

ABSENCE OF 5' METHYLGUANOSINE CAP IN POSTREPLICATIVE GENES' TRANSCRIPTS OF VACCINIA VIRUS

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The Vaccinia virus (VACV) played a pivotal role in the discovery of the 5' mRNA. Over the past decades, VACV investigation has contributed extensively to understanding the mechanism behind 5' mRNA cap synthesis. In this study, we conducted a comprehensive analysis of VACV transcripts at the level of individual mRNA molecules. Surprisingly, our findings reveal that postreplicative VACV mRNAs, which incorporate nontemplated 5' poly(A) leaders, lack the conventional 5' cap structure *in vivo*.

Remarkably, our research uncovers a notable trend wherein the occurrence of 5' caps in viral mRNAs gradually diminishes across successive gene time classes of VACV. This stands in contrast to the concurrent increase observed in the lengths of 5' poly(A) leaders. Strikingly, these two variables exhibit a mutually inverse correlation. Further exploration demonstrates a direct or indirect influence of the initiator region element (INR) on both the frequency of 5' mRNA capping and the presence of 5' poly(A) leaders, encompassing their varied lengths in postreplicative VACV mRNAs.

By amalgamating our observations, we posit a hypothesis suggesting a potential link between the extent of 5' mRNA polyadenylation and the synthesis of the 5' cap, mediated through an as-yet-undisclosed mechanism. This notion gains support from our identification that 5' poly(A) leaders in VACV late transcripts containing m⁷G caps are notably shorter than their counterparts, whose lengths were computed from an unbiased collection of all VACV late mRNAs.

In concert, our results underscore a compelling proposition: the regulatory framework governing VACV transcription orchestrates a gradual transition in viral mRNA translation initiation, shifting from a reliance on cap-dependent mechanisms to cap-independent ones. This intriguing phenomenon coincides with the virus-induced alteration of the host's translation machinery.

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