Specific transcription of Orthopox virus DNA by HeLa cell RNA polymerase II

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A HeLa cell extract was used to transcribe DNA isolated from cowpox virus. Truncated templates generate accurately initiated run-off transcripts of discrete sizes and whose sensitivity to inhibition by α-amanitin indicates synthesis by cell RNA polymerase II. A mapped restriction fragment of wild-type cowpox DNA contains specific sites of initiation which are not detected in the geographically equivalent fragment from a cowpox mutant having a defined sequence rearrangement in this region.

Orthopoxvirus *DNA* *Transcription* *RNA polymerase II*

1. INTRODUCTION

Poxviruses are large complex viruses which replicate and transcribe genomic DNA in the cytoplasm of infected cells [1]. This is made possible by the expression of numerous virus coded enzymes concerned with nucleic acid biosynthesis including a virus DNA-dependent RNA polymerase and a series of enzymes responsible for the subsequent modification of virus transcripts (review [2]). Transcription by the viral polymerase is resistant to α-amanitin [3] and is promoted by virus DNA sequences which differ from consensus sequences of either prokaryotic or eukaryotic systems [4].

However, numerous reports indicate that host nuclear functions are also required to complete Orthopoxvirus replication. Anucleate BSC-1 cells are unable to support formation of mature progeny vaccinia virus [5,6]. A proportion of total virus genomic DNA is replicated in association with the nucleus of vaccinia infected HeLa cells [7] and a restricted set of virus-specific transcripts is synthesized there [8]. Formation of infectious progeny vaccinia virus, like Herpes simplex virus, is inhibited by α-amanitin in normal but not in α-amanitin resistant cells [9], even after γ-irradiation suggesting a requirement for nuclear transcription of vaccinia virus DNA by the host RNA polymerase II [10]. Direct evidence is presented below that a specific DNA sequence from the related Orthopoxvirus cowpox is transcribed by HeLa cell RNA polymerase II. However, this is not an essential gene function in cowpox replication as the geographically equivalent DNA fragment from a cowpox phenotypic variant which has a sequence rearrangement at this locus is not transcribed.

2. MATERIALS AND METHODS

A HeLa cell lysate prepared by osmotic rupture [11] was used for in vitro transcription of genomic DNA from wild-type red cowpox strain Brighton (BRCP) or from a white pock mutant (BWCP clone C) in which sequences deleted from one terminus of the genome are replaced with a copy of sequences from the opposing terminus [12]. The wild-type sequence containing the site of the deletion and rearrangement which generate the white

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pock mutant was cloned and amplified as a 3.7 kb *Pst*I restriction fragment in plasmid pUC9 (BRCP/*Pst*I O/pUC9; fig.1c) as was the geographically equivalent, re-arranged 4.8 kb fragment from DNA of the mutant (BWCP C1 C/*Pst*I L/pUC9; fig.1d). These were transcribed before or after linearization by cleavage at the unique *Hin*dIII site immediately adjacent to the *Pst*I cloning site (fig.1e). HeLa cell lysate was obtained from Bethesda Research Laboratories (Gibco, Paisley, Scotland) and [α-^{32}P]UTP (spec. act. >3000 Ci/mmol) from Amersham International, England. Transcription reactions contained 0.5–2.0 μg DNA in 5 μl of 10 mM Tris 1 mM EDTA, pH 7.2, to which was added 10 μCi [α-^{32}P]UTP in 5 μl of a solution containing 0.25 mM UTP, 2.5 mM ATP, 2.5 mM CTP, 2.5 mM GTP and 20 mM creatine phosphate. Transcription was initiated by addition of 15 μl HeLa cell lysate and stopped after incubation for 1 h at 30°C by addition of 475 μl of a solution containing 5 mM EDTA, 1% SDS and 20 μg/ml yeast tRNA. After phenol extraction and ethanol precipitation, the nucleic acid pellet was dissolved in formamide/dye mix as for DNA sequencing [13]. Samples denatured by heating at 70°C for 10 min and cooling in ice were electrophoresed on 6% polyacrylamide/8 M urea gels and the radiolabelled RNA visualised by autoradiography at −70°C using preflashed Fuji Rx X-ray film. Denatured, kinase ^{32}P-labelled *Hae*III restriction fragments of ϕX 174 RF DNA were co-electrophoresed as size markers.

3. RESULTS

Transcription in this system is dependent on template concentration which is optimized empirically. There is no detectable endogenous RNA synthesis without addition of template (fig.2, lane 2). Addition of 0.5–1.0 μg genomic DNA from either wild-type cowpox or the white pock mutant generates heterogeneous high-M_r transcripts reflecting lack of accurate termination in this system (fig.2, lanes 13–16). The same is true of circular non-recombinant plasmid pUC9 (fig.2, lane 10) or

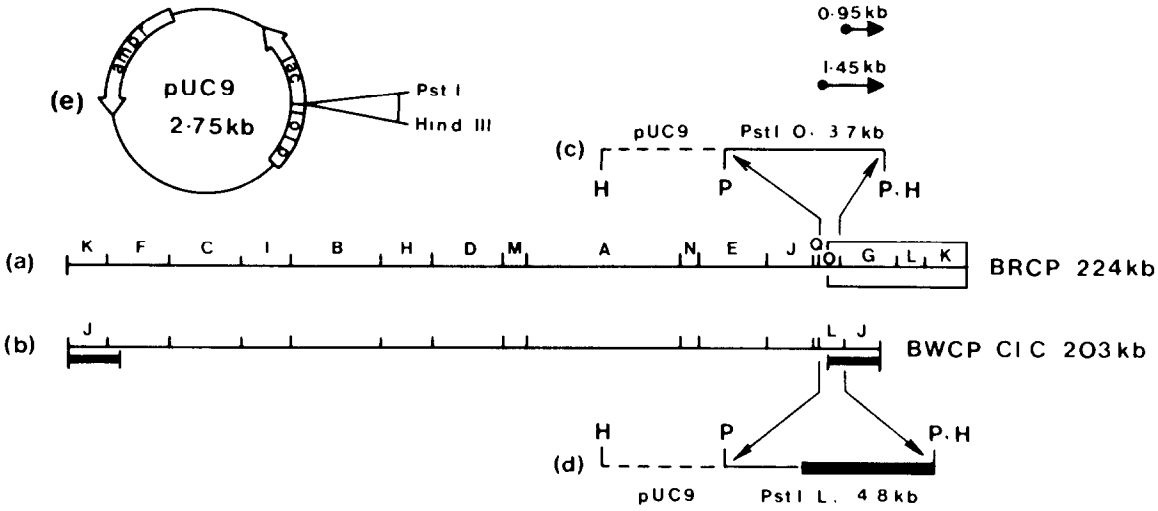

Fig.1. DNA templates employed for in vitro transcription. (a) Genomic DNA from wild-type red cowpox strain Brighton (BRCP) showing cleavage sites for restriction endonuclease *Pst*I with fragments lettered in order of decreasing size: the box indicates the size of the right-hand terminal sequence deleted in the generation of the white pock mutant. (b) Genomic DNA from Brighton white cowpox clone C (BWCP C1 C): the underlining indicates the size of the sequence copied from the left-hand terminus and replacing the deletion. (c) Linearized recombinant BRCP/*Pst*I O/pUC9 showing the proposed origins of virus-specific transcripts generated by HeLa cell RNA polymerase II. (d) Linearized recombinant BWCP/*Pst*I L/pUC9 showing the replacement of deleted sequences by sequences copied from the opposing terminus; P, site for endonuclease *Pst*I; H, site for endonuclease *Hin*dIII. (e) Plasmid vector pUC9. Virus genomic DNA, recombinants and vector are drawn to different scales.

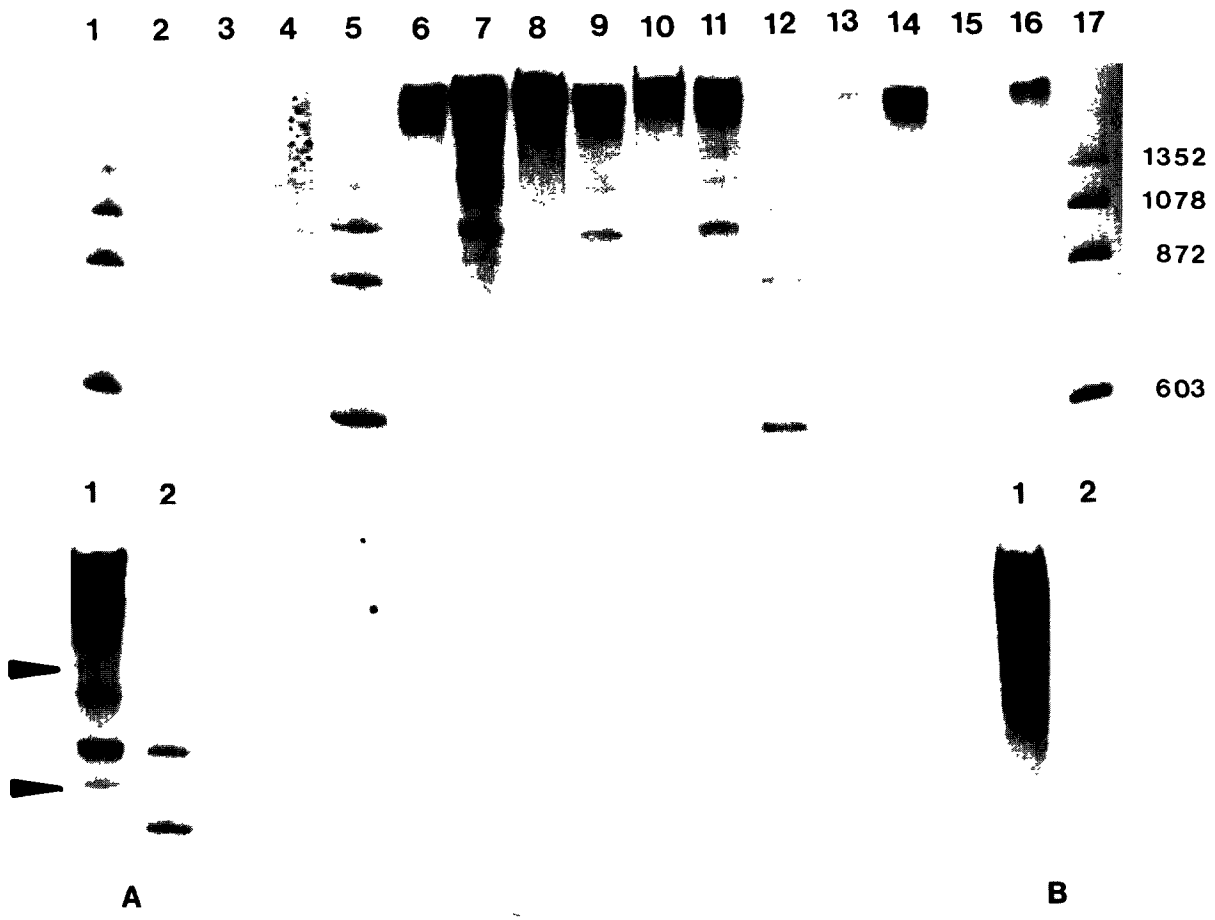

Fig.2. In vitro transcripts generated by HeLa cell RNA polymerase II from the following templates: 0.5 μg (lane 13) or 1.0 μg (lane 14) Brighton red cowpox genomic DNA; 0.5 μg (lane 15) or 1.0 μg (lane 16) Brighton white cowpox clone C genomic DNA; 2.5 μg each circular plasmid pUC9 (lane 10), circular recombinant BRCP/*Pst*I O/pUC9 (lanes 6 and 8), circular recombinant BWCP/*Pst*I L/pUC9 (lane 3), linearized plasmid pUC9 (lane 11), linearized recombinant BRCP/*Pst*I O/pUC9 (lanes 7 and 9) or linearized recombinant BWCP/*Pst*I L/pUC9 (lane 4). Absence of endogenous RNA synthesis before addition of template is shown in lane 2 and the positions of denatured *Hae*III restriction fragments of ϕX174 RF DNA (sizes 1352, 1078, 872 and 603 bp) in lanes 1, 5, 12 and 17. Inset A shows an enlargement from a separate gel of the transcripts derived from 2.5 μg linearized recombinant BRCP/*Pst*I O/pUC9 (lane 1) and ϕX174/*Hae*III size markers (lane 2). Virus-specific transcripts are indicated by arrows. Inset B shows the complete inhibition of transcription from 1.0 μg red cowpox genomic DNA (lane 1) in the presence of 2 μg/ml α-amanitin (lane 2) and is typical of all templates tested.

circular recombinants containing BRCP/*Pst*I O (fig.2, lanes 6 and 8) or BWCP/*Pst*I L (fig.2, lane 3) cloned in pUC9. In contrast, plasmid pUC9 linearized by cleavage at the unique *Hin*dIII site generates discrete run-off transcripts including prominent species of about 1350 and 1050 bp (fig.2, lane 11) indicating that this non-supercoiled prokaryotic DNA contains specific sequences recognised as initiation sites by HeLa cell RNA polymerase II. *Hin*dIII cleaved recombinant plasmid BRCP/*Pst*I O/pUC9 (fig.1c) generated additional transcripts of about 950 bp and less obviously, about 1450 bp (fig.2, lanes 7 and 9 and inset A) which are initiated in the wild-type Cowpox DNA insert. Transcription of *Hin*dIII cleaved recombinant plasmid BWCP/*Pst*I L/pUC9

(fig.1d) does not generate either of these additional virus specific transcripts (fig.2, lane 4), suggesting that their sites of initiation are located in that portion of the wild-type *Pst*I O fragment which is deleted in the generation of the white pock mutant BWCP clone C [12]. The intensity of radiolabelling of virus specific transcripts is less than that of transcripts derived from the vector sequences (fig.2, lanes 7 and 9) but is comparable to transcription from a purified restriction fragment of the human β-globin gene in the same system (not shown). Fig.2, lane 4 is underloaded compared with lanes 7 and 9 but increased loading on analogous gels confirms the absence of virus specific transcription from this template.

Transcription from all templates tested is inhibited completely in the presence of 2 μg/ml α-amanitin demonstrating that RNA synthesis in this system is due exclusively to cell RNA polymerase II (fig.2, inset B). Cell RNA polymerases I and III, like the virus transcriptase, are resistant to this concentration of α-amanitin [3,14].

These results demonstrate the possibility of in vivo nuclear transcription of Orthopox virus DNA by cell RNA polymerase II in addition to cytoplasmic transcription by the viral RNA polymerase. The transcripts concerned are not essential for virus replication but may be related to pathogenicity as white pock variants, which fail to suppress leucocyte infiltration, are less virulent than the wild-type virus [15].

This does not preclude the existence of host RNA polymerase II transcripts from other regions of the virus genome which represent essential genes.

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