

Highly pathogenic avian influenza virus nucleoprotein interacts with TREX complex adaptor protein Aly/REF

ABSTRACT

We constructed a novel chicken (*Gallus gallus*) lung cDNA library fused inside yeast acting domain vector (pGADT7). Using yeast two-hybrid screening with highly pathogenic avian influenza (HPAI) nucleoprotein (NP) from the strain (A/chicken/Malaysia/5858/2004(H5N1)) as bait, and the *Gallus gallus* lung cDNA library as prey, a novel interaction between the *Gallus gallus* cellular RNA export adaptor protein Aly/REF and the viral NP was identified. This interaction was confirmed and validated with mammalian two hybrid studies and co-immunoprecipitation assay. Cellular localization studies using confocal microscopy showed that NP and Aly/REF co-localize primarily in the nucleus. Further investigations by mammalian two hybrid studies into the binding of NP of other subtypes of influenza virus such as the swine A/New Jersey/1976/H1N1 and pandemic A/Malaysia/854/2009(H1N1) to human Aly/REF, also showed that the NP of these viruses interacts with human Aly/REF. Our findings are also supported by docking studies which showed tight and favorable binding between H5N1 NP and human Aly/REF, using crystal structures from Protein Data Bank. siRNA knockdown of Aly/REF had little effect on the export of HPAI NP and other viral RNA as it showed no significant reduction in virus titer. However, UAP56, another component of the TREX complex, which recruits Aly/REF to mRNA was found to interact even better with H5N1 NP through molecular docking studies. Both these proteins also co-localizes in the nucleus at early infection similar to Aly/REF. Intriguingly, knockdown of UAP56 in A549 infected cells shows significant reduction in viral titer (close to 10 fold reduction). Conclusively, our study have opened new avenues for research of other cellular RNA export adaptors crucial in aiding viral RNA export such as the SRSF3, 9G8 and ASF/SF2 that may play role in influenza virus RNA nucleocytoplasmic transport.

Keyword: Highly pathogenic avian influenza; Nucleoprotein; TREX complex; Protein Aly.