JOURNAL OF VIROLOGY, Dec. 1996, p. 8584–8589 Vol. 70, No. 12
0022-538X/96/$04.00+0


# Intermediate Class of mRNAs in African Swine Fever Virus

JAVIER M. RODRÍGUEZ,† MARÍA L. SALAS, AND ELADIO VIÑUELA*

*Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid), Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain*

Received 6 June 1996/Accepted 5 September 1996

**A transcriptional analysis of the African swine fever virus (ASFV) I226R and I243L genes is presented. Steady-state kinetics and transfection experiments showed the existence of a new temporal class of ASFV mRNAs transcribed from these genes, with the characteristics of the poxvirus intermediate transcripts. Transcription of the I226R gene gave rise to intermediate and late mRNAs that started from different sites, while the I243L gene produced early, intermediate, and late mRNAs, also starting from different sites. The presence of intermediate genes suggests a cascade model for the regulation of ASFV gene expression.**

African swine fever virus (ASFV) infects different species of swine as well as soft ticks of the genus *Ornithodoros*. In its natural swine host, the warthog (*Phacochoerus aethiopicus*), ASFV causes a mild disease with no clinical signs. By contrast, the infection of domestic pigs (*Sus scropha*) with virulent ASFV strains leads to a devastating disease, African swine fever, with a very high mortality rate and for which no effective vaccine is available (reviewed in references 21 and 24).

ASFV is a large enveloped DNA virus with an icosahedral morphology (reviewed in references 21 and 22). Its genome is a double-stranded DNA molecule with terminal inverted repeats (20) and hairpin loops (11), ranging in size from 170 to 190 kbp, depending on the virus strain (6). In the case of the BA71V isolate, whose complete DNA sequence is known (25), the genome is 170,101 nucleotides long and contains 151 major open reading frames (ORFs), which are closely spaced along the viral DNA. Both DNA chains are alternatively used as the coding strand.

The temporal expression of ASFV genes is strongly regulated, and the mature transcripts have a structure similar to that of eukaryotic mRNAs, containing capped 5′ ends and poly(A) tails at their 3′ termini (17). Experiments with cell-free translation of virus mRNAs selected by hybridization to different genomic fragments showed the presence of two main temporal classes of ASFV genes (18). Early genes are transcribed in cells infected in the presence of protein or DNA synthesis inhibitors by the virion-associated RNA polymerase. Many of the early mRNAs are present in an active form late in infection. An analysis of mRNA accumulation kinetics showed the presence of a subclass of early genes, named immediate-early genes, whose transcription is abrogated before the onset of virus DNA replication, in a process that requires protein synthesis (2). Late genes are expressed after the initiation of DNA replication. Late transcription is, like early RNA synthesis, independent of the host RNA polymerase II (16).

RNA mapping of several ASFV ORFs has shown that the individual genes are tightly regulated independent transcription units. Both early and late RNA syntheses start at a short distance from the initiating ATG of the corresponding ORF, giving rise to transcripts of defined sizes. Runs of seven or more thymidylate residues (7T motifs) on the coding strand have been reported to act as signals for 3′ end formation in both types of mRNAs (2, 3).

Although ASFV shares different properties with the other two families of large cytoplasmic deoxyviruses, poxviruses and iridoviruses, biochemical and morphological properties (reviewed in reference 22) as well as DNA sequence data (25) indicate an intermediate position for ASFV between these two viral families. Currently, ASFV is considered the only member of an unnamed virus family (7).

In a previous study we analyzed by Northern (RNA) blotting the transcription of ASFV genes present in the DNA fragment *Eco*RI I (14). The complex pattern of transcription found makes this region a good model to study the regulation of ASFV transcription. Therefore, we initiated a detailed transcriptional analysis of this fragment. Preliminary results showed that the pattern of synthesis of some of the mRNAs corresponding to the I226R and I243L genes was clearly distinguishable from those described for other species of mRNAs. In this report, we present the characterization of this fourth temporal class of ASFV mRNA, which possesses the characteristics of the poxvirus intermediate transcripts. The implications of this finding for the regulation of ASFV transcription are discussed.

## MATERIALS AND METHODS

**Cells and virus.** Vero cells, obtained from the American Type Culture Collection, were routinely grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum. ASFV strain BA71V was propagated and titrated as previously described (9). Virus inoculation of cell cultures grown in 100-mm-diameter plastic petri dishes was carried out at a multiplicity of infection of 25 PFU per cell. To synchronize virus penetration, the adsorption was performed at 4°C during 2 h in DMEM without sodium bicarbonate and supplemented with 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), pH 7.0. After adsorption, the monolayers were washed twice with DMEM containing 2% fetal bovine serum and incubated at 37°C under 5% $CO_2$.

**RNA purification.** Total cell RNA was obtained as previously described (19). Briefly, after removal of the medium, cells were scraped and resuspended in chilled phosphate-buffered saline. After centrifugation at a low speed for 5 min, the cell pellets were resuspended in lysis buffer (4 M guanidinium thiocyanate, 0.1 M Tris-HCl [pH 7.5], 1% β-mercaptoethanol), layered on top of 3-ml cushions of 5.7 M cesium chloride containing 10 mM EDTA, and spun at 300,000 × *g* for 18 h. The RNA pellets were resuspended in diethyl pyrocarbonate (0.2%)-treated $H_2O$ and ethanol precipitated twice.

Three different classes of total cell RNA were prepared from ASFV-infected Vero cells. Immediate-early RNA was obtained at 7 h postinfection by infecting the cells in the presence of 100 μg of cycloheximide per ml. Early RNA was obtained at 7 h postinfection in the presence of 40 μg of cytosine arabinoside per ml, and late RNA was obtained at 18 h postinfection in the absence of inhibitors. As a control for all the experiments, total cell RNA isolated from mock-infected Vero cells was used.

**Primer extension.** Primer extension analyses were done as previously de-

* Corresponding author. Phone: 34-1-3975070. Fax: 34-1-3974799. Electronic mail address: evinuela@mvax.cbm.uam.es.

† Present address: Institute of Virology and Environmental Microbiology, Oxford OX1 3SR, United Kingdom.

scribed (19) with minor modifications. Ten micrograms of RNA was mixed with approximately $50 \times 10^3$ cpm (approximately 0.1 pM) of the corresponding end-labeled primer (5′ TTCTCGCGGGATTAAAATCGTCTGTGTCCC 3′ for the I226R gene and 5′ TAAAGATTTGTCTCATAAATATGCATCCGC 3′ for the I243L gene) and then ethanol precipitated. The DNA-RNA pellets were resuspended in 20 μl of hybridization buffer [40 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) (pH 6.4), 1 mM EDTA, 0.4 M NaCl, 80% formamide], heated at 80°C for 10 min, and incubated at 22°C for 16 h. After hybridization, the samples were ethanol precipitated, resuspended in 20 μl of reaction buffer (50 mM Tris-HCl [pH 7.6], 60 mM KCl, 10 mM $MgCl_2$, 1 mM each deoxynucleoside triphosphate, 0.1 U of RNAsin [Promega] per ml, 1 mM dithiothreitol, 50 μg of actinomycin D per ml), and incubated for 2 h at 37°C in the presence of 20 U of avian myeloblastosis virus reverse transcriptase (Promega). The samples were phenol extracted and ethanol precipitated, and the pellets were resuspended in 15 μl of $H_2O$. After addition of 5 μl of loading buffer (50 mM Tris-borate [pH 8.3], 1 mM EDTA, 0.1% xylene cyanol, 0.1% bromophenol blue, 80% formamide), the samples were heated at 90°C for 5 min and loaded onto 6% polyacrylamide sequencing gels.

**Transfection of cell cultures.** Transfections were carried out by a liposome-mediated protocol (10) with the synthetic cationic lipid DOTMA [*N*-1-(2,3-dioleyloxy)propyl-*N*,*N*,*N*,-trimethylammonium chloride] and the neutral lipid PtdEtn (dioleoyl-L-α-phosphatidylethanolamine) as recommended by the supplier (BRL/Life Technologies, Inc.). Preconfluent monolayers of Vero cells grown in 100-mm-diameter plastic petri dishes were transfected with 1 μg of DNA per $10^5$ cells. Eighteen hours after transfection, the cell cultures were washed twice with DMEM and infected with ASFV at 20 PFU per cell in the presence of 40 μg of cytosine arabinoside per ml. The infected cells were harvested at 7 h postinfection, and total cell RNA was isolated as described above.

## RESULTS

**Determination of the transcription initiation sites of the I226R and I243L genes.** Figure 1A shows the *Eco*RI restriction map of ASFV BA71V DNA and the locations of ORFs I226R and I243L within the ASFV genome. 5′ end mapping of mRNAs was performed by primer extension analysis. The primer used for the I226R gene hybridized only with late RNA (Fig. 1B), in agreement with the previously reported data for the expression of this ORF (14). After extension with reverse transcriptase, two close clusters of bands were detected, corresponding to two groups of transcription initiation sites located at positions +1 and −1 and positions −8 to −11 with respect to the beginning of the first in-frame ATG codon of the ORF (Fig. 1C). The mRNAs starting at positions −8 to −11 correspond to true late transcripts, whereas positions +1 and −1 correspond to intermediate mRNAs (see below). Because of the postreplicative nature of the intermediate transcription, intermediate and late mRNAs are indistinguishable in this experiment.

The primer used for 5′ end mapping of the I243L gene hybridized with total cell RNA obtained at early and late times during virus infection (Fig. 1B), also in agreement with previous results (14). There are two different classes of extension products for this gene: (i) extensions observed with early as well as late RNA and corresponding to transcription initiation sites at positions +1 to +4 and (ii) extension products detected only with late RNA. In the latter case, a cluster is formed by four bands that correspond to transcription initiation sites at positions +8 to +11, and one extension corresponds to a start site at position −1 (Fig. 1C). This single initiation site corresponds to an intermediate mRNA (see below) that, as stated above, is indistinguishable from the true late mRNAs in this experiment.

Runs of seven or more thymidylate residues on the coding strand have been reported to act as signals for mRNA 3′ end formation in the case of both early and late ASFV mRNAs (2, 3). Taking into account the sizes of the transcripts of the I226R and I243L genes, determined by Northern analysis (1.0 and 0.8 kb, respectively) (14), and the positions of the initiation sites previously determined, the 3′ ends should map near the runs of 9 and 10 thymidylate residues located at distances of 985 and 884 nucleotides, respectively, from the first initiation site of each gene (Fig. 1A).

**Transcriptional kinetics of the I226R and I243L genes.** To further characterize the temporal regulation of the expression of the I226R and I243L genes, the kinetics of accumulation of their mRNAs were analyzed. For this, the relative amounts of the different transcripts were measured throughout the ASFV infectious cycle by primer extension analysis, using specific $^{32}$P-end-labeled primers and total cell RNA extracted from infected cells at 2-h intervals during the infection.

The analysis of the transcriptional kinetics of these genes showed that the different clusters of initiation sites are used at different stages of the virus cycle. In the case of the I226R gene (Fig. 2), while transcripts initiating at the +1 and −1 sites reached their highest level of accumulation at 6 to 8 h postinfection, the accumulation of mRNAs initiating at positions −8 to −11 followed a kinetics resembling that observed with late genes, reaching their highest level at 14 to 16 h postinfection.

On the other hand, the study of the I243L gene transcription kinetics (Fig. 3) showed that the mRNAs synthesized from three different groups of initiation sites accumulated with different kinetics. Initiations at positions +1 to +4, with respect to the first in-frame ATG of the ORF, are active only during the early phase of the infection, and the corresponding mRNAs showed an accumulation kinetics similar to that previously described for ASFV early genes (2), while the kinetics of transcription from the initiation sites at positions +8 to +11 is similar to that of the late I226R transcripts. Interestingly, the results of this analysis showed that transcription from position −1 of the I243L gene resembles that starting at positions +1 and −1 in the I226R gene, with a maximum accumulation of mRNA at 8 h postinfection. We conclude that I243L is transcribed throughout the infectious cycle and that the initiation sites used by the transcriptional apparatus vary with the phase of the replication cycle.

These studies show that both genes possess transcription initiation sites which are specifically active during a period clearly distinguishable from the early and late phases of the virus life cycle, suggesting the existence of a fourth, previously undetected, temporal class of ASFV genes that follow an expression pattern similar to that of the poxvirus intermediate genes.

**ASFV intermediate gene expression.** Poxvirus intermediate genes are expressed immediately after DNA replication and before the onset of late gene transcription (23). Intermediate transcription is dependent on *trans*-acting factors which are present in an active state in virus-infected cells prior to the onset of DNA replication. Expression of these genes leads to the synthesis of late virus polypeptides (12, 13).

To determine whether the intermediate expression of the ASFV I226R and I243L genes has the same characteristics as that of poxvirus intermediate genes, we have followed the method described by Vos and Stunnenberg (23) for the detection of these genes. This method consists of the transfection of plasmid vectors containing the gene under study into the cytoplasm of cells infected with the virus and treated with an inhibitor of DNA replication. Under these conditions, plasmid-borne intermediate genes are expressed, whereas the copies of the same genes within the viral genome are repressed. We therefore infected Vero cells with ASFV in the presence of cytosine arabinoside, an inhibitor of DNA replication, and then transfected the cells either with plasmid p(I)HindIII-2.3, which contains the complete sequences of the I226R and I243L genes as well as their putative transcriptional control regions (Fig. 4A), or with pUC19. Total cell RNA was purified at 7 h postinfection, and the presence of I226R- and I243L-specific

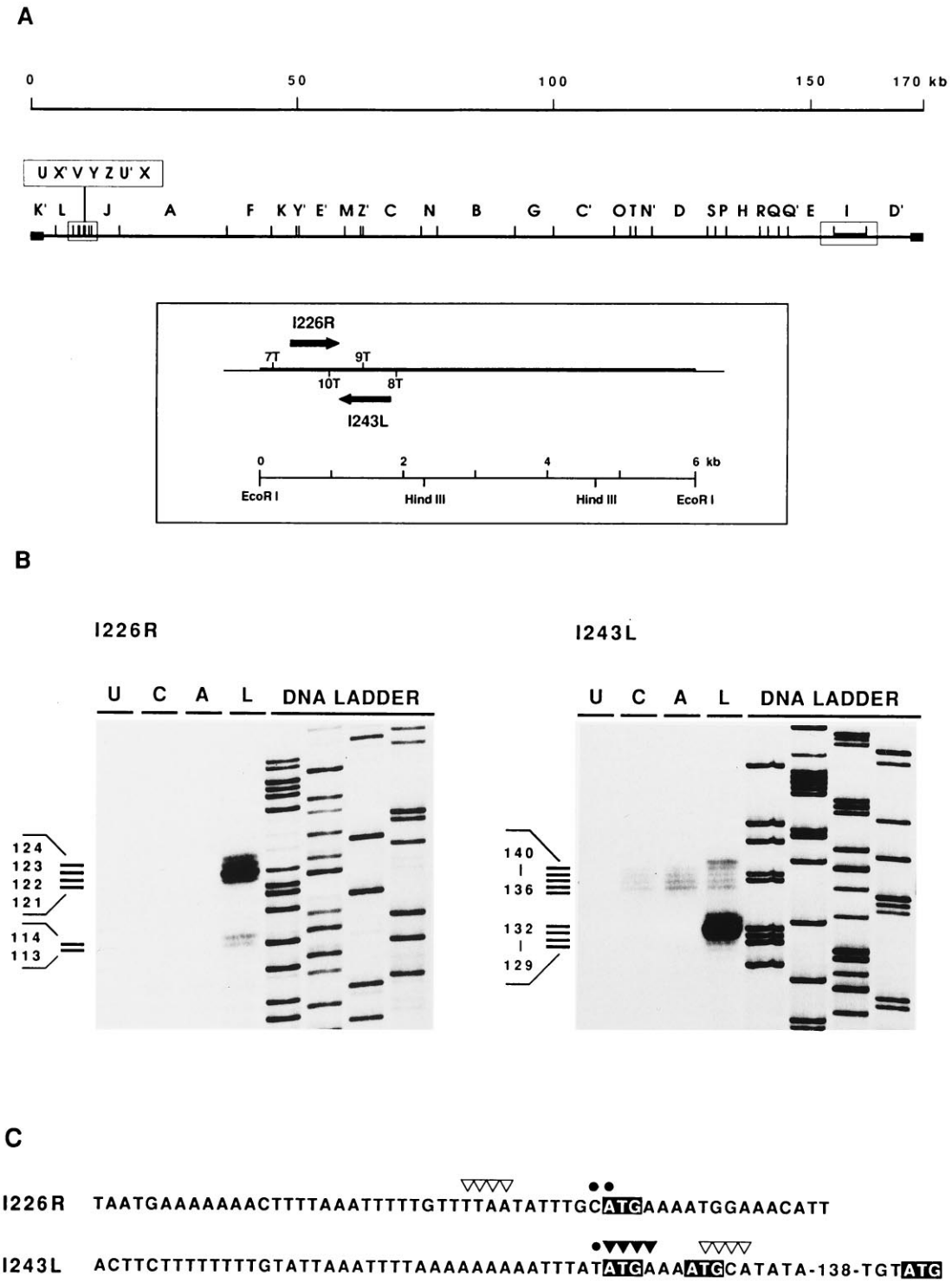


FIG. 1. 5′ end mapping of the I226R and I243L transcripts. (A) Locations of the I226R and I243L ORFs within the ASFV genome. An *Eco*RI map of ASFV BA71V DNA and the localization of the I226R and I243L ORFs, represented by solid arrows, within the *Eco*RI I fragment are shown. The locations of the runs of thymidylate residues around the I226R and I243L ORFs, potentially associated with mRNA 3′ end formation, are also shown. (B) Primer extension analyses of the 5′ ends of the I226R and I243L transcripts. Total RNA from mock-infected Vero cells (lanes U) and total immediate-early (lanes C), early (lanes A), and late (lanes L) RNAs from ASFV-infected cells were used. An unrelated DNA sequencing reaction (DNA ladder) was used as size marker. The sizes (in nucleotides) of the major bands are indicated. Only the informative areas of the gels are shown. (C) Sequences corresponding to the regions surrounding the I226R and I243L transcription start sites. The boxes enclose in-frame ATG codons. The positions of the early (▼), intermediate (●), and late (▽) transcription initiation sites are indicated.

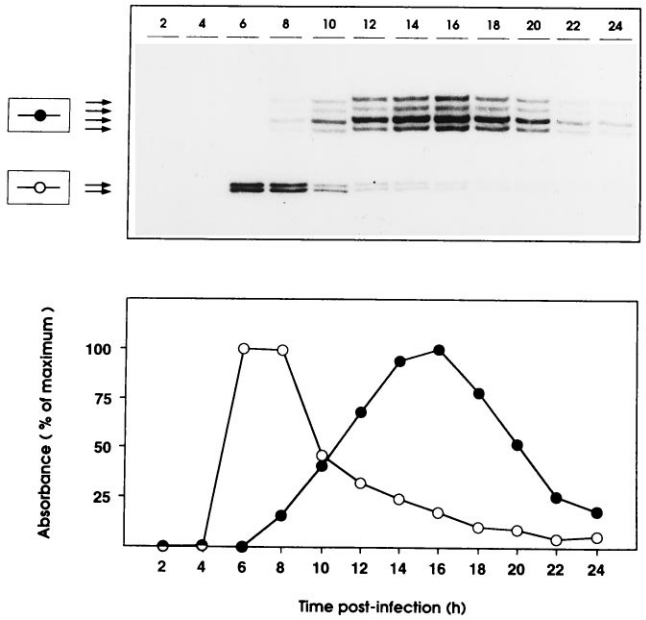


FIG. 2. Steady-state levels of the different I226R transcripts throughout the viral infection. (Upper panel) Detection of the I226R-specific transcripts by primer extension. Total cell RNA was isolated at the indicated times postinfection and hybridized with an oligonucleotide specific for I226R transcripts. After primer extension, the elongated fragments were analyzed by polyacrylamide gel electrophoresis and autoradiography. Only the informative area of the gel is shown. (Lower panel) Quantification of the primer extension analysis. The results shown in the upper panel were quantified by densitometry and plotted as the percentage of the maximum level of specific primer extension. Results for the two different temporal species were plotted independently.

mRNAs was determined by primer extension analysis. As shown in Fig. 4B, although mRNAs initiating at the early I243L transcription sites are clearly detectable in cultures transfected with either plasmid, the primer-extended product corresponding to the intermediate transcription initiation site is found only in the sample corresponding to the cells transfected with p(I)HindIII-2.3 (I243L, lane +). Similarly, products corresponding to mRNAs initiating at the I226R intermediate transcription sites are detected only in the case of cells transfected with p(I)HindIII-2.3 (Fig. 4B, I226R, lane +). It should be noted that, as expected, the presence of the inhibitor of DNA replication specifically prevented the synthesis of mRNAs from the late transcription initiation sites of either gene. The results indicate that the I226R and I243L genes belong to a new class of ASFV genes with characteristics identical to those of poxvirus intermediate genes.

**Conserved sequences upstream of the I226R and I243L ORFs.** A comparison of the sequences immediately upstream of the I226R and I243L ORFs shows a high degree of similarity (Fig. 5). Two regions, corresponding to positions −25 to −15 and −9 to +9, are highly conserved between the two sequences. Poxvirus intermediate promoters have conserved core and initiator sequences at similar locations (4), but they do not seem to have sequence similarity with the regions conserved upstream of the ASFV intermediate genes. Although this similarity is based on data for only two intermediate genes, it is worth noting that sequences upstream of the transcription initiation sites of ASFV early and late genes do not have significant stretches of similarity.

## DISCUSSION

To study the mechanisms controlling ASFV gene transcription, we have initiated a transcriptional analysis of the *Eco*RI I fragment of ASFV DNA. Previous RNA hybridization data (14) showed that this region was transcriptionally very complex, thus providing an interesting model to investigate the regulation of transcription in ASFV. Preliminary results revealed that two of the genes present in this region, I226R and I243L, gave rise to transcripts with a temporal pattern of synthesis clearly distinguishable from that previously described for other ASFV mRNAs. We therefore focused our analysis on the characterization of I226R and I243L gene expression.

I226R gene transcription is driven by two different temporal control elements giving rise to intermediate and late mRNAs. Sequence homology searches show no significant similarity between the I226R sequence and any other sequence in the data banks. However, its expression at intermediate times and the fact that several of the reported intermediate genes in poxviruses are late transcription factors (12) raise the possibility of a role for I226R in the regulation of late gene expression.

I243L transcription produces three clearly distinguishable temporal species of mRNA with the characteristics of those transcribed from early, intermediate, and late genes. We have previously reported the existence of a strong sequence similarity between I243L and the members of the TFIIS (SII) transcription factor family (14). The similarity with the E4L (rpo30) gene of vaccinia virus, which is also related to this family of transcription factors, is especially interesting. The product of the E4L gene was first described as the RNA polymerase subunit RP030, and later it was found to be also responsible for VITF-1 (intermediate transcription factor) activity (1, 8, 15).

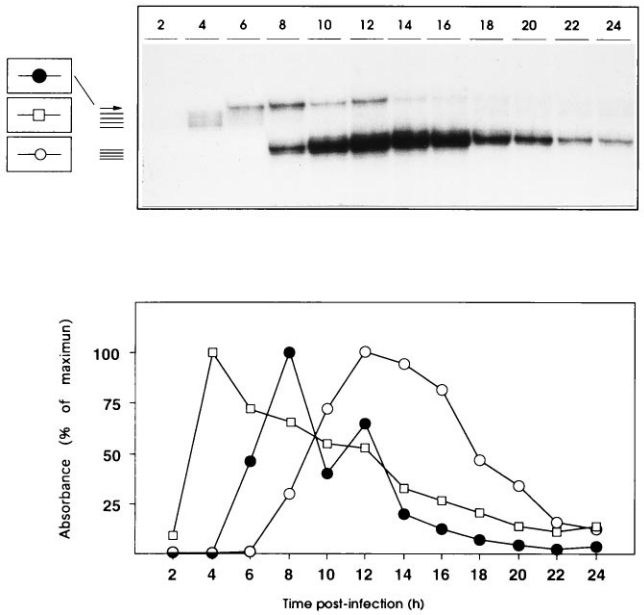


FIG. 3. Steady-state levels of the different I243L transcripts throughout the viral infection. (Upper panel) Detection of the I243L-specific transcripts by primer extension. Total cell RNA was isolated at the indicated times postinfection and hybridized with an oligonucleotide specific for I243L transcripts. After primer extension, the elongated fragments were analyzed by polyacrylamide gel electrophoresis and autoradiography. Only the informative area of the gel is shown. (Lower panel) Quantification of the primer extension analysis. The results shown in the upper panel were quantified by densitometry and plotted as the percentage of the maximum level of specific primer extension. Results for the three different temporal species were plotted independently.

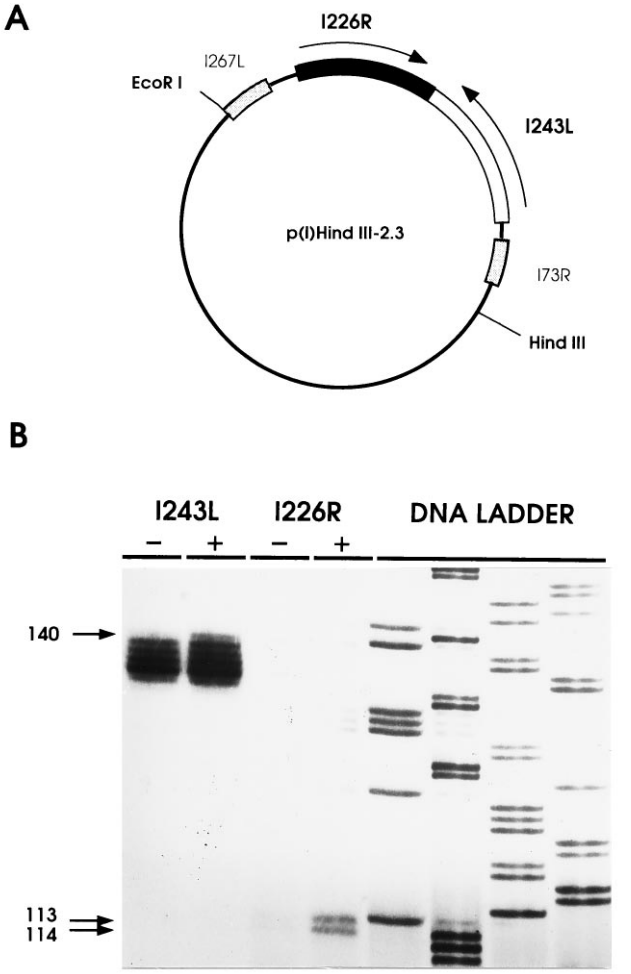


FIG. 4. Intermediate transcripts of the I226R and I243L genes. (A) Structure of plasmid p(I)HindIII-2.3. The positions of the different ORFs are indicated by boxes. Arrows show the directions of I226R and I243L transcription. (B) Primer extension analysis of RNA derived from genomic and transfected genes. Total cell RNA was isolated at 7 h postinfection from Vero cells infected in the presence of cytosine arabinoside and transfected with either plasmid p(I)HindIII-2.3 (+ lanes) or pUC19 (− lanes) as a control. Primer extensions were performed with oligonucleotides specific for the I226R or I243L transcripts. After the extension reaction, the elongated fragments were analyzed by polyacrylamide gel electrophoresis and autoradiography. An unrelated DNA sequencing reaction (DNA ladder) was used as size marker. Only the informative area of the gel is shown. Arrows show the extensions specific for intermediate transcripts in p(I) HindIII-2.3-transfected cells.

Although the E4L gene appears not to be transcribed from intermediate start sites, transcription of both I243L and E4L is initiated from different sites at early and late times of infection, giving rise to mRNAs with different first in-frame ATG codons. The remarkable similarity between I243L and E4L

```
                                                          +1
I226R  TAATGAAAAAAAACTTTTAAATTTTTGTTTTAATATTTGCATGAAAATGGAAACATT
I243L  ACTTCTTTTTTTTGTATTAAATTTTAAAAAAAAATTTATATGAAAATGCATATAGC
CON    ---T----------T-T-AA-TTTT-----AA-ATTT--ATGAAAATG-A-A-A--
```

FIG. 5. Alignment of the sequences surrounding the transcription initiation sites of the I226R and I243L genes. The sequences were aligned from the initiating ATG codon of each ORF. Position +1 corresponds to the first nucleotide of the ATG codon. A consensus sequence (CON) is indicated. The positions of the intermediate start sites are underlined.

argues in favor of a similar dual role for I243L in ASFV transcription.

The finding of intermediate genes in ASFV indicates that the temporal regulation of ASFV gene transcription is more complex than previously thought. ASFV gene expression starts immediately after infection by using the machinery present in the virus particles, giving rise to two types of early mRNAs: (i) immediate-early mRNAs show a peak at 2 to 3 h postinfection and then decrease abruptly in a process that requires protein synthesis; and (ii) early mRNA synthesis starts at the same time, but the maximum levels are reached at 4 to 6 h postinfection, declining at late times coincidentally with maximum DNA replication. The two intermediate mRNAs described in this report are first detected at 4 to 6 h postinfection, coincident with maximum expression of early genes. These intermediate mRNAs reached maximum levels of accumulation at 6 to 8 h postinfection, decreasing sharply at later times. The beginning of late gene expression is coincident with the maximum levels of intermediate mRNAs, and late mRNA levels peak at 12 to 16 h postinfection, decreasing slowly thereafter.

Poxvirus genes are expressed at early, intermediate, or late stages of infection, depending on their promoter sequences. The factors required for transcription of each class of genes are synthesized during the expression of the previous temporal classes. Thus, the expression of poxvirus genes appears as a cascade of transcriptional events. The intermediate class of poxvirus genes plays a key role in this cascade, acting as a switch for the entry into the late phase of the infectious cycle (4, 13). The data presented here support a similar view for the regulation of transcription of ASFV genes, because we have detected the same temporal classes of genes, with expression kinetics similar to those of their counterparts in poxvirus.

The similarity in transcription strategies between poxvirus and ASFV, together with the presence of strong similarities in DNA structure and in the enzymes involved in nucleic acid and nucleotide metabolism, poses the interesting questions of whether both groups of viruses evolved from a common ancestor and, if so, how they evolved to have such dramatic differences in virion structure and in the strategies used to evade the host immune system (5, 22, 25).

## ACKNOWLEDGMENTS

We thank J. F. Rodriguez for helpful discussions during the realization of this work; J. Salas, R. J. Yáñez, and S. de la Luna for critical reading of the manuscript; and F. Almazán for help with the preparation of figures.

This work was supported by grants from the Dirección General de Investigación Científica y Técnica (PB93-0160-C02-01) and the European Community (AIR-CT93-1332) and by an institutional grant from Fundación Ramón Areces.

## REFERENCES

1. **Ahn, B.-Y., P. D. Gershon, E. V. Jones, and B. Moss.** 1990. Identification of *rpo30*, a vaccinia virus RNA polymerase gene with structural similarity to a eucaryotic transcription elongation factor. Mol. Cell. Biol. **10:**5433–5441.
2. **Almazán, F., J. M. Rodríguez, G. Andrés, R. Pérez, E. Viñuela, and J. F. Rodriguez.** 1992. Transcriptional analysis of multigene family 110 of African swine fever virus. J. Virol. **66:**6655–6667.
3. **Almazán, F., J. M. Rodríguez, A. Angulo, E. Viñuela, and J. F. Rodriguez.** 1993. Transcriptional mapping of a late gene coding for the p12 attachment protein of African swine fever virus. J. Virol. **67:**553–556.
4. **Baldick, C. J., Jr., and B. Moss.** 1993. Characterization and temporal regulation of mRNAs encoded by vaccinia virus intermediate-stage genes. J. Virol. **67:**3515–3527.
5. **Blasco, R.** 1994. Evolution of poxviruses and African swine fever virus, p. 225–270. *In* A. J. Gibbs, C. H. Calisher, and F. García-Arenal (ed.), Molecular basis of virus evolution. Cambridge University Press, Cambridge.
6. **Blasco, R., M. Agüero, J. M. Almendral, and E. Viñuela.** 1989. Variable and constant regions in African swine fever virus DNA. Virology **168:**330–338.
7. **Brown, F.** 1986. The classification and nomenclature of viruses: summary of

results of meetings of the International Committee of Taxonomy of Viruses in Senday, September. Intervirology **25:**141–143.
8. **Broyles, S. S., and M. J. Pennington.** 1990. Vaccinia virus gene encoding a 30-kilodalton subunit of the viral DNA-dependent RNA polymerase. J. Virol. **64:**5376–5382.
9. **Enjuanes, L., A. L. Carrascosa, and E. Viñuela.** 1976. Titration of African swine fever (ASF) virus. J. Gen. Virol. **32:**471–477.
10. **Felgener, P. L., T. R. Gadek, M. Holm, R. Roman, H. W. Chan, M. Wenz, J. P. Northrop, G. M. Ringold, and M. Danielsen.** 1987. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc. Natl. Acad. Sci. USA **84:**7413–7417.
11. **González, A., A. Talavera, J. M. Almendral, and E. Viñuela.** 1986. Hairpin loop structure of African swine fever virus DNA. Nucleic Acids Res. **14:** 6835–6844.
12. **Keck, J. G., C. J. Baldick, Jr., and B. Moss.** 1990. Role of DNA replication in vaccinia virus gene expression: a naked template is required for transcription of three late trans-activator genes. Cell **61:**801–809.
13. **Moss, B.** 1990. Regulation of vaccinia virus transcription. Annu. Rev. Biochem. **59:**661–688.
14. **Rodríguez, J. M., M. L. Salas, and E. Viñuela.** 1992. Genes homologous to ubiquitin-conjugating proteins and eukaryotic transcription factor SII in African swine fever virus. Virology **186:**40–52.
15. **Rosales, R., N. Harris, B.-Y. Ahn, and B. Moss.** 1994. Purification and identification of a vaccinia virus-encoded intermediate-stage promoter-specific transcription factor that has homology to eukaryotic transcription factor SII (TFIIS) and an additional role as a viral RNA polymerase subunit. J. Biol. Chem. **269:**14260–14267.
16. **Salas, J., M. L. Salas, and E. Viñuela.** 1988. Effect of inhibitors of the host cell RNA polymerase II on African swine fever virus multiplication. Virology **164:**280–283.
17. **Salas, M. L., J. Kuznar, and E. Viñuela.** 1981. Polyadenylation, methylation and capping of the RNA synthesized *in vitro* by African swine fever virus. Virology **113:**484–491.
18. **Salas, M. L., J. Rey-Campos, J. M. Almendral, A. Talavera, and E. Viñuela.** 1986. Transcription and translation maps of African swine fever virus. Virology **152:**228–240.
19. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Sogo, J. M., J. M. Almendral, A. Talavera, and E. Viñuela.** 1984. Terminal and internal inverted repetitions in African swine fever virus DNA. Virology **133:**271–275.
21. **Viñuela, E.** 1985. African swine fever virus. Curr. Top. Microbiol. Immunol. **116:**155–170.
22. **Viñuela, E.** 1987. Molecular biology of African swine fever virus, p. 31–49. *In* Y. Becker (ed.), African swine fever. Martinus Nijhoff Publishing, Boston.
23. **Vos, J. C., and H. G. Stunnenberg.** 1988. Derepression of a novel class of vaccinia virus genes upon DNA replication. EMBO J. **7:**3487–3492.
24. **Wilkinson, P. J.** 1989. African swine fever virus, p. 17–37. *In* M. B. Pensaert (ed.). Virus infections of porcines. Elsevier Sciences Publishers B.V., Amsterdam.
25. **Yáñez, R. J., J. M. Rodríguez, M. L. Nogal, L. Yuste, C. Enríquez, J. F. Rodríguez, and E. Viñuela.** 1995. Analysis of the complete nucleotide sequence of African swine fever virus. Virology **208:**249–278.