African Swine Fever Virus \textit{trans}-Prenyltransferase*

(Received for publication, October 7, 1996, and in revised form, January 22, 1997)

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The present study describes the characterization of an African swine fever virus gene homologous to prenyltransferases. The gene, designated \textit{B318L}, is located within the EcORI B fragment in the central region of the virus genome, and encodes a polypeptide with a predicted molecular weight of 35,904. The protein is characterized by the presence of a putative hydrophobic transmembrane domain at the amino end. The gene is expressed at the late stage of virus infection, and transcription is initiated at positions $-118$, $-119$, $-120$, and $-122$ relative to the first nucleotide of the translation start codon. Protein B318L presents a colinear arrangement of the four highly conserved regions and the two aspartate-rich motifs characteristic of geranylgeranyl diphosphate synthases, farnesyl diphosphate synthases, and other prenyltransferases. Throughout these regions, the percentages of identity between protein B318L and various prenyltransferases range from 28.6 to 48.7%. The gene was cloned in vector pTrxFus without the amino-terminal hydrophobic region and expressed in \textit{Escherichia coli}. The recombinant protein, purified essentially to homogeneity by affinity chromatography, catalyzes the sequential condensation of isopentenyl diphosphate with different allylic diphosphates, farnesyl diphosphate being the best allylic substrate of the reaction. All-\textit{trans}-polyisoprenyl diphosphates containing 3–13 isoprene units are synthesized, which identifies the B318L protein as a \textit{trans}-prenyltransferase.

\textit{Prenyltransferase} is the generic name for a family of enzymes catalyzing the sequential condensation of isopentenyl diphosphate (IPP) with allylic diphosphates to form prenyl diphosphates of different chain length in the biosynthetic pathway of isoprenoid compounds (1–3). Farnesyl diphosphate (FPP) synthase and geranylgeranyl diphosphate (GGPP) synthase, the best characterized members of the prenyltransferase family, catalyze reactions at central positions in the isoprenoid pathway, giving rise to the formation of FPP and GGPP, respectively. These compounds are, in turn, precursors for the synthesis of a variety of products including cholesterol, carotenoids, and prenyl proteins, which are involved in basic cellular functions, such as membrane structure, synthesis of steroid hormones, and intracellular trafficking (3, 4). On the other hand, \textit{cis}- and \textit{trans}-prenyltransferases catalyze the \textit{cis} or \textit{trans} additions of IPP to \textit{trans}-FPP producing polyisoprenyl diphosphates, which are precursors of dolichols and ubiquinones, involved, respectively, in synthesis of glycoproteins and electron transport (5, 6).

Increasing evidence indicates that isoprenoid compounds also play essential roles during the life cycle of viruses. Thus, it has been recently shown that prenylation of hepatitis \textit{\delta} virus large antigen is required for the formation and release of virus particles (7, 8). In addition, other studies with murine leukemia virus have indicated the existence of a relationship between isoprenoid biosynthesis and intracellular transport and processing of the viral envelope protein (9). These authors have suggested that prenylation of cellular Rab proteins is required for the incorporation of viral envelope precursors into the virions. Despite this role of isoprenoids in the multiplication of certain viruses, no virus-encoded prenyltransferase has been described so far.

We report here the characterization of an African swine fever virus (ASFV) gene, designated \textit{B318L} (10), encoding a protein with homology to prenyltransferases. ASFV is a large, enveloped DNA virus, that causes a severe disease in domestic pigs (11, 12). Its genome is formed by a double-stranded, linear DNA molecule of 170–190 kilobase pairs with hairpin loops and terminal inverted repeats (13, 14), this structure being remarkably similar to that of the poxvirus DNA (15, 16). Also in common with the poxviruses (17), ASFV particles contain all of the enzymatic machinery required for early RNA synthesis (18–20). The virion has about 50 structural proteins (21) and consists of a nucleoprotein core surrounded by a lipoprotein envelope, the capsid, and an outer membrane where the virus attachment protein p12 is located (22, 23). The assembly of virions is known to occur in the cytoplasm of the infected cell, but the steps involved in the virus morphogenesis are still poorly understood.

We present a comparison of the amino acid sequences of the ASFV and cellular prenyltransferases, along with experiments describing the transcriptional expression of the viral gene during infection of cultured cells. We also show that the protein expressed in \textit{Escherichia coli} and purified catalyzes the synthesis of all-\textit{trans}-polyisoprenyl diphosphates, which identifies it as a \textit{trans}-prenyltransferase.

**EXPERIMENTAL PROCEDURES**

\textbf{Materials}—[1-\textit{\textsuperscript{14}}C]IPP (specific activity 56 Ci/mol) was obtained from Amersham Corp. Nonlabeled IPP, dimethylallyl diphosphate (DMAPP), geranyl diphosphate (GPP), all-\textit{trans}-FPP, all-\textit{trans}-GGPP, geraniol, farnesol (mixed isomers), all-\textit{trans}-geranylergosterol, solanesol, fica-

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* Grant AIR-CT93-1332, and an institutional grant from Fundación “Severo Ochoa”.

† This work was supported by Dirección General de Investigación Científica y Técnica Grant PB93-0160-C02-01, European Community Grant AIR-CT93-1332, and an institutional grant from Fundación Ramón Areces to the Centro de Biología Molecular “Severo Ochoa.”

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This paper is available on line at http://www-jbc.stanford.edu/jbc/

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nols C$_4$-C$_8$, and prenols C$_6$-C$_{12}$ were purchased from Sigma. 1-H-Labeled trans,trans,cis,geranylgeraniol was from American Radiolabeled Chemicals, Inc., and acid phosphatase was from Boehringer. Silica gel 60 and RP-18 thin layer plates were obtained from Merck. Prenols C$_6$-C$_{12}$ were kindly provided by Dr. T. Chojnacki (Polish Academy of Sciences, Warsaw).

**Cells and Virus**—Vero cells (CCL81) were obtained from the American Type Culture Collection and grown in Dulbecco's modified Eagle's medium containing 10% newborn calf serum. The Vero-adapted ASFV strain BA71V was propagated and titrated as described previously (24).

**Computer Analysis**—General analyses of DNA and protein sequences were performed with the software package of the University of Wisconsin Genetics Computer Group (25). Data base searches were done with programs FASTA and TFASTA (26). Computation was also performed at the National Center for Biotechnology Information using the BLAST (27) network service. Protein patterns were searched using the MacPatterns program (28) and the PROSITE (29) and BLOCKS (30) data bases.

**Preparation and Analysis of RNA**—Vero cells were mock-infected or infected with ASFV (BA71V strain) at a multiplicity of 20 plaque-forming units/cell. To obtain early RNA, the cells were infected in the presence of 100 μg/ml cycloheximide or 40 μg/ml cytosine arabinoside for 7 h. Late RNA was isolated from cells infected for 18 h in the absence of inhibitors. Whole cell RNA was prepared by the guanidinium isothiocyanate/cesium chloride extraction procedure (31). Northern blot hybridization was carried out as reported elsewhere (32), using as probes the 32P-end-labeled oligonucleotides 5'-GTATCCATGAAAAAGCCGACATCAGCC-3' (RB-8), 5'-GTTGGATCCTAGAAGCTTTGGG-3' (RB-3), or 5'-AATTCTGGATGATGATGATGGG-3' (RB-10), complementary to nucleotides +124 to +95, +91 to +62, and -200 to -229, respectively, of the B318L gene noncoding strand. Oligonucleotides RB-8 and RB-3 were used for primer extension analysis, performed essentially as described by Sambrook et al. (33). After 5'-end labeling with 32P, the primer was annealed, at 43 °C, to the different classes of RNA and extended with avian myeloblastosis virus reverse transcriptase for 15 min at 42 °C. The primer extension products were then electrophoresed in a 6% polyacrylamide gel.

**Cloning and Expression of Gene B318L**—The B318L gene lacking the first 62 nucleotides was amplified by polymerase chain reaction using oligonucleotides 5'-GCCGCTTCATAGCGGCAACCTTAAGTTTTT-3' and 5'-GCCGCTGTCAAGTGATCCAAATTGCAACT-3', and cloned in the expression vector pTrxFus (34). The first primer was designed with a CCCG tag and an XhoI restriction site, and the second primer contains a CCCG tag and a PsflI restriction site. Both restriction sites are absent in the viral sequence. The polymerase chain reaction product was digested with XhoI and PsflI and cloned into XhoI/PstI-cut pTrxFus vector under the control of the P$_e$ promoter from bacteriophage λ. The viral gene was thus expressed as a fusion to the E. coli thioredoxin. Sequencing of the cloned product revealed a silent mutation in Ala-117 (an A to T change in nucleotide 351 of the B318L open reading frame (ORF); third base on codon 117).

**E. coli strain GI724** cells (34) were transformed with either the wild-type pTrxFus vector or the recombinant plasmid pTrxFus-B318L, and protein expression was analyzed as indicated by the supplier (Invitrogen, ThioFusion Expression System Instruction Manual). Briefly, the transformed cells were grown overnight at 30°C in RM medium containing 100 μg/ml ampicillin. The cultures were then diluted 1:20 in induction medium and grown at 30°C to an A$_{600}$ of 0.5. The cells were induced with 100 μg/ml tetracyclin at 34°C for 2 h. Samples were analyzed in SDS-polyacrylamide gels, and the polypeptides were visualized by Coomassie Blue staining.

**Affinity Purification of the Recombinant B318L Protein**—A 250-ml culture of E. coli cells harboring the pTrxFus-B318L plasmid was induced with tetracyclin at 34°C for 2 h. The cells were harvested by centrifugation, suspended in 5 ml of running buffer containing 100 mM Tris-HCl, pH 7, 150 mM NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, and sonicated with five 15-s bursts. After centrifugation for 15 min at 10,000 × g, the supernatant was collected and incubated with 2 ml of ThioBond™ resin (Invitrogen), activated as indicated by the supplier, for 60 min at 4°C with rotation. The resin was allowed to settle, and, after collecting the flow-through, the column was washed with 30 bed volumes of running buffer containing 20 mM β-mercaptoethanol and eluted with 3 bed volumes of running buffer containing increasing concentrations of β-mercaptoethanol. The B318L protein eluted over a range of concentrations of β-mercaptoethanol (50, 100, 200, and 500 mM). The fractions containing the protein were pooled and dialyzed against 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA. This pool was then incubated with 1 ml of activated ThioBond™ resin for 1 h at 4°C with rotation, the resin was allowed to settle, and the column was washed with 10 bed volumes of running buffer containing 2 mM β-mercaptoethanol. The protein was "knocked" off the column with 3 volumes of a high β-mercaptoethanol concentration (1 M). The eluate was dialyzed as described before and concentrated using a Centricon-10 device. The concentrated sample was analyzed for purity by SDS-polyacrylamide gel electrophoresis with Coomassie Blue staining.

**Assay of Prenyltransferase Activity**—The assay mixture contained, in a final volume of 0.1 ml, 50 μM 1-[1-14C]IPP (specific activity 24 cpm/pmol), 100 μM all-trans-FPP or GPP, 2 mM MgCl$_2$, 1 mM dithiothreitol, 20 mM Tris-HCl, pH 7.5, 5.0 μg/ml of bovine serum albumin, and enzyme protein as indicated. After 15 min at 37°C, the reactions were stopped by chilling in ice, and the products were extracted as follows: 0.15 ml of H$_2$O saturated with n-butyl alcohol were added to the reaction mixtures, and the products were extracted with 0.5 ml of n-butyl alcohol saturated with H$_2$O. The aqueous phase was reextracted with 0.5 ml of n-butyl alcohol, and the pooled butanol extracts were back-washed with H$_2$O. The radioactivity in the butanol phase was determined by scintillation counting.

**Product Analysis**—The butanol extracts were treated with acid phosphatase by the method of Fujii et al. (35). The hydrolysates were extracted with hexane, and the hexane-soluble products were analyzed by reversed-phase RP-18 thin layer chromatography in acetone:H$_2$O (195:5) or by normal silica gel thin layer chromatography in benzene:ethyl acetate (91:1). The position of authentic standards was visualized with iodine vapor. For autoradiography, the thin layer plates were exposed on a Fujifilm BAS-IT 20405 imaging plate at room temperature. The exposed imaging plate was analyzed with a Fuji BAS 1500 analyzer.

**RESULTS**

**Properties and Transcriptional Analysis of ORF B318L**—ASVF ORF B318L, located within the EcoRI B fragment of the virus genome (Fig. 1A), encodes a protein of 318 amino acids, with a predicted molecular weight of 35,904, homologous to prenyltransferases (10). The hydrophyt profile shown in Fig. 1B predicts that the B318L protein contains a transmembrane region of 21 amino acids at the amino end of the protein. This region is followed by four positively charged amino acids (Arg or Lys residues at positions 22, 23, 25, and 28), thus classifying the B318L protein as a putative class III membrane protein,
with the carboxyl terminus facing the cytoplasm (36).

To study the expression of the B318L gene during the viral infection and to determine the transcription initiation site, Northern blot and primer extension analyses were carried out, using as a hybridization probe or as a primer the 32P-labeled oligonucleotide RB-3 shown. The samples in the right panel were electrophoresed beside a DNA sequencing reaction (DNA ladder) of the corresponding region of the gene performed with the same oligonucleotide used for primer extension. The sizes (in nucleotides) of the four DNA fragments obtained in the primer extension analysis are indicated. The transcription initiation sites of gene B318L are shown at the bottom and are indicated by open circles. The box encloses a consensus sequence for 3'-end formation of RNA.

A motif composed of seven consecutive thymidylate residues (the 7T motif), recently identified as a signal for 3'-end formation of ASFV RNAs (37, 38), is found 32 nucleotides downstream of the translation stop codon of ORF B318L. Transcription termination at this site would produce an RNA of approximately 1.1 kb, which is the size of the smaller RNA band detected by Northern hybridization. Termination of transcription at alternative 7T motifs located further downstream might account for the 6.6-, 8-, and 9.4-kb RNAs, while the remaining species might correspond to transcripts of upstream ORFs, running through the B318L gene (10). In agreement with this possibility, it has been found that oligonucleotide RB-10, corresponding to a region upstream of the transcription initiation sites determined above, hybridizes in Northern blots with this possibility, it has been found that oligonucleotide RB-10, corresponding to a region upstream of the transcription initiation sites determined above, hybridizes in Northern blots containing RNA from mock-infected cells and early RNA from cells infected with ASFV revealed eight RNA species with sizes ranging from 142 to 170, were obtained with the sequences of prenyltransferases, the ASFV protein was compared with the carboxyl terminus facing the cytoplasm (36).

Comparison of the Amino Acid Sequences of ASFV Protein B318L and Cellular Prenyltransferases—Searches in the data bases with the derived amino acid sequence of ORF B318L showed significant similarity to proteins of the prenyltransferase family. The highest optimized FASTA scores, with values ranging from 142 to 170, were obtained with the sequences of GGPP synthases from Capsicum annuum, Erwinia urendovora, and Erwinia herbicola and with the FPP synthase sequence from Bacillus steatorhombophilus.

To compare the amino acid sequence of protein B318L with the sequences of prenyltransferases, the ASFV protein was...
first aligned with 16 demonstrated or putative prenyltransferases by using the PILEUP program, and a dendrogram displaying clustering relationships was constructed. Representative members of the different clusters obtained were then selected for the comparison of conserved regions, as shown in Fig. 3. All of these regions, designated I to IV according to Kunz et al. (39), are also present in the B318L protein and have nearly the same spatial arrangement in the ASFV polypolyolipid and other prenyltransferases. Regions II and IV contain the PROSITE library signatures 1 and 2, used to identify proteins belonging to the prenyltransferase family. The consensus sequences of signatures 1 and 2 are L/IV/MI/XXDXX4_DXXRG and (L/IV/V/F/Y)GXFXQ/L/IV/V/M/XXXD/L/IV/M/F/Y/X/D/N, respectively, in which the amino acids enclosed in parentheses signify alternatives for that position found in different prenyltransferases, each X represents a non-conserved amino acid, and each subscript indicates the number of intervening residues. As can be seen in Fig. 3, these sequences are also conserved in the ASFV B318L protein, with only a substitution of the Gly residue for an Asn in signature 1.

It has been suggested that the motif DDXX4D in signatures 1 and 2 serves as binding site for the substrates and that this binding is facilitated by the formation of salt bridges between the aspartic residues and magnesium (39, 40). In keeping with this notion, mutational studies of the aspartate and arginine residues within signature 1 and of the two first aspartates within signature 2 of FPP synthase indicate that these amino acids are involved in enzyme catalysis (41–45). The proposed roles of these domains have been confirmed following crystallization of FPP synthase (44). On the other hand, sequence analysis of the FPP synthase gene of a yeast mutant defective in FPP synthase shows that the conserved lysine in region III is critical for catalytic activity (45). Conservation of these motifs and residues in the ASFV B318L protein strongly supports the proposal that the B318L gene encodes a prenyltransferase.

The percentages of identity and similarity between protein B318L and prenyltransferases throughout the regions aligned in Fig. 3 show that the ASFV protein is more similar to the GGPP synthases from C. annuum, R. capsulatus, and E. herbicola and to the FPP synthase and octaprenyl diphosphate synthase from B. stearothermophilus (Table I).

### Expression and Purification of the B318L Protein
To confirm that the B318L ORF encodes a prenyltransferase, the gene was cloned in the expression vector pTrxFus without the amino-terminal 21-amino acid hydrophobic region, to facilitate the expression of the protein in a soluble form. The resulting construction encodes a hybrid protein containing the E. coli thioredoxin followed in frame by 297 amino acids of the B318L protein.

As shown in Fig. 4A, the SDS-polyacrylamide gel electrophoresis of total cell lysates of E. coli G724 cells carrying the expression vector pTrxFus-B318L, harvested after induction with tetracyclin, showed a band of 47 kDa, which was not detected in uninduced cells or in cells harboring the control pTrxFus plasmid. The size of the induced protein is consistent with that calculated for the fusion protein (46.9 kDa).

To determine whether the protein encoded by ORF B318L has prenyltransferase activity, extracts from E. coli cells transformed with plasmid pTrxFus-B318L and induced with tetracyclin were prepared, and the protein was purified by affinity chromatography on ThioBond™ columns, as described under “Experimental Procedures.” Relative to cells transformed with the control pTrxFus plasmid, the pTrxFus-B318L-transformed cells have a 24-fold higher specific prenyltransferase activity (Table II). After affinity chromatography, the purified B318L protein was essentially homogeneous as estimated by SDS-polyacrylamide gel electrophoresis with Coomassie Blue staining (Fig. 4B) and had an specific activity of 167 nmol of IPP incorporated into prenyl diphosphates per 15 min per mg of protein (Table II).

### Characteristics of the Prenyltransferase Activity of Protein B318L
The B318L protein required a divalent metal for activity. Using FPP as substrate, the optimal Mg²⁺ concentration was 1–2 mM, while with GPP maximal stimulation was observed with Mg²⁺ 1:2.2:4.1:5, under the standard assay conditions. Thus, the best substrate of the enzyme was FPP.

The reaction products derived from [1-⁴⁰⁰]IPP and the various allylic substrates were analyzed by reversed-phase thin layer chromatography after butanol extraction and hydrolysis with acid phosphatase to the corresponding alcohols, as indicated under “Experimental Procedures.” As shown in Fig. 5, when the allylic substrate was DMAPP, a mixture of C₁₅–C₃₅ prenyl diphosphates was produced, while GPP yielded FPP and GGPP, as well as longer chain polyprenyl diphosphates containing 5–9 isoprene units. In the case of the reaction with FPP, GGPP, and polyprenyl diphosphates with chain lengths of C₂₅–C₃₅ were GPPed, and when GPP was used as substrate, polyprenyl diphosphates of 5–12 isoprene residues were synthesized. The product distribution obtained was very similar to that calculated for the fusion protein (46.9 kDa).

### Table I

| Protein          | FPSPS-ECOLI | GGPPS-RHOCA | GGPPS-ERWHE | HXPPS-SCHICE | B318L-ASFV | GGPPS-NEUCR | FPSPS-SCHECE | HPPPS-BSTEA | OPPS-ECOLI |
|------------------|-------------|-------------|-------------|--------------|------------|-------------|--------------|-------------|------------|
| GPPS-CAPAN       | 71.8 (80.8) | 61.5 (76.9) | 59.0 (78.2) | 57.9 (75.0)  | 44.9 (60.3) | 39.5 (64.5) | 35.1 (54.6)  | 52.6 (75.0) | 51.3 (68.4) |
| FPSPS-ECOLI      | 56.4 (71.8) | 57.7 (71.8) | 51.3 (65.8) | 47.4 (64.1)  | 38.2 (59.2) | 35.1 (53.3) | 54.0 (71.1)  | 51.3 (69.7) |           |
| GGPPS-RHOCA      | 56.4 (75.6) | 50.0 (72.4) | 48.2 (61.5) | 38.2 (60.5)  | 33.8 (49.4) | 52.6 (75.0) | 50.8 (65.8)  |           |           |
| GGPPS-ERWHE      |             | 48.1 (65.4) | 48.7 (64.1) | 42.1 (55.3)  | 36.4 (50.7) | 46.1 (69.7) | 47.4 (65.8)  |           |           |
| HXPPS-SCHICE     |             |             | 36.8 (54.0) | 35.5 (59.2)  | 36.8 (55.3) | 53.5 (76.3) | 60.5 (76.3)  |           |           |
| B318L-ASFV        |             |             |             | 30.3 (48.7)  | 28.6 (46.8) | 39.5 (63.2) | 43.4 (59.2)  |           |           |
| GGPPS-NEUCR      |             |             |             |             | 30.3 (50.0) | 35.5 (60.5) | 39.5 (57.9)  |           |           |
| FPSPS-SCHECE     |             |             |             |             | 34.2 (50.0) | 36.8 (54.0) | 50.0 (75.0)  |           |           |
| HPPPS-BSTEA      |             |             |             |             |             |             | 50.0 (75.0)  |           |           |
| Percentage of identity, and similarity between protein and DISTANCES (24), using threshold values of 1.5 and 0.6, respectively. Abbreviations for proteins are as indicated in the legend to Fig. 3. |
The B318L protein purified by affinity chromatography on ThioBond™ resin contains a putative transmembrane domain, the chain length of which is critical for catalytic activity. It is shown here that the ASFV protein encoded by ORF B318L is homologous to prenyltransferases, containing the four highly conserved amino acid regions characteristic of these enzymes, and, within these regions, several amino acids that are critical for catalytic activity.

To determine whether the B318L protein indeed had prenyltransferase activity, we undertook its expression in *E. coli*. Since attempts to express the complete protein in a soluble form failed, we cloned the gene without the amino-terminal hydrophobic region in the pTrxFus vector. The viral gene was thus expressed as a fusion to the *E. coli* protein thioredoxin, producing a soluble protein that could be purified essentially to homogeneity employing an affinity column.

The purified protein has been shown to catalyze the sequential condensation of IPP with allylic diphosphates to yield prenyl diphosphates containing 3–13 isoprene units. A study of the stereochemistry of the reaction showed that IPP is added to FPP in a trans configuration, suggesting that all-trans-polyisoprenyl diphosphates are synthesized.

Under the in vitro reaction conditions, the enzyme does not synthesize a single product of specific chain length. It is possible that in the in vivo environment the chain length distribution of the products might vary. Since the B318L protein contains a putative transmembrane domain, the chain length of the products might depend on its interaction with membranes, as appears to be the case for some prenyltransferases synthesizing long chain polyisoprenyl diphosphates (46). It will therefore be of interest in the future to investigate the possible associa-
fection of the B318L protein with membranes and to study its activity in a membrane-bound form. It has also been shown that some of these prenyltransferases require protein factors for the synthesis of the natural products (47–49). Purification of the B318L protein from ASFV-infected cells might reveal the existence of a similar factor for the viral enzyme.

To date, the ASFV B318L gene is the only prenyltransferase gene of viral origin identified. Its presence in ASFV raises a number of interesting questions about the significance of this enzyme in the virus life cycle. Since FPP and GGPP are formed in the reaction catalyzed by the viral enzyme, it could be argued that these products might be used as prenyl donors for the farnesylation or geranylgeranylation of cellular or viral proteins. These modifications might, in turn, be required for the assembly and release of virus particles, as has been described for other viruses (7–9). The existence of an ASFV ORF (L83L) encoding a protein with a consensus prenylation sequence (10) as well as the expression of the B318L gene at the late stage of infection, when virus morphogenesis occurs, would be consistent with such a role for the ASFV prenyltransferase.

However, it should be noted that the properties of the ASFV prenyltransferase clearly differ from those described for the FPP synthase or GGPP synthase involved in the synthesis of the prenyl donors for protein prenylation. Thus, these enzymes produce FPP (C15) or GGPP (C20) as the ultimate products and are unable to utilize GGPP as substrate (50, 51), while the viral enzyme can form longer products and uses GGPP efficiently. Furthermore, the cellular enzymes synthesizing the precursors for protein prenylation are cytosolic proteins (52–55), whereas the ASFV B318L protein is predicted to be membrane-bound.

On the other hand, all-trans-prenyltransferases, such as octaprenyl diphosphate synthase (56) and solanesyl diphosphate synthase (49), catalyze the sequential condensation of IPP to FPP to give long chain polypropenyl diphosphates, which are the precursors of quinone side chains. The ASFV B318L protein may have a similar role. If that is the case, the acquisition by ASFV of a prenyltransferase gene for the synthesis of the ubiquinone (coenzyme Q) prenyl chain could be related to an increase in mitochondrial function that might be required to provide extra energy for viral processes, such as macromolecular synthesis and morphogenesis. In this connection, it should be mentioned that mitochondrial DNA synthesis has been found to increase in herpes simplex type 1 virus-infected vero cells (57) and that adenovirus infection of human cells stimulates mitochondrial activity (58).

On the other hand, as has been previously discussed (10), macrophages and monocytes, the target cells in natural ASFV infection, generate reactive oxygen species for their microbiocidal functions, which may cause oxidative damage to the virus DNA and membranes. The reactive oxygen species can also trigger and/or mediate apoptotic cell death (59), whose control is important to develop a productive viral infection. In addition to its role as electron carrier in mitochondria, it has been suggested that coenzyme Q, which is also present in the nucleus, plasma membrane, endoplasmic reticulum-Golgi, and lysosomes (60, 61), can function outside the mitochondria in antioxidation (62–64). Thus, it can be speculated that the ASFV prenyltransferase might be involved in the synthesis of the prenyl chain of ubiquinone for the production of extra amounts of this compound and that this could be important to protect the virus membranes and/or DNA against oxidation and to prevent cell death by apoptosis of the infected cell.

Studies on the localization of protein B318L and on its prenyltransferase activity in ASFV-infected cells, as well as an examination of protein prenylation induction during the virus life cycle, may help to elucidate the role of the virus-encoded prenyltransferase in the biology of ASFV.

Acknowledgments—We thank T. Chojnacki for the gift of prenols and J. Salas for critical reading of the manuscript.

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J. Biol. Chem. 1997, 272:9417-9423.
doi: 10.1074/jbc.272.14.9417

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