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Binding of Initiation Factor 2 and Initiator tRNA to the *Escherichia coli* 30S Ribosomal Subunit Induces Allosteric Transitions in 16S rRNA†

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ABSTRACT: The specific effect of the binding of IF2 and initiator fMet-tRNA$_f^{Met}$ on *Escherichia coli* 16S rRNA has been probed by phosphate alkylation with ethylnitrosourea. The results show that IF2 does not significantly shield portions of 16S rRNA but induces both reductions and enhancements of reactivity scattered in the entire molecule. Most of them are topographically constrained in a region corresponding to the cleft, the lateral protrusion, and the part of the head facing the protrusion (positions 694, 771, 791, 1225, 1268, 1398, 1401, 1504, and 1527). Weak effects are also observed in distant parts of the subunit (positions 301, 302, 492, and 1428). All the reactivity changes induced by the binding of IF2 are still observed in the presence of the initiator tRNA and AUG as messenger. The additional changes induced by the tRNA are mostly centered around the cleft–head–lateral protrusion region, near positions affected by IF2 binding. Most of the changes correspond to reduced reactivities (positions 791, 1222, 1263, 1393, 1395, 1430, 1431, 1504, 1528, and 1529), while enhanced reactivities are observed at positions 708, 709, and 1398. Functional implications are discussed, which stress the dynamic properties of the ribosome.

Initiation of protein synthesis in *Escherichia coli* proceeds via the formation of a preinitiation complex involving fMet-tRNA$_f^{Met}$, the 30S subunit, the messenger RNA, and GTP. Three initiation factors (IF1, IF2, and IF3) promote this complex formation. IF2 is implicated in fMet-tRNA binding to the 30S subunit and possesses a ribosome-dependent GTPase activity [for reviews, see Maitra et al. (1982), Gualerzi et al. (1986), and Hershey (1987)]. IF2 is a large acidic protein, which exists in two forms IF2α (889 residues) and IF2β (732 residues) (Miller & Wahba, 1973). These two forms are

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expressed from the same gene by two independent translational initiation sites (Plumbridge et al., 1985). The primary structure of IF2 has been elucidated and a tertiary structure model of the G-binding domain has been predicted (Cenatiempo et al., 1987). The role devoted to IF2 is to promote the binding of fMet-tRNA$_f^{Met}$ in the ribosomal P site. The formation of a IF2/fMet-tRNA complex has been detected, but its stability is very weak [e.g., Majumbar et al. (1976), Sundari et al. (1976), and Van der Hofstad et al. (1977)]. Recently, it has been shown that IF2 shields the T loop and the minor groove of the stem and induces an increased flexibility in the anticodon arm of the initiator tRNA (Wakao et al., 1989). The initiator tRNA may bind either to the 30S subunit through a binary complex or to the three factors–30S ribosomal complex. From recent data, it appears that IF2 probably acts on the 30S subunit by kinetically favoring the binding of fMet-tRNA$_f^{Met}$ to the 30S subunit (Gualerzi & Wintermeyer, 1986; Canonaco et al., 1986). Also, it was shown that IF2 binds weakly to the 30S subunit but that one molecule of each of the three factors binds tightly when added together to the 30S ribosomal subunit [e.g., Benne et al. (1973), Weiel and Hershey (1982), and Wintermeyer and Gualerzi (1983)] and that both IF2 and IF3 exert an allosteric effect on the 30S ribosomal subunit (Wintermeyer & Gualerzi, 1983).

The initiator factor IF2 has been located by immune electron microscopy and by cross-linking experiments in the cleft between the head and the lateral protrusion of the 30S subunit [for reviews, see Lake (1985) and Hershey (1987)]. However, little information is available about the nucleotides of the 16S rRNA, which may interact with this factor, and about possible conformational adjustements induced by IF2 binding. During recent years, the conformation of the 16S rRNA, in its naked form, in interaction with individual ribosomal proteins or within the 30S subunit, has been extensively investigated by the use of several structure-specific probes [e.g., Douthwaite et al. (1983), Moazed et al. (1986), Stern et al. (1989), Baudin et al. (1987, 1989), and Mougel et al. (1987, 1988)] and by RNA–RNA and RNA–protein cross-linking [for a review, see Brimacombe et al. (1988)]. Compilation of these data together with the topographical localization of proteins (Capel et al., 1987) led to the construction of two three-dimensional models of 16S rRNA, which give a relative spatial orientation of the helices within the subunit (Brimacombe et al., 1988; Stern et al., 1988). Among the different structure-specific probes, ethylnitrosourea has a high affinity for the phosphate oxygen groups in nucleic acids (Vlassov et al., 1981). This reagent has the advantage of not being selective since its reactivity is sequence and secondary structure independent. Recently, ethylnitrosourea was used to probe the conformation of 16S rRNA, in its naked form, on the 30S subunit and in the 70S ribosome (Baudin et al., 1989). This made it possible to gain precise information on the accessibility of each phosphate group in the 16S rRNA and provided evidence for conformational adjustments induced by the subunit assembly and ribosome association.

The study presented here was designed to provide the 16S rRNA sites that are shielded or conformationally perturbed as a consequence of IF2 or fMet-tRNA$_f^{Met}$ binding within the 30S subunit. We have followed the reactivity of each phosphate group of the 16S rRNA within the 30S/IF2/GTP complex, and within the 30S/IF2/AUG/fMet-tRNA$_f^{Met}$/GTP initiation complex. The results are discussed in light of the knowledge of the higher order structure of 16S rRNA and of the 30S subunit topography. Functional implications for the initiation process are also discussed.

Experimental Procedures

Buffers. Buffer A: 20 mM Tris-HCl, pH 7.5; 60 mM NH_4Cl; 6 mM magnesium acetate; 6 mM β-mercaptoethanol. Buffer B: 20 mM Tris-HCl, pH 7.5; 60 mM NH_4Cl; 6 mM magnesium acetate; 6 mM β-mercaptoethanol; 400 mM NaCl. Buffer C: 50 mM sodium cacodylate, pH 7.6; 50 mM NH_4Cl; 5 mM magnesium acetate; 16 mM β-mercaptoethanol. Buffer D: 50 mM sodium cacodylate, pH 7.6; 1 mM EDTA. Buffer E: 20 mM Tris-HCl, pH 7.5; 100 mM LiCl; 1 mM EDTA; 0.5% SDS.

Chemicals and Enzymes. Ethylnitrosourea was from Sigma. RNases T_1 and U_2, T_4 RNA polynucleotide kinase, and T_4 RNA ligase were from Pharmacia. RAV-2 reverse transcriptase, [5′-^{32}P]pCp (3000 Ci/mmol), and [γ-^{32}P]ATP (5000 Ci/mmol) were from Amersham, and [^{3}H]methionine (25 Ci/mmol) was from CEA. *E. coli* tRNA$_f^{Met}$ was from Boehringer.

Preparation of 30S Subunits, IF2, and fMet-tRNA$_f^{Met}$. The 30S subunits were prepared from *E. coli* MRE 600 according to Baudin et al. (1987). The 70S tight couples were isolated on a 10–30% linear sucrose gradient in buffer A, and the 30S subunits were obtained by centrifugation through a sucrose gradient in buffer B. Naked 16S rRNA was obtained by deproteinization of the 30S subunits and fractionation through a 5–25% sucrose gradient (for 20 h at 10 °C at 25 000 rpm) in buffer E. The fractions containing the 16S rRNA were pooled and precipitated three times with three volumes of ethanol, washed with 80% ethanol, dissolved in 2 mM sodium acetate, pH 5.8, and stored in small fractions at −20 °C. The 30S subunits and the 16S rRNA were renatured by incubation at 40 °C for 20 min in buffer C and cooled on ice prior ethylnitrosourea alkylation.

Aminoacylation and formylation of the *E. coli* initiator tRNA were performed according to Wakao et al. (1989). IF2 was purified according to Dondon et al. (1985).

3′-End Labeling of 16S rRNA. The 3′-end labeling of 16S rRNA was done within the 30S subunit according to Gornicki et al. (1989), by using [5′-^{32}P]pCp and T_4 RNA ligase. The labeled 30S subunits were incubated in buffer A at 37 °C for 10 min prior to be incubated with a 3–5-fold excess of renatured 50S subunits at 37 °C for 30 min. A fraction of the labeled 70S ribosome was dissociated and the subunits were fractionated by centrifugation on a 10–25% sucrose gradient. The 3′-end-labeled 16S rRNA was obtained by phenol extraction of the 30S subunit in buffer E and was precipitated twice with ethanol from aqueous phase. The ribosomal particles and the RNA were renatured by incubation at 40 °C for 20 min in buffer C and then cooled on ice prior to alkylation.

Formation of 30S/IF2/GTP and 30S/IF2/fMet-tRNA$_f^{Met}$/AUG/GTP Complexes. The binding of IF2 to the 30S subunits was performed by incubating 20 pmol of 30S subunits with 140 pmol of IF2 and 1 mM GTP, in 40 μL of buffer C at 37 °C for 10 min. For the 30S initiation complex, 120 pmol of fMet-tRNA$_f^{Met}$ and 900 pmol of AUG triplet were added. Formation of the initiation complex and its stability after ethylnitrosourea alkylation were tested either by nitrocellulose filter binding assay or by sucrose gradient centrifugation using f[^{3}H]Met-tRNA$_f^{Met}$.

Alkylation of Phosphate Groups by Ethylnitrosourea. Phosphate alkylation was essentially as described by Vlassov et al. (1981) and Baudin et al. (1989). Reactions were performed on the free 30S subunits, on the 30S/IF2/GTP and

the 30S/IF2/AUG/GTP/fMet-tRNA$_f^{Met}$ complexes, in 40 μL of buffer C. Incubation was at 37 °C for 45 min, 1.5 h, and 2.5 h with 8 μL of an ethylnitrosourea-saturated ethanol solution. An incubation control was performed on the free and complexed subunits in the absence of ethylnitrosourea. Naked 16S rRNA (12.5 pmol) was incubated under denaturing conditions, in 15 μL of buffer D for 30 s and 1 min at 80 °C. When the 3′-end-labeled RNA was used, 2 μg of total tRNA was added as carrier. The reactions were stopped by ethanol precipitation of the RNA. The pellets were then resuspended in 60 μL of buffer E and extracted twice with a phenol–chloroform-saturated solution of 50 mM sodium acetate, pH 5.8. After precipitation of the RNA, the alkylated RNA was cleaved at phosphotriester positions by incubation at 50 °C for 10 min in 0.1 M Tris-HCl, pH 9.0. The resulting RNA fragments were then precipitated with ethanol and washed twice with 80% ethanol.

Detection and Analysis of the Alkylated Positions. Cleavage positions were detected by the primer extension method using reverse transcriptase as described by Mougel et al. (1987). Fourteen synthetic oligodeoxyribonucleotides complementary to nucleotides 110–124, 220–234, 330–344, 441–454, 550–564, 660–674, 761–777, 865–879, 986–1000, 1101–1116, 1220–1240, 1330–1347, 1435–1449, and 1498–1509 were synthesized with an Applied Biosystem apparatus. These oligomers were labeled at their 5′-end with [γ-^{32}P]ATP and T_4 polynucleotide kinase according to Silberklang et al. (1977). The generated cDNA fragments were sized at nucleotide resolution by electrophoresis on 8 or 10% polyacrylamide (1/20 bisacrylamide)/8 M urea slab gels at 1500 V for 2–4 h. Unmodified 16S rRNA was used as a template for the dideoxy-sequencing reactions (Sanger et al., 1977). When using 3′-end-labeled 16S rRNA, the resulting RNA fragments were sized by gel electrophoresis in parallel with RNases T_1 and U_2 and formamide ladders to identify the cleavage positions.

Results and Discussion

In this study, we have used ethylnitrosourea to map phosphate groups of 16S rRNA that undergo reactivity changes as a result of IF2 binding, either in the absence or in the presence of fMet-tRNA$_f^{Met}$ and AUG as messenger. Alkylation experiments were achieved under conditions in which more than 80% of the initiator tRNA was bound to the 30S subunit in the presence of IF2 and AUG. Probing experiments were repeated several times with a good reproducibility. Cleavage positions were detected by the primer extension method for the largest part of the molecule (nucleotides 1–1498). The 3′-terminal region was probed directly from 3′-end-labeled 16S rRNA, since primer extension could not be used due to the presence of two 2,6-dimethyladenines at positions 1518–1519 and of a 2-methylguanine at position 1516, which block the progress of reverse transcriptase. Control experiments were done in parallel in order to detect stops or pauses of reverse transcriptase. Some of these stops result from the methylation of certain residues or from proton-catalyzed hydrolysis, which reflects an intrinsic fragility and flexibility of the RNA molecule (Dock-Brégeon & Moras, 1987). Other pauses could also reflect the difficulty of the reverse transcriptase to melt a stable secondary structure. The relative reactivity of the phosphate groups was estimated by visual inspection, taking the reactivity of the 16S rRNA within the 30S subunit as a reference. It should be reminded that changes of reactivity to ethylnitrosourea are generally weak, since each phosphate group possesses two potential oxygen reactive sites. Thus, only protection involving the two oxygen atoms will result in strong reduction of reactivity. Interpretation of autoradiograms requires a careful examination of the relative intensity of bands in each lane. Only changes that have been reproducibly observed in several experiments have been taken into consideration.

Although the ionic conditions slightly differ from those used by Baudin et al. (1989), the reactivity of phosphate groups within the 30S subunit remains strictly identical with those reported by these authors. In summary, regions 1–51, 250–310, 567–612, 650–670, and 1307–1382 are particularly buried, whereas the 3′-terminal region and the 5′-proximal region (nucleotide residues 53–218) are exposed. Furthermore, the present results confirm the observations of Baudin et al. (1989) regarding the defined enhancement of reactivity specifically induced by the binding of ribosomal proteins. Thus, the same reactivity pattern was observed, especially in those regions in which conformational adjustments have been described. All observed reactivity changes are shown in Figure 1 and the results are summarized in Table I. The reactivity changes induced by the binding of IF2 and by subsequent addition of fMet-tRNA$_f^{Met}$ and AUG are reported on a schematic representation of the secondary structure of 16S rRNA (parts a and b of Figure 2, respectively).

Effect of IF2 Binding. The binding of IF2 on the 30S subunits causes significant reactivity changes in the 16S rRNA. These changes are rather limited and are not restricted to a defined part of the molecule but are scattered throughout the four domains of the RNA. The most sharply reduced reactivities are found in the central domain at phosphates 694 (Figure 1d) and 791 (Figure 1e) and in the 3′-terminal domain at phosphate 1504 (Figure 1h). Weak reductions of reactivity are also observed in the 5′-domain at phosphates 301, 302 (Figure 1a), and 492 (Figure 1c) and in the central domain at phosphate 771 (Figure 1e). The binding of IF2 also causes enhanced reactivity of several phosphate groups located in the 3′-terminal domain at positions 1398, 1401, 1428 (Figure 1g), and 1527 (Figure 1h) and in the 3′ major domain at positions 1225 (Figure 1f) and 1268 (Figure 1f). Strong bands also appear in the 5′-terminal domain, at positions 407 and 408, but it is not yet clear whether they arise from an enhanced reactivity or from uncontrolled stops of reverse trancriptase (Figure 1b). In some experiments, very weak reductions of reactivity have also been detected at phosphates 299 and 360, as well as faint enhanced reactivities at positions 61 and 101 (not shown). However, since it is not yet clear whether these alterations (as for phosphates 407–408) represent reliable alterations, they will not be considered in further discussion. Another striking consequence of the initiation factor binding is the appearance of new reverse transcriptase stops at defined positions in the central domain (at positions 606, 607, 693, 789, and 790). Note the absence of any reactivity changes in the 3′ upper major domain.

One of the first conclusions that emerges from the failure of IF2 (which is a large protein) to protect large and defined areas is that the factor does not tightly interact with RNA. More likely, the binding of IF2 slightly perturbs the conformation of the RNA in several defined regions. Reduced reactivity could be interpreted either by a direct shielding effect or by a conformational adjustment leading to a stabilization of a particular conformation of the RNA. The fact that these reduced reactivities are in most cases isolated and not clustered suggests that they reflect conformational perturbations rather than true protections. This is further confirmed by the fact that IF2 affects the phosphates by enhancing as much as reducing their reactivity toward ethylnitrosourea. Enhanced

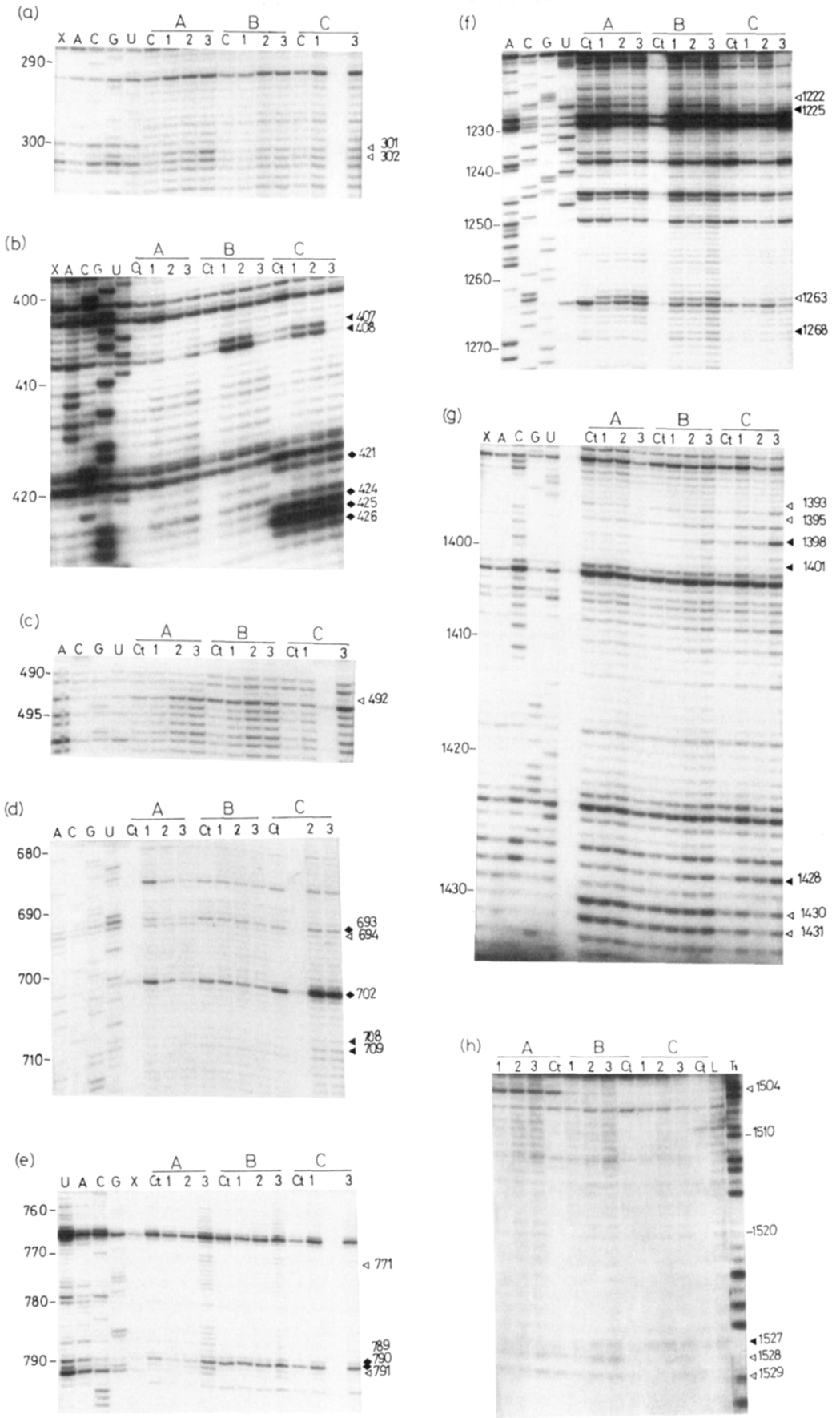

FIGURE 1: Autoradiograms showing the reactivity of phosphate groups in 16S rRNA toward ethylnitrosourea in the isolated 30S subunit (A), in the 30S/IF-2 complex (B), and in the 30S/IF-2/GTP/fMet-tRNA$_f^{Met}$/AUG complex (C). Incubation control (lane C); alkylation for 45 min (lane 1), 1 h 30 (lane 2), and 2 h 30 (lane 3). Lanes A, C, G, and U are sequencing products generated in the presence of ddTTP, ddGTP, ddCTP, and ddATP, respectively. Lane X is a sequencing incubation control. Arrowheads point to reactivity changes (△, reduced reactivity; ▲, enhanced reactivity) and diamonds to increased reverse transcriptase stops.

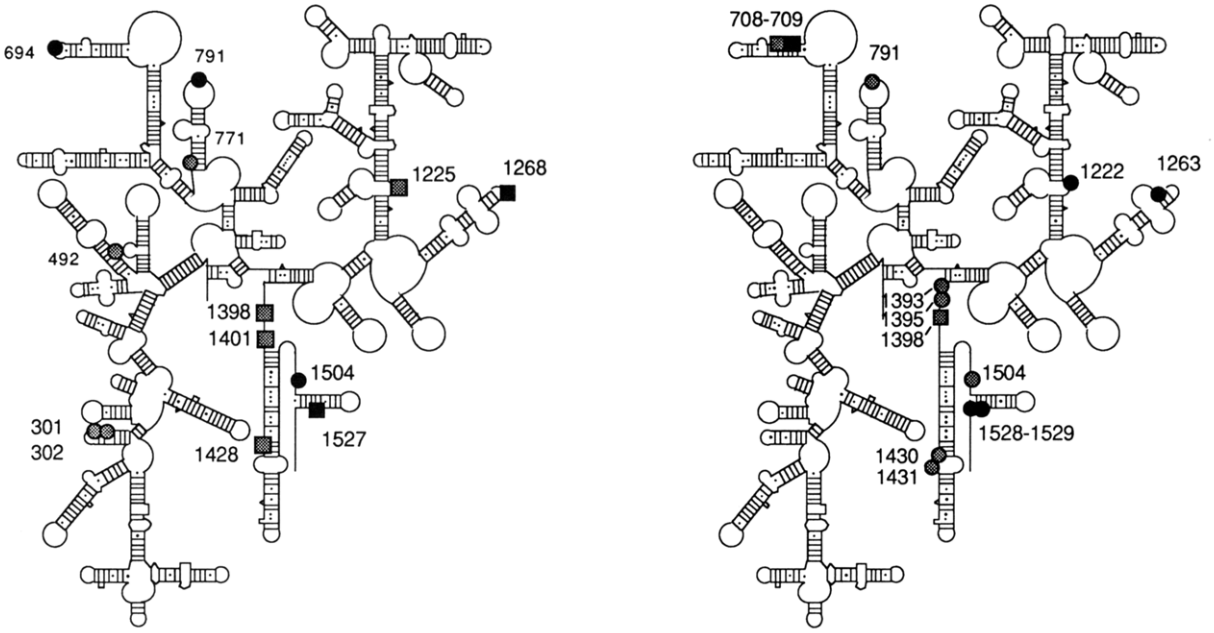

FIGURE 2: Schematic diagram of the *E. coli* 16S rRNA secondary structure summarizing the results of ethylnitrosourea probing experiments. Reactivity changes induced by (a) the binding of IF2 to the 30S relative to 30S and (b) the binding of the fMet-tRNA$_f^{Met}$ and the AUG triplet relative to the 30S/IF2 complex. Canonical base pairs are represented by bars and noncanonical base pairs (U·U, A·G, U·G) by dots. Weakly and strongly reduced reactivity (stippled and solid circles); weakly and strongly enhanced reactivity (stippled and solid boxes).

reactivity is most likely related to subtle conformational adjustment of the RNA or to ion release. Alternatively, it may reflect some modification in protein/RNA interaction, especially when enhancement affects phophate groups shown to display protein-induced protection. This might be the case of phosphate 1268, which is located in a cluster of phosphates showing a protein-induced protection (Baudin et al., 1989). It should be noted that this region falls in the S9–S19 binding regions (Stern et al., 1989). The case of phosphate 1401 is particularly interesting, since its reactivity is reduced in the naked RNA, enhanced by subunit assembly, and further enhanced as a consequence of IF2 binding. This strategic region has already been shown to display dynamic properties and protein-induced structural adjustments (Moazed et al., 1986; Baudin et al., 1987; Gornicki et al., 1989). Our results indicate that IF2 also affects the local conformation in this region.

The appearance of new reverse transcriptase pauses or stops induced by the binding of IF2 can probably be explained as the result of spontaneous degradations, reflecting an increased flexibility of the RNA backbone induced by protein binding. Remarkably, they are all found in the central domain of the molecule (at positions 606–607, 693, and 789–790). Such a phenomenon was also observed by Baudin et al. (1989) as a consequence of subunit assembly and association. Strikingly, several of these cleavages (at nucleotides 693 and 789–790, see Figure 1d,e) are observed near phosphates displaying a reduced reactivity. Of note is that IF2 also induces several spontaneous cleavages in the anticodon loop of the fMet-tRNA$_f^{Met}$, which do not occur when the tRNA is bound to the 30S subunit (Wakao et al., 1989).

As mentioned above, the IF2-induced reactivity changes appear to be distributed in the entire RNA molecule. However, these changes might be restricted in defined areas, if the three-dimensional folding of the RNA is considered. Thus, we compared our results to the three-dimensional map of 30S ribosomal proteins (Capel et al., 1987) and to the recent quaternary structure models of the 30S subunit proposed by Brimacombe et al. (1988) and Stern et al. (1988). They are

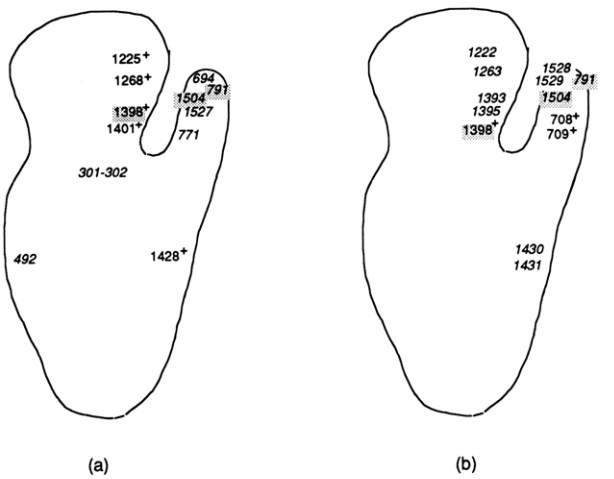

FIGURE 3: Results of probing experiments in a schematic representation of the 30S subunit. (a) IF2-induced reactivity changes; (b) initiator tRNA induced reactivity changes. The approximate localization of the phosphate groups is deduced from the three-dimensional models of Stern et al. (1988) and Brimacombe et al. (1988). Italic refers to reduced reactivity, + to enhanced reactivity, and shadowing to the reinforced effect in the presence of tRNA.

presented in a schematic representation of the 30S subunit in Figure 3. From these models, it appears that most phosphates (694, 771, 791, 1398, 1401, 1504, and 1527) are located in the lateral protrusion or in the cleft of the 30S subunit, while positions 1225 and 1268 are located in the head of the subunit facing the lateral protrusion. This precisely coincides with the localization of the factor by immune electron microscopy (Lake et al., 1985) and with the group of proteins found to be cross-linked to IF2: S1, S2, S11, S12, S13, S14, and S19 (Bollen et al., 1975; Boileau et al., 1983). This group of phosphates is directly located in the area covered by IF2, and some of the observed protections possibly result from a direct contact with the factor. The other phosphates are located in the body of the subunit. Note that phosphates 301–302 located in the central region are in the neighborhood of protein S16

Table I: Summary of the Effect of IF2 and fMet-tRNA$_f^{Met}$ Binding on the Reactivity of Phosphates in *E. coli* 16S rRNA[a]

position	observations	figure
	IF2 Effects	
strong reductions		
694		1d
791		1e
1504		1h
weak reductions		
301		1a
302		1a
492		1c
771		1e
strong enhancements		
1268	protected in 30S subunit	1f
1527		1h
weak enhancements		
407[b]	protected in 30S subunit	1b
408[b]	protected in 30S subunit	1b
1225		1f
1398	protected in 16S rRNA, strengthened in 30S subunit	1g
1401	protected in 16S rRNA, enhanced in 30S subunit	1g
1428		1g
	fMet-tRNA$_f^{Met}$ Effects	
strong reductions		
1222		1f
1263	strongly enhanced in 30S subunit	1f
1528		1h
1529		1h
weak reductions		
791		1e
1393	protected in both 16S rRNA and 30S subunit	1g
1395		1g
1430		1g
1431		1g
1504		1h
strong enhancements		
709		1d
weak enhancements		
708	protected in 30S subunit	1d
1398	protected in 16S rRNA, strengthened in 30S subunit	1g

[a] The observed reactivity changes (reduction or enhancements) are classified as specific IF2 and tRNA effects, by comparison with the reactivity of phosphates in the isolated 30S subunit and in the IF2-30S complex, respectively. The effects are averaged from independent experiments as weak or strong from visual inspection of autoradiograms (see Figure 1a–h as examples). The status of the concerned phosphates in the 30S subunit compared to the status of those in naked 16S rRNA is indicated [according to Baudin et al. (1989)]. [b] The case of phosphates 407 and 408 is ambiguous, since the observed enhancements do not necessarily reflect an increased reactivity but might result from a nonreproducible stop of reverse transcription. Such an effect is not understood.

(Stern et al., 1986). Their exact position is difficult to assign, since there are some discrepancies between the two models. Phosphate 492 (as well as phosphates 407–408) is in the binding area of protein S4 (Stern et al., 1986). These reactivity changes, occurring at a distance from the IF2 binding domain, most likely reflect allosteric transitions induced by IF2 binding. Such an effect at distance probably also accounts for the increased reactivity of phosphate 1428 in the penultimate stem, which appears to traverse the subunit from the cleft to the bottom.

The binding of IF2 causes conformational adjustments in topographically defined regions. One region, containing the most strongly affected phosphates, fits with the known binding area of the factor (the lateral protrusion and the part of the head facing this protrusion). However, a few weak reactivity changes are also induced in the center of the particle (not very far from the cleft) and on distant parts of the subunit, most likely reflecting subtle allosteric transitions. Also, the binding of IF2 may affect some specific interactions between the RNA and certain proteins. Thus, the enhanced reactivity of phosphate 1268 might result from an altered interaction with proteins S9–S19 (e.g., the loss of some contacts with the proteins). It is not yet clear whether the observed allosteric transitions are propagated through RNA or proteins, via RNA/protein interactions.

Effect of fMet-tRNA$_f^{Met}$ and AUG Triplet Binding. The stoichiometry of the 30S/IF2/AUG/GTP/fMet-tRNA$_f^{Met}$ initiation complex was checked by nitrocellulose filter binding assay or sucrose gradient centrifugation, using f[^{3}H]-Met-tRNA$_f^{Met}$. Under the conditions used, 70–80% of the 30S subunits were found to bind the initiator tRNA. All the IF2-induced reactivity changes are still observed in the presence of the 30S/IF2/AUG/GTP/fMet-tRNA$_f^{Met}$ complex, including both enhanced and reduced reactivities. However, a specific subset of phosphates shows additional reactivity changes as the result of fMet-tRNA$_f^{Met}$ and AUG binding (Figure 1 and Table I). The tRNA-induced modifications appear to be clustered in the 3′-terminal domain, the 3′ lower major domain, and the central domain. They mostly correspond to reduction of reactivity. The most sharply reduced reactivities occur at phosphates 1222, 1263 (Figure 1f), 1528, and 1529 (Figure 1h). Other weaker but significantly reduced reactivities are found at positions 791 (Figure 1e), 1393, 1395, 1430, 1431 (Figure 1g), and 1504 (Figure 1h). Otherwise, the reactivity of phosphates 708, 709 (Figure 1d), and 1398 (Figure 1g) is enhanced. Remarkably, the reactivity changes are mainly observed at or near positions that are already affected by IF2 binding. Thus, the reduction of reactivity at positions 791 and 1504 and the enhancement of reactivity at position 1398 are even more pronounced in the presence of the initiator tRNA. Also, protection often occurs near positions displaying IF2-induced enhancement (1222 near 1225, 1263 near 1268, 1430, 1421 near 1428, and 1393, 1395 near 1398, 1401). Another consequence of tRNA binding is the appearance of additional pauses or stops of reverse transcriptase at several defined positions, especially in loop regions (at positions 214, 421, 424–426, 702, and 1034–1035). One of the most striking examples regards phosphates 421 and 424–426 (Figure 1b).

The initiator tRNA induced reactivity changes are remarkably centered around a region comprising the lateral protrusion and the cleft of the subunit (Figure 3). Our results are in complete agreement with the localization of the decoding site deep in the cleft between the head and the lateral protrusion of the small subunit (Gornicki et al., 1984; Ofengand et al., 1986). The reduced reactivity of phosphates 1393 and 1395 is most likely related to the anticodon–codon interaction that takes place in the close vicinity of C1400, since this nucleotide has been directly cross-linked to the 5′-base of the anticodon of a tRNA bound in the P site (Prince et al., 1982; Ehresmann et al., 1984). It should be noted that C(N3)1399, C(N3)1400, and G(N7)1401 have been reported to be protected by P-site bound tRNA from chemical modification (Moazed & Noller, 1986, 1989). Also, a tRNA in the P site has been found to protect position 1397 from lead(II)-induced hydrolysis (Gornicki et al., 1989). Our results, together with other results obtained with different probes, provide strong support for an intimate contact between the phylogenetically conserved 1400 region in 16S rRNA and a P-site bound tRNA. Otherwise, it was shown that the 30S subunit essentially protects the anticodon stem–loop of the initiator tRNA

within the 30S/IF2/GTP/fMet-tRNA$_f^{Met}$/AUG complex (Wakao et al., 1989). However, the binding of the initiator tRNA also causes an enhanced reactivity at phosphate 1398, which was already found to be enhanced by IF2 alone. Note that an increased reactivity was reported by Moazed and Noller (1986) to be induced at C(N3)1400 by the binding of yeast tRNA^{Phe}. This enhancement, together with the increased protection at positions 791 and 1504, may reflect a stabilization of the IF2/30S subunit interaction in the presence of the initiator tRNA or a mutual adjustment between IF2 and the tRNA. Interestingly, nucleotides 793 and 795 (close to position 791) were found to be protected by edeine (Moazed & Noller, 1987), an oligopeptide antibiotic that blocks the P site binding of the tRNA (Szer et al., 1970).

The enhancement observed at phosphates 708–709, located in the lateral protrusion (as position 1504), most likely reflects conformational adjustments in 16S rRNA. However, it is difficult to ascertain whether the protection of phosphates 1528–1529, located on the superior part of the lateral protrusion, is the result of direct shielding or of a structural transition. It should be noted that no protection from base-specific probes has been detected in this region (Moazed & Noller, 1986, 1989). Remarkably, phosphates 1222 and 1263 located in the head of the subunit in the binding area of proteins S9–S19, found protected in the presence of the initiator tRNA, are located near phosphates 1225 and 1268, which show an IF2-induced enhancement. In this case again, the exact cause of the protection is ambiguous. It should be noted that the reactivity of phosphate 1263 is strongly enhanced as the result of subunit assembly (Table I). However, the reduced reactivity observed at positions 1430 and 1431, located rather far from the decoding site in the penultimate helix, more likely reflects a conformational transition. The enhanced stops of elongation, interpreted as increased spontaneous cleavages, also reflect such transitions. They are particularly important at positions 424–426 in the 5′-domain, in the proximity of the S4 binding domain. It might be relevant that they occur not far from positions 529–532, at which A- and P-site tRNA-induced protections have been reported (Moazed & Noller, 1986, 1989). Thus, our data, together with other studies (Moazed & Noller, 1986; Gornicki et al., 1989), provide further evidence for the existence of allosteric transitions induced by tRNA binding.

Concluding Remarks

The present study yields precise and new information on the regions of 16S rRNA affected by the binding of IF2 and of the initiator tRNA to the 30S subunit. One of the main conclusion is that IF2 does not tightly interact with 16S rRNA but induces allosteric transitions. IF2 not only affects the region corresponding to the recognition site mapped from immune electron microscopy (i.e., the lateral protrusion, the cleft and the head facing the protrusion) but also alters distant regions scattered in different parts of the particle, thus reflecting dynamic changes of the internal structure of the subunit. Possible alterations of RNA–protein interactions (presumably involving proteins S9–S19) can also be postulated. The effect of IF2 binding remains strictly identical in the presence of the initiator tRNA and the AUG triplet, some of the reactivity changes even being reinforced. Remarkably, the additional tRNA-induced reactivity changes are most often localized in close proximity to the positions that are affected by IF2. This appears to be consistent with the proposal that the binding of initiation factors precedes tRNA and mRNA binding (Gualerzi et al., 1986). In view of this, IF2 would induce specific allosteric transitions that should stimulate the binding of the initiator tRNA. The tRNA-induced additional changes are mostly centered around the cleft, the lateral protrusion, and the head region. On the other hand, other independent experiments imply that only the anticodon stem–loop region of the tRNA makes significant contacts with the 30S subunit (e.g., Rose et al., 1983; Moazed & Noller, 1986; Wakao et al., 1989). Our study provides additional support for a close contact between the anticodon region of the tRNA with residues of the 16S rRNA in the cleft of the subunit (around position 1400). If some of the changes induced by the tRNA binding most likely result from a direct shielding, others clearly reflect conformational transitions. It is probably significant that both IF2 and tRNA binding often affect positions that are sensitive to subunit assembly, being directly involved either in RNA/protein interactions or in protein-induced conformational adjustments. This stresses the crucial role of ribosomal proteins in stabilizing a particular conformation of 16S rRNA. Our results also emphasize the remarkable property of the ribosome to undergo dynamic adjustments accompanying the initiation steps.

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