An Isoform of Eukaryotic Initiation Factor 4E from Chrysanthemum morifolium Interacts with Chrysanthemum Virus B Coat Protein

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Abstract

Background: Eukaryotic translation initiation factor 4E (eIF4E) plays an important role in plant virus infection as well as the regulation of gene translation.

Methodology/Principal Findings: Here, we describe the isolation of a cDNA encoding CmeIF(iso)4E (GenBank accession no. JQ904592), an isoform of eIF4E from chrysanthemum, using RACE PCR. We used the CmeIF(iso)4E cDNA for expression profiling and to analyze the interaction between CmeIF(iso)4E and the Chrysanthemum virus B coat protein (CVBCP). Multiple sequence alignment and phylogenetic tree analysis showed that the sequence similarity of CmeIF(iso)4E with other reported plant eIF(iso)4E sequences varied between 69.12% and 89.18%, indicating that CmeIF(iso)4E belongs to the eIF(iso)4E subfamily of the eIF4E family. CmeIF(iso)4E was present in all chrysanthemum organs, but was particularly abundant in the roots and flowers. Confocal microscopy showed that a transiently transfected CmeIF(iso)4E-GFP fusion protein distributed throughout the whole cell in onion epidermis cells. A yeast two hybrid assay showed CVBCP interacted with CmeIF(iso)4E but not with CmeIF4E. BifC assay further demonstrated the interaction between CmeIF(iso)4E and CVBCP. Luminescence assay showed that CVBCP increased the RLU of Luc-CVB, suggesting CVBCP might participate in the translation of viral proteins.

Conclusions/Significance: These results inferred that CmeIF(iso)4E as the cap-binding subunit eIF(iso)4F may be involved in Chrysanthemum Virus B infection in chrysanthemum through its interaction with CVBCP in spatial.

Introduction

Eukaryotic translation initiation factor 4E (eIF4E) is a protein that plays a major role in the regulation of gene expression at the post-transcriptional level [1]. Biochemically, eIF4E functions at the levels of cap-dependent translation and nuclear mRNA export, both of which require its m7G cap-binding activity. eIF4E is therefore known as a cap-binding protein [2]. In translation, the initiation step is by far the most complex phase and at least 12 eukaryotic initiation factors are involved [3]. In the first step, eIF4E binds to the mRNA 5’ m7G cap structure and brings the mRNA into a complex with other protein synthesis initiation factors and ribosomes, resulting in mRNA circularization and translation initiation [4], [5]. eIF4F is a component of the heterotrimeric complex, eIF4F, which also includes the RNA helicase, eIF4A, and the large scaffolding protein, eIF4G [6]. It is becoming clear that most eukaryotic organisms encode and express multiple eIF4E family members, some for general translation and others for specific functions, including the control of translation [7]. The eIF4E family can vary significantly between different taxonomic groups; it is divided into two categories, eIF4E and its isoform eIF(iso)4E, in plants. Accordingly, isoforms of each component, eIF(iso)4F and eIF(iso)4G, also exist in plants [8], [9]. The eIF4E and eIF(iso)4E proteins are mechanistically equivalent during the process of translation, but exhibit differences in their expression in different tissues and their ability to bind to m7G and other cap analogues [10], [11]. Whereas eIF4E is present in all tissues except the root specialization zones, eIF(iso)4E is particularly abundant in floral tissues and young tissues [11]. Differences in their transcription patterns and binding affinities suggest that these isoforms might have complementary biological roles [12].

In addition to this traditional function, many studies have demonstrated that eIF4E and its isoform, eIF(iso)4E, often participate in the virus infection of plants [13]. Viruses target translation initiation factors to take over the protein synthesis...
germ. In the genera AMV coat protein interacts with eIF4F and eIF(iso)4F from wheat poly(A)-binding protein (PABP). GST pull-down revealed that genomic RNA is enhanced by binding of several coat protein with eIF4E, eIF(iso)4F, or subcellular localization of viruses. Furthermore, a mutation of Arabidopsis thaliana inhibits cucumber mosaic virus (CMV) multiplication by decreasing the accumulation of CMV 3a proteins, which is necessary for cell-to-cell movement of the virus. Therefore, eIF4E/eIF(iso)4F may not only participate in the infection and translation of viruses, but it may also affect virus movement.

The eIF4E and eIF(iso)4E proteins exhibit differences in their ability to bind mG and other cap analogues. Viruses selectively combine with either eIF4E or eIF(iso)4E. When mutated Arabidopsis thaliana eIF4E and eIF(iso)4E were tested for susceptibility to Closter yellow vein virus (CYVV) and Turnip mosaic virus (TuMV), eIF4E was shown to be necessary for infection by CYVV but not for TuMV, while eIF(iso)4E was in contrast. Different viruses infect the same plants, and the same virus infects different plants, by interaction with different members of the eIF-4E family. For example, Lettuce mosaic virus (LMV) and Tobacco etch virus (TEV) infect Arabidopsis by interacting with eIF(iso)4E, but infect pepper, tomato, and lettuce by interacting with eIF4E.

The mRNAs of many plant RNA viruses lack a cap structure, a poly(A) tail or both, yet they efficiently compete with host mRNAs for the translational machinery. Several host proteins that interact with viral coat proteins are involved in the cell-to-cell movement or subcellular localization of viruses. Some viruses selectively combine with either eIF4E or eIF(iso)4E. When mutated Arabidopsis thaliana eIF4E and eIF(iso)4E were tested for susceptibility to Closter yellow vein virus (CYVV) and Turnip mosaic virus (TuMV), eIF4E was shown to be necessary for infection by CYVV but not for TuMV, while eIF(iso)4E was in contrast.

Materials and Methods

Plants Materials

The chrysanthemum variety ‘Jinba’ and diseased leaves infected with CVB were obtained from the Chrysanthemum Germplasm Resource Preserving Centre, Nanjing Agricultural University, China. Arabidopsis thaliana Columbia-0 plants were grown in 8 h photoperiod at 23 °C in 50–60% humidity.

CVB CP cDNA Cloning and Sequencing

Total RNA was isolated from chrysanthemum ‘Jinba’ leaves using the RNAiso reagent (Takara, Japan) according to the manufacturer’s instructions. The cDNA first strand was synthesized from 1 µg of total RNA using the M-MLV RTase cDNA Synthesis kit (Takara, Japan) according to the manufacturer’s instructions. A gene-specific primer pair (i4E-F/R) was designed to amplify a fragment of CmeIF(iso)4E based on the sequences from other plants, and RACE PCR was then used to obtain the full-length cDNA. For the 3′ RACE reaction, the first strand cDNA was synthesized using an d(T)-adapter primer incorporating the sequence of the adaptor primer, and this was followed by a nested PCR using primer pair i4E-3′GSP 1/2 and the adaptor primer (Table 1). For the 5′ RACE, the nested PCR was performed using primers AMP and AUAP provided by the 5′ RACE System kit v2.0 (Invitrogen), along with gene-specific primer i4E-5′GSP1/2/3 (Table 1). PCR products were purified using a Bionspin Gel Extraction kit (BioFlux, China) and cloned into the pMD19-T easy vector (Takara) for sequencing. Finally, a pair of gene-specific primers (eIF(iso)4E-F and eIF(iso)4E-R) was designed from the putative 5′ and 3′ UTR sequences to amplify the complete CmeIF(iso)4E open reading frame (ORF). The sequences of all the above primers are given in Table 1. The CmeIF(iso)4E amino acid sequence was aligned with the sequences of its homologues using DNAMAN software. The phylogenetic tree file was produced using ClustalW (http://www.ebi.ac.uk/clustalw/).

C BV CP cDNA Cloning and Sequencing

Total RNA was isolated from infected leaves using the RNAiso reagent (Takara) according to the manufacturer’s instructions. The cDNA first strand was synthesized from 1 µg of total RNA using the M-MLV RTase cDNA Synthesis kit (Takara) and a random primer incorporating the sequence of the adaptor primer according to the manufacturer’s instructions. According to the reported cDNA sequence of the CVB coat protein (GenBank: AJ879077.1), we designed a pair of gene-specific primers (CVBCPF and CVBCPR-R) to amplify the complete CVBCP open reading frame (ORF). PCR products were purified using a Bionspin Gel Extraction kit (BioFlux) and cloned into the pMD19-T easy vector (Takara) for sequencing.

Quantitative Real-Time PCR (qRT-PCR)

To determine the expression pattern of CmeIF(iso)4E in different tissues in chrysanthemums, total RNA was extracted from roots, stems, leaves, tubular flowers, and ligulate flowers of chrysanthemum ‘Jinba’ using the RNAiso reagent (Takara), and potential contaminating genomic DNA was removed by DNase I treatment. cDNA was synthesized using the M-MLV RTase cDNA Synthesis kit (Takara), and quantitative real-time PCR (qRT-PCR) was performed using SYBR_Green I (TOYOBO, Japan) in a Rotor-Gene 3000 (Corbett, Australia). The primer pair i4E-RT-F/R (Table 1) was used to amplify a 158 bp fragment in the 3′ region of the gene, avoiding the more well-conserved segments of the gene. The chrysanthemum CmeGAPDH gene (GenBank accession num-

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Table 1. Names and sequences of primers used in this study.

| Primer name | Sequence (5'-3') |
|-------------|-----------------|
| i4E-F       | 5'CACCTCGACGCGTGGAAGANTTGGG 3' |
| i4E-R       | 5'CCGTCGACGCGTGGAAGANTTGGG 3' |
| dT-adaptor  | 5'AGCAGCTTTGCACTAGGATCT 3' |
| adapter     | 5'AGCAGCTTTGCACTAGGATCT 3' |
| i4E-3'SP1   | 5'TGGAAGAATTCTGTTGTTTATG 3' |
| i4E-3'SP2   | 5'GGATAGGAATCTGTTGTTTATG 3' |
| i4E-3'SP3   | 5'AGAAAGCAGCTAGGATCT 3' |
| AAP         | 5'GCCAGCCGCGTCACTAGGATCT 3' |
| AUAP        | 5'GCCAGCCGCGTCACTAGGATCT 3' |
| i4E-5'SP1   | 5'TGACATCTGCTTGATACATCGG 3' |
| i4E-5'SP2   | 5'GTCGACGAGCTTTACTGCTAC 3' |
| i4E-5'SP3   | 5'TAGCAACCAGCAGACGACGAGT 3' |
| eI4E-iso4E-F | 5'CCTGAAGAATAATCATGCGGCAG 3' |
| eI4E-iso4E-R | 5'ATTCTGTGATTCGCGAGAG 3' |
| CVBCP-F     | 5'AGTCCCTCCCAAGCGGC 3' |
| CVBCP-R     | 5'TTAATACGACGCTTATGACATTGGG 3' |
| ME-RT-F     | 5'TGAGCTGAGCTGCTGTAGGCGG 3' |
| ME-RT-R     | 5'TGACATCTGCTTGATACATCGG 3' |
| GAPDH-F     | 5'GCCGACACGCTATATCGACCATG 3' |
| GAPDH-R     | 5'AGAAGCCGAGCAGACTGAGG 3' |
| i4E-SL-F    | 5'GCCGACGCGTCACTAGGATCT 3' (SalI) |
| i4E-SL-R    | 5'TCCGACGCGTCACTAGGATCT 3' (NotI) |
| AD-4E-F     | 5'CCGGAATTCCGGTAGAAGGCAACTACCC 3' (EcoRI) |
| AD-4E-R     | 5'CCGGAATTCCGGTAGAAGGCAACTACCC 3' (EcoRI) |
| BD-CVBCP-F  | 5'CGGATATCGTCTTCAACCCGGGC 3' (EcoRI) |
| BD-CVBCP-R  | 5'ACGGGTACGTTATAGTTCTTTATAGACTGCTAC 3' (SalI) |
| BIFC-4E-F   | 5'CCGGAATTCCGGTAGAAGGCAACTACCC 3' (EcoRI) |
| BIFC-4E-R   | 5'CCGGAATTCCGGTAGAAGGCAACTACCC 3' (EcoRI) |
| BIFC-CVBCP-F| 5'CGGATATCGTCTTCAACCCGGGC 3' (EcoRI) |
| BIFC-CVBCP-R| 5'CGGATATCGTCTTCAACCCGGGC 3' (EcoRI) |
| CBB3UTR-1   | 5'GACGACTACTAGAATCATGCACTCTAC 3' |
| CBB3UTR-2   | 5'TATATAATGGTTTAGTTAAGACACTAATGAC 3' |
| CBB3UTR-3   | 5'-GGGTTTATTTGTTTAGTTAAGACACTAAC'-3' (KpnI) |
| Bif-Bif-F   | 5'CTGACAGATGAACAGACCCG 3' (NcoI) |

Note: underlined sequences indicate restriction enzyme sites, which are names in which are shown in brackets.
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Subcellular Localization of CmeIF(iso)4E

The plasmid used for transient transfection was generated using the Invitrogen Gateway system according to the manufacturer’s instructions. The CmeIF(iso)4E ORF, lacking its stop codon, was amplified using a Phusion® High-Fidelity PCR kit (New England Biolabs, USA) with the primer pair, 5'-TLF-F/-R, then subcloned into the pmD19-T vector (Takara) and confirmed by DNA sequencing. The confirmed gene from the pmD19-T vector was inserted into the pENTR™/I A dual selection vector (Invitrogen, USA) at Sol I and Not I with T4 DNA ligase (Fermentas, Canada), and then the construct was recombined with pEarleyGate 103 [42] to construct a CmeIF(iso)4E-GFP fusion vector, using the LR Clonase™ II enzyme mix (Invitrogen). Plasmid DNA was transiently introduced into onion Allium cepa) epidermal cells using a helium-driven particle accelerator (PDS-1000; Bio-Rad, USA) according to the manufacturer’s instructions. After bombardment, the onion peels were incubated for 16 h on Murashige and Skoog plates in the dark. Confocal laser microscopy (Leica SP2; Germany) was used to monitor the expression of GFP.

Yeast Two-Hybrid Assay

The yeast two-hybrid assay was performed with the Matchmaker™ Gold Yeast Two-Hybrid System (Clontech, USA). The coding region of CmeIF(iso)4E amplified with the primer pair, 5'-TLF-F/-R, was inserted into the pGADT7 prey vector at the EcoRI / BamHI sites (fused to the GAL4 activation domain) to produce pAD-CmeIF(iso)4E. The CVB CP gene was amplified using the primer pair, 5'-TLF-F/-R, and cloned into the yeast bait expression vector, pGBK7, at the EcoRI / Sol I sites (fused to the Gal4 DNA binding domain) to produce pBD-CVBCP. The interactions between CmeIF(iso)4E and CVBCP were identified by testing combinations of CVBCP-bait and CmeIF(iso)4E-prey constructs co-expressed in the yeast Y2HGold strain, according to the manufacturer’s instructions. Interactions were detected by growth on the selective media, SD/-Trp/-Leu and SD/-Trp/-Leu/-His/-Ade, according to the manufacturer’s instructions. Yeast diploids containing empty pGBK7 and pGAD7 were used as negative controls. z-Galactosidase activity was assayed on filters as described in the Yeast Protocol Handbook (Clontech). All primers used in the yeast two-hybrid assays are listed in Table 1.

Bimolecular Fluorescence Complementation Assay

In the BiFC constructs, the coding region of CmeIF(iso)4E was cloned as N-terminal fusions to the fluorescent protein fragments in the pSATA4a-eYFP-N1 vector at the EcoRI / BamHI cloning sites using the primer pair, 5'-TLF-F/-R. The full-length cDNA encoding the coat protein of CVB was cloned as C-terminal fusions to the fluorescent protein fragments in the pSATA4a-eYFP-N1 vector at the EcoRI / Smal cloning sites using the primer pair, 5'-TLF-F/-R. The transient gene expression in onion epidermal cells was performed using a Biolistic PDS-1000/ He Particle Delivery System (Bio-Rad) according to the manufacturer’s instructions. After bombardment, the onion peels were incubated for 16 h on Murashige and Skoog plates in the dark. YeF fluorescence images were acquired by confocal laser scanning microscopy (model TCS SP2; Leica). All primers used in bimolecular fluorescence complementation assays are listed in Table 1.

Luminescence assay

The Luc-CVB vector was constructed via adding the 3’UTR of CVB between the luciferase ORF and 35S terminator in the pCAMBIA99-1-Luc plasmid. The detailed construction proce-
Figure 1. Phylogenetic relationship between CmeIF(iso)4E and other eIF4E superfamily proteins. The phylogenetic tree file was produced using ClustalW (http://www.ebi.ac.uk/clustalW/). The GenBank accession numbers of the amino acid sequences used are: CmeIF_iso_4E (JQ904591, Chrysanthemum morifolium), LseIF_iso_4E (AAP86603.1, Lactuca sativa), SleIF_iso_4E (NP_001234772.1, Solanum lycopersicum), PseIF_iso_4E (BAK53449.1, Pisum sativum), NteIF_iso_4E (AAU06579.1, Nicotiana tabacum), PveIF_iso_4E (ABUS4807.1, Phaseolus vulgaris), CpeIF_iso_4E (ACM18197.1, Carica papaya), CseIF_iso_4E (ABY56102.1, Cucumis sativus), CmeIF4E (JQ904591, Chrysanthemum morifolium), Atel4E (NP_193538.1, Arabidopsis thaliana), CaeIF4E (AAN74644.1, Cayenne pepper), CpeIF4E (ACN38307.1, Carica papaya), HveIF4E (CAR92170.2, Hordeum vulgare), LseIF4E (AAP86602.1, Lactuca sativa), NteIF4E (DK22107.1, Nicotiana tabacum), SleIF4E (AAV88610.1, Solanum lycopersicum), PseIF4E (ABG35119.1, Pisum sativum) and ZmeIF4E (ACG34414.1, Zea mays).

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Figure 2. CmeIF(iso)4E expression in chrysanthemum as demonstrated by qRT-PCR.

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dures are described below. Triple nested PCR was amplified using a Phusion High-Fidelity PCR kit (New England Biolabs, USA) with the primer pairs (1st Luc-Nco-F/ CVB-3UTR-R1, 2nd Luc-Nco-F/ CVB-3UTR-R2, 3rd Luc-Nco-F/ CVB-3UTR-R3). The PCR products were purified using a Biospin Gel Extraction kit (BioFlux). The purified Luc-CVB fragment and pCAMBIA99-1-Luc plasmid were digested by NcoI/KpnI, then the digested Luc-CVB fragment was inserted into digested pCAMBIA99-1-Luc to replace Luc with Luc-CVB. The generated Luc-CVB vector was confirmed by sequencing.

Protoplasts were prepared and transfected based on the protocol described by Yoo et al. [43]. For Luc (pCAMBIA99-1-Luc), Luc-CVB (pCAMBIA99-1-Luc-CVB) and CVBCP (pSAT4A-CVBCP) transfection, 7.5 μg of pCAMBIA99-1-Luc, pCAMBIA99-1-Luc-CVB and pSAT4A-CVBCP plasmid was transfected respectively; while for Luc+CVBCP and Luc-CVB+CVBCP transfection, additional 7.5 μg pSAT4A-CVBCP plasmid was added. ddH2O was transfected as a mock.

The luciferase activities were measured 16 h after the transfection. Luminescence was detected according the protocol described by Fujikawa and Kato with minor modifications [44]. The beetle luciferin (Promega, Madison, Wisconsin, USA) was dissolved in sterile water to a final concentration of 7.8 mM and stored at -80°C. The stock solutions were diluted 10 times with the W5 buffer before each assay. 10 μl of diluted beetle luciferin was added to each well of a 96-well plate (PerkinElmer, Waltham, Massachusetts, USA), then added 150 μl transfected protoplasts, mixed well. After 15 min of incubation at room temperature in the dark, the luminescence in each well was quantified with 10 sec integration periods using a Glomax 96 microplate luminometer (Promega). Each assay repeated three times.

Results

Identification and characterization of CmeIF(iso)4E, an isoform of translation initiation factor 4E

Based on the amino acid sequences of eIF4E and eIF(iso)4E proteins from other plants, a eIF4E homologous gene was isolated by RT-PCR and RACE that was designated as CmeIF(iso)4E (GeneBank accession JQ904592). The cloned CmeIF(iso)4E cDNA consists of 818 bp with a 573 bp ORF encoding a 190-amino acid protein. A homology blast showed that CmeIF(iso)4E is substantially homologous to other eIF(iso)4E proteins from other species, with sequence identity between 69.12% and 89.18%, and highest similarity to CmeIF(iso)4E from Lactuca sativa. Phylogenetic analysis showed that CmeIF(iso)4E is clustered with the eIF(iso)4E subgroup and separated from the eIF4E subgroup (Fig. 1).

Expression profiling of CmeIF(iso)4E

The results of qRT-PCR showed that CmeIF(iso)4E is transcribed in the roots, stems, leaves, tubular florets, and ligulate florets, with the highest level of transcription being present in the root and the lowest in the stem (Fig. 2). To determine the distribution of CmeIF(iso)4E in cells, we used a transient assay involving the bombardment of CmeIF(iso)4E-GFP fusion constructs into onion (Allium cepa) epidermal cells. The results

Figure 3. Subcellular localization of the CmeIF(iso)4E protein in onion epidermis cells. The upper row shows the 35S::GFP signal alone as a positive control; the middle row displays the signal from 35S::CmeIF(iso)4E-GFP; the lower row shows 35S::CmeIF(iso)4E-GFP following treatment with 0.8 M sucrose to induce plasmolysis. The left panel shows bright field images; the middle panel shows green fluorescence signals detected at 488 nm; the right panel shows the merged GFP signals and bright field images. Bars = 50 μm.

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Figure 4. Interaction between CmeIF(iso)4E and CVBCP in vitro as demonstrated by Y2H assay. Yeast two-hybrid screen demonstrating the interaction between CVBCP and CmeIF(iso)4E. The left panel shows the growth of yeast cells containing both plasmids on SD-Leu-Trp medium (SD-2); the middle panel shows the selection of yeast colonies on SD-Leu-Trp-Ade-His medium (SD-4); the right panel shows the selection of yeast colonies on SD-4 containing α-X-Gal.

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CmeIF(iso)4E Interacts with CVB Coat Protein
demonstrated that the CmeIF(iso)4E-GFP fusion protein was present in the nucleus, cytoplasm, and cytomembrane (Fig. 3).

CmeIF(iso)4E interacts with CVBCP

Yeast two-hybrid heterologous expression was used to assess the possible interaction between CVBCP and CmeIF(iso)4E. The CVBCP gene was fused to the DNA-binding domain in the bait plasmid, pGBK7, and the CmeIF(iso)4E gene was cloned into the prey plasmid, pGADT7. In addition, empty vectors were transformed as negative controls for each recombinant plasmid. Screening for putative interactions was performed using SD/-Trp/-Leu/-His/-Ade media, as shown in the middle panel of Fig. 4. Fig. 4 shows that CmeIF(iso)4E grew significantly when co-transformed with CVBCP as bait, which was confirmed by positive -X-galactosidase assays. By contrast, pGADT7 co-transformed with CVBCP or pGBK7 as bait failed to induce growth on SD-4 media or show -X-galactosidase activity. The interaction of CVBCP and CmeIF(iso)4E in yeast was further confirmed by BiFC. A YFP fluorescence signal was observed in the onion epidermis cells co-expressing nEYFP-CmeIF(iso)4E and cEYFP-CVBPC (Fig. 5). In contrast, no fluorescence signal was generated in cells co-expressing nEYFP-CmeIF(iso)4E and cEYFP, nEYFP and cEYFP-CVBPC, or nEYFP and cEYFP, as controls. Thus, these observations demonstrate that CmeIF(iso)4E protein interacts with CVBCP in plant cells.
CVBCP involves in the translation process of CVB RNA

The relative light units (RLU) of Luc-CVB was lower than that of Luc transfectants in Arabidopsis prooplasts, which indicated that the 3' UTR of CVB RNA might be targeted by plant immune systems (Fig. 6). The cotransfection of CVBCP and Luc-CVB recovered the activity of Luc-CVB to that of Luc (Fig. 6), which inferred that CVBCP involves in the translation process of CVB RNA.

Discussion

Previous studies have demonstrated that the cap-binding protein, eIF4E/eIF(iso)4E, is responsible for conferring resistance to RNA viruses in plant systems. In this study, we cloned eIF(iso)4E from chrysanthemum using degenerate primers. Sequence phylogeny and phylogenetic analysis revealed that CmeIF(iso)4E is highly conserved with its orthologues in other plant species. The genetic relationship between related species coincides with plant taxonomy, with the closest relatives being chrysanthemum and lettuce.

The sequence identity of CmeIF(iso)4E to other reported plant eIF(iso)4E genes varied between 69.12 and 89.18%. Diversity between eIF(iso)4E proteins from different organisms mainly resides in their N-termini, which may have very different lengths and seem not to be directly involved in cap-binding [45], [46]. Functional differences also exist in the promotion of viral infection and accumulation in plant hosts. qRT-PCR showed that the transcription of CmeIF(iso)4E is highest in young roots and flowers, which is consistent with previous reports showing that eIF(iso)4E is particularly abundant in floral tissues and young tissues [11]. Other studies have shown that eIF4E and eIF(iso)4E both function in the selection of mRNA for translation, but differ in their expression in different tissues and their ability to bind to mRNA cap structures [4], [11]. Confocal microscopy showed that CmeIF(iso)4E is localized in the nucleus, cytoplasm, and cytomembrane in onion epidermal cells. In animals, eIF4E is localized predominantly in the cytoplasm, with between 12% and 25% of eIF4E being found in the nucleus [2], [47], [48]. In eukaryotic cells, eIF4E functions in the selective transport of specific mRNAs from the nucleus to the cytoplasm [49]. Here, we detected CmeIF(iso)4E on the cytomembrane by confocal microscopy, and this localization was confirmed by BiFC. Further research is needed to determine the function of CmeIF(iso)4E on the cytomembrane.

It has been shown that P. syringae uses eIF4E in one host and eIF(iso)4E in another host for protein translation; for example, Tobacco etch virus (TEV) uses eIF4E in pepper [9] and tomato [50], but eIF(iso)4E in A. thaliana [51]. Here, we report the interaction between the coat protein of CVB and eIF(iso)4E from chrysanthemum. In the yeast two-hybrid system, we found that CVBCP interacts with CmeIF(iso)4E. This interaction was confirmed by BIFC assays, in which the co-expression of CmeIF(iso)4E and CVBCP were observed by significant yellow fluorescent in the nucleus, cytoplasm, and cytomembrane.

Therefore, our results suggest that eIF4E and eIF(iso)4E are selectively involved in plant-virus interactions, and the isoforms may have complementary biological roles.

In P. syringae, the interaction between VPg and eIF4E may be important for the cellular transport and localization of RNA [51]. eIF4E based resistance has also been postulated to act at the level of virus cell-to-cell movement. In pea and pepper, eIF4E assists P. syringae cell-to-cell movement [19]. eIF(iso)4E is also involved in resistance to other viruses, such as CMV, and resistance to B. napus has also been reported [3], [23]. The recent demonstration of the binding of eIF4E and eIF(iso)4E to helper component proteinase (HCpro) of three potyviruses (Potato virus A, Potato virus Y, and Tobacco etch virus), further extends this potential interaction network [32]. It has been reported that extension of the 3' termini of AMV genomic RNAs with a poly(A) tail of 40 or 80 residues permits the initiation of infection of tobacco plants and protoplasts at a level that was 5% of the CP-mediated initiation of infection [33]. Therefore, binding of CP to the 3' termini of AMV RNAs is functionally equivalent to the binding of PABP to the poly(A) tail of cellular mRNAs [33]. The poly(A) tail of cellular messengers enhances translation synergistically with the cap structure by mediating the interaction of poly(A)-binding PABP with the cap-binding initiation factors, eIF4F and eIF4B [53]. The reported assays revealed that AMV CP interacts specifically with the eIF4G and eIF(iso)4G subunits from wheat eIF4E and eIF(iso)4F, respectively [33]. In the genera Aphanomyces and Barzirus, initiation of infection by viruses requires the addition of coat protein to a mixture of the genomic RNAs in multiple steps of the replication cycle [35]. CVBCP increased the RLU of Luc-CVB, suggesting CVBCP might participate in the translation of CVB viral proteins (Fig. 5, 6). The viral RNA of CVB has the 5' cap structure and 3' poly(A) tail [54]. The virus would recruit eIF4E of host plant cell to translate the viral protein [55]. Together with the observation of interaction between CVBCP and CmeIF(iso)4E, we suppose that CmeIF(iso)4E might regulate the viral translation via CVBCP.

The eukaryotic translation initiation factors are involved in virus translation, these host factors also participate in viral RNA replication [55]. Here, our results inferred that CmeIF(iso)4E as the cap-binding subunit eIF(iso)4F may be involved in Chrysanthemum Virus B infection in chrysanthemum through its interaction with CVBCP in spatial.

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Author Contributions

Conceived and designed the experiments: SAP LWH JF CSM SZX GZY FWM TNJ CFD. Performed the experiments: SAP LWH SZX. Analyzed the data: JF SAP LWH CSM SZX GZY FWM TNJ CFD. Contributed reagents/materials/analysis tools: JF SAP CSM GZY FWM TNJ CFD. Wrote the paper: JF SAP LWH CSM CFD.

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