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The acidic C-terminus of vaccinia virus I3 single-strand binding protein promotes proper assembly of DNA–protein complexes

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The vaccinia virus I3L gene encodes a single-stranded DNA binding protein (SSB) that is essential for virus DNA replication and is conserved in all Chordopoxviruses. The I3 protein contains a negatively charged C-terminal tail that is a common feature of SSBs. Such acidic tails are critical for SSB-dependent replication, recombination and repair. We cloned and purified variants of the I3 protein, along with a homolog from molluscum contagiosum virus, and tested how the acidic tail affected DNA–protein interactions. Deleting the C terminus of I3 enhanced the affinity for single-stranded DNA cellulose and gel shift analyses showed that it also altered the migration of I3-DNA complexes in agarose gels. Microinjecting an antibody against I3 into vaccinia-infected cells also selectively inhibited virus replication. We suggest that this domain promotes cooperative binding of I3 to DNA in a way that would maintain an open DNA configuration around a replication site.

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Introduction

Poxviruses are large DNA viruses that replicate in discrete cytoplasmic foci, or factories. The prototypical poxvirus, vaccinia virus (VACV), encodes most of its own proteins required for DNA replication. These include a DNA polymerase (E9) which associates with two additional proteins (A20+D4) in a trimeric complex (Stanitsa et al., 2006), a primase/helicase (D5) (De Silva et al., 2007), a DNA ligase (A50) (Kerr and Smith, 1991), enzymes catalyzing dNTP biogenesis (J2, F2, F4/I4, A48R) (Beaud, 1995; Gammon et al., 2010; Weir and Moss, 1983), a Holiday junction resolvase (A22) (Garcia et al., 2000), a single-stranded DNA binding protein (I3) (Rochester and Traktman, 1998; Tseng et al., 1999), a flap endonuclease (G5) (Senkevich et al., 2009), and other proteins of less certain function such as H5 (Boyle et al., 2015; Kay et al., 2013). Replication is also linked to virus recombination and uses a single-strand annealing reaction catalyzed by E9 and I3 (Gammon and Evans, 2009; Willer et al., 2000).

Several models have been proposed to explain the mechanism of poxvirus DNA replication with a “rolling hairpin” mechanism being long favored as a way of explaining the origins of concatemeric replication intermediates (Moyer and Graves, 1981). However, this model does not explain why VACV encodes a protein with DNA primase activity (De Silva et al., 2007). The fact that VACV encodes a primase as well as a flap endonuclease (Senkevich et al., 2009) suggests that it could also use a discontinuous mode of DNA replication requiring leading and lagging strand DNA synthesis.

Although these matters remain to be resolved, it is well established that single-stranded DNA (ssDNA) substrates are critical replication, recombination, and repair intermediates and ssDNA is always sequestered in complexes composed of the DNA plus single-strand DNA binding proteins (SSBs). VACV also encodes a SSB, called I3, encoded by the I3L gene (Rochester and Traktman, 1998). I3 is an essential gene product that exhibits early and intermediate expression kinetics, binds with high affinity to ssDNA, and localizes to factories (Rochester and Traktman, 1998; Welsch et al., 2003). S1 protection and gel-shift assays have detected at least two DNA binding sites (∼9 and ∼31 nt) and gel shift assays combined with protein titrations suggest that different structures are formed at different DNA-to-protein ratios (Tseng et al., 1999). Electron microscopy showed that compact DNA–protein complexes with a “beads on a string” appearance are formed at low protein densities, which are converted to more linear structures as the protein loading is increased (Tseng et al., 1999). The capacity to form such protein multimers seems to be essential for biological activity (Greseth et al., 2012). Although the gene cannot be disrupted, RNA interference shows that I3 is needed for both virus replication and recombination *in vivo* (Gammon and Evans, 2009; Greseth et al., 2012).

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The DNA-binding properties of I3 are reminiscent of the different DNA-binding modes exhibited by other SSBs even though there are no obvious similarities between poxviral and other bacterial, viral, or phage SSBs. Moreover, I3 encodes a patch of ~40 negatively charged residues at the C-terminus, a feature that is characteristic of many phage and bacterial SSBs and which serves several different functions. For example, the T4 bacteriophage GP32 protein also encodes a 46 aa acidic C-terminal tail. The tail can be excised by limited proteolysis to create a form called GP32*I, which binds more tightly to ssDNA than the native protein and acquires the capacity to melt duplex DNA (Lonberg et al., 1981; Moise and Hosoda, 1976). When DNA binds to native GP32, it displaces the C-terminus from where it occludes the DNA-binding cleft, and the displaced and exposed end can then serve as a binding site for other proteins (Kowalczykowski et al., 1981; Krassa et al., 1991; Lonberg et al., 1981). Interestingly, the C-terminus of GP32 is an immunodominant epitope (Krassa et al., 1991), a feature that may also characterize I3.

Bacteriophage T7 GP2.5 exhibits similar properties. The acidic C-terminus (~26 aa) also encodes a critically important aromatic amino acid residue and, if this domain and phenylalanine are deleted, it disrupts GP2.5 binding to the T7 DNA polymerase/thioredoxin complex and primase-helicase, as well as increasing the affinity of the truncated protein for ssDNA. Deleting the tail also prevents GP2.5 dimer formation by interfering with a domain swap interaction. A scheme very similar to the DNA binding and C-terminal tail displacement model, originally proposed for T4 GP32, has been suggested to explain these features of T7 GP2.5 (He et al., 2003; Hyland et al., 2003; Marintcheva et al., 2006, 2008).

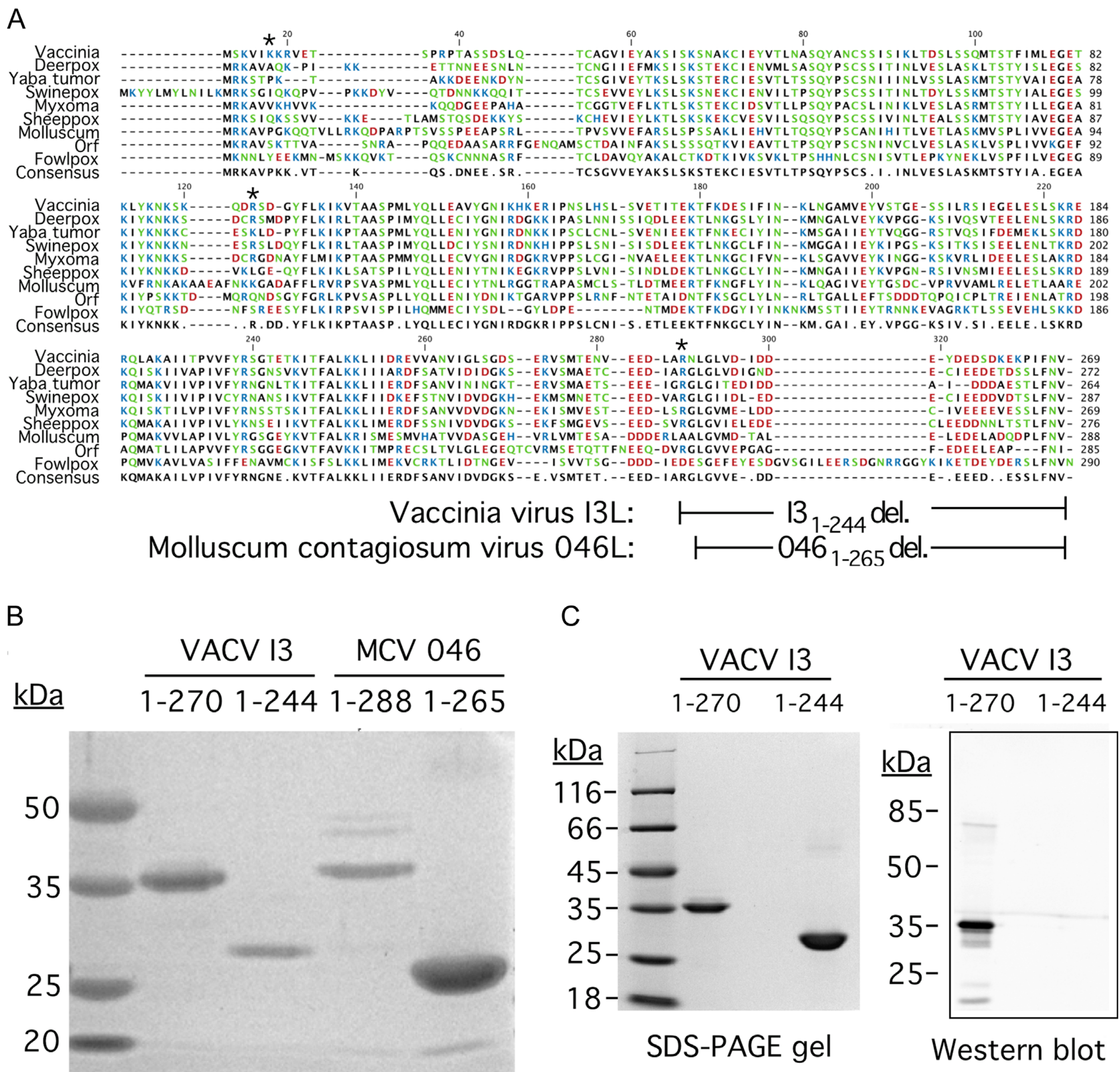

Fig. 1. Poxvirus single-strand binding proteins and the clones used in this study. *Panel A* shows an alignment of the vaccinia virus I3 protein with the homologs found in viruses representing other principle Chordopoxviruses. The asterisks (*) show trypsin hypersensitive sites that were previously mapped in I3 (Tseng, 2000). The figure also shows the sequences deleted from two sub-clones of the VACV I3L and molluscum contagiosum virus 046L genes. These encompass most of the acidic C-terminal tails of the two proteins. *Panel B* shows the purified recombinant proteins used in these studies, analyzed using SDS-PAGE. Note that although I3L encodes a 30 kDa protein, it generally migrates in SDS-PAGE gels at ~35 kDa (Tseng et al., 1999). Native MCV 046 protein (31.5 kDa) seems to exhibit the same property. *Panel C* illustrates the specificity of monoclonal antibody 10D11 determined by western blotting. It recognized an epitope deleted in form $I3_{1-244}$.

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