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EIF3i affects vesicular stomatitis virus growth by interacting with matrix protein

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ABSTRACT

The matrix protein of vesicular stomatitis virus (VSV) performs multiple functions during viral genome replication and virion production and is involved in modulating multiple host signaling pathways that favor virus replication. To perform numerous functions within infected cells, the M protein needs to recruit cellular partners. To better understand the role of M during VSV replication, we looked for interacting partners by using the two-hybrid system. The eukaryotic translation initiation factor 3, subunit i (eIF3i) was identified to be an M-binding partner, and this interaction was validated by GST pull-down and laser confocal assays. Through a mutagenesis analysis, we found that some mutants of M between amino acids 122 and 181 impaired but did not completely abolish the M–eIF3i interaction. Furthermore, the knockdown of eIF3i by RNA interference decreased viral replication and transcription in the early stages but led to increase in later stages. VSV transcription was increased at 4 h post-infection but was not changed at 8 and 12 h post-infection after the over-expression of eIF3i. Finally, we also demonstrated that VSV could inhibit the activity of Akt1 and that the knockdown of eIF3i inhibited the expression of the ISGs regulated by phospho-Akt1. These results indicated that eIF3i may affect VSV growth by regulating the host antiviral response in HeLa cells.

1. Introduction

Vesicular stomatitis (VS) is a highly contagious disease in swine, horses, cattle and other mammals. It is caused by the vesicular stomatitis virus (VSV) and characterized by widely erosive vesicles on the surface of the lips, tongue, gums and teats (Letchworth et al., 1999). Vesicular stomatitis virus (VSV) is a member of the *Vesiculovirus* genus, which belongs to the *Rhabdoviridae* family. The VSV genome is composed of a single negative polarity RNA strand that encodes five proteins: nucleocapsid (N), phosphoprotein (P), matrix (M) protein, glycoprotein (G) and large (L) viral polymerase (Barr et al., 2002). The nucleocapsid (N) protein tightly binds to the viral genomic and nascent RNA. The polymerase L is an RNA-dependent RNA polymerase (RdRP) that associates with phosphoprotein (P), nucleocapsid (N) and genomic RNA to form the transcriptionally active nucleocapsid (Barr et al., 2002). This complex is condensed by matrix protein (M) to generate a coiled helical structure and then enclosed within a lipid bilayer directed by the integral transmembrane glycoprotein (G) (Ge et al., 2010; Beilstein et al., 2015).

The matrix (M) protein of VSV is a multifunctional protein that plays an important role in the induction of cell rounding (Blondel et al., 1990), the modulation of apoptosis (Kopecky et al., 2001), the inhibition of host transcription (Black and Lyles, 1992), virion assembly/budding (Gaudin et al., 1995; Harty et al., 1999) and the blocking of nuclear-cytoplasmic transport of host RNAs (Her et al., 1997). To perform numerous functions, viral proteins may need to interact with specific host proteins. A number of M-interacting cellular proteins have been identified that contribute to these various functions during viral infection. It has been demonstrated that host E3 ubiquitin ligase Nedd4 interacts with M protein to promote efficient virus egress (Harty et al., 2001). In addition to facilitating virus egress, VSV M protein can deregulate nucleo-cytoplasmic transport to inhibit cellular gene expression by targeting Nup98 (von Kobbe et al., 2000). Furthermore, M protein can also interact with dynamin and inhibit clathrin-mediated endocytosis. Disrupting the M-dynamin interaction blocks VSV assembly and budding and affects the cellular localization of the N and G proteins (Raux et al., 2010). In addition, M protein has been shown to be associated with host factor-Rae1, and this complex serves as a

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"platform" to recruit cellular proteins involved in host transcription (Rajani et al., 2012). Thus, further exploration of the interaction of M with host cellular protein(s) is essential for understanding the roles of M in the replication and pathogenesis of VSV. In this study, we identified the host protein eIF3i as a novel M-interacting partner that regulates the growth of VSV.

EIF3i is a subunit of the eukaryotic translation initiation factor 3 complex, which was initially isolated as an interactor of TGF β-receptor type II (Chen et al., 1995). The EIF3 complex has been shown to affect the growth of different groups of viruses. For example, the rabies virus (RV) M protein can inhibit eukaryotic translation via a protein interaction with eIF3 h (Komarova et al., 2007). Furthermore, eIF3f can also interact with the S protein of SARS-CoV and infectious bronchitis virus (IBV) to inhibit expression of host genes (Xiao et al., 2008). In addition to inhibiting host translation, eIF3f can inhibit HIV replication by specifically impeding the 3′ end processing of HIV-1 mRNAs (Valente et al., 2009). However, no studies have investigated the role of eIF3 complex in the VSV life cycle. Here, we demonstrated that VSV M protein interacts with eIF3i and that this interaction affects the growth of VSV.

2. Materials and methods

2.1. Cells and virus

HeLa and BSR-T7/5 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Invitrogen), supplemented with 10% fetal bovine serum (FBS; HyClone), 2 mM L-glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin and maintained in a 37 °C, 5% CO_2 incubator. Vesicular stomatitis virus serotype Indiana (VSV-IND) was propagated in HeLa cells.

2.2. Construction of expression vectors

The eIF3i gene (GenBank accession no. NM_003757. 3) was amplified by using cDNA extracted from HeLa cells and inserted into the pcmv-flag vector (catalog no. 635688; Clontech) to generate the pcmv-flag-eIF3i plasmid. A Flag-M eukaryotic expression vector was constructed using the pMD-18T backbone vector that contains the T7 polymerase promoter, the encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES) and Flag-M, followed by a T7 terminator, which was designated pT-Flag-M. Other EGFP-M, EGFP, Dsred-eIF3i and Dsred eukaryotic expression plasmids were constructed as described above and designated pT-EGFP-M, pT-EGFP, pT-Dsred-eIF3i and pT-Dsred, respectively. The eIF3i and M ORFs were cloned into the C-terminus of GST in the vector pGEX-4T-1 to construct the prokaryotic expression plasmids. The primers sequences are listed in Table 1.

2.3. Yeast two-hybrid assay

VSV M was inserted into a pGBKT7 plasmid, fused to the DNA-binding domain and transformed into the yeast strain AH109 using LiAc. The auto activation and toxicity of the bait were verified on SDO/X (SD/-Trp/X-a-Gal) and SDO (SD/-Trp) plates. The normalized mouse brain cDNA library fused to the sequence encoding the GAL4 activation domain, pre-transformed in the Y187 yeast strain, was purchased from Clontech (catalog no. 630488; Clontech). Two-hybrid screens were performed using bait yeast mating with the library yeast strain and selected on DDO/X/A (SD/-Leu/-Trp/X-a-Gal/AbA) plates. Positive colonies were confirmed on QDO/X/A (SD/-Ade/-His/-Leu/-Trp/X-a-Gal/AbA) plates. Inserts of all putative positive clones were isolated, sequenced and analyzed through NCBI BLAST searches. To eliminate false-positive clones, the bait and prey plasmids were co-transformed into the AH109 yeast strain. AH109 co-transformed with pGBKT7-p53 (BD-p53) and pGADT7-T (AD-T) was used as a positive control, and AH109 co-transformed with pGBKT7-Lambda (BD-Lam) and AD-T was used as a negative control.

2.4. Plasmid transfection

Briefly, the cells were transfected with 1 ml of serum-free DMEM containing 4 μg of plasmids and 12 μl of Lipofectamine 2000 transfection reagent. At 4 h post-transfection, the transfection mixture was replaced with DMEM supplemented with 2% FBS and incubated for an additional 24 h before being assayed.

2.5. GST pull-down assay

For expression of the GST-tagged or GST-M fusion protein, *E. coli* BL21 (DE3) bacteria were transformed with the pGEX-4T-1 or pGEX-4T-M plasmids, and expression was induced by addition of 1 mM IPTG for 18 h at 16 °C. The collective bacteria pellet was resuspended in cold phosphate-buffered saline (PBS) containing 1 mg/ml protease inhibitor PMSF, followed by gentle sonication. The soluble proteins were obtained by centrifugation at 12,000 × *g* for 10 min at 4 °C. Subsequently, the soluble GST or GST-M protein was incubated with glutathione Sepharose 4B beads (catalog no. 17075601; GE Healthcare) for 6 h at 4 °C. The beads were washed three times with cold PBS. Cells transfected with the pcmv-flag-eIF3i or pT-M plasmid were washed with cold PBS and lysed with NP-40 (catalog no. P0013F; Beyotime, Shanghai,

Table 1
Primers used in this study.

Primer	Sequence(5′–3′)[a]	Purpose
BD-M-F	CCGGAATTCCGGATGAGTTCCTTAAAGAAGATTCTC	Amplification and cloning of M in PGBKT7 vector
BD-M-R	CGCGGATCCGCGTCATTTGAAGTGGCTGACAG	Amplification and cloning of M in PGBKT7 vector
AD-eIF3i-F	CCGGAATTCCGGATGAAGCCGATCCTACTGC	Amplification and cloning of eIF3i in pGADT7 vector
AD-eIF3i-R	CCGCTCGAGCGGTTAAGCCTCAAACTCAAATTC	Amplification and cloning of eIF3i in pGADT7 vector
GST-M-F	CGCGGATCCGCGATGAGTTCCTTAAAGAAGATTCTC	Amplification and cloning of M in PGEX-4T-1 vector
GST-M-R	CCGGAATTCCGGTCATTTGAAGTGGCTGACAG	Amplification and cloning of M in PGEX-4T-1 vector
GST-eIF3i-F	CCGGAATTCCGGATGAAGCCGATCCTACTGC	Amplification and cloning of eIF3i in pGEX-4T-1 vector
GST-eIF3i-R	CCGCTCGAGCGGTTAAGCCTCAAACTCAAATTC	Amplification and cloning of eIF3i in PGEX-4T-1 vector
Flag-eIF3i-F	CGGAATTCCGATGAAGCCGATCCTACTGC	Amplification and cloning of eIF3i in pcmv-flag vector
Flag-eIF3i-R	GGGGTACCCCTTAAGCCTCAAACTCAAATTC	Amplification and cloning of eIF3i in pcmv-flag vector
VSV P-F	GTGACGGACGAATGTCTCATAA	Amplification of VSV P mRNA
VSV P-R	TTTGACTCTCGCCTGATTGTAC	Amplification of VSV P mRNA
VSV 2795-F	GTGACGGACGAATGTCTCATAA	Amplification of VSV antigenomic RNA
VSV2955-R	TGATGAATGGATTGGGATAACA	Amplification of VSV antigenomic RNA
Actin-F	TGACGTGGACATCCGCAAAG	Amplification of actin mRNA
Actin-R	CTGGAAGGTGGACAGCGAGG	Amplification of actin mRNA

[a] Underlined sequences indicate restriction sites.