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Cap binding-independent recruitment of eIF4E to cytoplasmic foci

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ABSTRACT

Eukaryotic translation initiation factor 4E (eIF4E) is required for cap-dependent initiation. In addition, eIF4E occurs in cytoplasmic foci such as processing bodies (PB) and stress granules (SG). We examined the role of key functional amino acid residues of eIF4E in the recruitment of this protein to cytoplasmic foci. We demonstrate that tryptophan residues required for mRNA cap recognition are not required for the recruitment of eIF4E to SG or PB. We show that a tryptophan residue required for protein–protein interactions is essential for the accumulation of eIF4E in granules. Moreover, we show, by the analysis of two *Drosophila* eIF4E isoforms, that the tryptophan residue is the common feature for eIF4E for the transfer of active mRNA from polysomes to other ribonucleoprotein particles in the cytoplasm. This residue resides in a putative interaction domain different than the eIF4E-BP domain. We conclude that protein–protein interactions rather than interactions with the mRNA are essential for the recruitment of eIF4E and for a putative nucleation function.

1. Introduction

In most of the cases the initiation of translation requires the recognition of the 5′UTR of the mRNA by the cap-binding protein eIF4E. The recognition of the 5′ cap structure (m7GpppN) by eIF4E represents the canonical function of this translation factor. Binding of eIF4E to the cap requires two conserved tryptophan residues (56 and 102 in the murine eIF4E). Simultaneously, eIF4E interacts with other proteins, such as eIF4G, eIF4E-BP, and eIF4E-T, involving a third tryptophan residue (73 in the murine eIF4E). eIF4E integrates, along to the multi-adaptor protein eIF4G, the eIF4F complex, which facilitates the correct positioning of the small ribosome unit into the mRNA, while eIF4E-BP and eIF4E-T are regulators [1,2]

In addition to the canonical function, a translation-independent role has been demonstrated for eIF4E, such as the regulation of the nuclear export of specific mRNAs [3]. eIF4E has also been shown to be present in cytoplasmic foci like stress granules (SG) and processing bodies (PB) [4–6]. Stalled 48S pre-initiation complexes are the core constituents of SG, cytoplasmic foci that appears in response to stress. PB are ribonucleoprotein (RNP) aggregates that contain a variety of proteins related to mRNA degradation, the component of the RNAi machinery, and several factors with a still unknown function. In addition, microRNAs (miRNA) has been also found in PB bound to specific mRNA associated to the Argonaute and GW182 protein [7]. The nonsense-mediated decay pathway is also related to PB [8].

Although PB and SG are distinct structures, they share many components and interact with one another in stressed cells. The targeting of an active mRNA to these foci implies the remodeling of the mRNP. Especially in PB, it involves the exchange of factors present in the translational active complex for factors related to degradation, storage or silencing forming new protein complexes. Only one translation factor remains during this process, eIF4E. Although the function of eIF4E is not known, it might be a link between the remodeling of mRNP from polysomes to cytoplasmic granules, to protect the transcript from degradation and/or store it to reentry into new translational rounds. Studies on yeast have showed that eIF4E bound to the cap inhibits Dcp1 decapping activity [9,10].

In addition to the canonical eIF4E-1 [11,12] *Drosophila melanogaster* has seven other eIF4E isoforms [13,14]. In the canonical isoform eIF4E-1, the residues W100 and W146 (equivalent to W56 and W102 in the mouse ortholog) are required for cap recognition, while W117 (equivalent to W73 in mouse) is required for protein–protein interactions [11]. Here, we examine the residues required for the recruitment of eIF4E-1 to cytoplasmic foci. We show that the cap-binding residues are dispensable for localization while residue W117 is essential for recruitment. This implies that protein interactions, rather than cap recognition, are the key for localization.

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2. Materials and methods

2.1. Drosophila eIF4E homology modeling

Homology model was built for the protein eIF4E-1 encoded in the transcript eIF4E-PA (CG4035) using information of ortholog proteins as template and a suite of structural bioinformatics tools. Templates 1WKW and 1EJH were selected from Protein Data Bank using Blast P of NCBI-NIH. They correspond to human IF4E/M7GPPPA/4EBP1 peptide and mouse EIF4E/EIF4G PEPTIDE/7METHYL-GDP crystal structures of their ternary complexes respectively. Due to the lack of protein structural information for the first 70 N-terminal residues, they were not taken in consideration for modeling. The truncated form spans 189 amino acids, from Asp71 to Leu259. eIF4E and template sequences were aligned to build a tridimensional coordinates file. The identities of template alignments were more than fifty percent. These steps were performed with the software MODELLER (http://salilab.org/modeller/). Finally, the model was obtained from steps of energy minimization adjustment using VMD and NAMD software (http://www.ks.uiuc.edu/Research/vmd/) [15]. The model evaluation was performed by Ramachandran plot and DOPE score [16,17].

2.2. Plasmid construction

The open reading frame (ORF) of eIF4E-1, eIF4E-3 and deIF4E-HP was PCR-amplified using sequence specific primers creating the sites EcoRI before codon 1 and XhoI replacing the stop codon [11]. The PCR fragment was filled with Klenow (Promega Co, USA) and cloned onto both, the EcoRV site of vector pMT/V5-His C (Invitrogen) to produce a C-terminus fusion with the V5 epitope and the filled-in NcoI–EcoRI sites of vector pCS2 + MT vector to generate a N-terminus fusion with six Myc epitopes. V5 and Myc epitopes were used for immunodetection of the fusion proteins. C-terminus CFP fusions were created by cloning the same PCR products in the vector pECFP-C1 (Clontech, Mi, USA). All constructions were corroborated by sequencing (Macrogen Inc.).

2.3. Site directed mutagenesis

Site-directed mutagenesis of eIF4E-1 was carried out on the plasmids pMT-eIF4E1-V5/His to change tryptophan 100 and 146 to alanine to generate the plasmids pMT-eIF4E1-V5/His^{W100A}, pMT-eIF4E1-V5/His^{W146A}, and pMT-eIF4E1-V5/His$^{W100A/W146A}$. pCS-eIF4E-1 and pECFP-eIF4E3 were used to change tryptophan 117 or Phenylalanine103 to alanine and to generate pCS-eIF4E-1^{W117A} and pECFP-eIF4E3^{F103A}. PCR amplification of each template was performed using Quick Change Site Directed Mutagenesis Kit (Stratagene, USA) with following primers:

eIF4E-1^{W100A}—fwd: 5′-CGGTCCAAGAGCGCTGAGGACATGCAAAACGAG-3′
eIF4E-1^{W100A}—rev: 5′-CTGTTTGTGGGTTGCATCTTCGGCCAGGGGACG-3′
eIF4E-1^{W117A}—fwd: 5′-GATACCGTCGAGGACTTCGCGAGCCTATACAACCACATC-3′
eIF4E-1^{W117A}—rev: 5′-GATGTGGTTGTATAGGCTCGCGAAGTCCTCGACGGTATC-3′
eIF4E-1^{W146A}—fwd: 5′ GCTATTCAAGAACATTCGTCCCATGGCCGAGGATGCAGCCAACAA ACAGGGC-3′
eIF4E-1^{W146A}—rev: 5′-GCCCTGTTTGTTGGCTGCATCCTCGGCCATGGGACGAATTTCTTCTTGAATAGC-3′
eIF4E-3^{F103A}—fwd: 5′-ACTCCTGAAGCGGTCACACATGATGAAACAC-3′
eIF4E-3^{F103A}—rev: 5′-GTGTTTCATCATGTGTGACCGCTTCAGGAGT-3′.

Restriction sites for HaeII and EaeI, were created on eIF4E-1^{W100A} and eIF4E-1^{W146A} primers to identify the mutants. Plasmid extraction was performed using plasmid Prep Mini Spin Kit (GE Healthcare) and the mutation confirmed by sequencing (Macrogen Inc. Seoul, Korea).

2.4. Cell culture and transfections

S2 cells were grown in Schneider's medium (Sigma, USA) supplemented with 10% fetal bovine serum (Natocor, Córdoba, Argentina) and 1%antibiothic–antimycotic mixture (Invitrogen, USA) at 25 °C. HeLa cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, USA) supplemented with 10% fetal bovine serum (Natocor, Córdoba, Argentina) and 1% antibiotic–antimycotic (Invitrogen, USA) at 37 °C and 5% CO_2.

For transfections, 2×10^5 S2 cells or 1.5×10^5 HeLa cells were seeded onto 12 mm glass coverslips and allowed to grow during 4–12 h in 24× multiwells. 250 ng of purified plasmid was transiently transfected using Lipofectamine 2000 (Invitrogen, USA) in medium without serum, incubated during one hour and removed. Complete medium was added and further incubated overnight. Expression of pMT/V5/His was induced by addition of 500 μM of $CuSO_4$ to the medium. Cells were incubated during 48 h before fixation for immunostaining assays. Transfections using the vector pCS + MT-eIF4E1 were not induced as the CMV promoter is constitutively expressed in HeLa and S2 cells.

For experiments to study SG, transfected cells were stressed with 1 mM sodium arsenite for 30 min before cell fixation. Similarly, cells were treated with 100 μg/ml cycloheximide during 10 min to stabilize mRNA on polysomes either in untreated and after arsenite treatment. The culture medium was exchanged and cells were fixed 30 min after treatment.

Before the cellular analysis, the integrity of the recombinant products and the ability to recognize the cap were determined by Western Blot using anti-V5 antibody (Invitrogen, USA) and by purification in a cap-sepharose column and further anti-V5 Western Blot detection, respectively (data not shown).

2.5. Immunodetection and fluorescence microscopy

For immunostaining, the cells were fixed with 4% paraformaldehyde in PBS for 10 min, permeabilized with 0.5% (v/v) Triton X-100 in PBS for 5 min and blocked with 3% (w/v) BSA in PBS/0.1% (v/v) Tween 20 during 30 min. The cells were further incubated with antibodies against *Drosophila* eIF4E [12], GW182 (AbCam, Cambridge, UK; 1:500), V5 epitope (Invitrogen, USA; 1:500), and anti-TIA-1 (AbCam, Cambridge, UK; 1:500) for 1 h. Cells were washed with PBS and incubated with secondary anti-mouse, anti-goat and anti-rabbit antibodies conjugated to Cyanine dyes (Jackson Inc. Michigan, USA; 1:2000). Before imaging the cells were counterstained with DAPI and analyzed by epifluorescence to assess cell integrity. Images were acquired with a Carl Zeiss LSM 510-Meta confocal microscope using Argon (588/514 nm) and Helium/Neon (543/633 nm) lasers. The images were analyzed using the LSM software and Image J (http://rsbweb.nih.gov/ij/).

3. Results and discussion

3.1. Recruitment of eIF4E-1 to cytoplasmic granules is independent of the cap-binding activity

Structural studies of mammalian and yeast eIF4E demonstrated that eIF4E in complex with cap-analogs resembles “cupped-hands” in which the cap structure is stacked between two highly conserved tryptophan residues (W56 and W102 of human eIF4E) through π bound interactions. A third conserved tryptophan residue (W166 of human eIF4E) binds the N^7-methyl moiety of the cap-structure [18–21]. Residues W100 and W146 of *Drosophila* eIF4E-1 are the equivalent to W56 and W102 of human eIF4E. These residues are conserved in all *Drosophila* eIF4E cognates [13,14]. To investigate whether those

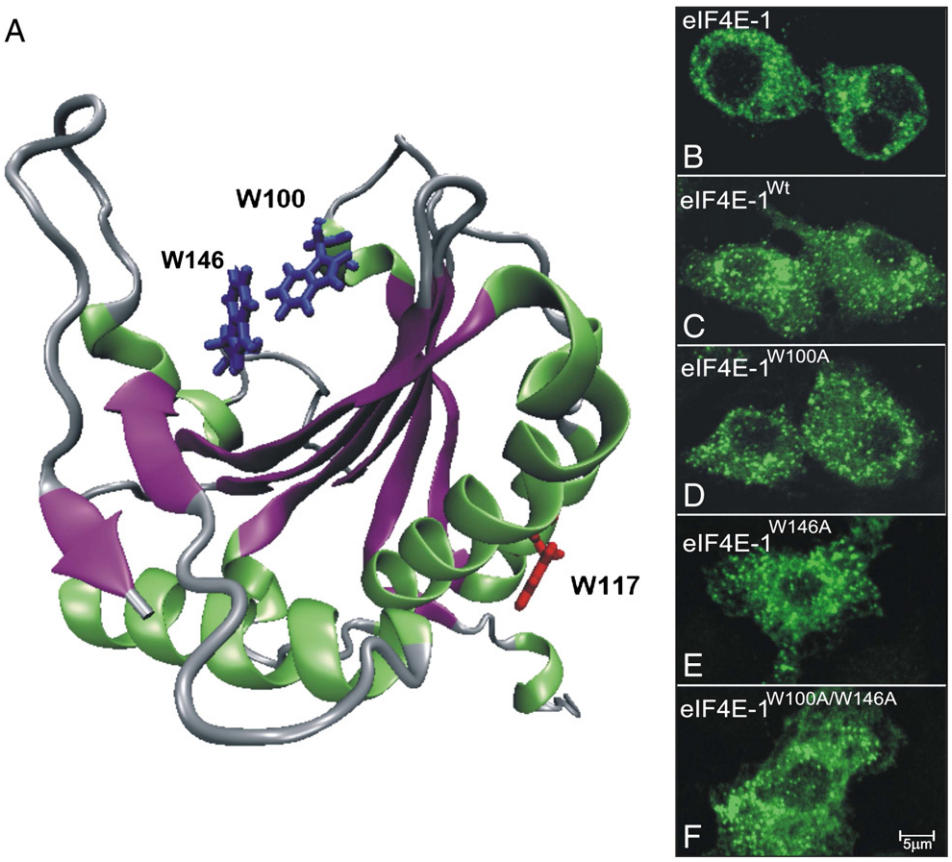

Fig. 1. Tryptophan residues involved in cap recognition are dispensable to localize eIF4E-1 in cytoplasmic foci. A. Molecular modeling of *Drosophila melanogaster* eIF4E-1. The model has been built from D71 to L259 spanning the conserved sequence among species (see Materials and methods). Purple backbone represents β-sheets, green backbone, the predicted α-helix, and white backbone, nonstructured turns. W100 and W146, the key cap recognition residues are shown in blue. W117 a conserved residue required for protein–protein interaction, is shown in red. B. Localization of endogen eIF4E-1 is visualized using anti-eIF4E-1 antibody [12]. eIF4E-1 distribution is enriched in cytoplasmic foci. C. Localization of recombinant eIF4E-1 in transfectedS2 cells as revealed with anti-V5 antibody. D–F. eIF4E-1 mutants on the cap binding sites (eIF4E^{W100A}, eIF4E^{W146A}, and the double mutant eIF4E$^{W100A/W146A}$) are also localized in cytoplasmic foci like the wild type protein and endogenous eIF4E-1. The cell integrity was assessed by DAPI counterstaining prior to confocal imaging. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

residues are involved in the localization of eIF4E-1 in cytoplasmic foci, we generated a molecular model based on the known eIF4E structures and the eIF4E-1 sequence. All residues analyzed (W100, W117 and W146) are predicted in the same spatial location as the mammalian counterpart (Fig. 1A). Based on this model we generated single and double mutants on the cap-binding residues and transfected them into *Drosophila* S2 cells. We observed that the endogenous eIF4E-1 protein localizes in cytoplasmic foci (Fig. 1B), as the expressed wild type eIF4E-1 (Fig. 1C). The mutants eIF4E^{W100A} (Fig. 1D) and eIF4E^{W146A} (Fig. 1E), and the double mutant eIF4E$^{W100A/W146A}$ (Fig. 1F), also localize in cytoplasmic granules, in an indistinguishable manner compared to the endogenous (Fig. 1B) and the transfected wild type protein (Fig. 1C). Similar results were obtained when HeLa cells were transfected with the Dm-eIF4E-1 mutants (Ref. [5] and data not shown). To assess the nature of the granules, the cells were transfected with the different mutant forms of eIF4E-1 and analyzed by immunocytochemistry using specific markers for the foci. A control using the empty vector is shown in Suppl. Fig. Fig. 1. The protein GW182, a component of the miRNA pathway was used as a marker for PB [22,23]. eIF4E-1 co-localize with GW182 in PB as well as eIF4E-1^{W100A}, eIF4E-1^{W146A} and eIF4E-1$^{W100A/146A}$ (Fig. 2). We also observed that eIF4E-1 mutants also aggregate in cytoplasmic granules that lack GW182. This could imply that cytoplasmic granules are a polymorphic family in which different functions and/or maturation stages might be simultaneously represented in the cells, as it has been proposed [24]. Recent evidence suggests that PB formation could precede SG formation, and PB could mature either into SG [24]. Thus, we next studied whether cap-binding residues also affect the accumulation of eIF4E in SG. We analyzed the localization of eIF4E-1 and mutants in transfected cells stressed with sodium arsenite and determined the co-localization of the eIF4E variants with TIA-1, a marker of SG [25]. After arsenite treatment, eIF4E-1, eIF4E-1^{W100A}, eIF4E-1^{W146A}, and eIF4E-1$^{W100A/W146A}$ exhibit a precise co-localization with TIA-1 (Fig. 3; a control for co-localization of PB and SG in non-stressed and arsenite-treated cells is shown in Suppl. Figs. Fig. 2 and Fig. 3). This indicates that eIF4E aggregation either in SG and PB is independent of the residues required for cap-binding. This led us to evaluate whether the mobilization of eIF4E-1 to cytoplasmic granules depends on the ribosome-associated or to the translation-independent fraction of eIF4E-1. We treated S2 cells with cycloheximide, which prevents mRNA translation by blocking the elongation step. Cycloheximide treatment prevented the accumulation of eIF4E-1, eIF4E-1^{W100A}, eIF4E-1^{W146A}, and eIF4E-1$^{W100A/W146A}$ in PB (Fig. 4). This suggests that at least a fraction of eIF4E-1 is directed to PB from the polysomes but they are not necessarily associated to the mRNA via cap-binding. Similar results were obtained when the cells were treated with cycloheximide before arsenite stress, indicating that the mobilization of the eIF4E-1 and the mutants to SG occurs from polysomes (Suppl. Fig. Fig. 4). These results support the notion

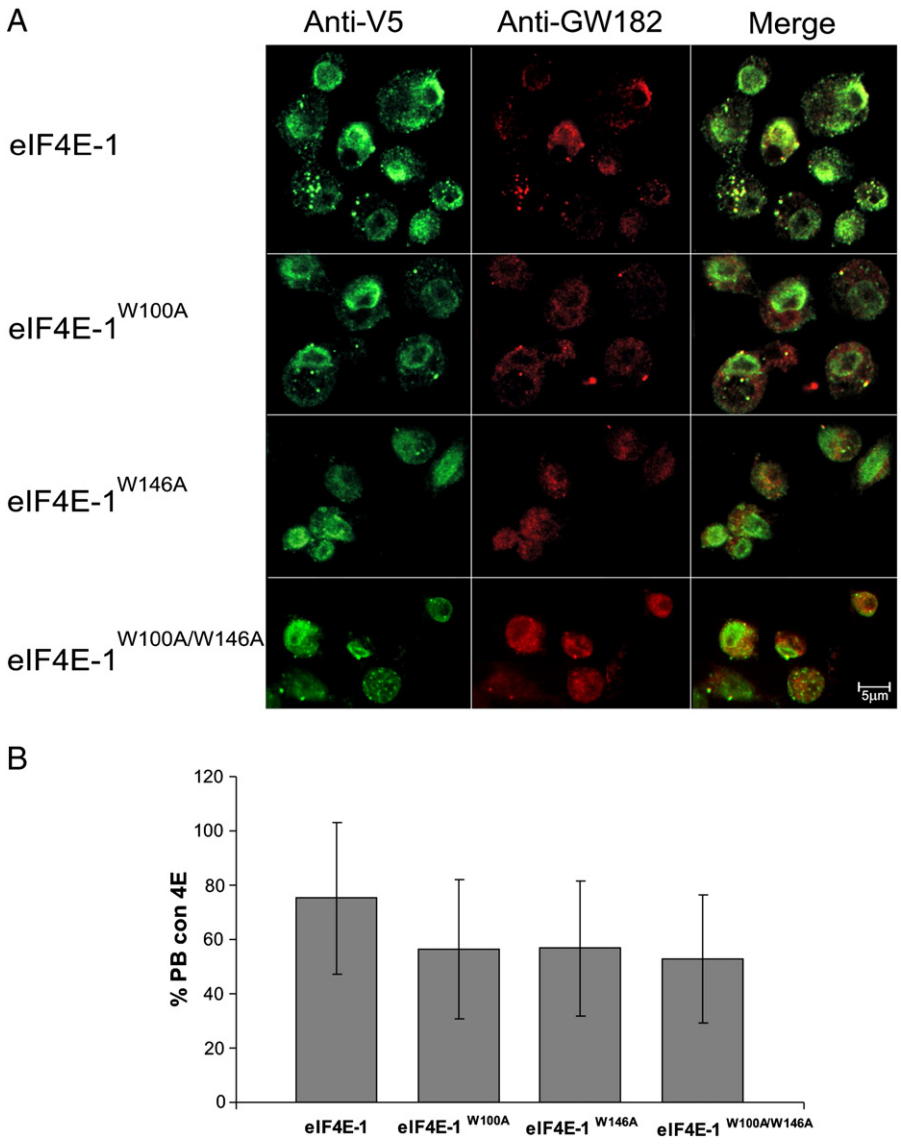

Fig. 2. Localization of eIF4E-1 and eIF4E-1 mutants in PB. A. S2 cell immunocytochemistry reveals the localization of eIF4E-1^{WT}, eIF4E-1^{W100A}, eIF4E-1^{W146A}, and eIF4E$^{W100A/W146A}$ in PB (anti-V5, green, left panel). PB are evidenced by the presence of the specific marker GW182 [22,23] using anti-GW182 (red, middle panel). The right panel shows the co-localization of both proteins in PB. Aggregation of eIF4E-1 and GW182 exhibits partial co-localization. The cell integrity was assessed by DAPI counterstaining prior to confocal imaging. B. Statistical analysis of the co-localization (ANOVA, $p<0.005$) shows that despite not all foci contains both proteins, there is no significant difference in the co-localization of the wt and mutant forms of eIF4E. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

that the remodeling from active to inactive ribonucleoprotein particles (mRNPs) requires eIF4E-1, but it may not necessarily involve the recognition of the mRNA cap.

3.2. eIF4E-1 recruitment to PBs requires the W117 residue

If the mobilization of eIF4E-1 from polysomes to PB and SG is independent of the cap-binding residues, we then asked whether W117, which is the equivalent to W73 of human eIF4E (Fig. 1A) is required for the localization. W73 and W117 are required for the interaction of eIF4E with eIF4G during translation initiation (eIF4G is absent in PB, [5]) or for eIF4E-T binding (present in PB, [6]). Consistent with this idea, previous studies demonstrated that eIF4E is transported to the nucleus via its association with eIF4E-T and it is retained by interaction with 4E-BPs [26]. These partners might be involved in the regulation of eIF4E localization. eIF4E-1^{W117A} does not evidence the localization of eIF4E-1 in foci and it is dispersed throughout the cytoplasm of S2 cells (Fig. 5). Equally, eIF4E-1^{W117A} is not recruited to SG in arsenite-treated cells (Suppl. Fig. Fig. 4). A similar pattern was observed in HeLa cells, transfected with the homologous mutant of the human eIF4E, eIF4E^{W73A} and eIF4E-1^{W117A} (data not shown). To further analyze the function of W117 we analyzed the localization of *Drosophila* eIF4E-3 in granules. eIF4E-3 binds to the cap structure and also to eIF4G and 4E-BP, and shares 59% identity in the carboxy-terminal moiety with eIF4E-1 [14]. eIF4E-3 contains a phenylalanine residue in position 103, which is the equivalent to W117 and W73 in *Drosophila* eIF4E-1 and human eIF4E, respectively (Fig. 6A). As it has been shown for eIF4E-1, eIF4E-3 localized in granules (Fig. 6B, left panel). This indicates that the natural substitution of the W117 residue to F103 does not affect the localization. Thus we mutated the F103 residue and analyze the localization of the resulting protein. The mutant eIF4E-3^{F103A}, like eIF4E-1^{W117A}, does not localize in foci (Fig. 6B, middle panel). These results show that F103 is functionally equivalent to W117 for the localization of the proteins in cytoplasmic

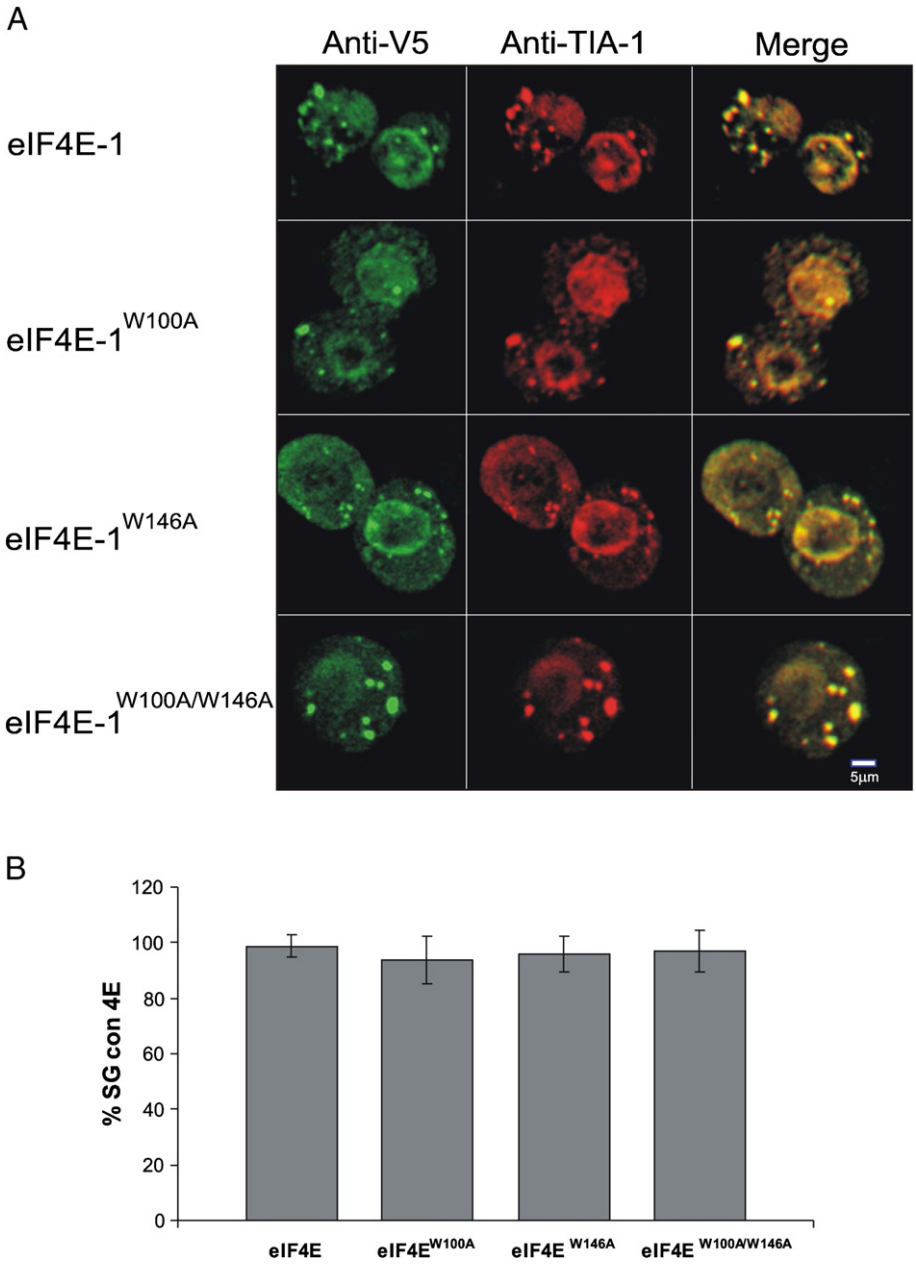

Fig. 3. Localization of eIF4E-1 and eIF4E-1 mutants in SG. A. Arsenite-stressed S2 cells reveal the localization of eIF4E-1^{WT}eIF4E-1^{W100A}, eIF4E-1^{W146A}, and eIF4E$^{W100A/W146A}$ in SG (anti-V5, green, left panel). SG are evidenced by the presence of the specific marker TIA-1 [14] using anti-TIA-1 (red, middle panel). The right panel shows the co-localization of both proteins. Aggregation of eIF4E-1 and TIA-1 in the arsenite-stressed cells shows complete co-localization. The cell integrity was assessed by DAPI counterstaining prior to confocal imaging. B. Statistical analysis of the co-localization (ANOVA, $p<0.005$) shows that there is no significant difference in the co-localization level of the wt and mutant forms of eIF4E. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

foci. Finally we analyzed the localization of *Drosophila* 4E-HP [27], a protein that diverges from all *Drosophila* eIF4E in most of the residues required for cap/eIF4G/4E-BP binding, but conserves the residue W85, the equivalent to W117 in eIF4E-1 ([14], Fig. 6A). Fig. 6B (right panel) shows that 4E-HP is located into cytoplasmic foci. This agrees with the results shown above, as 4E-HP weakly binds to the cap because it lacks basic residues involved in the recognition [13]. This implies that the residues required for the interaction with the cap structure of mRNA are not involved in protein localization, but the residue W117 of eIF4E-1 (and the equivalent residues in the other isoforms) is. Interestingly, 4E-HP has been shown to act as a repressor of translation by interacting with the protein Bicoid in a different domain that overlaps the eIF4G and eIF4E-BP binding domain [27]. Our results suggest that the localization of eIF4E into cytoplasmic foci requires the conserved residue W117 (or their equivalent residues in other eIF4E) rather than the residues required for cap binding.

An important consequence of our results is a re-evaluation of the role of eIF4E. Cap binding is absolutely required to initiate translation but, more importantly, the tethering of eIF4E to the mRNA is required to bring other translations factors to the 5′ end of the mRNA [28]. The recruitment of other translation factors is mediated by a domain that, modified in 4E-HP, is not required for the localization in cytoplasmic foci. Our data support the notion of a dual role for eIF4E, namely a function on translation that requires the cap-binding activity and the recruitment to PB or SG that is independent of the cap binding activity. We propose that the removal of the translation machinery and the assembly of PB-specific factors such as Rck/p54, eIF4E-T, and others would take over the translation-related eIF4E interactors. We support the notion that

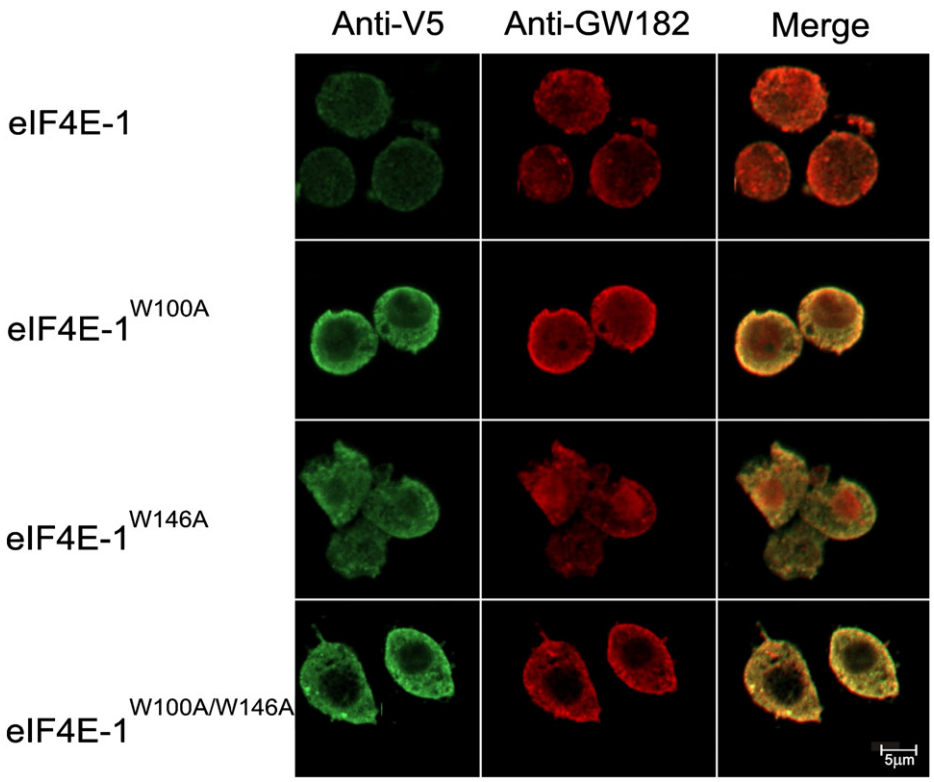

Fig. 4. Distribution of eIF4E-1 variants after cycloheximide treatment. S2 cells were transfected with the different plasmids and treated with cycloheximide (see Materials and methods). eIF4E-1^{WT}, eIF4E-1^{W100A}, eIF4E-1^{W146A}, and eIF4E$^{W100A/W146A}$ were revealed with anti-V5 (green, left panel) and PB with anti-GW182 (red, middle panel) and co-localization was studied (left panel). The number and size of PB with eIF4E are dramatically reduced. The cell integrity was assessed by DAPI counterstaining prior to confocal imaging. Statistical analysis was not performed due to the negligible number of cytoplasmic foci. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

eIF4E participates in the exchange of factors via protein–protein interactions that require W117. This agrees with the observation that human eIF4E simultaneously interacts with the translation repressor eIF4E-T and the helicase rck/p54 in PB of HeLa cells [5]. This does not rule out a role for eIF4E in preventing decapping as it has been postulated [29]. Our previous work suggested that there is a hierarchy of factors required for sequential assembly of PB formation [5] in which the lack of eIF4E, Rck/p54 or eIF4E-T prevented the recruitment of Dcp-1, Xrn-1, Ccr-4 and other processing factors. Recent evidence showed that Hsp90 also plays a role in PB formation and that is required for the presence of eIF4E and eIF4E-T [30,31]. Taken together, the current evidence supports the notion that during mRNP remodeling, eIF4E is maintained in the

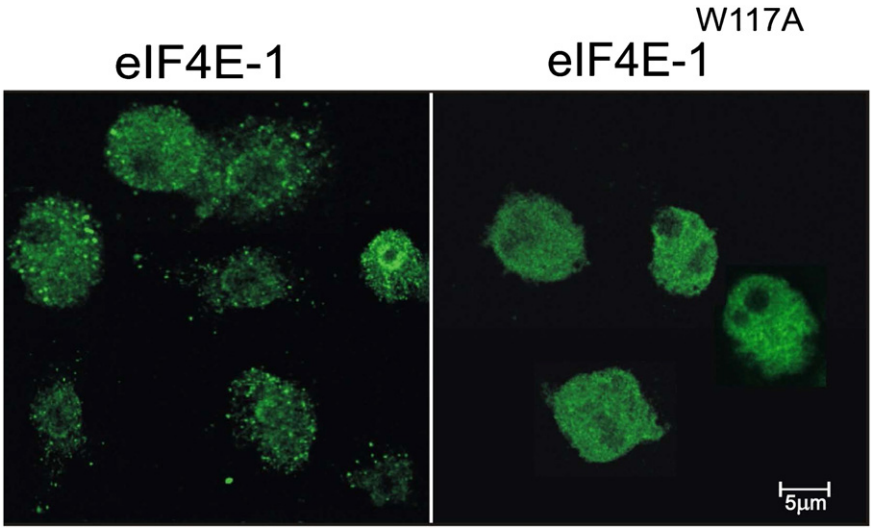

Fig. 5. W117 is essential for the localization of eIF4E-1 in cytoplasmic foci. S2 cells were transfected with a plasmid expressing eIF4E-1^{WT} (left panel) and the mutant eIF4E-1^{W117A} (right panel). The protein localization was revealed by immunostaining using anti-Myc antibody. eIF4E-1^{W117A} is homogeneously distributed and does not accumulate in foci, compared to eIF4E. Similar results were observed for the formation of SG in arsenite-stressed cells (see Suppl. Fig. Fig. 4). The cell integrity was assessed by DAPI counterstaining prior to confocal imaging.

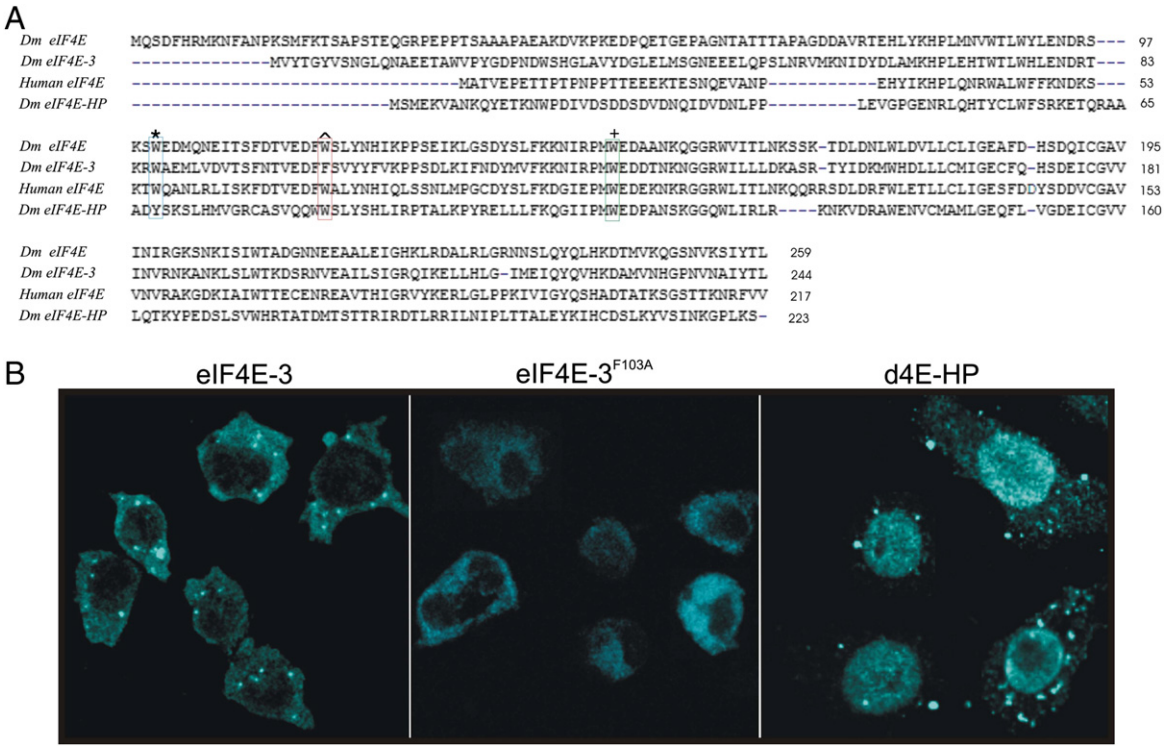

Fig. 6. W117, but not the 4E-BP interaction domain, is required for localization of eIF4E isoforms. A. Alignment of amino acid sequences of *Drosophila* eIF4E's isoforms (1, 3, HP) and *Human* eIF4E. Residues involved in the recognition of the cap are pointed as follows: the asterisk (*) indicates the position of residue W100 and the sign + marks the residue W146 of eIF4E-1. The analyzed W residue that interacts with eIF4G and eIF4E-BP (heIF4E W73, eIF4E-1 W117, eIF4E-3 F103, d4E-HP W85) is labeled with the sign ^. Note the dissimilarity of d4E-HP with respect to the other proteins. B. S2 cells were transfected with the isoform eIF4E-3 [14] and revealed as CFP fusion in cytoplasmic foci (left panel) similar to eIF4E-1. Mutation of F103 to A (eIF4E-3^{F103A}, middle panel) disrupts the localized distribution. The isoform eIF4E-HP (right panel) that lacks the ability to interact with eIF4G and eIF4E-BP, but conserves the residue W85, equivalent to W117 [14], shows the same pattern of distribution than eIF4E-1 and eIF4E-3. The cell integrity was assessed by DAPI counterstaining prior to confocal imaging.

mRNP complex independently of the binding to the cap, and that protein–protein interactions agglutinate the components that forms the cytoplasmic granules.

4. Conclusions

eIF4E is a widely studied translation factor. The occurrence of eIF4E in cytoplasmic foci as well as in the nucleus suggests new roles for it. The cytoplasmic foci (PB, SG and other uncharacterized ones) are structures with diverse components that share eIF4E. Our work suggests that eIF4E interacts with so far unidentified proteins in PB and SG during mRNP remodeling in a process that is independent of the eIF4E-cap interaction. Our current working hypothesis is that eIF4E might promote protein aggregates in which it plays a role as nucleation target. The analysis of new eIF4E interactors will serve to determine the dynamics of the foci formation.

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