Short Communication

Nuclear assortment of eIF4E coincides with shut-off of host protein synthesis upon poliovirus infection

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Eukaryotic initiation factor (eIF) 4E is a subunit of the cap-binding protein complex, eIF4F, which recognizes the cap structure of cellular mRNAs to facilitate translation initiation. eIF4E is assembled into the eIF4F complex via its interaction with eIF4G, an event that is under Akt/mTOR regulation. The eIF4E–eIF4G interaction is regulated by the eIF4E binding partners, eIF4E-binding proteins and eIF4E-transporter. Cleavage of eIF4G occurs upon poliovirus infection and is responsible for the shut-off of host-cell protein synthesis observed early in infection. Here, we document that relocalization of eIF4E to the nucleus occurs concomitantly with cleavage of eIF4G upon poliovirus infection. This event is not dependent upon virus replication, but is dependent on eIF4G cleavage. We postulate that eIF4E nuclear relocalization may contribute to the shut-off of host protein synthesis that is a hallmark of poliovirus infection by perturbing the circular status of actively translating mRNAs.

Binding of eukaryotic initiation factor (eIF) 4E to the 5′ mRNA cap structure (m7GpppN, where N is any nucleotide) is thought to be the rate-limiting step of cap-dependent translation (reviewed by Gingras et al., 1999). eIF4E is one of three subunits of the cap-binding protein complex eIF4F, which also includes eIF4A and eIF4G. eIF4A is an ATP-dependent RNA helicase required for unwinding of secondary structure in the 5′-untranslated region of mRNAs, in preparation for ribosome recruitment. eIF4G is a large scaffolding protein containing binding sites for eIF4E, eIF4A, and eIF3 – the latter probably mediating interactions between eIF4F and the small (40S) ribosomal subunit. A domain at the amino terminus of eIF4G also interacts with the poly(A)-binding protein (PABP) in mammals, plants and yeast. This interaction is thought to mediate mRNA circularization during translation (Wells et al., 1998).

The assembly of eIF4E into the eIF4F complex is regulated by a family of three proteins called eIF4E-binding proteins (4E-BPs), which compete with eIF4G for binding to eIF4E. Once bound to eIF4E, 4E-BPs prevent the formation of the eIF4F complex and inhibit translation (Haghighat et al., 1995). We have recently shown that 4E-BPs control the subcellular localization of eIF4E upon stress (Rong et al., 2008; Sukarieh et al., 2009). In the presence of 4E-BPs, a proportion of eIF4E is retained in the nucleus and its assembly into heat shock-induced stress granules (SGs) is reduced. Although 4E-BPs and eIF4G compete for the same binding site on eIF4E, the knockdown of 4E-BP1 and 4E-BP2 does not influence the localization of eIF4G to SGs, but does allow increased eIF4E localization to SGs (Sukarieh et al., 2009). These observations suggest that eIF4E binding partners play a general role in controlling the localization of eIF4E.

Two eIF4G isoforms, eIF4GI and eIF4GII (henceforth referred to collectively as eIF4G), have been identified in eukaryotic cells. eIF4GI and eIF4GII are 46% identical, have similar biochemical activities and are functionally interchangeable (Gradi et al., 1998a, b; Imataka et al., 1998). Infection of cells by poliovirus results in shut-off of host-cell protein synthesis, which is preceded by the cleavage of eIF4GI, then eIF4GII (Gradi et al., 1998b). Analysis of the kinetics of shut-off has shown that cleavage of both eIF4GI and eIF4GII appears to be required for the shut-off of host protein synthesis after poliovirus infection (Gradi et al., 1998b).

As eIF4G family members are direct binding partners of eIF4E, we sought to investigate whether eIF4G cleavage during poliovirus infection would also influence subcellular localization of eIF4E. To address this, we infected HeLa cells with poliovirus type 1 Mahoney and monitored the subcellular distribution of eIF4E by immunofluorescence as a function of time post-adsorption (Fig. 1a, b). Analysis of uninfected HeLa cells indicated that the majority of eIF4E was present in the cytoplasm (Fig. 1a, b). However, at approximately 1.5 h post-adsorption, there was a clear enrichment in the amount of eIF4E present in the nucleus and, by 2.5 h, a significant proportion of eIF4E had relocated to the nucleus (the mean cytoplasm/nuclear eIF4E ratio dropped by approx. 2.7-fold) (Fig. 1a, b).

To determine whether the redistribution of eIF4E to the nucleus coincided with host protein synthesis shut-off, we...
performed metabolic $[^{35}\text{S}]$methionine labelling at various time points post-infection (p.i.) (Fig. 1c). This experiment was performed concomitantly with those presented in Fig. 1(a). By 2.5 h p.i. of HeLa cells, the expected shut-off of host protein synthesis was observed (Fig. 1c) (Gradi et al., 1998b). Cleavage of eIF4GI was complete by 2 h p.i. and eIF4GII was completely cleaved by 2.5 h p.i. (data not shown), as reported previously (Gradi et al., 1998b).

We next asked whether other eIF4G-interacting proteins were retained in the nucleus upon poliovirus infection and whether the results that we observed with eIF4E were a non-specific consequence of altered nuclear permeability (Gustin & Sarnow, 2001). To test these possibilities, we monitored the behaviour of PABP – a protein that interacts with the N-terminal domains of eIF4GI and eIF4GII – upon poliovirus infection (Imataka et al., 1998). Our results indicate that PABP distribution was not altered up to 3 h following poliovirus infection (Fig. 2a, b). In order to rule out non-specific effects of poliovirus infection on nucleocytoplasmic trafficking, we compared the localization of eIF4E with that of the nuclear protein hnRNPA1, a protein previously documented to translocate to the cytoplasm upon poliovirus infection by 3 h p.i. (Gustin & Sarnow, 2001). We confirmed these findings and found that the majority of hnRNPA1 had indeed relocated to the cytoplasm 3 h post-adsorption, whereas PABP localization remained unchanged and eIF4E was redistributed equally between the nucleus and cytoplasm (Fig. 2c).

To address whether nuclear relocalization of eIF4E is dependent on poliovirus replication, we blocked replication by using the inhibitor guanidine–HCl [GuHCl]...
In the presence of GuHCl, only a partial shut-off of host protein synthesis is achieved in infected cells (Bonneau & Sonenberg, 1987). GuHCl blocks replication and therefore reduces the amount of newly translated viral protein in the cell, but does not prevent cleavage of eIF4GI or eIF4GII (data not shown), as reported previously (Bonneau & Sonenberg, 1987). Exposure of uninfected or poliovirus-infected cells to GuHCl had no effect on the subcellular distribution of eIF4E (Fig. 3a), indicating that the translocation of eIF4E was not dependent on virus replication.

Pyriothione, a zinc ionophore, is known to inhibit replication of picornaviruses by impairing viral polyprotein processing (Krenn et al., 2009). Hence, pyriothione can be used to block eIF4G cleavage by the 2A protease. In order to assess whether eIF4GI and eIF4GII cleavage during poliovirus infection is required for eIF4E nuclear relocation, we exposed mock- or poliovirus-infected HeLa cells to pyriothione. The translation of viral proteins (data not shown) and the cleavage of eIF4GI and eIF4GII were prevented by pyriothione (Fig. 3b). In mock-infected cells, pyriothione did not alter the subcellular distribution of eIF4E visibly (Fig. 3c). However, the nuclear retention of eIF4E in poliovirus-infected cells was clearly reduced by the presence of pyriothione (Fig. 3c). These results are consistent with cleavage of eIF4G being required for eIF4E nuclear translocation.

Cleavage of both eIF4GI and eIF4GII is associated with shut-off of host-cell protein synthesis upon poliovirus infection (Gradi et al., 1998b). Herein, we show that eIF4E translocation to the nucleus increased concomitantly with cleavage of eIF4G and onset of host protein synthesis shut-off (Fig. 1). Although PABP also binds to the N terminus of eIF4G near the eIF4E-binding site, poliovirus-induced cleavage of eIF4G does not induce

**Fig. 2.** Consequence of poliovirus infection on nucleocytoplasmic distribution of PABP and hnRNPA1. (a) Subcellular distribution of PABP in poliovirus-infected HeLa cells. HeLa S3 cells were infected with poliovirus, fixed and probed for PABP at the indicated times after adsorption. Bar, 20 μm. (b) Quantification of cytoplasmic and nuclear PABP. The y-axis represents the ratio of the mean intensity of the cytoplasm to the nucleus. n, Number of cells that were inspected in this experiment; the values ranged between 54 and 95. (c) Mock- or poliovirus-infected cells (3 h) were fixed and immunostained for eIF4E, PABP and hnRNP A1. Bars, 20 μm.
PABP translocation to the nucleus (Fig. 2a). Thus, eIF4E nuclear relocalization is not the consequence of nuclear-envelope breakdown. In this case, eIF4E translocation could depend on the N-terminally truncated eIF4G fragment. In addition, hnRNPA1 is actually transported out of the nucleus, suggesting that bidirectional transport is still active in poliovirus-infected cells. We suggest that depletion of eIF4E from the cytoplasm may play a role in poliovirus-mediated shut-off of host translation by stimulating linearization of mRNA templates. Cooperative binding of the eIF4F complex and PABP to the mRNA engenders a more stable eIF4E association with the cap structure than eIF4G/eIF4E binding alone, and is thought to maintain the mRNA in a circular state during translation (Kahvejian et al., 2005). mRNA circularization is thought to increase translation initiation, possibly by allowing for more efficient reinitiation of terminating ribosomes (Sachs, 2000). Cleavage of eIF4G between the eIF4E- and eIF4A-binding sites, as occurs during poliovirus infection (reviewed by Lloyd, 2006), is not predicted to perturb the circular state of the mRNA template, and such transcripts may continue to translate. Therefore, active reassignment of eIF4E from the cytoplasm to the nucleus upon virus infection as described herein would favour linearization of cellular transcripts and lead to a reduction in translation efficiency. Although we have not defined the mechanism by which eIF4E is relocalized to the nucleus, this could be mediated by the N-terminal fragment of eIF4G, which harbours a motif that is sufficient for nuclear localization (Coldwell et al., 2004). Other binding partners of eIF4E might be involved in the translocation and retention of eIF4E in the nucleus upon poliovirus infection. 4E-transporter (4E-T) was shown to be required for nuclear translocation of eIF4E under normal conditions (Dostie et al., 2000). 4E-BPs may also be involved, as these have been identified previously as modifiers of eIF4E localization (Sukarieh et al., 2009).

Our results suggest that eIF4E assortment to the nucleus, in conjunction with eIF4GI/II cleavage, during poliovirus infection could contribute to the host-cell translation shut-off to favour viral IRES-dependent translation.

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References


