

SUPPLEMENTARY RESULTS AND DISCUSSION

Further discussion of the monkeypox 'SPI-1' and 'C7L' orthologues

Based on computer analysis of ORFs (see Methods), the monkeypox West African/USA clade protein orthologues of the vaccinia SPI-1 and C7L expressed proteins are predicted to have an amino-terminal fusion (Fig. 5b and Supplementary Fig. S1e). Careful examination was made of regions upstream of the predicted fusion proteins to look for possible consensus promoter sequences, and literature was reviewed to determine if the promoters had been mapped in other orthopoxviruses. The vaccinia orthologues have been characterized to be expressed early (Kettle *et al.*, 1995; Oguiura *et al.*, 1993).

SPI-1

In vaccinia virus strain Western Reserve (WR), the SPI-1 transcription initiation site, a purine, has been mapped by primer extension to 28 nt upstream of the translation start site. An early promoter, with good homology to consensus (Broyles, 2003; Davison & Moss, 1989), is identified 13 nt upstream. This region, conserved between vaccinia virus strains Copenhagen and WR is boxed in the upper two lines of the alignment in Supplementary Fig. S2(a) and (b). Although this same promoter sequence is found upstream of the monkeypox SPI-1 homologues, in the Congo Basin orthologues MPX-ZAI-1996-016, MPX-RCG-2003-358 and MPX-ZAI-1979-005, it is ~200, 260 and 200 nt upstream of the translation initiation site, respectively. Intervening between this promoter and the translation initiation sites are variable strings of a 9 nt (ATTATATAC) repeat region. Longer strings, totaling ~290 nt (MPX-LIB-1970-184) or ~270 nt (MPXV-USA-2003-039), of this same repetitive nucleotide sequence are present upstream of the methionine of the West African/USA clade orthologues homologous to the translation initiation methionine of the vaccinia orthologues.

In a region (36–69 nt) upstream of the methionine homologous between all monkeypox orthologues (Supplementary Fig. S2a), similar to the vaccinia promoter/transcription/translation initiation site spacing, is a nucleotide sequence with reasonable consensus to an early promoter. However, the +1 transcription initiation site (13 nt from the promoter) would not be a purine in the West African/USA clade isolates (Supplementary Fig. S2a). As well, there is no central 'G' (position –20 or –21) within this possible early promoter element – at a minimum, this would be predicted to decrease the strength of the promoter. Looking upstream of the amino-terminal fusion protein West African/USA MPXV orthologue predicted by ORF analysis (Methods) is another possible early promoter element (Supplementary Fig. S2b). In this case, a consensus purine is found 13 nt downstream of the early promoter element, and the predicted promoter element contains a central 'G' (position –20 or –21) nucleotide. This promoter in the West African/USA clade could transcribe the predicted 'fusion' protein (Supplementary Fig. S1e). The alignment in Supplementary Fig. S2(b) depicts the mapped promoter element of vaccinia, the promoter element 36–69 nt upstream of the Congo Basin clade MPXV SPI-1 orthologues and the further upstream West African/USA clade predicted promoter element. In this case, if the fusion protein were transcribed and translated, the promoter element would not be conserved across all monkeypox isolate orthologues.

If the 'vaccinia-mapped' consensus early promoter is used to transcribe the MPXV orthologues, the amino-terminal fusion would not be translated in the West African/USA clade protein orthologues, as it falls ~20 nt downstream of the predicted translation initiation methionine for the fusion protein. The alignment of the SPI-1 orthologues, without the amino-terminal fusion in the West African/USA clade orthologues, is depicted in Supplementary Fig. S1(f). Ultimate determination of which promoter element is used awaits experimental determination; it may be, if the 'vaccinia-mapped' early promoter is used, that expression may be adversely affected by the number of nucleotides between transcription and translation start sites.

C7L

The promoter has not been mapped for the C7L orthologues within vaccinia or any other orthopoxvirus. However, the promoter of the Leporipoxvirus myxoma has been mapped. A promoter, with reasonable consensus to a vaccinia early promoter, precedes (by 13 nt) a transcription initiation site mapped to 44 nt upstream of the myxoma C7L orthologue translation initiation site (Jackson & Bults, 1992). No identical promoter can be identified upstream of the Orthopoxvirus C7L homologues. Instead, possible early promoter regions can be found either ~80 or ~40 nt upstream of the methionine conserved between all monkeypox clade orthologues; the predicted early promoter ~80 nt upstream of the conserved methionine is ~25 nt upstream of the translation initiation methionine belonging to the predicted fusion protein encoded by the West African/USA monkeypox clade isolates (Supplementary Fig. S3). The promoter more proximal to the 'conserved' methionine has poorer consensus to the vaccinia early promoter consensus, and the site 13 nt from the promoter element (optimally a purine) is not a purine in the West African/USA clade isolates (Supplementary Fig. S3). Thus, the early promoter further upstream would be predicted to be the preferred/stronger promoter, and could initiate transcription, leading to translation, of a fusion protein in the West African/USA clade isolates.

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