Seelig, J. (1978) *Biochim. Biophys. Acta 505*, 105–141.
Seelig, J., & Waespe-Šarčevič, N. (1978) *Biochemistry 17*, 3310–3315.
Seelig, J., & Seelig, A. (1980) *Q. Rev. Biophys. 13*, 19–61.
Seelig, J., Tamm, L., Hymel, L., & Fleischer, S. (1981) *Biochemistry 20*, 3922–3933.
Seelig, J., Seelig, A., & Tamm, L. (1982) in *Lipid-Protein Interactions* (Jost, P. C., & Griffith, O. H., Eds.) Vol. II, pp 127–148, Wiley-Interscience, New York.
Siminovitch, D. J., Rance, M., & Jeffrey, K. (1980) *FEBS Lett. 112*, 79–82.
Spiess, H. W., & Sillescu, H. (1981) *J. Magn. Reson. 42*, 381.
Taylor, M. G., Akiyama, T., Saito, H., & Smith, I. C. P. (1982) *Biochim. Biophys. Acta* (in press).
Vik, S. B., & Capaldi, R. A. (1977) *Biochemistry 16*, 5755–5759.
Wei, Y. H., & King, T. E. (1981) *Arch. Biochem. Biophys. 210*, 653–665.
Woessner, D. E., Snowden, B. S., & Meyer, G. H. (1969) *J. Chem. Phys. 51*, 2968–2976.
Yu, C. A., Yu, L., & King, T. E. (1975) *J. Biol. Chem. 250*, 1383–1392.

Photochemical Cross-Linking of Translation Initiation Factor 3 to *Escherichia coli* 50S Ribosomal Subunits†

Ira Schwartz,* Mark Vincent, William A. Strycharz,‡ and Lawrence Kahan

ABSTRACT: Translation initiation factor 3 (IF-3) was bound noncovalently to *Escherichia coli* 50S ribosomal subunits. Irradiation of such complexes with near-ultraviolet light (>285 nm) resulted in covalent attachment of initiation factor 3 to the 50S subunit. Photo-cross-linking attained its maximum level of 40% of that which was noncovalently bound after 90 min of irradiation. Cross-linking was abolished in the presence of either 0.5 M NH_4Cl or 0.25 mM aurintricarboxylic acid, indicating that specific binding of initiation factor 3 to the ribosome was a prerequisite for subsequent covalent attachment. Further analysis showed that all the IF-3 was covalently bound to a small number of 50S subunit proteins. The major cross-linked proteins were identified as L2, L7/L12, L11, and L27 by immunochemical techniques. These results are discussed in light of the proposed mechanism for IF-3 function.

Initiation of the synthesis of a peptide chain on ribosomes in *Escherichia coli* requires the participation of three protein factors (Grunberg-Manago & Gros, 1977; Revel, 1977). One of these, initiation factor 3 (IF-3),[1] serves two functions—it ensures the availability of free 30S subunits by preferentially binding to the free subunit and is required for the functional binding of mRNA to these subunits. A variety of studies have been interpreted as indicating that IF-3 carries out its function as an antiassociation factor (rather than a dissociation factor) by interacting solely with 30S subunits, thereby shifting the 70S $\rightleftarrows$ 30S + 50S equilibrium in favor of dissociation (Godefroy-Colburn et al., 1975; Grunberg-Manago & Gros, 1977; Chaires et al., 1981). Other biophysical evidence, however, is incompatible with this model and is more consistent with a 70S·IF-3 complex as an intermediate in initiation (Chaires et al., 1979; Goss et al., 1980a,b, 1982). In addition, there have been a number of studies which demonstrated a direct interaction between IF-3 and 70S couples. Thus, IF-3 was found to be required for efficient poly(U)-dependent polyphenylalanine synthesis at 18 mM Mg^{2+}, a concentration which does not favor ribosome dissociation (Schiff et al., 1974). Indeed, Hawley et al. (1974) have cross-linked IF-3 to both 50S subunits and 70S ribosomes by treatment of the appropriate complexes with dimethylsuberimidate, and periodate oxidation and reduction of 70S ribosome–IF-3 or 50S subunit–IF-3 mixtures resulted in covalent attachment of the IF-3 to the 3′ end of 23S RNA (Van Duin et al., 1976). These findings led Van Duin et al. (1976) to propose that IF-3 functions by disrupting complementary base pairs between 16S and 23S RNA which are at least partially responsible for 70S couple formation. Since the mRNA–16S RNA interaction occurs in the same region of the 16S RNA (Shine & Dalgarno, 1974; Steitz & Jakes, 1975), this single IF-3 binding event could explain its apparently dual function.

Previously, we reported the covalent cross-linking of IF-3 to its binding site on 30S subunits by irradiation of the complex with near-ultraviolet light (MacKeen et al., 1980). The target ribosomal proteins were identified immunochemically. Here we describe a similar cross-linking reaction between IF-3 and 50S subunits and present the identification of 50S ribosomal proteins which are in the neighborhood of the IF-3 binding site.

Experimental Procedures

Preparation of Ribosomes and Initiation Factor 3. The 70S ribosomes and IF-3 were prepared as described by Hershey

† From the Department of Biochemistry, New York Medical College, Valhalla, New York 10595, and University of Massachusetts, Amherst, Massachusetts 01003 (I.S. and M.V.), and Institute for Enzyme Research (W.A.S.) and Department of Physiological Chemistry (L.K.), University of Wisconsin, Madison, Wisconsin 53706. *Received August 4, 1982.* This work was supported in part by National Institutes of Health Grants GM25989 and GM29265 (to I.S.), GM22150 (to L.K.), and GM20247 (W.A.S., administered by M. Nomura). I.S. is a Sinsheimer Scholar Awardee of the Alexandrine and Alexander L. Sinsheimer Foundation.

‡ Present address: Genetics Institute, Boston, MA 02115.

[1] Abbreviations: IF, translation initiation factor; poly(U), poly(uridylic acid); Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $NaDodSO_4$, sodium dodecyl sulfate; RNase, ribonuclease; TP50, total protein extracted from the 50S ribosomal subunit; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid.

0006-2960/83/0422-1483$01.50/0

et al. (1977). The 50S subunits were prepared from the 70S ribosomes by separation of subunits on sucrose gradients in buffers containing 0.5 mM magnesium acetate followed by concentration of the 50S subunits by ethanol precipitation or centrifugation at 100000*g* through a 5% sucrose cushion for 14 h. The subunits were resuspended in 50 mM Tris-HCl (pH 7.4), 10 mM magnesium acetate, 5 mM 2-mercaptoethanol, and 100 mM NH_4Cl to a final concentration of 100–200 A_{260}/mL. IF-3 was radiolabeled by reductive methylation with [^{14}C]formaldehyde as described (MacKeen et al., 1979). Specific activities ranged from 20000 to 60000 cpm/μg of protein. The IF-3 was 92–95% pure as judged by NaDodSO$_4$–polyacrylamide gel electrophoresis.

Binding of [^{14}C]IF-3 to 50S Ribosomal Subunits. Binding mixtures (either 100 or 250 μL) containing 60–70 pmol of both IF-3 and 50S subunits in 50 mM NaHepes (pH 7.4), 6 mM magnesium acetate, 50 mM NH_4Cl, and 2.5% glycerol were incubated at 37 °C for 15 min. An equal volume of 0.5% glutaraldehyde in the above buffer was added and incubation continued on ice for 5 min. The mixtures were then immediately layered onto 15–30% sucrose gradients in the above buffer and centrifuged for 17.5 h at 24000 rpm in either a Beckman SW 27.1 or SW 41 rotor. The gradients were monitored for absorbance at 260 nm and fractionated, and the fractions were analyzed for radioactivity either by precipitation with trichloroacetic acid and filtration on glass-fiber filters or by direct analysis in Biofluor (New England Nuclear).

Irradiation of [^{14}C]IF-3·50S Subunit Mixtures. Reaction mixtures identical with those described above were prepared and incubated at 37 °C for 15 min. Samples, in siliconized Pyrex tubes, were then irradiated at 4 °C with light from a 450-W medium pressure mercury lamp (Hanovia) equipped with a Corex filter at a distance of 4 cm. After irradiation the samples were diluted with an equal volume of 50 mM NaHepes (pH 7.4), 6 mM magnesium acetate, 6 mM 2-mercaptoethanol, 500 mM NH_4Cl, and 2.5% glycerol, layered onto 15–30% sucrose gradients in the same buffer, centrifuged, and analyzed as described above.

Large-scale preparations of [^{14}C]IF-3·50S subunit covalent complexes employed in subsequent immunochemical experiments were prepared as described previously (MacKeen et al., 1980).

Ribonuclease Digestion of [^{14}C]IF-3·50S Subunit Complexes. Five to ten A_{260} units of [^{14}C]IF-3·50S complex in 100 μL was digested with a mixture of 10 μL of pancreatic ribonuclease (50 μg/mL), 10 μL of T_1 ribonuclease (50 μg/mL), and 20 μL of 0.3 M EDTA (pH 7.0), as described previously (MacKeen et al., 1980). The precipitates were solubilized by the addition of 0.75 × digestion volume (weight) of solid urea and dialyzed overnight against 40 mM Tris-HCl (pH 7.4) and 2 M KCl. Aliquots were removed for the measurement of concentration and radioactivity.

Immunochemical Identification of 50S Subunit Proteins Cross-Linked to [^{14}C]IF-3. Aliquots of 20 μL of ribonuclease-digested [^{14}C]IF-3·50S subunit complexes (containing 0.4 A_{260} equiv of protein) were tested against 20-μL aliquots of 4× concentrated antisera to each of the purified 50S ribosomal proteins essentially as described (MacKeen et al., 1980).

Results

Cross-Linking of [^{14}C]IF-3 to 50S Subunits. As a first step toward elucidating the topography of an IF-3 binding site on the 50S subunits, we examined the ability of IF-3 to bind noncovalently to the subunits. When equimolar amounts of [^{14}C]IF-3 and 50S subunits were incubated at 30 °C for 15 min, significant binding was detected. A typical result is presented in Figure 1A. Approximately 20% of the recovered IF-3 comigrated with the 50S subunits. In many binding experiments we have consistently found 20–30% of the IF-3 associated with 50S subunits.

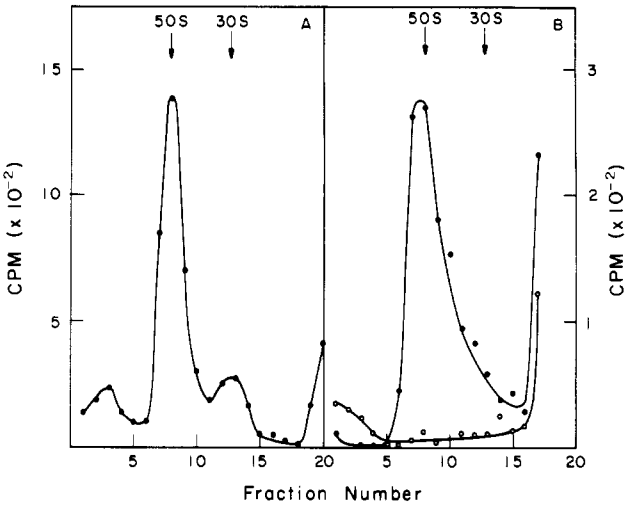

FIGURE 1: (A) Binding of [^{14}C]IF-3 to 50S subunits. Binding mixtures were incubated, fixed with glutaraldehyde, and centrifuged as described under Experimental Procedures. (B) Covalent cross-linking of [^{14}C]IF-3 to 50S subunits. Binding mixtures were incubated, irradiated for 45 min, centrifuged, and analyzed as described under Experimental Procedures (●); binding mixtures identical with those in (A) were incubated and centrifuged in gradients containing 0.5 M NH_4Cl (○). Arrows indicate migration positions of 30S and 50S subunits.

Irradiation of a noncovalent IF-3·50S subunit complex with near-ultraviolet light (>285 nm) resulted in covalent attachment of a significant portion of the bound IF-3 to the 50S subunit (Figure 1B). Cross-linking was monitored as comigration of [^{14}C]IF-3 with 50S subunits in sucrose gradients containing 0.5 M NH_4Cl (with omission of the fixation step). If an identically treated sample was not irradiated, no radioactivity was detected in the 50S region of the gradient (Figure 1B).

The following controls were performed in order to ascertain that the IF-3 was bound to 50S subunits in a specific manner. If incubation and irradiation were carried out in the presence of 0.5 M NH_4Cl, which disrupts noncovalent binding of IF-3 to 50S subunits, no cross-linking was detected (Figure 2). Furthermore, addition of 0.25 mM aurintricarboxylic acid to the reaction mixtures resulted in complete abolition of the cross-linking.

The kinetics of the reaction are shown in Figure 2. The extent of photo-cross-linking leveled off after 90 min of irradiation at a plateau value of 40%. The rate and extent of the reaction were identical in the absence or presence of 5% acetone [acetone has been shown by others (e.g., Hélène, 1976) to be an effective photosensitizer].

Characterization of Cross-Linked Products. The 50S subunit·IF-3 covalent complexes were isolated and analyzed in order to determine the site of IF-3 attachment. Both phenol extraction and acetic acid extraction indicated that essentially all the IF-3 was cross-linked to proteins of the 50S subunit. This was further established by electrophoresis of the 50S subunit·[^{14}C]IF-3 covalent complex on NaDodSO$_4$–polyacrylamide gels. The results showed that a portion of the input IF-3 migrated more slowly than the main band of IF-3, indicating that there was cross-linking of IF-3 to some 50S ribosomal proteins. The electrophoresis pattern was not altered by prior treatment of the covalent complex with a combination of RNases A and T_1 from which it is concluded that 5S RNA

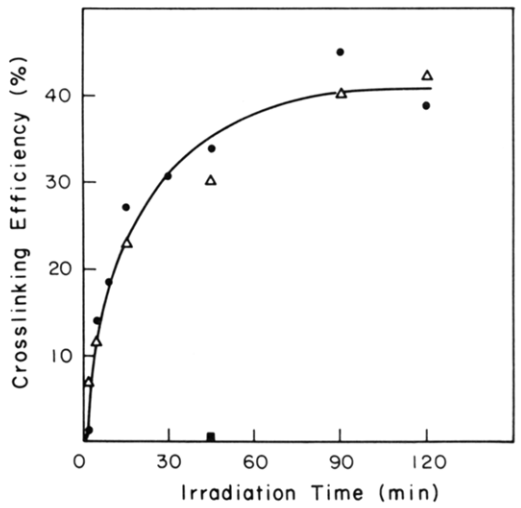

FIGURE 2: Kinetics of photo-cross-linking of [^{14}C]IF-3 to 50S subunits. Binding mixtures containing appropriate amounts of [^{14}C]IF-3 and 50S subunits were incubated, irradiated for the indicated times, and analyzed for covalent attachment of IF-3 as described. Irradiations were performed in the presence (●) or absence (△) of 5% acetone. (■) Incubation and irradiation were in the presence of 0.5 M NH_4Cl. Cross-linking efficiency, in percent, is defined as the fraction of noncovalently bound IF-3 which comigrates with 50S subunits.

is not a component of the cross-linked complexes. In addition, ribosomal protein–ribosomal protein cross-linking has not been detected under our conditions of irradiation, and thus the attachment of IF-3 appears to be directly to ribosomal proteins. Since most of the ribosomal proteins are the same size, it is essentially impossible to definitively identify specific ribosomal proteins by gel electrophoresis. This was accomplished by coprecipitation of [^{14}C]IF-3 with antisera specific for a given ribosomal protein.

Immunochemical Identification of Cross-Linked Proteins. [^{14}C]IF-3·50S subunit covalent complexes were digested with ribonuclease and diffused against specific antisera for each of the 50S subunit proteins. After being extensively washed to remove noncovalently bound [^{14}C]IF-3, the immunodiffusion gels were dried and subjected to autoradiography. The results of a representative experiment are presented in Figure 3. For this particular preparation it is clear that proteins L2, L7/L12, L11, L19, and L27 were covalently attached to [^{14}C]IF-3. A control experiment, in which 50S subunits and [^{14}C]IF-3 were mixed and treated in a manner similar to the [^{14}C]IF-3·50S subunit covalent complex, resulted in no coprecipitation of ^{14}C with any of the antisera. This confirms that the labeled IF-3 was not contaminated with any 50S subunit proteins.

The identities of the proteins covalently attached to IF-3 in three independently generated [^{14}C]IF-3·50S subunit covalent complexes are presented in Table I. In all the preparations which were analyzed, proteins L2, L7/L12, L11, and L27 were found complexed to IF-3. In addition, proteins L4, L19, L23, and L33 were observed in attachment to IF-3 in at least one of the samples. It is, therefore, concluded that these proteins define a neighborhood of the 50S subunit which constitutes the IF-3 binding site.

Discussion

The results presented here indicate that IF-3 is capable of binding and cross-linking to 50S subunits. This observation is in agreement with other studies in which IF-3·50S complex formation was observed (Vermeer et al., 1973; Hawley et al., 1974; Van Duin et al., 1976). A number of previous investigations have suggested that such an interaction does not occur (Sabol & Ochoa, 1971; Sabol et al., 1973; Gualerzi & Pon, 1973), although, in one of these reports, saturable binding of IF-3 to 50S subunits is observed [see Gualerzi & Pon (1973); Figure 3]. In the present case, glutaraldehyde fixation was used to stabilize the 50S·IF-3 complex such that it could be detected on sucrose density gradients. In addition, we employed low rotor speeds so as to minimize any possible pressure-induced dissociation of the complex. When the fixation step was omitted, no IF-3 was detected in the 50S region of the gradient. In contrast, the extent of complex formation between 30S subunits and IF-3 was identical with or without glutaraldehyde fixation (data not shown). Since, in the latter case, fixation did not affect the observed binding, it was

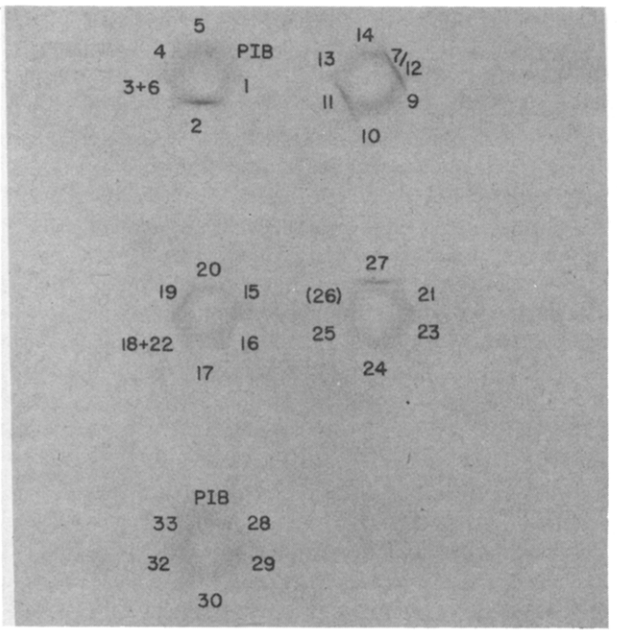

FIGURE 3: Identification of 50S ribosomal proteins cross-linked to [^{14}C]IF-3. [^{14}C]IF-3·50S subunit covalent complexes were digested with ribonuclease as described under Experimental Procedures. A total of 580 cpm of the digested complexes and 0.56 A_{260} unit of carrier TP50 were added to the center well and allowed to diffuse against antisera specific for the 50S subunit proteins in the outer wells as indicated. The figure is an autoradiograph after 6 weeks of exposure. Proteins were identified as cross-linked to IF-3 only if radioactivity coincided with the precipitin arc. PIB, preimmune bleeding.

Table I: Summary of 50S Ribosomal Proteins Cross-Linked to IF-3[a]

ribosomal protein	experiment 1	2	3
L2	+	+	+
L4	+	(+)	
L7/L12	+	+	(+)
L11	+	+	(+)
L19	+		
L23	+		
L27	+	+	+
L33	(+)	(+)	(+)

[a] Proteins cross-linked to IF-3 were identified immunochemically as described under Experimental Procedures. Figure 3 shows a typical analysis. The table summarizes results obtained for three independently prepared samples of IF-3·50S subunit covalent complex. Plus signs denote strong positive coprecipitation of [^{14}C]IF-3 with antiserum specific for the given 50S subunit protein in an analysis as shown in Figure 3; plus signs in parentheses denote weak coprecipitation (e.g., L2 showed strong coprecipitation in all three samples; L11 showed strong coprecipitation in two cases and weak coprecipitation in the third). The only antisera which induced any observable coprecipitation are those for the proteins listed.

concluded that under the conditions employed glutaraldehyde treatment simply stabilized the relatively weak binding between 50S subunits and IF-3 rather than promoted nonspecific interactions.

The photochemical procedure employed here is the same as that previously used for the study of the IF-3·30S subunit interaction (MacKeen et al., 1980). A major advantage of this approach is that it induces covalent attachment between ribosomes and IF-3 in the native complex without the introduction of extraneous chemical probes. In addition, the two participants in the cross-link must be within covalent bonding distance of each in order for cross-linking to occur. The irradiation conditions employed do not adversely affect ribosome function (MacKeen et al., 1980) nor do they result in protein–protein cross-linking within the isolated ribosomal subunit.

Three results attest to the specificity of the observed cross-linking. First, no covalant attachment was found when the irradiation was performed in the presence of 0.5 M NH_4Cl. This indicates that noncovalent binding of IF-3 to 50S subunits is necessary in order for subsequent cross-linking to occur. Second, the inclusion in the reaction mixture of 0.25 mM aurintricarboxylic acid, a specific inhibitor of the IF-3·ribosome interaction (Pon et al., 1972; Vazquez, 1974), led to total inhibition of photo-cross-linking. The most compelling evidence comes from the immunochemical results which indicated that only a small subset of the total 50S subunit proteins becomes covalently bound to IF-3. If the cross-linking were occurring from solution, or by some nonspecific electrostatic interaction, one would expect a more random distribution of IF-3 among the 50S proteins.

The present study represents the first report of covalent attachment of IF-3 to 50S subunits by application of photochemical techniques. There have been two previous instances of cross-linking of IF-3 to 50S subunits by using other approaches. Hawley et al. (1974) used the bifunctional protein cross-linking reagents dimethylsuberimidate and 2-iminothiolane to induce covalent complexes; proteins L2, L5, and L17 have been identified in the complex (Sobura et al., 1977). Van Duin et al. (1976) generated IF-3·23S RNA cross-links by periodate oxidation and reduction of either IF-3·70S or IF-3·50S complexes. The results presented here further extend the list of components of the 50S binding site for IF-3 to include proteins L7/L12, L11, and L27 and probably L4, L23, and L33. It should be pointed out that in a previous photo-cross-linking study of the IF-3·30S complex, covalent binding to 16S RNA was observed, in addition to the reaction with ribosomal protein (MacKeen et al., 1980). This indicates that the technique is capable of generating IF-3·rRNA cross-links if the appropriate components are within covalent bonding distance. Thus, the present results would appear to be in conflict with those of Van Duin et al. (1976). This point will be further discussed below.

A variety of sophisticated techniques have been employed in attempts to elucidate the structure of the 50S subunit and the roles of its components in ribosome function. The results obtained in this study are consistent with many of the previous investigations. Thus, a number of the 50S proteins cross-linked to IF-3 have been identified as near neighbors in the ribosome by bifunctional cross-linking. These cross-links include L2-L7/L12, L2-L11, L7/L12-L11, and L4-L11 (Kenny & Traut, 1979; Traut et al., 1980). Perhaps more relevant to IF-3's role in subunit dissociation is the identification of 50S proteins which can be cross-linked to proteins of the 30S subunit. These include L2, L27, L33, and L19 (Cover et al., 1981; Lambert & Traut, 1981)—proteins which were also covalently linked to IF-3. Of special interest were the protein pairs S11-L2, S12-L2, and S21-L33 since these 30S proteins were found to complex with IF-3 (MacKeen et al., 1980).

A number of the 50S proteins cross-linked to IF-3 have been localized at the peptidyltransferase center of the ribosome by (photo-) affinity labeling. L2, L11, L23, and L27 were cross-linked to various derivatives of puromycin (Cooperman et al., 1975; Jaynes et al., 1978; Nicholson & Cooperman, 1978), and native chloramphenicol was covalently bound to L11 and L27 (LeGoffic et al., 1980). L2, L11, and L27 and possible L33 have been found consistently in close proximity to the 3′ end of aminoacyl-tRNA (Ofengand, 1980). In addition, recent "single-omission" reconstitution experiments have resulted in the classification of L2 and L4 as essential for peptidyltransferase activity and L11 and L27 as strongly involved in this activity (Hampl et al., 1981). It has been suggested that the peptidyltransferase region of the 50S subunit contacts the 30S subunit (Lake, 1980; Lake & Strycharz, 1981), and this, therefore, lends further support for the proposed interaction of IF-3 with the interface region of both subunits.

Many of the IF-3 binding site proteins identified here are capable of binding to 23S RNA, and the regions of the 23S RNA have been identified. These include L2, L4, L7/L12, L11, L23, and L27 (Zimmermann, 1980; Röhl & Nierhaus, 1982). Of particular interest is the cross-linking of L27 to positions 2332–2337 of the 23S RNA (Wower et al., 1981) since guanine residues at positions 2307 and 2308 were previously shown to be rendered inacessible to kethoxal modification by association of the 50S subunit with 30S particles (Herr & Noller, 1979). These findings strengthen the assignment of L27 to the interface of the 50S subunit.

The work presented here and in a previous report (MacKeen et al., 1980) constitutes an analysis of the IF-3 binding site on both 30S and 50S subunits by a single experimental approach. This, in conjunction with other results, allows the construction of a model for IF-3 action. Immunoelectron microscopy has provided considerable information on the spatial arrangement of ribosomal proteins within the particle. Parts A and B of Figure 4 show diagrammatic representations of the 30S and 50S subunits as originally proposed by Lake (1976). The revised model of Stöffler and co-workers is now in substantial agreement with this view (Stöffler-Meilicke et al., 1981). For simplicity and consistency the locations of only those proteins to which IF-3 has been cross-linked by our photochemical technique are shown. In addition to those indicated in the figure, proteins S2, S3, and S19 have been cross-linked to IF-3 by others (Grunberg-Manago, 1980; Cooperman et al., 1981). It is clear that antigenic determinants for all of the proteins except S3 cluster in the platform region of the 30S subunit. The involvement of this region in the initiation of protein synthesis is supported by a variety of other experimental evidence [for review, see Lake (1980)]. Information on the spatial arrangement of the 50S subunit proteins is more sparse but still provides some useful landmarks. Protein L27 has been mapped to the central protuberance of the subunit (Lake & Strycharz, 1981). The position of L23 can be inferred from immumoelectron microscopic localization of the puromycin binding site on the subunit (Olson et al., 1982) based on the fact that puromycin cross-linked specifically to L23 (Jaynes et al., 1978). As shown in Figure 4B, both proteins map in close proximity to each other.

These results suggest a model for the role of IF-3 in subunit association as depicted in Figure 4C. The shaded area shows

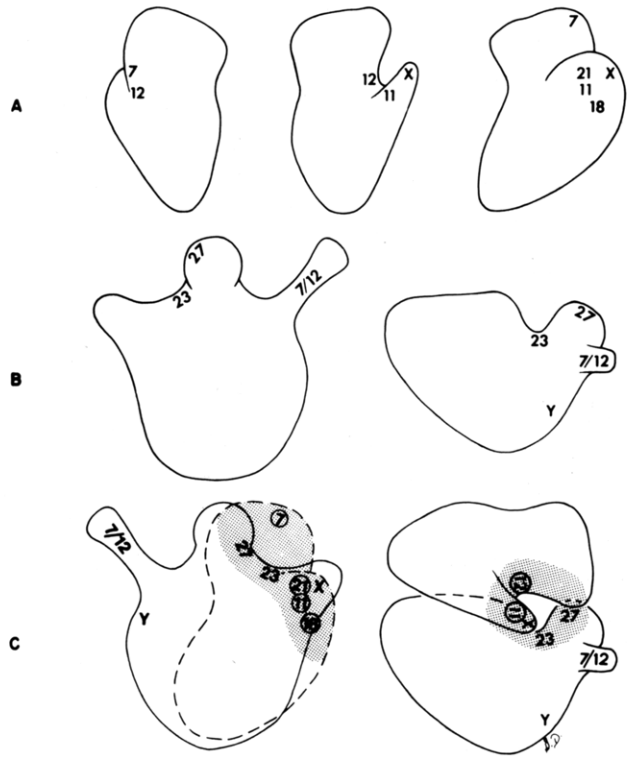

FIGURE 4: Models of the IF-3 binding domains on 30S (A), 50S (B), and 70S ribosomes (C). The models and spatial arrangements of the ribosomal proteins are based on Lake (1976, 1980) and Lake & Strycharz (1981). The three views of the 30S subunit (A) correspond to, from left to right, intermediate (−40°), intermediate (+50°), and asymmetric (+110°) views (Lake, 1976). Positions of determinants for the 30S subunit proteins cross-linked to IF-3 (MacKeen et al., 1980) are indicated by number. × denotes the 3′ terminus of 16S RNA as determined by Olson & Glitz (1979). The drawings of the 50S subunit (B) correspond to the quasi-symmetric (left) and asymmetric (right) projections (Lake, 1976). Positions of antigenic determinants for L7/L12 and L27 are from Strycharz et al. (1978) and Lake & Strycharz (1981) and are denoted by number. The position of L23 is deduced from the puromycin binding site (Olson et al., 1982) as described in the text. Y denotes the 3′ terminus of 23S RNA (Stöffler-Meilicke et al., 1981). The two representations of the 70S couples (C) are the overlap (left) and nonoverlap (right) views (Lake, 1976). The 30S proteins are circled. In the overlap view the 30S subunit lies below the 50S subunit and is outlined by the dashes. In the nonoverlap view the dashes indicate regions of the 50S subunit which are behind the 30S subunit. The shaded region is the proposed IF-3 binding domain (see text).

the proposed region of IF-3 binding in the 70S ribosome. This region includes proteins S7, S11, S12, S18, S21, L23, and L27, as well as the 3′ end of 16S RNA. Thus, antigenic determinants for most of the proteins which have been photo-cross-linked to IF-3 in our studies cluster in a topographically distinct area of the ribosome. This model of the IF-3 binding site would be consistent with either the antiassociation or dissociation mode of action for IF-3; i.e., binding of IF-3 to the platform and upper one-third positions of the 30S subunit would preclude its interaction with the appropriate region of the 50S subunit. Alternatively, IF-3 could interact directly with the 70S ribosome at the subunit interface and thereby induce dissociation. This might be accomplished by insertion of the IF-3 into the "hole" between the subunits which is visible when the 70S couple is observed in the nonoverlap view (Figure 4C, right). As can be seen, this "hole" is surrounded by IF-3 binding elements of both subunits.

The only protein (among those which have been mapped) that was photo-cross-linked to IF-3 and does not fall within the proposed IF-3 binding site is L7/L12. However, the involvement of L7/L12 in initiation is supported by its cross-linking to IF-2 (Heimark et al., 1976). In addition, there are assembly relationships between L7/L12, L11, and L27 (Röhl & Nierhaus, 1982). It must also be borne in mind that both cross-linking and immunoelectron microscopy recognize specific sites within a given protein. Since it is likely that the recognition sites will differ in the two methods, it is possible that a single protein will be labeled at two spatially distant regions. Thus, although the accessible antigenic determinants for L7/L12 are distant from L27, there may be portions of L7/L12 which lie close to the shaded region of Figure 4. This point is particularly relevant to L7/L12 since it is present in four copies in the 50S subunit (Hardy, 1975) and is an extremely elongated protein whose long axis measures 180 Å in the dimer form (Österberg et al., 1976; Luer & Wong, 1979). Thus, the localized antigenic determinants and the IF-3 cross-linking sites might even be associated with different L7/L12 molecules in the subunit.

A number of studies have demonstrated a direct role for ribosomal RNA in the mechanism of subunit association (Herr & Noller, 1979; Herr et al., 1979). Van Duin et al. (1976) proposed that interaction between complementary sequences at the 3′ termini of 16S RNA and 23S RNA were partially responsible for subunit association. The model in Figure 4 is not consistent with this view since the 3′ terminus of 23S RNA maps a great distance from the 3′ terminus of 16S RNA. The role of the 3′ terminus of 16S RNA in initiation of protein synthesis has been firmly established (Grunberg-Manago, 1980; Steitz, 1980) and this site falls within the proposed binding region for IF-3 (Figure 4). It must be concluded at this point, therefore, that the 3′-terminal region of 23S RNA plays no direct role in the initiation process. As pointed out earlier, Herr & Noller (1979) implicated residues 2307, 2308, and 2458 in 23S RNA as being involved in subunit association, and Noller et al. (1981) have identified residues 2447, 2451, 2503, and 2504 as part of the peptidyltransferase. In conjunction with the observed cross-linking of L27 to residues 2332–2337 (Wower et al., 1981), these results strongly suggest that residues 2307–2458 (or 2504), which constitute a distinct region of domain V in the 23S RNA secondary structure model of Noller et al. (1981), are localized to the left side of the central protuberance of the 50S subunit (see Figure 4B, left) and fall within the proposed IF-3 binding domain. In addition, positions 2308–2313 in 23S RNA and 817–822 in 16S RNA, both of which contain kethoxal-reactive sites which are rendered resistant in 70S couples, are complementary to each other (Herr et al., 1979). This would place 817–822 of 16S RNA in the platform region of the ribosome and suggests that this portion of the 16S RNA, as well as the 3′ end, plays a role in the initiation of protein synthesis.

IF-3 functions as a modulator of ribosomal subunit association. The proposed 70S·IF-3 binding domain presented here not only suggests possible mechanisms for the action of IF-3 but also provides additional insight into the process of ribosomal subunit interactions. When the spatial organization of the other IF-3 binding proteins on the 50S subunit is elucidated, it will be possible to further refine this model for IF-3 action and its role in ribosomal subunit association.

After this work was completed and submitted, Chaires et al. (1982) presented spectrophotometric evidence for an interaction between IF-3 and 70S ribosomes. This lends further support to the existence of an IF-3 binding domain on 70S couples which is comprised of structural elements from both the 30S and 50S subunits. In addition, these authors reported that L2, L5, and L17 were specifically cross-linked to IF-3 after treatment of 50S·IF-3 complexes with 2-iminothiolane.

Thus, L2 has been identified as an IF-3 binding protein by two different cross-linking methodologies. L5 is a 5S RNA binding protein (Zimmermann, 1980), and 5S RNA has been localized on the central protuberance of the 50S subunit (Stöffler-Meilicke et al., 1981). This would place L5 within the proposed IF-3 binding region shown in Figure 4C. The identification of L17 covalently linked to IF-3, however, is inconsistent with the immunoelectron microscopic localization of L17 to the lower portion of the 50S subunit (Lake & Strycharz, 1981).

Acknowledgments

We acknowledge the participation of Michael Savage and Sheryl Brown in some of the early aspects of this work.

References

Chaires, J. B., Kegeles, C., & Wahba, A. J. (1979) *Biophys. Chem. 9*, 405–412.

Chaires, J. B., Pande, C., & Wishnia, A. (1981) *J. Biol. Chem. 256*, 6600–6607.

Chaires, J. B., Hawley, D. A., & Wahba, A. J. (1982) *Nucleic Acids Res. 10*, 5681–5693.

Cooperman, B. S., Jaynes, E. N., Brunswick, D. J., & Luddy, M. A. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2974–2978.

Cooperman, B. S., Expert-Bezancon, A., Kahan, L., Dondon, J., & Grunberg-Manago, M. (1981) *Arch. Biochem. Biophys. 208*, 554–562.

Cover, J. A., Lambert, J. M., Norman, C. M., & Traut, R. R. (1981) *Biochemistry 20*, 2843–2852.

Godefroy-Colburn, T., Wolfe, A. D., Dondon, J., Grunberg-Manago, M., Dessen, P., & Pantaloni, D. (1975) *J. Mol. Biol. 94*, 461–487.

Goss, D. J., Parkhurst, L. J., & Wahba, A. J. (1980a) *J. Biol. Chem. 255*, 225–229.

Goss, D. J., Parkhurst, L. J., & Wahba, A. J. (1980b) *Biophys. J. 32*, 283–295.

Goss, D. J., Parkhurst, L. J., & Wahba, A. J. (1982) *J. Biol. Chem. 257*, 10119–10127.

Grunberg-Manago, M. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 445–477, University Park Press, Baltimore, MD.

Grunberg-Manago, M., & Gros, F. (1977) *Prog. Nucleic Acid Res. Mol. Biol. 20*, 209–284.

Gualerzi, C., & Pon, C. L. (1973) *Biochem. Biophys. Res. Commun. 52*, 792–799.

Hampl, H., Schulze, H., & Nierhaus, K. H. (1981) *J. Biol. Chem. 256*, 2284–2288.

Hardy, S. J. S. (1975) *Mol. Gen. Genet. 140*, 253–274.

Hawley, D. A., Miller, M. J., Slobin, L. I., & Wahba, A. J. (1974) *Biochem. Biophys. Res. Commun. 61*, 329–337.

Heimark, R. L., Hershey, J. W. B., & Traut, R. R. (1976) *J. Biol. Chem. 251*, 7779–7784.

Hêlène, C. (1976) in *Aging, Carcinogenesis and Radiation Biology* (Smith, K. C., Ed.) pp 149–163, Plenum Press, New York.

Herr, W., & Noller, H. F. (1979) *J. Mol. Biol. 130*, 421–432.

Herr, W., Chapman, N. M., & Noller, H. F. (1979) *J. Mol. Biol. 130*, 433–449.

Hershey, J. W. B., Yanov, J., Johnston, K., & Fakunding, J. L. (1977) *Arch. Biochem. Biophys. 182*, 626–638.

Jaynes, E. N., Grant, P. G., Giangrande, G., Wieder, R., & Cooperman, B. S. (1978) *Biochemistry 17*, 561–569.

Kenny, J. W., & Traut, R. R. (1979) *J. Mol. Biol. 127*, 243–263.

Lake, J. A. (1976) *J. Mol. Biol. 105*, 131–159.

Lake, J. A. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 207–236, University Park Press, Balitmore, MD.

Lake, J. A., & Strycharz, W. A. (1981) *J. Mol. Biol. 153*, 979–992.

Lambert, J. M., & Traut, R. R. (1981) *J. Mol. Biol. 149*, 451–476.

LeGoffic, G., Capman, M.-L., Chausson, L., & Bonnet, D. (1980) *Eur. J. Biochem. 106*, 667–674.

Luer, C. A., & Wong, K. P. (1979) *Biochemistry 18*, 2019–2027.

MacKeen, L. A., DiPeri, C., & Schwartz, I. (1979) *FEBS Lett. 101*, 387–390.

MacKeen, L. A., Kahan, L., Wahba, A. J., & Schwartz, I. (1980) *J. Biol. Chem. 255*, 10526–10531.

Nicholson, A. S., & Cooperman, B. S. (1978) *FEBS Lett. 90*, 203–208.

Noller, H. F., Kop, J., Wheaton, V., Brosius, J., Gutell, R. R., Kopylov, A. M., Dohme, F., Herr, W., Stahl, D. A., Gupta, R., & Woese, C. R. (1981) *Nucleic Acids Res. 9*, 6167–6189.

Ofengand, J. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 497–529, University Park Press, Baltimore, MD.

Olson, H. M., & Glitz, D. G. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 3769–3773.

Olson, H. M., Grant, P. G., Cooperman, B. S., & Glitz, D. G. (1982) *J. Biol. Chem. 257*, 2649–2656.

Österberg, R., Sjöberg, B., Liljas, A., & Pettersson, I. (1976) *FEBS Lett. 66*, 48–51.

Pon, C. L., Friedman, S. M., & Gualerzi, C. (1972) *Mol. Gen. Genet. 116*, 192–198.

Revel, M. (1977) in *Molecular Mechanisms of Protein Biosynthesis* (Weissbach, H., & Pestka, S., Eds.) pp 254–321, Academic Press, New York.

Röhl, R., & Nierhaus, K. H. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 729–733.

Sabol, S., & Ochoa, S. (1971) *Nature (London), New Biol. 234*, 233–236.

Sabol, S., Meier, D., & Ochoa, S. (1973) *Eur. J. Biochem. 33*, 332–340.

Schiff, N., Miller, M. J., & Wahba, A. J. (1974) *J. Biol. Chem. 249*, 3797–3802.

Shine, J., & Dalgarno, L. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 1342–1346.

Sobura, J., Chowdhury, R., Hawley, D. A., & Wahba, A. J. (1977) *Nucleic Acids Res. 4*, 17–29.

Steitz, J. A. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 479–495, University Park Press, Baltimore, MD.

Steitz, J. A., & Jakes, K. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4734–4738.

Stöffler-Meilicke, M., Stöffler, G., Odom, O. W., Zinn, A., Kramer, G., & Hardesty, B. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 5538–5542.

Strycharz, W. A., Nomura, M., & Lake, J. A. (1978) *J. Mol. Biol. 126*, 123–140.

Traut, R. R., Lambert, J. M., Boileau, G., & Kenny, J. W. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 89–110, University Park Press, Baltimore, MD.

Van Duin, J., Kurland, C. G., Dondon, J., Grunberg-Manago, M., Brantlant, C., & Ebel, J. P. (1976) *FEBS Lett. 62*, 111–114.
Vazquez, D. (1974) *FEBS Lett. 40*, 563–584.
Vermeer, C., Boon, J., Talens, A., & Bosch, L. (1973) *Eur. J. Biochem. 40*, 283–293.
Wower, I., Wower, J., Meinke, M., & Brimacombe, R. (1981) *Nucleic Acids Res. 9*, 4285–4302.
Zimmermann, R. A. (1980) in *Ribosomes: Structure, Function and Genetics* (Chambliss, G., Craven, G. R., Davies, J., Davis, K., Kahan, L., & Nomura, M., Eds.) pp 135–169, University Park Press, Baltimore, MD.

Patterns of Histone Acetylation in the Cell Cycle of *Physarum polycephalum*†

Jaap H. Waterborg and Harry R. Matthews*

ABSTRACT: Labeling of histones in the naturally synchronous cell cycle of *Physarum polycephalum* with short pulses of tritiated acetate in vivo clearly showed three distinct patterns of histone acetate turnover. In *G2 phase*, turnover of acetate was observed only in histones H3 and H4, predominantly on the multiple acetylated forms. No acetate turnover was found in histones H2A and H2B. This indicates different functional roles of histones H2A and H2B compared with histones H3 and H4. In *S phase*, intense labeling was seen in all four core histones, in histones H3 and H4 predominantly in the low acetylated forms. In addition, cotranslational acetylation of the amino-terminal serines of histones H4 and H1 was observed during S phase. During *mitosis*, from condensation at prophase to decondensation after telophase, acetate turnover is almost zero. This suggests that within the mitotically condensed chromosomes all potential histone acetylation sites are masked. In G2 phase, when transcription is occurring, only histones H3 and H4 are available for acetate turnover, but in S phase, when both transcription and replication occur, all four histones are available for acetate turnover.

Chromatin is involved in several of the key processes of living cells, particularly transcription, replication, and distribution of the genetic material to daughter cells. These processes are associated with different structural states of the chromatin, and although very substantial progress has been made in understanding the structure of the underlying nucleosome subunit, there remains much speculation about how the nucleosome structure is modified to accommodate different chromatin functions and about higher order packing of nucleosomes in the different chromatin structures [for a review, see Igo-Kemenes et al. (1982)]. There are two key questions being asked about the chromosome structural transitions. First, what signals determine when a particular transition should occur and where it occurs, relative to specific DNA sequences in the chromatin. Second, what are the characteristics of each specific state of the chromatin that stabilize that state and enable it to function in its specific role? We are concerned, here, with the second question and with the role of histone modification in stabilizing specific chromatin structures. Acetylation of core histones has previously been correlated with chromosome replication and transcription and negatively correlated with mitosis [for a review, see Matthews & Bradbury (1982)]. However, many of the systems used to establish these correlations did not have total separation of replicating from transcribing chromatin, and results from chromatin fractionation procedures have shown variable amounts of acetylated histones associated with "active" chromatin. We have reinvestigated the relationship between histone acetylation and chromatin function by using an in vivo, unperturbed system in which three situations are available with a purity of 98–99%. These are the phases of the naturally synchronous cell cycle in the true slime mold *Physarum polycephalum*: mitosis (chromosome condensation and separation; no transcription or replication); S phase (chromosome replication and transcription); and G2 phase (transcription only). The results confirm the correlation of histone acetylation with chromosome structure and function and show, for the first time, that the pattern of acetylation associated with transcription is quite distinct from the pattern associated with replication.

Materials and Methods

Physarum Culture. Physarum polycephalum, strain M3c, was cultured as microplasmodia in shaking flasks or as macroplasmodia on filter papers in petri dishes, essentially as described (Daniel & Baldwin, 1964; Guttes & Guttes, 1964). The semidefined growth medium described by Daniel & Baldwin (1964) was used, with a final hematin concentration of 2.5 mg/L. Exponentially growing microplasmodia were used for fusion to macroplasmodia. Mitosis was observed by phase contrast microscopy in smears taken from the edges of macroplasmodia and fixed in ethanol (Mohberg & Rusch, 1971). The time between the second and third mitoses after fusion was in the range of 8–10 h. All cell cycle times are given relative to metaphase.

For labeling with [^{3}H]acetate at the desired time of the cell cycle ranging from M2 −1 h (1 h before the second metaphase after fusion) to M3 +1 h (1 h after the third metaphase after fusion), we removed the filter paper with the macroplasmodium from the growth medium, allowed it to drain for a few seconds, and then placed the filter paper on 1 mL of semidefined growth medium on a clean petri dish. The medium contained 6.25 mCi of sodium [^{3}H]acetate (New England Nuclear, 2.3 Ci/mmol) per mL. Culture was continued for the desired length of time, as given in the text and figure legends, and the plasmodium was harvested by dropping the

† From the Department of Biological Chemistry, School of Medicine, University of California, Davis, California 95616. *Received October 19, 1982.* This work was supported by National Institutes of Health Grant 26901 and by a NATO Science Fellowship to J.H.W. from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

0006-2960/83/0422-1489$01.50/0