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African swine fever virus transcription

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ABSTRACT

African swine fever virus (ASFV), a large, enveloped, icosahedral dsDNA virus, is currently the only known DNA-containing arbovirus and the only recognized member of the family *Asfarviridae*. Its genome encodes more than 150 open reading frames that are densely distributed, separated by short intergenic regions. ASFV gene expression follows a complex temporal programming. Four classes of mRNAs have been identified by its distinctive accumulation kinetics. Gene transcription is coordinated with DNA replication that acts as the main switch on ASFV gene expression. Immediate early and early genes are expressed before the onset of DNA replication, whereas intermediate and late genes are expressed afterwards. ASFV mRNAs have a cap 1 structure at its 5′-end and a short poly(A) tail on its 3′-end. Transcription initiation and termination occurs at very precise positions within the genome, producing transcripts of definite length throughout the expression program. ASFV devotes approximately 20% of its genome to encode the 20 genes currently considered to be involved in the transcription and modification of its mRNAs. This transcriptional machinery gives to ASFV a remarkable independence from its host and an accurate positional and temporal control of its gene expression. Here, we review the components of the ASFV transcriptional apparatus, its expression strategies and the relevant data about the transcriptional cis-acting control sequences.

1. Introduction

African swine fever virus (ASFV) is a large (≈200 nm), enveloped, icosahedral double-stranded DNA virus with a lineal genome that varies in size from 170 to 190 kbp depending on the strain, and contains terminal inverted repeats and covalently closed ends (Sogo et al., 1984; González et al., 1986; reviewed in Tulman et al., 2009).

The natural hosts of this virus are the wild swine warthogs, bushpigs and the argasid ticks of the genus *Ornithodoros*. The infection of ASFV in these hosts results in a mild disease, often asymptomatic, with low viremia titers, that in many cases develops into a persistent infection (Anderson et al., 1998; Boinas et al., 2011; Kleiboeker et al., 1998; Thomson et al., 1980; reviewed by Penrith and Vosloo, 2009). In contrast, infection of domestic pigs leads to a lethal hemorrhagic fever for which the only available methods of disease control are the quarantine of the affected area and the elimination of the infected animals (Penrith and Vosloo, 2009).

The strains completely sequenced encode between 151 and 167 open reading frames closely spaced along both chains of the viral DNA. About half of them lack any known or predictable function (Chapman et al., 2008; Yáñez et al., 1995). It is currently the only known DNA-containing arbovirus and the only recognized member of the family *Asfarviridae* (Dixon et al., 2012). It has been recently reported the complete sequence of the type B DNA polymerase (PolB) gene of *Heterocapsa circularisquama* DNA virus (HcDNAV, Ogata et al., 2009) that shows a remarkable similarity with the PolB sequence of ASFV. HcDNAV is a marine virus that lytically infects the dinoflagellate *H. circularisquama* (Nagasaki et al., 2003; Tarutani et al., 2001), an abundant and ubiquitous unicellular eukaryotic component of the marine environments (Falkowski et al., 2004). HcDNAV is also a large (180–210 nm) icosahedral dsDNA virus, but possesses a larger genome than ASFV (≈356-kpb). Sequence searches and phylogenetic analyses with a partial sequence of the putative RNA Polymerase gene of HcDNAV also produce a monophyletic grouping between ASFV and HcDNAV, suggesting that HcDNAV is a new member of the *Asfarviridae* family (Ogata et al., 2009). These results, together with the data from a recent survey of the oceanic virome that found several PolB-like sequences closely related to the PolB sequence of ASFV, suggest that ASFV had its evolutionary origin in the marine environments (Monier et al., 2008).

Comparative genome analysis indicates that ASFV belongs to nucleocytoplasmic large DNA viruses (NCLDVs), a apparently monophyletic viral group infecting a broad variety of eukaryotes, that currently includes the six virus families *Asfarviridae, Ascoviridae, Iridoviridae, Phycodnaviridae, Poxviridae, Mimiviridae* and the

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proposed family *Marseilleviridae* (Iyer et al., 2001, 2006; Koonin and Yutin, 2010). The members of these families either have and exclusively cytoplasmic replication, or it is initiated in the nucleus and is later completed in the cytoplasm of the infected cell; some of them are relatively independent of the host cell transcriptional machinery for replication. Lineage-specific gene loss and gain within the NCLDV families, including horizontal gene transfer across the three cellular domains (Filée et al., 2008; Moreira and Brochier-Armanet, 2008)), and possibly horizontal gene exchange of essential genes among viruses from different families (Yutin et al., 2009), is thought to have contributed to the highly diverse characteristics of present-day forms, which complicates the establishment of their true evolutionary relationship. Although this is particularly true for ASFV, being the only available representative of the *Asfarviridae* family, ASFV is consistently paired along with poxvirus as sister groups (Iyer et al., 2001, 2006; Koonin and Yutin, 2010).

Despite the fact that they are morphologically very different, poxvirus and ASFV share numerous biological characteristics like its genome structure and their almost complete independence on their hosts for genome replication and gene transcription.

Poxvirus replication is strictly cytoplasmic, although some nuclear proteins are an essential part of the poxviral transcription machinery (Broyles et al., 1999; Rosales et al., 1994b; Wright et al., 2001). Despite the fact that a brief nuclear phase has been proposed for ASFV (Ballester et al., 2011; Garcia-Beato et al., 1992a; Rojo et al., 1999), most of the replication and all the viral morphogenesis takes place in the cytoplasm of the infected cell. Neither virus possesses introns.

ASFV devotes approximately 20% of its genome to encode the 20 genes currently considered to be involved in the transcription and modification of its mRNAs. This transcriptional machinery gives ASFV a remarkable independence from its host and an accurate positional and temporal control of its gene expression. Here, we review the components of the ASFV transcriptional apparatus, its expression strategies and the relevant data about the transcriptional cis-acting control sequences.

2. ASFV proteins implicated in RNA transcription and modification

Very little experimental characterization has been conducted on the genes of the proteins involved in ASFV transcription. With the exception of the identification of the guanylyltransferase activity on protein pNP868R (Pena et al., 1993) and the mRNA decapping activity on protein pD250R (Parrish et al., 2009) most of the information gathered on Table 1 comes from the comparative analysis of sequence databases (Chapman et al., 2008; Dixon et al., 1994; Yáñez et al., 1995). Particularly helpful in this regard has been the effort realized in the establishment and characterization of the NCLDV group (Iyer et al., 2001, 2006; Yutin et al., 2009). For most of the putative homologies thus identified, the degree of similarity between the ASFV protein and the database protein is enough to be confident that the ASFV protein has a similar or closely related activity during the infectious cycle. In other cases, however, the sequence similarity is scarce or limited to a small protein domain and, although this does not preclude evolutionary relationship, there is a reasonable possibility that evolution has reshaped the ancestral protein for a different function in ASFV. Nonetheless, we believe that, without experimental data, this information is important as a starting point for the experimental characterization of the ASFV transcriptional machinery.

The majority of the experimental work on ASFV transcription has been realized using the BA71V strain, thus the nomenclature of the BA71V genes and proteins has been maintained (Yáñez et al., 1995). Table 1 lists the ASFV genes, the genes to whom they are similar or, if there are experimental data, the activities identified in the ASFV genes. It also lists the relevant references. For some of the genes listed in the table, the nomenclature proposed by Iyer et al. (2001) based on the putative orthologues of the model orthopoxvirus vaccinia virus (VV) has been maintained.

2.1. Core components of the DNA dependent RNA polymerase

The majority of the components of the DNA dependent RNA polymerase show a higher degree of similarity with eukaryotic polymerases than with the polymerases of members of the NCLDV. An outline of the subunits that compose the ASFV RNA polymerase in comparison with those of the eukaryotic RNA polymerase II (Pol II) is shown in Fig. 1.

2.1.1. RPB1 (Gene NP1450L of the BA71V strain) and RPB2 (EP1242L)

The ASFV virion contains an alpha-amanitine resistant DNA-dependent RNA polymerase activity (Kuznar et al., 1980). Experiments using inhibitors of the cellular Pol II show that the cellular activity is not essential for viral replication (Salas et al., 1988), which indicates that all temporal classes of ASFV genes are transcribed by the virus-encoded RNA polymerase. Sequence analysis identified two ASFV genes, NP1450L and EP1242L, that encode proteins homologous to the two largest subunits (RPB1 and RPB2, respectively) of the DNA-dependent RNA polymerases of eukaryotes and members of the NCLDV viruses such as VV (Yáñez et al., 1993a). The similarity of both ASFV subunits is higher with the polymerases of eukaryotes and the similarity domains conserved between the eukaryotic and *Escherichia coli* subunits are basically maintained in the ASFV proteins. However, both the VV and the ASFV largest subunit lack the repetitive C-terminal domain of the eukaryotic subunit (Broyles and Moss, 1986).

Expression of the two subunits occurs late during the infection, thus the early transcription is realized by the structural RNA polymerase (Yáñez et al., 1993a). In VV, it has been demonstrated that newly synthesized RNA polymerase is necessary for the transcription of the postreplicative genes (Hooda-Dhingra et al., 1989).

2.1.2. RPB3 (H359L)

Gene H359L shows a high degree of similarity with the subunit 3 of the Pol II of eukaryotes (RPB3). RPB3 is a core subunit of Pol II that, together with the RPB11 subunit, forms the heterodimer considered as a functional counterpart of the bacterial alpha subunit homodimer involved in promoter recognition. RPB1, RPB2 along with RPB3 and RPB11 are largely responsible for Pol II RNA catalysis (Lee and Young, 2000). No similar protein is found in the genome of poxvirus.

2.1.3. RPB5 (D205R)

The sequence of the carboxyterminal half of protein pD205R is very similar to the eukaryotic subunit 5 of Pol II (RPB5) and contains the signature domain of this protein superfamily, RNA_pol_Rpb5_C superfamily (CCD; Marchler-Bauer et al., 2011). The RPB5 is a highly conserved subunit shared by the three eukaryotic RNA polymerases, Pol I–III. This subunit is in close contact to promoter DNA in the preinitiation complex and has been shown to interact with the basal transcription factor TFIIB and to be one of the potential interaction targets for transcriptional activators (Todone et al., 2000). ASFV encodes also a protein similar to the TFIIB factor. The presence of a gene similar to RPB5 is one of the defining features of NCLDVs (Iyer et al., 2006).

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Table 1
ASFV genes involved in RNA transcription and modification.

Similarity/activity	ASFV gene	References
RPB1	NP1450L	Yáñez et al. (1993a)
RPB2	EP1242L	Yáñez et al. (1993a)
RPB3	H359L	Yáñez et al. (1995)
RPB5	D205R	Dixon et al. (1994) and Yáñez et al. (1995)
RPB6	C147L	Lu et al. (1993) and Yáñez et al. (1995)
RPB7	D339L	Yáñez et al. (1995)
RPB10	CP80R	Yáñez et al. (1995)
TFIIB	C315R	Iyer et al. (2006)
TFIIS	I243L	Rodríguez et al. (1992)
D6/D11-like	D1133L/Q706L	Baylis et al. (1993b), Iyer et al. (2001), Roberts et al. (1993) and Yáñez et al. (1993b)
A7-like	G1340L	Iyer et al. (2006)
I8-like	B962L	Yáñez et al. (1995)
A1-like	B175L	Iyer et al. (2001)
A2-like	B385R	Iyer et al. (2006)
A18-like	QP509L/A859L	Baylis et al. (1993b), Iyer et al. (2001), Roberts et al. (1993), Yáñez et al. (1995) and Yutin et al. (2009)
Poly(A) polymerase	C475L	Iyer et al. (2006)
Capping enzyme	NP868R	Pena et al. (1993)
mRNA decapping enzyme	D250R	Cartwright et al. (2002), Parrish et al. (2009)
DNA topoisomerase II	P1192R	Baylis et al. (1992) and Garcia-Beato et al. (1992b)
RNA ligase	M448R	Iyer et al. (2006)

2.1.4. RPB6 (C147L)

Protein pC147L is highly similar to the subunit 6 of the RNA polymerase of eukaryotes (RPB6) (Lu et al., 1993). RPB6 is an essential subunit in the three eukaryotic polymerases. This family also contains the bacterial equivalent to RPB6, the omega subunit. RPB6 and omega are structurally conserved and both function in polymerase assembly (Minakhin et al., 2001). Experimental evidence indicates that RPB6 plays an important role in the interaction between the Pol II and the transcription elongation factor TFIIS (Ishiguro et al., 2000). Interestingly, ASFV also encodes a protein similar to the TFIIS that is thought to be an intrinsic component of the polymerase (Rodríguez et al., 1992). No similar protein is found in the genome of poxviruses.

2.1.5. RPB7 (D339L)

Amino acids 9–79 of protein pD339L contain a protein–protein interaction SHS2 domain (Anantharaman and Aravind, 2004) with an architecture SHS2_Rpb7-N (pfam03876), that is characteristic of the SHS2 domains found in N-terminus of the archaeo-eukaryotic RNA polymerase II subunit 7 (RPB7). RPB7 bind to the RNA polymerase II subunit 4 to form a heterodimer. The complex RPB4/7 has numerous functions. It mediates both transcription (Choder, 2004) and the two major cytoplasmic mRNA decay pathways (Lotan et al., 2007), it interacts physically and functionally with components of the translation initiation factor 3, and is required for efficient translation initiation (Harel-Sharvit et al., 2010). No similar protein is found in the genome of poxvirus.

2.1.6. RPB10 (CP80R)

ASFV gene CP80R encodes a protein highly similar to the archaeo-eukaryotic RNA polymerase II subunit 10 (RPB10). The RPB10 subunit is a zinc-binding protein with an atypical CX2CXnCC metal binding motif that is conserved in CP80R (Woychik and Young, 1990). In yeast, the mutation of any of the four completely conserved Cys residues results in a lethal phenotype (Gadal et al., 1999). RPB10 is essential for cell growth and is shared by all three yeast RNA polymerases (Woychik et al., 1990). Given its small size (55–80 amino acids) and the identification of mutants in RBP10 with assembly defects in yeast (Gadal et al., 1999), it has been suggested that RPB10 plays a structural role in the polymerase complexes (Mackereth et al., 2000). Iridovirus and poxvirus have a conserved gene similar to RPB10.

2.2. Proteins similar to RNA polymerase II transcription factors

2.2.1. TFIIB (C315R)

Protein pC315R shows moderate similarity with the homologue to the transcription factor TFIIB of mimivirus and large unclassified

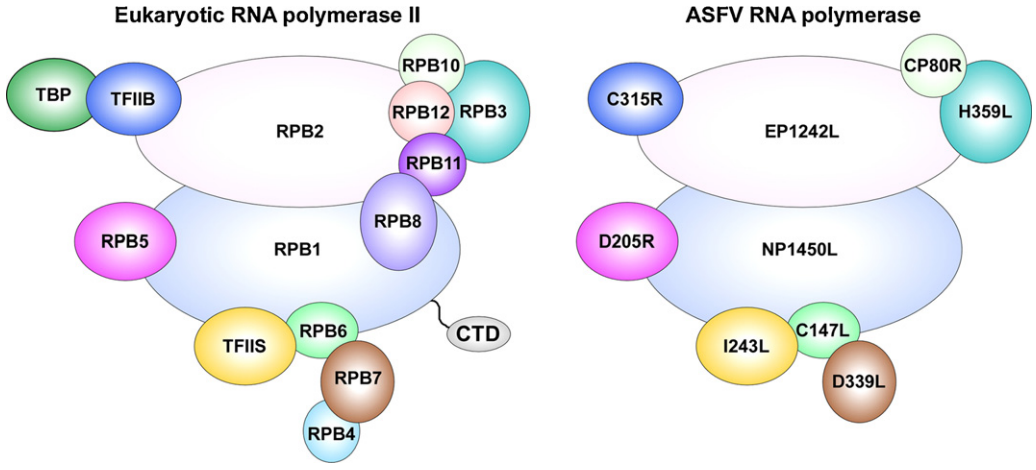

Fig. 1. Putative subunits of the ASFV RNA polymerase compared with those of the eukaryotic RNA polymerase II. The composition of eukaryotic RNA polymerase is adapted from Zhang et al. (2012).

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DNA viruses (Iyer et al., 2006). A similar protein does not seem to be present in the genome of poxviruses. TFIIB is one of several general transcription factors that make up the RNA polymerase II preinitiation complex. It interacts with the TATA binding protein (TBP) at the TATA element (Nikolov et al., 1995), and is one of two indispensable factors (along with TBP) for promoter-directed transcription in vitro in two domains of life (eukaryotes and the archaea) (Parvin and Sharp, 1993; Bell et al., 2001).

2.2.2. TFIIS (I243L)

Protein pI243L is similar to the E4L protein of VV and both of them are similar to the eukaryotic transcription elongation factor TFIIS (Kim et al., 2007). A TFIIS-like protein is part of the core set of conserved genes among the NCLDV families (Yutin et al., 2009; NCVOG0272). The similarity of I243L is slightly greater to the eukaryotic transcription factor than to the E4L protein, and, like the eukaryotic factors, it lacks the carboxy-terminal extension rich in proline and acidic residues characteristic of the E4L protein. TFIIS are extrinsic components of the polymerase whereas E4L has a dual role as a subunit (rpo30) of the VV RNA polymerase (Ahn et al., 1990), and as a transcription factor for the intermediate gene expression (VITF-1) (Rosales et al., 1994a). Experimental evidence strongly suggests that both activities are independent, i.e. the E4L protein associated with the polymerase as rpo30 is unable to act as the VITF-1 (Rosales et al., 1994a). Transcription of the E4L gene is initiated from two closely spaced sites at early times and a third at late times (Ahn et al., 1990). The different forms of the E4L polypeptide appear to be incorporated into RNA polymerase as rpo30. Similarly, transcription of gene I243L is initiated at early, intermediate and late times of infection. Each of the three types of transcripts may give rise to three different polypeptides by using three in-frame ATG codons (Rodríguez et al., 1996). This reinforces the similarity between I243L and E4L and supports a similar role in transcription.

2.3. Proteins similar to poxviral transcription factors

The genes belonging to this functional category are among the most characteristic proteins of NCLDV. Thus, A1-, A2- and A7-like orthologous bear no significant similarity with any other eukaryotic or viral protein present in the databases (Yutin et al., 2009). Several decades of continuous research on the molecular biology of VV, the prototypical poxvirus, has resulted in a wealth of knowledge that makes this viral family the natural reference on the functions of the proteins that any NCLDV virus shares with it. We will examine the putative ASFV transcription factors and the roles of the orthologous VV protein in transcription.

2.3.1. Proteins similar to prereplicative transcription factors

All the factors necessary for VV prereplicative transcription are encoded in the viral genome, are expressed late during the infection and are encapsidated in the viral particle. ASFV encodes proteins with various degrees of similarity to all of them. Among these is ASFV protein NP868R, orthologous to the catalytic subunit of the VV capping enzyme. Besides its role in the construction of the 5′ cap structure of VV mRNAs (Shuman et al., 1980), this VV enzyme has been shown to participate in the prereplicative transcription termination (Shuman and Moss, 1988) and in the initiation of the intermediate transcription (Vos et al., 1991). We will discuss NP868R below, as the capping enzyme, along with other proteins involved in mRNA modification.

2.3.1.1. D6/D11-like proteins (D1133L/Q706L). Although not classified as true orthologous to the VV proteins encoded by genes D6R and D11L, ASFV genes D1133L and Q706L belong, like the VV genes, to the DEAD_SNF2_like family of helicases (Yutin et al., 2009). Genes of this family are present in the genome of all members of the NCLDV. Very little is known about these ASFV genes, except that D1133L is expressed after DNA replication (Yáñez et al., 1993b).

D6R encodes the small subunit of the VV early transcription factor (VETF) (Broyles and Fesler, 1990), and D11L the nucleoside triphosphate phosphohydrolase I (NPH-I) (Deng and Shuman, 1998; Christen et al., 1998). NPH I is a DNA-dependent ATPase with no helicase activity. Five of the six helicase motifs in NPH I are essential for termination factor activity (Christen et al., 1998). It plays two different roles in transcription, it acts as a polymerase elongation factor to facilitate readthrough of intrinsic pause sites, and in conjunction with the VV termination factor, catalyzes the release of the nascent RNA from the elongation complex at the signal of transcription termination (Deng and Shuman, 1998).

VV VETF is a heterodimeric protein composed by the products of the D6L and A7L genes (Broyles and Fesler, 1990; Gershon and Moss, 1990). During transcription initiation, the complex recruits RNA polymerase to the template (Li and Broyles, 1993) and induces promoter bending (Broyles et al., 1991). The intrinsic DNA-dependent ATPase activity of VETF is due to the protein encoded in D6L (Broyles and Fesler, 1990).

2.3.1.2. A7-like (G1340L). ASFV gene G1340L is orthologous to the A7L gene of VV; proteins belonging to this cluster are present in all NCLDV families except phycodnavirus (Yutin et al., 2009). A7L product is the larger component of the VETF (Gershon and Moss, 1990), the only known poxvirus-encoded promoter-binding protein, that has no apparent enzymatic activity. Cross-linking experiments (Cassetti and Moss, 1996) showed that both subunits of VETF interact with DNA at the promoter sequence; the larger subunit binds primarily with the core region (−29 to −12), in a sequence specific manner (Broyles et al., 1991) and directs the binding of the small subunit at the downstream (+8 to +10) region of the promoter.

2.3.1.3. I8-like (B962L). ASFV late gene B962L is orthologous to the I8-like group on NCLDV proteins (Yáñez et al., 1993b). Members of this group are also present in poxvirus and mimivirus (Yutin et al., 2009). The VV orthologous I8L gene encodes the nucleoside triphosphate phosphohydrolase II (NPH-II; Shuman, 1992). It belongs to the superfamily II of helicases and its biochemical analysis has contributed to define the characteristics of the helicase family NS3/NPHII (Fairman-Williams et al., 2010). Although the specific function of NPH-II remains unknown, this enzyme possesses RNA and DNA helicase activities (Bayliss and Smith, 1996), and is thought to play an important accessory role in transcriptional termination of early viral genes in vivo.

2.3.2. Proteins similar to intermediate transcription factors

ASFV encodes two orthologous to two VV intermediate transcription factors, gene I243L and the large subunit of the capping enzyme. I243L has been already mentioned as the homologous to the TFIIS transcription elongation factor and the capping enzyme will be discussed below.

2.3.3. Proteins similar to the late transcription factors A1- and A2-like (B175L, B385R)

ASFV encodes two genes orthologous to the late transcription factors of VV, B175L, which is an A1-like gene, and B385R, that belongs to the A2-like cluster of orthologous genes. All the analyzed genomes of the NCLDVs have genes that belong to these clusters (Yutin et al., 2009).

A1L and A2L are regulated by intermediate promoters, and do not have any known enzymatic activity. The products of these two genes along with that of the G8R gene were necessary and sufficient

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for transactivation of a late promoter in cells infected with VV that were blocked in DNA replication (Keck et al., 1990).

2.3.4. Proteins similar to the intermediate/late termination factor (QP509L, A859L)

ASFV encodes two genes orthologous to the A18R gene of VV that are grouped along with proteins of each of the members of the NCLDV, except for Ascovirus, in cluster NCVOG0076 (Yutin et al., 2009). They have the motifs characteristic of the superfamily II of helicases. VV A18R encodes a protein that is expressed throughout infection and is packaged into virions (Simpson and Condit, 1994). It has helicase and ATPase activities (Simpson and Condit, 1995; Bayliss and Condit, 1995; Bayliss and Smith, 1996). Mutation of the A18R gene results in read through transcription from intermediate promoters into downstream genes (Xiang et al., 1998) suggesting that it could function as a transcript termination factor of the intermediate genes. It has also been shown to act in vitro as a transcript release factor dependent on ATP hydrolysis (Lackner and Condit, 2000).

2.4. Proteins involved in mRNA modification

2.4.1. Poly(A) polymerase (C475L)

ASFV mRNAs are characterized by the presence of poly(A) tails at their 3′-ends. The protein encoded in the ASFV C475L gene has been recently identified as having a moderate sequence similarity with the poly(A) polymerases of members of the NCLDV (Iyer et al., 2006; Yutin et al., 2009; NVCOG1165). Orthologous to this protein are present in poxvirus and mimivirus, although the sequence similarity is higher with the mimivirus genes. They are highly divergent members of the polß nucleotidyltransferase superfamily, typified by the eukaryotic DNA polymerase ß (Aravind and Koonin, 1999). In the poxvirus VV, the poly(A) polymerase is an heterodimer of VP55, the catalytic component, and VP39, that acts as a processivity factor. Without VP39, VP55 adds short (≈35 nucleotides) poly(A) tails before dissociating from the RNA substrate, whereas in the presence of the processivity factor, VP55 does not dissociate, producing longer (up to 600 nt in vitro) poly(A) tails (Gershon and Moss, 1990). No protein similar to VP39 has been identified in ASFV. Interestingly, the mean length of the poly(A) tails of the in vitro transcribed ASFV mRNAs is 33 nucleotides (Salas et al., 1981), similar to that produced by the catalytic subunit of VV in the absence of the processivity factor.

2.4.2. Capping enzyme (NP868R)

The presence of a cap structure in the 5′-end of the mRNAs is a common characteristic of the mRNAs of eukaryotes and of many viruses that infect eukaryotes. It consists on a N^7-methylguanosine moiety linked to the first transcribed nucleotide (N) by a 5′–5′ triphosphate bridge, resulting in the structure m^7GpppN (cap 0 structure). Commonly, a second methylation is found in the ribose of the N nucleotide, at the 2′-O position (cap 1 structure), and in some cases additional methyl groups are added to the first and second transcribed nucleotides (cap 2 structure). The 5′-cap facilitates export, splicing, translation initiation and protects mRNAs from degradation by 5′-exonucleases. The cap 0 structure is constructed by the coordinate action of three enzymatic activities in the 5′-triphosphate end of the nascent RNA: (1) an RNA triphosphatase (RTPase) removes the γ-phosphate of the transcript; (2) an RNA guanylyltransferase (GTase) caps the 5′-diphosphate RNA with GMP; and (3) an RNA methyltransferase (MTase) adds a methyl group at the 5′-guanine base at the N7 position (reviewed in Shuman, 2002). Gene NP868R encodes the ASFV capping enzyme. Experimentally, protein pN868R has been shown to have guanylyltransferase activity (Pena et al., 1993). The sequence of this protein resembles the domain organization of the D1 subunit of the VV capping enzyme and the mimivirus capping enzyme (Benarroch et al., 2008). While in eukaryotes these three activities are encoded by two or three different genes (Shuman, 2002), the capping enzymes of these three viral families show a remarkable compactation. They are multifuntional polypeptides composed of N-terminal RTPase, central GTase, and C-terminal MTase domains (Benarroch et al., 2008). The VV capping machinery is composed of two subunits, D1 and D12 (Shuman, 1990), however, the D12 subunit, that has an stimulatory role on the MTase activity of the D1 subunit, shows no sequence similarity with any other known protein except to homologous present in other poxviruses.

VV mRNAs acquire its final 5′-cap 1 structure by 2′-O-methylation of the first transcribed nucleotide by the VP39 protein, which, as seen before, is also a processivity subunit for the viral poly-A polymerase (Schnierle et al., 1992). ASFV mRNAs have also a cap 1 structure in the 5′-end, but as indicated before, the protein responsible for the 2′-O-methyltransferase activity in ASFV is currently unknown.

2.4.3. mRNA decapping enzyme (D250R)

Gene D250R (or g5R in other ASFV strains) encodes a protein with a NUDIX (NUcleoside DIphosphate linked to some other moiety X) hydrolase motif (Bessman et al., 1996; Koonin, 1993). This motif characterizes a widely distributed family of proteins with genes present in viruses, prokaryotes, and eukaryotes, and includes enzymes such as Ap4AP (adenosine 5′-tetraphospho-5′-adenosine pyrophosphatase), MutT pyrophosphohydrolase, GDPMH (GDP-mannose mannosyl hydrolase) and decapping enzymes (reviewed in McLennan, 2006). In viruses, the presence of NUDIX hydrolases is almost restricted to the members of the NCLDV, where it is one of the core set of genes common to most families (Iyer et al., 2006; Yutin et al., 2009).

Protein pD250R has been shown to have two different enzymatic activities in vitro; it has a diadenosine hexaphosphate (Ap6A) hydrolase activity (Cartwright et al., 2002), and hydrolyzes the mRNA cap, liberating m^7GDP as a product (Parrish et al., 2009).

Its diadenosine hexaphosphate (Ap6A) hydrolase activity is able to hydrolyze a range of guanine and adenine nucleoside and dinucleoside polyphosphates but exhibits highest substrate efficiency with diphosphoinositol pentakisphosphate (PP-InsP5). Although the functional significance of this reduction in PP-InsP5 during the infection remains unclear, it has been suggested that the control of the metabolism of PP-InsP5 could be related to the manipulation by the virus of the components of cellular secretory pathways during viral morphogenesis (Cartwright et al., 2002).

The decapping activity of pD250R does not cleave free cap analogues in vitro (Cartwright et al., 2002), but is able to hydrolyze efficiently the mRNA cap when attached to a RNA molecule (Parrish et al., 2009). It is abolished by mutations on the Nudix motif. Protein pD250R binds to RNA, this being inhibited by uncapped RNA but not by methylated cap analogs, suggesting that it recognizes the RNA moiety on the target substrate. The D250R gene shows a greater sequence similarity with the G10 gene of VV, which encodes one of the two mRNA decapping enzymes (along with D9) present in the genome of this virus. Over-expression of the D9 or D10 genes during VV infection produces an enhanced turnover of capped mRNA molecules (Shors et al., 1999). The deletion of the D10R gene from the VV genome results in the persistence of cellular and viral transcripts and a delay in the shutoff of host protein synthesis (Parrish and Moss, 2006). These results indicate that the two decapping activities increase the turnover of cellular and viral mRNA during the infection, which might contribute to an acceleration of the shutoff of host proteins synthesis and to the generation of the sequential cascade of viral gene expression. A similar role has been proposed

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for the decapping activity of pD250R during ASFV infection (Parrish et al., 2009).

2.4.4. Topoisomerasa II (P1192R)

Protein pP1192R of ASFV has an extensive sequence similarity with Type II topoisomerases (Baylis et al., 1992; Garcia-Beato et al., 1992b). Genes encoding these enzymes are also present in the genome of all the members of the NCLDV families. Thus, iridovirus, phycodnavirus and marseillevirus also have a Type II enzyme, whereas poxviruses encode a Topoisomerase IB and mimivirus encode both types of enzymes. Topoisomerases deal with topological problems that arise due to the double helical nature of DNA during replication, transcription and recombination. The possible involvement of the viral topoisomerase II in early transcription is suggested by the finding that the specific inhibitor of this enzyme, coumermycin A1, inhibits in vitro transcription by ASFV virions (Salas et al., 1983).

2.4.5. RNA ligase (M448R)

ASFV gene M448R encodes a protein with similarity to the T4 RNA ligase 1 (Rnl1) family of RNA ligases (Silber et al., 1972). Orthologues to M448R are found in iridovirus, ascovirus and marseillevirus, although some catalytic residues are not maintained (Iyer et al., 2006; Yutin et al., 2009; NCVOG1088). It is also similar to the aminoterminal RNA ligase domain of the baculovirus AcNPV ORF86 (BacRnl1), a baculovirus trifunctional enzyme with RNA ligase, polynucleotide 5′-kinase, and polynucleotide 3′-phosphatase activities (Martins and Shuman, 2004). The function of Rnl1 in vivo is to counteract the RNA damage-based immune response of *E. coli* to virus infection. Bacteriophage T4 infection activates a host-encoded anticodon nuclease, PrrC, that produces a break in the anticodon loop of *E. coli* tRNALys (Blanga-Kanfi, 2006). The depletion of the tRNALys blocks phage protein synthesis and arrests the infection before it can spread. However, the end-healing and end-sealing reactions catalyzed by the T4 bifunctional polynucleotide kinase/phosphatase (Pnkp) and T4 Rnl1 repair the broken tRNAs bypassing the host defense mechanism (Amitsur et al., 1987). The single trifunctional baculovirus enzyme BacRnl1 catalyzes the same repertoire of RNA repair reactions that the two enzymes that compose the T4-encoded tRNA repair system. The limited distribution of BacRnl1 among known baculovirus genera and the inessentiality of BacRnl1 for AcNPV replication in cell culture imply that RNA repair is a recent acquired capacity that may be relevant to baculovirus host range, by providing AcNPV with a means to evade an RNA damage-based host response (Martins and Shuman, 2004). There are no experimental data regarding the role of M448R during ASFV infection.

3. Temporal regulation of ASFV gene expression

ASFV gene expression occurs under a strict temporal regulation through a tight control at the level of transcription initiation and a fast rate of mRNA degradation (Fig. 2). DNA replication divides the infection cycle in an early phase, before the onset of DNA replication, and a late phase, after DNA replication begins. To avoid confusion, we will refer as prereplicative and postreplicative to genes expressed before and after DNA replication.

Four classes of transcripts with a distinct accumulation kinetics have been detected by primer-extension and nuclease S1 analysis of mRNA obtained at different times after infection and in the presence of inhibitors of DNA and protein synthesis (Almazán et al., 1992, 1993; Rodríguez et al., 1996). Transcription of prereplicative genes starts almost immediately after virus infection and for the *early* genes it continues until the initiation of DNA replication. However, the expression of a subset of the prereplicative genes called *immediately early* is repressed before, in a process that requires protein synthesis. Two classes of transcripts are expressed in the postreplicative stage of the infection, *intermediate* transcripts are synthesized immediately after DNA replication is initiated whereas the transcription of the *late* genes occurs after the intermediate ones and continues until the end of the infection. This fine regulation is thought to occur by a cascade mechanism similar to that of poxviruses (Broyles, 2003), where the transcription factors necessary for the expression of the intermediate genes are expressed at early times, the factors necessary for the expression of the late genes are encoded on the intermediate genes, and those necessary for the transcription of the early genes are expressed at late times and are packaged into the viral particles.

3.1. Prereplicative phase of gene expression

Experimentally, ASFV prereplicative genes are defined by its expression in cells infected in the presence of inhibitors of DNA replication (Salas et al., 1986). Early and immediate early genes are differenced by comparing their relative expression in cells infected in the presence of inhibitors of DNA replication and protein expression. Early genes are found at a similar extent in both types of RNAs, whereas bona fide immediately early genes are barely detectable in mRNA from cells infected in the presence of inhibitors of DNA replication (Almazán et al., 1992).

The transcription initiation sites have been determined for approximately 20 ASFV prereplicative genes (Fig. 3). The majority of them (14) belong to the multigenic families, which are thought to be involved in the control of the host response to the infection (Afonso et al., 2004), others are involved in DNA replication (2), or encode proteins with unknown function. In the case of VV, for which the complete transcriptome has been determined, 118 of its proximately 200 genes are expressed before the onset of DNA replication. They encode proteins involved in transcription, DNA replication and in the control of the host response to the infection (Yang et al., 2010).

3.1.1. In vitro transcribed mRNAs

The characterization of the structure and composition of ASFV mRNAs was performed using in vitro transcribed mRNA (Salas et al., 1981). When purified ASFV particles are permeabilized with detergents and incubated with ribonucleotides, they are able to synthesize viral mRNAs with methylated CAP structures at their 5′-ends and poly(A) tails at their 3′-ends.

Among the enzymatic activities identified in the viral particles directly involved in RNA trascription are DNA-dependent RNA polymerase (Kuznar et al., 1980), RNA triphosphatase, guanylyltransferase, (guanine-N7) methyltransferase, (nucleoside-2′-O-)methyltransferase, Poly(A) polymerase and a viral topoisomerase II (Salas et al., 1981, 1983). With the exception of the nucleoside-2′-O methyltransferase, these activities have been assigned to viral genes (Table 1) in base to their sequence similarities to functionally characterized genes.

Additionally, protein kinase (Baylis et al., 1993a; Polatnick et al., 1974; Salas et al., 1988) and nucleic-acid-dependent ATPase activities (Kuznar et al., 1981) have been identified in the viral particles, although the role of these enzymes in transcription remains to be determined.

The in vitro transcribed mRNAs show no internal methylation, and have an average length of their 3′-poly(A) chains of 33 nucleotides. The majority (91%) of the transcripts have cap 1 structures ($m^7G(5')ppp(5')(A/G)^m$), of which 76% have an A as the penultimate nucleotide. However, in a small percentage of the transcripts (8%) the methylation of the first transcribed nucleotide does not require a methylated terminal guanosine, unlike the VV 2′-O-methyltransferase (Barbosa and Moss, 1978; Salas et al., 1981).

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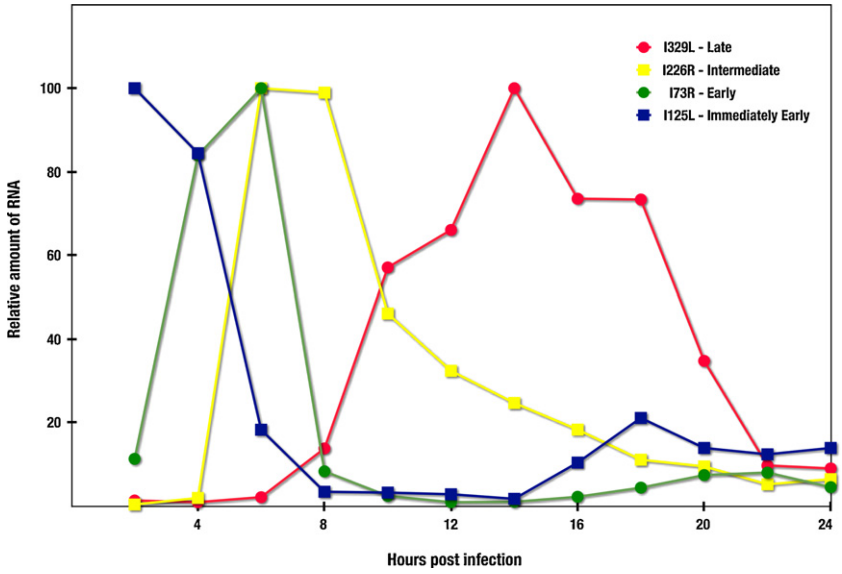

Fig. 2. Accumulation kinetics for immediately early (blue line, gene I215L), early (green line, gene I73R), intermediate (yellow line, intermediate transcripts of gene I226R) and late (red line, gene I329L) transcripts throughout the viral infection. Primer extension assays were used to detect and measure steady state RNA levels for the different transcripts. The quantity of each transcript is plotted as the percentage of the maximum level. Data from Rodríguez (1993) and Rodríguez et al. (1996). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

It is important to note the ribose 2′-O-methylation of the ASFV cap, since this modification has been shown to play an essential role in evading antiviral responses of the cells to some viruses, including the poxviruses (Daffis et al., 2010; Zust et al., 2011). Unfortunately, as mentioned before the gene encoding the 2′-O-methyltransferase of ASFV has no been identified so far.

The in vitro transcribed mRNAs resemble those obtained by infections in the presence of inhibitors of DNA replication, i.e. early and immediately-early genes, mapping to similar regions in the viral genome and yielding translation products of similar size distribution (Salas et al., 1986), suggesting that ASFV initiates early gene transcription immediately upon infection.

3.1.2. Early transcription

ASFV early gene expression in infected cells is detectable using primer extension assays as early as 1 h p.i. (JMR, unpublished results), but in general transcripts are abundant at 2 h post infection (hpi), with a plateau in accumulation at 2–6 hpi (Almazán et al., 1992; Rodríguez, 1993; and Fig. 2) depending on the gene, and a sharp decrease in concentration from 8 to 10 hpi, coincidental with the increase in DNA replication. The fast reduction observed in the abundance of prereplicative transcripts between 8 and 10 h post-infection indicates that they are rapidly degraded. However, a low, but detectable level of prereplicative transcripts has been observed through the late times, and has been attributed to a basal activity of their promoters throughout the postreplicative phase

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          −50        −40        −30        −20        −10         +1
A151R     AGATAAAAAATTTAAGTAAAACAAATTTGAATAAAAAAAATAATAGTTATGATGG
A240L     AAATAGATTTGTAAGAAATGTTAAAAACTGAACTCTTTTTACCCATTTATTAATC
A280R     AAAATTCAATAGATATACATCATTAATATTGATTATATTTTCGAATATTATCTTC
A498R     TAGAAACTCTGTTGAAATGTGAGTAAAATTTATTTTTTTGATCAGAGTAAGAAAT
A505R     TTTTATCATACATTAAAATTCCAGTAAAATTTATATTTTTTTTGGTAAACAAATG
A506R     ATACTTTTTAAAAACCCTCCATAAAAATTTATTTTTTTTTCATAAAAGTAGAGAA
A528R     TTAGGTAGGTTGTGAAAAACAGATTAAACTTAAAATTATGTGTATTATGTAAAAT
A542R     ACATTAGTTCTGTTAAGATAATAAAAATTTATTTTTTTTTCATCAAGGTAGAGAA
CP204L    CATCTTCTTATTATTTTATAATTTTAAAATTGAATGGATTTTATTTTAAATATAT
DP238L    ATATATTTAAAACTAAAAATTGATTTGCAATCAAATTTCATAGAATTGTCACAGC
G1207R    TCCATTATACCCGGTATAGAAAATAAAATTTAAAATAAAAAACGGATGATATCTA
I73R      GCATTTTCATATAAATTTTTTTTTAAAATTTAATACAAAAAAAAGAAGTATACTC
I215L     ATTTTTCCAGTTCATAAATTTAGTAAAAATGAACCCAATAAAAAACAAAAGAGGT
I267L     CCATTTTCATGCAAATATTAAAACAAAAATTTAAAAGTTTTTTTCATTAATGCAT
J268L     CCAAATAATAAAATCTCGATTGTTAAAACTAAAAACCTATTTTTTTTAGTAAAGAC
L270L     AGAAAACTCGTTATTTTTTTTATCAAACTGAATTCTCCTTTCAAGGGTATGGCCC
U104L     TCTATAAAAAGGGCTTTTTTTTCCAAATTTTAACATATTTTTTCGTAATATAACA
V82L      GTAAAACGTTGGAAAAATGTTGGACTCAATCTTAAATCATCATATAAAGAATAGA
Y118L     AAACTATTGAAAAAATGTCGACCGAATCTTAAATGATCTTATGAAAAGTACAGTG
XP124L    AAACACAGGAAAAATGCTGAATCGAATCTTAAATTTTCATATTAAGAGTAGAGGG
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Fig. 3. Alignment of 5′-flanking sequences of prereplicative ASFV genes. Sequences were aligned by their transcription initiation site closest to the ATG of the ORF, which was designated as −1. The positions of the transcription initiation sites are shown in bold and underlined. References are as follows: A151R (JMR, unpublished data), A240L (Yáñez et al., 1993c), A280R, A498R, A505R, A506R, A528R and A542R (Rodriguez et al., 1994), CP204L, DP238L, I73R, I215L and I267L (Rodríguez, 1993), G1207R (Rodríguez et al., 1993b), J268L (Almazán et al., 1995), L270L, U104L, V82L, Y118L and XP124L (Almazán et al., 1992).

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of the infection (Almazán et al., 1992). Finally, the reactivation of the expression very late during the infection (20 hpi) is a common phenotype shared by the majority of the early and immediately early genes that have been characterized in detail (Almazán et al., 1992; Rodríguez, 1993; and Fig. 2). A similar phenomenon of reactivation of early gene expression late during the infection has been described in VV (Garces et al., 1993; Masternak and Wittek, 1996), where it has been regarded as a consequence of the presence of all the machinery necessary for the early transcription and the template DNA during the viral morphogenesis. A similar mechanism is likely to apply to the reactivation observed in ASFV early genes.

The transcriptional mapping of the members of multigene family 110 provided a clear image of the structure of the prereplicative ASFV transcriptional units. The five genes analyzed are organized as five tightly packed non overlapping transcriptional units. Their transcripts have a defined length, initiating at 8–70 bp upstream of the corresponding ORF and terminating at one or more sites located downstream of the termination codon of the ORF, thus indicating the existence of an accurate transcriptional control. In all detected transcripts of these genes, the first AUG codon from the 5′-end coincides with the predicted translational initiation site of the gene. The 3′-ends map within a strictly conserved sequence motif, consisting on a run of seven or more consecutive thymidylate residues (7T), found within the intergenic regions. Four to six thymidylate residues where incorporated in the 3′-end of the sequenced mRNAs followed by a poly(A) tail of 15–54 nucleotides consistent with a model in which the 3′-end of ASFV genes are generated by transcriptional termination in the 7T signal, followed by poly(A) addition (Almazán et al., 1992).

3.1.3. Immediate early transcription

Two genes have been identified as belonging to the immediate early temporal class, L270L, a member of the multigene family 110 (Almazán et al., 1992), and I215L, a protein similar to the ubiquitin conjugating enzymes (Rodríguez et al., 1992; Rodríguez, 1993; Fig. 2). Both genes share the same defining characteristics: they are barely detected when analyzed on mRNA obtained from cells infected with ASFV in the presence of DNA replication inhibitors but are strongly detected when the infection is carried out in the presence of an inhibitor of protein synthesis. Comparison of detailed accumulation kinetics (1 h-interval from 1 to 6 hpi) show that both temporal classes of mRNA accumulate up to 3 hpi, when, in cells untreated and in cells treated with an inhibitor of DNA replication, the synthesis of immediate early transcripts is silenced. The silencing was completely prevented when the infected cells were incubated with an inhibitor of protein synthesis (Almazán et al., 1992; JMR, unpublished results). Although there is no evidence about the mechanism of control of these immediate early genes, it has been suggested that they may require the specific environment provided by the subvirion structure in which the virus DNA is enclosed during the initial steps of infection. Thus, silencing of these genes would be coincidental with disruption of the virus cores (Almazán et al., 1992). Comparative 2D gel analysis of the polypeptide products obtained after in vitro translation of mRNAs obtained from infected cells treated with inhibitors of protein synthesis or DNA replication showed that at least three virus-induced polypeptides are encoded by genes that can be classified as part of a subset of the early gene class whose transcription is abrogated before the onset of DNA replication.

A subclass of early proteins that are expressed only transiently prior to DNA synthesis has been previously described (Carvalho and Rodrigues-Pousada, 1986; Esteves et al., 1986; Salas et al., 1986; Santarén and Viñuela, 1986; Urzainqui et al., 1987). By 2D gel analysis, up to 30 polypeptides displaying these characteristics were identified of a total of 100 virus induced polypeptides in macrophages infected with the BA71 virulent isolate, whereas 25 out of a total of 129 were found in Vero cells infected with the attenuated BA71V strain (Rodríguez et al., 2001). Although these results could not be directly extrapolated to the number of genes belonging to this temporal class, it does suggest that this temporal class of genes could be more abundant that the previous data suggested.

It is also worth mentioning that the definition of immediate early genes used in ASFV is different from the "classical" definition used in other DNA viruses like baculovirus (Ross and Guarino, 1997) or herpesvirus (Honess and Roizman, 1975). There, it is applied to a subset of genes that are expressed in the presence of protein synthesis inhibitors, and whose translation is needed for the expression of the early genes. Thus far, all the studied prereplicative ASFV genes are expressed in the presence of proteins synthesis inhibitors, thus all could be classified as immediately early according to the "classical" definition.

In VV, Assarsson et al. (2008) have defined a "kinetic" class of immediately early genes that differs from early genes because of its preferential expression before them. A similar result was found during the analysis of the complete VV transcriptome (Yang et al., 2010). Two subclusters of prereplicative genes were identified by their differences on the timing and the intensity of expression. Thus, genes classified in the subcluster E1.1 were expressed earlier and at higher levels that those of E1.2. However, the genes present in both clusters are expressed in the presence of inhibitors of DNA replication and protein synthesis. Thus, as it occurs in ASFV, all the prereplicative genes of VV are immediate early genes in the "classical" sense. Interestingly, Yang et al. (2010) detected transcripts that were more abundant on the RNA obtained in the presence of inhibitors of protein synthesis than on the RNA obtained in the presence of inhibitors of DNA replication, thus matching the characteristics used to define the immediate early genes in ASFV. However, this class of genes was not further characterized, and its particular accumulation kinetics was attributed to a particular sensitivity of these transcripts to the degradation induced by early protein(s) encoded by the virus, such as the VV early decapping enzyme D9 (Parrish and Moss, 2007). Since ASFV encodes a protein with a similar activity, pD250L (g5R; Parrish et al., 2009), a similar explanation could also be applied to the kinetic observed for the immediate early genes of ASFV.

3.2. Postreplicative phase of gene expression

Experimentally, ASFV post replicative genes are defined by its dependence on the DNA replication for its expression, thus they are not detected when cells are infected in the presence of inhibitors of DNA replication. Intermediate genes are experimentally differentiated from bona-fide late genes by its expression in cells infected by ASFV in the presence of inhibitors of DNA replication when the intermediate gene is expressed from a transfected plasmid (Vos and Stunnenberg, 1988; Rodríguez et al., 1996).

The structure of the ASFV postreplicative transcriptional units is very similar to that seen previously for the prereplicative transcriptional units. They are found tightly packed in the genome with very short intergenic regions. Transcripts initiate from these units at very precise sites and terminate in definite positions giving rise to mRNAs of precise length. This is in acute contrast with the structure of the postreplicative transcripts of poxvirus. Poxviral intermediate and late transcripts have in common the presence of poly(A) leaders on their 5′ ends, considered to be the result of RNA polymerase slippage (Yang et al., 2012; Baldick and Moss, 1993; de Magistris and Stunnenberg, 1988), and an imprecise transcript termination, since the early termination motif is not recognized despite its frequent presence. In consequence, postreplicative mRNAs in poxvirus are heterogeneous in length,

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overlap adjacent ORFs (Cooper et al., 1981; Mahr and Roberts, 1984) and cover essentially every nucleotide of the genome (Yang et al., 2010, 2011).

3.2.1. Intermediate transcription

ASFV intermediate class of genes was defined by its similitude with that of poxvirus (Keck et al., 1990; Rodríguez et al., 1996; Vos and Stunnenberg, 1988). Intermediate genes are a very distinct class of genes shared by poxvirus and ASFV that are expressed immediately after DNA replication and before the onset of late gene transcription. Intermediate transcription is dependent on transacting 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.

Two ASFV genes, I226R and I243L, have transcription initiation sites that belong to the intermediate temporal class of ASFV mRNAs. Both genes possess complex promoter elements that drive the expression of transcripts during different temporal stages of transcription. Each temporal class initiates at different initiation sites. Thus, I226R gene transcription is driven by two different temporal control elements giving rise to intermediate and late mRNAs whereas I243L transcription produces three clearly distinguishable temporal species of mRNA with the characteristics of those transcribed from early, intermediate, and late genes (Rodríguez et al., 1996).

Two kinds of experiments have been used to characterize the ASFV intermediate transcripts: (i) detailed accumulation kinetics. The two ASFV intermediate mRNAs showed distinctive accumulation kinetics (Fig. 2): they are first detected at 4–6 h post infection, coincident with maximum expression of early genes, reached maximum levels of accumulation at 6–8 h post infection, and decreased sharply at later times. The beginning of late gene expression is coincident with the maximum levels of intermediate mRNAs. (ii) Expression from transfected plasmids in the presence of DNA replication inhibitors. As has been shown in VV, intermediate genes require of DNA replication for their expression although the intermediate transcription factors are present in the infected cell before DNA replication. This has been explained as a result of the inability of the newly synthesized transcription factors to reach the genome within the infective particle, and it is consistent with the fact that DNA isolated from viral particles, or plasmids containing intermediate genes, can serve as templates for intermediate transcription when transfected to infected cells in the presence of DNA replication inhibitors (Keck et al., 1990; Vos and Stunnenberg, 1988). When this kind of experiment is performed with ASFV genes I226R and I243L, the plasmid-borne intermediate genes are expressed, whereas the copies of the same genes within the viral genome are repressed (Rodríguez et al., 1996).

Initially it was thought that intermediate genes encoded mainly transcription factors necessary for the synthesis of late genes, and in ASFV one of the two intermediate genes, I243L, is homologous to the transcription elongation factor TFIIS. However, in a recent genome-wide characterization of the postreplicative VV genes, 53 out of the 91 postreplicative genes were classified as intermediate, a number higher than expected, although some of the intermediate genes continued to be expressed at late times. In addition to late transcription factors, the intermediate genes encode proteins involved in genome packaging and core formation (Yang et al., 2011).

3.2.2. Late transcription

Late gene expression initiates at 6–8 hpi when expression of intermediate transcripts is maximal and reaches a maximum at 12–16 hpi (Almazán et al., 1993; Rodríguez, 1993), decreasing slowly thereafter (Fig. 2).

The transcriptional mapping of the 3′ termination of the late gene O61R indicates that this class of genes also terminates at polythymidylate tracks (Almazán et al., 1992). In general, for all the transcriptional classes of genes found in ASFV, when transcription initiation data are available, and the total size of the transcripts is known by northern blot experiments, there is a strong correlation between the presence of runs of 7T in the genome sequence and the putative positions of transcript termination (Alejo et al., 1997; Galindo et al., 2000b; García et al., 1995; Rodríguez et al., 1993a,b, 1994, 1996). Thus, although a formal proof has not been yet published, the 7T motif is considered to be the termination signal for all the temporal classes of ASFV transcripts. The recognition of the first available 7T signal is not absolute; thus, transcripts with identical initiation sites can have different 3′-ends that map to alternative 7T motifs.

Remarkably, this 7T motif would act as a termination signal of early genes if inserted within the context of a poxvirus genome. Transcription of VV early genes terminates heterogeneously about 30–50 nt downstream in response to the sequence TTTTTNT (where N is any nucleotide) on the non-template strand of the DNA (Yuen and Moss, 1987). The termination signal is actually sensed in the form of the sequence UUUUUNU in the nascent RNA (Shuman and Moss, 1988). To induce termination and transcript release, the VV RNA polymerase requires the VV termination factor (VTF), nucleoside triphosphate phosphohydrolase I (NPH I, encoded in gene D11L) and ATP (Deng and Shuman, 1998). VTF is a heterodimer of the products of the genes D1R and D12L that also acts as the viral capping enzyme (Shuman and Moss, 1988). ASFV encodes genes similar to the D1R subunit of the VTF and to the NPHI (NP868R and D1133L, respectively). Although the similarity of the recognition sequence and the presence of similar proteins in the genome points to a common evolutionary origin for the machinery of transcriptional termination, the 3′-ends of ASFV transcripts fall within the 7T sequence, instead of 30–50 nt downstream, as the poxviral 3′-ends, and no gene similar to the VV small subunit of the capping enzyme/VTF, D12L, has been identified in the ASFV genome, thus indicating the existence of significant differences between the mechanisms of 3′-end formation used by the two virus groups.

4. Promoter sequences

Very little is known about the cis-acting elements controlling ASFV gene transcription. All classes of temporal ASFV genes have in common that they are tightly regulated independent transcription units whose mRNA start sites are located at a short distance from the corresponding translation initiation codon. The comparison of promoter sequences is complicated by the fact that most of the classes of genes have not been subject to a thorough analysis. Thus, it is possible that some genes classified to the late class could possess intermediate promoters.

4.1. Promoter sequence of the prereplicative genes

Figs. 3 and 4 show an alignment of the sequences from −50 to +5 relative to the transcription start site (−1) closest to the initiation codon of the ORF for the majority of the genes whose transcription initiation site has been mapped. An analysis of the base composition of these regions shows that they are rich in A-T sequences. Thus, the average A-T content found is significantly higher (78% for the prereplicative genes, and 80% for the late genes) than the mean content of the BA71V genome (61%).

Except for that compositional bias, the sequences in 5′ of the prereplicative genes do not show any apparent sequence similarity.

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Fig. 4. Alignment of 5′-flanking sequences of late ASFV genes. Sequences were aligned by their transcription initiation site closest to the ATG of the ORF, which was designated as −1. The sequences of the minimal promoter and the essential −18 to −14 region of gene B646L are boxed and highlighted in orange, respectively (García-Escudero and Viñuela, 2000). TATA sequences close to the transcription initiation sites are highlighted in blue. The positions of the transcription initiation sites are shown in bold and underlined. References are as follows: B646L, A137R (García-Escudero and Viñuela, 2000), A224L (Chacón et al., 1995), A489R (Rodriguez et al., 1994), B318L (Alejo et al., 1997), B438L (Galindo et al., 2000b), C129R, K78R (JMR unpublished data), CP530R, CP2745L (Simón-Mateo, 1993), D117L (Simón-Mateo et al., 1995), E165R (Oliveros et al., 1999), E183L (Rodriguez et al., 1994), EP153R (Galindo et al., 2000a), EP402R (Rodríguez et al., 1993a), I177L, I196L, I329L (Rodríguez, 1993), J154R (Almazán et al., 1995), L83L (Alejo, 1999), O61R (Almazán et al., 1993), S273R (Andrés et al., 2001).

4.2. Promoter sequence of the postreplicative genes

An alignment of the two characterized genes with intermediate transcripts show highly conserved sequences at positions −25 to −15 and −9 to +9 relative to the translational start codon (Rodríguez et al., 1996). However, these promoters are very complex since they drive the expression of two (I226R) and three (I243L) temporal classes of transcripts, making impossible to draw any conclusion from this small set of complex samples about the nature of the cis acting control elements of the intermediate genes.

A detailed characterization of the promoter element for the late gene B646L, encoding the major capsid protein p72, has been published (García-Escudero and Viñuela, 2000). This late gene utilizes a promoter region contained within positions –39 to +2 relative to the transcription initiation site closest to the translation start codon (Fig. 4). The results of the analysis of deletions, linker scan substitutions, and point mutations in this promoter defined two regions essential for activity. One region is located at positions −18 to −14 (5′-TATTT) and the second is located at positions −4 to +2 (5′-TATATA). Although there is no evident nucleotide conservation in the region from −18 to −14 among the sequences of the late genes whose transcription initiation is known (Fig. 4), the replacement of these nucleotides by a GCGCG sequence abolished the promoter activity. Whether this effect is a consequence of the deletion of some essential sequence motif is unknown. The second essential region of the p72 promoter, localized at positions −4 to +2, includes sites for mRNA initiation. This region around the transcription initiation site of late promoters shows some degree of similarity; thus, TATA sequences are present close to the transcription initiation site in 19 out of the 22 sequences shown in Fig. 4. Single-nucleotide substitutions at this region in the B646L promoter indicate that position -1 is notable for its intolerance to any deviation from the wild-type sequence. Changes at nucleotides +1 and +2 reduced the activity of the promoter about threefold, while only a small effect was observed when positions −2 to −4 were replaced. Thus, the TATA sequence located at −2 to +2 appears to be critical for B646L promoter activity. Importantly, the replacement, by GCGC, of the equivalent TATA sequence on the late genes K78R, EP402R and A137R was also deleterious for activity, suggesting that the TATA sequence could be a motif for late promoter function.

The bipartite structure of the late ASFV promoter is similar to that of the VV late and intermediate promoters that contain a core and an initiator region. The TAAAT motif in the initiator region, usually followed by a G, is a signature of VV late promoters, and the pure intermediate promoters invariably contain TAAA upstream of the ORF (Yang et al., 2012; Baldick et al., 1992; Hänggi et al., 1986). There are no experimental data regarding the identity of the ASFV transcription factors that regulate the expression of viral late genes. However, the similarity of some ASFV proteins with transcription factors of VV suggests that B175L (A1L, VLTF-2, Keck et al., 1990) and B385R (A2L, VLTF-3, Keck et al., 1990) are likely candidates to play a role in the transcription of ASFV late genes.

Interestingly, ASFV promoter regions can be recognized, and ASFV genes expressed, in the context of VV infection, suggesting some degree of functionality for the ASFV promoter sequences in VV transcription (Hammond and Dixon, 1991). However, it has been recently shown that there is a widespread initiation during VV postreplicative transcription, and that almost 25% of the AAA sequences are used as transcription initiation sites (Yang et al., 2012). The special richness on AT sequences of the promoter regions of ASFV genes hampers the interpretation of these findings. More experimental data are necessary to ascertain whether there are functional similarities between ASFV and VV cis-acting sequences, despite a lack of obvious sequence similarity, or if the compositional bias along with the pervasive VV postreplicative initiation are a simpler explanation for these results.

5. Concluding remarks

There is experimental evidence that suggests the presence of a brief nuclear phase at the beginning of the ASFV infection. Thus, ASFV DNA has been detected by in situ hybridization in the nucleus of infected cells, and the virus is incapable of replicating in enucleated cells (Ballester et al., 2011; Garcia-Beato et al., 1992a,b;

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Ortin and Viñuela, 1977; Rojo et al., 1999). The beginning of viral replication in the nucleus would have a profound effect in our understanding of the prereplicative and intermediate phases of transcription. However, the lack of information regarding the coupling of transcription with the putative nuclear phase makes it difficult to merge the transcriptional data with the nuclear phase hypothesis in a coherent model. Both the genetic resources and the transcriptional strategies are very similar between ASFV and poxvirus, which argues in favor of a purely cytoplasmic model for the transcription of ASFV. Further experimental data should solve this apparent contradiction.

The similarities observed in the transcriptional strategies reinforce the genetic data indicating a close relationship between poxviruses and ASFV that point to a common evolutionary ancestor for both viral families. The differences observed, specially the different degree of control in the postreplicative gene transcription, suggest that the lax control of poxvirus over initiation and termination gives them an evolutionary advantage over the strict control displayed by ASFV.

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References

Afonso, C.L., Piccone, M.E., Zaffuto, K.M., Neilan, J., Kutish, G.F., Lu, Z., Balinsky, C.A., Gibb, T.R., Bean, T.J., Zsak, L., Rock, D.L., 2004. African swine fever virus multigene family 360 and 530 genes affect host interferon response. Journal of Virology 78, 1858–1864.

Ahn, B.Y., Gershon, P.D., Jones, E.V., Moss, B., 1990. Identification of rpo30, a vaccinia virus RNA polymerase gene with structural similarity to a eucaryotic transcription elongation factor. Molecular and Cellular Biology 10, 5433–5441.

Alejo, A., 1999. Ph.D. Thesis. Universidad Autónoma de Madrid, Madrid, Spain.

Alejo, A., Yáñez, R.J., Rodríguez, J.M., Viñuela, E., Salas, M.L., 1997. African swine fever virus trans-prenyltransferase. Journal of Biological Chemistry 272, 9417–9423.

Almazán, F., Rodríguez, J.M., Andrés, G., Pérez, R., Viñuela, E., Rodríguez, J.F., 1992. Transcriptional analysis of multigene family 110 of African swine fever virus. Journal of Virology 66, 6655–6667.

Almazán, F., Rodríguez, J.M., Angulo, A., Viñuela, E., Rodriguez, J.F., 1993. Transcriptional mapping of a late gene coding for the p12 attachment protein of African swine fever virus. Journal of Virology 67, 553–556.

Almazán, F., Murguía, J.R., Rodríguez, J.M., la Vega, de, I., Viñuela, E., 1995. A set of African swine fever virus tandem repeats shares similarities with SAR-like sequences. Journal of General Virology 76, 729–740.

Amitsur, M., Levitz, R., Kaufmann, G., 1987. Bacteriophage T4 anticodon nuclease, polynucleotide kinase and RNA ligase reprocess the host lysine tRNA. EMBO Journal 6, 2499–2503.

Anantharaman, V., Aravind, L., 2004. The SHS2 module is a common structural theme in functionally diverse protein groups, like Rpb7p FtsA, GyrI, and MTH1598/TM1083 superfamilies. Proteins 56, 795–807.

Anderson, E.C., Hutchings, G.H., Mukarati, N., Wilkinson, P.J., 1998. African swine fever virus infection of the bushpig (*Potamochoerus porcus*) and its significance in the epidemiology of the disease. Veterinary Microbiology 62, 1–15.

Andrés, G., Alejo, A., Simón-Mateo, C., Salas, M.L., 2001. African swine fever virus protease, a new viral member of the SUMO-1-specific protease family. Journal of Biological Chemistry 276, 780–787.

Aravind, L., Koonin, E.V., 1999. DNA polymerase beta-like nucleotidyltransferase superfamily: identification of three new families, classification and evolutionary history. Nucleic Acids Research 27, 1609–1618.

Assarsson, E., Greenbaum, J.A., Sundström, M., Schaffer, L., Hammond, J.A., Pasquetto, V., Oseroff, C., Hendrickson, R.C., Lefkowitz, E.J., Tscharke, D.C., Sidney, J., Grey, H.M., Head, S.R., Peters, B., Sette, A., 2008. Kinetic analysis of a complete poxvirus transcriptome reveals an immediate-early class of genes. Proceedings of the National Academy of Sciences 105, 2140–2145.

Baldick, C.J., Moss, B., 1993. Characterization and temporal regulation of mRNAs encoded by vaccinia virus intermediate-stage genes. Journal of Virology 67, 3515–3527.

Baldick, C.J., Keck, J.G., Moss, B., 1992. Mutational analysis of the core, spacer and initiator regions of vaccinia virus intermediate class promoters. Journal of Virology 66, 4710–4719.

Ballester, M., Rodriguez-Carino, C., Perez, M., Gallardo, C., Rodríguez, J.M., Salas, M.L., Rodriguez, F., 2011. Disruption of nuclear organization during the initial phase of African swine fever virus infection. Journal of Virology 85, 8263–8269.

Barbosa, E., Moss, B., 1978. mRNA(nucleoside-2′-)-methyltransferase from vaccinia virus. Characteristics and substrate specificity. Journal of Biological Chemistry 253, 7698–7702.

Baylis, S.A., Dixon, L.K., Vydelingum, S., Smith, G.L., 1992. African swine fever virus encodes a gene with extensive homology to type II DNA topoisomerases. Journal of Molecular Biology 228, 1003–1010.

Baylis, S.A., Banham, A.H., Vydelingum, S., Dixon, L.K., Smith, G.L., 1993a. African swine fever virus encodes a serine protein kinase, which is packaged into virions. Journal of Virology 67, 4549–4556.

Baylis, S.A., Twigg, S.R., Vydelingum, S., Dixon, L.K., Smith, G.L., 1993b. Three African swine fever virus genes encoding proteins with homology to putative helicases of vaccinia virus. Journal of General Virology 74, 1969–1974.

Bayliss, C.D., Condit, R.C., 1995. The vaccinia virus A18R gene product is a DNA-dependent ATPase. Journal of Biological Chemistry 270, 1550–1556.

Bayliss, C.D., Smith, G.L., 1996. Vaccinia virion protein I8R has both DNA and RNA helicase activities: implications for vaccinia virus transcription. Journal of Virology 70, 794–800.

Bell, S.D., Magill, C.P., Jackson, S.P., 2001. Basal and regulated transcription in Archaea. Biochemical Society Transactions 29, 392.

Benarroch, D., Smith, P., Shuman, S., 2008. Characterization of a trifunctional mimivirus mRNA capping enzyme and crystal structure of the RNA triphosphatase domain. Structure 16, 501–512.

Bessman, M.J., Frick, D.N., O′Handley, S.F., 1996. The MutT proteins or "Nudix" hydrolases, a family of versatile, widely distributed, "housecleaning" enzymes. Journal of Biological Chemistry 271, 25059.

Blanga-Kanfi, S., 2006. PrrC-anticodon nuclease: functional organization of a prototypical bacterial restriction RNase. Nucleic Acids Research 34, 3209–3219.

Boinas, F.S., Wilson, A.J., Hutchings, G.H., Martins, C., Dixon, L.J., 2011. The persistence of African swine fever virus in field-infected *Ornithodoros erraticus* during the asf endemic period in Portugal. PLoS One 6, e20383.

Broyles, S.S., 2003. Vaccinia virus transcription. Journal of General Virology 84, 2293–2303.

Broyles, S.S., Fesler, B.S., 1990. Vaccinia virus gene encoding a component of the viral early transcription factor. Journal of Virology 64, 1523–1529.

Broyles, S.S., Moss, B., 1986. Homology between RNA polymerases of poxviruses, prokaryotes, and eukaryotes: nucleotide sequence and transcriptional analysis of vaccinia virus genes encoding 147-kDa and 22-kDa subunits. Proceedings of the National Academy of Sciences of the United States of America 83, 3141–3145.

Broyles, S.S., Li, J., Moss, B., 1991. Promoter DNA contacts made by the vaccinia virus early transcription factor. Journal of Biological Chemistry 266, 15539–15544.

Broyles, S.S., Liu, X., Zhu, M., Kremer, M., 1999. Transcription factor YY1 is a vaccinia virus late promoter activator. Journal of Biological Chemistry 274, 35662–35667.

Cartwright, J.L., Safrany, S.T., Dixon, L.K., Darzynkiewicz, E., Stepinski, J., Burke, R., McLennan, A.G., 2002. The g5R (D250) gene of African swine fever virus encodes a Nudix hydrolase that preferentially degrades diphosphoinositol polyphosphates. Journal of Virology 76, 1415–1421.

Carvalho, Z.G., Rodrigues-Pousada, C., 1986. African swine fever virus gene expression in infected Vero cells. Journal of General Virology 67 (Pt 7), 1343–1350.

Cassetti, M.A., Moss, B., 1996. Interaction of the 82-kDa subunit of the vaccinia virus early transcription factor heterodimer with the promoter core sequence directs downstream DNA binding of the 70-kDa subunit. Proceedings of the National Academy of Sciences of the United States of America 93, 7540–7545.

Chacón, M.R., Almazán, F., Nogal, M.L., Viñuela, E., Rodriguez, J.F., 1995. The African swine fever virus IAP homolog is a late structural polypeptide. Virology 214, 670–674.

Chapman, D.A.G., Tcherepanov, V., Upton, C., Dixon, L.K., 2008. Comparison of the genome sequences of non-pathogenic and pathogenic African swine fever virus isolates. Journal of General Virology 89, 397–408.

Choder, M., 2004. Rpb4 and Rpb7: subunits of RNA polymerase II and beyond. Trends in Biochemical Sciences 29, 674–681.

Christen, L.M., Sanders, M., Wiler, C., Niles, E.G., 1998. Vaccinia virus nucleoside triphosphate phosphohydrolase I is an essential viral early gene transcription termination factor. Virology 245, 360–371.

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.

Daffis, S., Szretter, K.J., Schriewer, J., Li, J., Youn, S., Errett, J., Lin, T.-Y., Schneller, S., Zust, R., Dong, H., Thiel, V., Sen, G.C., Fensterl, V., Klimstra, W.B., Pierson, T.C., Buller, R.M., Gale, M., Shi, P.-Y., Diamond, M.S., 2010. 2′-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature 468, 452–456.

de Magistris, L., Stunnenberg, H.G., 1988. Cis-acting sequences affecting the length of the poly(A) head of vaccinia virus late transcripts. Nucleic Acids Research 16, 3141–3156.

Deng, L., Shuman, S., 1998. Vaccinia NPH-I, a DExH-box ATPase, is the energy coupling factor for mRNA transcription termination. Genes and Development 12, 538–546.

Dixon, L.K., Twigg, S.R., Baylis, S.A., Vydelingum, S., Bristow, C., Hammond, J.M., Smith, G.L., 1994. Nucleotide sequence of a 55 kbp region from the right end of the genome of a pathogenic African swine fever virus isolate (Malawi LIL20/1). Journal of General Virology 75, 1655–1684.

Dixon, L.K., Alonso, C., Escribano, J.M., Martins, C., Revilla, Y., Salas, M.L., Takamatsu, H., 2012. The *Asfarviridae*. In: King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. (Eds.), Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, pp. 153–162.

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Esteves, A., Marques, M.I., Costa, J.V., 1986. Two-dimensional analysis of African swine fever virus proteins and proteins induced in infected cells. Virology 152, 192–206.
Fairman-Williams, M.E., Guenther, U.-P., Jankowsky, E., 2010. SF1 and SF2 helicases: family matters. Current Opinion in Structural Biology 20, 313–324.
Falkowski, P.G., Katz, M.E., Knoll, A.H., Quigg, A., Raven, J.A., Schofield, O., Taylor, F.J.R., 2004. The evolution of modern eukaryotic phytoplankton. Science 305, 354–360.
Filée, J., Pouget, N., Chandler, M., 2008. Phylogenetic evidence for extensive lateral acquisition of cellular genes by nucleocytoplasmic large DNA viruses. BMC Evolutionary Biology 8, 320.
Gadal, O., Shpakovski, G.V., Thuriaux, P., 1999. Mutants in ABC10beta, a conserved subunit shared by all three yeast RNA polymerases, specifically affect RNA polymerase I assembly. Journal of Biological Chemistry 274, 8421–8427.
Galindo, I., Almazan, F., Bustos, M.J., Viñuela, E., Carrascosa, A.L., 2000a. African swine fever virus EP153R open reading frame encodes a glycoprotein involved in the hemadsorption of infected cells. Virology 266, 340–351.
Galindo, I., Viñuela, E., Carrascosa, A.L., 2000b. Characterization of the African swine fever virus protein p49: a new late structural polypeptide. Journal of General Virology 81, 59–65.
Garces, J., Masternak, K., Kunz, B., Wittek, R., 1993. Reactivation of transcription from a vaccinia virus early promoter late in infection. Journal of Virology 67, 5394–5401.
García, R., Almaz n, F., Rodríguez, J.M., Alonso, M., Viñuela, E., Rodriguez, J.F., 1995. Vectors for the genetic manipulation of African swine fever virus. Journal of Biotechnology 40, 121–131.
Garcia-Beato, R., Salas, M.L., Viñuela, E., Salas, J., 1992a. Role of the host cell nucleus in the replication of African swine fever virus DNA. Virology 188, 637–649.
Garcia-Beato, R., Freije, J.M., López-Otín, C., Blasco, R., Viñuela, E., Salas, M.L., 1992b. A gene homologous to topoisomerase II in African swine fever virus. Virology 188, 938–947.
García-Escudero, R., Viñuela, E., 2000. Structure of African swine fever virus late promoters: requirement of a TATA sequence at the initiation region. Journal of Virology 74, 8176–8182.
Gershon, P.D., Moss, B., 1990. Early transcription factor subunits are encoded by vaccinia virus late genes. Proceedings of the National Academy of Sciences of the United States of America 87, 4401–4405.
González, A., Talavera, A., Almendral, J.M., Viñuela, E., 1986. Hairpin loop structure of African swine fever virus DNA. Nucleic Acids Research 14, 6835–6844.
Hammond, J.M., Dixon, L.K., 1991. Vaccinia virus-mediated expression of African swine fever virus genes. Virology 181, 778–782.
Hänggi, M., Bannwarth, W., Stunnenberg, H.G., 1986. Conserved TAAAT motif in vaccinia virus late promoters: overlapping TATA box and site of transcription initiation. EMBO Journal 5, 1071–1076.
Harel-Sharvit, L., Eldad, N., Haimovich, G., Barkai, O., Duek, L., Choder, M., 2010. RNA polymerase II subunits link transcription and mrna decay to translation. Cell 143, 552–563.
Honess, R.W., Roizman, B., 1975. Regulation of herpesvirus macromolecular synthesis: sequential transition of polypeptide synthesis requires functional viral polypeptides. Proceedings of the National Academy of Sciences of the United States of America 72, 1276–1280.
Hooda-Dhingra, U., Thompson, C.L., Condit, R.C., 1989. Detailed phenotypic characterization of five temperature-sensitive mutants in the 22- and 147-kilodalton subunits of vaccinia virus DNA-dependent RNA polymerase. Journal of Virology 63, 714–729.
Ishiguro, A., Nogi, Y., Hisatake, K., Muramatsu, M., Ishihama, A., 2000. The Rpb6 subunit of fission yeast RNA polymerase II is a contact target of the transcription elongation factor TFIIS. Molecular and Cellular Biology 20, 1263–1270.
Iyer, L.M., Aravind, L., Koonin, E.V., 2001. Common origin of four diverse families of large eukaryotic DNA viruses. Journal of Virology 75, 11720–11734.
Iyer, L.M., Balaji, S., Koonin, E.V., Aravind, L., 2006. Evolutionary genomics of nucleocytoplasmic large DNA viruses. Virus Research 117, 156–184.
Keck, J.G., Baldick, C.J., Moss, B., 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.
Kim, B., Nesvizhskii, A.I., Rani, P.G., Hahn, S., Aebersold, R., Ranish, J.A., 2007. The transcription elongation factor TFIIS is a component of RNA polymerase II preinitiation complexes. Proceedings of the National Academy of Sciences of the United States of America 104, 16068–16073.
Kleiboeker, S.B., Burrage, T.G., Scoles, G.A., Fish, D., Rock, D.L., 1998. African swine fever virus infection in the argasid host *Ornithodoros porcinus porcinus*. Journal of Virology 72, 1711–1724.
Koonin, E.V., 1993. A highly conserved sequence motif defining the family of MutT-related proteins from eubacteria, eukaryotes and viruses. Nucleic Acids Research 21, 4847.
Koonin, E.V., Yutin, N., 2010. Origin and evolution of eukaryotic large nucleocytoplasmic DNA viruses. Intervirology 53, 284–292.
Kuznar, J., Salas, M.L., Viñuela, E., 1980. DNA-dependent RNA polymerase in African swine fever virus. Virology 101, 169–175.
Kuznar, J., Salas, M.L., Viñuela, E., 1981. Nucleoside triphosphate phosphohydrolase activities in African swine fever virus. Archives of Virology 69, 307–310.
Lackner, C.A., Condit, R.C., 2000. Vaccinia virus gene A18R DNA helicase is a transcript release factor. Journal of Biological Chemistry 275, 1485–1494.
Lee, T.I., Young, R.A., 2000. Transcription of eukaryotic protein-coding genes. Annual Review of Genetics 34, 77–137.
Li, J., Broyles, S.S., 1993. The DNA-dependent ATPase activity of vaccinia virus early gene transcription factor is essential for its transcription activation function. Journal of Biological Chemistry 268, 20016–20021.
Lotan, R., Goler-Baron, V., Duek, L., Haimovich, G., Choder, M., 2007. The Rpb7p subunit of yeast RNA polymerase II plays roles in the two major cytoplasmic mRNA decay mechanisms. Journal of Cell Biology 178, 1133–1143.
Lu, Z., Kutish, G.F., Sussman, M.D., Rock, D.L., 1993. An African swine fever virus gene with a similarity to eukaryotic RNA polymerase subunit 6. Nucleic Acids Research 21, 2940.
Mackereth, C.D., Arrowsmith, C.H., Edwards, A.M., McIntosh, L.P., 2000. Zinc-bundle structure of the essential RNA polymerase subunit RPB10 from *Methanobacterium thermoautotrophicum*. Proceedings of the National Academy of Sciences of the United States of America 97, 6316–6321.
Mahr, A., Roberts, B.E., 1984. Arrangement of late RNAs transcribed from a 7.1-kilobase EcoRI vaccinia virus DNA fragment. Journal of Virology 49, 510–520.
Marchler-Bauer, A., Lu, S., Anderson, J.B., Chitsaz, F., Derbyshire, M.K., DeWeese-Scott, C., Fong, J.H., Geer, L.Y., Geer, R.C., Gonzales, N.R., Gwadz, M., Hurwitz, D.I., Jackson, J.D., Ke, Z., Lanczycki, C.J., Lu, F., Marchler, G.H., Mullokandov, M., Omelchenko, M.V., Robertson, C.L., Song, J.S., Thanki, N., Yamashita, R.A., Zhang, D., Zhang, N., Zheng, C., Bryant, S.H., 2011. CDD: a conserved domain database for the functional annotation of proteins. Nucleic Acids Research 39, D225–D229.
Martins, A., Shuman, S., 2004. Characterization of a baculovirus enzyme with RNA ligase, polynucleotide 5′-kinase, and polynucleotide 3′-phosphatase activities. Journal of Biological Chemistry 279, 18220–18231.
Masternak, K., Wittek, R., 1996. Cis- and trans-acting elements involved in reactivation of vaccinia virus early transcription. Journal of Virology 70, 8737–8746.
McLennan, A.G., 2006. The nudix hydrolase superfamily. Cellular and Molecular Life Sciences 63, 123–143.
Minakhin, L., Bhagat, S., Brunning, A., Campbell, E.A., Darst, S.A., Ebright, R.H., Severinov, K., 2001. Bacterial RNA polymerase subunit omega and eukaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly. Proceedings of the National Academy of Sciences of the United States of America 98, 892–897.
Monier, A., Claverie, J.-M., Ogata, H., 2008. Taxonomic distribution of large DNA viruses in the sea. Genome Biology 9, R106.
Moreira, D., Brochier-Armanet, C., 2008. Giant viruses, giant chimeras: the multiple evolutionary histories of mimivirus genes. BMC Evolutionary Biology 8, 12.
Nagasaki, K., Tomaru, Y., Tarutani, K., Katanozaka, N., Yamanaka, S., Tanabe, H., Yamaguchi, M., 2003. Growth characteristics and intraspecies host specificity of a large virus infecting the dinoflagellate *Heterocapsa circularisquama*. Applied and Environmental Microbiology 69, 2580–2586.
Nikolov, D.B., Chen, H., Halay, E.D., Usheva, A.A., Hisatake, K., Lee, D.K., Roeder, R.G., Burley, S.K., 1995. Crystal structure of a TFIIB–TBP–TATA-element ternary complex. Nature 377, 119–128.
Ogata, H., Toyoda, K., Tomaru, Y., Nakayama, N., Shirai, Y., Claverie, J.-M., Nagasaki, K., 2009. Remarkable sequence similarity between the dinoflagellate-infecting marine girus and the terrestrial pathogen African swine fever virus. Journal of Virology 6, 178.
Oliveros, M., García-Escudero, R., Alejo, A., Viñuela, E., Salas, M.L., Salas, J., 1999. African swine fever virus dUTPase is a highly specific enzyme required for efficient replication in swine macrophages. Journal of Virology 73, 8934–8943.
Ortin, J., Viñuela, E., 1977. Requirement of cell nucleus for African swine fever virus replication in Vero cells. Journal of Virology 21, 902–905.
Parrish, S., Moss, B., 2006. Characterization of a vaccinia virus mutant with a deletion of the D10R gene encoding a putative negative regulator of gene expression. Journal of Virology 80, 553–561.
Parrish, S., Moss, B., 2007. Characterization of a second vaccinia virus mRNA-decapping enzyme conserved in poxviruses. Journal of Virology 81, 12973–12978.
Parrish, S., Hurchalla, M., Liu, S.-W., Moss, B., 2009. The African swine fever virus g5R protein possesses mRNA decapping activity. Virology 393, 177–182.
Parvin, J.D., Sharp, P.A., 1993. DNA topology and a minimal set of basal factors for transcription by RNA polymerase II. Cell 73, 533–540.
Pena, L., Yáñez, R.J., Revilla, Y., Viñuela, E., Salas, M.L., 1993. African swine fever virus guanylyltransferase. Virology 193, 319–328.
Penrith, M.L., Vosloo, W., 2009. Review of African swine fever: transmission, spread and control. Journal of the South African Veterinary Association 80, 58–62.
Polatnick, J., Pan, I.C., Gravell, M., 1974. Protein kinase activity in African swine fever virus. Archiv fur Die Gesamte Virusforschung 44, 156–159.
Roberts, P.C., Lu, Z., Kutish, G.F., Rock, D.L., 1993. Three adjacent genes of African swine fever virus with similarity to essential poxvirus genes. Archives of Virology 132, 331–342.
Rodríguez, J.M., 1993. Ph.D. Thesis, Universidad Autónoma de Madrid, Madrid, Spain.
Rodriguez, F., Alcaraz, C., Eiras, A., Yáñez, R.J., Rodríguez, J.M., Alonso, C., Rodriguez, J.F., Escribano, J.M., 1994. Characterization and molecular basis of heterogeneity of the African swine fever virus envelope protein p54. Journal of Virology 68, 7244–7252.
Rodríguez, J.M., Salas, M.L., Viñuela, E., 1992. Genes homologous to ubiquitin-conjugating proteins and eukaryotic transcription factor-Sii in African swine fever virus. Virology 186, 40–52.
Rodríguez, J.M., Yáñez, R.J., Almazán, F., Viñuela, E., Rodriguez, J.F., 1993a. African swine fever virus encodes a CD2 homolog responsible for the adhesion of erythrocytes to infected cells. Journal of Virology 67, 5312–5320.

Please cite this article in press as: Rodríguez, J.M., Salas, M.L., African swine fever virus transcription. Virus Res. (2012), http://dx.doi.org/10.1016/j.virusres.2012.09.014

Rodríguez, J.M., Yáñez, R.J., Rodriguez, J.F., Viñuela, E., Salas, M.L., 1993b. The DNA polymerase-encoding gene of African swine fever virus: sequence and transcriptional analysis. Gene 136, 103–110.
Rodríguez, J.M., Yáñez, R.J., Pan, R., Rodriguez, J.F., Salas, M.L., Viñuela, E., 1994. Multigene families in African swine fever virus: family 505. Journal of Virology 68, 2746–2751.
Rodríguez, J.M., Salas, M.L., Viñuela, E., 1996. Intermediate class of mRNAs in African swine fever virus. Journal of Virology 70, 8584–8589.
Rodríguez, J.M., Salas, M.L., Santarén, J.F., 2001. African swine fever virus-induced polypeptides in porcine alveolar macrophages and in Vero cells: two-dimensional gel analysis. Proteomics 1, 1447–1456.
Rojo, G., Garcia-Beato, R., Viñuela, E., Salas, M., Salas, J., 1999. Replication of African swine fever virus DNA in infected cells. Virology 257, 524–536.
Rosales, R., Harris, N., Ahn, B.Y., Moss, B., 1994a. 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. Journal of Biological Chemistry 269, 14260–14267.
Rosales, R., Sutter, G., Moss, B., 1994b. A cellular factor is required for transcription of vaccinia viral intermediate-stage genes. Proceedings of the National Academy of Sciences of the United States of America 91, 3794–3798.
Ross, L., Guarino, L.A., 1997. Cycloheximide inhibition of delayed early gene expression in baculovirus-infected cells. Virology 232, 105–113.
Salas, M.L., Kuznar, J., Viñuela, E., 1981. Polyadenylation, methylation, and capping of the RNA synthesized in vitro by African swine fever virus. Virology 113, 484–491.
Salas, M.L., Kuznar, J., Viñuela, E., 1983. Effect of rifamycin derivatives and coumermycin A1 on in vitro RNA synthesis by African swine fever virus. Brief report. Archives of Virology 77, 77–80.
Salas, M.L., Rey-Campos, J., Almendral, J.M., Talavera, A., Viñuela, E., 1986. Transcription and translation maps of African swine fever virus. Virology 152, 228–240.
Salas, J., Salas, M.L., Viñuela, E., 1988. Effect of inhibitors of the host cell RNA polymerase II on African swine fever virus multiplication. Virology 164, 280–283.
Santarén, J.F., Viñuela, E., 1986. African swine fever virus-induced polypeptides in Vero cells. Virus Research 5, 391–405.
Schnierle, B.S., Gershon, P.D., Moss, B., 1992. Cap-specific mRNA (nucleoside-O2′-)-methyltransferase and poly(A) polymerase stimulatory activities of vaccinia virus are mediated by a single protein. Proceedings of the National Academy of Sciences of the United States of America 89, 2897–2901.
Shors, T., Keck, J.G., Moss, B., 1999. Down regulation of gene expression by the vaccinia virus D10 protein. Journal of Virology 73, 791–796.
Shuman, S., 1990. Catalytic activity of vaccinia mRNA capping enzyme subunits coexpressed in *Escherichia coli*. Journal of Biological Chemistry 265, 11960–11966.
Shuman, S., 1992. Vaccinia virus RNA helicase: an essential enzyme related to the DE-H family of RNA-dependent NTPases. Proceedings of the National Academy of Sciences of the United States of America 89, 10935–10939.
Shuman, S., 2002. What messenger RNA capping tells us about eukaryotic evolution. Nature Reviews Molecular Cell Biology 3, 619–625.
Shuman, S., Moss, B., 1988. Factor-dependent transcription termination by vaccinia virus RNA polymerase. Evidence that the cis-acting termination signal is in nascent RNA. Journal of Biological Chemistry 263, 6220–6225.
Shuman, S., Surks, M., Furneaux, H., Hurwitz, J., 1980. Purification and characterization of a GTP-pyrophosphate exchange activity from vaccinia virions. Journal of Biological Chemistry 255, 11588–11598.
Silber, R., Malathi, V.G., Hurwitz, J., 1972. Purification and properties of bacteriophage T4-induced RNA ligase. Proceedings of the National Academy of Sciences of the United States of America 69, 3009–3013.
Simón-Mateo, 1993. Ph.D. Thesis, Universidad Autónoma de Madrid, Madrid, Spain.
Simón-Mateo, C., Freije, J.M., Andrés, G., López-Otín, C., Viñuela, E., 1995. Mapping and sequence of the gene encoding protein p17, a major African swine fever virus structural protein. Virology 206, 1140–1144.
Simpson, D.A., Condit, R.C., 1994. The vaccinia virus A18R protein plays a role in viral transcription during both the early and the late phases of infection. Journal of Virology 68, 3642–3649.
Simpson, D.A., Condit, R.C., 1995. Vaccinia virus gene A18R encodes an essential DNA helicase. Journal of Virology 69, 6131–6139.
Sogo, J.M., Almendral, J.M., Talavera, A., Viñuela, E., 1984. Terminal and internal inverted repetitions in African swine fever virus DNA. Virology 133, 271–275.
Tarutani, K., Nagasak, i.K., Itakura, S., Yamaguchi, M., 2001. Isolation of a virus infecting the novel shellfish-killing dinoflagellate *Heterocapsa circularisquama*. Aquatic Microbial Ecology 23, 103–111.
Thomson, G.R., Gainaru, M.D., van Dellen, A.F., 1980. Experimental infection of warthogs (*Phacochoerus aethiopicus*) with African swine fever virus. Onderstepoort Journal of Veterinary Research 47, 19–22.
Todone, F., Weinzierl, R.O., Brick, P., Onesti, S., 2000. Crystal structure of RPB5, a universal eukaryotic RNA polymerase subunit and transcription factor interaction target. Proceedings of the National Academy of Sciences of the United States of America 97, 6306–6310.
Tulman, E.R., Delhon, G.A., Ku, B.K., Rock, D.L., 2009. African swine fever virus. Current Topics in Microbiology and Immunology 328, 43–87.
Urzainqui, A., Tabares, E., Carrasco, L., 1987. Proteins synthesized in African swine fever virus-infected cells analyzed by two-dimensional gel electrophoresis. Virology 160, 286–291.
Vos, J.C., Stunnenberg, H.G., 1988. Derepression of a novel class of vaccinia virus genes upon DNA replication. EMBO Journal 7, 3487–3492.
Vos, J.C., Sasker, M., Stunnenberg, H.G., 1991. Vaccinia virus capping enzyme is a transcription initiation factor. EMBO Journal 10, 2553–2558.
Woychik, N.A., Young, R.A., 1990. RNA polymerase II subunit RPB10 is essential for yeast cell viability. Journal of Biological Chemistry 265, 17816–17819.
Woychik, N.A., Liao, S.M., Kolodziej, P.A., Young, R.A., 1990. Subunits shared by eukaryotic nuclear RNA polymerases. Genes and Development 4, 313–323.
Wright, C.F., Oswald, B.W., Dellis, S., 2001. Vaccinia virus late transcription is activated in vitro by cellular heterogeneous nuclear ribonucleoproteins. Journal of Biological Chemistry 276, 40680–40686.
Xiang, Y., Simpson, D.A., Spiegel, J., Zhou, A., Silverman, R.H., Condit, R.C., 1998. The vaccinia virus A18R DNA helicase is a postreplicative negative transcription elongation factor. Journal of Virology 72, 7012–7023.
Yáñez, R.J., Boursnell, M., Nogal, M.L., Yuste, L., Viñuela, E., 1993a. African swine fever virus encodes two genes which share significant homology with the two largest subunits of DNA-dependent RNA polymerases. Nucleic Acids Research 21, 2423–2427.
Yáñez, R.J., Rodríguez, J.M., Boursnell, M., Rodriguez, J.F., Viñuela, E., 1993b. Two putative African swine fever virus helicases similar to yeast "DEAH" pre-mRNA processing proteins and vaccinia virus ATPases D11L and D6R. Gene 134, 161–174.
Yáñez, R.J., Rodríguez, J.M., Rodriguez, J.F., Salas, M.L., Viñuela, E., 1993c. African swine fever virus thymidylate kinase gene: sequence and transcriptional mapping. Journal of General Virology 74 (Pt 8), 1633–1638.
Yáñez, R.J., Rodríguez, J.M., Nogal, M.L., Yuste, L., Enríquez, C., Rodriguez, J.F., Viñuela, E., 1995. Analysis of the complete nucleotide sequence of African swine fever virus. Virology 208, 249–278.
Yang, Z., Bruno, D.P., Martens, C.A., Porcella, S.F., Moss, B., 2010. Simultaneous high-resolution analysis of vaccinia virus and host cell transcriptomes by deep RNA sequencing-Supple. Proceedings of the National Academy of Sciences of the United States of America 107, 11513–11518.
Yang, Z., Reynolds, S.E., Martens, C.A., Bruno, D.P., Porcella, S.F., Moss, B., 2011. Expression profiling of the intermediate and late stages of poxvirus replication. Journal of Virology 85, 9899–9908.
Yang, Z., Martens, C.A., Bruno, D.P., Porcella, S.F., Moss, B., 2012. Pervasive initiation and 3′-end formation of poxvirus postreplicative RNAs. Journal of Biological Chemistry 287, 31050–31060.
Yuen, L., Moss, B., 1987. Oligonucleotide sequence signaling transcriptional termination of vaccinia virus early genes. Proceedings of the National Academy of Sciences of the United States of America 84, 6417–6421.
Yutin, N., Wolf, Y.I., Raoult, D., Koonin, E.V., 2009. Eukaryotic large nucleocytoplasmic DNA viruses: clusters of orthologous genes and reconstruction of viral genome evolution. Virol Journal 6, 223.
Zhang, D.W., Rodríguez-Molina, J.B., Tietjen, J.R., Nemec, C.M., Ansari, A.Z., 2012. Emerging views on the CTD code. Genetics Research International 2012, 347214.
Zust, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., Siddell, S.G., Ludewig, B., Thiel, V., 2011. Ribose 2′-O-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. Nature Immunology 12, 137–143.

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