FEBS Letters 455 (1999) 130–134 FEBS 22309

Characterization of the domains of *E. coli* initiation factor IF2 responsible for recognition of the ribosome

Juan Manuel Palacios Moreno, Lars Dyrskjøtersen, Janni Egebjerg Kristensen, Kim Kusk Mortensen, Hans Uffe Sperling-Petersen*

Department of Biostructural Chemistry, Institute of Molecular and Structural Biology, Aarhus University, Gustav Wieds vej 10, DK-8000 Aarhus C, Denmark

Received 1 June 1999

Abstract We have studied the interactions between the ribosome and the domains of *Escherichia coli* translation initiation factor 2, using an in vitro ribosomal binding assay with wild-type forms, N- and C-terminal truncated forms of IF2 as well as isolated structural domains. A deletion mutant of the factor consisting of the two N-terminal domains of IF2, binds to both 30S and 50S ribosomal subunits as well as to 70S ribosomes. Furthermore, a truncated form of IF2, lacking the two N-terminal domains, binds to 30S ribosomal subunits in the presence of IF1. In addition, this N-terminal deletion mutant IF2 possess a low but significant affinity for the 70S ribosome which is increased by addition of IF1. The isolated C-terminal domain of IF2 has no intrinsic affinity for the ribosome nor does the deletion of this domain from IF2 affect the ribosomal binding capability of IF2. We conclude that the N-terminus of IF2 is required for optimal interaction of the factor with both 30S and 50S ribosomal subunits. A structural model for the interaction of IF2 with the ribosome is presented.

Key words: Translation initiation factor 2; IF2; IF1; IF3; In vitro ribosomal binding assay

1. Introduction

In prokaryotes, three initiation factors IF1, IF2 and IF3 interact with the 30S ribosomal subunit to promote the formation of a protein synthesis initiation complex between the small ribosomal subunit, mRNA and fMet-tRNA$_f^{Met}$. The initiation complex formation as well as the postulated role of all three factors have been extensively reviewed [1–4]. IF2 is the largest of the factors and is expressed, in *E. coli*, in three different forms in vivo differing in the N-termini: IF2α (97.3 kDa), IF2β (79.7 kDa) and IF2γ (78.8 kDa) [5][1]. The function of IF2 is to stimulate the binding of fMet-tRNA$_f^{Met}$ to ribosomes during formation of 30S and 70S initiation complexes followed by the hydrolysis of GTP to GDP and Pi. The binding site(s) of IF2 on the 30S and 50S ribosomal subunits has been studied for more than two decades by cross-linking methods [7–12]. It appears that IF2 binds to the ribosome essentially by protein-protein interactions covering a large part of the head and platform of the 30S ribosomal subunit and, in addition, interacts with the L7/L12 stalk in the 50S subunit. On the contrary, less is known concerning which regions of IF2 are involved in the binding of the factor to the ribosome. Since IF2 exists in *E. coli* in three forms differing in only the N-terminus and different N-terminal truncated forms have been shown to possess similar activities in vitro and in vivo, it has been proposed that the active centers for interaction with fMet-tRNA$_f^{Met}$, ribosomes and GTP would reside within the C-terminal half of the protein leaving the N-terminus of IF2 functionally uncharacterized [13–15].

In a recent paper we have shown the interaction of a series of truncated forms and isolated domains of IF2 (Fig. 1) and the 30S ribosomal subunits in the absence of fMet-tRNA$_f^{Met}$, mRNA, IF1 and IF3 [16]. We concluded that the N-terminus of IF2 had affinity per se to bind the 30S ribosomal subunits with domain II being directly involved in the interaction. Moreover, we could unexpectedly conclude that the mutant IF2 domains III–VI (see Fig. 1) showed a negligible affinity for the ribosomal subunit. In the present paper we have extended our studies of the domains of IF2 involved in the binding to the ribosome by including 50S and 70S ribosomes as well as IF1 and IF3 aiming at defining a model for the interaction of IF2 with the ribosome and, moreover, revisiting the functions of IF1 and IF3. The discussion of the results obtained will be related to our six-domain structural model for IF2 described in [17].

2. Materials and methods

2.1. Chemicals

All the chemicals used were of analytical grade from Merck or Sigma unless otherwise indicated.

2.2. Buffers

Ribosome binding buffer: 10 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 100 mM NH_4Cl, 1 mM DTT, 2 mM GTP.

2.3. IF2α and IF2β

Both native forms of the initiation factor were overexpressed and purified essentially as described by Mortensen et al. [18].

2.4. Truncated forms of IF2

Construction, overexpression and purification of N-terminal and C-terminal deletion mutants as well as isolated domains of IF2 were carried out essentially as described in [16–18].

2.5. Cloning, expression and purification of IF1

The gene *infA*, encoding IF1, was amplified from *E. coli* MRE600 genomic DNA by PCR using degenerate primers introducing restriction sites for *Bam*HI and *Eco*RI and a protease factor Xa cleavage site. The PCR product was cloned into the gene fusion system pGEX-1 [19]. The plasmid was transformed into *E. coli* UT5600 cells [20] and

*Corresponding author. Fax: +45 86182812.
E-mail: husp@biobase.dk

[1] A new nomenclature for translation factors has been proposed by IUBMB [6]. According to this, IF2α and IF2β named IF2-1 and IF2-2 respectively and IF2γ would be IF2-3.

0014-5793/99/$20.00
PII: S0014-5793(99)00858-3

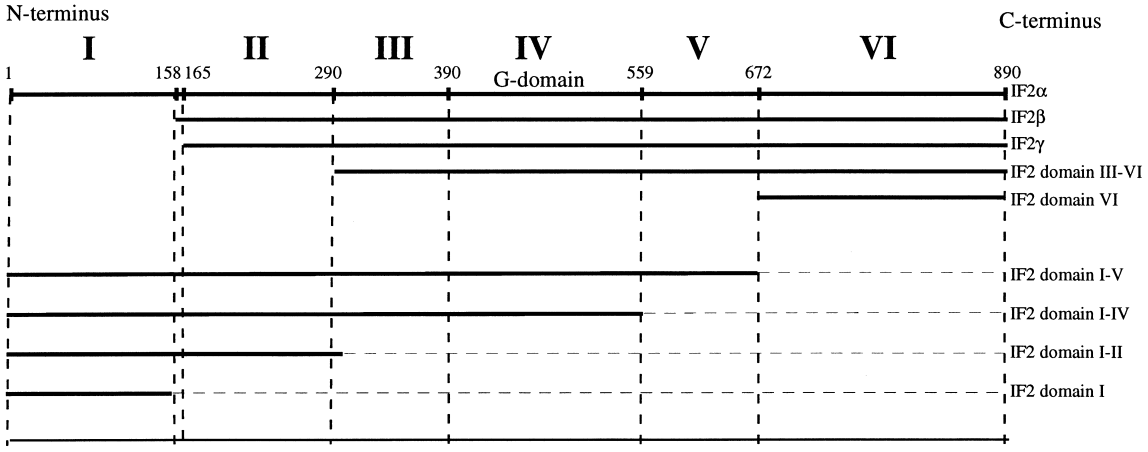

Fig. 1. Schematic representation of the three naturally existing forms of IF2: IF2α, β and γ, as well as the N-terminal and C-terminal deletion mutants. The domains of IF2 are numbered I–VI according to the six domain model proposed in [16], where domain IV is the GTP binding domain.

overexpression and purification was performed essentially as described in [19].

2.6. Expression and purification of IF3

E. coli UT5600[pCI857] cells were transformed with plasmid pIM201 carrying the gene *infC* (kindly provided by M. Springer) [21]. IF3 was overexpressed essentially as described for IF2 in [18] and purified as in [22].

2.7. Preparation of 30S and 50S ribosomal subunits

Ribosomes were prepared from MRE600 *E. coli* cells essentially as described in [23] and 30S and 50S ribosomal subunits isolated by zonal centrifugation as described in [16].

2.8. 30S, 50S and 70S binding assays

The ribosomal binding assays were performed essentially as described in [16]. Ribosomal subunits were incubated in *ribosomal binding buffer* (final reaction volume 50 μl) for 10 min at 37°C prior to mixing with different truncated forms of IF2 and equimolar amounts of IF1 and IF3. Incubation, ultracentrifugation and subsequent analysis of supernatants and pellets was performed as described in [16].

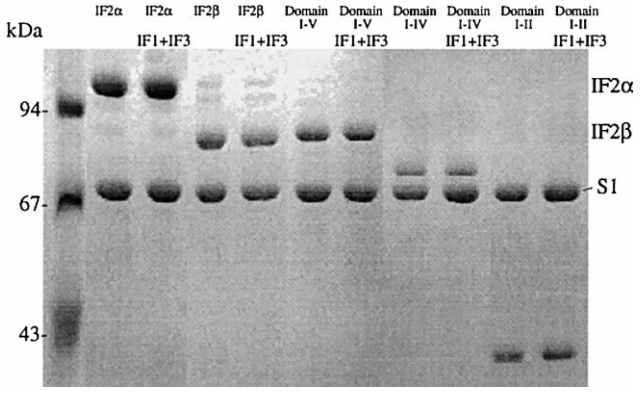

Fig. 2. SDS-PAGE and Coomassie brilliant blue G staining of pellets after ultracentrifugation of 30S ribosomal assay. The different recombinant IF2 proteins, as well as IF1 or IF3 when present, were incubated with 30S ribosomal subunits in an equimolar amount (2 μM) and the incubation was subsequently ultracentrifuged. In the absence of ribosomes no truncated proteins were found in the pellet (not shown). IF3 (20.5 kDa) and IF1 (8.1 kDa) are not seen in the gel.

3. Results

It was recently shown that IF2 domains I–II (see Fig. 1) bind to the 30S ribosomal subunits whereas IF2 domains III–VI do not [16]. In this work we have studied the effect of IF1 and IF3 on the binding of truncated forms of IF2 to the 30S and 50S ribosomal subunits and to 70S reconstituted ribosomes.

3.1. Interaction of IF2 with the 30S ribosomal subunits

Results are shown in Figs. 2 and 3 and summarized in Table 1. The addition of IF1 and IF3 do not further stimulate the binding of wild-type IF2 (IF2α and IF2β) or C-terminal deletion mutants IF2 domains I–V, IF2 domains I–IV and IF2 domains I–II to the 30S ribosomal subunit. Furthermore, neither IF2 domain I nor IF2 domain VI can bind to the 30S ribosomal subunits under any experimental conditions including IF1 and/or IF3. Results including IF2 domains III–VI in the ribosomal binding assay are striking since this mutant of IF2, which is lacking the two N-terminal domains, can only bind to the 30S ribosomal subunit in the presence of IF1 (see Fig. 3), whereas IF3 has no stimulatory binding effect either alone or together with IF1. Therefore, the significant stimulatory binding effect of IF1 may be caused by interactions with regions of IF2 located within the C-terminal two thirds of the molecule and, in addition, the specific strong interaction between the N-terminus of IF2 and the ribosome, would mask

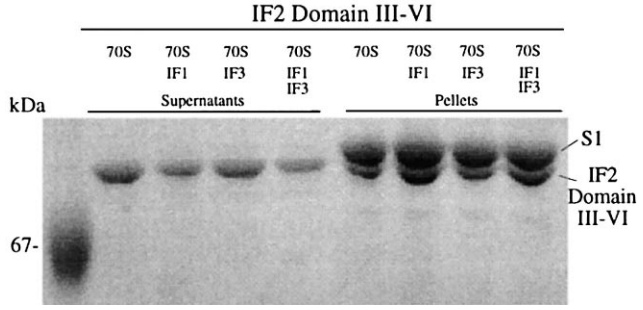

Fig. 3. SDS-PAGE and Coomassie brilliant blue G staining of supernatant and pellet samples after ultracentrifugation of IF2 domains III–VI 30S ribosomal assay.

Table 1
Binding efficiency to the 30S, 50S and 70S ribosomes of different native and truncated forms of IF2 in absence (−) or presence (+) of IF1

Protein studied	Schematic primary structure	30S binding		50S binding		70S binding	
		- IF1	+ IF1	- IF1	+ IF1	- IF1	+ IF1
IF2α = IF2 Domain I-VI		+++	+++	+++	+++	+++	+++
IF2β = IF2 Domain II-VI		+++	+++	+++	+++	+++	+++
IF2 Domain III-VI		-	+++	+	+	++	+++
IF2 Domain VI		-	-	-	-	-	-
IF2 Domain I-V		+++	+++	+++	+++	+++	+++
IF2 Domain I-IV		+++	+++	+++	+++	+++	+++
IF2 Domain I-II		+++	+++	+++	+++	+++	+++
IF2 Domain I		-	-	-	-	-	-
BSA, Trypsin inhibitor, Ovalbumin, Lysosym		-	n.d.	-	n.d.	-	n.d.

Values represented come from the analysis of the protein bound to the ribosomes in the pellet as well as the non-bound fraction present in the supernatant, subsequently being compared with experiments in the absence of ribosomal subunits. +++, 80–100%; ++, 40–80%; +, 10–40%; −, 0–10%; n.d., not determined.

the stimulatory effect of IF1 on IF2 forms containing N-terminal domains.

3.2. Interaction of IF2 with the 50S ribosomal subunits

E. coli IF2 binds less efficiently to the 50S ribosomal subunits than to the 30S as previously reported for *Bacillus stearothermophilus* IF2 in [24]. However, it was possible clearly to detect the binding of wild-type IF2 forms and moreover study the binding efficiency of truncated forms of the factor to the large ribosomal subunit. Deletion of 600 C-terminal amino acids does not significantly decrease the ability of IF2 to interact with the 50S ribosomal subunit (see Fig. 4 and summary in Table 1).

On the contrary, the binding of IF2 domains III–VI, where the two N-terminal domains are deleted, to the 50S subunits was drastically reduced although not completely abolished as

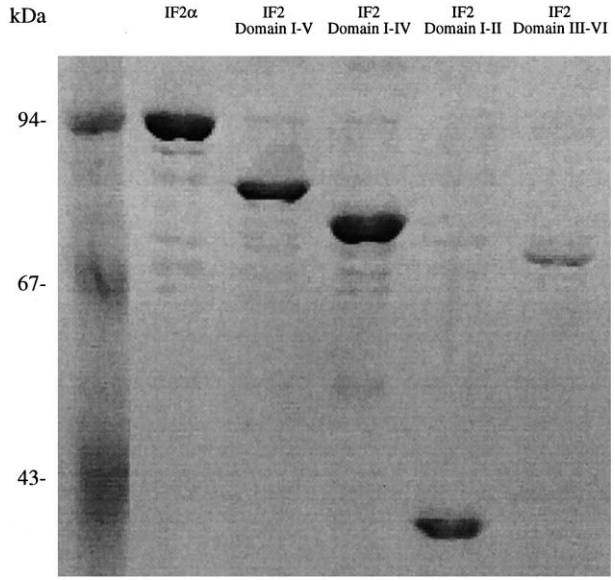

Fig. 4. SDS-PAGE and Coomassie brilliant blue G staining of pellets after ultracentrifugation of 50S ribosomal assay. In the absence of ribosomes no truncated proteins were found in the pellet (not shown).

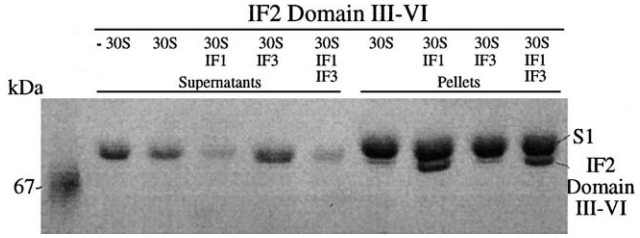

Fig. 5. SDS-PAGE and Coomassie brilliant blue G staining of supernatant and pellet samples after ultracentrifugation of IF2 domains III–VI 70S ribosomal assay.

described for the binding to 30S ribosomal subunits (see Fig. 3). This result implies a closer contact between C-terminal domains of IF2 and the 50S ribosomal subunit than to the 30S subunit. Neither IF1 nor IF3 promoted the binding of IF2 domains III–VI and, in addition, neither IF2 domain I nor IF2 domain VI interacts with the large ribosomal subunit as reported for the 30S subunits.

3.3. Interaction of IF2 with the 70S ribosomes

Similar binding patterns as found for IF2 to the ribosomal subunits are found with the 70S ribosome, e.g. C-terminal sequential deletions do not significantly affect the binding of IF2 and the C-terminal domain VI and N-terminal domain I are not interacting with the ribosome. Despite of the lack of affinity of IF2α domains III–VI for the 30S, and low affinity for the 50S, ribosomal subunits, this mutant IF2 can bind to the 70S reconstituted ribosome in absence of IF3 or IF1 (Fig. 5). However, IF1 stimulates the binding of IF2 domains III–VI fragment as shown for the 30S ribosomal subunits (see Figs. 3 and 5). Consequently, it can be postulated a synergetic effect which would, somehow, stabilizes the IF2 mutant bound to the reconstituted 70S ribosome.

4. Discussion

The present study together with [16] present new information concerning the interaction of IF2 with the ribosome. A model is proposed in which the main active center for the binding of IF2α to the ribosome is within the N-terminal domain II. The N-terminal domain I and the C-terminal domain VI are devoid of interaction with the ribosome and in addition, the two third C-terminal domains do not directly interact with the 30S ribosomal subunit, but interacts weakly with 50S and 70S ribosomes. Furthermore, IF1 is involved in the binding of IF2 to the 30S ribosome by direct interaction with regions located within IF2 domains III–V. The proposed model corrects earlier conclusions that the active centers on IF2 for the interaction with fMet-tRNA$_f^{Met}$, ribosomes and GTP would reside within the C-terminal two thirds of IF2, leaving the N-terminus of IF2 functionally uncharacterized [4,13,14]. It was observed that IF1 and IF3 greatly influence the activity, in vitro, of the IF2 domains III–VI, in that work termed IF2γ, as compared to IF2α [13], and, in vivo, the lack of N-terminal domains of IF2α abolish the cell growth at 37°C [14]. These results are consistent with the model we propose in which IF1 stimulates the binding of IF2 and where the N-terminus of IF2 is important for the binding to the ribosome.

Based on these results together with published data of IF2

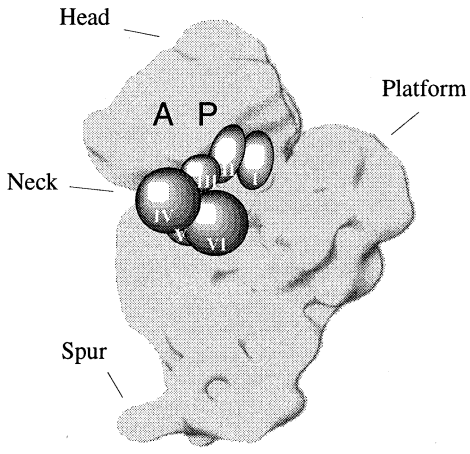

Fig. 6. Model for the interaction of *E. coli* IF2 and the ribosome. The 30S ribosomal subunit is schematically represented from the side that faces the 50S subunit (adapted from [25]). Transfer RNA binding sites, A and P are moreover included. The six-domain model of IF2 is adapted from [17]. Whereas domain IV is in close contact with the 50S L7/L12 stalk, the N-terminal domains would be located covering part of the head and platform of the 30S ribosomal subunit.

cross-linking and three-dimensional structure of ribosomes and translation factors, we are proposing a structural model for the interaction of IF2 and the 30S ribosome (Fig. 6). No NMR or X-ray crystallographic model exists for IF2. However, from the three-dimensional reconstruction of the ribosome [26,27] it can be deduced that IF2 must be a relatively large molecule to achieve the interaction with proteins located in the head as well as the platform of the 30S ribosomal subunit [7,10] and with proteins L7 and L12 in the 50S ribosomal stalk [8], covering a distance of approximately 10 nm [26]. This interaction may be archieved stericly by an IF2 with the overall dimension of $9.0\times9.9\times9.6$ nm, which was estimated from the radius of gyration determined by small angle neutron scattering experiments on *E. coli* IF2α [28]. The binding location of the different domains of IF2 on the ribosome are proposed as follows:

4.1. IF2 domain I

The N-terminal domain of IF2α does not possess intrinsic affinity for the 30S, 50S or 70S ribosome. Moreover, monoclonal antibodies with epitopes mapped within domain I do not affect the binding of IF2 to the 30S ribosomal subunit [16]. The fact that IF2α and IF2β are both active in vitro and in vivo, leaves domain I functionally uncharacterized [5,29]. The N-terminal domain I would be located between the head and the platform in a solvent exposed area, consistent with the high hydrophilicity proposed for the N-terminus of IF2 [30].

4.2. IF2 domain II

Cross-linking results from [7,10,11] showed that IF2 covers part of the head and platform of the 30S ribosomal subunit. Since IF2 domain II was shown to be directly involved in the binding of the factor to the ribosome [16], we belief that domain II covers the cross-linked areas. This can explain the results of Yusupova et al. [31], who found cross-linking between amino acid residues in IF2 domain II and the anticodon arm of the fMet-tRNA$_f^{Met}$ located in the ribosomal P-site. Furthermore, the binding of IF2 domains I–II to the 50S ribosomal subunit is feasible considering that the association between 30S and 50S ribosomal subunits involves the part of the 30S platform [32], where the N-terminus of IF2 is located in the proposed model.

4.3. IF2 domain III

This domain is the link between domain IV and domain II [17].

4.4. IF2 domain IV

Earlier results have shown that protein L7/L12 was directly involved in the GTPase activity of IF2 [33,34], and that L7/L12 could be cross-linked to IF2 [8]. More recently, the visualization of other GTP binding translation factors on the ribosome has been able to confirm the location of the structurally homologous GTP binding domain close to the L7/L12 stalk [35,36]. Therefore, in our model (Fig. 6), domain IV of IF2 is located opposite of the 30S platform which will permit close contact with the 50S L7/L12 stalk.

4.5. IF2 domain V

A model based on the analysis of sequence homologies in which *Thermus thermophilus* IF2 domain III (*E. coli* IF2 domain V) together with IF1 would mimic the structure of the elongation factor G at the ribosomal A-site was recently proposed [37,38]. This hypothesis, together with the cross-linking results reported for IF2-IF1 [10], are consistent with the results presented in this work showing that IF1 stimulates the binding of IF2 to the 30S ribosomal subunit by interaction with regions located within domains III–V. Furthermore, the finding that amino acid residues within IF2 domain V cross-link to the T-arm of the fMet-tRNA$_f^{Met}$ [31], and the putative arrangement of the initiator tRNA on the ribosome [25], makes it feasible to locate IF2 domain V close to the ribosomal A-site, on the 50S ribosomal side.

4.6. IF2 domain VI

The C-terminal domain is believed to contain the fMet-tRNA$_f^{Met}$ recognition site of IF2 [39]. Supporting this hypothesis, we found that a deletion mutant of IF2 lacking the C-terminal 90 amino acid residues was unable to protect the fMet-tRNA$_f^{Met}$ against spontaneous deacylation or promote its binding to the ribosome (data not shown). In addition, foot-printing experiments have revealed that IF2 covers the T-loop and the acceptor stem of the initiator tRNA [40]. Therefore, we locate the C-terminal domain of IF2 in close contact with the fMet-tRNA$_f^{Met}$ which occupies the P-site on the ribosome but not necessarily binding to it, as revealed from the lack of affinity of domain VI for the ribosome.

A structural model for the interaction of IF2 with the ribosome is proposed in which IF2 is initially recognized from the solvent by interactions between domain II and the 30S ribosomal subunit and subsequently positioned at the correct location by IF1. The interaction with the 50S subunit occurs through IF2 domain II and the G domain. IF3 is not involved in the interaction.

Guanosine nucleotides were not involved in the assays. However, further experiments are going on to refine the proposed model.

Acknowledgements: This work was supported by grants from the Commission of the European Communities (Contract no CHRX-CT-94-0529), Familien Hede Nielsens Fund and the Biotechnology Programme of the Danish Natural Sciences Research Council (28807-9502036, 9602401) to H.U.S.-P. J.M.P.M. received a visiting fellowship from the Danish Forskerakademi.

References

[1] Maitra, U., Stringer, E.A. and Chauduri, A. (1982) Annu. Rev. Biochem. 51, 869–900.
[2] Hershey, J.W.B. (1987) in: F.C. Neidhardt, J.L. Ingraham, K.B. Low, B. Magasanik, M. Schaechter and H.E. Umbarger (Eds.), *Escherichia coli* and *Salmonella typhimurium*. Cellular and Molecular Biology, American Society for Microbiology, Washington, DC, pp. 1386–1409.
[3] Gold, L. (1988) Annu. Rev. Biochem. 57, 199–233.
[4] Gualerzi, C.O. and Pon, C.L. (1990) Biochemistry 29, 5881–5889.
[5] Nyengaard, N.R., Mortensen, K.K., Lassen, S.F., Hershey, J.W.B. and Sperling-Petersen, H.U. (1991) Biochem. Biophys. Res. Commun. 181, 1572–1579.
[6] International Union of Biochemistry, IUBMB (1996) Biochimie 78, 1119–1122.
[7] Bollen, A., Heimark, R.L., Cozzone, A., Traut, R.R., Hershey, J.W.B. and Kahan, L. (1975) J. Biol. Chem. 250, 4310–4314.
[8] Heimark, R.L., Hershey, J.W.B. and Traut, R.R. (1976) J. Biol. Chem. 251, 7779–7784.
[9] Girshovich, A.S., Dondon, J. and Grunberg-Manago, M. (1980) Biochimie 62, 509–512.
[10] Boileau, G., Butler, P., Hershey, J.W.B. and Traut, R.R. (1983) Biochemistry 22, 3162–3170.
[11] Wakao, H., Romby, P., Ebel, J.P., Grunberg-Manago, M., Ehresman, C. and Ehresman, B. (1991) Biochimie 73, 991–1000.
[12] Moazed, D., Samaha, R.R., Gualerzi, C. and Noller, H.F. (1995) J. Mol. Biol. 248, 207–210.
[13] Cenatiempo, Y., Deville, F., Dondon, J., Grunberg-Manago, M., Sacerdot, C., Hershey, J.W.B., Hansen, H.F., Petersen, H.U., Clark, B.F.C., Kjeldgaard, M., la Cour, T.F.M., Mortensen, K.K. and Nyborg, Jens (1987) Biochemistry 26, 5070–5076.
[14] Laalami, S., Putzer, H., Plumbridge, J.A. and Grunberg-Manago, M. (1991) J. Mol. Biol. 220, 335–349.
[15] Spurio, R., Severini, M., La Teana, A., Canonaco, M.A., Pawlik, R.T., Gualerzi, C.O. and Pon, C.L. (1993) in: K.H. Nierhaus et al. (Eds.), The Translational Apparatus: Structure, Function, Regulation and Evolution, Plenum Press, New York, pp. 241–252.
[16] Palacios Moreno, J.M., Kildsgaard, J., Siwanowicz, I., Mortensen, K.K. and Sperling-Petersen, H.U. (1998) Biochem. Biophys. Res. Commun. 252, 465–471.
[17] Mortensen, K.K., Kildsgaard, J., Palacios Moreno, J.M., A. de A. Steffensen, S., Egebjerg, J. and Sperling-Petersen, H.U. (1998) Biochem. Mol. Biol. Int. 46, 1027–1041.
[18] Mortensen, K.K., Nyengaard, N.R., Hershey, J.W.B., Laalami, S. and Sperling-Petersen, H.U. (1991) Biochimie 73, 983–989.
[19] Smith, D.B. and Jonson, K.S. (1988) Gene 67, 31–40.
[20] Elish, M.E., Pierce, J.R. and Earhart, C.T. (1988) J. Gen. Microbiol. 134, 1355–1364.
[21] Brombach, M. and Pon, C.L. (1987) Mol. Gen. Genet. 208, 94–100.
[22] Soffientini, A., Lorenzetti, R., Gastaldo, L., Parlett, J.H., Spurio, R., La Teana, A. and Islam, K. (1994) Protein Expr. Purif. 5, 118–124.
[23] Spedding, G. (1990) in: G. Spedding (Ed.), Ribosomes and Protein Synthesis, Oxford University Press, Oxford, pp. 1–29.
[24] Pon, C.L., Paci, M., Pawlik, R.T. and Gualerzi, C.O. (1985) J. Biol. Chem. 260, 8918–8924.
[25] Nierhaus, K.H., Wadzack, J., Burkhardt, N., Jünemann, R., Meerwinck, W., Willumeit, R. and Stuhrmann, H.B. (1998) Proc. Natl. Acad. Sci. USA 95, 945–950.
[26] Stark, H., Orlova, E.V., Rinke-Appel, J., Jünke, N., Mueller, F., Rodnina, M., Wintermeyer, W., Brimacombe, R. and van Heel, M. (1997) Cell 88, 19–28.
[27] Malhotra, A., Penczek, P., Agrawal, R.K., Gabashvili, I.S., Grassucci, R.A., Jünemann, R., Burkhardt, N., Nierhaus, K.H. and Frank, J. (1998) J. Mol. Biol. 280, 103–116.
[28] Sperling-Petersen, H.U. (1985) Mat. Fys. Medd. Dan. Vid. Selsk. 41, 299–335.
[29] Sacerdot, C., Vachon, G., Laalami, S., Morel-Deville, F., Cenatiempo, Y. and Grunberg-Manago, M. (1992) J. Mol. Biol. 225, 67–80.
[30] Sacerdot, C., Dessen, P., Hershey, J.W.B., Plumbridge, J.A. and Grunberg-Manago, M. (1984) Proc. Natl. Acad. Sci. USA 81, 7787–7791.
[31] Yusupova, G., Reinbolt, J., Wakao, H., Laalami, S., Grunberg-Manago, M., Romby, P., Ehresmann, B. and Ehresmann, C. (1996) Biochemistry 35, 2978–2984.
[32] Lata, K.R., Agrawal, R.K., Penczek, P., Grassucci, R., Zhu, J. and Frank, J. (1996) J. Mol. Biol. 262, 43–52.
[33] Kay, A., Sander, G. and Grunberg-Manago, M. (1973) Biochem. Biophys. Res. Commun. 51, 979–986.
[34] Fakunding, J.L., Traut, R.R. and Hershey, J.W.B. (1973) J. Biol. Chem. 248, 8555–8559.
[35] Stark, H., Rodnina, M., Rinke-Appel, J., Brimacombe, R., Wintermeyer, W. and van Heel, M. (1997) Nature 389, 403–406.
[36] Agrawal, R.K., Penczek, P., Grassucci, R.A. and Frank, J. (1998) Proc. Natl. Acad. Sci. USA 95, 6134–6138.
[37] Ævarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Y., Al-Karadaghi, S., Svensson, L.A. and Liljas, A. (1994) EMBO J. 13, 3669–3677.
[38] Brock, S., Szkaradkiewicz, K. and Sprinzl, M. (1998) Mol. Microbiol. 29, 409–417.
[39] Gualerzi, C.O., Severini, M., Spurio, R., La Teana, A. and Pon, C.L. (1991) J. Biol. Chem. 266, 16356–16362.
[40] Wakao, H., Romby, P., Westhof, E., Laalami, S., Grunberg-Manago, M., Ebel, J.P., Ehresmann, C. and Ehresmann, B. (1989) J. Biol. Chem. 264, 20363–20371.