P-4

The Minute Virus of Canines NP1 Protein Affects Multiple Aspects of Viral Gene Expression via its Role in Alternative RNA Processing

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The Bocaparvovirus Minute Virus of Canines (MVC) encodes a small, genus-specific protein, NP1, which governs access to the viral capsid gene via its role in alternative polyadenylation and alternative splicing of the single MVC pre-mRNA. Additionally, three essential nonstructural proteins are encoded by mRNAs that excise the NP1-regulated MVC intron immediately upstream of the internal polyadenylation site (pA)p, and so generation of these proteins is regulated by NP1. Thus, in addition to controlling capsid gene access, NP1 also controls expression of three of the five identified MVC NS proteins via its effect on pre-mRNA splicing. *In vivo* UV cross-linking and RNA-immunoprecipitation has recently shown that NP1 can bind MVC viral RNA. In addition, we found that cleavage and polyadenylation specificity factor subunit 6 (CPSF6), had a role in processing MVC RNA independent of viral replication. Specifically, depletion of CPSF6 enhanced splicing of the NP1-dependent 3rd intron from MVC pre-mRNAs. This resulted in decreased expression of the NS-66 protein, but not NP1. In addition, depletion of CPSF6 also led to a relative decrease in the percentage of polyadenylation at the NP1-dependent internal p(A)p site. Internal polyadenylation of MVC RNAs was independent of NP1 when (pA)p was replaced by the strong polyA site from the bovine growth hormone (bGH). Interestingly, under these conditions, the effects of CPSF6 on 3rd intron splicing was also independent of NP1. This suggested that CPSF6 inhibition of MVC alternative RNA processing was suppressed by factors recruited by, or associated with, the heterologous bGH signal. CPSF6 also had an effect on export of MVC RNAs. In CPSF6 depleted cells, export of internally polyadenylated (pA)p RNAs, relative to RNAs polyadenylated distally at (pA)d, as well as export of spliced, relative to unspliced RNAs, was decreased. This observation could explain why depletion of CPSF6 enhanced rather than diminished NP1 levels.