *C. pasteurianum* ribosomes. Similar to the result in the *B. stearothermophilus* system, *C. pasteurianum* ribosomes demonstrated species-specific translation (i.e., inability to translate f2 RNA) in the presence of either *E. coli* or *C. pasteurianum* initiation factors.

In somewhat more general studies, it was found that although ribosomes from *E. coli* could translate f2 RNA, formaldehyde-treated f2 RNA, T4 early mRNA, *E. coli* mRNA, and *Clostridium pasteurianum* mRNA, a ribosome system from *C. pasteurianum* could translate *C. pasteurianum* mRNA but not the other four messengers (Stallcup and Rabinowitz, 1973a,b). These studies were extended to other gram-negative (*Pseudomonas fluorescens* and *Azotobacter vinelandii*) as well as to gram-positive bacteria (*B. subtilis*, *C. acidi-urici*, *C. tetanomorphum*, *Streptococcus faecalis*, and *Peptococcus aerogenes*), with results that suggested that protein synthesis systems derived from *E. coli* and other gram-negative

bacteria were capable of translating mRNA derived from any bacterial source or phage, whereas systems derived from gram-positive organisms could only translate mRNA derived from gram-positive organisms (Stallcup *et al.*, 1974, 1976). This phenomenon was referred to as "translational specificity." This specificity was also noted in translational systems derived from *B. subtilis*, which could translate mRNA derived from *B. subtilis* or other gram-positive organisms, as well as mRNA from phages related to *B. subtilis* such as ϕ29 or SP82, but not mRNA related to coliphage f2 or early T4 RNA (Legault-Demare and Chambliss, 1975; Stallcup *et al.*, 1976; Leventhal and Chambliss, 1979).

II. Translational Machinery

In seeking the molecular basis of the translational specificity observed in prokaryotes, each of the components of the translational machinery could be considered as a potential determinant of that specificity. These components include

- Ribosomes
  - Ribosomal proteins
  - Ribosomal ribonucleic acids (rRNAs)
- Initiation factors
- Transfer RNAs and activating enzymes
- Messenger RNAs (mRNAs)

We shall consider the possible effect of each of these components on translational specificity from a consideration of their specific function in the translational process. Much of this information is based on investigations of the process in *E. coli*. In a previous chapter in this treatise, Smith (Volume 1, Chapter 4) described the genetic determinants of the translational apparatus in *B. subtilis* and the regulation of their response to environmental factors.

A. Ribosomes

Lodish (1970a) demonstrated that the 30S subunit of the ribosome plays the key role in cistron selection in species-specific translation. The identification of the individual components of the 30S subunit responsible for this species-specific translation followed Nomura's pioneering work on the reassembly of the 30S subunit. Nomura *et al.* (1968) showed that the 30S subunit of ribosomes from *E. coli*, *Micrococcus lysodeikticus*, *Azotobacter vinelandii*, and *B. stearothermophilus* could be reconstituted from rRNA and protein fractions (Nomura *et al.*, 1968). In addition, the rRNA and protein fractions could be heterologously mixed to produce 30S particles active in a poly(U)-primed translation assay. These and other single-protein replacement studies (Higo, 1973) indicated the

highly conserved nature of the prokaryotic translation machinery; however, the translation of poly(U) is not a stringent test of function. Other workers with Nomura went on to demonstrate species-specific translation with reconstituted *B. stearothermophilus* 30S particles (Held *et al.*, 1974). Their measure of species-specific translation was the relative ratio of activity using poly(U) and R17 (a close relative of f2) RNA as substrates for translation. They observed that *E. coli* 16S rRNA combined with *B. stearothermophilus* 30S proteins showed reduced activity on R17 RNA. Indeed, the ratio of activity was indistinguishable from that of the homologous *B. stearothermophilus* 30S subunit, indicating that the protein component of the 30S subunit determines species-specific translation. However, the converse experiment using *E. coli* 30S proteins and the *B. stearothermophilus* 16S rRNA also showed a reduced activity on R17 RNA, indicating that the 16S rRNA plays some role in cistron selection. Similar studies were done by Goldberg and Steitz (1974) with homologous and heterologous 30S subunits from the 16S rRNA and 30S protein fractions of *E. coli* and *B. stearothermophilus*. They measured 30S binding to the three cistrons of R17 RNA and also measured dipeptide synthesis from R17 RNA. Their results were similar to the work of Held *et al.* (1974), that is, species-specific translation was found to be primarily associated with the protein fraction of the 30S subunit, and the role of the 16S rRNA was less significant.

B. Ribosomal Proteins

The primary amino acid sequence of all the 52 ribosomal proteins of *E. coli* has been determined (Wittmann, 1982). Such complete, detailed information is not available for other bacteria, although the complete sequences of several ribosomal proteins of *B. subtilis* and *B. stearothermophilus* are available. However, the 20 proteins of the 30S subunit of *B. subtilis* ribosomes have been isolated and their amino acid compositions and N-terminal amino acid sequences were determined (Higo *et al.*, 1982). This information was sufficient to demonstrate the occurrence of closely related 30S ribosomal proteins in *E. coli* for all 20 of the *B. subtilis* proteins. By replacing 17 individual 30S proteins from *E. coli* with their *B. stearothermophilus* counterparts, Held *et al.* (1974) were able to demonstrate that the single most important protein affecting species-specific translation is S12. When S12 and the 16S rRNA of *B. stearothermophilus* were substituted into an otherwise homologous *E. coli* 30S subunit, the ratio of activity on poly(U) to R17 was 0.15, compared to 1.0 for the *E. coli* 30S and 0.06 for the *B. stearothermophilus* 30S.

Although homologies have been shown between the 30S ribosomal proteins of *B. subtilis* and those of *E. coli*, no equivalent of the S1 protein of *E. coli* could be detected in *B. subtilis* (Higo *et al.*, 1982) or in *B. stearothermophilus* (Isono and Isono, 1976). These findings suggest that bacilli in general do not contain S1.

The absence of S1 from bacilli is of particular relevance to the phenomenon of translational specificity in view of the postulated role of this protein (Subramanian, 1983). The translation of poly(U) is S1 dependent only at low concentrations of poly(U). As the concentration of poly(U) increases, S1 has progressively less effect, until it no longer stimulates. In contrast, the translation of natural mRNA is more strongly dependent on S1 (van Dieijen *et al.*, 1975, 1976, 1977). S1 may act as an RNA-binding protein to bring mRNA into proximity with the ribosome. This would account for the elongated shape of S1, its RNA binding site or sites (Draper and von Hippel, 1978a,b), and its stimulation of translation at low concentrations of mRNA. In addition, S1 does not dissociate from the ribosome during translation, so it may also act to hold on to the mRNA during the course of translation. *Escherichia coli* S1 was shown to stimulate translation of f2 RNA by *B. stearothermophilus* ribosomes (Isono and Isono, 1975) and to bind to the *B. stearothermophilus* 30S subunit (Isono and Isono, 1976). S1 stimulation of f2 translation by *B. stearothermophilus* ribosomes might reflect the role of S1 in assisting mRNA binding. Attempts to show an effect of S1 on translation by *B. subtilis* ribosomes have not been successful (McLaughlin *et al.*, 1981a). One interpretation is that although *E. coli* S1 interacts with *B. stearothermophilus* ribosomes to facilitate translation, it does not bind to *B. subtilis* ribosomes. The evidence for a role of S1 in translational specificity remains equivocal. Because information is not available concerning the absence of S1 from bacterial species other than those mentioned, it is not possible to correlate translational specificity with the presence or absence of this ribosomal component. The experimental evidence shows that S1 plays a role in the binding of mRNA to the 30S ribosomal subunit; however, it is not clear whether S1 is a determinant of the site of translation initiation or a nonspecific binding protein that increases the ribosome's affinity for any mRNA.

C. Ribosomal RNAs

Three species of rRNAs are recognized in *E. coli*. They are designated and differentiated on the basis of their size as 5, 16, and 23S. They function in the binding of mRNA and tRNA and in the association of the ribosomal subunits, processes that might be related to translational specificity. *Bacillus stearothermophilus* 16S rRNA can be used for the reconstitution of "functional" 30S ribosomes with *E. coli* 30S ribosomal proteins (Smith, chapter 4, volume 1 of this treatise; Nomura, 1973); *E. coli* 5 and 23S RNA can likewise be used to reconsitute 50S ribosomes in the presence of *B. stearothermophilus* 50S ribosomal proteins (Nomura and Erdmann, 1970).

Models for the structure of *E. coli* 5S rRNA based on sequence determination and responses to extensive physical and chemical perturbations exist. The function of this RNA species is uncertain (Wittmann, 1982), although interactions

with various ribosomal components and reactants related to protein synthesis have been demonstrated (Erdmann, 1976). The primary structures of many 5S RNAs have been determined (Erdmann *et al.*, 1983). It is of interest that the 5S RNAs of eubacteria may be classified as the 16-N-type characteristic of gram-positive bacteria or as the 21-N-type characteristic of gram-negative bacteria (Hori and Osawa, 1979). Since translational specificity is related to the 30S ribosomal subunit, both the 5S and 23S rRNAs are unlikely determinants of translational specificity.

Shine and Dalgarno (1975a,b) suggested that differences in the 3′ end of the 16S rRNA might account for species-specific translation. They postulated that the 3′ termini of *E. coli* and *Pseudomonas aeruginosa*, which differ from the 3′ termini of *B. subtilis*, *B. stearothermophilus*, and *Caulobacter crescentus*, could explain the differential recognition of phage RNA cistrons, since the different sequences would have different abilities to base pair with the pyrimidine-rich sequence of the mRNA. More complete sequencing has effectively destroyed this hypothesis, since the 3′ end of the *B. stearothermophilus* 16S rRNA contains the same CCUCC sequence found in *E. coli*, with a difference of three extra bases inserted at the penultimate nucleotide (Sprague *et al.*, 1977). Although it is possible that the additions to the 3′ end of the 16S rRNA in *B. stearothermophilus* and *B. subtilis* influence binding to mRNA, the analysis of the bases of the *B. subtilis* 16S rRNA that can pair to the purine-rich region of the gram-positive translation initiation sites has revealed that the extreme end of the 16S rRNA does not play a major role in binding to the mRNA, as will be discussed below.

The total sequence of the 16S rRNA of *E. coli* and other bacteria has been determined, and a structure based on these results has been proposed (Noller, 1980; Woese *et al.*, 1983). Several functions of the 16S rRNA have been recognized. These include assembly, subunit association, tRNA binding, and initiation. These functions have been assigned to various domains of the proposed secondary structure model. Domain V is associated with the initiation function of the 16S rRNA and is located at the 3′ end of the RNA. Detailed examination of the 3′ minor domain of the 16S rRNA from both *E. coli* and *B. stearothermophilus* provide evidence for a high degree of structural conservation throughout the evolutionary divergence of the gram-positive and gram-negative eubacteria (Douthwaite *et al.*, 1983; Woese *et al.*, 1983).

The eubacterial sequence CCUCC found near the 3′ terminus of the 16S rRNA is involved in mRNA binding (Sprague *et al.*, 1977). This sequence appears to be conserved in eubacteria, although the exact nature of the 3′ end of the 16S rRNA shows some variations (Woese *et al.*, 1983). Thus, assuming that the base sequence from the 3′ end of the 16S rRNA to the CCUCC sequence does not have a specific function in translation, the base sequences of the 16S rRNA would not appear to be responsible for translational specificity.

There is little direct evidence concerning the possible role of the 23S rRNA as a determinant of translational specificity. Attempts to obtain active heterologous ribosome couples from *E. coli* and *Clostridium pasteurianum* were frustrated because of the inactivity of 50S subunits derived from *C. pasteurianum* (Himes *et al.*, 1972). Analogous reconstitution of heterologous ribosome couples with components of *E. coli* and *B. subtilis* has not been reported.

D. Initiation Factors

Preferential effects of *E. coli* initiation factors on the expression of different cistrons in R17 RNA have been noted (Steitz *et al.*, 1977). It was also reported that translations of mRNAs derived from gram-positive sources show less dependence on initiation factors than do *E. coli* mRNAs translated by systems from both *E. coli* and *B. subtilis* (Stallcup *et al.*, 1976; McLaughlin *et al.*, 1981a). Nevertheless, it appears that, in general, initiation factors facilitate an interaction for which the specificity is determined by the 30S subunit and features in the mRNA. Most of the information concerning the structure and function of prokaryotic initiation factors is based on examination of the *E. coli* factors. These are all associated with the 30S ribosomal subunit and function in different steps of the initiation process (Hershey, 1980). Three initiation factors are recognized: IF-1, IF-2, and IF-3. They are usually associated with the ribosomes but can be dissociated with 1 *M* NH_4Cl into a ribosomal salt wash fraction. The individual proteins of *E. coli* have been purified to homogeneity by conventional means (Hershey, 1980). Their activity is usually measured by their stimulation of formylmethionyl-tRNA (fMet-tRNA) binding to 70S ribosomes in the presence of mRNAs. IF-2 functions in the binding of fMet-tRNA to the 30S ribosomal subunit, IF-3 functions in mRNA binding and ribosome dissociation, and IF-1 stimulates the activity of the other two factors. A review by Maitra *et al.* (1982) provides a detailed description of the specific interactions of the initiation factors with the other components involved in the initiation process in *E. coli* as far as they are known.

Very limited information exists concerning the initiation factors of prokaryotes other than *E. coli*. IF-1 (Leffler and Szer, 1974b) and IF-3 (Leffler and Szer, 1974a) have been purified from *Caulobacter crescentus*. The proteins from either *E. coli* or *C. crescentus* are active in reactions tested that require the initiation factors. Purification of the initiation factors from *B. stearothermophilus* by chromatography of the ribosomal salt wash fraction resulted in the purification of factors corresponding in properties to IF-1 and IF-2 (Kay and Grunberg-Manago, 1972). *Bacillus stearothermophilus* IF-2 functions with the thermophile ribosomes at 60°C or with *E. coli* ribosomes at 37°C, but the IF-1 derived from the thermophile is active only at the elevated temperatures. No evidence was obtained for the occurrence of IF-3 in *B. stearothermophilus* (Kay