Onderstepoort Journal of Veterinary Research, 61:255–261 (1994)

Early and late transcriptional phases in the replication of lumpy-skin-disease virus

W.C. FICK and G.J. VILJOEN
Biochemistry Division, Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa

ABSTRACT

W.C. FICK & VILJOEN, G.J. 1994. Early and late transcriptional phases in the replication of lumpy-skin-disease virus. *Onderstepoort Journal of Veterinary Research*, 61:255–261

Approximately 71 of the estimated 145 kilobase pairs of the genome of the South African isolate of lumpy-skin-disease virus, Neethling strain, was cloned into the plasmid vector pBluescribe. Selected clones were used in Northern blot analysis to investigate the replication cycle of the virus. The synthesis of early mRNA was initiated immediately after infection, and continued for approximately 9 h. The transition to late-gene transcription occurred approximately 10 h post-infection and required DNA replication. Transcription of endogenous late genes cannot occur when viral DNA replication is prevented, while it is activated after DNA replication has occurred. The cloned fragments of the genome are expected to help in the identification of important promoter elements.

INTRODUCTION

Lumpy-skin-disease virus (LSDV) is a member of the capripoxvirus genus of the subfamily Chordopoxvirinae. While LSDV affects cattle, other members of this genus infect sheep and goats, causing either sheep- or goatpox (Matthews 1982). They are the most important group of animal poxviruses, with serious consequences in terms of mortality, lost productivity and hide damage. Capripoxviruses infect only ungulates, and most strains cause clinical disease in only one species (Weiss 1968). Since the capripoxviruses have a limited host range, they have the potential to be developed as practical vaccine vectors.

Much progress has been made in ascertaining the vector potential of species-specific poxviruses such as fowlpox virus (Taylor & Paoletti 1988; Boursnell 1992), canarypox virus (Taylor, Weinberg, Kawaoka, Desmettere & Paoletti 1988; Tartaglia, Jarret, Neil, Desmettere & Paoletti 1993) and swinepox virus (Massung & Moyer 1991a; b). A major advantage of host-restricted poxviruses is that, outside their host range, infection leads to incomplete replication which, nevertheless, appears to be sufficient for expression of foreign genes and the induction of a protective immune response (Taylor & Paoletti 1988).

Received 21 July 1994—Editor

Although some progress has been made in characterizing the viruses responsible for sheep- and goatpox, little is known about the molecular biology of LSDV. Detailed restriction-enzyme maps of various sheep and goat isolates, and a Kenyan cattle isolate (KC-1) have nonetheless been compiled (Gershon & Black 1987; Gershon & Black 1988). In contrast, only a *Hind* III restriction map is available for a South African strain of LSDV (SAC-1; Gershon & Black 1988). Gershon & Black (1988) demonstrated the existence of a close relationship between the viruses causing sheep- and goatpox and lumpy-skin disease. Their DNA genomes vary between 143 and 147 kilobase

pairs (kbp) in length, and enzyme-restriction digest patterns indicate a 95,8–97 % homology at the nucleotide level. The cattle- and sheep isolates, however, were more closely related to each other than to the goat isolates.

A comparison of the genomes of capripoxviruses with those of orthopoxviruses has shown that the two genera share a centrally placed collinear region of 105–120 kbp, with divergence in both sequence and size towards their termini (Gershon, Ansell & Black 1989). This is found in all poxviruses. The central core element contains the genes essential for maintaining the normal poxvirus life cycle (Weir & Moss 1984; Wittek, Hanggi & Hiller 1984a; Wittek, Richner & Hiller 1984b; Hirt, Hiller & Wittek 1986), while the genes involved in pathogenicity and host-range specificity are situated towards the terminal regions of the genome.

In South Africa, a strain of LSDV has been attenuated and routinely used as a vaccine since the 1960s (Weiss 1968). This isolate, originally named the Neethling strain, was described by Alexander, Plowright & Haig (1957) in the late 1950s. Over the years, valuable experience concerning its efficacy and safety has been gained. It is therefore a primary candidate for development as a vector. In this communication we present *Pst* I and *Bam* HI restriction-enzyme profiles of the genome of this virus, which we designate SA-LSDV. Both enzymes yielded fragments of a manageable size, and it was possible to clone approximately 50 % of the genome into the plasmid vector pBluescribe (pBS). These cloned fragments were, in turn, used as probes to investigate the growth cycle of SA-LSDV. In this study, mRNA transcription was examined in order to establish when the early and late transcriptional phases occurred. In addition, viral DNA was monitored to determine the onset of DNA replication, confirming the temporal character of LSDV transcription.

MATERIALS AND METHODS

Virus and cell culture

The attenuated LSDV vaccine strain (Neethling strain) of South Africa (SA-LSDV), was obtained from Dr B.J. Erasmus of the Onderstepoort Biological Products and grown in Madin Darby bovine-kidney (MDBK) cells maintained as monolayers in modified Eagles medium (MacPherson & Stoker 1962), supplemented with 5 % foetal-calf serum. Cells were infected at a multiplicity of infection (MOI) of 0,01 focus-forming units (ffu) per cell and harvested 5–7 d post-infection (pi). Titrations were performed in six-well polystyrene plates (Nunc) on monolayers of primary calf-foetal-kidney (CFK) cells to estimate the virus concentration, calculated as ffu/mℓ.

Viral DNA isolation

DNA was isolated from semi-purified virions, based on a procedure described for vaccinia virus (Mackett, Smith & Moss 1985). The isolated SA-LSDV DNA was purified by extracting it twice with 100 mM Tris-HCl (pH 8) -saturated phenol and twice with chloroform and ethanol precipitated. The DNA was collected by centrifugation at 4 000 rpm for 30 min, rinsed with 70 % EtOH, lyophilized, resuspended in 1 x TE buffer (10 mM Tris-HCl pH 7,4; 1 mM EDTA) and stored at 4 °C until use.

Enzymes, plasmids and molecular cloning of LSDV

Restriction enzymes were obtained from commercial suppliers and used according to the manufacturer's instructions. Purified SA-LSDV DNA was digested with *Pst* I or *Bam* HI and cloned into pBluescribe (pBS) (Stratagene, USA). To clone the larger fragments (5–12,9 kbp), the supernatant was enriched by high-salt-sucrose-gradient centrifugation (Sambrook, Fritsch & Maniates 1989), followed by their cloning into pBS (Sambrook *et al.* 1989).

Gel electrophoresis, DNA transfer and hybridization

DNA (1 μg) was analysed either by 0,8 % agarose (Type V, Sigma) or pulsed field gel electrophoresis (PFGE) (Rotavor^R Type V, Biometra system) in 0,5 x TBE (44,5 mM Tris base, 44,5 mM Boric acid, 0,5 mM EDTA). PFGE was performed as described for swinepox virus (Massung & Moyer 1991a). After electrophoresis, the DNA was visualized by ethidium-bromide (EtBr) staining (0,5 μg/mℓ) and photographed. The DNA in the gel was denatured and neutralized (Sambrook *et al.* 1989), after which the DNA was transferred to Hybond-N$^+$ membranes (Amersham) by vacuum blotting for 45 min, by means of a Hoefer vacuum-blot apparatus (San Francisco). The procedure followed was according to the Hoefer instruction manual and 2 x SSC (300 mM NaCl, 30 mM Na_3 citrate) was used as blotting buffer. The membranes were removed and air-dried, and the DNA fixed by UV-light exposure. Cloned viral fragments were used as probes for restriction-enzyme-digested SA-LSDV DNA, while genomic viral DNA was used in the case of undigested DNA. Probes were α-^{32}P radio-labelled by the random oligonucleotide primer extension method (Feinberg & Vogelstein 1983) and a multiprime labelling system (Amersham) was used. The membranes were washed (three times for 20 min each) with 0,1 x SSC/0,1 % SDS at 65 °C, allowing homology hybridization of > 92 %, and exposed to Cronex MRF31 X-ray film at –70 °C.

RNA isolation and Northern blot analysis

Monolayers of MDBK cells on 25 cm^2 Nunc plastic flasks were infected with SA-LSDV at a MOI of 10

ffu/cell in the presence or absence of 40 μg/mℓ cytosine arabinoside (Sigma), a DNA-replication inhibitor. After a 1-h adsorption period, the virus inoculum was removed and replaced with fresh medium. Total RNA was isolated from the flasks at different times pi, with the use of guanidinium isothiocyanate (Glisin, Crkvenjakov & Byus 1974). RNA samples (5 μg) were denatured and electrophoresed on a 1,4 % formaldehyde/agarose gel (Brown 1989). Overnight capillary transfer of the RNA to Hybond N^+ (Amersham) with 20 x SSC used as transfer buffer, was followed by UV fixation of the membranes. Prehybridization was performed for at least 4 h at 42 °C in 50 % deionized formamide, 2 x Denhardt's solution, 5 x SSPE, 0,1 % SDS and 100 μg/mℓ denatured herring-sperm DNA. SA-LSDV clones used as probes were radio-labelled with α-^{32}P by the random primer oligonucleotide extension method (Feinberg & Vogelstein 1983), with the use of a multiprime labelling system (Amersham). Hybridization was performed for 16 h at 42 °C in 50 % deionized formamide, 5 x Denhardt's solution, 5 x SSPE, 0,5 % SDS and 10 % dextran sulphate. Membranes were washed at stringency conditions allowing for > 72 % homology (1 x SSC/0,1 % SDS at 55 °C), and autoradiographed.

RESULTS

Restriction-enzyme digestion of DNA of SA-LSDV

Genomic DNA was extracted from semi-purified SA-LSDV virions and digested with *Pst* I and *Bam* HI. Fourteen *Pst* I and 16 *Bam* HI fragments were identified by their migration in agarose gel electrophoresis (Fig. 1). Their sizes were estimated by comparison with a *Hind* III digest of bacteriophage λ DNA. (Table 1). Letters were assigned alphabetically to each of the fragments in descending order, according to size. The sizes of 11 of the *Pst* I fragments, ranging between 2,3 and 12,7 kbp, and 13 of the *Bam* HI fragments, ranging between 1,1 and 12,9 kbp, were appropriate to be cloned into a plasmid vector. The estimated total size of the SA-LSDV genome, based on the *Pst* I and *Bam* HI fragment analysis, was 144,4 kbp and 145,7 kbp, respectively (Table 1). This yielded an average estimate of 145,05 kbp.

Identification of cloned fragments

Pst I or *Bam* HI fragments derived from the restriction-digested SA-LSDV DNA, were cloned into pBS and screened for their ability to hybridize with authentic viral DNA. In this experiment, recombinant plasmids were digested with either enzyme to excise the cloned inserts. These inserts were then electrophoresed in parallel with genomic DNA that had been digested with either *Pst* I or *Bam* HI. The separated fragments were then transferred from the gel to nylon membranes and probed with radioactively labelled viral DNA. Five *Pst* I clones (pKV10, –18, –21, –9 and –6) and seven *Bam* HI clones (pKV56, –52, –43, –28, –36, –4 and –46) represented specific SA-LSDV-restriction fragments (summarized in Table 1).

The first step was to determine whether each generated SA-LSDV clone represented a different part of

TABLE 1 Cloning and restriction-enzyme analysis of SA-LSDV DNA

A

Pst I fragment	Size (kbp)	Plasmid	No. of sites		
			Eco RI	*Cla* I	*Hind* III
A	33,0	NC			
B	23,0	NC			
C	19,0	NC			
D	12,7	NC			
E	12,2	NC			
F	8,9	pKV10	3	1	4
G	8,2	pJV18	2	2	4
H	6,4	pJV21	0	0	2
I	4,7	NC			
J	4,6	NC			
K	3,5	pKV9	0	1	0
L	3,2	NC			
M	2,7	NC			
N	2,3	pKV6	0	1	1
Total	144,4				

B

Bam HI fragment	Size (kbp)	Plasmid	No. of sites		
			Eco RI	*Cla* I	*Hind* III
A	35,0	NC			
B	21,0	NC			
C	15,0	NC			
D	12,9	pKV56	0	4	3
E	11,5	pJV52[a]	3	4	5
F	10,0	NC			
G	8,6	NC			
H	8,2	pKV43	1	2	3
I	6,0	NC			
J	4,7	NC			
K	4,2	NC			
L	2,8	pKV52[a]			
M	1,8	pKV28	1	0	1
N	1,6	pKV36	0	0	0
O	1,3	pKV4	0	0	1
P	1,1	pKV46	0	1	1
Total	145,7				

a Plasmid constructed with a partial digest of the E and L fragment

NC No clones obtained

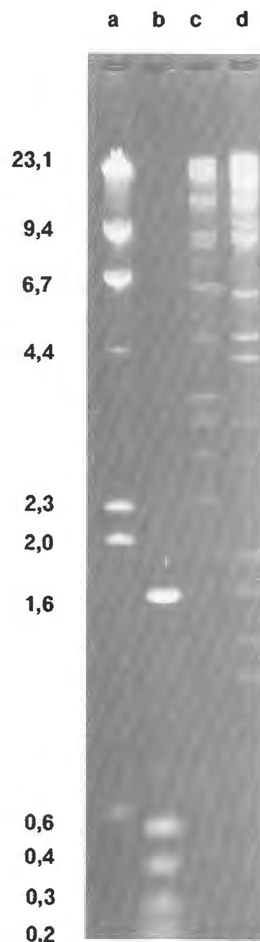

FIG. 1 Agarose gel (0,8 %) electrophoresis of SA-LSDV DNA digested with restriction enzymes. (a) Bacteriophage λ DNA digested with *Hind* III (size marker); (b) pAT153 digested with *Hind* fI (size marker); (c) *Pst* I fragments; (d) *Bam* HI fragments

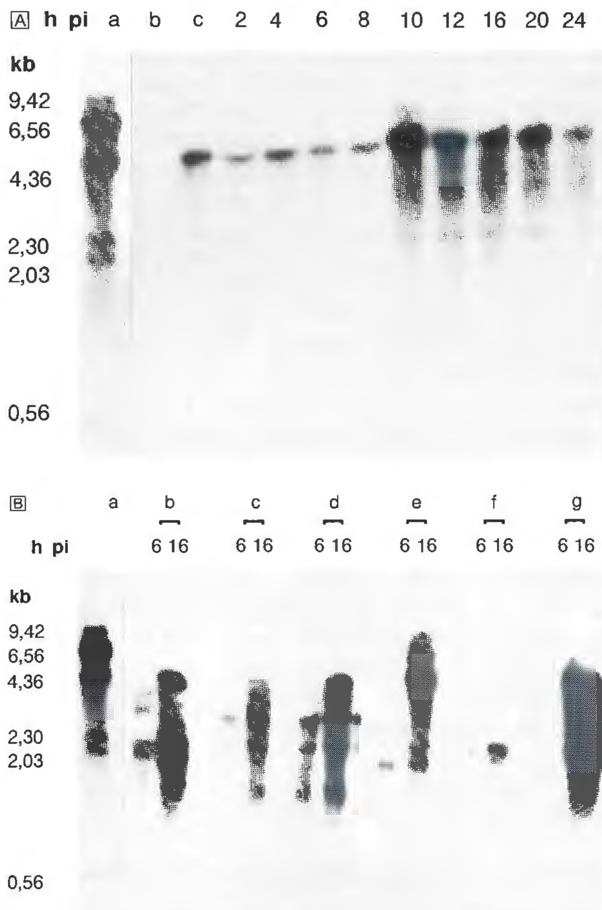

FIG. 2 Northern blot analysis of total RNA isolated from MDBK cells infected with SA-LSDV, at different times pi. (A) SA-LSDV RNA analysed from 2–24 h pi. The membrane was hybridized to clone pKV10. (a) Bacteriophage λ DNA digested with *Hind* III (size marker); (b) RNA from uninfected MDBK cells; (c) SA-LSDV-infected MDBK cells maintained in the presence of 40 μg/mℓ cytosine arabinoside, 24 h pi. (B) SA-LSDV RNA analysed at six and 16 h pi, respectively. Membranes were hybridized to various SA-LSDV clones. (a) bacteriophage λ DNA digested with *Hind* III (size marker); (b) pKV43; (c) pKV56; (d) pKV52; (e) pKV46; (f) pKV4; (g) pKV21 (h pi: hours post infection)

the genome, i.e. a unique sequence. The *Pst* I clones were cross-hybridized with the *Bam* HI clones in a Southern blot experiment (Southern 1975). The *Pst* I cloned fragments did not cross-hybridize with the *Bam* HI cloned fragments (results not shown), confirming that each SA-LSDV clone was unique and did therefore not represent overlapping sequences.

Addition of the molecular weights of the cloned fragments indicated that the clones together represented a total of 70,5 kbp, which was approximately 48 % of the SA-LSDV genome. Each of the clones was subjected to restriction-enzyme digestion to identify potential internal sites for subcloning.

Synthesis of mRNA

Transcription of early and late viral mRNA was investigated to help establish the time course of mRNA synthesis during the growth cycle of SA-LSDV. Clones (pKV43, –56, –52, –46, –4 and –21) were used to probe transcribed mRNA. Confluent MDBK cells were infected with SA-LSDV (MOI of 10/cell), and total RNA was extracted at different time intervals. Viral mRNA synthesis was analyzed by Northern blotting. In the case of clone pKV10, illustrated as an example in Fig. 2A, only a single band, which migrated slightly faster than a 4,36 kbp λ DNA/*Hind* III restriction fragment, was initially detected in the autoradiogragph. The number and intensity (indicating more or less abundant mRNA, and hence promoter strength) of bands, however, depended upon the clone used for probing (Fig. 2B, 6 h pi.). These defined bands were visible until 8–10 h pi. From 10 h pi onwards, the appearance of the transcribed mRNA in the autoradiographs changed. Although a general heterodispersity

was observed, several new transcripts also appeared (Fig. 2A, lanes 10–24 and Fig. 2B, 16 h pi), while others disappeared. When DNA replication was inhibited by the addition of cytosine arabinoside, transcription was restricted to the early transcriptional phase. In the case of pKV10, a single, discrete, early mRNA transcript was observed 24 h pi (Fig. 2A, lane c). In contrast, indications are that pKV21 contains only late promoters (Fig. 2B, lane g). This will be confirmed by sequence analysis.

DNA accumulation in cell culture

Macroscopically, the first signs of infection with SA-LSDV were manifested as foci in cell-culture monolayers after 4–5 d. The presence and relative amounts of viral DNA in infected cells were investigated to confirm the temporal regulation of LSDV transcription. Generally, poxvirus DNA replication marks the end of the early mRNA and the onset of the late mRNA transcriptional phase (Oda & Joklik 1967; Moss & Salzman 1968; Pennington 1974). Once DNA replication is initiated, DNA will accumulate (assuming that, initially, DNA synthesis will occur at a higher rate than DNA degradation) in the infected cells and become detectable above the background level (Massung & Moyer 1991b). SA-LSDV-infected MDBK cells were harvested at different time intervals pi and the DNA was analyzed by conventional agarose gel electrophoresis and PFGE (Fig. 3A and 3B). Viral DNA could be detected 18 h pi by EtBr staining (not shown) and from 12 h pi onwards using Southern blot analysis. The amount of DNA increased throughout the 48-h duration of the experiment. The size of the SA-LSDV DNA was confirmed as being approximately 145 kbp, by PFGE (Fig. 3B).

DISCUSSION

The size of the SA-LSDV-Neethling-strain genome was estimated at approximately 145 kbp. The sizing was calculated from restriction-enzyme profiles of the genome by conventional agarose-gel electrophoresis. The total size of the LSDV genome was later confirmed by PFGE. Although the genomic size determination is probably an under-estimation (due to the limitations of gel electrophoresis), it correlates well with sizes reported for KC-1 (Gershon & Black 1987) and for the strain designated SAC-1 (Gershon & Black 1988). Another laboratory (K.R. Dumbell, personal communication), determined the SA-LSDV genome size to be 152 kbp when PFGE was used. Five of the *Pst* I fragments, representing approximately 20 % of the genome, and seven of the *Bam* HI fragments, representing nearly 28 % of the viral DNA, were cloned into pBS. Surprisingly, all the cloned fragments were unique, there was no cross-hybridization between the *Pst* I- and the *Bam* HI-cloned fragments. This means that nearly one half of the SA-LSDV genome had been cloned.

In order to develop SA-LSDV as an expression vector of foreign proteins, a knowledge of the kinetics of its growth cycle is essential. The first signs of a morphological change in MDBK cells were observed approximately 4–5 d pi, when virions produced localized foci. This is in accordance with swinepox virus, where foci appeared from 4 d pi (Massung & Moyer 1991b). LSDV therefore has a relatively slow growth cycle, comparable to that of swinepox virus (Massung & Moyer 1991b). Both these viruses replicate more slowly than the prototypical poxvirus, vaccinia virus (Moss 1990).

In the study of the growth cycle of capripoxviruses at the molecular level, two aspects of viral development are of importance; firstly, the mRNA transcriptional switch from early to late and, secondly, DNA replication. Most of the current knowledge of poxvirus-gene expression and mRNA synthesis is derived from studies on vaccinia virus (Moss 1990; 1992). It was found that gene expression can be divided into two phases, namely an early and a late phase. Early transcripts are synthesized prior to DNA replication and encoded for functional proteins, some of which are necessary for DNA replication. Early transcripts, which are less abundant than late transcripts, are of a defined length (Venkatesan, Baroudy & Moss 1981) and can be visualized as discrete bands by Northern blot analysis. This is due to the fact that early mRNA transcription is terminated by a specific early transcriptional stop signal, TTTTTNT (Rohrmann, Yuen & Moss 1986; Yuen & Moss 1987). In contrast, poxvirus late-mRNA transcripts are in abundance (when compared with early transcripts) and have heterologous ends due to insufficient mRNA termination. This results in transcriptional read-through of the late genes by the DNA-dependent RNA polymerase (Wittek, Cooper, Barbosa & Moss 1980; Cooper, Wittek & Moss 1981), leading to heterodisperse transcripts and higher background. The switch from early to late transcription is therefore detectable by Northern blot analysis.

In the case of SA-LSDV, the appearance of multiple new as well as non-discrete transcripts (Fig. 2A) indicated that this switch occurred at approximately 9 h pi. Furthermore, multiple mRNAs of varying abundance were detected, indicating a number of weak to strong cis-activating elements. The Northern blot analysis of the transcriptional switch from early to late, was confirmed by Southern blot analysis of the viral DNA replication. Since viral DNA was detected from as early as 12 h pi, it is likely that SA-LSDV DNA replication had commenced somewhat earlier, confirming the transcriptional switch at approximately 9 h pi. The amount of DNA continued to increase throughout the 48-h observation period. These findings are

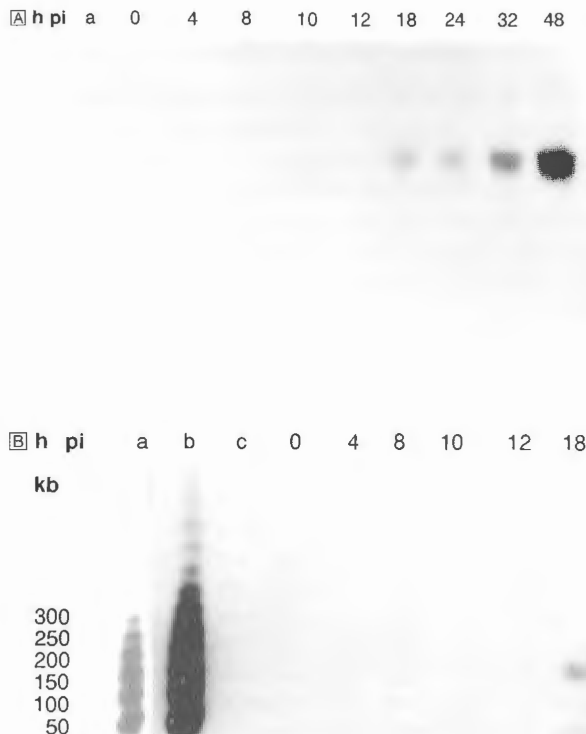

FIG. 3 Southern blot analysis of SA-LSDV DNA, collected from infected MDBK cells at various times pi and separated by (A) 0,8 % agarose gel electrophoresis and (B) PFGE. (a) and (b) Under-exposure and an overnight exposure of the λ DNA ladder (size marker). (c) DNA from uninfected MDBK cells; (h pi: h post-infection)

analogous to those reported for swinepox virus, where late transcripts were first detected at 8 h pi and DNA presence at 12 h pi (Massung & Moyer 1991b). When treated with cytosine arabinoside, no late transcripts were detected (Fig. 2A, lane c), showing that, as with other poxviruses (Moss 1992), DNA replication was required for late transcription to be initiated.

Renewed interest in LSDV vaccines comes at a time when the disease is extending beyond its traditional boundaries (Carn 1993). It was formerly considered a disease of southern and eastern Africa, and its establishment in Egypt causes concern about the potential spread of disease outside Africa. Capripoxviruses are ideal vector vaccine candidates as they not only have the potential for expression of multiple foreign antigens, (because of the large size of their genome), but are also useful as vaccines in their own right. Romero, Barrett, Evans, Kitching, Gershon, Bostock & Black (1993), have constructed a Kenyan sheep (KS-1) recombinant expressing the fusion protein gene of rinderpest virus. This recombinant virus protected cattle against clinical rinderpest. In addition, the vaccine protected the cattle against lumpy-skin disease.

This study provides the first information on the early and late transcriptional phases in the growth cycle of a capripoxvirus. It is anticipated that the availability of genomic DNA clones will facilitate the localization of foreign gene-insertion sites as well as cis-acting elements that may allow for the optimal expression of foreign genes. This is now being investigated.

ACKNOWLEDGEMENTS

We acknowledge Dr Albie van Dijk and Prof. Keith Dumbell for initiating this project. Their continued encouragement and interest are appreciated. We thank Dr Dion du Plessis and Prof. Keith Dumbell for their assistance in preparing the manuscript.

REFERENCES

ALEXANDER, R.A., PLOWRIGHT, W. & HAIG, D.A. 1957. Cytopathic agents associated with lumpy skin disease of cattle. *Bulletin of Epizootic Diseases in Africa,* 5:489–492.

BOURSNELL, M.E.G. 1992. Avipoxvirus vectors, in *Recombinant poxviruses*, edited by M.M. Binns & G.L. Smith. Boca Raton: CRC Press: 269–283.

BROWN, T. 1989. Analysis of RNA by Northern and slot blot hybridization, in *Current protocols in molecular biology*, vol. 1, edited by F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, & K. Struhl. New York: Greene Publishing & Wiley Interscience.

CARN, V.M. 1993. Control of Capripoxvirus infections. *Vaccine*, 11:1275–1279.

COOPER, J.A., WITTEK, R. & MOSS, B. 1981. Extension of the transcriptional and translational map of the left end of the vaccinia virus genome to 21 kilobase pairs. *Journal of Virology*, 39: 733–745.

FEINBERG, A.P. & VOGELSTEIN, B. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry*, 132:6–13.

GERSHON, P.D. & BLACK, D.N. 1987. Physical characterisation of the genome of a cattle isolate of capripoxvirus. *Virology*, 160: 473–476.

GERSHON, P.D. & BLACK, D.N. 1988. A comparison of the genomes of capripoxvirus isolates of sheep, goats and cattle. *Virology*, 164:341–349.

GERSHON, P.D., ANSELL, D.M. & BLACK, D.N. 1989. A comparison of the genome organization of capripoxvirus with that of the orthopoxviruses. *Journal of Virology*, 63:4703–4708.

GLISIN, V., CRKVENJAKOV, R. & BYUS, C. 1974. Ribonucleic acid isolated by cesium chloride centrifugation. *Biochemistry*, 13:2633–2636.

HIRT, P., HILLER, G. & WITTEK, R. 1986. Localization and fine structure of a vaccinia virus gene encoding an envelope antigen. *Journal of Virology*, 58:757–764.

MACPHERSON, I. & STOKER, M.P.G. 1962. Polyoma transforming of hamster cell clones—an investigation of genetic factors affecting cell competence. *Virology*, 16:147–151.

MACKETT, M., SMITH, G.L. & MOSS, B. 1985. In *DNA Cloning: A practical approach,* edited by D.M. Glover. Washington, D.C.: IRL Press, 2:191–211.

MASSUNG, R.F. & MOYER, R.W. 1991a. The molecular biology of swinepox virus. I. A characterization of the viral DNA. *Virology*, 180:347–354.

MASSUNG, R.F. & MOYER, R.W. 1991b. The molecular biology of swinepox virus. II. The infectious cycle. *Virology*, 180:355–364.

MATTHEWS, R.E.F. 1982. Classification and nomenclature of viruses. *Intervirology,* 17:42–46.

MOSS, B. & SALZMAN, P. 1968. Sequential protein synthesis following vaccinia virus infection. *Journal of Virology*, 2:1016–1027.

MOSS, B. 1990. Regulation of vaccinia virus transcription. *Annual Reviews of Biochemistry*, 59:661–688.

MOSS, B. 1992. Molecular biology of poxviruses, in *Recombinant Poxviruses*, edited by M.M. Binns & G.L. Smith. Boca Raton: CRC Press: 45–80.

ODA, K. & JOKLIK, W.K. 1967. Hybridisation and sedimentation studies on "early" and "late" vaccinia messenger RNA. *Journal of Molecular Biology*, 27:395–419.

PENNINGTON, T.H. 1974. Vaccinia virus polypeptide synthesis: sequential appearance and stability of pre- and post-replicative polypeptides. *Journal of General Virology*, 25:433–444.

ROHRMANN, G., YUEN, L. & MOSS, B. 1986. Transcription of vaccinia virus early genes by enzymes isolated from vaccinia virions terminates downstream of a regulatory sequence. *Cell*, 46:1029–1035.

ROMERO, C.H., BARRETT, T., EVANS, S.A., KITCHING, R.P., GERSHON, P.D., BOSTOCK, C. & BLACK, D.N. 1993. Single capripoxvirus recombinant vaccine for the protection of cattle against rinderpest and lumpy skin disease virus. *Vaccine*, 11: 737–742.

SAMBROOK, J., FRITSCH, E.F. & MANIATES, T. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

SOUTHERN, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology*, 98:503–517.

TARTAGLIA, J., JARRET, O., NEIL, J.C., DESMETTERE, P. & PAOLETTI, E. 1993. Protection of cats against feline leukemia virus by vaccination with a canarypox virus recombinant, ALVAC-FL. *Journal of Virology*, 67:2370–2375.

TAYLOR, J. & PAOLETTI, E. 1988. Fowlpox virus as a vector for non-avian species. *Vaccine,* 6:466–472.

TAYLOR, L., WEINBERG, R., KAWAOKA, Y., DESMETTRE, R., & PAOLETTI, E. 1988. Efficacy studies on a canarypox-rabies recombinant virus. *Vaccine*, 9:190–193.

VENKATESAN, S., BAROUDY, B.M. & MOSS, B. 1981. Distinctive nucleotide sequences adjacent to multiple initiation and termination sites of an early vaccinia virus gene. *Cell*, 25: 805–813.

WEIR, J.P. & MOSS, B. 1984. Regulation of expression and nucleotide sequence of a late vaccinia virus gene. *Journal of Virology*, 51:662–669.

WEISS, K.E. 1968. Lumpy skin disease virus. *Virology Monographs*, 3:111–131.

WITTEK, R., COOPER, J., BARBOSA, E. & MOSS, B. 1980. Expression of the vaccinia virus genome—analysis and mapping of mRNAs encoded within the inverted terminal repetition. *Cell*, 21:487–493.

WITTEK, R., HANGGI, M. & HILLER, G. 1984a. Mapping of a gene coding for a major late structural polypeptide on the vaccinia virus genome. *Journal of Virology*, 49:371–378.

WITTEK, R., RICHNER, B. & HILLER, G. 1984b. Mapping of the genes coding for the two major vaccinia virus core polypeptides. *Nucleic Acids Research*, 12:4835–4848.

YUEN, L. & MOSS, B. 1987. Oligonucleotide sequence signaling transcriptional termination of vaccinia virus early genes. *Proceedings of the National Academy of Sciences U.S.A.*, 84:6417–6421.