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Initiation factor-independent translation of mRNAs from Gram-positive bacteria

(DNA-directed protein synthesis/*Escherichia coli* and *Bacillus subtilis* cell-free systems/phage ϕ29/phage T7)

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ABSTRACT **Initiation factor-independent translation of mRNA derived from bacillus phage ϕ29 DNA occurs with translation systems derived from *Bacillus subtilis* or *Escherichia coli*. This is in sharp contrast to the strict dependence on ribosome salt wash fraction of *E. coli* ribosomes for the translation of T7 and other mRNAs derived from Gram-negative organisms.**

Although the classification of bacteria as Gram-negative or Gram-positive is based on differences in cell wall architecture (1), additional distinctions between these two groups of bacteria are known. Evolutionary trees based on 16S and 5S RNA sequences (2, 3) indicate that the Gram-positive bacteria represent some of the most primitive species, such as the clostridia, that are characterized by fermentative metabolism and the ability to form endospores in a hostile environment. The Gram-positive bacilli appeared somewhat later with perhaps the first use of aerobic respiratory metabolism, but they retain spore-forming ability. The Gram-negative bacteria evolved even later. They are often capable of facultative respiratory metabolism but have lost the ability to sporulate. Instead, the Gram-negative bacteria, such as *Escherichia coli*, are equipped to adapt to a changing environment.

One manifestation of the increased adaptability of *E. coli* is its ability to express genes from a wide variety of bacteria including both Gram-positive and Gram-negative species. In contrast, many *E. coli* genes carried on hybrid plasmids are not expressed in the Gram-positive species *Bacillus subtilis* (4–6). These *in vivo* restrictions of heterospecific gene expression in *B. subtilis* could result from limitations in transcription, translation, or posttranslational processing of genetic information.

There is increasing evidence that the translational machinery isolated from *E. coli* differs significantly from that of its Gram-positive predecessor, *B. subtilis*. Studies from several laboratories indicate that ribosomes isolated from Gram-positive bacteria are extremely inefficient in translating mRNAs derived from Gram-negative bacteria (7–13). Ribosomes from *E. coli*, on the other hand, show comparable translational efficiency in response to mRNAs from Gram-positive or Gram-negative bacteria.

Reconstitution of hybrid 30S ribosomal subunits has revealed differences in the protein components which affect the efficiencies with which Gram-negative mRNAs are translated (11, 14, 15). For example, Isono and Isono (16) reported that translation of f2 RNA by *Bacillus stearothermophilus* ribosomes is stimulated by the addition of *E. coli* ribosomal protein S1. We have demonstrated here that *E. coli* ribosomal protein S1 has very little effect on the efficiency with which f2 RNA and T7 mRNA are translated by the *B. subtilis* translation system and concluded that S1 is not the sole determinant of species-specific initiator recognition.

Another approach to understanding the basis for selective mRNA recognition by *B. subtilis* ribosomes involves characterization of their interaction with mRNAs from Gram-positive organisms which is, after all, their only productive interaction. The difficulty in obtaining defined populations of mRNAs from Gram-positive bacteria has prevented comparison of specific product formation by the *B. subtilis* and *E. coli* translation systems. Recently, a procedure was devised for the purification of highly active preparations of RNA polymerase holoenzyme from *B. subtilis* in order to generate defined mRNAs from known Gram-positive DNA phages such as ϕ29 (17). This purified RNA polymerase can be used to make RNA which is then added to a translation system. We refer to this system as an "uncoupled reaction." Alternatively, RNA and protein synthesis can be carried out concurrently in a system that more closely resembles the *in vivo* situation. We refer to this as a "coupled reaction." Leventhal and Chambliss (18) have also recently reported an *in vitro* transcription and translation system from *B. subtilis*.

A distinctive feature of the Gram-positive mRNA-ribosome interaction demonstrated by our analysis is the extent to which productive initiation occurs in the absence of salt wash (10, 19). We have utilized cell-free transcription and translation systems from the Gram-positive bacterium *B. subtilis* and the Gram-negative bacterium *E. coli* to analyze the protein products formed in the absence of the salt wash fraction from bacillus phage ϕ29 and *E. coli* phage T7. Phage ϕ29 mRNA-directed translation shows extensive salt wash-independent product formation. This report supports the idea that mRNAs derived from Gram-positive bacteria have a unique character that facilitates protein synthesis in the absence of salt wash fraction (19, 20).

MATERIALS AND METHODS

Materials. The sources of many of the materials have been reported (21, 22). T7 DNA was a gift of George A. Kassavetis and Michael J. Chamberlin of this department. ϕ29 DNA was prepared as described (17). L-[3,4-^{3}H]Valine (32.6 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was purchased from Amersham. Purified *E. coli* initiation factors were prepared by William J. Sharrock (formerly of this laboratory) according to Hershey *et al.* (23).

***In Vitro* ϕ29 DNA-Directed Protein Synthesis.** For uncoupled reactions, ϕ29 mRNA was synthesized in a 50-μl reaction mixture containing ϕ29 DNA at 250 μg/ml, 6.4 pmol of RNA polymerase, 100 mM Tris·HCl (pH 8), 12 mM $MgCl_2$, 0.1 mM EDTA, 1.6 mM spermidine, 0.2 mM dithiothreitol, ATP, GTP, UTP, and CTP each at 1 mM, and 160 mM KCl. RNA synthesis was initiated at 4°C by addition of either *B. subtilis* RNA polymerase (17) or *E. coli* MRE600 RNA polymerase (24) followed

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Abbreviation: IF, initiation factor.

by incubation for 30 min at 37°C. Rifampicin (Sigma) was added to 150 μg/ml and the reaction tubes were placed on ice until used for the translation assay. These conditions consistently result in the incorporation of 4 nmol of labeled ribonucleotides with both sources of polymerase.

Equal volumes (15 μl) of the transcription reactions were added to the homologous translation system to start the reaction. All 60-μl translation assays contained 64 mM Tris·HCl (pH 7.6), 12 mM $Mg(OAc)_2$, 2 mM ATP, 0.5 mM GTP, 6.3 mM phospho*enol*pyruvate (monopotassium salt), pyruvate kinase at 16 μg/ml, 10 mM 2-mercaptoethanol, 100 mM KCl, 50 mM NH_4Cl, 16 μM 10-formyltetrahydrofolate (25), *E. coli* MRE600 tRNA at 840 μg/ml, 19 unlabeled amino acids (lysine omitted) at 0.17 mM each, and 43 μM L-[4,5-^{3}H]lysine (ICN; 54 Ci/mmol). *B. subtilis* translation assays contained 1 A_{260} unit of vacant ribosomal couples, 55 μg of salt wash protein from *B. subtilis* ribosomes (20) (when included), and 250 μg of S150 protein prepared as described (10) except for the omission of the DEAE-cellulose column as the last step. *E. coli* translation assays contained 1 A_{260} unit of vacant ribosomal couples (20), 42 μg of salt wash protein from *E. coli* ribosomes (20) (when included), and 210 μg of S150T protein (10). Assays were incubated at 37°C for 25 min and then cooled on ice. A 10-μl aliquot of each was assayed on a filter paper disc as described (7).

Coupled φ29 DNA-directed assays were performed in 60 μl containing 6 μg of φ29 DNA (100 μg/ml), 3.5 pmol *B. subtilis* or *E. coli* RNA polymerase, 64 mM Tris·HCl (pH 7.6), 12 mM $Mg(OAc)_2$, 2 mM ATP, GTP, CTP, and UTP each at 0.5 mM, 0.1 mM EDTA, 1.6 mM spermidine, 100 mM KCl, 50 mM NH_4Cl, pyruvate kinase at 16 μg/ml, *E. coli* MRE600 tRNA at 840 μg/ml, 16 μM 10-formyltetrahydrofolate, 6.3 mM phospho*enol*pyruvate, 10 mM 2-mercaptoethanol, 19 unlabeled amino acids (lysine omitted) at 0.17 mM each, 43 μM L-[4,5-^{3}H] lysine, and the same quantities of ribosomes, S150, and salt wash (when included) as for uncoupled reactions. Assays were incubated at 37°C for 25 min and then cooled on ice. Aliquots were assayed as described above.

***In Vitro* T7 DNA-Directed Protein Synthesis.** For uncoupled reactions, T7 mRNA was synthesized under conditions similar to those described for φ29 mRNA synthesis except that T7 DNA at 240 μg/ml was used and the KCl was omitted in order to permit more efficient transcription by *B. subtilis* RNA polymerase. Generally, 1.4 nmol of labeled ribonucleotide were incorporated by *B. subtilis* RNA polymerase and 4.5 nmol of labeled ribonucleotide were incorporated by *E. coli* RNA polymerase. Consequently, 15 μl of the *B. subtilis* RNA polymerase transcription reaction was added to translation assays of *B. subtilis* components and 10 μl of the *E. coli* RNA polymerase transcription reaction was added to *E. coli* translation assays. This represents the addition of half as much T7 mRNA to the *B. subtilis* uncoupled translations as to the *E. coli* translations. Addition of T7 mRNA synthesized by *E. coli* RNA polymerase (10 μl) has no effect on the extent of amino acid incorporation by the *B. subtilis* translation system. Coupled T7 DNA-directed assays were performed as described for the φ29 DNA-directed assays except that 3.5 pmol of *E. coli* RNA polymerase was added to all reactions because *B. subtilis* RNA polymerase is inactive under the high salt conditions of the translation assay.

Gel Electrophoresis. Proteins synthesized in *in vitro* assays were analyzed by $NaDodSO_4$/polyacrylamide slab gel electrophoresis (26) with a discontinuous buffer system (27) and an acrylamide/bisacrylamide ratio of 30:0.8. Gel dimensions were 19 × 16 × 0.08 cm. Samples were prepared by adding 16 μl of 3× sample buffer [0.16 M Tris·HCl, pH 6.8/30% (vol/vol) glycerol/3% (wt/vol) $NaDodSO_4$/0.45 M 2-mercaptoethanol/0.05% bromphenol blue] to the remaining 50 μl of the protein synthesis reaction mixtures and boiling for 2 min. Labeled proteins were visualized by fluorography (28) with Kodak Blue Brand film.

Table 1. [^{3}H]Lysine incorporation in response to T7 DNA

Translation system	Incorporation, pmol			
	E. coli		*B. subtilis*	
	−	+	−	+
Control (no DNA)	7	9	13	25
Uncoupled	8	490	17	58
Coupled	32	1110	16	41

Uncoupled and coupled T7 DNA-directed protein synthesis reactions were performed with ribosomes from *E. coli* and *B. subtilis* in the absence (−) and presence (+) of the ribosomal high salt wash fraction. [^{3}H]Lysine specific activity was 240 cpm/pmol.

RESULTS

Translation of T7. The *E. coli* translation system was strictly dependent upon the addition of salt wash for the formation of T7 directed proteins (Table 1). Indeed, few T7-specified proteins were synthesized in the absence of the initiation factor fraction (Fig. 1) by either the *B. subtilis* or *E. coli* translation system. The heterologous combination of *E. coli* ribosomes and *B. subtilis* S150 also was inactive in the absence of the initiation factor fraction (data not shown). The product most efficiently synthesized by *E. coli* ribosomes in the absence of the salt wash was a protein of M_r 41,700 that we have identified as T7 ligase (Fig. 1, lane j) through the use of a ligase-deficient phage mutant (unpublished results). Several other barely detectable products were formed by the *E. coli* system operating in the absence of salt wash. These tended to be those proteins synthesized most

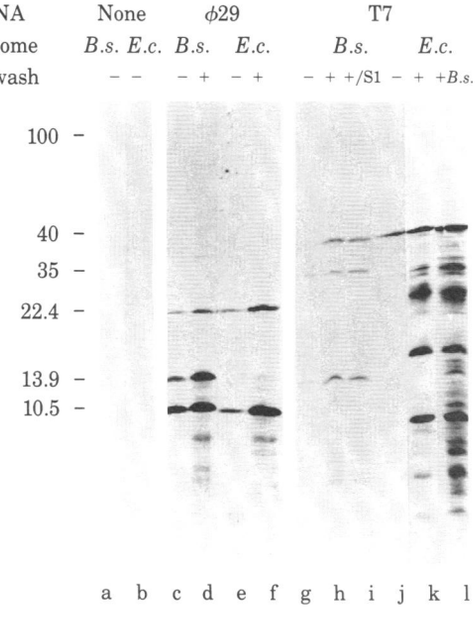

FIG. 1. The uncoupled reactions from the experiments described in Tables 1 and 2 were analyzed. Because the sample aliquot (40 μl) was the same in all lanes shown here, the radioactivity detected by fluorography is directly proportional to the incorporation activity of a given assay. The counts per min applied in lanes a to l, respectively, were 1540, 910, 14,100, 32,700, 9600, 42,300, 2100, 7100, 7300, 950, 60,300, and 73,800. To the sample loaded into lane i, 20 pmol of purified *E. coli* ribosomal protein S1 was added. In lane l, 55μg of *B. subtilis* salt wash protein was substituted for *E. coli* salt wash protein. The M_r of the standard proteins (shown × 10^{-3}) ranged from 10,500 to 100,000. Exposure time was 6 days.

efficiently by *B. subtilis* ribosomes in the presence of salt wash. That is, in general, the initiation sites on mRNAs from Gram-negative organisms that are detectably utilized by *B. subtilis* ribosomes also show the most extensive salt wash-independent translation by *E. coli* ribosomes.

Amino acid incorporation in response to T7 mRNA by *B. subtilis* ribosomes was about 2% in the coupled and 7% in the uncoupled system compared to that shown by *E. coli* ribosomes (Table 1). Because *B. subtilis* RNA polymerase is inactive on a linear Gram-negative DNA template under the conditions of the translation assay (21), *E. coli* RNA polymerase was used in the coupled assay with the *B. subtilis* translation system. If this coupling of heterologous RNA polymerase and ribosomes were roughly half as efficient as the homologous combination would be on T7 (see following section), the coupled T7 DNA-directed *B. subtilis* incorporation also would increase, but only to about 4% of what was observed for the *E. coli* system. At any rate, coupling transcription and translation had no alleviating effect on the observed restricted translation of T7 mRNA by *B. subtilis* ribosomes. The relative inability of the protein-synthesizing system derived from *B. subtilis* to respond to T7 mRNA illustrates the discriminatory nature of systems derived from Gram-positive organisms that has been noted (8).

ϕ29 Directed Translation. In marked contrast to the results obtained with T7 DNA, the translation system derived from *B. subtilis* incorporated amino acids at an efficiency similar to the system derived from *E. coli* (Table 2) in both uncoupled and coupled reactions directed by ϕ29 DNA. Substitution of the heterologous RNA polymerase resulted in a 40% decrease in incorporation but did not affect the pattern of products synthesized (data not shown). Three major proteins corresponding to *in vivo* phage specific products (29–33) were produced by the *B. subtilis* translation system (Fig. 1, lanes c and d). Two of these three proteins made *in vitro* by *B. subtilis* ribosomes also were made efficiently by *E. coli* ribosomes (Fig. 1, lanes e and f). The intermediate molecular weight product is an example of a mRNA derived from a Gram-positive organism that is utilized less efficiently by *E. coli* ribosomes (unpublished data).

Salt Wash-Independent Translation of ϕ29 DNA. Protein-synthesizing systems containing salt-washed ribosomes from *E. coli*, *B. subtilis*, *Pseudomonas fluorescens*, *Clostridium pasteurianum*, or *Streptococcus faecalis* were found to synthesize up to 30% the amount of protein in the absence of salt wash that was synthesized in the presence of salt wash in response to whole cellular RNA from five Gram-positive bacteria. This salt wash-independent translation was not observed in response to mRNAs from Gram-negative bacteria (10). Subsequent analysis confirmed that the formation of pressure-resistant initiation complexes of *E. coli* ribosomes with f2 RNA is completely dependent upon addition of salt wash, whereas significant complex formation occurs with *B. subtilis* RNA in the absence of salt wash (19).

The ϕ29 DNA-directed system was utilized to determine whether specific protein products are synthesized in the absence of the initiation factor fraction. Ribosomes from both *B. subtilis* and *E. coli* directed substantial salt wash-independent translation when presented with ϕ29 mRNA (Table 2). Both *B. subtilis* and *E. coli* coupled systems operate at about 40% efficiency in the absence of the initiation factor fraction. Substitution of heterologous S150 components did not affect this extent of initiation factor-independent translation (data not shown). Uncoupling of transcription and translation (presentation of the ribosome with a preformed mRNA) had little effect on the ability of the *B. subtilis* translation system to synthesize protein in the absence of salt wash but apparently decreased this capacity in the *E. coli* translational system. Polyacrylamide gel analysis of salt wash-independent translation products is shown in lanes c and e of Fig. 1. The proteins made in the absence of the initiation factor fraction were the same as the major products synthesized in the presence of salt wash by both *E. coli* and *B. subtilis* translation systems.

Table 2. [^{3}H]Lysine incorporation in response to ϕ29 DNA

Translation system	Incorporation, pmol			
	E. coli		*B. subtilis*	
	−	+	−	+
Control (no DNA)	7	9	13	25
Uncoupled	78	340	120	270
Coupled	490	1140	370	930

Uncoupled and coupled ϕ29 DNA-directed protein synthesis reactions were performed as in Table 1. [^{3}H]Lysine specific activity was 240 cpm/pmol.

The results of addition of purified *E. coli* initiation factors (IF) (23) to the salt wash-depleted *E. coli* translation system suggested that the lack of IF 3 limits amino acid incorporation in response to ϕ29 mRNA (Table 3). Addition of IF 1, IF 2, or both in amounts equivalent to the molar amounts of ribosomes increased amino acid incorporation very little relative to the amino acid incorporation obtained without added factors. However, addition of the same molar equivalent of IF 3 gave 77% of the amino acid incorporation of the complete system.

Effect of *E. coli* S1 on Translation by *B. subtilis* Ribosomes. *E. coli* ribosomal protein S1 was purified by a modification of Tal's procedure (34) involving ammonium sulfate fractionation. The purified S1 obtained was >95% pure and was characterized chromatographically, by molecular weight, by poly(U)binding, by inhibition of translation when present in excess of the molar amount of ribosomes, and by stimulation of a partially S1-depleted *in vitro* *E. coli* translation system with f2 RNA (34, 35) (data not shown). Isono and Isono (16) reported that S1 stimulates translation of the f2 coat and replicase cistrons by *B. stearothermophilus* ribosomes at 39°C, a temperature that is not optimal for growth of either the f2 phage or the thermophile. We investigated the effect of S1 on the translation of f2 and T7 mRNA by *B. subtilis* ribosomes at 37°C. Addition of 19–94 μg of f2 RNA to 60 μl of reaction mixture containing 1 A_{260} unit of *B. subtilis* vacant couples (20) stimulated amino acid incor-

Table 3. Stimulation of purified *E. coli* initiation factors (IF)

Addition	Incorporation of [^{3}H]valine			
	Reaction directed by ϕ29 DNA		Reaction directed by T7 DNA	
	pmol	%	pmol	%
None	180	34	27	10
IF 1	260	49	35	13
IF 2	240	45	27	10
IF 3	410	77	130	46
IF 1+2	290	55	42	15
IF 1+3	450	85	180	64
IF 2+3	490	92	130	46
IF 1+2+3	530	100	280	100
Crude salt wash (40 μg)	780	—	500	—

Coupled assays were performed with the *E. coli* translation system described in Table 1, except that 0.05 mM L-[3,4-^{3}H]valine (Amersham; 32.6 Ci/mmol diluted to 54 cpm/pmol in the assay) was substituted for [^{3}H]lysine and purified *E. coli* initiation factors were substituted for crude salt wash protein [0.2 μg (22 pmol) of IF 1, 2.2 μg (19 pmol) of IF 2, and 0.6 μg (27 pmol) of IF 3]. The background value (17 pmol), determined in the absence of added DNA, was subtracted from each assay result.

poration up to 2-fold over background. This represents about 1% of the [^{3}H]valine incorporation observed in the analogous *E. coli* translation system. The addition of molar amounts of S1 stimulated the system somewhat more, but the level obtained was still only 2–7% of that obtained with the *E. coli* system. Because of the low level of incorporation, we were unable to analyze the products of the reaction in order to characterize the proteins made.

The effect of S1 was also tested with T7 mRNA in a *B. subtilis* translational system (Fig. 1, lane i). There was no difference in amino acid incorporation in the presence of a molar amount of S1 and there was no effect on the relative amounts of protein products synthesized (Fig. 1, lanes h and i). Thus, the addition of S1 to a *B. subtilis* translation system does not significantly affect its inability to use Gram-negative mRNA.

DISCUSSION

There is considerable evidence to suggest that the interaction between mRNA and ribosomes is different in homologous translation systems prepared from *E. coli* and *B. subtilis*. We have observed that, in the absence of ribosomal salt wash fraction, the synthesis of specific proteins encoded by mRNA derived from the bacillus phage ϕ29 is 40% of that in the presence of factors present in a salt wash of ribosomes in translation systems derived from both *E. coli* and *B. subtilis*. This contrasts sharply with the complete dependence on the salt wash for translation by *E. coli* ribosomes of T7 and other mRNAs derived from Gram-negative organisms (10, 36–38).

It is important to consider whether sources of initiation factors other than the salt wash are present that could account for the salt wash-independent activity we have observed. That the *E. coli* vacant couple ribosomes (39) used in these studies are free of initiation factors is indicated by the fact that the addition of a ribosome salt wash stimulates amino acid incorporation in response to T7 mRNA 50- to 60-fold (Table 1). In contrast, only a 2- to 4-fold stimulation of activity is obtained in response to ϕ29 mRNA when salt wash is added (Table 2). Qualitatively similar results are obtained when salt-washed ribosomes are used (data not shown), but high endogenous activity prevents conclusive product analysis. Little is known about the IF proteins in *B. subtilis*; however, a salt wash preparation from *B. subtilis* ribosomes (20) substitutes fully for *E. coli* salt wash in the translation of T7 mRNA by *E. coli* ribosomes (Fig. 1, lane l). Thus, factors with similar initiation functions must be present in the *B. subtilis* salt wash. Two factors similar to *E. coli* IF 2 and IF 3 have been reported in *B. stearothermophilus* and were also found to be interchangeable with the *E. coli* factors (40). It cannot be ruled out, however, that there are factors associated with *B. subtilis* ribosomes that are not removed by salt washing procedures and are not required by *E. coli* for efficient translation of mRNAs from Gram-negative organisms. This would be another interesting difference between the two systems.

The other possible source of IFs is the S150 fraction. In contrast to IF 1 and IF 3, IF 2 is reported to be present in the *E. coli* supernatant fraction (41, 42). It is not surprising, therefore, that addition of purified IF 2 has little stimulatory effect on the translation of ϕ29 or T7 mRNAs by *E. coli* ribosomes (Table 3). We also observe that addition of purified IF 1 alone has little effect, as noted by previous investigators (23, 38, 41). This incomplete dependence on IF 1 may reflect the cooperative nature of its function rather than its presence in the supernatant fraction. Furthermore, the reported absence of IF 1 in the salt wash of *B. stearothermophilus* (40) and *Caulobacter crescentus* (12) ribosomes has led to speculation that this factor may be somewhat dispensible in some prokaryotic systems (43). The most dramatic stimulation of both ϕ29 and T7 mRNA-directed translation results from the addition of purified IF 3 (Table 3). This suggests that, without addition of salt wash, the system is relatively free of IF 3. Consistent with this interpretation is the virtual lack of T7 mRNA-directed protein synthesis observed in the absence of salt wash (Table 1). We are able to detect some synthesis of T7 ligase in the absence of salt wash, and it is reported (44) that synthesis of T7 RNA polymerase as detected by enzymatic activity is also independent of addition of IF 3. Even when synthesis of T7 RNA polymerase is detectable in our system in the presence of salt wash (detectability varies with choice of the labeled amino acids), we do not detect its production in the absence of salt wash. The synthesis of picogram quantities of T7 RNA polymerase observed by Benne and Pouwels (44), however, would not be detectable in our fluorographic gel analysis. At any rate, as reported for R17 RNA (37), there seems to be a small group of initiation sites on mRNAs from Gram-negative organisms that are recognized by ribosomes with less dependence on IF 3.

A much greater extent of salt wash-independent translation is observed with ϕ29 mRNA (Table 2). The initiation of protein synthesis on this mRNA is much less dependent on IF 3 than is the case with T7 mRNA. Because the pattern of protein products formed in the presence or absence of salt wash factors from mRNAs derived from Gram-positive organisms is similar, it is likely that the selection of initiation sites for protein synthesis is occurring by similar mechanisms both in the absence and presence of the salt wash fraction and that the salt wash provides a kinetic advantage (45). This advantage is much less dramatic in the *in vitro* translation of mRNAs from Gram-positive relative to Gram-negative sources.

Consideration of the proposed functions of IF 3 may provide some clues concerning the characteristics of mRNA from Gram-positive organisms that facilitates IF-independent translation. IF 3 functions in at least three capacities: (*i*) dissociating the 70S ribosome subunits; (*ii*) binding to the 30S subunit to prevent subunit reassociation; and (*iii*) binding to the initiator region of mRNA to facilitate the 30S–mRNA complex formation (42). Because the mRNA appears to determine the extent of IF 3-independent translation, the following possibilities are suggested: (*i*) dissociation of the 70S ribosome is caused by mRNA from Gram-positive sources; (*ii*) subunit reassociation is essentially prevented by the efficiency with which mRNA from Gram-positive sources binds 30S subunits; and (*iii*) the structure of the mRNA from Gram-positive sources is such that the 30S–mRNA complex is more stable than the average 30S–mRNA complex derived from Gram-negative sources and is therefore less dependent upon IF 3.

It has been observed that the *B. subtilis* ribosomal subunit association equilibrium is shifted toward dissociation not only by the salt wash fraction but also by *B. subtilis* cellular mRNA (20). The dissociative effect of *B. subtilis* mRNA alone is unexpected on the basis of results with *E. coli* and suggests that mRNA is interacting with one or both subunits to prevent reassociation. This property of mRNAs of Gram-positive origin may contribute to the observed decreased dependence upon IF 3.

The stability of the 30S–mRNA complex is dependent upon several structural features of mRNA including the extent of Shine–Dalgarno complementarity with the 3′ end of the 16S RNA, the presence of a codon that can interact with the fMet-tRNA^{fMet} (43, 46), and possibly the distance or sequence between these two regions (47, 48). *E. coli* ribosome binding to the three R17 RNA initiator regions has been shown to have different dependencies upon IFs and S1 ribosomal protein. Ribosome recognition of the coat and replicase sites, each of which has a Shine–Dalgarno complementarity of four base pairs, is severalfold more dependent on the presence of initiation fac-

tors and S1 than is recognition of the A protein site which has a complementarity of seven base pairs (37). It has been suggested that the high degree of complementarity between the A site and the 3′ end of 16S rRNA may provide sufficient binding energy to obviate the usual factor requirements (37). It is possible, therefore, that a characteristic of mRNAs from Gram-positive organisms is the potential for stronger Shine–Dalgarno complementarity that is required for efficient translation by *B. subtilis* ribosomes. Consequently, *B. subtilis* ribosomes would recognize only those mRNAs from a Gram-negative source that possess the requisite complementarity such as R17 A protein. Efficient translation in the Gram-positive system may have additional requirements for less variability in the orientation of the Shine–Dalgarno region and the initiation codon of a mRNA to aid in correct positioning of the mRNA without the assistance of IF 3.

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