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Areca palm velarivirus 1 encoded P25 hijacks host eIF4E and enhances viral translation through strengthening the interaction of eIF4E with 3' cap-independent translation enhancer

Siyu Wan¹, Hongxing Wang^{1*} and Xi Huang^{1*}

Abstract

Plant viruses employ diverse strategies to hijack host machinery for viral translation initiation. Cap-independent translation enhancers located in the 3' untranslated region (3'-CITEs) have been identified in many members of the families *Tombusviridae* and *Luteoviridae*. However, viral proteins that regulate 3'-CITE-mediated translation remain unreported, and the underlying mechanisms are still poorly understood. Closteroviruses are important pathogens that infect many economically significant crops, yet their translation initiation mechanisms remain largely unexplored. Areca palm velarivirus 1 (APV1), a member of the family *Closteroviridae*, is the causative agent of yellow leaf disease (YLD) in areca palms. Here, we demonstrate that eukaryotic initiation factor 4E (eIF4E) specifically binds to the 3'-UTR of APV1. The APV1-encoded protein P25 interacts with eIF4E, enhances its stability, alters its subcellular localization, and promotes its recruitment to APV1 virions. Notably, P25 significantly enhances cap-independent viral translation. Knockout of *eIF4E* in *Nicotiana benthamiana* or mutation of key residues in APV1-P25 required for eIF4E interaction impairs APV1 infection. We identify the 3'-UTR of APV1 as the first reported 3'-CITE in the family *Closteroviridae*. Our study reveals P25 as the first viral regulator of 3'-CITE-mediated translation initiation, providing new insights into the molecular mechanisms underlying viral infection.

Keywords Areca palm velarivirus 1, 3'-CITE, Translation initiation, eIF4E, P25

Background

In eukaryotic cells, mRNAs typically possess a 5'-cap (m⁷GpppN) and a 3'-poly(A)-tail, both essential for canonical translation. The eukaryotic translation initiation factor (eIF) and poly(A)-binding protein (PABP) cooperate to initiate translation. In plants, eIF4E, a heterodimer composed of eIF4E and the scaffolding protein

eIF4G, forms the initiation complex (Hinnebusch and Lorsch 2012; Hinnebusch 2014). eIF4G binds both eIF4E and PABP, which interacts with the poly(A)-tail, resulting in mRNA circularization (Aitken and Lorsch 2012). eIF4G also associates with eIF3 to recruit the 40S ribosomal subunit and initiate mRNA scanning in the 5'-to-3' direction (Jackson et al. 2010).

Unlike host mRNAs, many positive-strand RNA plant viruses lack conventional 5'-caps or poly(A) tails, necessitating alternative translation strategies. These include internal ribosome entry sites (IRESs), tRNA-like structures (TLS), and 3'-cap-independent translation enhancers (3'-CITEs), which allow viral RNAs to hijack host machinery efficiently (Nicholson and White 2011; Simon

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and Miller 2013). Among these, 3'-CITEs are particularly notable for their ability to recruit translation factors to the 3'-UTR, which are then translocated to the 5' end via long-distance RNA interactions (Karetnikov et al. 2006; Miras et al. 2017a; Truniger et al. 2017). The long-distance pairing facilitates the recruitment of the 40S ribosome complex to the 5'-UTR for scanning to the start codon (Miras et al. 2017a). While structurally diverse, 3'-CITEs are functionally conserved and have been extensively characterized in *Tombusviridae* and *Luteoviridae* (Nicholson and White 2011; Miras et al. 2017a). However, 3'-CITEs have been rarely reported in other virus families. The virus-encoded proteins regulating 3'-CITE-mediated translation remain poorly understood.

The family *Closteroviridae* consists of seven genera and 58 species according to the current ICTV (International Committee on Taxonomy of Viruses) taxonomy release. Many members of this family are known to be pathogens that afflict economically important crops such as citrus (Dawson et al. 2013), grapevine (Naidu et al. 2015), cherry (Candresse et al. 2013), pineapple (Sether and Hu 2002), and areca palm (Wang et al. 2020). Due to the lack of natural resistance, developing disease resistance through conventional breeding is a significant challenge. The application of virus-induced gene silencing (VIGS) and novel genome-editing technologies should be considered as alternatives to classical breeding and transgenic approaches for the targeted genome modification of cultivars with resistance to viruses (Folimonov et al. 2007; Dawson and Folimonova 2013; Naidu et al. 2015). Both naturally occurring recessive *eIF4E* and CRISPR/Cas9-edited *eIF4E* have demonstrated durable resistance in many crops without reducing yield (Nieto et al. 2006; Yeam et al. 2007; Bastet et al. 2019; Ruffel et al. 2002; Kan et al. 2023). Understanding of the translation initiation mechanism of the viral protein synthesis in the infected host cell is crucial for developing strategies to effectively control viral diseases. However, the translation initiation mechanisms of *Closteroviridae* remain unknown.

Areca palm velarivirus 1 (APV1), first discovered in yellow leaf disease (YLD) of areca palm (Yu et al. 2015; Wang et al. 2020), belongs to the *Velarivirus* genus of the *Closteroviridae* family. APV1 is a major cause of YLD in areca palms, manifesting in symptoms such as chloroplast disassembly, leaf yellowing, and reduced yield (Zhang et al. 2022; Khan et al. 2023; Cao et al. 2024). The APV1 genome encodes 11 open reading frames (ORFs), of which three ORFs at the 5' terminal (ORF1a, ORF1b, and ORF2) encode proteins for viral RNA replication, while the virion assembly and systemic movement of closteroviruses primarily rely on the quintuple gene block (ORF3 to ORF7) located downstream of the replication module (Dolja et al. 1994). The three ORFs (ORF8 to

ORF10) located at the 3' end of the genome are involved in interactions with the host (Yu et al. 2015; Wang et al. 2020; Cao et al. 2021). Here, we demonstrate that APV1-ORF10-encoded P25 interacts with host eIF4E, alters eIF4E subcellular localization, facilitates the recruitment of eIF4E to APV1 virions and enhances viral translation. APV1 3'-UTR functions as the first identified 3'-CITE in *Closteroviridae*. Our findings reveal a novel cap-independent translation mechanism orchestrated by P25, providing critical insights into viral translation regulation and potential targets for disease control.

Results

APV1-ORF10-encoded P25 interacts with eIF4E

To identify potential host factors interacting with APV1, a bait vector expressing the APV1-ORF10 encoded P25 protein was constructed and used in yeast two-hybrid (Y2H) screening against a Y2H cDNA library from areca palm. This screening identified eight candidate proteins, including the eIF4E (GenBank accession number PQ874234), that interacted with P25 (Additional file 1: Table S1). The P25-eIF4E interaction was further validated through pairwise Y2H assays (Fig. 1a). To confirm this interaction *in planta*, a bimolecular fluorescence complementation (BiFC) experiment was performed. eIF4E and P25 were fused to the N-terminal and C-terminal halves of yellow fluorescent protein (YFP), respectively. Positive interactions were evidenced by the presence of YFP fluorescence, demonstrating that P25 interacts with eIF4E *in vivo* (Fig. 1b). Further validation was provided by luciferase complementation assays (LCA), which detected luciferase activity in leaves transiently expressing cLuc-P25 and nLuc-eIF4E (Fig. 1c). Co-immunoprecipitation (Co-IP) assays also confirmed that Flag-P25 co-precipitated with GFP-eIF4E when using an antibody against GFP (Fig. 1d). Finally, glutathione S-transferase (GST) pull-down assays demonstrated that GST-eIF4E could capture His-tagged P25, confirming that P25 interacts with eIF4E *in vitro* (Fig. 1e). Taken together, these assays provide robust evidence that P25 interacts with eIF4E both *in vivo* and *in vitro*.

Three-dimensional prediction model of the interaction between P25 and eIF4E

To investigate the potential interaction mechanism between eIF4E and P25, the multimer module of AlphaFold v2.3.2 was employed to predict the three-dimensional structure of the eIF4E-P25 complex, with eIF4E and P25 represented in green and blue, respectively. The overall structure of P25 complements the shape of the eIF4E binding pocket, indicating the specificity and stability of the interaction (Additional file 2: Figure S1a, b). The predicted multimeric structure was further

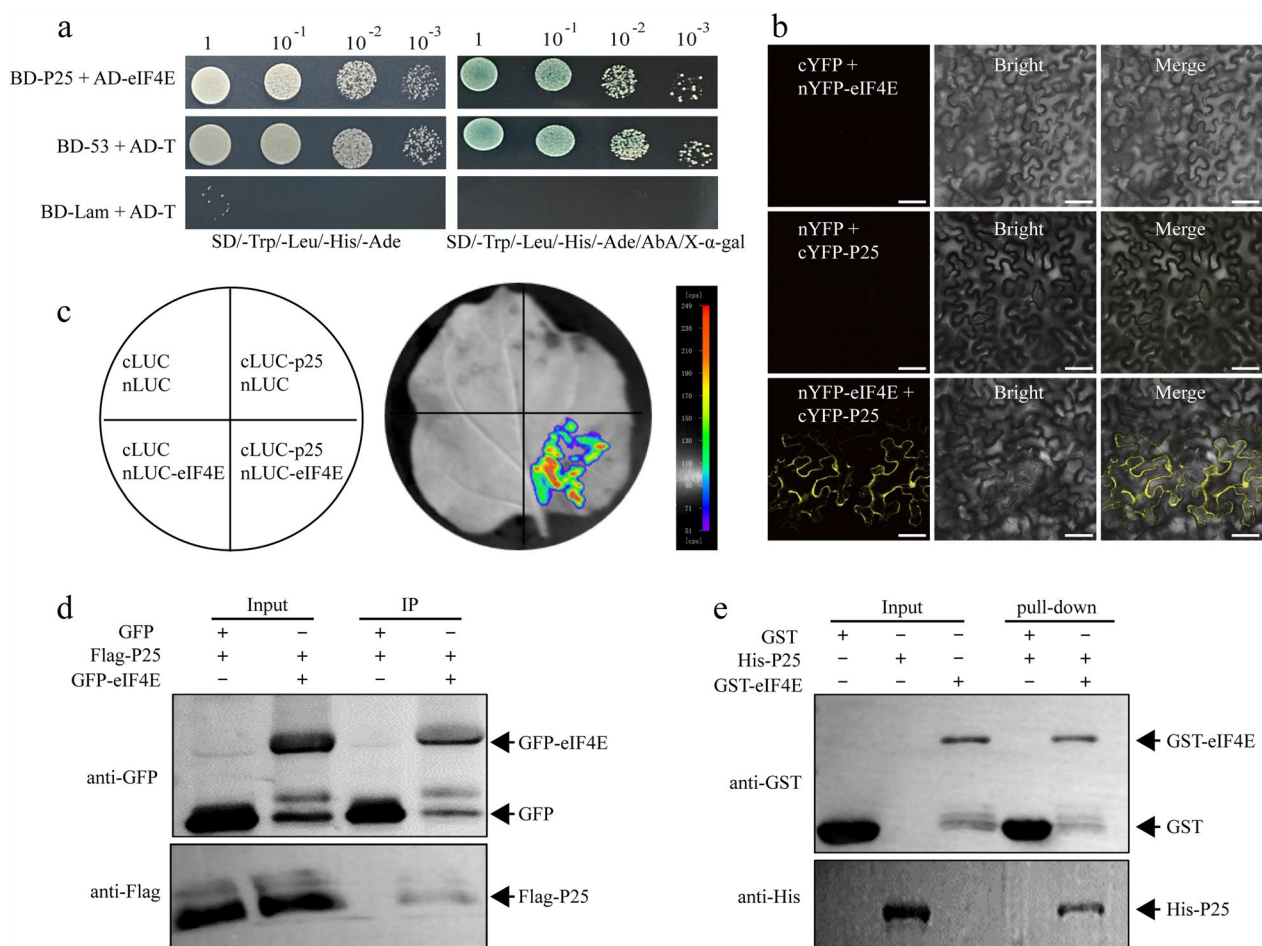


Fig. 1 Interaction between P25 and eIF4E. **a** Yeast two-hybrid assay on QDO medium (SD/-Trp/-Leu/-His/-Ade) with/without AbA and X-α-Gal. pGBK-Lam + pGAD-T (negative control), pGBK-53 + pGAD-T (positive control). **b** Bimolecular fluorescence complementation (BiFC) in *N. benthamiana* leaves. YFP fragments were fused to P25 and eIF4E; fluorescence was visualized at 72 hpi. Scale bar = 50 μm. **c** Luciferase complementation assay (LCA) in *N. benthamiana*. Combinations tested: cLuc-P25 + nLuc-eIF4E, cLuc-P25 + nLuc, cLuc + nLuc-eIF4E, and cLuc + nLuc (negative control). Luminescence was quantified at 72 hpi. **d** Co-IP of eIF4E-GFP and Flag-P25 co-expressed in *N. benthamiana*. Lysates were immunoprecipitated with anti-GFP beads and probed with anti-Flag antibodies. **e** GST pull-down assay with GST-eIF4E and P25-6×His. Blots were probed with anti-GST (upper) and anti-His (lower) antibodies

visualized and analyzed using PyMOL v2.5.0, which indicates that the key residues W61 and Y64 of eIF4E (red-circled) form a highly specific interaction interface through π - π stacking and hydrophobic interactions with critical residues of P25, such as I8 and C11 (blue-circled), via their aromatic ring structures. Additionally, P25 residues I8 and C11 interact with hydrophobic residues of eIF4E, such as V51 and T53, further enhancing the stability of the interaction. Other residues, such as T113 and D112 of eIF4E, form hydrogen bonds with N89 of P25, providing additional support for the overall stability of the interaction interface (Additional file 2: Figure S1c). The interaction interface is primarily concentrated around the binding pocket region and its adjacent critical sites. This interaction model demonstrates the synergy

among residues at the interaction interface, highlighting the central role of W61 and Y64 in forming a highly efficient and stable complex. Furthermore, the P25 protein does not directly mimic the RNA cap structure but may instead provide a unique interaction interface that facilitates eIF4E's interaction with the 3'-UTR. To assess the reliability of the prediction, the PRODIGY tool (<https://prodi.gy/>) was used to calculate the binding energy of the multimeric structure, yielding a value of -18.5 kcal/mol, indicating a highly stable interaction between eIF4E and P25.

To verify the domain of P25 that interacts with eIF4E, truncated versions of P25 were fused with cYFP and co-expressed with nYFP-CP through agroinfiltration in *Nicotiana benthamiana*. BiFC results revealed that the

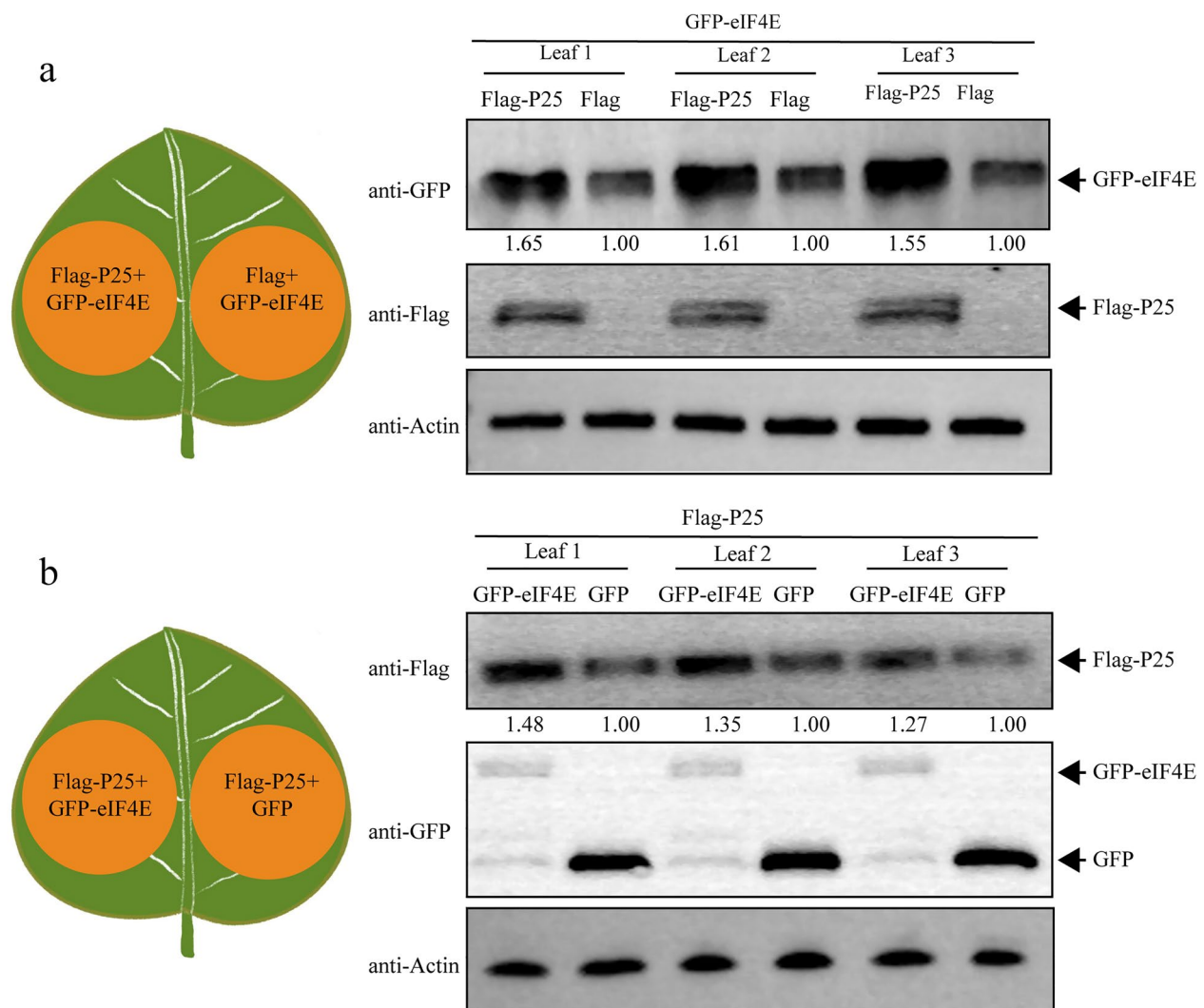


Fig. 2 P25 and eIF4E mutually stabilize both proteins. **a** GFP-eIF4E was transiently co-expressed with Flag-P25 and with Flag separately on each half of the same leaf of *N. benthamiana* through agroinfiltration. **b** Flag-P25 was transiently co-expressed with GFP-eIF4E and with GFP in *N. benthamiana*. The infiltrated leaves were then treated with cycloheximide (CHX), and leaf samples were collected for Western blotting using antibodies against Flag and GFP at 6 h after treatment. An anti-actin antibody was used to show the loading. Protein band intensities were quantified using ImageJ software and are presented as relative protein levels normalized to the corresponding control

1–35 aa and 1–50 aa fragments of P25 interacted with eIF4E, displaying stronger fluorescence (Additional file 2: Figure S2), while the 36–215 aa and 51–215 aa fragments did not interact with eIF4E (Additional file 2: Figure S2), confirming that the interaction site of P25 is located in the 1–35 aa region. This was further corroborated by a Y2H assay (Additional file 2: Figure S3). Additionally, the Y2H truncation assay identified that the interaction site of eIF4E is located in the eIF4E domain (24–176 aa) (Additional file 2: Figure S3b).

The interaction between P25 and eIF4E mutually stabilizes both proteins

To investigate the impact of the P25 interaction on the eIF4E stability, Flag-P25 and GFP-eIF4E were transiently co-expressed in *N. benthamiana* via agroinfiltration. The infiltrated leaves were subsequently treated with cycloheximide (CHX), a known protein synthesis inhibitor. Upon CHX treatment, synthesis of Flag-P25 and GFP-eIF4E was inhibited, but degradation persisted. Immunoblotting revealed that GFP-eIF4E accumulated at higher levels in the leaf half co-expressing Flag-P25 compared to the half with Flag alone (Fig. 2a). Similarly, GFP-eIF4E co-expression enhanced Flag-P25 stability

(Fig. 2b), while actin levels remained unchanged, indicating a mutual stabilization through the P25-eIF4E interaction.

P25 alters the subcellular localization of eIF4E

To investigate the subcellular localization of eIF4E and P25, eIF4E-GFP and P25-GFP were transiently expressed in *N. benthamiana* via agroinfiltration. Co-expression with the membrane marker Gw5-RFP and the nuclear marker Ghd7-RFP revealed that eIF4E localized to both the nucleus and membrane (Fig. 3a), whereas P25 was diffusely distributed in the cytoplasm

at uncharacterized locations (Fig. 3b). When eIF4E-RFP was co-expressed with P25-GFP, the localization of eIF4E shifted from the nucleus to the cytoplasm, where it colocalized with P25 in distinct but unidentified cytoplasmic structures (Fig. 3c). These results demonstrate that P25 mediates the relocalization of eIF4E from the nucleus to the cytoplasm through direct interaction.

eIF4E binds specifically to the 3'-UTR of APV1

The interaction of P25 with eIF4E hints at the possibility that P25 might hijack eIF4E to initiate APV1 translation. To monitor the APV1 translation, 5'- and 3'-UTR

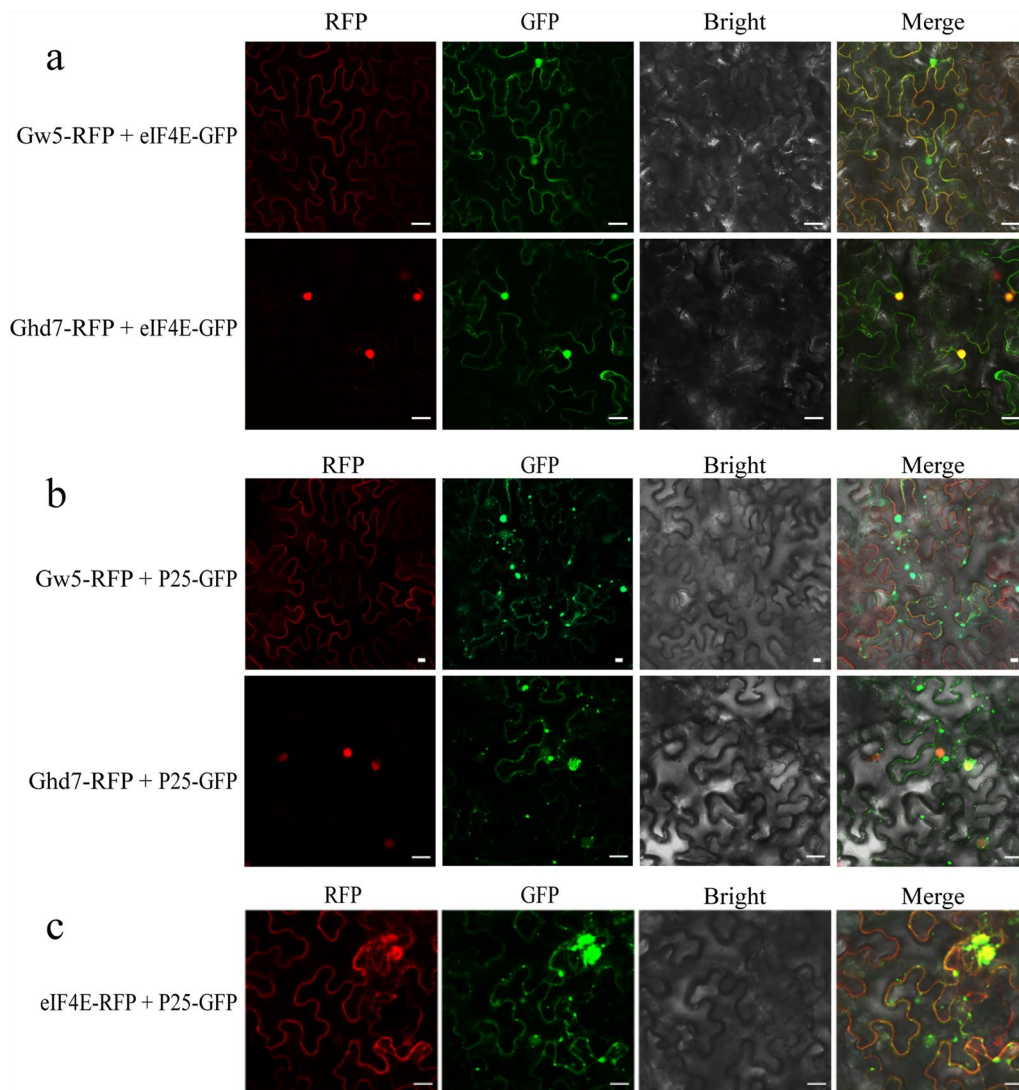


Fig. 3 P25 alters the subcellular localization of eIF4E. **a, b** Subcellular localization of eIF4E and P25. The fusion protein **a** eIF4E-GFP and **b** P25-GFP were transiently expressed in *N. benthamiana*, respectively. Gw5-RFP and Ghd7-RFP were co-expressed as a cell membrane marker and a nucleus marker, respectively. Localization of fluorescence signal in leaves of *N. benthamiana* was imaged by confocal microscopy at 60 hpi. Scale bars = 50 μ m. **c** P25 alters the subcellular localization of eIF4E. The fusion protein eIF4E-RFP and P25-GFP were transiently co-expressed through agroinfiltration in *N. benthamiana*, and leaves of *N. benthamiana* were imaged by confocal microscopy at 60 hpi. Scale bars = 50 μ m

of APV1 were inserted into the pSP-Luc+NF vector to generate chimeric luciferase constructs, including 5'-plasmid-Luc-3'-plasmid, 5'-UTR-Luc-3'-plasmid, 5'-plasmid-Luc-3'-UTR, and 5'-UTR-Luc-3'-UTR. The RNA was reverse-transcribed in vitro, and the translation efficiency was assessed in wheat germ extract. For uncapped RNA, 5'-plasmid-Luc-3'-plasmid exhibited minimal luciferase activity. Replacement of the 5' plasmid sequence with the APV1 5'-UTR (5'-UTR-Luc-3'-plasmid) slightly increased the activity, while substitution of the 3'-plasmid with the APV1 3'-UTR (5'-plasmid-Luc-3'-UTR) significantly enhanced translation. The 5'-UTR-Luc-3'-UTR construct showed the highest activity, confirming the importance of both UTRs (Fig. 4a). These results indicate that the 3'-UTR is more essential than the 5'-UTR for APV1 translation initiation.

To further investigate which region of APV1 genomic RNA was bound by eIF4E. RNA sequences of the entire 5'-UTR and 3'-UTR of APV1 were in vitro reverse-transcribed using SP6 RNA Polymerase. eIF4E-GST was synthesized and purified from *E. coli*. Electrophoretic mobility shift assay (EMSA) revealed that eIF4E-GST interacted with the 3'-UTR, but not with the 5'-UTR of APV1, resulting in a noticeable band shift (Fig. 4b). The band shift was reduced upon the addition of cold competitor 3'-UTR (Fig. 4c), indicating that eIF4E bound specifically to the 3'-UTR of APV1.

To further study the interaction between eIF4E and the 3'-UTR, the secondary structure of APV1-3'-UTR was analyzed using RNAfold, which revealed several stem-loop structures potentially involved in translation regulation and RNA-protein interactions. The red, green, and blue regions in the 3'-UTR are key functional areas likely affecting RNA stability, translation efficiency, and interactions with host factors (Additional file 2: Figure S4a). Energy and nucleotide conservation analyses highlight the levels of stable RNA secondary structure in the 3'-UTR. The greatest heights of all three curves, e.g., red (Minimum Free Energy, MFE), green (Partition Function Boltzmann ensemble, PF), and blue (Centroid), are distributed between 100–200 nt region (peak at 150 nt), indicating the most reliable folding region (Additional file 2: Figure S4b). The 3'-UTR-eIF4E binding model was further predicted by Alphafold Multimer, revealing a strong interaction, with the terminal region being crucial for eIF4E binding. This model provides insights into the role of APV1-3'-UTR in translation regulation (Additional file 2: Figure S4c). An enlarged view displays the critical interactions between APV1-3'-UTR and eIF4E. Hydrogen bonds and π - π stacking interactions stabilize the binding interface between G225 and the surrounding nucleotides in the RNA and the surface amino acids, such as W90 and E91 (green-circled) of eIF4E, suggesting that the 3' end structure of APV1-3'-UTR plays a key role

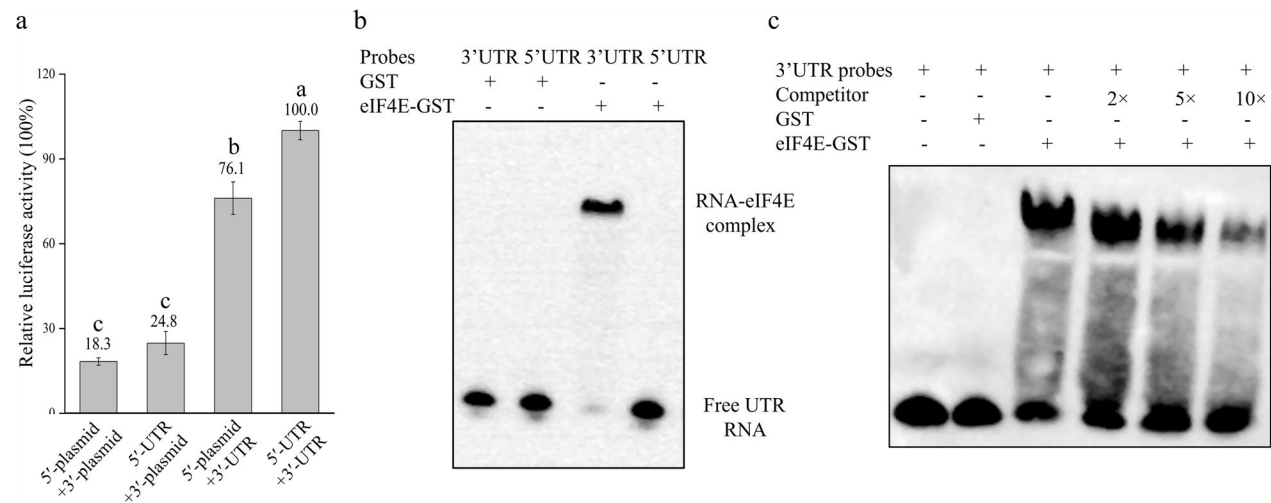


Fig. 4 eIF4E binds specifically to the 3'-UTR of APV1. **a** Monitoring the APV1 translation in wheat germ extract. 5'- and 3'-UTR of APV1 were inserted into pSP-luc+NF vector to generate chimeric luciferase constructs, including 5'-plasmid-Luc-3'-plasmid, 5'-UTR-Luc-3'-plasmid, 5'-plasmid-Luc-3'-UTR, and 5'-UTR-Luc-3'-UTR. The RNA was reverse-transcribed in vitro, and the translation efficiency was assessed in wheat germ extract. The reactions were incubated at 22 °C for 2 h with varying concentrations of P25 (0–64 ng). Luciferase activity (normalized to maximum) was measured after 2 h at 22 °C. A one-way ANOVA analysis was conducted using SPSS 26.0, and multiple comparisons were performed using the Tukey method. Different subtitles indicate significant differences ($P < 0.05$). **b, c** Electrophoretic mobility shift assay (EMSA) analysis of eIF4E binding to the 3'-UTR of APV1. **b** Binding of eIF4E-GST to biotin-labeled 5'-UTR and 3'-UTR of APV1. **c** Competition assay with unlabeled 3'-UTR. The protein-RNA complexes were resolved on a 6% native polyacrylamide gel after 5 min incubation and imaged using an Azure 200 Gel Documentation System (Azure Biosystems)

in eIF4E binding. Specifically, key amino acids in eIF4E, such as E91 and W90, directly interact with G225 and C224 (blue-circled) in the RNA, which may be vital for stable RNA binding. Additionally, amino acid residues in eIF4E, such as R195 and K189, form hydrogen bonds with A150 and U141 in the RNA, further strengthening the stability of the RNA-eIF4E interaction (Additional file 2: Figure S4d).

P25 enhances cap-independent translation efficiency

To investigate the role of P25 in APV1 translation, we added P25 into a wheat germ extract system to assess its effect on translation. When uncapped RNA was used, the addition of P25 significantly increased luciferase activity in constructs containing the 3'-UTR (5'-plasmid-Luc-3'-UTR and 5'-UTR-Luc-3'-UTR), whereas no significant increase was observed in constructs lacking the 3'-UTR (5'-plasmid-Luc-3'-plasmid and 5'-UTR-Luc-3'-plasmid) across the same P25 concentration range (Fig. 5a), suggesting that P25 enhances viral cap-independent translation. In contrast, with capped RNA, luciferase activity increased uniformly across all constructs, although the differences between them were less pronounced. These results indicate that the 3'-UTR is essential for APV1 translation initiation and that P25 modulates translation via its interaction with the 3'-UTR. Furthermore, P25 significantly enhanced translation in constructs containing the 3'-UTR (Fig. 5b), further supporting its role in promoting cap-independent translation.

To determine whether the interaction between eIF4E and P25 is essential for P25-mediated enhancement of cap-independent translation, a truncated form of P25 lacking the N-terminal region ($\Delta 1-35$ aa) interacting with eIF4E was added to the wheat germ translation system, no enhancement of translation efficiency was observed with either uncapped (Fig. 5c) or capped RNA (Fig. 5d). These results strongly demonstrate that the interaction between eIF4E and P25 is indispensable for the cap-independent translation enhancement mediated by P25.

P25 binds to APV1 3'-UTR and enhances the recruitment of eIF4E to 3'-UTR

To decipher the mechanism of cap-independent translation mediated by P25, we further investigated whether P25 binds to the 3'-UTR. EMSA results showed that P25 interacts with the 3'-UTR, but not with 5'-UTR, resulting in a prominent 3'-UTR band shift (Fig. 6a). The band shift was reduced upon the addition of cold competitor 3'-UTR, indicating the binding specificity (Fig. 6b). To determine whether P25 could affect the recruitment of eIF4E to 3'-UTR, P25 protein was gradually added to the eIF4E-3'-UTR complex. EMSA results clearly indicated that the addition of P25 significantly enhanced the

binding of eIF4E to the 3'-UTR (Fig. 6c). Therefore, it is reasonable to speculate that P25 enhances the cap-independent translation through enhancing the recruitment of eIF4E to the 3'-UTR.

AlphaFold was further used to predict a three-dimensional model of the interaction among APV1-3'-UTR, P25, and eIF4E. The predicted structure suggests that the APV1 3'-UTR (brown), P25 (blue), and eIF4E (green) may form a stable ternary complex. P25 is positioned between eIF4E and the 3'-UTR, forming distinct interaction interfaces on both sides, implying its potential role as a bridging molecule to stabilize the complex. Notably, the model reveals limited direct contact between eIF4E and the viral 3'-UTR, suggesting that P25 may not enhance eIF4E's intrinsic RNA-binding ability. Instead, it likely facilitates the spatial positioning or recruitment of eIF4E through its own RNA-binding activity, thereby promoting the assembly of the translation initiation complex (Additional file 2: Figure S5). This conformation supports a proposed "translational bridge" model, in which P25 mediates the functional coupling of eIF4E and the viral 3'-UTR. The 3'-UTR functions as a 3'-CITE for APV1 translation.

P25 enhances the recruitment of eIF4E to APV1 virion

To investigate whether P25 enhances the recruitment of eIF4E to APV1 virion other than 3'-UTR, APV1 virions were extracted from APV1-infected plant samples by density gradient centrifugation. Fusion proteins GST-eIF4E, His-P25, and APV1-CP were purified from *E. coli* using prokaryotic expression systems. The microtiter plate was initially coated with BSA, GST, GST-eIF4E, His-P25, or a combination of GST-eIF4E and His-P25. The extracted APV1 virions or APV1-CP were then added, and the captured APV1 virions or CP were detected using a monoclonal antibody against APV1 CP. The enzyme-linked immunosorbent assay (ELISA) revealed that both GST-eIF4E and His-P25 could capture APV1 virions at low levels, while CP protein was not captured by the GST-eIF4E coating, suggesting that eIF4E binds directly to the RNA genome of the APV1 virion rather than the coating CP. Adding P25 significantly enhanced virion capture by GST-eIF4E (Fig. 7a). The finding was further supported by GST pull-down assays. GST-eIF4E was able to capture APV1 virions from the virion extract, both in the presence and absence of His-P25. However, the addition of His-P25 significantly increased the amount of co-precipitated APV1 virions (Fig. 7b). To explore the role of P25 in recruiting eIF4E to the APV1 virion, increasing amounts of His-P25 were added to the GST-eIF4E pull-down system. Western blotting revealed a direct correlation between the amount of His-P25 added and the quantity of APV1 virions co-precipitated by GST-eIF4E

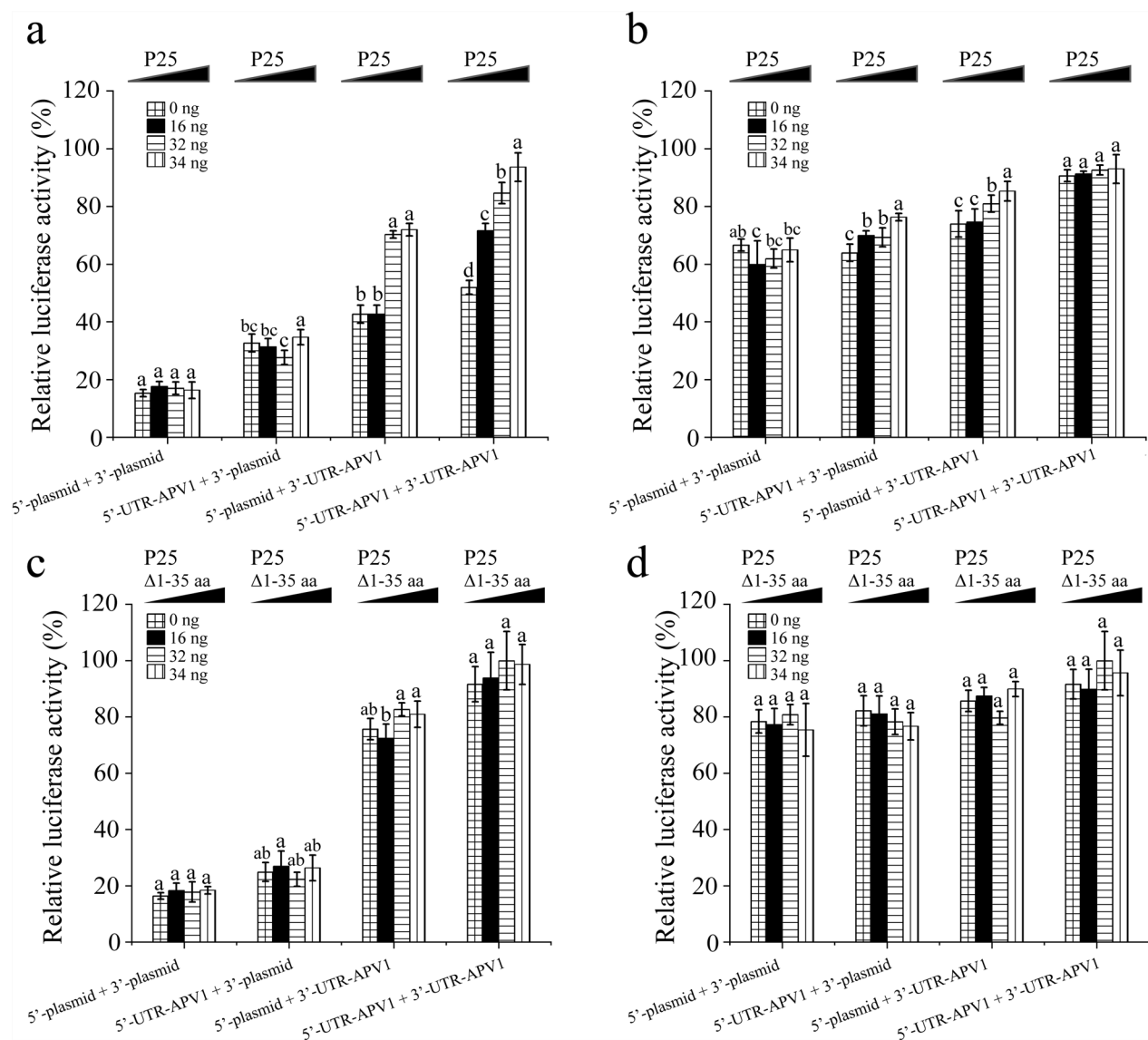


Fig. 5 P25 enhances cap-independent translation efficiency. Luciferase constructs under the SP6 promoter, e.g., 5'-plasmid-Luc-3'-plasmid, 5'-UTR-Luc-3'-plasmid, 5'-plasmid-Luc-3'-UTR, and 5'-UTR-Luc-3'-UTR were in vitro transcribed. **a, c** Luciferase relative light units were measured for uncapped and **b, d** capped 5'-plasmid-Luc-3'-plasmid, 5'-UTR-Luc-3'-plasmid, 5'-plasmid + 3'-UTR-APV1, and 5'-UTR-APV1 + 3'-UTR-APV1 in wheat germ translation extract. **a, b** The translation systems were added with 0, 16, 32, and 64 ng P25 protein or **c, d** the truncated P25_{36-215aa} corresponding to the final concentrations of 0, 0.32, 0.64, and 1.28 ng/μL, respectively. Luciferase activity was measured as in Fig. 4a

(Fig. 7c). This result better demonstrates the effect of P25 on enhancing the recruitment of eIF4E to APV1 virions. To further clarify whether GST-eIF4E captured the APV1 virion or APV1-CP, Y2H, LCA, and BiFC assays were used to examine the interactions between CP and eIF4E. No interaction was detected (Additional file 2: Figure S6), while a strong interaction between P25 and CP was observed in all three assays (Additional file 2: Figure S7). These findings suggest that P25 enhances the recruitment of eIF4E to the APV1 virion by anchoring eIF4E to the

APV1 virion through its interaction with CP and binding to 3'-UTR.

eIF4E knockout or P25^{I8A+C11A} mutation inhibits APV1 infection

To investigate whether P25-mediated translation is essential for APV1 infection, a Cas9/CRISPR-mediated *NbeIF4E* mutant plant was generated. This *NbeIF4E* mutant plant has a nucleotide insertion in the target sequence, resulting in a frameshift mutation (Additional

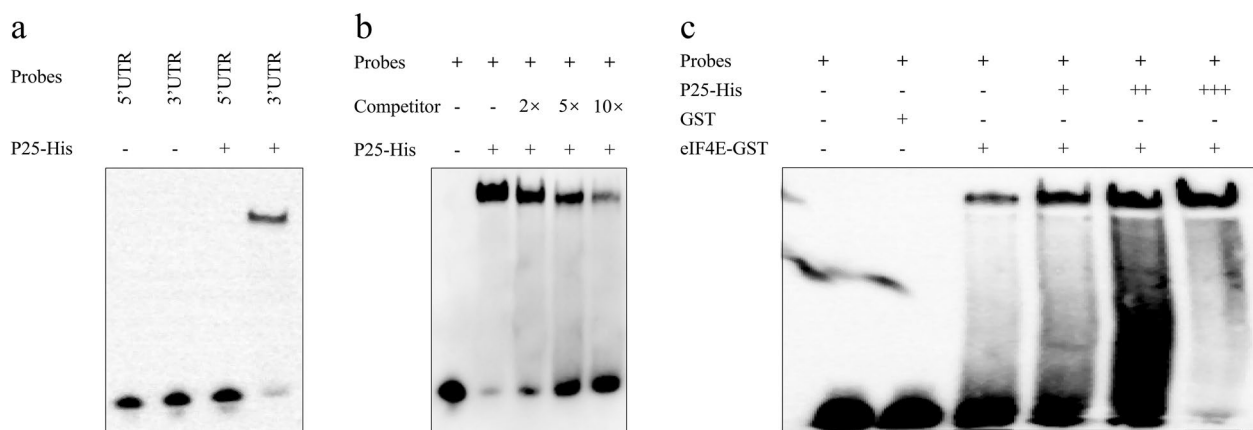


Fig. 6 P25 binds to 3'-UTR of APV1. **a** The 5'-UTR and 3'-UTR of APV1 were incubated with P25-His. **b, c** The 3'-UTR of APV1 were incubated with P25-His with competed unlabeled 3'-UTR, The 3'-UTR of APV1 were incubated with eIF4E-GST and with increasing P25-His. The protein-RNA complexes were resolved and imaged as in Fig. 4

file 2: Figure S8). When a GFP-tagged infectious cDNA clone of APV1 was agroinfiltrated into the *eIF4E* knockout *N. benthamiana* line, GFP signal density and intensity were markedly reduced compared with wild-type plants, underscoring the critical role of eIF4E in APV1 infection (Fig. 8a, b). Based on earlier predictions, I8 and C11 are key residues of P25 that form the specific interaction interface with eIF4E (Additional file 2: Figure S1). To test whether this interaction is essential for APV1 infection, we introduced a P25^{I8A+C11A} double mutation into the GFP-tagged APV1 cDNA clone. Yeast-two-hybrid and LCA results showed that mutation of both residues of P25 abolishes the interaction with eIF4E (Additional file 2: Figure S9). This mutation strongly inhibited APV1 infection (Fig. 8c). Moreover, when the P25^{I8A+C11A} mutant clone was infiltrated into *eIF4E* knockout plants, viral infection was nearly abolished (Fig. 8d, e). Western blot analysis using anti-GFP and anti-APV1-CP antibodies further showed that both *eIF4E* knockout and P25^{I8A+C11A} mutation reduced the accumulation of GFP and CP proteins, indicating the inhibition of APV1 infection (Fig. 8f).

Discussion

To date, seven distinct classes of 3'-CITEs have been identified and classified primarily based on the shapes of their RNA secondary structures (Nicholson and White 2011; Truniger et al. 2017). The first reported 3'-CITE, the translation enhancer domain (TED) from Satellite tobacco necrosis virus (STNV), adopts an elongated stem-loop structure with internal bulges and a terminal loop (Danthinne et al. 1993). Subsequent studies revealed further structural diversity: the BYDV-like element (BTE) in Barley yellow dwarf virus exhibits a

cloverleaf-like architecture, with radiating helices varying in number across viral genera, e.g., two in necroviruses, three in luteoviruses, and five in dianthoviruses (Miras et al. 2017a; Wang et al. 2010). In Panicum mosaic virus (PMV), the Panicum mosaic virus translation element (PTE) class features two short helices flanked by a pyrimidine-rich C-domain and a guanylate-rich G-domain, with its pseudoknot proposed to recruit eIF4E (Wang et al. 2009). Additional 3'-CITE structural classes include the compact I-shaped structures (ISS) in *Tomoviridae* (Miras et al. 2017b; Truniger et al. 2008), the Y-shaped structures (YSS) with extended helices in *Tomovirus* spp. (Danthinne et al. 1993), the three-dimensional T-shaped structure (TSS) in Turnip crinkle virus (TCV) (McCormack et al. 2008), and the small (55 nt) CXTE with a bipartite helical design (Miras et al. 2014). The 3'-UTR of APV1 exhibits a unique architecture distinct from known 3'-CITEs. While its predicted secondary structure shares superficial similarities with the YSS of Tomato bushy stunt virus (TBSV) (Fabian and White 2006), such as a Y-shaped branch, key differences exist: (1) APV1's branching arms are markedly shorter; (2) it contains a large central loop absent in YSS, and (3) its putative eIF4E-binding residues localize to the terminal loop (Additional file 2: Figure S4), contrasting with YSS's branch-localized eIF4E interaction site. These structural and functional disparities suggest that APV1's 3'-UTR represents a novel 3'-CITE class existing in the *Closteroviridae* family, which we propose to designate the *Areca palm velarivirus element* (APVE).

In most characterized 3'-CITEs, translation initiation requires formation of intramolecular RNA-RNA interactions between complementary sequences in the 3'-CITE and 5'-UTR of viral genomes (Nicholson and

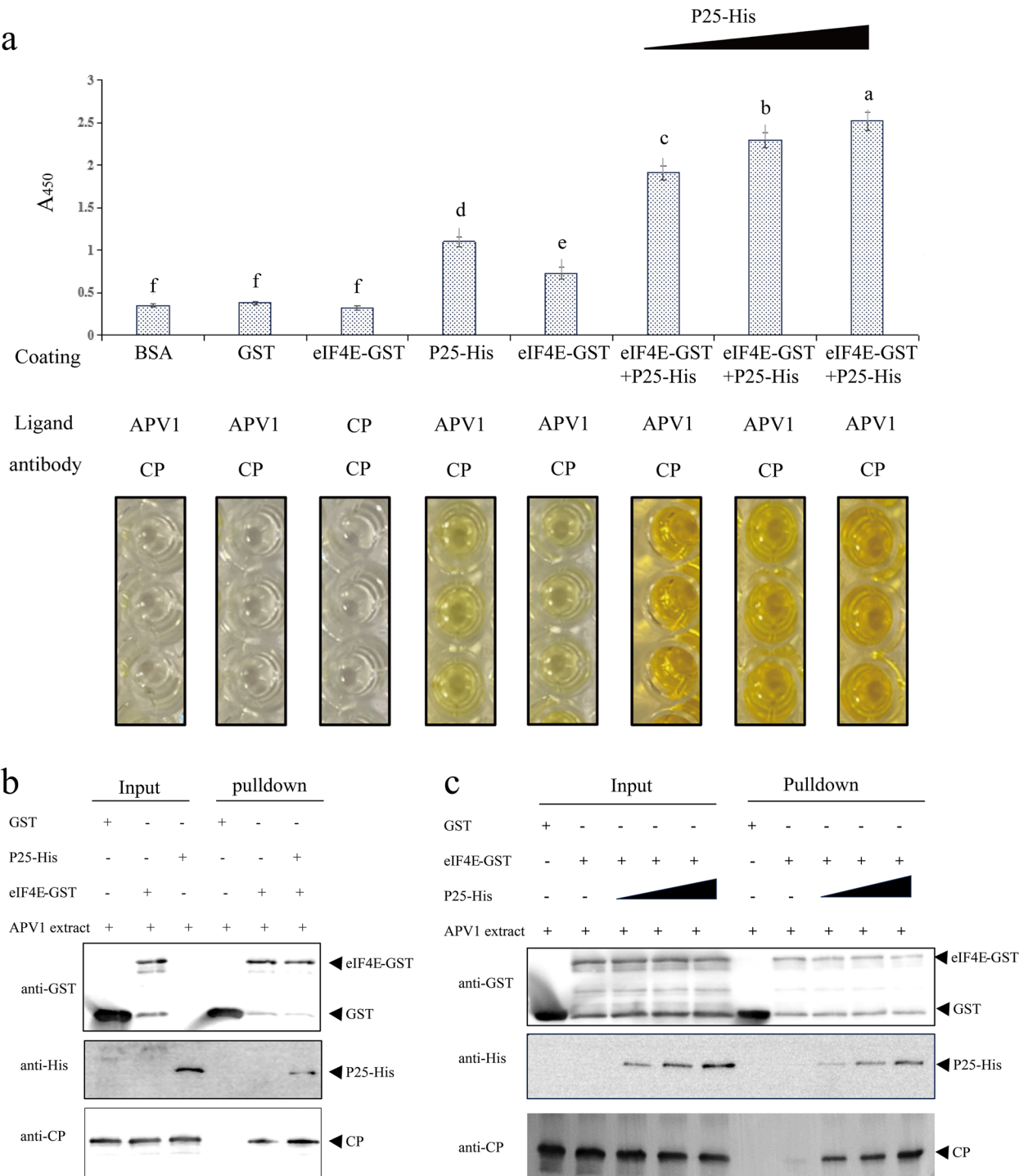


Fig. 7 P25 enhances the recruitment of eIF4E to APV1 virion. **a** The enzyme-linked immunosorbent assay (ELISA) for detection of in vitro interaction between eIF4E and APV1 virion extract from leaves of *N. benthamiana* infected by APV1 infectious clone. The microtiter plate was first coated with BSA, GST, GST-eIF4E, His-P25, or a combination of both GST-eIF4E and His-P25, and then incubated with APV1 virion extract or APV1 coat protein (CP). The captured virion or CP was detected using a monoclonal antibody against APV1 CP as primary antibody followed by incubation with secondary antibodies conjugated to horseradish peroxidase (HRP). A one-way ANOVA analysis was conducted using SPSS 26.0, and multiple comparisons were performed using the Tukey method. Different subtitles indicate significant differences ($P < 0.05$). **b** GST pull-down assay for detection of in vitro interaction of eIF4E with APV1 virion. **c** The pull-down assay was repeated with the gradually increased P25 protein. GST-eIF4E immobilized on glutathione-Sepharose beads was incubated with virion extract with or without P25 protein. Beads were washed and proteins were analyzed by Western blot assays using anti-GST (upper panel), anti-His (middle panel), and anti-CP (lower panel) antibodies

White 2011; Miras et al. 2017a 2011). For instance, BTE-mediated translation strictly depends on long-range base-pairing between its terminal loop and the 5'-UTR (Guo et al. 2001). While we identified potential complementary sequences between APV1's 5'- and 3'-UTRs, these sequences were notably absent from loop regions, suggesting such RNA-RNA interactions may not occur under physiological conditions. How to initiate the translation of APV1 requires further investigation.

Viruses lack the capacity to encode proteins for viral mRNA translation and must instead hijack the host's protein synthesis machinery to produce viral proteins. However, few viral proteins involved in the recruitment of translation initiation factors have been identified, with the exception of genome-linked viral proteins (VPgs) reported in *Potyviridae*. The VPg covalently binds to the 5' end of potyviral RNAs, mimicking a cap structure to interact with translation initiation factors and directly enhancing translational efficiency (Khan et al. 2008; Miyoshi et al. 2008). To the best of our knowledge, APV1-encoded P25 is the first viral protein identified to play a role in 3'-CITE-mediated translation initiation. P25 interacts with eIF4E both in vivo and in vitro (Fig. 1). When transiently expressed in *N. benthamiana*, eIF4E localized to the nucleus, while P25 was found in both the nucleus (Fig. 3a) and an undefined cytoplasmic location (Fig. 3b). However, when eIF4E and P25 were co-expressed, eIF4E relocated to the cytoplasm (Fig. 3c), indicating that P25 binding alters eIF4E's cellular distribution. Additionally, both P25 and eIF4E alone could bind APV1 particles from viral extracts, but their combination significantly increased virion capture in ELISA assays, which was further confirmed by pull-down assays (Fig. 7). The AlphaFold model reveals limited direct contact between eIF4E and the viral 3'-UTR. P25 likely facilitates the spatial positioning or recruitment of eIF4E through its own RNA-binding activity. P25 mediates the functional coupling of eIF4E and the viral 3'-UTR (Additional file 2: Figure S5). These results strongly suggest that P25 "hijacks" eIF4E from the nucleus to the cytoplasm, facilitating viral genome recruitment for translation initiation. Furthermore, the addition of P25 to a wheat germ extract

system markedly enhanced APV1 translation efficiency in a cap-independent manner. These findings highlight P25 as a key viral regulator of APV1 translation initiation, offering new insights into the molecular mechanisms of *Closterovirus* infection.

Naturally occurring *eIF4E* resistance alleles have been identified in crops such as pepper, tomato, and lettuce (Robaglia and Caranta 2006). These alleles often confer durable resistance by maintaining functionality while harboring mutations that disrupt viral interactions. Notably, some recessive *eIF4E* genes have been successfully deployed in agriculture for over 50 years without resistance breakdown (van Schie and Takken 2014). Although certain *eIF4E* knockouts can lead to yield loss or sterility (Hoffie et al. 2021; Pechar et al. 2022), other cases—such as the *eIF(iso)4E* knockout in pepper—demonstrate that such modifications can be both viable and fertile (Ruffel et al. 2006; Chandrasekaran et al. 2016). Moreover, CRISPR/Cas9-mediated editing has enhanced wheat resistance to yellow mosaic virus without compromising yield (Kan et al. 2023). However, CRISPR/Cas9-mediated editing of *eIF4E* knock-out did inhibit APV1 infection, but not completely (Fig. 8), suggesting that additional eIF4E isoforms might be involved in APV1 translation. This study provides a foundational framework for developing *eIF4E*-based genome editing strategies to combat closteroviruses, further investigation is still required.

In this study, we demonstrate that P25, encoded by APV1, interacts with eIF4E, enhances its stability, redirects it from the nucleus to the cytoplasm, and promotes its recruitment into APV1 virions. Furthermore, P25 significantly enhances cap-independent viral translation. Structural modeling reveals that P25 facilitates eIF4E recruitment via its intrinsic RNA-binding activity, thereby promoting the assembly of the translation initiation complex. These findings support a proposed "translational bridge" model, wherein P25 mediates functional coupling between eIF4E and the viral 3'-UTR. In summary, P25 hijacks host eIF4E and enhances viral translation by reinforcing the interaction between eIF4E and the 3'-CITE.

(See figure on next page.)

Fig. 8 *eIF4E* knockout or P25^{18A+C11A} mutation inhibits APV1 infection. The expression cassette of full-length APV1 genomic RNA is integrated into a mini-binary vector pCB301 backbone. GFP CDS sequence was inserted at the junction of the ORF5 and ORF6. The GFP-tagged infectious cDNA clone of WT APV1 and APV1 with P25^{18A+C11A} mutation were agroinfiltrated into **a, c** WT *N. benthamiana* or **b, d** *eIF4E* knockout plant, respectively. **e** The APV1 accumulation was indicated by the GFP fluorescence density and intensity at 15 dpi. **f** Western-blot was used to analyze GFP and APV1 CP accumulation using anti-GFP and anti-APV1-CP monoclonal antibodies at 15 dpi, respectively. The level of actin was used as control. A one-way ANOVA analysis was conducted using SPSS 26.0, and multiple comparisons were performed using the Tukey method. Different subtitles indicate significant differences ($P < 0.05$)

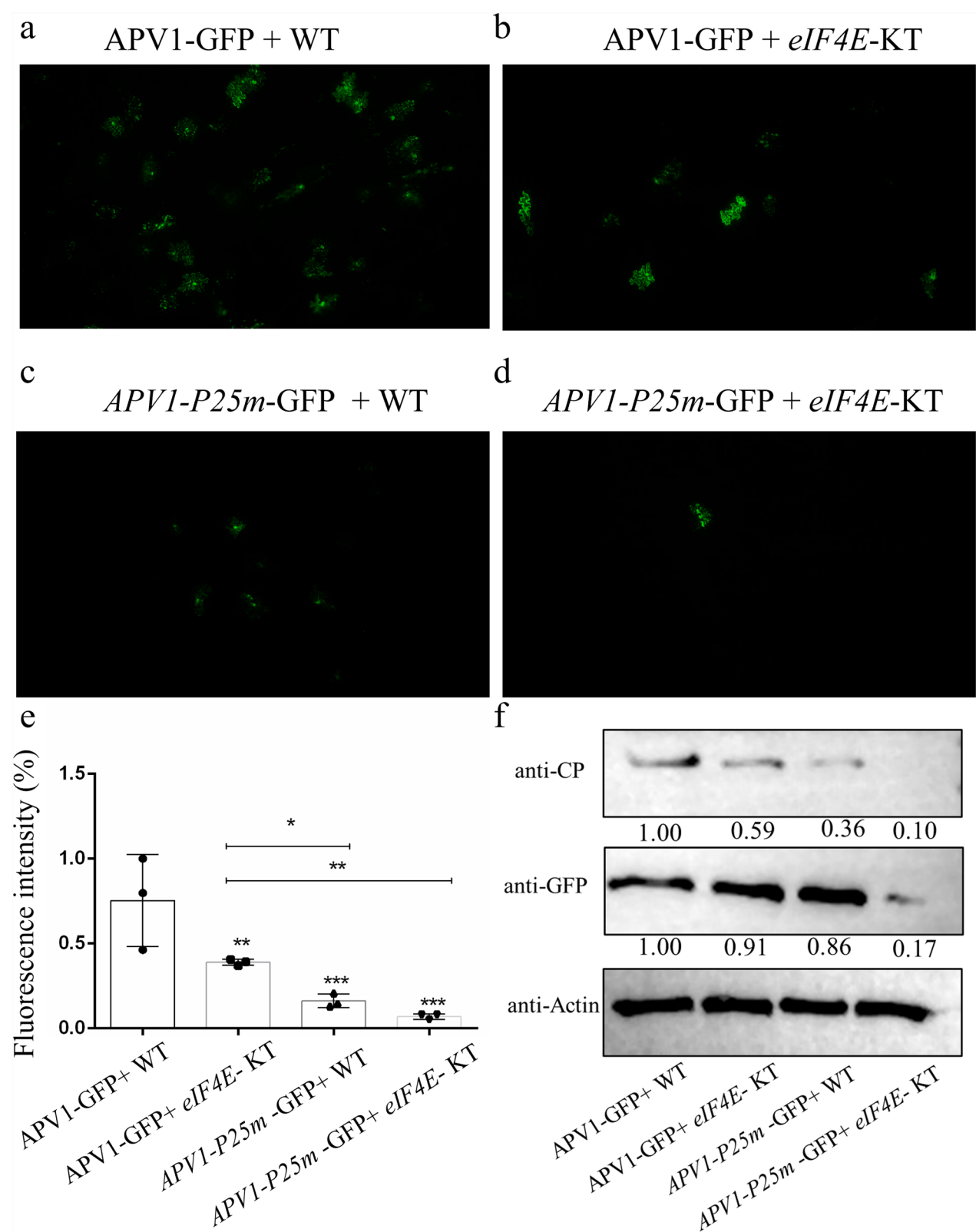


Fig. 8 (See legend on previous page.)

Conclusion

The P25 protein encoded by Areca palm velarivirus 1 (APV1) interacts with eIF4E, enhances its stability, alters its subcellular localization, and facilitates the recruitment of eIF4E to APV1 virions through its intrinsic RNA-binding activity. Moreover, P25 enhances cap-independent viral translation. This study identifies P25 as the first viral regulator of 3'-CITE-mediated translation initiation, offering new insights into the molecular mechanisms of viral infection.

Methods

Plant materials and virus inoculation

Areca palm seedlings were inoculated with mealybugs (*Ferrisia virgata* or *Pseudococcus cryptus*) carrying APV1, following previously established protocols (Zhang et al. 2022; Zhao et al. 2024). *N. benthamiana* plants were inoculated using an infectious cDNA clone of APV1 through agroinfiltration. Transgenic lines of *N. benthamiana* expressing either the coat protein (CP) or the P25 protein of APV1 are maintained in our laboratory. The P25 protein transgenic lines were developed to investigate its role in viral infection and host interaction. All plants were cultivated in a growth chamber under controlled conditions of 25 °C with a 16-h light period and 18 °C with an 8-h dark period, with a relative humidity of 75%. Regular monitoring was performed to ensure uniform growth and infection status across all experimental groups.

Subcellular localization

pCambia1302 is a binary vector for the expression of proteins from the 35S promoter. The coding sequences of *eIF4E* and *P25* were amplified using RT-PCR and subsequently cloned into the pCambia1302-GFP expression vector, yielding the constructs pCambia1302-GFP-eIF4E and pCambia1302-GFP-P25. To further investigate localization, the coding sequence of *eIF4E* was fused to the RFP protein under the control of the CaMV 35S promoter, creating the construct 35S::RFP-AceIF4E. The resulting vectors were agroinfiltrated into the leaves of *N. benthamiana* using standard procedures. Fluorescence imaging was performed 72 hpi using a Zeiss LSM 780 confocal laser scanning microscope. Primers utilized for amplification are detailed in Additional file 1: Table S2. Gene amplification was conducted with PrimeSTAR® GXL DNA Polymerase (TaKaRa, Dalian), and vector construction was carried out using the ClonExpress® Ultra One Step Cloning Kit (Vazyme, Nanjing, China; Cat #C115).

Yeast two-hybrid assay

To identify potential proteins interacting with APV1 P25, a cDNA library from the areca palm was constructed using the yeast two-hybrid (Y2H) system by OE Biotech (Shanghai, China). The bait vector pGBKT7-P25 was constructed, and Y2H screening was performed using the Yeastmaker™ Yeast Transformation System 2 (Clontech, USA). To confirm the interaction of candidate proteins in yeast, the prey vector pGADT7-eIF4E was co-transformed with the bait vector pGBKT7-P25 into the yeast strain Y187. The transformed yeast cells were plated on minimal media with triple dropout (TDO, SD/-Trp/-Leu/-His) and quadruple dropout (QDO, SD/-Trp/-Leu/-His/-Ade) media, supplemented with Aureobasidin A (AbA) and X-α-Gal, respectively, for selection and interaction detection. Positive control plasmids, pGBKT7-53 and pGADT7-T, and negative control plasmids, pGBKT7-Lam and pGADT7-T, were included in the screening. Three independent experiments were conducted to validate the reproducibility and reliability of the results.

BiFC assay and CLA assays

Vectors pFGC-YN173-eIF4E and pFGC-CN155-P25 were constructed to study the interaction between eIF4E and P25. The YN-CN vector combinations were agroinfiltrated into *N. benthamiana* leaves. At 72 hpi, epidermal cells from 1 cm of the infiltrated leaf explants were collected for confocal microscopy observation using a Zeiss LSM 780 microscope.

For the complementary luciferase assay (CLA), constructs AccLUC-P25 and AcnLUC-eIF4E were used as previously outlined. The nLUC/cLUC vector pairs were co-infiltrated into *N. benthamiana* leaves via agroinfiltration. The leaves were harvested at 48 hpi, treated with 1 mM luciferin, and subjected to imaging using the NightShade LB 985 In Vivo Plant Imaging System (Berthold Technologies, Germany), two minutes after luciferin treatment.

In vitro GST pull-down assay

The coding sequence of AceIF4E was cloned into the pGEX-6p vector, and the open reading frame (ORF) of P25 was cloned into the pET28a vector. Recombinant GST-AceIF4E and His-P25 proteins were expressed in *E. coli* BL21 (DE3) and purified using the GST-tag and His-tag Protein Purification Kit (Beyotime, Shanghai, China, Cat #P2226). The pull-down assay was conducted using the BersinBio™ GST-pulldown Kit (Cat #Bes3012), following the manufacturer's protocol. For each assay, 5 µg of His-P25 protein was incubated with an equal amount of GST-AceIF4E and GST on BersinBio GSH magnetic

beads at 4 °C for 12 h. The proteins bound to the beads were collected, separated by SDS-PAGE, and analyzed by Western blot using anti-GST antibody (TransGen Biotech, Beijing, China, Cat #HT601) and anti-His antibody (TransGen Biotech, Beijing, China, Cat #HT501).

Co-immunoprecipitation (Co-IP) assay

Co-IP assays were performed by agroinfiltration of pCambia1302-GFP-eIF4E together with pCambia1302-Flag-P25 into *N. benthamiana* leaves. At 72 hpi, the infiltrated leaves were harvested, and total proteins were extracted from frozen, ground tissue using an ice-cold extraction buffer containing 50 mM HEPES (pH 7.5), 150 mM KCl, 1 mM EDTA, and 0.5% Triton X-100, supplemented with 1 mM dithiothreitol (DTT) and a 1× protease inhibitor cocktail (Roche). The protein lysates were clarified by centrifugation at 11,000×g for 15 min at 4 °C, and the supernatants were incubated with Anti-GFP Magnetic Beads (Yamei, Shanghai, China, Cat #YJ010) at room temperature for three hours with gentle agitation. After incubation, the magnetic beads were washed three times with the extraction buffer. The bound proteins were eluted and analyzed via Western blotting using anti-Flag antibodies.

Protein extraction and Western blot analysis

Total proteins from plant leaf tissues were extracted using a lysis buffer comprising 50 mM Tris-HCl (pH 6.8), 4.5% SDS, 7.5% β-mercaptoethanol, and 9 M urea. The lysates were centrifuged at 10,000×g for 15 min at 4 °C, and the supernatant containing soluble proteins was carefully collected. Protein detection was carried out using specific monoclonal antibodies, including anti-GFP (HT801, TransGen Biotech, Beijing, China), anti-His (HT501, TransGen), anti-GST (HT601, TransGen), and anti-Flag (R24091, ZEN-BIOSCIENCE, Chengdu, China). Primary antibody detection was followed by incubation with secondary antibodies conjugated to horseradish peroxidase (HRP), including HRP-conjugated goat anti-mouse IgG (HS201, TransGen) and HRP-conjugated goat anti-rabbit IgG (AS014, ABclonal, China). Protein bands were visualized using a chemiluminescence substrate (SuperSignal™ West Pico PLUS Chemiluminescent Substrate, Thermo Fisher Scientific) and documented on chemiluminescent film.

Confocal imaging analysis

Fluorescence signals from eGFP, YFP, and RFP fluorophores were captured under optimized excitation and emission parameters: eGFP was excited at 488 nm with emission collected between 490–540 nm; YFP was excited at 514 nm with emission detected from 520–560 nm; and RFP was excited at 561 nm with emission

captured within the range of 560–620 nm. Imaging was conducted using a laser scanning confocal microscope (LSM 780, Carl Zeiss), and the images were subsequently processed and analyzed using the ZEN 2012 software suite (Carl Zeiss).

APV1 virion purification method

Leaf samples from *N. benthamiana* infected with APV1 were ground in liquid nitrogen and resuspended with 5 mL of pre-chilled extraction buffer (0.1 M Tris-HCl buffer at pH 7.4 with 0.5% Na₂SO₃, 0.5% Triton X-100, 0.5% β-mercaptoethanol, and 5% sucrose) per gram of powder. The mixture was homogenized on a magnetic stirrer for one hour. The resulting suspension was filtered through gauze and then centrifuged at 13,400 g for 15 min at 4 °C to remove the pellet. PEG600 (final 5%) and NaCl (final 0.6% W/V) were added to the supernatant and stirred for one hour on a magnetic stirrer. The mixture was then centrifuged at 8000 g for 15 min at 4 °C. The pellet was resuspended in 1/10th volume of resuspension buffer (0.01 M Tris-HCl buffer at pH 7.4, 1 mM EDTA) and dialyzed overnight in a dialysis bag. The next day, the solution was centrifuged at 25,000 g for 10 min at 4 °C, and the supernatant was retained. The pellet was resuspended again in resuspension buffer and centrifuged at 25,000 g for 10 min at 4 °C, and the supernatant was again retained. The two supernatants were combined to obtain the purified APV1 extract.

Luc constructs and in vitro transcription

APV1-5'UTR was obtained by PCR amplification using primers containing the T7 promoter sequence. The APV1-5'UTR sequence was directionally cloned into the *Kpn*I and *Bst*EII sites of the pSP-luc+NF plasmid. The APV1-3'UTR was also amplified by PCR and directionally cloned into the *Xba*I and *Hpa*I sites of the pSP-luc plasmid. The in vitro RNA transcription reaction was carried out using Roche's in vitro transcription kit, with the following reagents and conditions: 2 µL of nuclease-free water, 1.5 µL of 10×T7 reaction buffer (final 0.75×), 1.5 µL of ATP (100 mM, final 7.5 mM), 1.5 µL of GTP (100 mM, final 7.5 mM), 1.5 µL of UTP (100 mM, final 7.5 mM), 1.5 µL of CTP (100 mM, final 7.5 mM), 2 µL of template DNA (0.05 µg/µL), 0.5 µL of RNase inhibitor, and 2 µL of T7 RNA polymerase. The total reaction volume was 20 µL, and the reaction was incubated at 37 °C for four hours. RNA cap addition was performed using reagents from Roche under the following conditions: nuclease-free water 5 µL, 10×T7 reaction buffer 2 µL (final concentration 1×), ATP 10 mM, UTP 10 mM, CTP 10 mM, GTP 2 mM, m7(3'-O-methyl)-G(5')ppp(5')G 8 mM, template DNA 2 0.05 µg/µL, RNase inhibitor 0.5 µL, and T7 RNA polymerase 2 µL. The total reaction

volume was 20 μ L. The reaction was incubated at 37 °C for 2–4 h, and after transcription, RNA products were purified. The molar ratio of cap analog to GTP is 4:1.

RNA electrophoretic mobility shift assay (EMSA)

Approximately 200 ng of the APV1-5'-UTR or APV1-3'-UTR RNA was dissolved in water and then refolded in a buffer containing 10 mM Tris–HCl (pH 7.4), 5 mM $MgCl_2$, and 300 mM NaCl. For the refolding process, the RNA was heated at 90 °C for one minute, followed by the addition of an appropriate volume of refolding buffer, and then incubated at 50 °C for 10 min, with a final incubation on ice for 5 min. The refolded RNA samples were incubated at room temperature for 30 min with equimolar amounts of Areca palm eIF4E or P25 protein (5 μ g), ensuring adequate binding of the protein to the RNA. Subsequently, the protein-RNA complex samples were mixed with an equal volume of gel loading buffer containing 30% glycerol and 0.1% bromophenol blue, preparing the samples for electrophoresis. Electrophoresis was performed using a 6% native polyacrylamide gel, with the following conditions: the gel was run at 100 V for two hours at 4 °C, and a transfer was performed at 380 mA for 50 min. The Beyotime Chemiluminescent RNA EMSA Kit (Catalog No. GS606) was used during electrophoresis. After electrophoresis, the gel was stained with ethidium bromide (EtBr) and imaged using the Azure 200 Gel Documentation System (Azure Biosystems).

In vitro translation

The in vitro-transcribed mRNA was used for translation using the Wheat Germ Extract System (Catalog No. L4380, Promega). The translation reaction mixture was prepared as follows: 25 μ L of Wheat Germ Extract, 10 μ L of mRNA (final amount 100 μ g), 1 μ L of RNasin® Ribonuclease Inhibitor (40 U/ μ L), 1 μ L of 1 M potassium acetate, and nuclease-free water to a final volume of 50 μ L. The reaction mixture was incubated at 25 °C for 180 min.

CRISPR editing *eIF4E* knockout in *N. benthamiana*

Gene sequence of *N. benthamiana eIF4E* (*NbeIF4E*, NbS00018008g0003) was blasted against *N. benthamiana* genome, the specific CRISPR editing target sequence was determined and inserted into pEGCas9Pubi-B vector to generate the CRISPR vector, which was transformed into *N. benthamiana* through *Agrobacterium*. The knockout T1 seedlings were verified by Sanger-sequencing and used for APV1 infection.

Abbreviations

APV1	Areca palm velarivirus 1
BiFC	Bimolecular fluorescence complementation
CHX	Cycloheximide
Co-IP	Co-immunoprecipitation
CP	Coat protein

eIF4E	Eukaryotic initiation factor 4E
GST	Glutathione S-transferase
LCA	Luciferase complementation assays
3'-CITEs	3' cap-independent translation enhancer
UTR	Untranslated region
YLD	Yellow leaf disease

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42483-025-00409-2>.

Additional file 1: Table S1. Interacted proteins identified by yeast two hybrid against cDNA library of Areca palm using pGBKT7-APV1-P25 as prey. **Table S2.** Primers and sequences used in this study.

Additional file 2: Figure S1. Three-dimensional prediction model of the interaction between P25 and eIF4E. **Figure S2.** Interaction between eIF4E and truncated P25. **Figure S3.** Determination of the domains of interaction between eIF4E and P25. **Figure S4.** Prediction of secondary structure APV1-3'-UTR and the interaction with eIF4E. **Figure S5.** Predicted structural model of the ternary complex of APV1-3'-UTR, P25, and eIF4E. **Figure S6.** Determination of the interaction between eIF4E and CP. **Figure S7.** Determination of the interaction between CP and P25. **Figure S8.** Alignment of the Cas9/CRISPR-mediated NbeIF4E mutant plant with the wild-type sequence.

Author contributions

HW and XH designed the research; SW conducted the experiments; SW analyzed the data; SW wrote the original draft; HW and XH reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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