Biochem. J. (2002) **362**, 539–544 (Printed in Great Britain)

RESEARCH COMMUNICATION

Crystal structures of 7-methylguanosine 5′-triphosphate (m^{7}GTP)- and P^1-7-methylguanosine-P^3-adenosine-5′,5′-triphosphate (m^{7}GpppA)-bound human full-length eukaryotic initiation factor 4E: biological importance of the C-terminal flexible region

Koji TOMOO*[1], Xu SHEN*, Koumei OKABE*, Yoshiaki NOZOE*, Shoichi FUKUHARA*, Shigenobu MORINO*, Toshimasa ISHIDA*, Taizo TANIGUCHI†, Hiroshi HASEGAWA†, Akira TERASHIMA†, Masahiro SASAKI†, Yoshio KATSUYA‡, Kunihiro KITAMURA§, Hiroshi MIYOSHI‖, Masahide ISHIKAWA‖ and Kin-ichiro MIURA‖

*Department of Physical Chemistry, Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan, †Hyogo Institute for Aging Brain and Cognitive Disorders, 520 Saisho-koh, Himeji 670-0981, Japan, ‡Hyogo Prefectural Institute of Industrial Research, 1479-6 Kanaji, Kamigori-cho, Ako-gun, Hyogo 678-1201, Japan, §Research Center, Taisho Pharmaceutical Co., Ltd., 1-403 Yoshino-cho, Ohmiya, Saitama 330-0031, Japan, and ‖Institute for Biomolecular Science, Gakushuin University, Toshima-ku, Tokyo 171-8588, Japan

The crystal structures of the full-length human eukaryotic initiation factor (eIF) 4E complexed with two mRNA cap analogues [7-methylguanosine 5′-triphosphate (m^{7}GTP) and P^1-7-methylguanosine-P^3-adenosine-5′,5′-triphosphate (m^{7}GpppA)] were determined at 2.0 Å resolution (where 1 Å = 0.1 nm). The flexibility of the C-terminal loop region of eIF4E complexed with m^{7}GTP was significantly reduced when complexed with m^{7}GpppA, suggesting the importance of the second nucleotide in the mRNA cap structure for the biological function of eIF4E, especially the fixation and orientation of the C-terminal loop region, including the eIF4E phosphorylation residue. The present results provide the structural basis for the biological function of both N- and C-terminal mobile regions of eIF4E in translation initiation, especially the regulatory function through the switch-on/off of eIF4E-binding protein–eIF4E phosphorylation.

Key words: cap analogues, eIF4E, flexibility, translation initiation.

INTRODUCTION

Translation initiation in eukaryotes requires a complex biochemical pathway, catalysed by a large number of initiation factors. The first step of the translation process involves binding of the small ribosomal subunit to mRNA. This is the rate-limiting step in translation initiation, and is also a target for translational control (reviewed in [1]). Eukaryotic mRNA has a common cap structure at the 5′-terminal end. The cap structure is important for stabilizing mRNA and facilitating its binding to ribosomes in the translation process [2,3]. In cap-dependent translation, an interaction is required between the cap structure and the eukaryotic initiation factor (eIF) 4F for the efficient translation of mRNA [4,5]. eIF4E, the smallest subunit in eIF4F, binds specifically to the mRNA cap structure and plays an important role in the initiation of protein synthesis [6]. Elucidation of the interaction between eIF4E and the mRNA cap structure is therefore necessary to understand the initiation mechanism of protein synthesis.

Recently, two 7-methylguanosine 5′-diphosphate (m^{7}GDP)-bound eIF4E structures from different sources were analysed by X-ray crystallography [7] and NMR spectroscopy [8], and the structural basis of eIF4E for m^{7}G cap recognition was made clear, together with the tertiary structure in the core region. However, the structures of the N- and C-terminal regions are still unclear, because of the high flexibility of these regions in eIF4E. Furthermore, the binding mode between eIF4E and the capped mRNA molecule has not yet been fully elucidated.

In the present study we have determined the X-ray structures of human full-length eIF4E complexed with mRNA cap analogues [7-methylguanosine 5′-triphosphate (m^{7}GTP) and P^1-7-methylguanosine-P^3-adenosine-5′,5′-triphosphate (m^{7}GpppA)]. These structures reveal that the flexible C-terminal loop region in eIF4E could be important for binding with capped mRNA. The analyses of their complete structures at the atomic level may provide a more detailed insight into the initiation mechanism of eIF4E.

MATERIALS AND METHODS

Expression and purification of eIF4E complexed with mRNA cap analogues

Recombinant human eIF4E expression in *Escherichia coli* and purification were carried out according to the previously reported methods [9]. *E. coli* BL21(DE3) cells transformed with the pDUCBP vector encoding the expression of eIF4E were grown in Luria–Bertani medium containing 100 μg/ml ampicillin at

Abbreviations used: eIF, eukaryotic initiation factor; 4E-BP, eIF4E-binding protein; m^{7}GDP, 7-methylguanosine 5′-diphosphate; m^{7}GTP, 7-methylguanosine 5′-triphosphate; m^{7}GpppA, P^1-7-methylguanosine-P^3-adenosine-5′,5′-triphosphate.

[1] To whom correspondence should be addressed (e-mail tomoo@gly.oups.ac.jp).

The coordinates of human full-length eIF4E complexed with m^{7}GTP and m^{7}GpppA have been deposited in the PDB (accession codes 1IPC and 1IPB respectively).

© 2002 Biochemical Society

Table 1 Data collection and refinement statistics

Statistics	$+m^7GTP$	$+m^7GpppA$
Data collection		
Resolution (Å)	30–1.9	30–1.9
Unique reflection	21848	20089
Completeness (%)	89.8	82.6
R_{merge} (%)	7.86	8.36
Space group	$P4_3$	$P4_3$
Cell dimensions (Å)	a = b = 88.18	a = b = 87.98
	c = 38.27	c = 38.21
Refinement		
Resolution (Å)	30–2.0	30–2.0
Reflection	16787	16683
R-factor (%)	19.2	19.6
R_{free} (%)	22.8	22.9
Number of atoms		
Protein	1540	1572
Cap analogue	33	51
Water	153	139
Root-mean-square deviation from ideal geometry		
Bond (Å)	0.0057	0.0058
Angle	1.28	1.28
Improper	0.68	0.68
Average B factor (Å^2)	24.14	23.76

37 °C. The expression of wild-type eIF4E was induced by the addition of 0.1 mM isopropyl β-D-thiogalactoside. After induction for 12 h, the cells were harvested and disrupted by sonication. The supernatant was applied to an m^{7}GTP–Sepharose 4B column, and the bound protein was eluted with elution buffer containing 100 μM m^{7}GTP or m^{7}GpppA in order to obtain eIF4E complexed with mRNA cap analogues.

Crystallization and data collection

The m^{7}GTP- and m^{7}GpppA-bound eIF4Es were crystallized by hanging drop vapour diffusion at 15 °C. The protein solution was equilibrated against the reservoir solution containing 100 mM Hepes/KOH (pH 7.5), 27 % (w/v) PEG-6000 and 0.2 M $(NH_4)_2SO_4$. Long needle-shaped crystals were obtained after one week. X-ray diffraction data were collected at 100 K on a Rigaku R-AXISIV detector using synchrotron radiation at BL-24XU in Spring-8. Data processing was performed using the program PROCESS [10]. The crystals diffracted up to 1.9 Å resolution (where 1 Å = 0.1 nm; see Table 1).

Structure determination and refinement

The initial structure of the human eIF4E–m^{7}GTP complex was determined by the molecular replacement method with the program CNS [11] using the starting models of the yeast and mouse eIF4E. The best solution of molecular replacement was obtained using 10–4 Å resolution data. The atomic model was constructed using the graphics program TURBO-FRODO [12], and the structure was refined by the CNS program package. The statistics are shown in Table 1.

RESULTS AND DISCUSSION

Conserved cap-recognition pocket

Human full-length eIF4E (Figure 1) consists of an α,β structure motif similar to RNA-binding protein [13]. As compared with the overall structure of m^{7}GDP-bound mouse eIF4E [7], no remarkable difference is found in the core region of the human eIF4E structure; the average Cα deviation between the m^{7}GDP and m^{7}GpppA complexes is 0.38 Å. The electron densities of m^{7}GTP and m^{7}GpppA bound to eIF4E are shown in Figures 2(a) and 2(b) respectively. The structure of the cap-recognition pocket is almost conserved between human and mouse eIF4E. The schematic interaction mode in the m^{7}GpppA complex is depicted in Figure 3; nearly the same mode is also observed in the m^{7}GTP complex, except for the adenosine moiety. As has already been proposed from the model studies [14,15], the m^{7}G moiety of the cap structure is sandwiched between the two aromatic side chains of Trp^{56} and Trp^{102}. Two (cap m^{7}G)NH · · · O(Glu^{103} carboxy group) hydrogen bonds stabilize the stacking interaction.

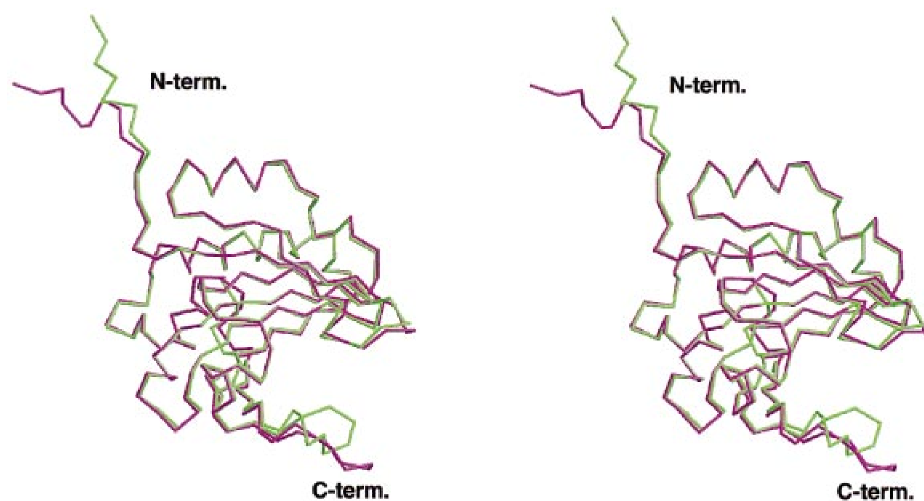

Figure 1 Stereoscopic superposition of Cα traces of the human full-length eIF4E–m^{7}GpppA complex (magenta) and mouse eIF4E (truncated at N-terminal 27 residues) complexed with m^{7}GDP (green)

In contrast with large flexibility at both N- and C-terminal regions, the core regions consisting of eight-stranded anti-parallel β-sheets and three long α-helices are very similar to each other. The Figure was prepared using MOLSCRIPT [20] and Raster3D [21].

© 2002 Biochemical Society

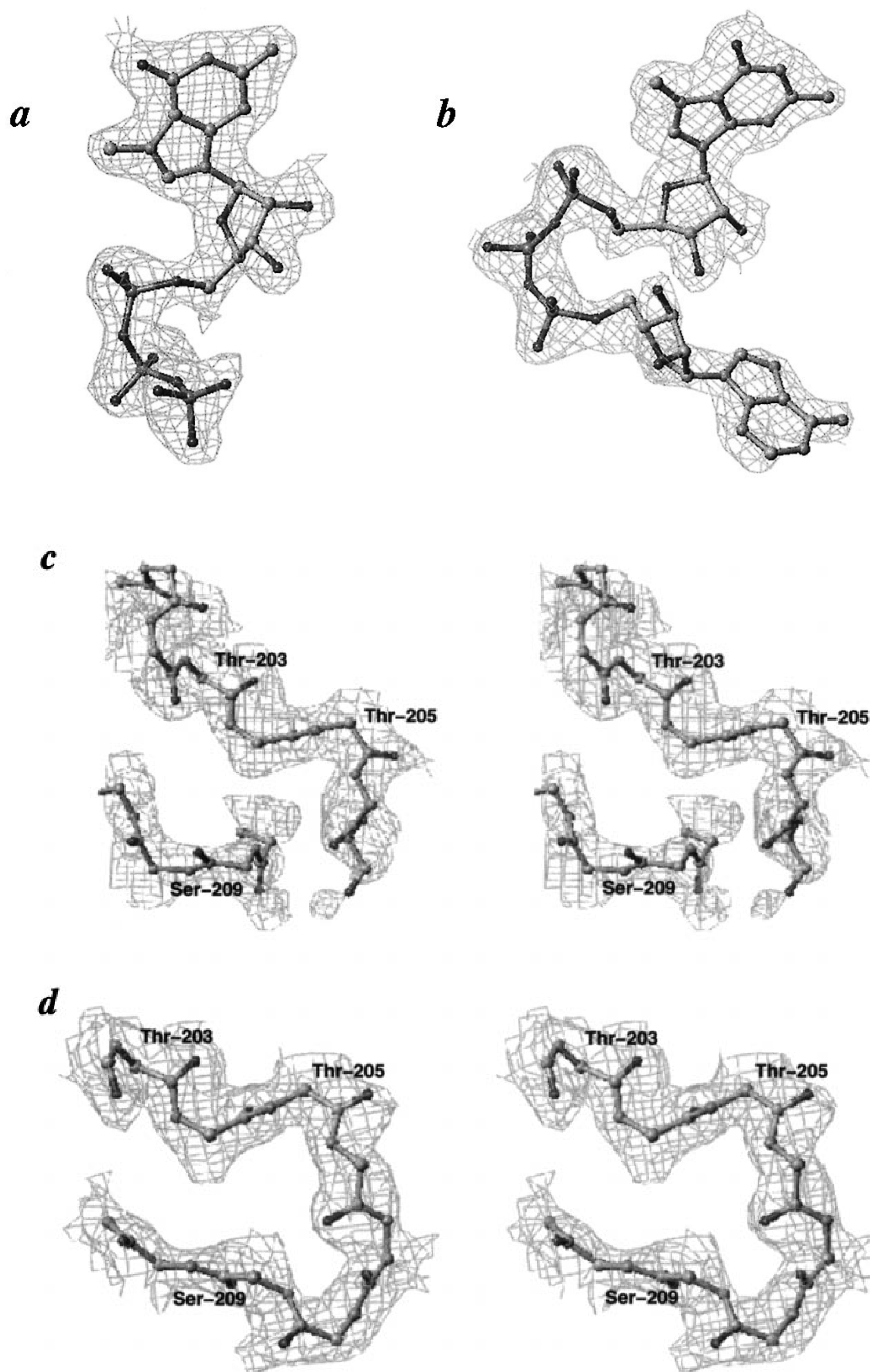

Figure 2 Final electron density maps

2Fo-Fc omit maps of m^{7}GTP (**a**) and m^{7}GpppA (**b**) bound to human eIF4E, and 2Fo-Fc maps and backbone models (in stereo) of C-terminal loop regions of human eIF4E complexed with m^{7}GTP (**c**) and m^{7}GpppA (**d**). The continuity of electron densities in these C-terminal loop regions is clearly different, and this resulted from the absence or presence of the second nucleotide in the mRNA cap analogue.

© 2002 Biochemical Society

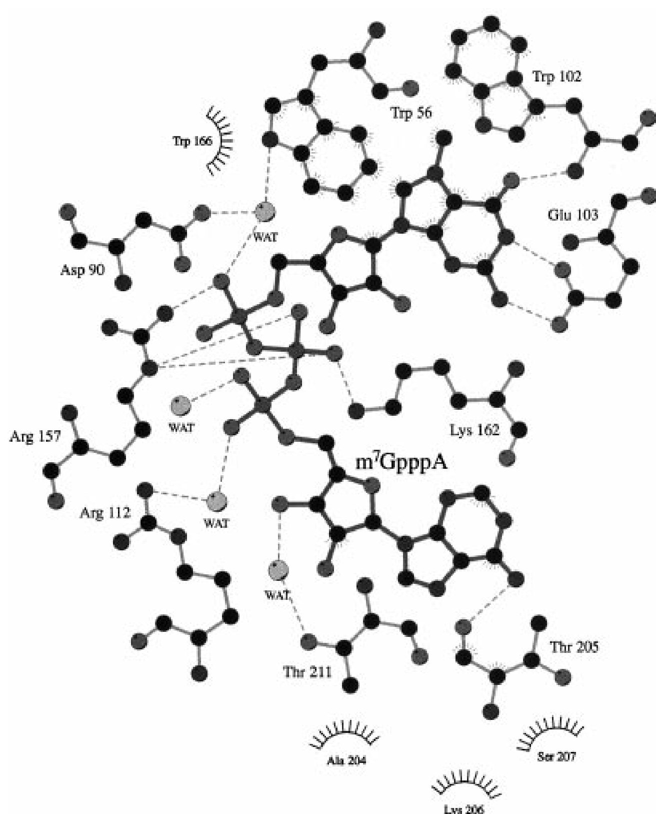

Figure 3 Schematic representation of the interaction modes between m^7GpppA and human eIF4E

Possible hydrogen bonds and short contacts are shown with broken lines, and van der Waals contacts are indicated by arcs. The Figure was generated using the program LIGPLOT [22].

The first and second phosphate groups of the m^7GTP/m^7GpppA were linked to the basic amino acids located at the cap-binding pocket (Arg^{112}, Arg^{157} and Lys^{162}) through the direct or water-mediated hydrogen bonds (Figure 3). These interactions including water molecules are also well conserved in the mouse eIF4E–m^7GDP complex. The third phosphate group of m^7GTP/m^7GpppA forms a hydrogen bond with the Arg^{112} side chain via a water molecule, whereas the corresponding space in the eIF4E–m^7GDP complex is occupied by one water molecule.

Importance of the C-terminal loop for cap binding and functional regulation

In contrast with the well conserved core structure, the C-terminal region shows conformational flexibility depending on the structure of the mRNA cap analogue. In the m^7GpppA complex, however, the adenine nucleoside located in the C-terminal loop region is hydrogen-bonded to Thr^{205} and Thr^{211} (via a water molecule) and is stabilized by some van der Waals contacts with Ser^{207} and its neighbouring residues (Figure 3). Consequently, the C-terminal loop (Ala^{203}–Thr^{211}), which was not determined in the structure of human eIF4E–m^7GTP (Figure 2c), showed clear electron density in the structure of the m^7GpppA complex (Figure 2d).

The crystal structure of mouse eIF4E in complex with m^7GDP contains two crystallographically independent molecules in the asymmetric unit. One molecule of mouse eIF4E can trace the backbone of the C-terminal loop region, although the backbone trace for residues 208–211 is missing for the other molecule. When we compare the structure of the human eIF4E–m^7GpppA complex with that of the mouse m^7GDP complex, these two structures show a large difference at the C-terminal loop region (Figures 1 and 4), where the root-mean-square deviation is 0.9 Å for the $C\alpha$ position of residues 200–217. This is obviously due to the interactions of the flexible C-terminal loop with the adenosine moiety of m^7GpppA (Figure 3), indicating that this nucleotide or its sequence linked to the m^7Gppp moiety of the cap structure could regulate the flexibility and orientation of the C-terminal loop region. For example, this region is moved to widen the cap-binding slot of eIF4E by the adenosine moiety of m^7GpppA (Figure 4).

It is generally believed [16,17] that the phosphorylation of eIF4E mainly occurs at the O^{γ} atom of Ser^{209} and plays a role in controlling the switch-on/off of association between eIF4E and mRNA. The adenosine moiety of m^7GpppA is located far from Ser^{209} in the m^7GpppA complex (Figure 4). Thus this suggests that the adenosine moiety does not contribute directly to the Ser^{209} phosphorylation, but through the fixation and orientation of the flexible loop region of Ala^{203}–Thr^{211}. On the other hand, it is still unclear how the phosphorylation affects eIF4E activity. It has been considered that the phosphorylated Ser^{209} could form a retractable salt bridge with Lys^{159} to clamp the cap moiety of mRNA. However, the distance between the $C\alpha$ positions of Ser^{209} and Lys^{159} is approx. 19 Å in the m^7GpppA complex. This distance would be too long to form such a retractable salt bridge, even though the loop region and Lys^{159} side chain are both flexible (Figure 4). We have observed that the $Ser^{209} \rightarrow$ Glu mutant of eIF4E has much higher affinity for the cap analogue than the wild-type. Thus it may be reasonable to assume that the charge change around the cap-binding site by Ser^{209} phosphorylation increases the binding ability with capped mRNA.

N-terminal flexible region

As is shown in Figure 1, we could determine the structure from Glu^{27} in both complexes of human full-length eIF4E; it was impossible to determine the structure from the N-terminus to Gln^{26} because of the high flexibility. No specific secondary structural element was observed in the Glu^{27}–His^{37} sequence. In human eIF4E, Ala^{29}, Tyr^{34} and Lys^{36} form hydrogen bonds with Leu^{62}, Gln^{80} and His^{78} of the neighbouring protein respectively, thus stabilizing this region.

It is known that the biological function of eIF4E is performed through binding with eIF4G and is regulated by binding with endogenous eIF4E-binding protein (4E-BP) [18]. The crystal structures of mouse eIF4E complexed with eIF4G- or 4E-BP-mimic peptide showed that His^{37}, Val^{69} and Trp^{73} in eIF4E play an important role in binding with eIF4G or 4E-BP [19]. On the other hand, it is also known that phosphorylation of Ser^{65} and Thr^{70} of 4E-BP weakens its binding ability to eIF4E, leading to its dissociation from the complex. Although the actual binding mode between eIF4E and 4E-BP has not yet been determined, the present results suggest that two acidic amino acids, Glu^{32} and Glu^{70}, in the N-terminal region of eIF4E are located in the neighbourhood of Ser^{65} and Thr^{70} in 4E-BP. Phosphorylation of Ser^{65} and Thr^{70} will cause electrostatic repulsion with these two acidic residues in eIF4E and thus lead to the decrease in binding ability with eIF4E (Figure 5).

The present results indicate that the flexible N- and C-terminal moieties of eIF4E shift the structure so as to just fit to the structures of biological partners, i.e. 4E-BP/eIF4G and the mRNA cap structure, leading to a functionally active supermolecule.

© 2002 Biochemical Society

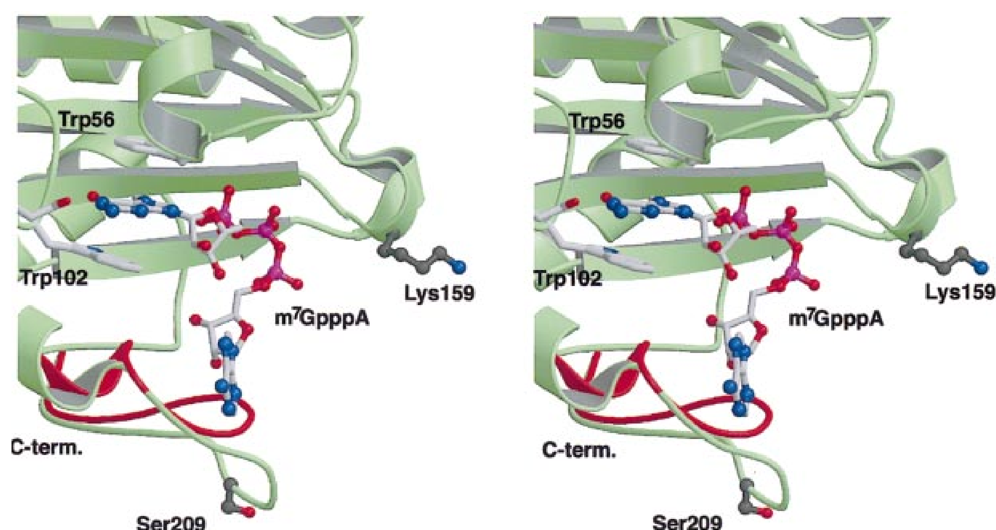

Figure 4 Comparison between C-terminal loop regions of human eIF4E–m^{7}GpppA (green) and mouse eIF4E–m^{7}GDP (red line) complexes

The only major difference is found in the C-terminal loop region. This difference is due to the presence of the second nucleoside in m^{7}GpppA. Prepared using MOLSCRIPT [20] and Raster3D [21].

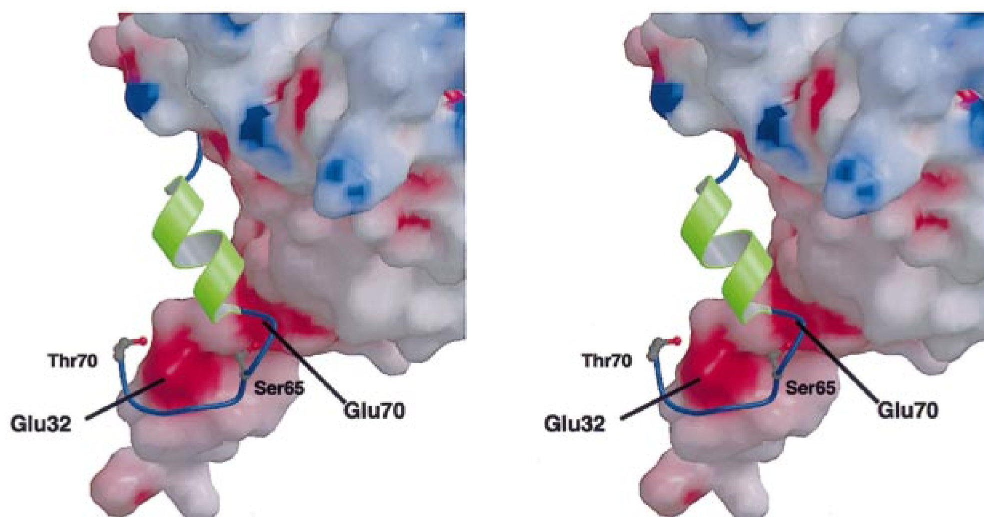

Figure 5 Probable binding mode of 4E-BP fragment to human eIF4E

4E-BP peptide (PDB code 1EJ4 [11]) was fitted to human eIF4E, although the structure of Ser^{65}–Thr^{70} was not determined. The molecular surface of human eIF4E is coloured according to its electrostatic potential (GRASP [23]); blue and red represent positively and negatively charged regions respectively.

We thank Dr Norbert Strater (Institute for Crystallography, Free University of Berlin, Germany) for helping prepare Figures 4 and 5.

REFERENCES

1 Mathews, M. B., Sonenberg, N. and Hershey, J. W. B. (1996) Origins and targets of translational control. In Transcriptional Control (Hershey, J. W. B., Mathews, M. B. and Sonenberg, N., eds.), pp. 1–29, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
2 Muthukrishnan, S., Both, G. W., Furuichi, Y. and Shatkin, A. J. (1975) 5′-Terminal 7-methylguanosine in eukaryotic mRNA is required for translation. Nature (London) **255**, 33–37
3 Shatkin, A. J. (1976) Capping of eucaryotic mRNAs. Cell (Cambridge, Mass.) **9**, 645–653
4 Haghighat, A. and Sonenberg, N. (1997) eIF4G dramatically enhances the binding of eIF4E to the mRNA 5′-cap structure. J. Biol. Chem. **272**, 21677–21680
5 Morino, S., Imataka, H., Svitkin, Y. V., Pestova, T. V. and Sonenberg, N. (2000) Eukaryotic translation initiation factor 4E (eIF4E) binding site and the middle one-third of eIF4G constitute the core domain for cap-dependent translation, and the C-terminal one-third functions as a modulatory region. Mol. Cell. Biol. **20**, 468–477
6 Sonenberg, N. (1996) mRNA 5′ cap-binding protein eIF4E and control of cell growth. In Transcriptional Control (Hershey, J. W. B, Mathews, M. B. and Sonenberg, N., eds.), pp. 245–269, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
7 Marcotrigiano, J., Gingras, A. C., Sonenberg, N. and Burley, S. K. (1997) Cocrystal structure of the messenger RNA 5′ cap-binding protein (eIF4E) bound to 7-methyl-GDP. Cell (Cambridge, Mass.) **89**, 951–961
8 Matsuo, H., Li, H., McGuire, A. M., Fletcher, C. M., Gingras, A. C., Sonenberg, N. and Wagner, G. (1997) Structure of translation factor eIF4E bound to m^{7}GDP and interaction with 4E-binding protein. Nat. Struct. Biol. **4**, 717–724

© 2002 Biochemical Society

9 Morino, S., Hazama, H., Ozaki, M., Teraoka, Y., Shibata, S., Doi, M., Ueda, H., Ishida, T. and Uesugi, S. (1996) Analysis of the mRNA cap-binding ability of human eukaryotic initiation factor-4E by use of recombinant wild-type and mutant forms. Eur. J. Biochem. **239**, 597–601
10 Higashi, T. (1989) The processing of diffraction data taken on a screenless Weissenberg camera for macromolecular crystallography. J. Appl. Crystallogr. **22**, 9–18
11 Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S. et al. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination. Acta Crystallogr., Sect. D: Biol. Crystallogr. **54**, 905–921
12 Jones, T. A. (1978) A graphics model building and refinement system for macromolecules. J. Appl. Crystallogr. **11**, 268–272
13 Nagai, K. (1996) RNA–protein complexes. Curr. Opin. Struct. Biol. **6**, 53–61
14 Ishida, T., Doi, M., Ueda, H., Inoue, M. and Scheldrick, G. M. (1988) Specific ring stacking interaction on the tryptophan-7-methylguanine system: Comparative crystallographic studies of indole derivatives-7-methylguanine base, nucleoside, and nucleotide complexes. J. Am. Chem. Soc. **110**, 2286–2294
15 Ishida, T., Iyo, H., Ueda, H., Doi, M., Inoue, M., Nishimura, S. and Kitamura, K. (1991) Importance of simultaneous co-operation of hydrogen bond pairing and stacking interactions for recognition of guanine base by a peptide: X-ray crystal analysis of 7-methylguanosine-5′-phosphate-tryptophanylglutamic acid complex. J. Chem. Soc., Perkin Trans. **1**, 1847–1853
16 Joshi, B., Cai, A. L., Keiper, B. D., Minich, W. B., Mendez, R., Beach, C. M., Stepinski, J., Stolarski, R., Darzynkiewicz, E. and Rhoads, R. E. (1995) Phosphorylation of eukaryotic protein synthesis initiation factor 4E at Ser-209. J. Biol. Chem. **270**, 14597–14603
17 Fukunaga, R. and Hunter, T. (1997) MNK1, a new MAP kinase-activated protein kinase, isolated by a novel expression screening method for identifying protein kinase substrates. EMBO J. **16**, 1921–1933
18 Sonenberg, N. and Gingras, A. C. (1998) The mRNA 5′ cap-binding protein eIF4E and control of cell growth. Curr. Opin. Cell Biol. **10**, 268–275
19 Marcotrigiano, J., Gingras, A. C., Sonenberg, N. and Burley, S. K. (1999) Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. Mol. Cell. **3**, 707–716
20 Kraulis, P. J. (1991) MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. J. Appl. Crystallogr. **24**, 946–950
21 Meritt, E. A. and Bacon, D. J. (1997) Raster3D: photorealistic molecular graphics. Methods Enzymol. **277**, 505–524
22 Wallace, A. C., Laskowski, R. A. and Thornton, J. M. (1995) LIGPLOT: a program to generate schematic diagrams of protein--ligand interactions. Protein Eng. **8**, 127–134
23 Nicholls, A., Sharp, K. A. and Honig, B. (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. Proteins **11**, 281–296

Received 18 October 2001/10 January 2002; accepted 17 January 2002

© 2002 Biochemical Society