

Identification of Neighbouring Components in the Quaternary Eukaryotic Protein Synthesis Initiation Complex, eIF-2.GTP.Met-tRNA_f.small ribosomal subunit *

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Eukaryotic protein synthesis initiation factor eIF-2 consists of three subunits α , β and γ . Together with GTP and initiator tRNA (Met-tRNA_f) the factor forms a ternary initiation complex, eIF-2.GTP.Met-tRNA_f.¹⁻² Upon addition of 40S ribosomal subunits a quaternary complex, eIF-2.GTP.Met-tRNA_f.40S ribosomal subunit, is formed.³⁻⁴ In both the ternary and quaternary initiation complexes Met-tRNA_f is protected by the factor against enzymatic deacylation.⁵

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The spatial arrangement of the subunits of eIF-2 and individual ribosomal proteins within the quaternary initiation complex was studied by use of the bifunctional and heterobifunctional cross-linking reagents listed in Table 1.

Initiation factor eIF-2 from rat liver was purified to homogeneity by affinity chromatography on ribosomal RNA cellulose.¹ The purified factor was covalently attached to the heterobifunctional reagents ABAI or APTPI and bound to 40S ribosomal subunits,⁶ GTP and [³⁵S]Met-tRNA_f⁷ in the quaternary complex. After the removal of uncomplexed components by gel filtration, the quaternary complex was cross-linked by irradiation with ultraviolet light. Alternatively, the quaternary complex was formed with unmodified eIF-2 and directly cross-linked with the bifunctional reagents DBI or DEB. After labelling of the proteins in the complex with ¹²⁵I,⁸ the appropriate macromolecular conjugates were purified under denaturing conditions by gel filtration, sucrose gradient centrifugation or LiBr density gradient centrifugation. The cross-linked components were identified by two-dimensional gel electrophoresis⁹ or by one-dimensional sodium dodecylsulfate gel electrophoresis.¹⁰

The experiments with ABAI and APTPI indicate that all three subunits of eIF-2 are located within a distance of less than 12 Å from Met-tRNA_f in the ternary initiation complex² (Table 2). With the much shorter cross-linking reagent, DEB, only the β subunit of eIF-2 was found covalently attached to

Table 1. Cross-linking reagents used to determine neighbouring components in the quaternary initiation complex, eIF-2.GTP.Met-tRNA_f.small ribosomal subunit.

Reagent		Distance of reactive groups Å
DEB	1,2:3,4-Diepoxybutane $\begin{array}{c} \text{H}_2\text{C} - \text{HC} - \text{CH} - \text{CH}_2 \\ \diagdown \quad \diagup \\ \text{O} \quad \text{O} \end{array}$	4
ABAI	Methyl- <i>p</i> -azidobenzoylaminoacetimidate $\text{N}_3 - \text{Ph} - \text{CO} - \text{NH} - \text{CH}_2 - \text{C} \begin{array}{l} \diagup \text{NH} \\ \diagdown \text{OCH}_3 \end{array}$	10
APTPI	Methyl-5-(<i>p</i> -azidophenyl)-4,5-dithiapentanimidate $\text{N}_3 - \text{Ph} - \text{S} - \text{S} - \text{CH}_2 - \text{CH}_2 - \text{C} \begin{array}{l} \diagup \text{NH} \\ \diagdown \text{OCH}_3 \end{array}$	12
DBI	Dimethyl-5,6-dihydroxy-4,7-dioxo-3,8-diazadecanbisimidate $\begin{array}{c} \text{HN} \\ \diagdown \\ \text{H}_3\text{CO} - \text{C} - \text{CH}_2 - \text{NH} - \text{CHOH} - \text{CHOH} - \text{NH} - \text{CH}_2 - \text{C} \begin{array}{l} \diagup \text{NH} \\ \diagdown \text{OCH}_3 \end{array} \end{array}$	14

Table 2. Composition of cross-linked conjugates isolated from ABAl, APTPI, DEB or DBI-treated initiation complexes.

Cross-linked complex	Reagent	Neighbouring components A and B		Ref.
		A	B	
eIF-2.GTP.Met-tRNA _f	ABAl or APTPI	α, β and γ subunit of eIF-2	Met-tRNA _f	2
	DEB	β subunit of eIF-2	Met-tRNA _f	2
eIF-2.GTP.Met-tRNA _f .40S ribosomal subunit	DBI	α and γ subunit of eIF-2	ribosomal proteins S3, S3a, S6, S13/16, S15 and S15a	3
	ABAl or APTPI	α and γ subunit of eIF-2	18S rRNA	4
		18S rRNA	ribosomal proteins S2, S3, S3a, S4, S6, S8, S9, S11, S16/18, S23/24, S25 and S26	14
	DEB	α and γ subunit of eIF-2	18S rRNA	4
			18S rRNA	ribosomal proteins S3a, S6, S7, S8, S11, S16/18, S23/24 and S25
ABAl		Met-tRNA _f	ribosomal proteins S3a and S6	14
DEB		Met-tRNA _f	ribosomal proteins S3a and S6	14
		Met-tRNA _f	β subunit of eIF-2	14

the initiator tRNA within either of the ternary or quaternary initiation complexes. In the latter complex, Met-tRNA_f was in close proximity to the ribosomal proteins S3a and S6. Thus, in the binding site for eIF-2 on the 40S ribosomal subunit, Met-tRNA_f is at a distance of less than 4 Å from both the β subunit of eIF-2 and the ribosomal proteins S3a and S6. The suggestion that the β subunit of eIF-2 is responsible for the binding of the initiator tRNA is further supported by the observation that the isolated β subunit has RNA binding activity.¹¹

Proteins S3a and S6 as well as proteins S3, S13/16, S15 and S15a, were also located at a distance of less than 14 Å from the α and γ subunits of eIF-2 within the quaternary initiation complex (Table 2).

When eIF-2 is attached to its binding site on the 40S ribosomal subunit, the factor subunits α and γ are within a distance of less than 4 Å from the 18S ribosomal RNA. This suggests that sequences of the 18S rRNA are located near the surface of the 40S ribosomal particle at the binding site for eIF-2. It was therefore not surprising that some of

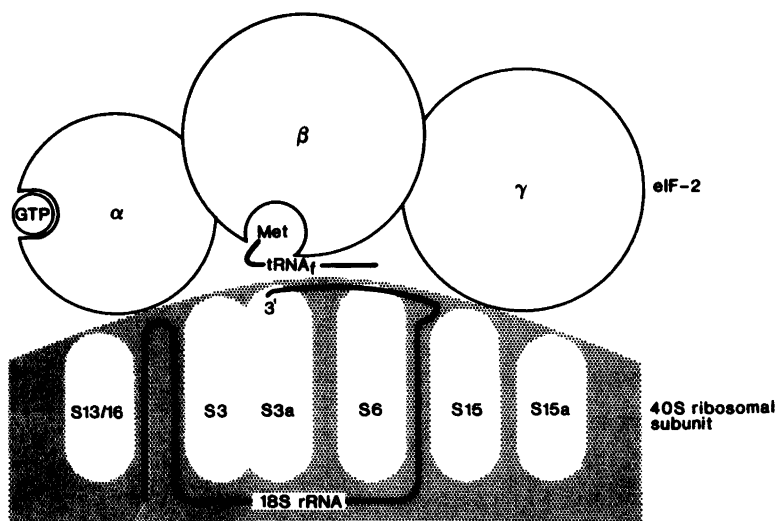


Fig. 1. Spatial arrangement of interacting components within the quaternary initiation complex, eIF-2.GTP.Met-tRNA_f.40S ribosomal subunit.

the ribosomal proteins which cross-linked to the α and γ subunits of eIF-2 could also be covalently attached to the 18S rRNA. Proteins S3a and S6, together with a limited number of other proteins, were found to be located at a distance of less than 4 Å from 18S rRNA (Table 2). Protein S3 was detected at a somewhat greater distance (10 Å) from 18S rRNA. The close proximity of S3a and 18S rRNA is further supported by the observation that protein S3a can be cross-linked to the 3' end of 18S rRNA after oxidation with sodium periodate.¹² In addition, proteins S3 and S3a are closely adjacent to each other as shown by cross-linking with DBI.¹³

Fig. 1 summarizes the available cross-linking data obtained for the quaternary initiation complex. The α subunit of eIF-2 has GTP-binding activity.¹¹ The α and γ subunits represent the areas of eIF-2 in closest contact with various components of the eIF-2 binding site on the 40S particle (S3, S3a, S6, S13/16, S15, S15a and 18S rRNA). Within the binding site, Met-tRNA_i is at contact distance of both the β subunit of eIF-2 and the ribosomal proteins S3a and S6.

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