Channel catfish virus ORF25 and ORF63 genes are essential for viral DNA replication and infection

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Background

- The channel catfish virus (CCV) is one of the most important viral pathogens in fish industry, Molecular mechanisms of CCV infection and pathogenesis are still less known.
- Genomic DNA replication is a necessary and key event for CCV life cycle. The impacts of putative helicase and primase encoded by viral ORF25 and ORF63 on the CCV genome replication and infection were evaluated in channel catfish ovary (CCO) cells.
- ORF25 and ORF63 are essential for regulating CCV genome replication and CCV-induced infection. Our findings will provide benefit for understanding the replication mechanisms of CCV and contribute to the development of antiviral strategy for controlling CCV infection in channel catfish culture.

Methods

- Cell culture and virus propagation
- Plasmid and small interfering RNA construction
- Construction of viral standards for quantification
- Viral DNA replication assay
- Detection of viral true-late gene
- Cytopathic effect and virus titration assays
- Morphogenesis of CCV in CCO cells
- RNA isolation, cDNA synthesis and RT-qPCR analysis

Results

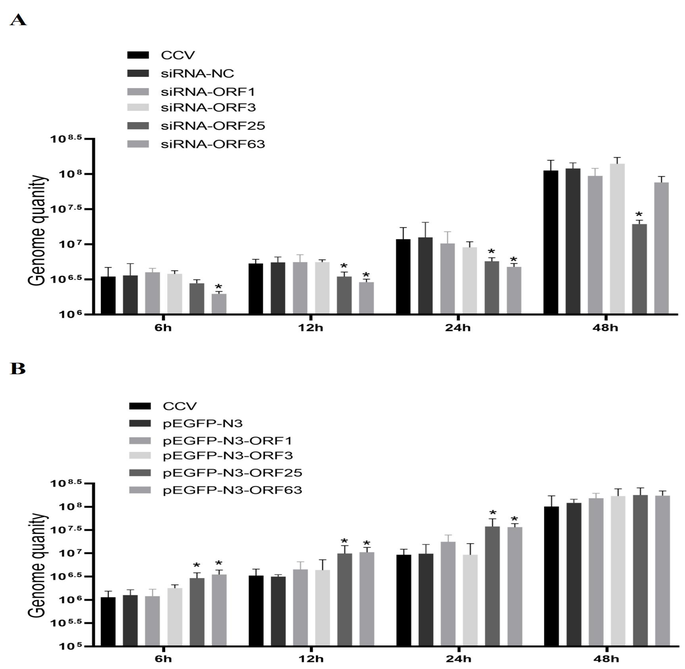

Figure 1: The effects of ORF25 and ORF63 on CCV genome replication in CCO cells.

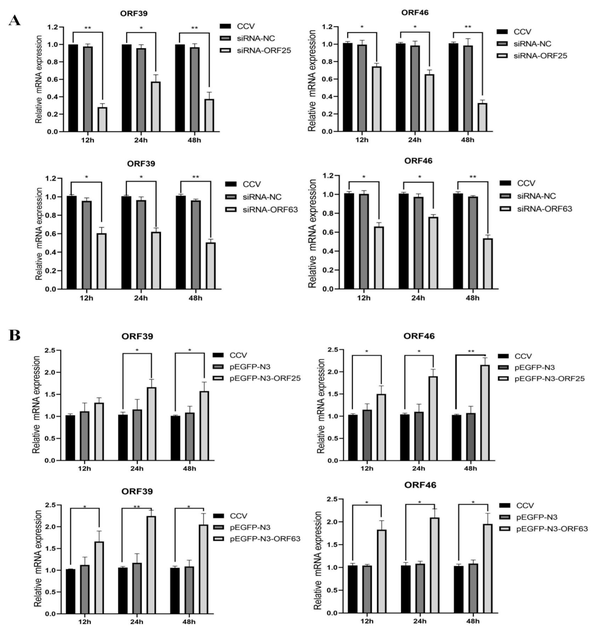

Figure 2: The true-late genes expression level during CCV infection in CCO cells.

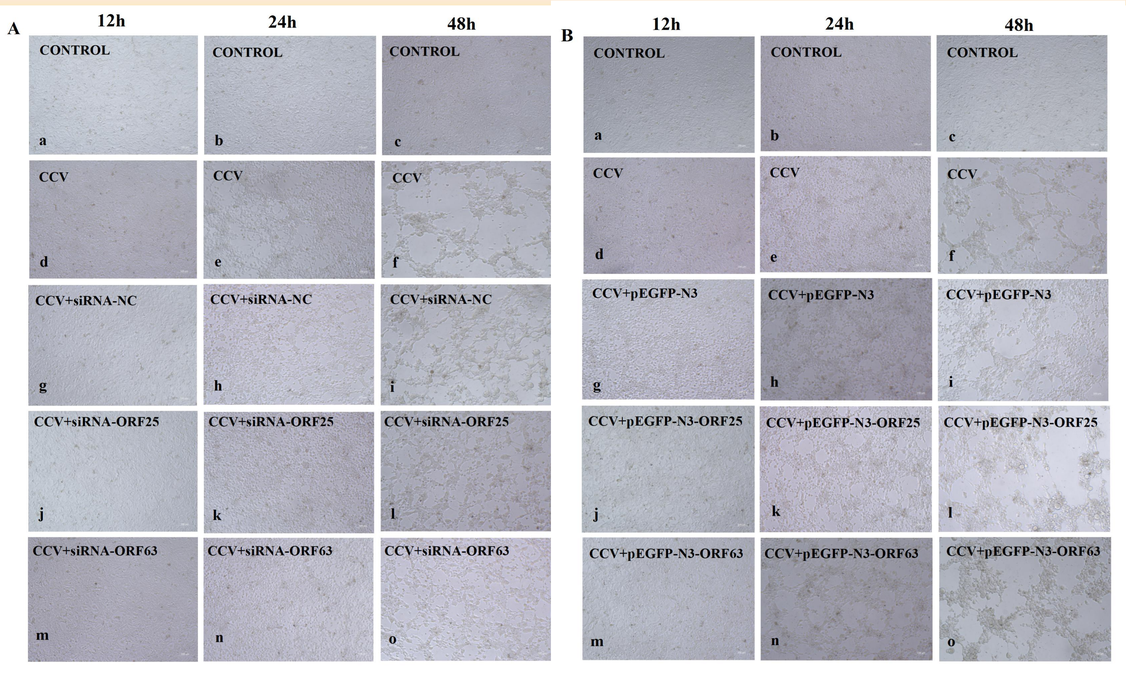

Figure 3: Changes in CCV-induced CPE by RNA interference or overexpression ORF25 and ORF63.

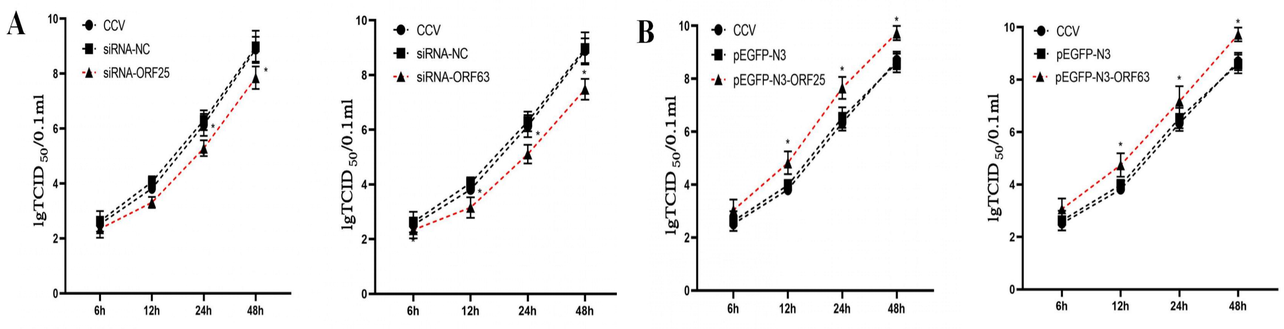

Figure 4: Analysis of virus titer. (A) CCV titer of progeny virus reduced via siRNA targeted ORF25 and ORF63. (B) CCV titer of progeny virus witnessed growing trends by overexpression ORF25 and ORF63.

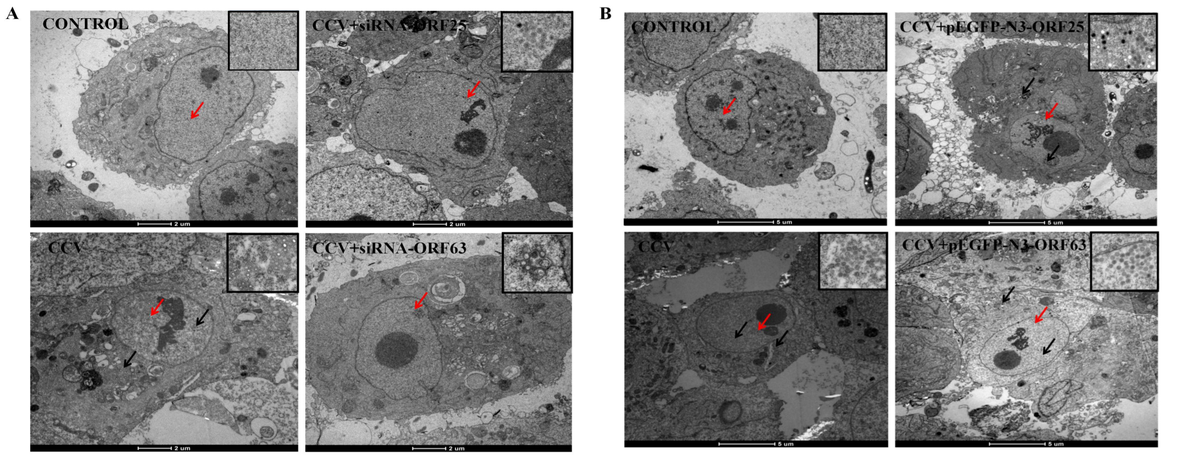

Figure 5: Ultrastructural changes of CCV particles. (A) Transmission electron microscopy image showed the virion changes in CCO cells by siRNA targeted ORF25 and ORF63. (B) Transmission electron microscopy image showed the virion changes in CCO cells by overexpression ORF25 and ORF63.

Conclusion

- We provide the evidence that the putative helicase and primase encoded by CCV ORF25 and ORF63 genes participate in the DNA replication of CCV in vitro experiment.
- After knockdown via small interfering RNA targeted viral ORF25 and ORF63 genes, the observed antiviral activity was found to be associated with reduced CPE, viral titer and the production of mature virion.
- Our findings will provide benefit for understanding the replication mechanisms of CCV and contribute to the development of antiviral strategy for controlling CCV infection in channel catfish culture.

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