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# Eukaryotic translational initiation factor 4AII reduces the replication of infectious bursal disease virus by inhibiting VP1 polymerase activity

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## ABSTRACT

Infectious bursal disease (IBD) is an acute, highly contagious, and immunosuppressive avian disease caused by IBD virus (IBDV). Although an interaction between eukaryotic translational initiation factor 4AII (eIF4AII) of the host and viral protein 1 (VP1), the RNA-dependent RNA polymerase (RdRp) of IBDV, has been established, the underlying effects of this interaction on IBDV and the molecular mechanism remain unclear. We here report that interaction of the host eIF4AII with VP1 inhibits the RNA polymerase activity of IBDV to reduce its replication in host cells. We found that ectopically expressed eIF4AII markedly inhibited IBDV growth in DF1 cells, and knockdown of eIF4AII by small interfering RNA significantly enhanced viral replication in CEF cells. Furthermore, IBDV infection led to an increase in host eIF4AII expression, suggesting a feedback mechanism between the host and virus infection both *in vitro* and *in vivo*, which further confirmed the involvement of the host eIF4AII in the IBDV life cycle. Thus, via the interaction with VP1, eIF4AII plays a critical role in the IBDV life cycle, by inhibiting viral RNA polymerase activity, leading to a reduction of IBDV replication in cells.

## 1. Introduction

Infectious bursal disease (IBD) is an acute, highly contagious viral disease causing damage in the lymphoid organs of birds, especially the bursa of Fabricius (Müller et al., 2003). Importantly, chickens infected with IBD virus (IBDV) suffer from immunosuppression with compromised humoral and cellular immune responses (Sharma et al., 2000; Tp et al., 2000), leading to increased susceptibility to other diseases. Thus, IBD remains a threat to the poultry industry worldwide.

IBDV is an *Avibirnavirus* belonging to the *Birnaviridae* family, which is composed of nonenveloped viruses containing two segments of dsRNA: segments A and B (Azad et al., 1985). The larger segment A contains two partially overlapping ORFs, encoding the nonstructural protein VP5, and a polyprotein that gives rise to pVP2, VP3, and VP4 (Birghan et al., 2000; Lombardo et al., 2000). Segment B contains one large ORF encoding VP1. This 90-kDa protein shares a number of primary sequence features with RNA polymerases from diverse origins (Bruenn, 1991; von Einem et al., 2004). Based on its size, low copy number in virions, and the presence of several conserved domains associated with RNA-dependent RNA polymerases (RdRps) of other RNA viruses, VP1 is believed to be the virion-associated RdRp (Dobos et al., 1979; Duncan et al., 1991; Gorbalenya and Koonin, 1988; Macreadie and Azad, 1993), and is responsible for the replication of the genome and synthesis of mRNAs (Pan et al., 2007; von Einem et al., 2004). Indeed, the polymerase activity of VP1 has been unequivocally characterized *in vitro* (Macreadie and Azad, 1993), and its crystal structure and activation mechanism have been described (Garriga et al., 2007; Pan et al., 2007).

Identification of the cellular interaction partners of viral proteins is likely to provide a better understanding of the dynamics of viral replication, virus-mediated cellular modulation, and host-range restriction. VP1 was recently reported to interact with the carboxy-terminal domain of eIF4AII using yeast two-hybrid screening (Tacken et al., 2004). However, the interaction between the full-length eIF4AII protein and VP1 has not been confirmed.

eIF4AII plays an essential role in the initiation of the translation of both capped and uncapped mRNAs. Although its association with

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IBDV VP1 suggests involvement of this viral protein in IBDV replication, the exact molecular mechanism underlying such interaction remains elusive. In this study, we examined the effect of eIF4AII and its interaction with VP1 on the RNA polymerase activity and replication of IBDV. We further examined the specific role of eIF4AII on IBDV replication, by knocking down eIF4AII expression with small interfering RNA (siRNA).

## 2. Materials and methods

### 2.1. Cells, viruses, and animals

DF1 (immortal chicken embryo fibroblast) and E6/Vero cells were conserved in our laboratory, and cultured in Dulbecco's modified Eagle medium (DMEM) (Invitrogen, USA) supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO2. Primary chick embryo fibroblasts (CEFs) were prepared from 10-day-old specific pathogen-free (SPF) chicken embryos and used for virus propagation and titration. The IBDV strain Gt was used in this experiment, which was cell culture-adapted from the strain Gx after blind passaged five times in SPF embryos and 20 times in the cell cultures (Wang et al., 2004). rGtNEG, rGtTEG, and rGtTDN viruses were rescued using IBDV reverse genetics as described in our previous study (Gao et al., 2014). SPF chicken embryos and SPF White Leghorn chickens were obtained from the Experimental Animal Center of the Harbin Veterinary Research Institute, the Chinese Academy of Agricultural Science, China, and housed in negative pressure-filtered air isolators. Animal experiments were approved by the Animal Ethics Committee of the Institute and performed in accordance with animal ethics guidelines and approved protocols.

### 2.2. Construction of plasmids

IBDV VP1 was cloned from the IBDV strain Gt (GenBank accession no. DQ403249) using specific primers of Table 1. The plasmids pCAGGVP1-Flag and pCAGGVP1-Myc were constructed by introducing a 3 × Flag tag or a c-Myc tag into the 3’-terminus of VP1, respectively, and cloned in pCAGGS vectors. Chicken eIF4AII was cloned from the bursa of 3-week-old SPF chickens using specific primers shown in Table 1, with reference to the sequence in GenBank (accession no. AF515726). pCAGGeIF4AII-Flag and pCAGGeIF4AII-Myc were constructed by introducing a 3 × Flag tag or c-Myc tag into the 3’-terminus of eIF4AII, respectively, and cloned in pCAGGS vectors. All the primers were synthesized by COME Company (Chang chun, China).

**Table 1**
Sequences of specific primers, probe, and siRNAs used in this text.

| Names | Orientation | Sequences (5' → 3') |
|---|---|---|
| Specific primers for VP1 PCR | sense | ATGAGTGACATTTTCAACAGT |
| | antisense | TTAGCGGCTCTCCTTTTGGCG |
| Specific primers for eIF4AII PCR | sense | ATGTCAGGCGGCTCCGCGGAT |
| | antisense | AATGAGATCAGCCACATTCAT |
| Specific primers and probe for eIF4AII qRT-PCR | sense | CCAGAAGGAGCGGGATGT |
| | antisense | CACGATTGGTCGGCAGGT |
| | probe | FAM-ATCACTACTGACTTGCTGGCTCGT-BHQ1 |
| Specific primers and probe for β-ctin qRT-PCR | sense | CTGGCATTGCTGACAGGAT |
| | antisense | GCCTCCAATCCAGACAGAGT |
| | probe | ROX-AGAAGGAGATCACAGCCCTGGCACC-BHQ2 |
| siRNA-479 | sense | GGCCGUGUGUUUGAUAUGUTT |
| | antisense | TTCCGGCACACAAACUAUACA |
| siRNA-570 | sense | GAGGAUUUAAGGAUCAAAUTT |
| | antisense | TTCUCCUAAAUUCCUAGUUUA |
| siRNA-1104 | sense | GGAAGGGUGUGGGCUAUAAATT |
| | antisense | TTCCUUCCCACACCGAUAUUU |

### 2.3. RNA isolation and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis

Total RNA was prepared from the tissues (liver, spleen, lung, kidney, glandular stomach, heart, bursa, and thymus) and cells (CEF, E6/Vero, and DF1) using RNAiso Plus (TaKaRa). The total RNA was used for cDNA synthesis by reverse transcription with the M-MLV RT-PCR kit (Invitrogen). The specific primers and probes for chicken eIF4AII and β-actin qRT-PCR were shown in Table 1 and synthesized by Invitrogen (Shanghai, China). Real-time PCR was carried out with a Light Cycler 480 system (Roche, Indianapolis, IN, USA) in a total volume of 25 μl containing 2.5 μl of 10 × Ex Taq Buffer, 2 μl of dNTP (2.5 mM), 3 μl of $MgCl_2$ (25 mM), 1 μl of each primer (10 pM), 0.5 μl of probe (10 pM), 1 U of Ex Taq HS (TaKaRa, China), 1 μl of DNA, and an appropriate amount of $ddH_2O$. Thermal cycling parameters were as follows: 95 °C for 5 min; 40 cycles of 94 °C for 10 s, 55 °C for 30 s, and 72 °C for 20 s. The fluorescent signals were measured during the elongation step. The negative control was set up by substituting the DNA template with $ddH_2O$. The mean β-actin copy number extracted from 0.1 g of tissue or 200 μl of the cell lysate was defined as A; thus, the relative amount of eIF4AII was calculated as: eIF4AII RNA copies/g tissue (or ml of cell lysate) = (eIF4AII copies of one tissue sample/β-actin copies of the same sample) $\times A \times 10$ for tissue or $\times 5$ for cell lysate. All samples were run in triplicate on the same plate, and the β-actin gene was utilized as the reference gene.

### 2.4. Measurement of Gt growth in CEF and DF1 cells

Confluent monolayers of CEF or DF1 cells in cell culture flasks ($25\ cm^2$) were infected with Gt at a multiplicity of infection (MOI) of 0.01. Infected cell cultures were harvested at different time points (24, 36, 48, 60, and 72 h) after infection, and the titer of infectious progeny present in the cultures was determined as the number of plaque-forming units (PFU)/ml using the Reed-Muench formula (Piotrowski and Krawczyk, 1950). The mean and standard deviation (SD) were calculated from three independent experiments.

### 2.5. Distribution of eIF4AII in different cells and tissues

Total RNA was prepared from tissues and cells as described above. The RNA copy numbers of eIF4AII were calculated as described above. To detect the expression level of eIF4AII in cultured cells, CEF, DF1, or E6/Vero cells were lysed with RIPA buffer (Beyotime, China) containing 1 mM phenylmethylsulfonyl fluoride. Protein samples were separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Hybond-C Super; GE Healthcare). The nonspecific antibody-binding sites were blocked with 5% (m/v) skim milk (232100, BD) in PBS with Tween, and then reacted with the following primary antibodies: anti-eIF4AII polyclonal antibody (ab166801; Abcam) or anti-β-actin monoclonal antibodies (A5316; Sigma). The blots were incubated with IRDye 800CW donkey anti-goat IgG (926–32214; LiCor BioSciences) or IRDye800CW goat anti-mouse IgG (926–32210; LiCor BioSciences), and scanned using an Odyssey infrared imaging system (LiCor BioSciences) for visualization.