Characterization of *Ictalurid herpesvirus 1* glycoprotein ORF59 and its blocking role on viral infection in catfish cells

Shuxing Li, Fei Yu , Zhe Zhao

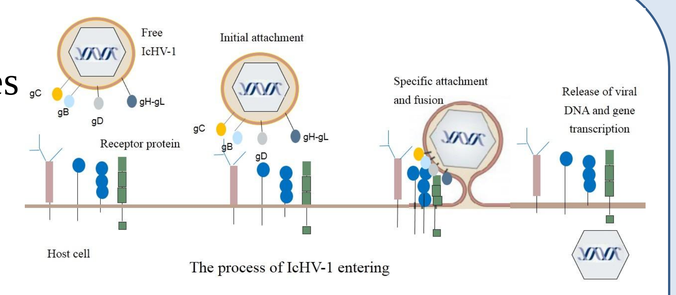
Department of Marine Biology, College of Oceanography, Hohai University, Nanjing 210098, PR China

Contact:191311040007@hhu.edu.cn

Introduction

The CCV (*Ictalurid herpesvirus 1*) has caused sustained economic losses in the fish industry because of its short replication cycle and strong infectivity. In order to control virus infection, it is necessary to understand the interaction mechanism between the virus protein and host cells in the process of infection and immune stimulation.

This study was undertaken to explore the impact of CCV envelope protein ORF59 blocking cell receptors on virus invasion of host cells.



The process of IcHV-1 entering

CCV ORF59 is a membrane associated protein.

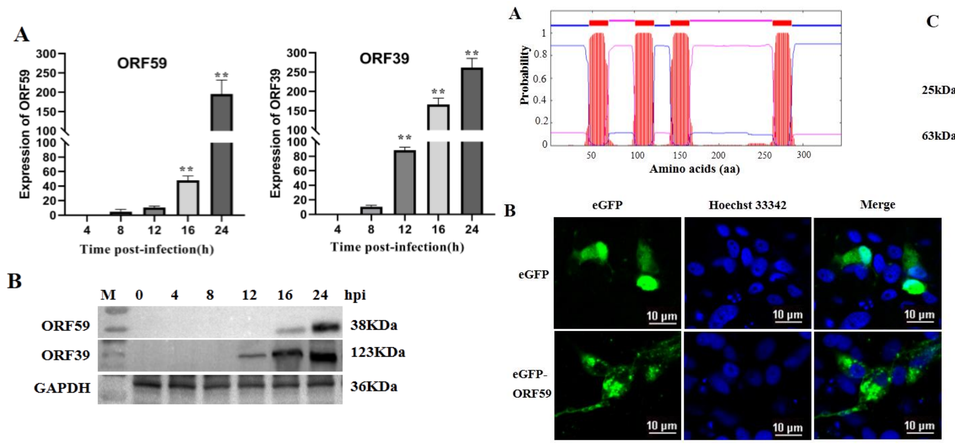


Figure 1: Kinetics of ORF59 expression in infected cells.（AB）

Figure 2: ORF59 is a membrane protein in CCO cells.

(A): Prediction of transmembrane regions of the ORF59 protein.

(B): Intracellular localization of ORF59 detected by expression of an eGFP-ORF59 fusion protein.

(C): Analysis of ORF59 expression using west blotting. Membrane proteins (MP) and non-membrane proteins (Non-MP) were extracted from CCO cells transfected with pEGFP-ORF59.

ORF59 knockdown reduces production of infectious virus particles in CCO cells.

★ shRNA-948 exhibits the highest knockdown efficiency against ORF59 mRNA

★The result revealed that ORF59 silencing reduced the CCV titers at 12, 24, and 36 hpi respectively.

★The results showed that mRNA levels of three CCV genes (ORF3, ORF5, and ORF39) were obviously decreased in infected CCO cell at 12, 24, and 36 hpi, compared with those of the shRNA-NC group

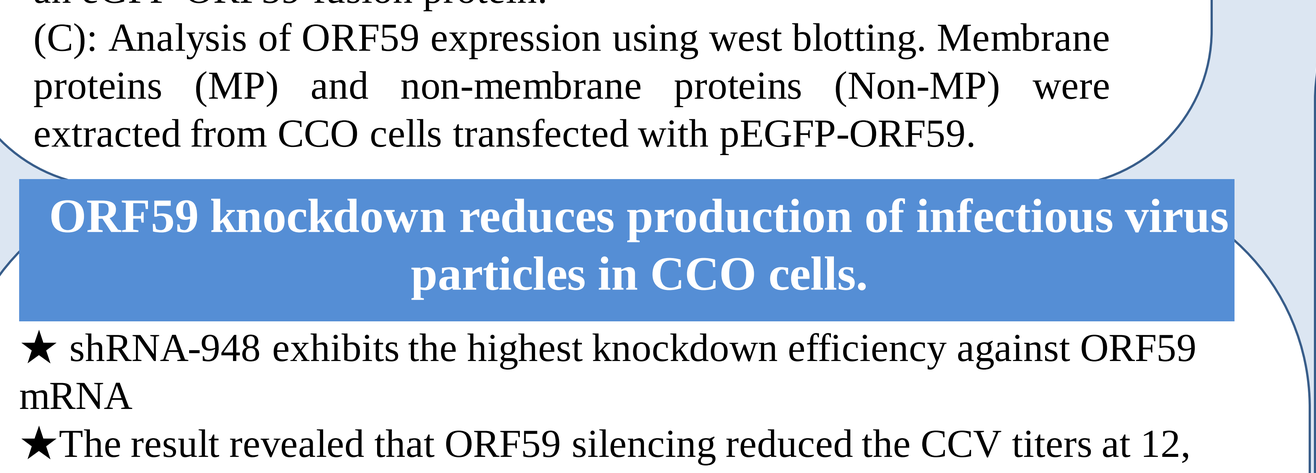
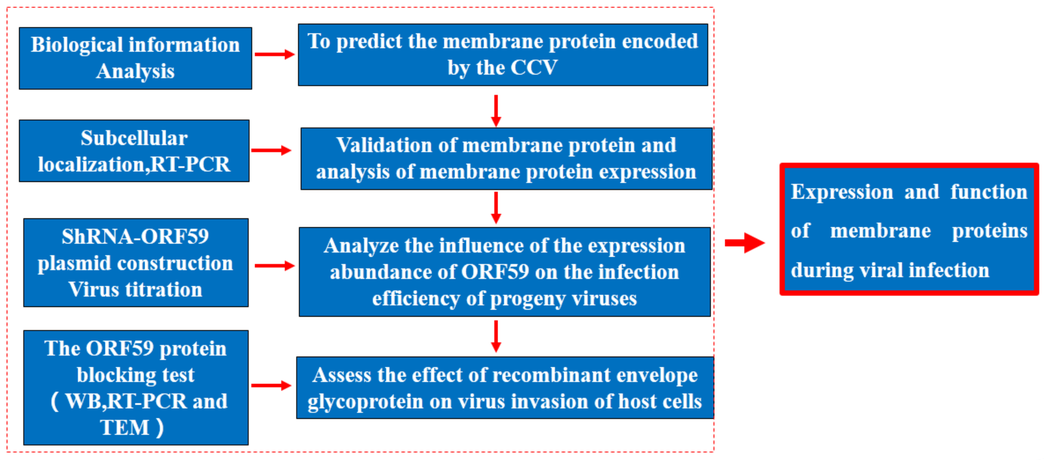


Figure 3:shRNA silencing of ORF59 affect CCV infection of cells.

Figure 4:The expression levels of genes closely related with replication of virus were decreased when CCV ORF59 gene knockdown.

Methods



Expression of ORF59 Protein in Sf9 Cells.

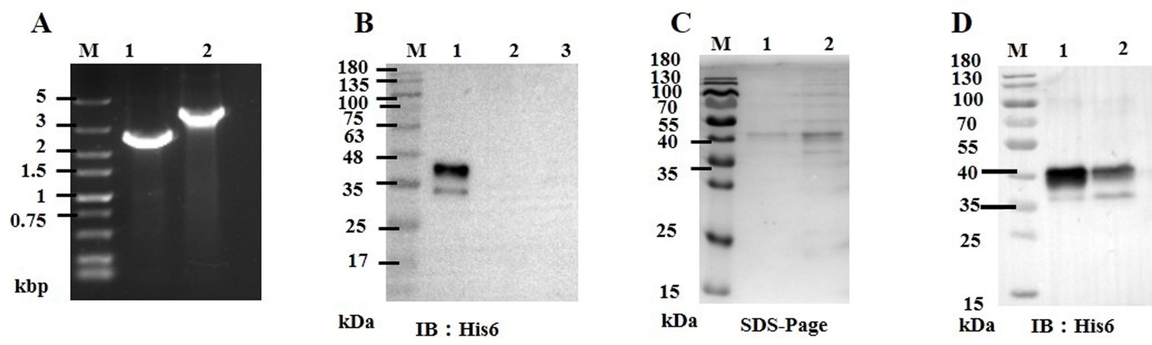


Figure 5:. Expression of ORF59 Protein in Sf9 Cells.

(A)Identification of recombinant Bacmid-ORF59 by PCR

(B)SDS-PAGE analysis of ORF59 gene expression in recombinant baculovirus infected Sf9 cells.

(C) SDS-PAGE of expressed and purified tagged fusion protein.

(D)Western blot of expressed and purified tagged fusion protein.

Recombinant ORF59 protein blocks CCV infection by abrogation of cellular binding.

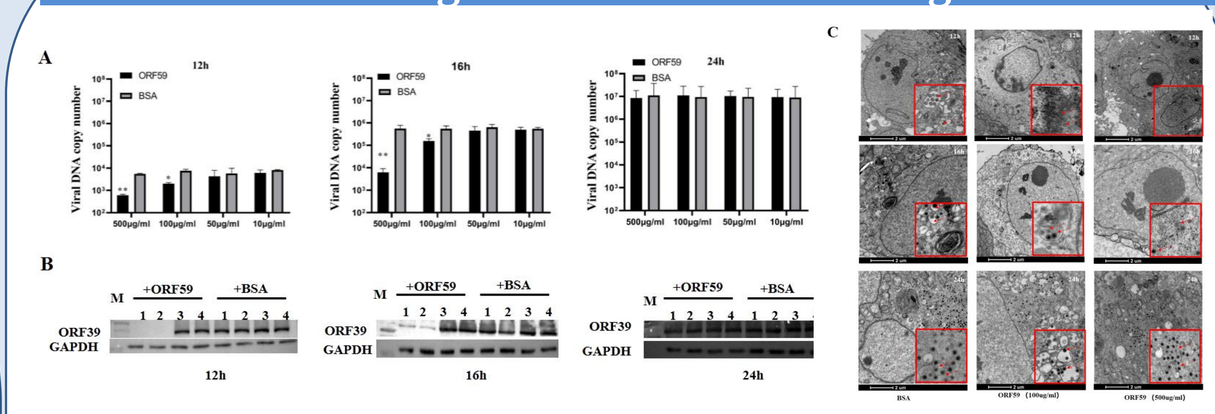


Figure 6:Recombinant ORF59 protein blocks CCV infection.

★ The results showed that preincubation of CCO cells with ORF59 protein at 500 μg/ml or 100 μg/ml significantly reduced the viral genomic copy number and protein expression level of ORF39 at 12 and 16 h.

★The data showed that there were fewer virus particles in the ORF59 protein-treated group at 12 and 16 h, while many unenveloped particles were seen in the cytoplasm of the con-trol group cells. Likewise, there were no significant differences at the late stage of virus in-fection (24 h).

conclusion

- CCV ORF59 protein is a membrane protein expressed at the late-stage of the virus infection cycle
- ORF59 protein blocking had an inhibitory effect on virus adsorption
- ORF59 silencing inhibited the production of infectious CCV particles in CCO cells.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 32002433), and the Natural Science Foundation of Jiangsu Province (Nos. BK20200522, BK20190484), and the Earmarked Fund for Jiangsu Agricultural Industry Technology System (No. JATS [2020] 465).