Poliovirus 2A proteinase cleaves directly the eIF-4G subunit of eIF-4F complex

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Abstract The initiation of translation on eukaryotic mRNA is governed by the concerted action of polypeptides of the eIF-4F complex. One of these polypeptides, eIF-4G, is proteolytically inactivated upon infection with several members of the *Picornaviridae* family. This cleavage occurs by the action of virus-encoded proteinases: $2A^{pro}$ (entero- and rhinovirus) or L^{pro} (aphthovirus). An indirect mode of eIF-4G cleavage through the activation of a second cellular proteinase has been proposed in the case of poliovirus. Although cleavage of eIF-4G by rhino- and coxsackievirus $2A^{pro}$ has been achieved directly in vitro, a similar activity has not been documented to date for poliovirus $2A^{pro}$. We report here that a recombinant form of poliovirus $2A^{pro}$ fused to maltose binding protein (MBP) directly cleaves human eIF-4G from a highly purified eIF-4F complex. Efficient cleavage of eIF-4G requires magnesium ions. The presence of other initiation factors such as eIF-3, eIF-4A or eIF-4B mimics in part the stimulatory effect of magnesium ions and probably stabilizes the cleavage products of eIF-4G generated by $2A^{pro}$. These results suggest that efficient cleavage of eIF-4G by MBP-$2A^{pro}$ requires a proper conformation of this factor. Finally, MBP-$2A^{pro}$ protein cleaves an eIF-4G-derived synthetic peptide at the same site as rhino- and coxsackievirus $2A^{pro}$ (R485-G486).

Key words: Poliovirus $2A^{pro}$; eIF-4G cleavage; Translation initiation factor; Inhibition of translation; Cytotoxic protease

1. Introduction

Translation of eukaryotic mRNAs requires the concerted action of the initiation complex 4F (eIF-4F), together with other components that participate in the initiation event. The eIF-4F complex recognizes the cap structure present at the 5′ end of mRNAs, unwinds the secondary structure of the leader sequence and promotes the binding of mRNAs to ribosomes [1–3]. Mammalian eIF-4F has been purified as a complex of three polypeptides: eIF-4E (24 kDa, cap-binding protein), eIF-4A (50 kDa, RNA helicase) and eIF-4G (220 kDa) [4,5]. The eIF-4F complex interacts with other initiation factors, such as eIF-3 and eIF-4B to promote the entry of the small 40S ribosomal subunit to the mRNA [6–9].

Host translation is rapidly halted upon infection of susceptible cells with poliovirus as well as with other members of the *Picornaviridae* family. In the case of entero-, rhino-, and aphthovirus genera, shut-off of translation has been related to the functional inactivation of the eIF-4F complex. Indeed, soon after infection of susceptible cells with these viruses, eIF-4G (220 kDa) is cleaved giving rise to an amino-terminal fragment of 13 kDa and to a carboxy fragment of 100 kDa [10,7,11,12]. Both genetic and biochemical evidence supports the notion that virus-encoded proteinases $2A^{pro}$ (L^{pro} in aphthovirus) are responsible for the proteolytic bissection of eIF-4G [13–15]. However, the molecular details of the proteolytic process of eIF-4G are still very much debated. For poliovirus $2A^{pro}$, a model of indirect cleavage through the activation of a quiescent cellular proteinase has been proposed [16,17]. This latent cellular proteinase would be, in the last instance, that responsible for eIF-4G cleavage following a cascade mechanism triggered by poliovirus $2A^{pro}$. This model was proposed on the basis of early observations where poliovirus $2A^{pro}$ did not copurify with eIF-4G cleavage activity obtained from poliovirus-infected HeLa extracts [18]. Moreover, extracts from *E. coli* obtained expressing poliovirus $2A^{pro}$ require a cellular component to mediate cleavage of a partially purified eIF-4G [16]. This putative cellular proteinase was initially identified as initiation factor eIF-3. Further work resolved the putative protease from eIF-3 that was reidentified as a protein of 55–60 kDa unrelated to eIF-3 [17]. However, the role of these two cellular factors in $2A^{pro}$-mediated cleavage of eIF-4G remains uncertain.

In contrast, direct cleavage of eIF-4G has been achieved using recombinant rhino- and coxsackievirus $2A^{pro}$ and purified eIF-4F complex obtained from rabbit reticulocytes [19,20]. More recently, eIF-4G assembled into the 4F complex has been proposed as the natural substrate for rhinovirus $2A^{pro}$, suggesting that the conformational state of eIF-4G when bound to the other eIF-4F subunits determines its susceptibility to be cleaved by picornaviral 2A proteinases [21]. Strikingly, such an activity has not been analyzed for poliovirus $2A^{pro}$, perhaps due to the difficulty to obtain this proteinase in an active form. Recently, we have purified a recombinant and active form of poliovirus $2A^{pro}$ fused to maltose binding protein (MBP). In the present work, we examine if this recombinant poliovirus $2A^{pro}$ is able to cleave directly the eIF-4G subunit from the eIF-4F complex.

2. Materials and methods

2.1. Purification of initiation factors from HeLa cells

eIF-4F complex used as substrate in cleavage experiments was purified from the ribosomal salt wash (RSW) of HeLa S3 cells as described previously [22]. Briefly, RSW was precipitated with 40% ammonium persulfate and the resulting pellet was resuspended and dialyzed against H100 buffer (20 mM HEPES (pH 7.5), 100 mM KCl, 2 mM EDTA, 5% glycerol, 10 mM β-mercaptoethanol and 1 μg/ml aprotinin). This extract, enriched in eIF-4F and eIF-3 factors, was further purified by two chromatographic steps. First, a ^{7}m-GTP-Sepharose column (Pharmacia) was used to retain the eIF-4F complex. Bound proteins were eluted in H100 buffer plus ^{7}m-GTP and fractions containing eIF-4F (monitored by Western blotting against

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the eIF-4G subunit) were pooled and loaded on a Mono Q 5/5 column (Pharmacia) that had been equilibrated with buffer H100. eIF-4F complex was eluted at approximately 360 mM KCl and dialyzed against buffer H100. The degree of eIF-4F purification was determined by silver staining of gels and by Western blotting using antisera against eIF-4G, eIF-4A, eIF-4E and eIF-3 factors. Purified eIF-3, eIF-4A and eIF-4B were obtained from HeLa cells as described previously [6].

2.2. Purification of MBP-2A^{pro}

Poliovirus 2A^{pro} was purified as fusion protein with MBP (maltose binding protein) following the instructions recommended by the manufacturer (New England Biolabs). After binding of MBP-2A^{pro} expressing bacterial extracts to the amylose column, the fusion protein was eluted in buffer MBP (10 mM HEPES (pH 7.4), 50 mM KCl, 2 mM DTT, 5% glycerol and 10 mM maltose). The resulting fractions contained homogeneously pure MBP-2A^{pro} at 0.5 μg/ml. MBP protein alone was purified in parallel.

2.3. Cleavage experiment of eIF-4G

Cleavage reactions were carried out in buffer MBP (without maltose) in a final volume of 15 μl, containing 100 ng of purified eIF-4F and the indicated amounts of MBP-2A^{pro}. The reactions were incubated at 30°C for 75 min and stopped by addition of sample buffer (62 mM Tris-HCl (pH 6.8), 2% SDS, 0.1 M DTT, 17% glycerol and 0.024% bromophenol blue), boiled for 5 min and analyzed by SDS-PAGE and Western blotting. When indicated, reactions were supplemented with 5 mM $Mg(AcO)_2$. In some cases, purified initiation factors eIF-3, 4A or 4B obtained from Hela cells were added to the reactions and incubated for 5 min prior to the addition of MBP-2A^{pro}.

2.4. Electrophoresis and Western blotting

Usually, proteins were separated on a 7.5%-acrylamide gel (SDS-PAGE), transferred to nitrocellulose membrane and blotted against the indicated antisera as described previously [10]. A mixture of rabbit antisera generated against synthetic peptides from the amino-terminus and carboxy-terminus of eIF-4G was used.

2.5. Synthesis and analysis of peptides

Peptide 4G (GRTTLSTRGPPRGG) encompassing amino acids 478–491 of human eIF-4G was synthesized by a peptide synthesizer apparatus (Applied Biosystems, model 431A) using the Fmoc procedure. Cleavage products generated after incubation of peptide 4G with MBP-2A^{pro} were separated by reverse HPLC and the molecular weight verified by plasma desorption mass spectroscopy.

3. Results

3.1. Purification of human eIF-4F complex

To address whether or not poliovirus 2A^{pro} cleaves directly eIF-4G factor, eIF-4F complex was first purified from HeLa cells as described previously [22]. The purified fractions contained equimolar amounts of eIF-4G (220 kDa) and eIF-4E (24 kDa), but lacked eIF-4A polypeptide (50 kDa) (data not shown). This composition of eIF-4F was not surprising, because eIF-4A may copurify or not with eIF-4F depending on the ionic strength of the buffers and the type of column employed [22].

Wyckoff et al. [16] provided evidence that eIF-3 is required for poliovirus 2A^{pro}-mediated cleavage of eIF-4G. Therefore, we first tested if our preparation of eIF-4F contained eIF-3 factor. To this end, eIF-4F preparations were probed with an antiserum against human eIF-3. This factor, with an estimated molecular weight of 550 kDa, is composed of 10 subunits [23]. As shown in Fig. 1, even at the highest concentration of eIF-4F, no apparent bands of eIF-3 subunits are detected. Longer exposures of the immunoblot did not reveal any band relative to eIF-3 (result not shown). Antiserum raised against eIF-3 reacts weakly with eIF-4F (220 kDa) as

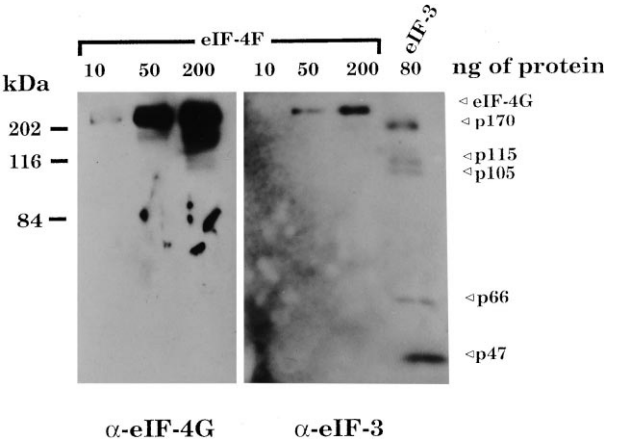

Fig. 1. Purified eIF-4F is free of eIF-3 factor. The indicated amounts of purified eIF-4F factor obtained from HeLa cells were analyzed by Western blotting using an antiserum against eIF-4F (left panel) or an antiserum against human eIF-3 (right panel). Lane denoted as eIF-3 contains 80 ng of purified human eIF-3. The bands corresponding to eIF-4G and five of the 10 subunits of eIF3 factor (p170, p115, p105, p66 and p47) are indicated.

described previously [6]. Therefore, our preparation of eIF-4F is essentially free of eIF-3.

3.2. Cleavage of eIF-4G by MBP-2A^{pro}

Lamphear et al. [19] reported that recombinant 2A^{pro} from rhino- and coxsackievirus directly cleaved purified eIF-4F from rabbit reticulocyte. Under these conditions, addition of purified eIF-3 did not enhance eIF-4G cleavage. Since 2A^{pro} proteinases from polio-, rhino- and coxsackieviruses share a substantial degree of sequence homology (more than 60%), it is unlikely that these proteinases follow different mechanisms to cleave the same cellular factor, i.e. eIF-4G. To address this possibility, recombinant poliovirus 2A^{pro} was purified from *E. coli* as a fusion protein with MBP (MBP-2A^{pro}) as described [24]. Preliminary experiments showed that this recombinant proteinase cleaves eIF-4G factor from HeLa S10 extracts. Moreover, we have observed that cleavage of eIF-4G by MBP-2A^{pro} is inhibited by low concentrations of sodium ions (results not shown). This result agrees with those obtained by [25], but contrasts with those reported for rhinovirus 2A^{pro}, where optimal proteinase activity is reached at 150–250 mM NaCl [26]. Therefore, potassium ions were included in all buffers instead of sodium ions.

Addition of 2 μg of MBP-2A^{pro} to eIF-4F preparation in the presence of 5 mM Mg $(AcO)_2$ results in an almost complete cleavage of eIF-4G (Fig. 2A, lane 4). Under these conditions, addition of purified eIF-3 or eIF-4A does not exert any effect on this cleavage (Fig. 2A, lanes 7 and 8). Parallel incubations of eIF-4F with 2 μg of control protein MBP does not affect the integrity of eIF-4G (Fig. 2A, lane 3), strongly suggesting that cleavage of this factor is a specific property of poliovirus 2A^{pro}. Interestingly, the cleavage products derived from eIF-4G upon treatment with MBP-2A^{pro}, especially the carboxy-terminus, are poorly detected by the anti-eIF-4G antibodies as compared with those originated after treatment of HeLa RSW with MBP-2A^{pro}. The reason of this observation is unclear, but it can be explained by the instability of the carboxy-terminal fragment of eIF-4G leading to its further cleavage. Interestingly, a similar observation has been re-

ported recently using both recombinant rhinovirus $2A^{pro}$ and human eIF-4G [21].

In the absence of magnesium ions eIF-4G is poorly cleaved by MBP-$2A^{pro}$ (Fig. 2A, lane 12). Under these conditions, only 10–20% of eIF-4F is bissected by poliovirus $2A^{pro}$, suggesting that magnesium ions play a key role to make eIF-4G accessible to MBP-$2A^{pro}$. The addition of eIF-3, eIF-4A or eIF-4B separately to the reaction does not exert any effect on eIF-4G proteolysis. However, simultaneous addition of the three initiation factors to the reaction considerably enhances eIF-4G cleavage (Fig. 2A, lane 16). Moreover, the eIF-4G fragments generated under these conditions are more easily detected with the anti-eIF-4G antibodies. Therefore, addition of this set of initiation factors to the cleavage reaction mimics, at least in part, the effect observed with magnesium ions. Interestingly, Lamphear et al. [9] have recently mapped the regions of the interaction of eIF-3 and eIF-4A with eIF-4G. The amino-terminal moiety of eIF-4G interacts with eIF-4E, while the carboxy-terminus binds eIF-3 and eIF-4A. Possibly magnesium ions as well as the interaction of these factors with eIF-4G could hold eIF-4G in a conformation accessible to the enzymatic attack of MBP-$2A^{pro}$. In addition, the interaction of eIF-3 and eIF-4A with the carboxy-terminal fragment of eIF-4G upon MBP-$2A^{pro}$ cleavage could protect this fragment from further degradation. This is consistent with the fact that the cleavage products of eIF-4G are stable in poliovirus-infected cells [27].

3.3. Cleavage of an eIF-4G-derived synthetic peptide by MBP-$2A^{pro}$

To get further insight into the direct cleavage mechanism of eIF-4G by poliovirus $2A^{pro}$, we tested the activity of MBP-$2A^{pro}$ on a synthetic peptide matching the cleavage site mapped on human eIF-4G for rhino- and coxsackievirus $2A^{pro}$ [19]. Thus, a 14-mer peptide encompassing the amino acid sequence from 478 to 491 of eIF-4G was synthesized [12]. This peptide was incubated with MBP-$2A^{pro}$ either in the presence or absence of magnesium ions and cleavage products were analyzed by HPLC. As shown in Fig. 3, the parent peptide dissappears and a novel product with a lower retention time on the HPLC column is obtained upon incubation with MBP-$2A^{pro}$. Data from plasma desorption mass spectrometry unambiguously probed that the peptide product corresponds to the sequence GRTTLSTR, which results from a single cleavage of the parent peptide at its first Arg-Gly bond. The second digestion product (GPPRGG) is not observed in the chromatogram, most probably because this short peptide eluates in the first fractions with DTT and ions. Addition of

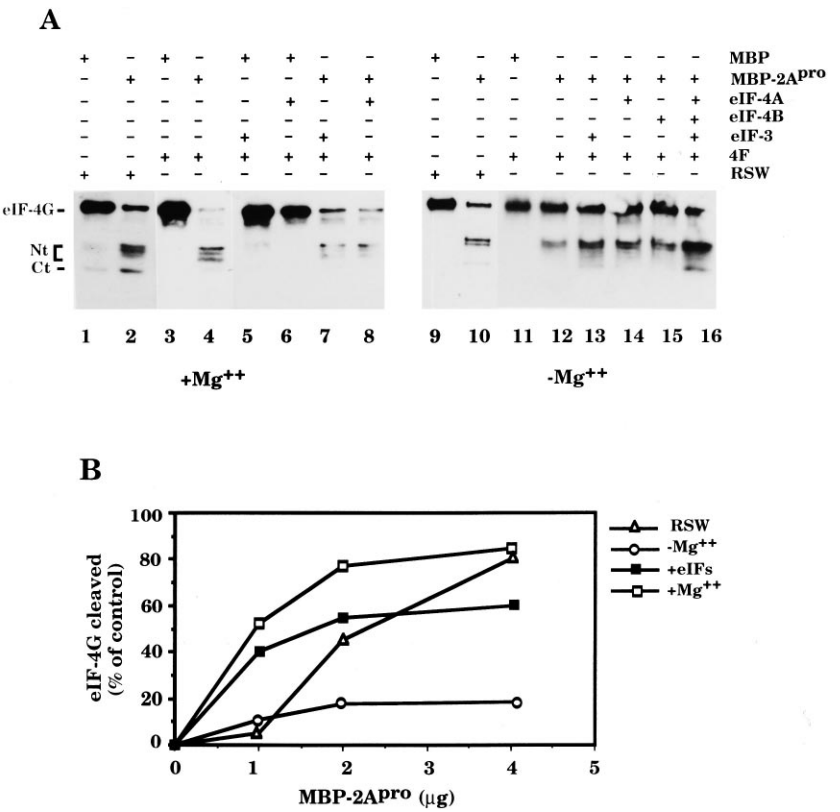

Fig. 2. Cleavage of eIF-4G factor by MBP-$2A^{pro}$. Influence of magnesium ions and several eukaryotic initiation factors. A: Reactions containing 100 ng of purified eIF-4F and 2 μg of MBP-$2A^{pro}$ were incubated in the presence (left panel) or absence (right panel) of 5 mM $Mg(AcO)_2$ for 75 min at 30°C. Where indicated, 400 ng of eIF-3, 250 ng of eIF-4B or 250 ng of eIF-4A were added to the reaction mixture 5 min prior to the addition of MBP-$2A^{pro}$. 30 μg of ribosomal salt wash (RSW) from HeLa cells were incubated with or without MBP-$2A^{pro}$ in lanes 1 and 2 as indicated. Samples were separated on a 7.5% acrylamide gel (SDS-PAGE), transferred to a nitrocellulose membrane and probed with an antiserum against eIF-4G. Intact eIF-4G as well as its amino-terminal (Nt) and carboxy-terminal (Ct) cleavage fragments are indicated. B: Effect of different concentrations of MBP-$2A^{pro}$ on eIF-4G cleavage. The reaction was carried out as described above, except that the concentration of MBP-$2A^{pro}$ was varied. Hydrolysis of eIF-4G was estimated by densitometric quantitation of bands corresponding to intact eIF-4G that remain after the incubations. The data are expressed as cleavage with respect to a control (incubation with MBP, 100% intactness of eIF-4G) (O). RSW was used as source of eIF-4G.

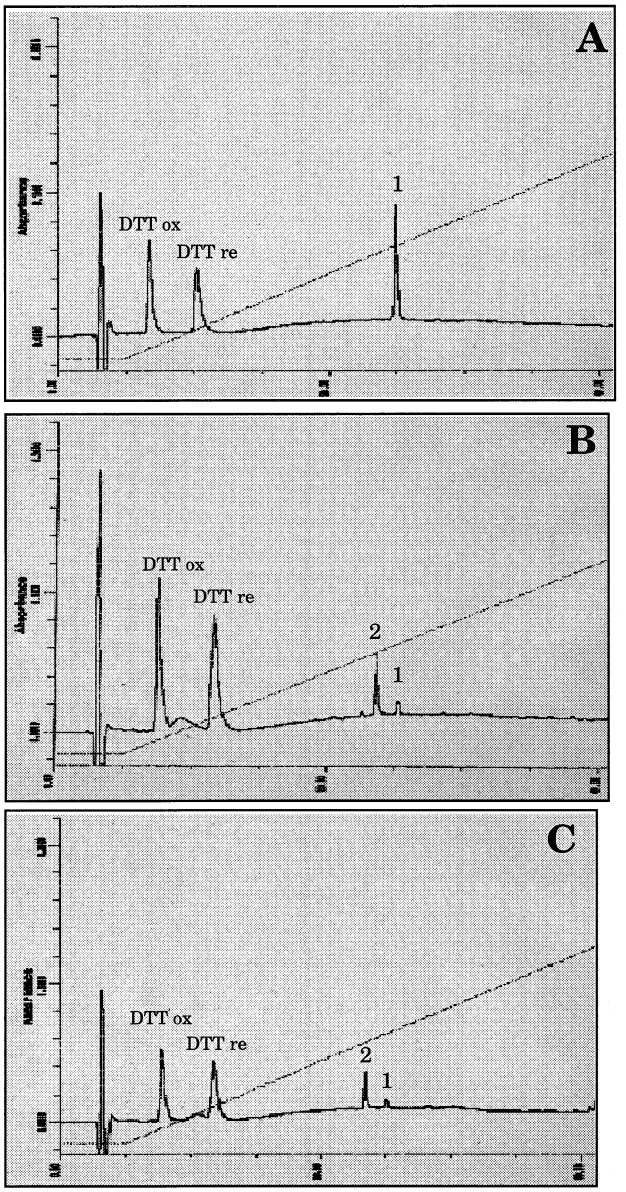

Fig. 3. MBP-2A^{pro} hydrolyzes an eIF-4G-derived synthetic peptide. Chromatogram of peptides analyzed by reverse-phase HPLC. 50 μM of peptide 4G (GRTTLSTRGPPRGG) was incubated alone (panel A) or with MBP-2A^{pro} (6 μM) either in the absence (panel B) or presence of 5 mM $Mg(AcO)_2$ (panel C) during 10 h at 30°C. Products generated after digestion were separated on a HPLC column and detected by absorption at 214 nm. Dashed line indicates the TFA gradient employed as the mobile phase. Peak denoted as 1 corresponds to parent peptide 4G whereas peak denoted as 2 corresponds to the cleavage product (GRTTLSTR) generated after digestion with MBP-2A^{pro}. The peaks corresponding to DTT (either oxidized or reduced) are also indicated.

magnesium ions did not affect the cleavage efficiency of this peptide.

4. Discussion

Taken together, the results presented here provide evidence for a direct cleavage of eIF-4G by poliovirus 2A^{pro}. In addition to previous findings on rhino- and coxsackievirus 2A proteinases, direct interaction and subsequent cleavage of eIF-4G by poliovirus 2A^{pro} with eIF-4G can also be achieved in vitro using the purified components. Furthermore, the use of an eIF-4G-derived synthetic peptide suggests that poliovirus 2A^{pro} cleaves eIF-4G at Arg^{485}-Gly^{486} bond. This is the cleavage site within eIF-4G mapped for rhino- and coxsackievirus cognate 2A^{pro} [19]. Therefore, 2A proteinases from entero- and rhinoviruses appear to share the same sequence specificity on eIF-4G.

Our data contrast in part with those reported by Wyckoff et al. [16] where cleavage of eIF-4G by poliovirus 2A^{pro} was observed only when additional factor(s) from HeLa cells were present. This apparent discrepancy could be explained by the use of different protocols to obtain recombinant poliovirus 2A^{pro} from *E. coli* and eIF-4F complexes from HeLa cells. We have tested a highly purified and active form of poliovirus 2A^{pro} (MBP-2A^{pro}) instead of the crude bacterial extracts expressing 2A^{pro} used by [16]. In addition, the eIF-4F complex employed in the present work as substrate for MBP-2A^{pro} contains the eIF-4E factor. Recently, it has been reported that this factor drasticaly increases the rate of cleavage of eIF-4G by rhino- and coxsackievirus 2A^{pro} and by aphthovirus L^{pro} [21,28]. We do not know if eIF-4G preparations employed by Wyckoff et al. [16] contained eIF-4E or not. However, the lack of eIF-4E in such preparations could explain the dependence of 2A^{pro}-mediated cleavage of eIF-4G for eIF-3, that is usually contaminated with detectable amounts of eIF-4G and eIF-4E when purified from HeLa cells using the traditional protocols [6].

On the other hand, our data show that cleavage of eIF-4G by poliovirus 2A^{pro} is greatly dependent upon the ionic conditions used. We believe that magnesium ions, more than to serve as cofactor for MBP-2A^{pro}, could serve to keep eIF-4G under a proper conformation accessible to protease attack. This is supported by the fact that when a synthetic peptide was used as substrate instead of eIF-4G, no effect of magnesium ions on cleavage was observed, most probably because the cleavage site for MBP-2A^{pro} is exposed in the short peptide. Perhaps magnesium ions stabilize the eIF-4G-4E interaction, the most probable substrate for picornaviral 2A^{pro} and L^{pro} [21].

Note added in proof: When this manuscript was in progress we learnt that Bovee et al. Virology 246, 241–249 (1998) have reported results that agree with our present findings.

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References

[1] Hershey, J.W. (1991) Annu. Rev. Biochem. 60, 717–755.
[2] Hershey, J.W.B., Mathews, M.B. and Sonenberg, N. (1996) Translational Control, pp. 1–794, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[3] Pain, V.M. (1996) Eur. J. Biochem. 236, 747–771.
[4] Edery, I., Humbelin, M., Darveau, A., Lee, K.A., Milburn, S., Hershey, J.W., Trachsel, H. and Sonenberg, N. (1983) J. Biol. Chem. 258, 11398–11403.

[5] Tahara, S.M., Morgan, M.A. and Shatkin, A.J. (1981) J. Biol. Chem. 256, 7691–7694.
[6] Brown-Luedi, M.L., Meyer, L.J., Milburn, S., Yau, C.P.M.-P., Corbett, S. and Hershey, J.W.B. (1982) Biochemistry 21, 4202–4206.
[7] Etchison, D., Molburn, S.C., Edery, I., Sonenberg, N. and Hershey, J.W.B. (1982) J. Biol. Chem. 257, 14806–14810.
[8] Hansen, J., Etchison, D., Hershey, J.W.B. and Ehrenfeld, E. (1982) J. Virol. 42, 200–207.
[9] Lamphear, B.J., Kirchweger, R., Skern, T. and Rhoads, R.E. (1995) J. Biol. Chem. 270, 21975–21983.
[10] Aldabe, R., Feduchi, E., Novoa, I. and Carrasco, L. (1995) Biochem. Biophys. Res. Commun. 215, 928–936.
[11] Lloyd, R.E., Grubman, M.J. and Ehrenfeld, E. (1988) J. Virol. 62, 4216–4223.
[12] Yan, R., Rychlik, W., Etchison, D. and Rhoads, R.E. (1992) J. Biol. Chem. 267, 23226–23231.
[13] Bernstein, H.D., Sonenberg, N. and Baltimore, D. (1985) Mol. Cell. Biol. 5, 2913–2923.
[14] Devaney, M.A., Vakharia, V.N., Lloyd, R.E., Ehrenfeld, E. and Grubman, M.J. (1988) J. Virol. 62, 4407–4409.
[15] Krausslich, H.G., Nicklin, M.J., Toyoda, H., Etchison, D. and Wimmer, E. (1987) J. Virol. 61, 2711–2718.
[16] Wyckhoff, E.E., Hershey, J.W.B. and Ehrenfeld, E. (1990) Proc. Natl. Acad. Sci. USA 87, 9529–9533.
[17] Wyckoff, E.E., Lloyd, R.E. and Ehrenfeld, E. (1992) J. Virol. 66, 2943–2951.
[18] Lloyd, R.E., Toyoda, H., Etchison, D., Wimmer, E. and Ehrenfeld, E. (1986) Virology 150, 299–303.
[19] Lamphear, B.J., Yan, R., Yang, F., Waters, D., Liebig, H.-D., Klump, H., Kuechler, E., Skern, T. and Rhoads, R.E. (1993) J. Biol. Chem. 268, 19200–19203.
[20] Liebig, H.-D., Ziegler, E., Yan, R., Hartmuth, K., Klump, H., Kowalski, H., Blaas, D., Sommergruber, W., Frasel, L., Lamphear, B., Rhoads, R., Kuechler, E. and Skern, T. (1993) Biochemistry 32, 7581–7588.
[21] Haghighat, A., Svitkin, Y., Novoa, I., Kuechler, E., Skern, T. and Sonenberg, N. (1996) J. Virol. 70, 8444–8450.
[22] Lamphear, B.J. and Panniers, R. (1990) J. Biol. Chem. 265, 5333–5336.
[23] Asano, K., Goss Kinzy, T., Merrick, W.C. and Hershey, J.W.B. (1996) J. Biol. Chem. 272, 1101–1109.
[24] Novoa, I., Martínez-Abarca, F., Fortes, P., Ortín, J. and Carrasco, L. (1997) Biochemistry 36, 7802–7809.
[25] Konig, H. and Rosenwirth, B. (1988) J. Virol. 62, 1243–1250.
[26] Sommergruber, W., Ahorn, H., Zöphel, A., Maurer-Fogy, I., Fessl, F., Schnorrenberg, G., Liebig, H.-D., Blaas, D., Kuechler, E. and Skern, T. (1992) J. Biol. Chem. 267, 22639–22644.
[27] Irurzun, A., Sanchez-Palomino, S., Novoa, I. and Carrasco, L. (1995) J. Virol. 69, 7453–7460.
[28] Ohlmann, T., Pain, V.M., Wood, W., Rau, M. and Morley, S.J. (1997) EMBO J. 16, 844–855.