

Mutation of the First ATG ORF-3 of Chicken Anemia Virus into ACG Completely Abolish the Apoptin Production

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Abstrak

To test the effect of one point mutation on the first initiation codon of Chicken Anemia Virus (CAV) open reading frame-3 (ORF-3), an apoptin knocked out expressor plasmid pCLS-VP3(-) and a CAV apoptin knocked out plasmid pCAV/Ap(-) were constructed. In both plasmids, the first ATG in ORF-3 was mutated into ACG by a reversed long PCR. No apoptin detected in COS-1 cells transfected with pCLS-VP3(-) using western blotting and immunofluorescence assay, while apoptin was detected in COS-1 cells transfected with pCLS wild type. After released from pCAV/Ap(-), the complete genome of CAV/Ap(-) was ligated to form the replicative form. The apoptin production was completely abolished in MDCC-MSB1 cells transfected with replicate form of CAV/Ap(-). The apoptin production was fully regained after a reverse mutation into CAV/Ap(-)RM. These data shows the first evidence that mutation of the first ATG of ORF-3 into ACG could completely abolish the production of apoptin.