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Biochemical analysis of the multifunctional vaccinia mRNA capping enzyme encoded by a temperature sensitive virus mutant

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Prior biochemical analysis of the heterodimeric vaccinia virus mRNA capping enzyme suggests roles not only in mRNA capping but also in early viral gene transcription termination and intermediate viral gene transcription initiation. Prior phenotypic characterization of Dts36, a temperature sensitive virus mutant affecting the large subunit of the capping enzyme was consistent with the multifunctional roles of the capping enzyme in vivo. We report a biochemical analysis of the capping enzyme encoded by Dts36. Of the three enzymatic activities required for mRNA capping, the guanylyltransferase and methyltransferase activities are compromised while the triphosphatase activity and the D12 subunit interaction are unaffected. The mutant enzyme is also defective in stimulating early gene transcription termination and intermediate gene transcription initiation in vitro. These results confirm that the vaccinia virus mRNA capping enzyme functions not only in mRNA capping but also early gene transcription termination and intermediate gene transcription initiation in vivo.

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Introduction

The poxvirus vaccinia contains a 200 kb dsDNA genome encoding approximately 200 annotated gene products, and replicates in the cell cytoplasm (Moss, 2013). The cytoplasmic site of replication demands that the virus encode and package in the virion an entire complement of enzymes required for production of capped and polyadenylated mRNA, including a multisubunit RNA polymerase, a heterodimeric poly(A) polymerase and a heterodimeric mRNA capping enzyme. For this reason, vaccinia has a long been utilized as a simple model system for understanding basic mechanisms of mRNA metabolism. Interestingly, despite a relatively large coding capacity, vaccinia has nevertheless apparently evolved to economize on genetic content by elaborating multiple roles for several viral enzymes. Notable in this regard is the viral mRNA capping enzyme, which serves not only to cap the mRNA 5′ end, but also as an early gene transcription termination factor and an intermediate gene transcription initiation factor. Understanding the mechanisms of this multifunctionality is of intrinsic interest in the context of virus biology and evolution.

Transcription during vaccinia infection is regulated to yield three waves of gene expression: early, intermediate and late (Broyles, 2003). The viral RNA polymerase exists in two forms, one specific for early gene transcription, and one for intermediate and late (together called postreplicative) gene transcription. Both forms of the RNA polymerase contain eight core subunits. The core enzyme transcribes postreplicative genes. The early gene-specific form of the RNA polymerase contains an additional subunit, encoded by gene H4, which interacts with an early promoter-binding viral early transcription factor (Yang and Moss, 2009). Early mRNAs are synthesized from infecting virion core particles using transcription and mRNA modification enzymes packaged in the virion. Early transcripts encode both viral DNA replication enzymes and intermediate transcription factors, which together with newly synthesized viral RNA polymerase activate transcription from intermediate gene promoters on replicating viral DNA released during uncoating of the virion cores. Intermediate genes encode late transcription factors which in turn activate transcription from late gene promoters. Late genes encode virion structural proteins plus the enzymes required for early gene transcription, which are packaged into maturing virions for the subsequent round of infection.

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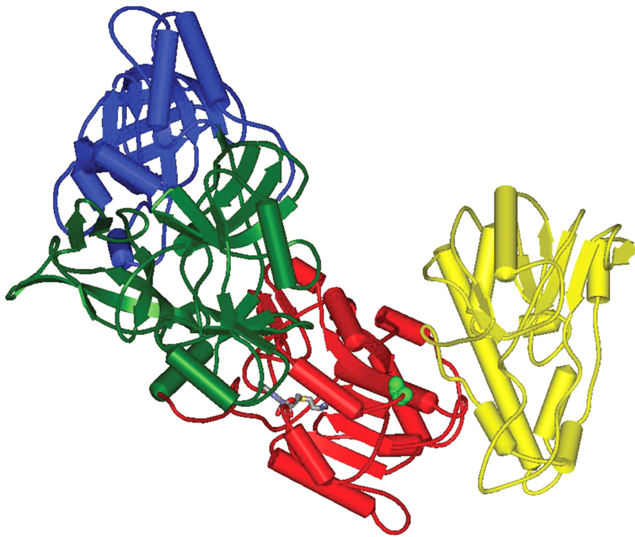

Fig. 1. Structure of the vaccinia virus capping enzyme. Ribbon diagram representation of the structure of CE. The three domains of the large D1 subunit are represented in different colors. The TPase domain (residues 1–225) is blue, the GTase domain (residues 226–545) is green, and the MTase domain (residues 548–844) is red. The small D12 subunit is yellow. The bound S-adenosyl-L-homocysteine in the MTase active site is shown in stick models colored by element. Gly 705, which is replaced with Asp in the Dts36 mutant, is depicted in space-filling models colored light green.

The viral mRNA capping enzyme (CE) is comprised of two subunits of 97 kDa and 33 kDa, the products of gene D1 and D12 respectively (Martin et al., 1975; Morgan et al., 1984; Niles et al., 1989; Guo and Moss, 1990). Biochemical and genetic analysis of the enzyme coupled with a recent crystal structure (Kyrieleis et al., 2014) (Fig. 1) reveal three separate and distinct catalytic domains in the D1 subunit which together carry out the three reactions (Martin and Moss, 1975, 1976; Shuman and Hurwitz, 1981) required for cap formation on a nascent 5′ triphosphorylated RNA shown below:

I	pppRNA → ppRNA + Pi
IIa	GTP + enz → enz-GMP + Pi
IIb	enz-GMP + ppRNA → GpppRNA + enz
III	GpppRNA + SAM → meGpppRNA + SAH

The first reaction, removal of the gamma phosphate from the triphosphorylated RNA to yield a 5′ diphosphorylated RNA, is catalyzed by the N-terminal RNA triphosphatase (TPase) domain of D1 (Yu and Shuman, 1996; Myette and Niles, 1996a; Gong and Shuman, 2003). The second reaction, addition of a GMP to the 5′ diphosphorylated RNA in a 5′–5′ linkage to yield an unmethylated cap structure, proceeds through a covalent enzyme GMP complex (Shuman and Hurwitz, 1981) and is catalyzed by the internal guanylyltransferase domain (GTase) of D1 (Niles and Christen, 1993; Cong and Shuman, 1995; Myette and Niles, 1996b). The third reaction, transfer of a methyl group from S-adenosylmethionine to the 7 position of the cap guanine to yield a cap 0 structure and S-adenosylhomocysteine, is catalyzed by the C-terminal methyltransferase domain (MTase) of D1 (Higman et al., 1994; Mao and Shuman, 1994). The D12 subunit forms a stable complex with the methyltransferase domain of D1. D12 has no known enzymatic activity but serves to stimulate the otherwise relatively weak intrinsic methyltransferase activity of D1.

The D1/D12 heterodimer is a required cofactor for viral early gene transcription termination (Shuman et al., 1987; Luo et al., 1991). Early gene transcription termination is a sequence specific event triggered by the sequence U5NU found 20–50 nt upstream of early gene 3′ ends (before polyadenylation) (Yuen and Moss, 1987). During viral early gene transcription termination, the sequence U5NU is recognized by CE via the N-terminal TPase domain of D1 (Christen et al., 2008). Termination itself is catalyzed by a DNA-dependent nucleoside triphosphate phosphohydrolase (NPHI, gene D11), which interacts with the early form of the RNA polymerase through contacts with the early gene specific H4 subunit (Deng and Shuman, 1996, 1998; Christen et al., 1998; Mohamed and Niles, 2000). CE can induce pausing by elongating RNA polymerase, which could theoretically provide a properly configured ternary complex of polymerase, DNA and RNA, primed for NPHI termination activity (Tate and Gollnick, 2011). The mRNA capping activity of CE is not required for early gene transcription termination, however both the D1 and D12 subunits must be present (Luo et al., 1995; Condit et al., 1996; Yu and Shuman, 1996).

CE is one of three viral factors required for intermediate gene transcription initiation (Vos et al., 1991b). The other factors are VITF1, comprised of the E4 core subunit of RNA polymerase, and VITF3, a heterodimer of the viral A8 and A23 proteins (Rosales et al., 1994; Sanz and Moss, 1999). A cellular factor consisting of G3BP and p137 has also been reported to stimulate intermediate gene transcription in vitro (Katsafanas and Moss, 2004). Formation of a stable complex between capping enzyme and RNA polymerase seems to be a prerequisite for intermediate gene transcription, otherwise little is known about the mechanism of action of intermediate gene transcription initiation factors (Vos et al., 1991a). The mRNA capping activity of CE is not required for intermediate gene transcription initiation, however both the D1 and D12 subunits must be present (Harris et al., 1993; Condit et al., 1996).

We have described previously a temperature sensitive mutant in gene D1R, Dts36, the phenotype of which suggests effects on all three roles of CE: mRNA capping, early gene transcription elongation and intermediate gene transcription initiation (Shatzer et al., 2008). Specifically, although mRNA capping was not examined directly, steady state levels of some early viral mRNAs were decreased, consistent with a defect in mRNA capping. The mutant also displayed transcriptional readthrough of early viral genes consistent with a defect in early gene transcription termination, and decreased steady state levels of specific intermediate but not late gene transcripts, consistent with a defect in intermediate gene transcription initiation. The mutation comprises a glycine to aspartic acid substitution on the surface of the MTase domain of D1, distant from either the catalytic site or the D12 interaction domain. Our goal in the work presented here was to perform a biochemical analysis of the Dts36 mutant CE in order to substantiate the phenotypic analysis and provide additional mechanistic insight into the multifunctional nature of the enzyme. We find that the mutant enzyme displays defects in both MTase and GTase activities while the TPase activity and the D12 subunit interaction are unaffected. The mutant enzyme is also defective in stimulating early gene transcription termination and intermediate gene transcription initiation in vitro. The findings are interpreted in light of the 3D structure of the enzyme.

Results

CE abundance in WT and Dts36 virions; enzyme purification

WT and Dts36 virions were purified from BSC-40 cells grown at 30 °C. The overall protein content in the virion preparations was examined by SDS-PAGE analysis of varying amounts of WT virions and Dts36 virions, visualized by Coomassie stain (Fig. 2A). Similar polypeptide staining patterns were observed between WT virions

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