Veterinary Microbiology 208 (2017) 82–88

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

The matrix protein of vesicular stomatitis virus inhibits host-directed transcription of target genes via interaction with the TFIIH subunit p8

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ARTICLE INFO

Keywords:
VSV
Transcription inhibition
TFIIH
p8

ABSTRACT

In response to viral infection, the host innate antiviral response is elicited to limit viral replication. Many viruses have evolved various strategies to circumvent the host antiviral response. It has been reported that matrix (M) protein of vesicular stomatitis virus (VSV) can inhibit host gene expression to evade the host innate immune response. However, the molecular mechanism remains unclear. Here, we demonstrated that VSV M protein inhibited transcription of a reporter gene transfected into BSR-T7/5 cells. To further investigate the underlying mechanism, a yeast two-hybrid screen was performed to search for host proteins that interact with the M protein. The subunit of transcription/repair factor TFIIH, p8, was identified as an M binding partner, and the interaction was validated with a GST pull-down assay and laser confocal microscopy. Through a mutagenesis analysis, we found that the p8-M interaction was impaired when I96, E156, R159 and R160 residues on M were replaced with Ala. These mutants reduced the inhibitory effect on transcription of the reporter gene. Furthermore, the transcription inhibition mediated by M was impaired when co-expressed with p8. These results indicate that the p8-M interaction plays an important role in inhibiting transcription of host genes.

1. Introduction

Vesicular stomatitis (VS) is a highly contagious disease in swine, horses, cattle and other mammals. It is caused by vesicular stomatitis virus (VSV) and characterized by widely erosive and vesicular lesions on the surface of the lips, tongue, gums, and teats (Letchworth et al., 1999). 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: large (L) viral polymerase, glycoprotein (G), matrix (M) protein, phosphoprotein (P) and nucleocapsid (N). 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 with a lipid bilayer directed by integral transmembrane glycoprotein (G) (Ge et al., 2010; Raux et al., 2010).

Upon viral infection, the pathogen-associated molecular patterns (PAMPs) within viral products are detected by the pathogen recognition receptors (PRRs) of host cells, and intracellular antiviral signaling pathways are activated to inhibit viral replication. Transcription and translation of host antiviral genes are essential steps in the innate antiviral immune response. Some viruses, such as Rift Valley fever virus (RVFV) and poliovirus, have been shown to inhibit host RNA pol II-dependent transcription to evade the host innate immune response (Crawford et al., 1981; Le May et al., 2004). In the case of VSV, it has been reported that the matrix protein plays a key role in inhibiting expression of host genes at multiple levels to restrain antiviral responses in infected cells (Black et al., 1994; Ahmed and Lyles, 1998; Petersen et al., 2000, 2001; Connor and Lyles, 2002). A previous study has shown that host Rae1 can interact with VSV M protein, and Rae1-M complexes can recruit other proteins that participate in host transcription to inhibit host transcription (Rajani et al., 2012). To further explore other transcription factors that mediate the inhibition of host transcription, a yeast two-hybrid assay was conducted using M as bait, and the p8 subunit of TFIIH was identified as a binding partner targeted by M protein.

TFIIH is one of the best-studied basal transcription complexes, which play important roles in DNA transcription, repair and cell cycle control (Zurita and Merino, 2003). The TFIIH multiprotein complex

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http://dx.doi.org/10.1016/j.vetmic.2017.07.020
Received 9 March 2017; Received in revised form 16 July 2017; Accepted 18 July 2017

consists of 10 subunits, a 7-subunit core (p8, p34, p44, p52, p62 and XPB) associated with a 3-subunit CDK-activating kinase module (CAK) (Cdk7, Cyclin H and MAT1) (Egly and Coin, 2011). Here, we demonstrated that TFIIH subunit p8 interacts with the M protein of VSV, and this interaction, mediated by the M protein, was responsible for inhibiting the transcription of a reporter gene.

2. Materials and methods

2.1. Cells and viruses

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 BSR-T7/5 cells.

2.2. Construction of expression vectors

The luciferase gene was amplified from pGL3-Basic vector (catalog no. E1741; Promega) using the following primers: 5′-CGGAATT CCGATGGAAGACGCCAAAAAC-3′ (forward) and 5′-GGGGTACCCCTT ACACGGCGATCTTTC-3′ (reverse) and cloned into a pcmv-flag vector (catalog no. 635688; Clontech) to generate the pcmv-flag-luciferase plasmid. The VSV M protein eukaryotic expression vector was constructed using a pMD-18T backbone vector (catalog no. D101A; TaKaRa, Dalian, China) containing the T7 polymerase promoter, the encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES), and flag-labeled M followed by the T7 terminator and designated pT-flag-M. The EGFP-M, EGFP, EGFP-p8, Dsred-p8, Dsred, flag, myc-p8 and myc eukaryotic expression vectors were constructed as described above and designated pT-EGFP-M, pT-EGFP, pT-EGFP-p8, pT-Dsred-p8, pT-Dsred, pT-flag, pT-myc-p8, and pT-myc, respectively. The p8 and M ORFs were cloned into the C-terminus of GST in the vector pGEX-4T-1 using the follow primers: 5′-CGCGGATCCGCGATGGT CAACGTCTTGAAAG-3′ (forward) and 5′-CCGGAATTCCGGTCAT TTCTGGGTAAGGGAAAA-3′ (forward) for p8 and 5′-CGCGGATCC GCGATGAGTAAAGAAGATTCTC-3′ (forward) and 5′-CCGGAATTCCG GTCATTTGAAGTGGCTGACAG-3′ (reverse) for M to construct the prokaryotic expression plasmids.

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 autoactivation and toxicity of the bait was verified on SDO/X (SD/-Trp/X-a-Gal) and SDO (SD/-Trp) plates. The yeast strain Y187 pretransformed with a normalized mouse brain cDNA library fused to the sequence encoding the GAL4 activation domain was purchased from Clontech (catalog no. 630488; Clontech). Two-hybrid screens were conducted using bait yeast mated 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. VSV M mutants were constructed in a pGBKT7 plasmid. ß-galactosidase assays were performed as described by Raux et al. (2000).

2.4. In vitro transcription and transfection

Capped and polyadenylated mRNA of M and its mutants were synthesized using a mMESSAGE mMACHINE T7 Ultra kit (Ambion, Austin, TX) according to the manufacturer's protocol. BSR-T7/5 cells seeded in 6-well plates (Corning, USA) were transfected with 4 μg of plasmid or 2 μg of mRNA and 2 μg of plasmid per well using Lipofectamine 2000 (Life Technology; USA) according to the manufacturer's instructions. 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 tested.

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 pGEX-4T-1 or pGEX-4T-M plasmid, and expression was induced by addition of 1 mM IPTG for 18 h at 16 °C. The collected 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 the Glutathione Sepharose 4B beads (catalog no. 17075601; GE Healthcare) for 6 h at 4 °C. The beads were washed three times with cold PBS. BSRT-7/5 cells transfected with pT-EGFP-p8 plasmid were washed with cold PBS and lysed with NP-40 (catalog no. P0013F; Beyotime, Shanghai, China). The cell lysate was centrifuged at 12,000*g* for 10 min at 4 °C, and the supernatant was incubated with Glutathione Sepharose 4B beads for 3 h at 4 °C. The beads were again washed three times, and the bead-bound protein complexes were separated by SDS-PAGE followed by western blotting using an anti-EGFP MAb (1:2000) (catalog no. 66002-1-Ig; Proteintech) and an anti-GST MAb (1:2000) (catalog no. HRP-66001; Proteintech). GST-p8 expressed in *E. coli* BL21(DE3) and EGFP-M expressed in BSR-T7/5 cells were also included in the GST pull-down assay to further validate the interaction as described above.

2.6. Confocal microscopy

BSR-T7/5 cells seeded on glass coverslips in 12-well plates were co-transfected with the indicated plasmids using Lipofectamine 2000 (Life Technology; USA). At 24 h post-transfection, cells were washed with PBS and fixed with 4% paraformaldehyde for 20 min. After being stained with Hoechst (0.2 mg/ml, 10 min) and washed with PBS, the cells were visualized with laser scanning confocal microscopy.

2.7. Western blotting

Proteins were separated by SDS-PAGE in 12% gels, followed by electrotransfer to PVDF membranes. Then, the blots were blocked with 5% skim milk and incubated with respective primary antibodies diluted with PBST. After washing with PBST (0.05% Tween-20 in PBS), the blots were incubated with secondary goat anti-rabbit (mouse) IgG-(HRP). Finally, they were washed four times with PBST and visualized by enhanced chemiluminescence (ECL) (Thermo Fisher Scientific).

2.8. Real-time PCR analysis

Total RNA was extracted from the BSR-T7/5 cells using a TriPure RNA isolation reagent (catalog no. 11667165001; Roche). First-strand cDNA was generated with 1 μg of total RNA using reverse transcriptase M-MLV (catalog no. 2641A; TaKaRa, Dalian, China) according to the manufacturer's protocol. Oligo(dT) primers were used for synthesis of all cDNAs. Real-time PCR was performed on a Bio-Rad CFX Connect Real-Time system (Bio-Rad). The specific primer sequences were as