NEW ASPECTS OF THE IF3–RIBOSOME INTERACTION

J. VAN DUIN, C. G. KURLAND, J. DONDON*, M. GRUNBERG-MANGAGO*, C. BRANLANT**, and J. P. EBEL**

*Wallenberg Laboratory, University of Uppsala, Uppsala, Sweden, *Institut de Biologie Physico-Chimique, Rue P. et M. Curie, 75005 Paris, France, and **Institut de Biologie Moleculaire et Cellulaire, Rue Descartes, 67000 Strasbourg, France*

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1. Introduction

Considerable evidence indicates that IF3 together with several ribosomal components is responsible for the acquisition of an mRNA during the initiation of protein synthesis. Here, nucleotide sequences in mRNA are thought to interact with complementary sequences at the 3′ end of the 16S RNA [1–3]. The proteins S1 and IF3 seem to be involved in this interaction [4–6], and together with S7 as well as S21 make up a protein neighborhood organized around the 3′ region of the 16S RNA [7,8].

IF3 also influences the distribution of 70S couples by blocking the association of 30S and 50S subunits [9–11]. Significantly, 70S couples which are cross-linked so that they can not dissociate into subunits are unable to initiate protein synthesis even though they are capable of polypeptide elongation [12,13]. Such observations suggest that the mRNA binding function of IF3 may be related to its effect on the association of the ribosomal subunits.

Most of our previous studies of the IF3–30S complex were performed in the absence of 50S subunits [8]. For this reason it was important to determine whether or not the presence of the 50S subunits would influence the association of IF3 with the 3′ end of the 16S RNA. In particular we have noticed that two tetra nucleotide sequences at the 3′ end of 16S RNA are homologous with sequences at the 3′ end of 23S RNA [14,15]. It, therefore, seemed possible that IF3 could interact with both 30S and 50S subunits at these homologous sequences. The data reported here verify this expectation, and together with recently obtained 23S RNA sequence information lead to a simple model to describe the dual function of IF3.

2. Materials and methods

A clarified lysate of *Escherichia coli* A19 was prepared as described earlier [8] in 10 mM Tris, 10 mM magnesium acetate, 60 mM ammonium-chloride, 6 mM 2-mercaptoethanol, pH 7.4. The lysate was then layered on a 10–30% sucrose gradient containing 10 mM Tris, 4 mM magnesium acetate, 60 mM ammonium acetate, pH 7.4, and then centrifuged in an SW 27 rotor (Beckman) for 16 h at 19 000 rev/min. The 70S peak was recovered from the gradient and used as a source of 'tight couples'. All other preparative and analytical techniques are described in reference [8].

3. Results and discussion

The strategy of the present experiments is the same as that reported earlier [7,8]: periodate is used to specifically oxidize the 3′ ribose of the ribosomal RNA molecules in situ. The aldehydes so created will then react with near neighboring proteins to form a covalent crosslink. After the RNA is extracted from the oxidized/reduced ribosomes, the proteins cross-linked to the RNA 3′ termini can be identified by relatively simple means.

Accordingly, 70S 'tight couples' were oxidized with periodate and subsequently reduced with borohydride in the presence of various additions

including [^{14}C]IF3, as described previously [8]. The RNA extracted from these incubation mixtures was then fractionated on SDS-containing sucrose gradients. As can be seen from the data displayed in fig.1a, the 23S RNA as well as 16S RNA is labelled by [^{14}C]IF3 under the normal conditions of periodate oxidation. However, if the oxidation is omitted, none of the RNA peaks are labelled (fig.1a).

The site-specificity of the RNA–IF3 complexes formed in these experiments was tested in the same way as for the 30S–IF3 complexes described earlier [8]. First, experiments were done in the presence of 0.8 M lithium chloride, which destabilizes the IF3–ribosomes complexes. When lithium chloride is present, no IF3–RNA can be recovered after oxidation/reduction (data not shown). Such a result

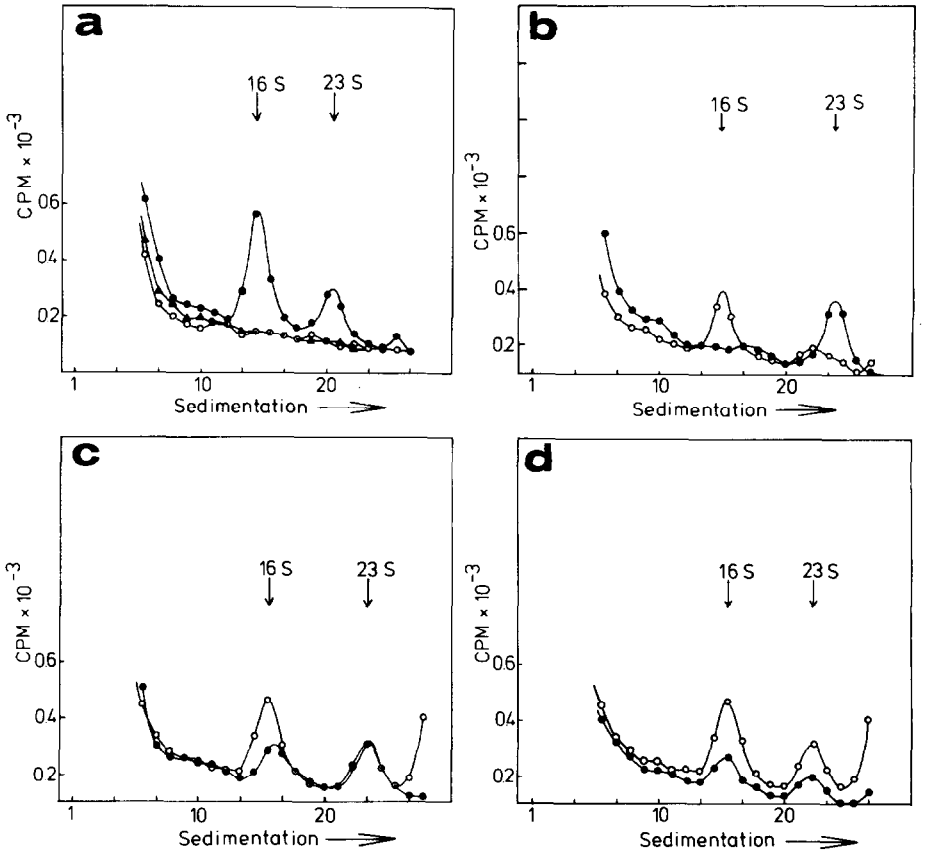

Fig.1. Ribosomes incubated with [^{14}C]IF3 were oxidized/reduced and then fractionated in SDS-containing sucrose gradients as described previously [8] in order to identify factor complex with the 16S and 23S RNA molecules. When 70S tight couples were used, the incubation mixtures contained 10A_{260} units of these, 6 micrograms [^{14}C]IF3 (8000 cpm/microgram), 10 mM Hepes, 40 mM sodium chloride, 0.1 mM EDTA, pH 6.2 and magnesium as specified below. When ribosomal subunits were used, they were present in amounts of 6 A_{260} units for 50S and 3 A_{260} units for 30S subunits in the above incubation mixture in place of the couples. Oxidation was done with 20 mM sodium periodate for 45 min at room temperature in the dark. After dialysis and treatment with borohydride, the samples were layered on the gradients and fractionated [8]. (a) Tight couples were incubated under standard conditions which include 3 mM magnesium acetate (–•–), in the presence of 0.2 mM ATA (–▲–), and treated in the standard way with the omission of periodate (–○–). (b) 50S subunits were incubated under the standard conditions in the presence (–○–), and absence (–•–) of 30S subunits. (c) The labelling of RNA was compared for mixtures of 50S and 30S subunits incubated at 3 mM magnesium acetate (–○–), and at 0.3 mM magnesium acetate (–•–). (d) The labelling of RNA was compared for mixtures of 50S and 30S subunits incubated at 3 mM magnesium acetate (–○–), and at 20 mM magnesium acetate (–•–).

indicates that random collisions between the factor and ribosome would not produce significant amounts of the IF3–RNA complexes. Similarly, the initiation-specific inhibitor [16,8] aurintricarboxylic acid (ATA), which inhibits formation of the IF3–16S RNA complex also abolishes the formation of the complex with 23S RNA (fig.1a). The data indicate that IF3 can form a site-specific complex in the immediate neighborhood of the 3′ end of 23S RNA.

We next attempted to determine whether or not the IF3–23S RNA complex was formed on the 70S couple or on the free 50S subunits. First, 50S subunits were incubated with IF3 in the absence of 30S subunits and it was observed that IF3–23S RNA complexes could be recovered from such incubation mixtures after oxidation/reduction (fig.1b). Finally, the magnesium concentration dependence of complex formation was tested. It is observed that excessively low (fig.1c) or high (fig.1d) magnesium concentration depress the recovery of the IF3–RNA complexes. This inhibitory effect is expressed preferentially on the 16S RNA, and therefore, a correlation between the degree of labelling of 23S RNA and magnesium concentration is not apparent.

In summary, the data show that the presence of 30S subunits is not obligatory for the formation of the IF3–23S RNA complex. However, there is evidence that more than one IF3 molecule will bind to ribosomes at high factor concentrations [11,18]. Therefore, much more exacting experiments are required to decide whether or not the factor can react with 23S RNA on the 70S ribosome as well as on the free 50S subunit. The data showing that both 16S RNA and 23S RNA have two homologous tetranucleotide sequences at their 3′ termini (see fig.2a) could explain our finding that IF3 can be crosslinked to both RNA molecules by oxidation of their 3′ riboses. Thus, part of the binding site for IF3 on both of these subunits may consist of these homologous sequences.

It has been argued [17] that an interaction between the RNAs of each subunit could provide a convenient way of forming a 70S couple. If, for example, the 3′ ends of 16S and 23S RNA must be in close proximity to each other in the 70S couple, the binding of factor to either of these sites could interfere with the association of the subunits. Indeed, new sequence data for the 3′ end of 23S RNA, which will be published in detail elsewhere [19], reveal the presence of two complementary sequences between nucleotides near the 3′ termini of 16S and 23S RNA. This provides the basis for a simple model to account for the function of IF3.

We assume for the moment that the interaction of 30S and 50S subunits in a 70S 'tight couple' is at least in part stabilized by these complementary interactions between 16S and 23S RNA. One possible way of visualizing the interaction between the

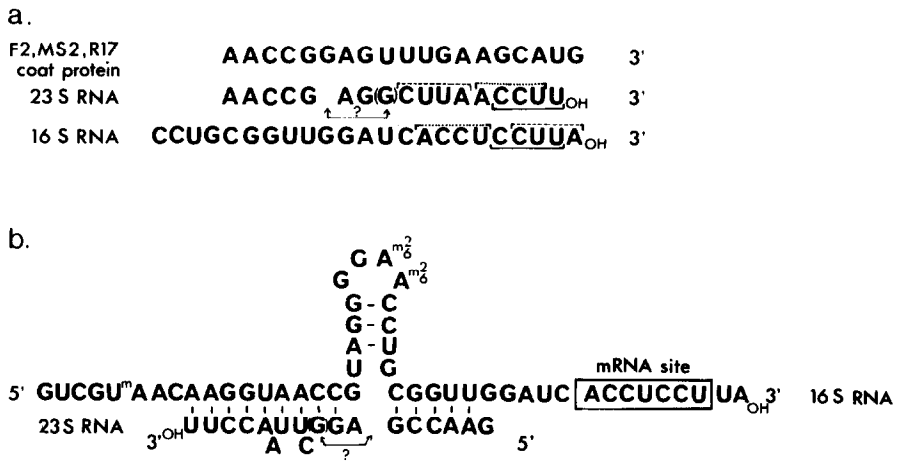

Fig.2. (a) the nucleotide sequences of the 3′ end of the 16S RNA and 23S RNA [14,15] are compared with those of the bacteriophage RNA ribosomal binding site associated with the coat protein cistron [1,2]. The hexa nucleotide sequence of the 23S RNA distal to the 3′ end are from [19]. (b) Hydrogen bonding schemes are depicted for the 16S–23S RNA interaction.

ribosomal RNAs is depicted in fig.2b. Since the mRNA–16S RNA interaction [1–3] is thought to take place in the same region of the 16S RNA as that with the 23S RNA, the two sorts of interactions could be mutually exclusive. Another possibility for such mutually exclusive interactions, but with little generality, is provided by the striking homology displayed between a sequence near the 3′ end of 23S RNA and that of the ribosomal binding site of the coat protein cistron for coliphage RNA (see fig.2a).

In the presence of IF3 the ribosomal subunits are preferentially distributed as free subunits rather than couples [9,10]. It therefore follows that IF3 will favor formation of a mRNA–30S preinitiation complex over the formation of 70S couples. In effect, IF3 could be either directly or indirectly responsible for an exchange of complementary nucleotide interactions from those between 16S RNA and 23S RNA to those between 16S RNA and mRNA. From the point of view of this model, the different in vitro effects of IF3 on the stability of 70S couples and the binding of mRNA are seen as but two different aspects of a single function.

The formation of the 70S couple prior to the elongation phase of protein synthesis would presumably require displacement of IF3 from the 30S subunit interaction. We expect to be able to determine whether the other initiation factors, GTP, or Fmet-tRNA contribute to these rearrangements.

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References

[1] Shine, J. and Dalgarno, L. (1974) Proc. Natl. Acad. Sci. USA 71, 1342–1346.
[2] Shine, J. and Dalgarno, L. (1975) Nature 254, 34–37.
[3] Steitz, J. and Jakes, K. (1975) Proc. Natl. Acad. Sci. USA, in the press.
[4] Van Duin, J. and Kurland, C. G. (1970) Mol. Gen. Genet. 109, 169–176.
[5] Van Dieijen, G., Van der Laken, C. J., Van Knippenberg, P. H. and Van Duin, J. (1975) J. Mol. Biol. 351–366.
[6] Revel, M., Herzberg, M. and Greenshpan, H. C. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 261–275.
[7] Czernilofsky, A. P., Kurland, C. G. and Stöffler, G. (1975) FEBS Lett., in the press.
[8] Van Duin, J., Kurland, C. G., Dondon, J. and Grunberg-Manago, M. (1975) FEBS Lett., in the press.
[9] Kaempfer, R. (1975) J. Mol. Biol. 71, 583–598.
[10] Noll, M. and Noll, H. (1972) Nature New Biology 238, 225–228.
[11] Godefrey-Colburn, T., Wolfe, A. D. Dondon, J., Grunberg-Manago, M., Dessen, P. and Pantaloni, D. (1975) J. Mol. Biol. 94, 461–478.
[12] Slobin, L. I. (1972) Proc. Nat. Acad. Sci. USA 69, 3769–3773.
[13] Hawley, D. A., Miller, M. J., Slobin, L. I. and Wahba, A. J. Biochem. Biophys. Res. Common. 61, 329–337.
[14] Ehresmann, C., Stiegler, P., Mackie, G. A., Zimmermann, R. A. Ebel, J. P. and Fellner, P. (1975) Nue Acid Res. 2, 265–278.
[15] Fellner, P., Ebel, J-P. and Blasi, O. (1970) FEBS Lett., 6, 102–104.
[16] Pon, C. L., Friedman, M. and Gualerizi, C. (1972) Molec. Gen. Genet. 116, 192–198.
[17] Kurland, C. G. (1974) in: Ribosomes (M. Nomura, A. Tissieres, P. Lengyel, eds.) Cold Spring Harbor Laboratory, New York, pp. 309–331.
[18] Vermaer, V., Boon, J., Talens, A. and Bosch, L. (1973) J. Mol. Biol. 75, 391–400.
[19] Branlant, C. and Ebel, J. P., in preparation.