INTRODUCTION

Plant viruses have limited genome sizes, can encode only a few proteins, and thus must recruit host factor(s) to survive and replicate in infected cells (Ahlquist et al., 2003; Nagy, 2008; Noueiry & Ahlquist, 2003; Sanfaçon, 2015). Numerous host factors that influence plant virus infections in plants have been identified in recent years (Kang et al., 2005), but how these host factor(s) regulate virus replications in cells remains to be determined.

Eukaryotic elongation factors (eEFs), such as eEF1A and eEF1B, are key enzymes involved in protein elongation during translation in eukaryotes (Andersen & Nyborg, 2001). eEF1A is a highly abundant and conserved protein that delivers aminoacylated tRNA (aa-tRNA) to the ribosome in a GTP-dependent manner (Andersen et al., 2003). The noncanonical functions of eEF1A are vital for several
cellular processes, including protein translation, cytoskeleton organization, nuclear export, and ubiquitin-dependent protein degradation (Andersen et al., 2003; Vera et al., 2014). During protein synthesis, the ternary complex of eEF1A binds to and delivers aa-tRNAs to the ribosome. When the aa-tRNA anticodon in the ribosome matches the mRNA codon bound to the ribosome, GTP is hydrolysed to GDP and then combined with eEF1A. eEF1A is restored to active GTP under the action of the nucleotide exchange factor eEF1B (EF-Ts). Finally, EF2 (EF-G) assists the translocation of tRNA and mRNA through a codon on the ribosome. Moreover, eEF1A can also promote replication of certain RNA viruses through direct interaction with viral RNA-dependent RNA polymerases (RdRp) or through interaction with viral genomic RNAs (Blackwell & Brinton, 1997; Davis et al., 2007). For example, eEF1A has been found to enhance the translation of turnip yellow mosaic virus (TYMV) RNA through binding to the 3′-untranslated region (3′-UTR) of viral genomic RNAs (Matsuda & Dreher, 2004). eEF1A can also bind to the upstream pseudoknot domain in the 3′-UTR of brome mosaic virus (BMV) RNAs and the 3′-UTR of tobacco mosaic virus (TMV) genomic RNA (Bastin & Hall, 1976; Joshi et al., 1986; Matsuda & Dreher, 2004; Zeenko et al., 2002). eEF1A can recruit TMV RdRp and the RdRp and VPg-protease (VPg-Pro) of turnip mosaic virus (TuMV) to form virus replication complexes (VRC) (Blumenthal et al., 1976; Thivierge et al., 2008; Yamaji et al., 2006). Furthermore, eEF1A can interact with the RdRp of tomato bushy stunt virus (TBSV) or West Nile virus (Davis et al., 2007; De Nova-Ocampo et al., 2002; Rodnina & Wintermeyer, 2009; Sasvari et al., 2011).

Chinese wheat mosaic virus (CWMV) has two single-strand positive-sense genomic RNAs. Chinese wheat mosaic virus is a member of the genus Furovirus, family Virgaviroidae. CWMV does not contain a poly(A) sequence at the RNA 3′ end of the genome (Adams et al., 2009). CWMV RNA1 consists of 7,147 nucleotides (nt) and encodes three proteins: a 153-kDa replication-associated protein, a 212-kDa RdRp, and a 37-kDa movement protein (MP). CWMV RNA2 comprises 3,564 nt and encodes four proteins: a 19-kDa major capsid protein (CP), two minor CP-related proteins (a 23-kDa N-CP and an 84-kDa CP-RT), produced through an initiation of translation at the noncanonical CUG start codon or through occasional read-through of the UGA termination codon, respectively, and a 19-kDa RNA silencing suppressor (Andika et al., 2013; Diao et al., 1999; Sun et al., 2013; Yang et al., 2001). Both the 5′ and 3′ termini of RNA1 and RNA2 contain UTRs. The 3′-UTR of RNA1 and RNA2 includes a highly conserved tRNA-like structure, but the function of this structure is unclear. Full-length CWMV infectious clones have recently been developed and used successfully to infect Triticum aestivum (Yang et al., 2016). Very few host factors have been identified to participate in CWMV infections or in other furovirus infections in plants.

Because eEF1A has been shown to regulate the replication of several RNA viruses, we decided to investigate the function of eEF1A in CWMV infection. We used in vivo assays to investigate whether eEF1A can positively influence CWMV infection in plants, as well as in vitro and reverse-genetic assays to determine whether eEF1A can facilitate CWMV infection in plants via its binding to the 3′-UTR of CWMV genomic RNAs.

2 | RESULTS

2.1 | CWMV infection induces eEF1A expression in T. aestivum and N. benthamiana

To investigate the relationship between the expression of eEFs and CWMV infection in wheat plants, we studied three T. aestivum genes encoding eEFs: TaeEF1A (GenBank accession number AK334915), TaeEF1B (GenBank accession number AK332529), and TaeEF2 (GenBank accession number AK452406). Barley stripe mosaic virus (BSMV, family Hordevirus) and wheat yellow mosaic virus (WYMV, family Potyviridae) are positive-sense RNA viruses that can infect wheat. Quantitative reverse transcription PCR (RT-qPCR) analyses were conducted using total RNA extracted from leaf samples harvested from CWMV-, BSMV- or WYMV-inoculated T. aestivum plants at 14 days postinoculation (dpi). Wheat plants mock inoculated with the empty vector were used as controls. Compared with the relative expression of TaeEFs in mock-inoculated plants, the expression of TaeEF1B was downregulated by 0.6-fold after BSMV infection and upregulated by 1.8-fold after WYMV infection while no significant effects were observed after CWMV infection; the expression of TaeEF2 was 2.3-fold upregulated after BSMV infection while no significant effects were observed after CWMV or WYMV infection; and only the expression of TaeEF1A was significantly upregulated by approximately 1.4- to 2.3-fold after CWMV, BSMV, or WYMV infection (Figure 1a). A homology-based analysis revealed that the amino acid sequences of eEF1As from Capsicum annuum, Solanum lycopersicum, Solanum pennelli, Solanum tuberosum, N. benthamiana, Ipomoea nil, Orzya sativa, Tarenaya hassleriana, and T. aestivum share approximately 98% sequence identity (Figure S1). It has been reported that NbeEF1A is required both for TMV and TuMV infection (Thivierge et al., 2008; Zeenko et al., 2002). Thus, we decided to analyse the expression of NbeEF1A (XM_009784954) in N. benthamiana plants with or without viral infection by performing RT-qPCR. The relative expression of NbeEF1A in CWMV-, TMV-, or TuMV-infected N. benthamiana plants was significantly increased compared with expression in control plants that were mock infiltrated with the empty vector at 14 dpi (Figure 1b). Furthermore, we also analysed the relative expression of NbeEF1A in N. benthamiana plants at 7–28 days after CWMV infection by performing RT-qPCR. The relative expression of NbeEF1A in CWMV-infected plants was approximately 2.2-fold higher than that of mock-treated plants 14 to 28 dpi after infection with CWMV (Figure 1c).

Western blot and northern blot assays further showed that this correlated with increases in CWMV CP and CWMV genomic RNAs concentrations (Figure 1d,e). Thus, because N. benthamiana is an excellent experimental host plant for CWMV studies, and because an efficient and reliable agroinfiltration method has been developed for reverse-genetic assays for CWMV infection (Yang...
et al., 2016, 2017), we decided to focus our research on the role of NbeEF1A in CWMV infection in subsequent experiments.

2.2 | Silencing of NbeEF1A expression inhibits CWMV infection

A 400 nt sequence fragment of NbeEF1A was RT-PCR amplified and cloned into a tobacco rattle virus (TRV)-based vector to produce TRV:NbeEF1A. This vector was used to perform virus-induced gene silencing (VIGS) in N. benthamiana plants via agroinfiltration. At 7 dpi, five assayed plants were sampled and analysed by performing RT-qPCR. The results showed that NbeEF1A transcript levels in assayed plants #1, #3, and #4 were approximately 0.4- to 0.26-fold lower than that in TRV:00-inoculated control plants (Figure S2). To investigate the role of NbeEF1A in CWMV infection, we inoculated assayed plants #1, #3, and #4 with CWMV. Plants inoculated with TRV:00 and then CWMV (TRV:00 + CWMV) served as controls. At 21 dpi with CWMV, NbeEF1A-silenced plants showed milder CWMV symptoms than TRV:00 + CWMV-inoculated plants (Figure 2a). At 7 days after infection with CWMV, western blot and northern blot assays showed that significantly lower levels of CWMV CP and genomic RNAs were detected in CWMV-inoculated #1, #3, and #4 plants than in TRV:00 + CWMV-inoculated plants (Figure 2b,c). In addition, RT-qPCR analyses also showed that the replication level of CWMV RNA1 and RNA2 in CWMV-inoculated #1, #3, and #4 plants was significantly lower than that in leaves of TRV:00 + CWMV plants (Figure 2d). Due to the critical role of eEF1A in promoting mRNA translation, the effect of eEF1A on translation efficiency of CWMV genes was evaluated by RT-qPCR analysis. The results showed that the translation efficiency of CWMV CP in CWMV-inoculated NbeEF1A-silenced plants was 0.54-fold lower than that in TRV:00+CWMV plants (Figure 2e).
2.3 Overexpression of NbeEF1A in plants enhances CWMV accumulation

To further elucidate the role of NbeEF1A during CWMV infection, we constructed a p35S:NbeEF1A-GFP expression vector to transiently overexpress NbeEF1A in *N. benthamiana* leaves. Leaves were cocultivated with *Agrobacterium* containing this expression vector and CWMV using agroinfiltration. Plants inoculated with TRV:00 + CWMV acted as controls. The Coomassie Brilliant blue (CBB)-stained RuBisCO gel is used to show the protein loadings. Viral protein was quantified using ImageJ software. (c) Northern blot assay for CWMV RNA accumulation in TRV:NbeEF1A + CWMV-inoculated leaves of assayed plants #1, #3, and #4. Plants inoculated with TRV:00 + CWMV acted as controls. Ethidium bromide-stained gel was used to visualize RNA loadings. Viral RNAs were quantified using ImageJ software. (d) Relative transcript level of viral RNA in TRV:00 + CWMV-inoculated or assayed *N. benthamiana* plants #1, #3, and #4 inoculated with TRV:NbeEF1A + CWMV. (e) Translation efficiency assay for CWMV CP in TRV:00 + CWMV-inoculated plants and TRV:NbeEF1A + CWMV-inoculated plants acted as control. Translation efficiency calculated as the relative expression in polysomal/total RNA fractions. Relative transcript levels are the mean ± SD of three biological samples; each biological sample had three technical replicates. Significant differences between treatments were determined using Student’s t test (*p < 0.05)

2.4 eEF1A binds to the 3’-UTR of CWMV genomic RNAs but not CWMV RdRp

The successful infection of a plant by an RNA virus and disease symptom development are dependent on complicated molecular interactions between viral and host factors. Numerous reports have indicated that eEFs can interact with viral RdRps and/or viral genomic RNAs (Dufresne et al., 2008; Li et al., 2010, 2015; Matsuda & Dreher, 2004; Thivierge et al., 2008; Yamaji et al., 2006). To determine whether CWMV replication-associated proteins can interact with NbeEF1A, we performed yeast two-hybrid assays, communo-precipitation (Co-IP) assays, and pull-down assays. Because the full-length replication-associated protein has a large molecular weight, the CWMV replication-associated protein (Rep) was divided into three fragments according to their functional domains: amino acid positions 1–670 referred to as Rep1–670 (methyltransferase, Met), amino acid positions 670–1430 referred to as Rep670–1430 (helicase,
Hel), and amino acid positions 1430–1840 referred to as Rep1430–1840 (RdRp) (Figure 4a). As shown in Figure 4b, all the yeasts harbouring a plasmid expressing BD-eEF1A together with a plasmid expressing AD-replicase, -Met, -Hel, or -RdRp, did not show any growth on the selection medium compared with the positive control. Additionally, the interaction between Met-, Hel-, or RdRp-His and NbeEF1A-GFP in planta was confirmed by a Co-IP assay after transiently coexpressing Met-, Hel-, or RdRp-His, and NbeEF1A-GFP in N. benthamiana leaves, respectively. The interaction of Met-, Hel-, or RdRp-His with NbeEF1A-GST was also observed by pull-down assays. The VPg-Pro protein of TuMV was used as a positive control in these assays. Our results showed that although the VPg-Pro protein interacted with NbeEF1A, none of the CWMV replication-associated protein domains interacted with NbeEF1A (Figure 4b–d). Further analyses also showed that these three domains did not interact with TaeEF1A in yeast cells (Figure S4). eEF1A has been reported to interact with viral RNAs to ensure RNA virus replication in cells (Blackwell & Brinton, 1997; Davis et al., 2007). Here, we decided to explore whether NbeEF1A can bind to CWMV genomic RNAs by performing electrophoretic mobility shift assays (EMSA). We prepared in vitro transcripts of the CWMV (+/−) 3’-UTR and (+/−) 5’-UTR of RNA1 and RNA2, which were biotin labelled (BL) at their 3’ ends (+/−). In

**FIGURE 3** Transient overexpression of NbeEF1A in *Nicotiana benthamiana* plants enhances CWMV RNA accumulation. (a) Western blot assay for CWMV coat protein (CP) expression in plants coinoculated with CWMV and p35S:NbeEF1A-GFP (NbeEF1A-GFP + CWMV) through agroinfiltration using a CP-specific antibody at 7 days postinoculation (dpi). Plants coinoculated with CWMV and p35S:GFP (GFP + CWMV) acted as controls. Proteins were resolved using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) stained with Coomassie Brilliant blue (CBB) to visualize protein loadings. Viral protein was quantified using ImageJ software. (b) Northern blot assay for CWMV RNA accumulation. Ethidium bromide-stained gel was used to visualize RNA loadings. Three plants from the same treatment were used for the assay. Viral RNA was quantified using ImageJ software. (c) Relative transcript level of viral RNA in NbeEF1A-GFP + CWMV-inoculated plants and GFP + CWMV-inoculated plants acted as controls. (d) Translation efficiency assay for CWMV CP in GFP + CWMV-inoculated plants and NbeEF1A-GFP + CWMV-inoculated plants served as controls. Translation efficiency calculated as the relative expression in polysomal/total RNA fractions. Relative transcripts levels are the mean ± SD of three biological samples; each biological sample had three technical replicates. Significant differences between treatments were determined using Student’s t test (*p < 0.05)
addition, CWMV\textsuperscript{301–781} of RNA1 and (+/−) CWMV\textsuperscript{681–981} of RNA2 (regions covering nucleotides 301–781 or 681–981, respectively, in the CWMV coding region) were randomly selected and BL at their 3′ ends. Binding of eEF1A to the 3′-UTRs of RNA1 and RNA2 was analysed by EMSA. Shift of the 3′-UTR bands was observed on addition of eEF1A and disappeared after adding competitive cold unlabelled RNA (UL-RNA) (Figure 4e), but not in other treated reactions (Figure S5a,b). We examined the effects of different concentrations of BL-3′-UTR transcripts on the formation of the 3′-UTR RNA1 (R1)/NbeEF1A and 3′-UTR RNA2 (R2)/NbeEF1A complexes. The results showed an increase in the retarded band when 3′-UTR RNA or NbeEF1A concentrations were increased from 0.125 to 20 nM or 5 to 60 µM, respectively, supporting this interaction (Figure 5a–d).

The assays also showed no effect on the formation of the 3′-UTR RNA/NbeEF1A complex when unlabelled CWMV\textsuperscript{301–781} RNA1 (UL-CWMV\textsuperscript{301–781}) or CWMV\textsuperscript{681–981} RNA2 (UL-CWMV\textsuperscript{681–981}) concentrations were increased from 0 to 40 nM (Figure 5e,f). Moreover, the binding activity between NbeEF1A and 3′-UTR RNA was weakened following the addition of excess UL-3′-UTR RNA from 0 to 48 nM (Figure 5g,h). These findings suggest that eEF1A can bind to the 3′-UTR of CWMV genomic RNAs but cannot interact with CWMV RdRp.

**FIGURE 4** Analysis of the interaction between eEF1A and CWMV RdRp. (a) A schematic diagram showing CWMV replicase domains. The three segments (amino acid residues 1–670, 670–1430, and 1430–1840) represent the three conserved domains (Met, Hel, and RdRp) in CWMV replicase. (b) The right-hand column shows that yeast cells coexpressing BD:NbeEF1A + AD:Replicase, BD:NbeEF1A + AD:Met, BD:NbeEF1A + AD:Hel, or BD:NbeEF1A + AD:RdRp grow well on SD/-Trp−Leu selective medium. The left-hand column shows that none of the transformed yeast cells can grow on the SD/-Trp−Leu−His−Ade selective medium, indicating no positive interaction between NbeEF1A and the replicase domains. Yeast cells coexpressing AD-T+BD-Lam acted as a negative control. (c) Immunoprecipitation assay for binding of Met, Hel, and RdRp regions of CWMV to NbeEF1A, showing that binding failed in vivo. Immunoprecipitation was performed with anti-GFP agarose beads and immunoblotting was carried out with anti-His. GFP proteins acted as negative controls and TuMV Vpg-Pro-His acted as positive controls. (d) Immunoprecipitation assay for binding of CWMV Met, Hel, and RdRp domains to NbeEF1A, showing that binding failed in vitro. A GST-fused inactive NbeEF1A was first bound to glutathione–sepharose beads and incubated with Met-His, Hel-His, or RdRp-His as indicated. His proteins acted as a negative control and TuMV Vpg-Pro acted as a positive control. (e) Electrophoretic mobility shift assay for NbeEF1A binding activity to (+) 3′-UTR of CWMV RNA1 (R1) or (+) 3′-UTR of CWMV RNA2 (R2). Each treatment has three components: a biotin-labelled (BL) RNA probe, an unlabelled (UL) RNA probe (the UL-RNA corresponds to the labelled RNA), and purified recombinant NbeEF1A or other protein. BL-RNA + NbeEF1A was used to show the binding, UL-RNA + BL-RNA + NbeEF1A was used to show the competitive binding, and bovine serum albumin (BSA) and green fluorescent protein (GFP) acted as negative controls.
2.5 | A conserved region in the 3′-UTR is crucial for CWMV infection

The sequences of the 3′-UTRs of CWMV RNA1 and RNA2 have an identity of approximately 57%, but interestingly a region of about 160 nucleotides of RNA1 and RNA2 (nucleotide positions 6988–7147 and 3407–3569) shows higher similarity (Figure S6a). Secondary structure predictions using Mfold software (http://unafold.rna.albany.edu/?q=mfold) indicated that there is a tRNA-like structure (TLS) at the conserved region of the 3′-UTR of CWMV RNA1 or RNA2 (Figure S6b). We conducted a microscale thermophoresis (MST) of NbeEF1A and the RNA1 3′-UTR, RNA2 FIGURE 5 Electrophoretic mobility shift assays for competitive binding between NbeEF1A and (+) 3′-UTR (R1), (+) 3′-UTR (R2), UL-CWMV301-781, or UL-CWMV681-981. (a) Protein concentration-dependent binding activity of NbeEF1A for CWMV (+) 3′-UTR of RNA1 (R1). The amount added to each component is indicated at the top. (b) Biotin-labelled (BL)-RNA concentration-dependent binding activity of NbeEF1A for CWMV (+) 3′-UTR (R1). The amount added to each component is indicated at the top. (c) Protein concentration-dependent binding activity of NbeEF1A for CWMV (+) 3′-UTR of RNA2 (R2). The amount added to each component is indicated at the top. (d) BL-RNA concentration-dependent binding activity of NbeEF1A for CWMV (+) 3′-UTR (R2). The amount added to each component is indicated at the top. (e) Assay for competitive binding of BL-3′-UTR (R1) + NbeEF1A and unlabelled (UL)-CWMV301-781 of RNA1. The amount added to each component is indicated at the top. (f) Assay for competitive binding of BL-3′-UTR (R1) + NbeEF1A and UL-CWMV681-981 of RNA2. The amount added to each component is indicated at the top. (g) Titration of a UL-3′-UTR (R1) with NbeEF1A + BL-3′-UTR (R1). The amount added to each component is indicated at the top. (h) Titration of a UL-3′-UTR (R2) with NbeEF1A + BL-3′-UTR (R2). The amount added to each component is indicated at the top.
3′-UTR, RNA1 3′-UTRΔ6730–6987, RNA2 3′-UTRΔ3174–3406, RNA1 3′-UTRΔ6988–7147, or RNA2 3′-UTRΔ3407–3567, respectively. The binding equilibrium dissociation constant (K_{D}) values between NbeEF1A and the RNA1 3′-UTR, RNA2 3′-UTR, RNA1 3′-UTRΔ6730–6987, RNA2 3′-UTRΔ3174–3406, RNA1 3′-UTRΔ6988–7147, or RNA2 3′-UTRΔ3407–3567 were 7.32 ± 2.1 nM, 12.53 ± 3.7 nM, 67.70 ± 4.6 nM, 68.48 ± 4.4 nM, 964.11 ± 63.4 nM, or 997.36 ± 57.1 nM, respectively (Figure 6a). The CWMVΔ301-781 of RNA1 was used as a control. Our analyses demonstrated that the conserved TLS in the 3′-UTR is a key domain response for NbeEF1A binding CWMV genomic RNAs. To elucidate the function of the TLS during CWMV infection, we generated a mutant CWMV ΔR1 with the TLS deleted in its 3′-UTR (RNA1 3′-UTRΔ6988–7147) and a ΔR2 with the TLS deleted in its 3′-UTR (RNA2 3′-UTRΔ3407–3567) (Figure 6b). Next, CWMV ΔR1 and ΔR2 were co-inoculated with the wildtype (WT) CWMV RNA2 or RNA1, respectively, into N. benthamiana plants using agroinfiltration. WT CWMV RNA1 and RNA2 co-inoculated plants served as controls. At 7 dpi, CP and viral RNA accumulations were much lower in CWMV ΔR2-inoculated plants than those in WT CWMV-inoculated plants and were hardly detected in CWMV ΔR1-inoculated plants (Figure 6c,d). RT-qPCR analyses also showed that CWMV RNA1 and RNA2 replication levels were 0.20- to 0.31-fold lower in CWMV ΔR1-inoculated plants and 0.50- to 0.58-fold lower in CWMV ΔR2-inoculated plants than in WT CWMV-inoculated plants (Figure 6e). Consistent with these findings, the translation efficiency of CWMV CP was 0.62- to 0.74-fold lower in CWMV ΔR1-inoculated plants or CWMV ΔR2-inoculated plants, respectively, than in WT CWMV-inoculated plants (Figure 6f). By 21 dpi, CWMV ΔR2-inoculated N. benthamiana plants showed much milder disease symptoms than WT CWMV-inoculated plants, whereas CWMV ΔR1-inoculated plants showed mild disease symptoms that were similar to those of WT plants (Figure 6g). Taken together, our analyses demonstrated that the conserved TLS in the 3′-UTR is a key domain for NbeEF1A binding CWMV genomic RNAs, which is crucial for CWMV infection.

2.6 | Role of stem-loop structures in CWMV RNA2 3′-UTR in the interaction between NbeEF1A and 3′-UTR

Given that the TLS is highly conserved in CWMV RNA1 and RNA2 and that the deletion of the TLS from CWMV RNA1 3′-UTR affected CWMV infection, we decided to further investigate the TLS of CWMV RNA2. Predictions using CWMV RNA2 3′-UTR (3419–3569 nucleotides) revealed six stem-loop (SL)-like structures that form the TLS (Figure 7a). To determine which SL structure affects the binding affinity of CWMV 3′-UTR for NbeEF1A, we prepared six CWMV RNA2 3′-UTR deletion mutants i.e., ΔSL-1 for 3′-UTRΔ3419–3429, ΔSL-2 for 3′-UTRΔ3469–3471, ΔSL-3 for 3′-UTRΔ3500–3503, ΔSL-4 for 3′-UTRΔ3505–3509, ΔSL-5 for 3′-UTRΔ3532–3535, and ΔSL-6 for 3′-UTRΔ3561–3565 (Figure 7b). We analysed the binding activity between these mutants and NbeEF1A, which indicated that all of these mutants did affect the binding activity. Compared with the WT 3′-UTR, the shifts of the ΔSL-1, ΔSL-2, ΔSL-3, ΔSL-4, and ΔSL-5 bands were significantly weakened, and the most reduced ΔSL-6 was only 6% of the WT 3′-UTR (Figure 7c). To further measure the binding affinity between NbeEF1A and 3′-UTR, we conducted an MST assay using the same concentration of NbeEF1A and the indicated amounts of these six mutant transcripts. The assays revealed a K_{D} between NbeEF1A and the WT 3′-UTR, ΔSL-1, ΔSL-2, ΔSL-3, ΔSL-4, or ΔSL-5 of 6.51 ± 1.14 nM, 116.69 ± 22.53 nM, 91.71 ± 13.21 nM, 33.48 ± 10.03 nM, 113.45 ± 33.48 nM, and 36.02 ± 7.57 nM, respectively (Figure 7d), indicating that these mutant 3′-UTRs all had lower binding capacities for NbeEF1A than that of the WT 3′-UTR. In addition, the mutant ΔSL-6 had the highest K_{D} value (997.76 ± 59.74 nM), which supports the above finding that the TLS structure is required for the interaction between NbeEF1A and 3′-UTR, and that SL-6 may be the key region in the TLS for this interaction.

2.7 | SL-6 in 3′-UTR is crucial for CWMV accumulation

To investigate the function of SL-6 in eEF1A-regulated CWMV accumulation, we changed the ACCGGCC of SL-6 to ugaacau for producing the mutant m3′-UTRugaacau, and then complementary mutations in the opposite strand to produce the mutant 3′-UTRIns(auguuca) or by deleting nucleotide positions 3561–3567 of the RNA2 3′-UTR to produce the mutant m3′-UTRΔ3561–3567. As shown in Figure 8a, the stem structure was changed in the mutant m3′-UTRugaacau and m3′-UTRΔ3561–3567, while it was restored in the complementary mutant 3′-UTRIns(auguuca). Agrobacterium harbouring the CWMV RNA1 and RNA2 clones (CWMV) or the CWMV RNA1 and the m3′-UTRugaacau clones (CWMVugaacau) or the CWMV RNA1 and the m3′-UTRΔ3561–3567 clones (CWMVΔ3561–3567) or the CWMV RNA1 and the 3′-UTRIns(auguuca) clones (CWMVins(auguuca)) were inoculated into N. benthamiana plants using agroinfiltration. By 21 dpi, both CWMVugaacau- and CWMVΔ3561–3567-inoculated N. benthamiana plants showed much milder disease symptoms than CWMV-inoculated plants (Figure 8b) and accumulated significantly lower levels of viral RNAs and CP (Figure 8c,d). Interestingly, CWMVins(auguuca)-inoculated N. benthamiana plants showed similar disease symptoms at 21 dpi (Figure 8b). Western blot and northern blot assays showed greater CWMV RNA and CP accumulations in WT CWMV- and CWMVins(auguuca)-inoculated N. benthamiana plants at 21 dpi than in other plants, which was consistent with these observations (Figure 8c,d). In addition, RT-qPCR also showed that the replication level of CWMV RNA1 and RNA2 in the CWMVugaacau- or CWMVΔ3561–3567-inoculated plants was 0.50- to 0.51-fold lower, respectively, than that in CWMV-inoculated plants (Figure 8e). By contrast, replication levels of CWMV RNA1 and RNA2 in CWMVins(auguuca)-inoculated plants were higher than those in CWMVugaacau-, or CWMVΔ3561–3567-inoculated plants and accumulation levels were similar to that in CWMV-inoculated plants (Figure 8e). The translation efficiencies of CWMV CP in CWMVugaacau- or CWMVΔ3561–3567-inoculated plants were 0.72- to
The 3′-UTR of CWMV RNA is necessary for viral infection. (a) A microscale thermophoresis assay of NbeEF1A binding affinity for RNA1 3′-UTR, RNA1 3′-UTRΔ6988-7147, RNA1 3′-UTRΔ6730-6987, RNA2 3′-UTR, RNA2 3′-UTRΔ3407-3569, or RNA2 3′-UTRΔ3174-3406 transcripts. RNA1 5′-UTR transcripts acted as a negative control. This experiment was performed three times and similar results were obtained. Bars represent standard errors. NA, no affinity. (b) Schematic diagrams showing the difference between the wild type (WT) and the deletion mutants CWMV ΔRNA1 and CWMV ΔRNA2. (c) Western blot assay for CWMV coat protein (CP) accumulation in WT CWMV-, CWMV ΔRNA1-, or CWMV ΔRNA2-inoculated Nicotiana benthamiana plants. Coomassie Brilliant blue (CBB) was used to visualize protein loadings. Three plants from the same treatment were used. The relative intensity of the blot signal quantified by ImageJ is shown above the lane. (d) Northern blot assay for CWMV RNA accumulation in WT CWMV-, CWMV ΔRNA1-, or CWMV ΔRNA2-inoculated N. benthamiana plants. Ethidium bromide-stained gel was used to visualize RNA loadings. Three plants from the same experiment were used. The relative intensity of the blot signal quantified by ImageJ is shown in the image. (e) Relative levels of CWMV RNA1 and RNA2 replication assessed by quantitative reverse transcription PCR in WT CWMV-, CWMV ΔRNA1-, or CWMV ΔRNA2-inoculated N. benthamiana leaves. (f) Translation efficiency assay for CWMV CP in CWMV ΔRNA1- or CWMV ΔRNA2-inoculated N. benthamiana plants and WT CWMV-inoculated plants served as controls. Translation efficiency calculated as the relative expression in polysomal/total RNA fractions. (g) Symptoms in WT CWMV-, CWMV ΔRNA1-, or CWMV ΔRNA2-inoculated N. benthamiana plants at 21 days postinoculation (dpi) with CWMV. Data presented are the mean ± SD from three biological samples per treatment; each biological sample had three technical replicates. Significant differences between treatments were determined using Student’s t test (*p < 0.05)
0.63-fold lower while that in CWMV \textsuperscript{Ins}\textsubscript{auguca} inoculated plants was similar than that in WT CWMV-inoculated plants (Figure 8f). Thus, we conclude that the SL-6 in the 3\textsuperscript{′}-UTR is important for CWMV multiplication in infected cells.

3 | DISCUSSION

Because viruses have limited coding capacities, they must rely on host factor(s) to complete their infection cycles in cells (Nagy & Pogany, 2011). One of the best-studied host factors is eEF1A. Many studies on the roles of eEF1A during virus infections of plants have demonstrated that eEF1A can positively regulate the multiplication of viruses such as TMV, BSMV, TuMV, and tomato spotted wilt virus (Komoda et al., 2014; Li et al., 2009, 2010; Yamaji et al., 2010).

Our analyses showed that TaeEF1A is also an important host factor for CWMV, BSMV, or WYMV infection in wheat plants (Figure 1a). In addition, the expression of NbeEF1A was upregulated under CWMV, TMV, or TuMV infection in \textit{N. benthamiana} (Figure 1b). These results suggest that eEF1A may be a general host factor required for infection by different viruses. eEF1A is known to be highly conserved throughout the eukaryotes. Our amino acid sequence analysis also showed that eEF1As in \textit{N. benthamiana} and \textit{T. aestivum} are highly conserved (Figure S1), suggesting that these two eEF1As share similar properties. \textit{N. benthamiana} is an excellent experimental host plant for studies of virus infections, including rice stripe virus (RSV), bamboo mosaic virus (BaMV), and BSMV (Alazem et al., 2017; Shi et al., 2016; Zhang et al., 2018). Thus, we reasoned that \textit{N. benthamiana} would be a suitable host plant for studying the effect of NbeEF1A during CWMV infection. Indeed,
we also demonstrated that the expression of NbeEF1A was positively correlated with the accumulation of CWMV CP and genomic RNAs during CWMV infection from 7 to 28 dpi (Figure 1c–e). When NbeEF1A expression in N. benthamiana plants was silenced through VIGS, we found that accumulation of CWMV CP and genomic RNAs was significantly reduced (Figure 2). By contrast, transient overexpression of NbeEF1A in N. benthamiana plants significantly increased the accumulation of CWMV CP and genomic RNAs (Figure 3a,b). We also found that the replication and translation efficiency of CWMV exhibited significant changes when NbeEF1A was silenced or overexpressed in N. benthamiana plants (Figures 2d,e and 3c,d). Based on these findings, we conclude that eEF1A is required for CWMV infection in plants. To our knowledge, this is the first report to show that eEF1A regulates CWMV infection in plants.

Virus replication complexes (VRC) are sites where viral RNAs are synthesized in cells (Cotton et al., 2009). Previous studies have reported that eEF1A is a component of VRCs and can control the replication of different viruses via interactions with viral RdRps (Thivierge et al., 2008; Yamaji et al., 2006). In this study, we found that NbeEF1A cannot interact with CWMV RdRp in vivo or in vitro (Figure 4b–d). VRCs are known to contain ribosomes, tubulin-like structures, endoplasmic reticulum, RdRp and other viral proteins, and viral RNAs (Asurmendi et al., 2004; Liu et al., 2005). Moreover, eEF1A has been shown to form complexes with RdRp and other proteins encoded by TuMV (Thivierge et al., 2008) or to help VRC formation through interactions with viral VPg-Pro (Thivierge et al., 2008). Although eEF1A does not interact directly with CWMV RdRp, it may nonetheless promote CWMV multiplication through its participation in VRC formation. Shamovsky et al. have shown that
eEF1A can bind to aminoacylated tRNAs and other mammalian RNA species (Shamovsky et al., 2004). In a separate report, Zeenko et al. have shown that eEF1A can interact with the 3′-UTR of TMV genomic RNA to regulate virus replication in N. benthamiana (Zeenko et al., 2002). TYMV and BMV also have TLSs in their RNAs and these TLSs can all bind to eEF1A to promote viral replication (Annamalai & Rao, 2007; Matsuda & Dreher, 2004; Matsuda et al., 2004). These previous reports encouraged us to investigate whether NbeEF1A can bind to the TLSs in UTRs of CWMV RNAs. Our results show that NbeEF1A is indeed capable of interacting with the 3′-UTR of CWMV genomic RNAs (Figure 4e). Several studies have also shown that eEF1A can bind to RNA structures outside 3′-UTRs. For example, eEF1A has been shown to bind to a region in the Hepatitis delta virus genomic RNA to promote HDAg mRNA transcription and the synthesis of its negative-strand RNA (Sikora et al., 2009). The 5′-UTR of HIV-1 genomic RNA has been shown to interact with eEF1A and this interaction is important for late DNA synthesis during reverse transcription (Li et al., 2015). It has been demonstrated that for some plant RNA viruses the VRC could specifically recognize the 3′-terminal portion of the viral genomic RNA, which contains a unique promoter to enhance viral replication (Osmann et al., 2000; Volot et al., 2001). The specific binding of NbeEF1A to the 3′-UTR of CWMV genomic RNAs (Figure 5) suggests a role for eEF1A-binding in both assembly of the CWMV VRC and template recognition for viral replication. However, other potential roles of the eEF1A interaction to CWMV RNA 3′-UTR, such as anchoring the CWMV VRC to a specific membrane or cytoskeleton in a host cell or participation in cell-to-cell spread of viral RNA, should also be examined in future studies. Taken together, our results show that the 3′-UTR of CWMV genomic RNAs is required for CWMV RNA replication.

The 3′-UTRs often are composed of SLs, pseudoknots (PKs), or TLSs, and tend to be conserved among different RNA viruses and within virus groups. Here, we also used the RNA structure Web to predict the PKs in the 3′-UTR of RNA1 and RNA2 of CWMV. The results showed that the 3′-UTR of RNA1, but not of RNA2, contains a potential PK (Figure 5c). Several studies have also shown that PKs in the TLS of turnip yellow mosaic virus, BMV, and TMV are involved in infectivity of these viruses (Chandrika et al., 2000; Deiman et al., 1998; Dreher et al., 1996). The PK region and TL regions interact with eEF1A independently or simultaneously during TMV infection (Zeenko et al., 2002). Thus, we speculate that the potential PKs may be responsible for the weak infectivity of CWMV ΔR1 (Figure 6c, d), which also requires further experimental analysis in the future. MST analysis also showed that the TLS in the CWMV 3′-UTR is essential for the binding between NbeEF1A and CWMV genomic RNAs (Figure 5a). The binding ability of CWMV RNA with a mutagenized 3′-UTR showed a 65% reduction compared with the WT CWMV RNA, possibly due to the weak affinity of eEF1A for RNA. Thus, eEF1A binding to the CWMV 3′-UTR might be required for both the initiation of viral negative-strand RNA synthesis and enhancing viral replication. Secondary structures in viral RNAs play pivotal roles in viral life cycles. The secondary or more advanced structures in virus genomic RNAs can stabilize RNAs and allow specific interactions between different RNA molecules or between RNAs and proteins. An early investigation of turnip yellow mosaic virus showed that the TLS at the 3′ end of viral RNA participates in virus replication (Matsuda & Dreher, 2004). Here, six TLS structures were predicted in the CWMV RNA2 3′-UTR (Figure 5d). Our mutational analyses showed that SL-6 was important for binding eEF1A and the CWMV 3′-UTR (Figure 7). Because the affinity of eEF1A for viral RNA is independent of GTP and other RNA binding sites that are specific for binding to aa-tRNA (Slobin, 1983), we considered that the binding of eEF1A to the SL-6 of the CWMV 3′-UTR plays an important role in CWMV infection. To confirm this idea, we generated a series of CWMV mutants with an altered SL-6 (CWMVΔ6accau), a deleted SL-6 (CWMVΔ3561-3567), and a compensatory SL-6 (CWMVΔ3561-3567 InsACCGGCC). N. benthamiana plants inoculated with either the CWMVΔ6accau or the CWMVΔ3561-3567 InsACCGGCC mutant virus accumulated significantly lower levels of viral RNA and protein than plants inoculated with WT CWMV, whereas plants inoculated with the CWMVΔ3561-3567 InsACCGGCC mutant virus, which had a restored stem structure, accumulated similar levels of viral RNA and protein to that of the WT CWMV (Figure 8). Mutation in the TLS can affect RNA affinity for eEF1A (Matsuda & Dreher, 2004). This finding suggests that the mutation in SL-6 of CWMV 3′-UTR interferes with its ability to bind to eEF1A and disrupts the synthesis of the CWMV negative-strand, thereby impeding the initiation of viral RNA replication.

eEF1A is one of the most abundant proteins in eukaryotic cells and is one of the most characterized proteins of the translational machinery (Andersen et al., 2003). Our results have demonstrated that eEF1A is involved in the replication and translation of CWMV through binding to the CWMV 3′-UTR. Thus, it seems likely that CWMV might have evolved to use it in various ways. Future studies should reveal the relevance of eEF1A not only for CWMV replication but also for translation or other CWMV infection steps, which should contribute to our understanding of host–virus interactions, propagation strategies, and the adaptive evolution of RNA viruses.

4 | EXPERIMENTAL PROCEDURES

4.1 | Plant growth and virus inoculation

N. benthamiana plants were grown inside a growth chamber maintained at 25 °C and a 14 hr light/10 hr dark cycle. Agrobacterium tumefaciens GV3101 harbouring CWMV RNA1 (pCB-35S-R1) or RNA2 (pCB-35S-R2) infectious clones were obtained from a previously reported source (Yang et al., 2016). The mutant CWMV RNA2 constructs produced in this study were also transformed individually into A. tumefaciens GV3101, and the Agrobacterium cultures were grown individually overnight at 28 °C in a yeast extract peptone medium containing kanamycin (50 µg/ml) and rifampicin (50 µg/ml). The resulting Agrobacterium cultures were pelleted and then resuspended in an infiltration buffer (100 mM MES, pH 5.2, 10 mM MgCl₂, 200 mM acetosyringone) to obtain an OD600 of 0.6 followed by >2 hr incubation at 25 °C. Agrobacterium harbouring pCB-35S-R1
was mixed with Agrobacterium harbouring pcB-35S-R2 in a 1:1 ratio or with one of its derivatives prior to infiltration into N. benthamiana leaves. The infiltrated plants were grown inside a growth chamber maintained at 15 ± 2 °C, with a 14 hr/10 hr (light/dark) cycle and 70% relative humidity.

Wheat cv. Yangmai 158 plants were grown to the two-leaf stage in a greenhouse and then inoculated with in vitro transcripts of CWMV, BSMV, or WYMV after adding 1 vol of FES buffer (1% wt/vol sodium pyrophosphate, 1% wt/vol macaloid, 1% wt/vol celite, 0.5 M glycine, 0.3 M K2HPO4, pH 8.5, with phosphoric acid). Inoculum was applied by gently rubbing it on the surface of leaves that had been abraded with carborundum.

4.2 | Plasmid construction

Full-length TaeEF1A or NbeEF1A sequences were RT-PCR amplified from a T. aestivum or a N. benthamiana leaf sample, respectively. The resulting PCR products were purified using a Gene JET gel extraction kit (Thermo Fisher Scientific) and cloned individually into a pGW-B5C vector (Invitrogen) to produce p35S:TaeEF1A and p35S:NbeEF1A using Gateway cloning technology and following the manufacturer’s instructions (Invitrogen).

For VIGS, a partial sequence of NbeEF1A (nucleotide positions 1451–1750) was RT-PCR amplified, double digested with BamHI and Smal restriction enzymes (New England Biolabs) and cloned into the vector pTRV (Liu et al., 2002), a TRV RNA2-based vector, to produce pTRV:NbeEF1A. Agrobacterium harbouring the pYL196 vector (i.e., the TRV RNA1-based vector) was mixed with an equal amount of Agrobacterium harbouring the pTRV:NbeEF1A vector (the mixed Agrobacterium is referred to as TRV:NbeEF1A hereafter) and infiltrated into N. benthamiana leaves.

To determine the effect of SL structures in the CWMV 3′-UTR on viral replication, we deleted the SL-6 from the 3′-UTR of CWMV and cloned into the pGADT7 vector to produce pAD-Rep1–670 (Met), pAD-Rep670–1430 (Hel), and pAD-Rep1430–1840 (RdRp), respectively. The construction of these three activation domain vectors has been described previously (Yang et al., 2017). The primers used in this study are listed in Table S1.

4.3 | Silencing NbeEF1A expression through virus-induced gene silencing

To silence NbeEF1A expression in N. benthamiana plants, leaves were infiltrated with Agrobacterium harbouring TRV:NbeEF1A. The VIGS procedure used in this study was similar to that reported previously (Ratcliff et al., 2001). The TRV:NbeEF1A- or TRV:00-infected (control) plants were grown at 25 °C for 7 days and then inoculated again with CWMV through agroinfiltration and maintained at 15 °C.

4.4 | RNA extraction and RT-qPCR analysis

Total RNA was extracted from T. aestivum and N. benthamiana leaf samples with a HiPure plant RNA mini kit (Magen). First-strand cDNA was synthesized using a first-strand cDNA synthesis kit (TOYOBO) followed by quantitative PCR using an Applied Biosystems QuantStudio 6 Flex system (Applied Biosystems) and a SYBR Green Master Mix kit (Vazyme). Each treatment had three biological replicates and each biological replicate had four technical replicates. The relative expression levels of assayd genes or the CWMV CP gene were calculated using the 2^-ΔΔCT method (Livak & Schmittgen, 2001). The primers used in this study are listed in Table S1.

4.5 | Northern blot assays

Northern blot assays were performed as previously described (Yang et al., 2016). Briefly, total RNA (c.5 μg per sample) was separated in a 2% formaldehyde agarose gel through electrophoresis (60 V for 1.5 hr). The separated RNA bands were transferred onto a Hybond-N* membrane (Amersham Biosciences) followed by a 10 min crosslink under a UV light. The blot was hybridized with a digoxigenin-labelled probe specific for the 3′ end of CWMV genomic RNAs. The probe was produced using a Detection Starter Kit II following the manufacturer’s instructions (Roche).

4.6 | Western blot assays

Western blot assays were conducted as previously described (Yang et al., 2016). Protein samples were separated using SDS-PAGE and transferred onto nitrocellulose membranes. Blots were blocked using phosphate-buffered saline (PBS) containing 5% skimmed milk, rinsed several times with PBS, and then probed with a specific primary mouse antibody (at a 1:5,000 dilution) followed by an anti-mouse (at
4.7 | Translation efficiency assay

Translation efficiency was assayed as previously described with minor modifications (Merchant et al., 2015). First, 10 g of N. benthamiana leaves was ground into fine powder in liquid nitrogen. Then, 2 g of sample was used to extract the total RNA with a HiPure plant RNA mini kit (Magen). Next, the other 8 g of sample was suspended in 20 ml of polysome extraction buffer (200 mM Tris-HCl, pH 9.0, 35 mM MgCl₂, 200 mM KCl, 25 mM EGTA, 1% vol/vol Triton X-100, 1% vol/vol IGEPAL CA-630, 5 mM dithiothreitol [DTT], 1 mM phenylmethylsulfonyl fluoride [PMSF], 50 μg/ml chloramphenicol, 100 μg/ml cycloheximide) at 4 °C for 20 min with slight shaking. The mixture was centrifuged twice at 16,000 x g for 20 min at 4 °C. Then, 16 ml of supernatant was slowly transferred onto 15 ml of sucrose buffer (1.75 M sucrose, 400 mM Tris-HCl, pH 9.0, 35 mM MgCl₂, 5 mM EGTA, 200 mM KCl, 5 mM DTT, 50 μg/ml chloramphenicol, 50 μg/ml cycloheximide). After centrifugation at 200,000 x g for 4 hr at 4 °C, the supernatant was carefully removed and the polysomes in the bottom were resuspended in 300 μl of diethylpyrocarbonate [DEPC]-treated water. The polysomes RNA was isolated using TRIzol reagent (Life Technologies) and then subjected to reverse transcription and real-time PCR analysis.

4.8 | Yeast two-hybrid assay

The yeast two-hybrid assay was performed using a Matchmaker Gold yeast two-hybrid system and a Yeastmaker yeast transformation system 2 according to the manufacturer’s instructions (Clontech Laboratories).

4.9 | In vitro transcription

PCR products containing a T7 promoter followed by a specific probe sequence (i.e., sense or antisense [+]−/−) CWMV 3′-UTR, [+]/−/− CWMV 5′-UTR, and [+]/−/− CWMV 3′-UTR and [+]−/− CWMV 5′-UTR were prepared using a T7 in vitro transcription kit following the manufacturer’s instructions (Thermo Fisher Scientific) and used as unlabelled (UL) RNA probes.

4.10 | Electrophoretic mobility shift assay

Biotin was integrated at the 3′ end of the UL RNA probes using a Pierce RNA 3′ end biotinylation kit (Thermo Fisher Scientific) to produce BL RNA probes. The EMSA binding reaction and chemiluminescence detection were conducted using a LightShift chemiluminescent RNA EMSA (REMSA) kit (Thermo Fisher Scientific). Briefly, a BL RNA probe was incubated with a purified protein sample for 30 min at 25 °C. The mixture was analysed in a 5% native polyacrylamide gel by performing electrophoresis, transferred onto a Hybond-N+ membrane, and crosslinked for 45 s under an UV light. The detection signal was scanned using an Amersham Imager 680 machine.

4.11 | Microscale thermophoresis assay

The affinity of the purified NbeEF1A for RNA was determined using Monolith NT.115 (NanoTemper Technologies). Microscale thermophoresis (MST) labelling of NbeEF1A was conducted in PBS solution containing a Monolith NT protein labelling kit RED according to the manufacturer’s instructions (NanoTemper Technologies). Samples were then loaded into NanoTemper hydrophilic-treated capillaries. The resulting samples were analysed by the manufacturer using NanoTemper analytical software to estimate their equilibrium dissociation constant (Kd) values.

4.12 | Pull-down

Purified GST-NbeEF1A protein was incubated with purified His, TuMV VPg-Pro-His, CWMV Met-His, CWMV Hel-His, or CWMV RdRp-His at room temperature for 15 min. Next, 25 μl of GST-Trap agarose (ChromoTek) was added to each reaction system, which was then incubated at 4 °C for 2 hr. After beads were collected and washed three times with Tris-buffered saline solution (TBS; 10 mM Tris-HCl pH 8.0, 150 mM NaCl), the reaction mixtures were run using SDS-PAGE and immunoblotted with an anti-GFP antibody (TransGene) and an anti-His antibody (TransGene).

4.13 | Co-immunoprecipitation assay

Proteins were transiently coexpressed in leaves of N. benthamiana by Agrobacterium infiltration. Coimmunoprecipitation assays were performed on N. benthamiana leaves that were harvested 2 days after infiltration, pooled, and ground in liquid nitrogen. Total protein was extracted with an extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM MgCl₂, 5 mM DTT, 0.1% Triton X-100). Protein extracts were incubated with 25 μl GFP-Trap agarose (ChromoTek) for immunoprecipitation ranging from 2 hr to overnight at 4 °C. Finally, beads were collected and washed three times with TBS solution, then the reaction mixtures were run using SDS-PAGE and immunoblotted with an anti-GFP antibody and an anti-His antibody.

ACKNOWLEDGEMENTS

This work was supported by the National Key R&D Plan in China (2017YFD-0201701, 2018YFD0200507, 2018YFD0200408), the Natural Science Foundation of Ningbo City (2019A610415, 201901A610415).
Andersen, G.R., Nissen, P. & Nyborg, J. (2003) Elongation factors in pro-

Jian Yang

CHEN E et al.

Blackwell, J.L. & Brinton, M.A. (1997) Translation elongation factor- 1

Ahlquist, P., Noueiry, A.O., Lee, W.-M., Kushner, D.B. & Dye, B.T. (2003)

Disease Control (2010DS700124-KF1811), the Ningbo 3315 Talents

Project for Research on Transgenic Biology (2016ZX08002-001), the

State Key Laboratory Breeding Base for Zhejiang Sustainable Pest and

Dr Xinshun Ding for his help during the preparation of this manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

J.Y., H.Z., and J.C. conceived the project and designed the experi-

ments. X.C. carried out the experiments with assistance from M.X.,

L.H., J.L., T.Z., J.Y., and Q.L. All authors analysed and discussed the

results, and J.Y. wrote the manuscript. All authors confirm that they

have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from

the corresponding author upon reasonable request.

ORCID

Xuan Chen  https://orcid.org/0000-0001-9299-7079

Long He  https://orcid.org/0000-0002-2814-6541

Jian Yang  https://orcid.org/0000-0002-4456-7231

REFERENCES

Adams, M.J., Antoniw, J.F. & Kreuze, J. (2009) Virgaviridae: a new family

of rod-shaped plant viruses. Archives of Virology, 154, 1967–1972.

Ahliquist, P., Noueiry, A.O., Lee, W.-M., Kushner, D.B. & Dye, B.T. (2003)

Host factors in positive-strand RNA virus genome replication. Journal of

Virology, 77, 8181–8186.

Alazem, M., He, M.H., Moffett, P. & Lin, N.S. (2017) Abscissic acid induces

resistance against Bamboo mosaic virus through Argonaute2 and 3.

Plant Physiology, 174, 339–355.

Andersen, G.R., Nissen, P. & Nyborg, J. (2003) Elongation factors in pro-
intrinsic biosynthesis. Trends in Biochemical Sciences, 28, 434–441.

Andersen, G.R. & Nyborg, J. (2001) Structural studies of eukaryotic elonga-
tion factors. Cold Spring Harbor Symposium on Quantitative Biology, 66, 425–437.

Andika, I.B., Sun, L., Xiang, R., Li, J. & Chen, J. (2013) Root-specific role

of Nicotiana benthamiana RDR6 in the inhibition of Chinese wheat
mosaic virus accumulation at higher temperatures. Molecular Plant-

Microbe Interactions, 26, 1165–1175.

Annamalai, P. & Rao, A.L. (2007) In vivo packaging of brome mosaic virus

RNA3, but not RNAs 1 and 2, is dependent on a cis-acting 3' tRNA-
like structure. Journal of Virology, 81, 173–181.

Asurmedini, S., Berg, R.H., Koo, J.C. & Beachy, R.N. (2004) Coat protein

regulates formation of replication complexes during tobacco mosaic

virus infection. Proceedings of the National Academy of Sciences of the

United States of America, 101, 1415–1420.

Bastin, M. & Hall, T.C. (1976) Interaction of elongation factor 1 with amni-
ocylated brome mosaic virus and tRNAs. Journal of Virology, 20, 117–122.

Blackwell, J.L. & Brinton, M.A. (1997) Translation elongation factor-1e

interacts with the 3' stem-loop region of West Nile virus genomic

RNA. Journal of Virology, 71, 6433–6444.

Blumenthal, T., Young, R.A. & Brown, S. (1976) Function and structure in

phase Qbeta RNA replicase. Association of EF-Tu-Ts with the other

enzyme subunits. Journal of Biological Chemistry, 251, 2740–2743.

Chandrika, R., Rabindran, S., Lewandowski, D.J., Manjunath, K.L. &

Dawson, W.O. (2000) Full-length tobacco mosaic virus RNAs and
defective RNAs have different 3' replication signals. Virology, 273, 198–209.

Cotton, S., Grangeon, R., Thivierge, K., Mathieu, I., Ide, C., Wei, T. et al.

(2009) Turnip mosaic virus RNA replication complex vesicles are mo-

 bile, align with microfilaments, and are each derived from a single

viral genome. Journal of Virology, 83, 10460–10471.

Davis, W.G., Blackwell, J.L., Shi, P.Y. & Brinton, M.A. (2007) Interaction

between the cellular protein eEF1A and the 3'-terminal stem-loop of

West Nile virus genomic RNA facilitates viral minus-strand RNA

synthesis. Journal of Virology, 81, 10172–10187.

De Nova-Ocampo, M., Villegas-Sepulveda, N. & del Angel, R.M. (2002)

Translation elongation factor-1a, La, and PTB interact with the 3'

untranslated region of dengue 4 virus RNA. Virology, 295, 337–347.

Deiman, B.A., Koenen, A.K., Verlaan, P.W. & Pleij, C.W. (1998) Minimal

template requirements for initiation of minus-strand synthesis in vitro

by the RNA-dependent RNA polymerase of turnip yellow mo-

saic virus. Journal of Virology, 72, 3965–3972.

Diao, A., Chen, J., Ye, R., Zheng, T., Yu, S., Antoniw, J.F. et al. (1999) Complete

sequence and genome properties of Chinese wheat mosaic virus, a new

furovirus from China. Journal of General Virology, 80, 1141–1145.

Dreher, T.W., Tsai, C.H. & Skuzeski, J.M. (1996) Aminoacylation identity

switch of turnip yellow mosaic virus RNA from valine to methionine

results in an infectious virus. Proceedings of the National Academy of

Sciences of the United States of America, 93, 12212–12216.

Dufresne, P.J., Thivierge, K., Cotton, S., Beauchemin, C., Ide, C., Ubalijoro,

E. et al. (2008) Heat shock 70 protein interaction with Turnip mosaic

virus RNA-dependent RNA polymerase within virus-induced mem-

brane vesicles. Virology, 374, 217–227.

Joshi, R.L., Ravel, J.M. & Haenni, A.L. (1986) Interaction of turnip yellow

mosaic virus Val-RNA with eukaryotic elongation factor EF-1a.

Search for a function. EMBO Journal, 5, 1143–1148.

Kang, B.C., Yeam, I. & Jahn, M.M. (2005) Genetic of plant virus resis-

tance. Annual Review of Phytopathology, 43, 581–621.

Komoda, K., Ishibashi, K., Kawamura-Nagaya, K. & Ishikawa, M. (2014)

Possible involvement of eEF1A in Tomato spotted wilt virus RNA

synthesis. Virology, 468–470, 81–87.

Li, D., Wei, T., Jin, H., Rose, A., Wang, R., Lin, M.-H. et al. (2015) Binding

of the eukaryotic translation elongation factor 1A with the 5'UTR of

HIV-1 genomic RNA is important for reverse transcription. Virology

Journal, 12, 118.

Li, Z., Pogany, J., Panavas, T., Xu, K., Esposito, A.M., Kinzy, T.G. et al.

(2009) Translation elongation factor 1A is a component of the tomb-

busvirus replicase complex and affects the stability of the p33 repli-

cation co-factor. Virology, 385, 245–260.

Li, Z., Pogany, J., Tupman, S., Esposito, A.M., Kinzy, T.G. & Nagy, P.D.

(2010) Translation elongation factor 1A facilitates the assembly of the

tombusvirus replicase and stimulates minus-strand synthesis.

PLoS Pathogens, 6, e1001175.

Liu, J.Z., Blancaflor, E.B. & Nelson, R.S. (2005) The tobacco mosaic virus

126-kilodalton protein, a constituent of the virus replication com-

plex, alone or within the complex aligns with and traffics along mi-

crofilaments. Plant Physiology, 138, 1853–1865.

Liu, Y., Schif, M. & Dinesh-Kumar, S.P. (2002) Virus-induced gene silenc-

ing in tomato. The Plant Journal, 31, 777–786.

Livak, K.J. & Schmittgen, T.D. (2001) Analysis of relative gene expres-

sion data using real-time quantitative PCR and the 2−ΔΔCT method.

Methods, 25, 402–408.

Matsuda, D. & Dreher, T.W. (2004) The tRNA-like structure of Turnip

yellow mosaic virus RNA is a 3'-translational enhancer. Virology, 321, 36–46.
Matsuda, D., Yoshinari, S. & Dreher, T.W. (2004) eEF1A binding to aminoacylated viral RNA represses minus strand synthesis by TYMV RNA-dependent RNA polymerase. Virology, 321, 47–56.

Merchant, C., Brunos, J., Yun, J., Hu, Q., Spencer, K., Enriquez, P., et al. (2015) Gene-specific translation regulation mediated by the hormone-signaling molecule EIN2. Cell, 163, 684–697.

Nagy, P.D. (2008) Yeast as a model host to explore plant virus–host interactions. Annual Review of Phytopathology, 46, 217.

Nagy, P.D. & Pogany, J. (2011) The dependence of viral RNA replication on co-opted host factors. Nature Reviews Microbiology, 10, 137–149.

Noueiry, A.O. & Ahlquist, P. (2003) Brome mosaic virus RNA replication: revealing the role of the host in viral RNA replication. Annual Review of Phytopathology, 41, 77–98.

Osman, T.A.M., Hemenway, C.L. & Buck, K.W. (2000) Role of the 3′-tRNA-like structure in tobacco mosaic virus minus-strand RNA synthesis by the viral RNA-dependent RNA polymerase in vitro. Journal of Virology, 74, 11671–11680.

Ratcliff, F., Martin-Hernandez, A.M. & Baulcombe, D.C. (2001) Technical advance. Tobacco rattle virus as a vector for analysis of gene function by silencing. The Plant Journal, 25, 237–245.

Rodríguez, M.V. & Wintermeyer, W. (2009) Recent mechanistic insights into eukaryotic ribosomes. Current Opinion in Cell Biology, 21, 435–443.

Sanfaçon, H. (2015) Plant translation factors and virus resistance. Viruses, 7, 3392–3419.

Sasvari, Z., Izotova, L., Kinzy, T.G. & Nagy, P.D. (2011) Synergistic roles of eukaryotic translation elongation factors 1Bγ and 1A in stimulation of tombusvirus minus-strand synthesis. PLoS Pathogens, 7, e1002438.

Shamovsky, I., Ivannikov, M., Kandel, E.S., Gershon, D. & Nudler, E. (2006) RNA-mediated response to heat shock in mammalian cells. Nature, 440, 556–560.

Shi, B., Lin, L., Wang, S., Guo, Q., Zhou, H., Rong, L. et al. (2016) Identification and regulation of host genes related to Rice stripe virus symptom production. New Phytologist, 209, 1106–1119.

Sikora, D., Greco-Stewart, V.S., Miron, P. & Pelchat, M. (2009) The hepatitis delta virus RNA genome interacts with eEF1A1, p54(nr), hnRNPL, GAPDH and ASF/SF2. Virology, 390, 71–78.

Slobin, L.I. (1983) Binding of eucaryotic elongation factor Tu to nucleic acids. Journal of Biological Chemistry, 258, 4895.

Sun, L., Andika, I.B., Kondo, H. & Chen, J. (2013) Identification of the amino acid residues and domains in the cysteine-rich protein of Chinese wheat mosaic virus that are important for RNA silencing suppression and subcellular localization. Molecular Plant Pathology, 14, 265–278.

Thivierge, K., Cotton, S., Dufresne, P.J., Mathieu, I., Beauchemin, C., Ide, C. et al. (2008) Eukaryotic elongation factor 1A interacts with Turnip mosaic virus RNA-dependent RNA polymerase and VPg-Pro in virus-induced vesicles. Virology, 377, 216–225.

Vera, M., Pani, B., Griffiths, L.A., Muchardt, C., Abbott, C.M., Singer, R.H. et al. (2014) The translation elongation factor eEF1A1 couples transcription to translation during heat shock response. eLife, 3. e03164.

Vlot, A.C., Neeleman, L., Linthorst, H.J.M. & Bol, J.F. (2001) Role of the 3′-untranslated regions of alfalfa mosaic virus RNAs in the formation of a transiently expressed replicase in plants and in the assembly of virions. Journal of Virology, 75, 6440–6449.

Yamaji, Y., Kobayashi, T., Hamada, K., Sakurai, K., Yoshii, A., Suzuki, M. et al. (2004) In vivo interaction between Tobacco mosaic virus RNA-dependent RNA polymerase and host translation elongation factor 1A. Virology, 347, 100–108.

Yamaji, Y., Sakurai, K., Hamada, K., Komatsu, K., Ozeki, J., Yoshida, A. et al. (2010) Significance of eukaryotic translation elongation factor 1A in tobacco mosaic virus infection. Archives of Virology, 155, 263–268.

Yang, J., Chen, J., Jiang, H., Zhao, Q. & Adams, M.J. (2001) Sequence of a second isolate of Chinese wheat mosaic furovirus. Journal of Phytopathology, 149, 135–140.

Yang, J., Zhang, F., Cai, N.-J., Wu, N.e., Chen, X., Li, J. et al. (2017) A furoviral replicase recruits host HSP70 to membranes for viral RNA replication. Scientific Reports, 7, 45590.

Yang, J., Zhang, F., Xie, L.i., Song, X.-J., Li, J., Chen, J.-P. et al. (2016) Functional identification of two minor capsid proteins from Chinese wheat mosaic virus using its infectious full-length cDNA clones. Journal of General Virology, 97, 2441.

Zeenko, V.V., Ryabova, L.A., Spirin, A.S., Rothnie, H.M., Hess, D., Browning, K.S. et al. (2002) Eukaryotic elongation factor 1A interacts with the upstream pseudoknot domain in the 3′ untranslated region of tobacco mosaic virus RNA. Journal of Virology, 76, 5678–5691.

Zhang, X., Dong, K., Xu, K., Zhang, K., Jin, X., Yang, M. et al. (2018) Barley stripe mosaic virus infection requires PKA-mediated phosphorylation of γb for suppression of both RNA silencing and the host cell death response. New Phytologist, 218, 1570–1585.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Chen, X., He, L., Xu, M., Yang, J., Li, J., Zhang, T., et al (2021) Binding between elongation factor 1A and the 3′-UTR of Chinese wheat mosaic virus is crucial for virus infection. Molecular Plant Pathology, 22, 1383–1398. https://doi.org/10.1111/mpp.13120