EXPERIMENTAL WORKS

Analysis of the African Swine Fever Virus Immunomodulatory Proteins

M. V. Nefedeva^{a, *}, I. A. Titov^{a, **}, K. A. Mima^{a, ***}, and A. S. Malogolovkin^{a, ****}

^aMolecular Virology Laboratory Federal Research Center for Virology and Microbiology, Volginsky, Vladimir oblast, 601125 Russia

*e-mail: masha67111@mail.ru

**e-mail: titoffia@yandex.ru

***e-mail: mima89@ya.ru

****e-mail: Malogolovkin@inbox.ru

Received March 29, 2018; revised June 6, 2018; accepted September 7, 2018

Abstract— Molecular epidemiology of viral infections traditionally based on the analysis of changes in individual genes or genetic markers. The analysis of the African swine fever virus (ASFV) genes encoding immunomodulatory proteins is an important tool for studying the diversity and evolution of the virus. In this work, we carried out a structural and phylogenetic analysis of the ASF virus immunomodulatory proteins 5EL (*A238L* gene), I14L (*Dp71L* gene), K11L (*I329L* gene). The degree of nucleotide substitutions of the ASFV concatenated genes *A238L*, *I329L* and *Dp71L* revealed purifying (stabilizing) selection at the nucleotide sequences level. The variability characteristic of the selected group of ASFV genes is of great interest for the genetic differences search in immunomodulatory proteins. The sequencing results of the *A238L*, *I329L* and *Dp71L* genes and their phylogenetic analysis showed that these genes are conservative among a large group of ASFV genes. The *I329L* gene is a genetic marker of common origin. The East African strains (Genotype X) of *Dp71L* gene have two forms: a long (184 amino acids) and a short (from 70 to 72 amino acids) and is formed by fusion of the I3L and I4L. All ASF virus Russian isolates isolated in 2016–2017 were identical to the reference strain ASFV/Georgia/wb/2007. Characterization of variability 5EL protein, I14L, K11L may be serve to identify target sites in the ASFV genome and to develop vaccines. The obtained data allow to evaluate the genetic diversity of the ASFV immunomodulatory proteins and the dynamics of their evolution, to predict the possible participation of the *A238L*, *I329L* and *Dp71L* genes in the virulence of various ASFV strains.

Keywords: African swine fever virus, sequencing, phylogenetic analysis, immunomodulating proteins, analysis of synonymous and nonsynonymous substitutions

DOI: 10.3103/S0891416819010075

INTRODUCTION

African swine fever (ASF) is a hemorrhagic deadly disease of domestic pigs and boars caused by the complex shell deoxivirus of the *Asfarviridae* family. Mortality in infected herds reaches 100%. The disease is transmitted from sick animals and virus carriers by alimentary, contact and transplacental [1]. The ASFV genome is a linear double-stranded DNA molecule with covalently closed ends and terminal inverted repeats (TIR) [2].

Domestic pigs of all age groups and breeds and wild boars are susceptible to the ASFV. Among the African members of the *Suidae* family, wild African and bush pigs, warthogs, may be infected with ASFV but without showing clinical signs. The main virus reservoir in the wild in East African countries are soft mites of the genus *Ornithodoros* [3].

Depending on the virus strain, virulence and mode of infection, the observed clinical signs are diverse in a peracute, acute, subacute and chronic forms of infection [4]. The ASF epidemiology is very complex and varies depending on the geographical features of the area and susceptible species of animals (wild boar, domestic pigs, ticks) [3].

Effective means of protection against the ASFV has not yet been developed. Historical attempts to protect animals with inactivated vaccines have either failed or yielded conflicting results. Studies using attenuated vaccines have demonstrated their potential to protect pigs from experimental infection with a homologous virulent virus, but rarely against heterologous viruses [5–7]. The study of antigenic diversity among naturally occurring of ASFV isolates is of great interest for vaccine development [8].

Recent studies indicate that the virus genetic diversity determined by sequencing the major capsid pro-

tein P72 (*B646L* gene) is highest in Central and East Africa [3]. The high level of the ASFV genetic variability between different isolates is explained by the difference in the genome size, but most genetic variation is due to changes in the number and sequence of multi-genic families members (MGF) located at both ends of the genome, where they limit the left and right variable regions (LVR and RVR, respectively) [9].

The ASFV genome contains a set of genes encoding the proteins responsible for the immune evasion mechanisms. The modulation activity range of viral proteins is very diverse and includes: inhibition of humoral response, interference with interferons, inhibition of cytokines and chemokines, evasion of cytotoxic T lymphocytes (CTLs) and natural killer cells (NKs), and control of the major histocompatibility complex (MHC I and II) function, changes in the effector function of dendritic cells and inhibition of apoptosis [10].

The ASFV is a macrophage-tropic virus and can manipulate both innate and adaptive immune response by modulating macrophages functions. According to L. Dixon et al., several ASFV proteins have been identified which prevent the development of the host's immune defense [11]. These include the *A238L* gene that encodes a 5EL protein which acts to inhibit transcriptional activation of host immunomodulatory genes, K11L protein (*I329L* gene) acting as inhibitor of Toll-like receptors signaling pathways and I14L protein (*Dp71L* gene) similar to the herpes simplex virus-encoded virulence factor ICP34.5 which regulates the phosphatase activity of the host cell during infection displacing inhibitory substrates from PP1 host phosphatase and determining the range of sensitive hosts [12–14]. In addition, *A238L* inhibits the host calcineurin dependent pathways by directly binding calcineurin phosphatase [14].

However, not all known ASFV isolates have the same immunomodulatory activity [15]. The genetic background of these differences remains unclear. Thus, the detection of genetic markers of evolutionary variability in ASFV immunomodulating proteins will allow to study the ASFV immune evasion mechanisms.

The aim of the study is to conduct a comparative analysis of nucleotide sequences of ASFV *A238L*, *I329L* and *Dp71L* genes involved in immune evasion.

MATERIAL AND METHODS

At the first stage, the 5EL, K11L and I14L proteins variability was analyzed using the web service Protein Variability Server by Simpson method (<http://www.expasy.org/proteomics>) [16]. Region for which the values obtained exceeded the threshold value (0.46) were considered variable. SignalP 4.1 web server was used to predict the presence and location of signal peptide cleavage sites in amino acid sequences. (<http://www.cbs.dtu.dk/services/SignalP-4.1>) [17].

The CCTOP (Constrained Consensus TOPology prediction server) web application provided prediction of the transmembrane protein topology (<http://cctop.enzim.ttk.mta.hu>) [18].

At the next stage of work, 12 isolates isolated from different regions of the Russian Federation in 2016 and 15 strains belonging to different genotypes [19, 20] and serotypes of the ASFV [21] were selected from the State Collection of Microorganisms of the FRCVM. For each seroimmunotype, attenuated and virulent ASFV strains are used as indicated in the Table 1.

Selection of primers for the copies amplification of the *A238L*, *I329L* and *Dp71L* genes was carried out by comparing and analyzing the nucleotide sequences of these genes from strains and various ASFV isolates published in the international GenBank database (<http://www.ncbi.nlm.nih.gov/>) using BioEdit 7.0.1, Oligo 6.0. and the IDT DNA web server (<https://eu.idtdna.com/site>).

DNA extraction from blood and organ suspension was performed using DNA-sorb-B kit (Interlabservice, Russia) in accordance with the manufacturer's instructions. Thermal cycler T100 (Bio-Rad Laboratories, USA) was used to optimize the temperature regimes of PCR and gene sequences amplification.

Analysis of the reaction products was carried out by electrophoresis in 1.5% agarose gel and taken into account on ChemiDoc MP (Bio-Rad Laboratories, USA) by detection specific bands in the tracks with test samples relative to the molecular weight marker fragment and the calculated length of the PCR product. PCR products purification from agarose gel was performed by QIAquick Gel Extraction (QIAGEN) and Cleanup—standard (Evrogen, Russia) commercial kit. The sequencing reaction was performed using the Bigdye terminator v3.1 cycle sequencing kit (Applied Biosystems, USA). Genetic Analyzer 3130 sequencer (Applied Biosystems, USA) was used for sequencing.

Multiple alignment of ASFV gene sequences was performed using variants of the ClustalW method and MUSCLE [22]. To construct phylogenetic trees, the maximum likelihood method was used with an additional bootstrap—analysis of 1000 random samples using the Mega 6.0 program [23].

For the analysis of synonymous (synonymous distance, dS) and non-synonymous substitutions (non-synonymous distance, dN) per site in the *A238L*, *I329L* and *Dp71L* genes, as well as the number of potentially synonymous and non-synonymous sites for each codon based on the hypothesis of equal frequencies of all nucleotide substitutions, the SNAP (Synonymous Non-synonymous Analysis Program) software package was used (www.hiv.lanl.gov).

Table 1. ASFV strains used in the work

Strain/isolate	Country	Isolated year	Geno- type	Seroimmu- notype	A238L	I329L	DP71L
Lisbon-57 (L-57)	Portugal	1957	I	1	MG209277	MG209292	MF589625
LK-111	Portugal	1978	I	1	MG209278	MG209293	MF589627
Kongo-49 (K-49)	Zaire	1949	I	2	MG209275	MG209290	MF589624
KK-262	Zaire	—	I	2	MG209276	MG209291	MF589626
France-32 (F-32)	France	1964	I	4	MG209272	MG209288	MF589621
FK-32/135	France	Passaged from F-32	I	4	MG209273	MG209289	MF589622
CKA-2015	Russia	Passaged from Stavropol 01/08	II	8	MG010372	MG209287	MF589620
Irkutsk-2017	Russia	2017	II	8	MG209274	—	MF589623
Mozambique-78 (M-78)	Mozambique	1978	V	3	MG209279	MG209294	MF589628
MK-200	Mozambique	Passaged from M-78	V	3	MG209280	MG209295	MF589630
TKF	Tanzania	—	X	3	MG209282	MG209297	MF589631
TSP-80	Tanzania	Passaged in 1980	X	5	MG209285	MG209300	MF589634
TSP-80/300	Tanzania	—	X	5	MG209286	MG209301	MF589635
TS-7	Tanzania	Passaged in 1984	X	6	MG209283	MG209298	MF589632
TS-7/27-230	Tanzania	Passaged in 1986	X	6	MG209284	MG209299	MF589633
Nanyuki	Kenya	1960		8	MG209281	MG209296	MF589629
Lipetsk-2016	Russia	2016	II	8	—	—	—
Tambov-2016							
Moscow-2016							
Penza-2016							
Pskov-2016							
Voronezh-2016							
Arkhangelsk-2016							
Kursk-2016							
Krasnodar-2016							

RESULTS AND DISCUSSION

The results of the proteins 5EL, I14L and K11L variability analysis of different ASFV strains showed that the 5EL protein has 13 variable sites, some of which are located in the region of IκB—a similar domain consisting of 3 ankyrin repeats (from 87 to 178 amino acids) and C-terminal end of the P_xI_xI_Tx_C/S motif, which binds the catalytic subunit of calcineurin—serintreonin phosphatase (from 200 to 213 amino acids) (Fig. 1). The ankyrin repetitive proteins although absent in most viruses, are also common among poxviruses. Conservative amino acid residues of ankyrins are crucial for folding and protein stability, and are also involved in the attachment of other proteins to different parts of the cell membrane [24].

Analysis of the I14L protein shows that this protein is conservative among the different ASFV strains, but has 7 variable sites. All proteins encoded by this gene contain a central region with a highly conserved 56-amino acid domain and a PP1c_bdg motif (from 7 to 57 amino

acids in short form and from 123 to 170 amino acids in long form) of the regulatory subunits (15A and 15B) of protein phosphatase 1, located at the carboxyl end, common to MyD116 and ICP34.5. This conserved C-terminus appears to be the binding region of the catalytic subunit of protein phosphatase-1 (PP1C), which is found in both the herpes simplex virus and in mice and humans [25].

The highly glycosylated K11L protein expressed in the cell membrane and on its surface, containing leucine-rich repeats similar to TLR3, is conservative and has no pronounced variability. For him, there are only 8 variable sites, some of which fall into the extracellular topological domain (from 17 to 239 amino acid), as well as the intracellular topological domain (from 261 to 329 amino acid). The transmembrane region of the protein is located at the C-terminus (from 240 to 260 amino acids) (see Fig. 1).

As a result of observations D. Chapman et al. the 5EL and I14L proteins are attributed to variable, in the

Fig. 1. The results of the variability analysis of proteins 5EL—Ik β homolog and inhibitor calcineurin phosphatase; I14L—a protein similar to HSV neurovirulence factor ICP34.5; K11L—a TLR signaling inhibitor protein.

work of V. de Oliveira et al. the K11L protein is conservative, and in the works of J. Neilan et al. and C. Abrams et al., the 5EL protein, on the contrary, was recognized as conservative [12, 26–28]. According to the results of our studies (see Fig. 1), the studied proteins of the ASFV are conservative, especially in comparison with other ASFV variable proteins (CD2V, P54) [29].

In order to determine the genetic relationship at the nucleotide sequences level between ASFV strains and isolates, we performed sequencing and multiple alignment of the concatenated set of *A238L*, *I329L* and *Dp71L* genes.

According to the sequencing results of the *A238L* and *I329L* genes of ASFV isolates, which were isolated on the Russian Federation territory in 2016, are identical to the Georgia_2007/1 parent strain, which is representative of the genotype II.

According to the results obtained for the *I329L* gene, we can divide the existing isolates into 5 main clusters, which correlate with their genotypes (Fig. 2). The first cluster includes strains from South-East

Africa of genotype V, seroimmunotypes 3 and 7, and full-genome isolates Warthog and Pretorisuskop/96/4 genotypes XIX and XX, seroimmunotype 2. The second cluster consists of the representatives genotype II seroimmunotype 8 from the Russian Federation. A separate cluster contains strains of genotype I seroimmunotype 2. The fourth cluster consists of strains and isolates of European origin seroimmunotypes 1 and 4, with the exception of isolate Benin 97/1 of African origin belonging to genotype I. The fifth cluster included strains and isolates of genotype X isolated on the territory of Tanzania and Kenya, as well as some representatives of genotype VIII and IX. Isolates from South Africa and Portugal with indeterminate and seroimmunotype 2 were not included in any of the groups of listed clusters.

An interesting fact is that in 6 East African strains of the genotype X (TKF, Nanyuki, TSP-80, TSP-80/300, TS-7, TS-7/27-230), as in the full-genome isolates Ken05/Tk1, Kenya 1950, Malawi Lil-20/1 and Ken06.Bus strain, the *Dp71L* gene is formed by

Fig. 3. Electrophoregram of the amplification products of the *Dp71L* gene encoding I14L protein. M = 100 b.p.

possible that many mutations that occur inside the host are removed during transmission between them due to the strong purifying selection, primarily due to the mismatch of the cytotoxic host immune response carried out by T-lymphocytes.

In the work of E. de Villiers et al. 4 models of codon replacement (M1, M2, M7 and M8) were investigated. The M2 model identified 14 ASFV genes under positive selection. These include proteins of the 360 and 505 multigenic families, several hypothetical proteins, a CD2v homologue, several enzymes and viral chaperone *B602L* which ensures the correct folding of the basic protein capsid P72. The strictest model for positive selection M8 identified eighteen genes, eight of which are under positive selection and are genes that can participate in modulating the host cell functions [3].

Data analysis of the ratio of non-synonymous and synonymous mutations at a broad level of the genome show several genes that may be under selective pressure.

CONCLUSIONS

The range of group variability of proteins was previously studied by various groups of researchers on the example of the P17 proteins [34], P54 and CD2v [15, 26, 33].

Characteristics of the ASFV genes variability of the selected group are object of great interest for searching for genetic differences and markers of evolutionary variability in immunomodulatory proteins and can also serve to identify target sites in the ASFV genome for vaccine development.

The results obtained from bioinformatics analysis showed that the 5EL and I14L proteins have intracellular regions, the K11L protein has extracellular and intracellular topological domains and a transmembrane region. The presence of single nucleotide substitutions in these sites may indicate a greater conservatism of these proteins.

The degree of nucleotide substitutions of the concatenated *A238L*, *I329L* and *Dp71L* ASFV genes, as determined in this study, revealed a purifying (stabilizing) selection at the level of the nucleotide sequences. As a result of the nucleotide sequences analysis obtained as a result of sequencing of the *A238L*, *I329L* and *Dp71L* genes, it can be concluded that these genes are conservative, with variable regions within them. All Russian ASFV isolates obtained in 2016–2017 have identical sequences of the *A238L* and *I329L* genes, which indicate their common origin. The topology of the phylogenetic tree according to the *I329L* gene completely corresponds with the phylogenetic tree constructed on the basis of a pseudosequence of 7 immunomodulatory genes. Thus, the *I329L* gene may be a genetic marker of common origin. ASFV I14L protein has two forms: a long one (184 amino acids) and a short one (from 70 to 72 amino acids), which is unique among the ASFV variants.

Using the data obtained, it is possible to predict the possible involvement of these genes in changing the level of virulence inherent in the selected strains, as well as the ability to be factors for the ASFV virulence.

FUNDING

This work was carried out within the framework of state task and was supported by the Ministry of Science and Higher Education of the Russian Federation. The work was performed as part of the state assignment 0615-2017-0001.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

AUTHOR CONTRIBUTIONS

Writing the text, collecting and processing the material, performing individual stages of the experimental part by Nefedeva M.V., gene sequencing by Titov I.A., statistical processing of protein variability by Mima K.A., conception and design of the study, and text editing by Malogolovkin A.S.

REFERENCES

1. Sanchez-Vizcaino, J.M., African swine fever, in: *Diseases of Swine*, Straw, B., D'Allaire, S., Mengeling, W., and Taylor, D., Eds., Iowa State Univ., 2006.
2. Rodriguez, J.M., Moreno, L.T., Alejo, A., Lacasta, A., Rodriguez, F., and Salas, M.L., Genome sequence of African swine fever virus BA71, the virulent parental strain of the nonpathogenic and tissue-culture adapted BA71V, *PLoS One*, 2015, vol. 10, no. 11, p. e0142889. <https://doi.org/10.1371/journal.pone.0142889>
3. De Villiers, E.P., Gallardo, C., Arias, M., Da Silva, M., Upton, C., Martin, R., et al., Phylogenomic analysis of 11 complete African swine fever virus genome

- sequences, *Virology*, 2010, vol. 400, pp. 128–136.
<https://doi.org/10.1016/j.virol.2010.01.019>
4. Sanchez-Vizcano, J.M., Mur, L., Gomez-Villamandos, J.C., and Carrasco, J.L., An update on the epidemiology and pathology of African swine fever, *J. Comp. Pathol.*, 2015, vol. 152, pp. 9–21.
<https://doi.org/10.1016/j.jcpa.2014.09.003>
 5. Rock, D.L., Challenges for African swine fever vaccine development—“...perhaps the end of the beginning”, *Vet. Microbiol.*, 2017, vol. 206, pp. 52–58.
 6. Kolbasov, D.V., Balyshev, V.M., and Sereda, A.D., Overall results of the development of African swine fever live vaccines, *Veterinariya*, 2014, vol. 8, pp. 3–8.
 7. King, K., Chapman, D., Argilaguet, J.M., Fishbourne, E., Hutet, E., Cariolet, R., et al., Protection of european domestic pigs from virulent African isolates of African swine fever virus by experimental immunization, *Vaccine*, 2011, vol. 29, no. 28, pp. 4593–4600.
<https://doi.org/10.1159/000170936>
 8. Dixon, L.K., Abrams, C.C., Chapman, D.D., Goatley, L.C., Netherton, C.L., Taylor, G., et al., Prospects for development of African swine fever virus vaccines, *Dev. Biol. (Basel, Switz.)*, 2013, vol. 135, pp. 147–157.
<https://doi.org/10.1159/000170936>
 9. Nix, R.J., Gallardo, C., Hutchings, G., Blanco, E., and Dixon, L.K., Molecular epidemiology of African swine fever virus studied by analysis of four variable genome regions, *Arch. Virol.*, 2006, vol. 151, no. 12, pp. 2475–2494.
<https://doi.org/10.1007/s00705-006-0794-z>
 10. Dixon, L.K., Baylis, S.A., Vydeingum, S., Twigg, S.R.F., Hammond, J.M., Hingamp, P.M., et al., African swine fever virus genome content and variability, *Arch. Virol.*, 1993, vol. 7, pp. 185–199.
https://doi.org/10.1007/978-3-7091-9300-6_15
 11. Dixon, L.K., Abrams, C.C., Bowick, G., Goatley, L.C., Kay-Jackson, P.C., Chapman, D., et al., African swine fever virus proteins involved in evading host defense systems, *Vet. Immunol. Immunopathol.*, 2004, vol. 100, nos. 3–4, pp. 117–134.
<https://doi.org/10.1016/j.vetimm.2004.04.002>
 12. De Oliveira, V.L., Almeida, S.C., Soares, H.R., Crespo, A., Marshall-Clarke, S., and Parkhouse, R.M.E., A novel TLR3 inhibitor encoded by African swine fever virus (ASFV), *Arch. Virol.*, 2011, vol. 156, pp. 597–609.
<https://doi.org/10.1007/s00705-010-0894-7>
 13. Zsak, L., Lu, Z., Kutish, G.F., Neilan, J.G., and Rock, D.L., An African swine fever virus virulence-associated gene NL-S with similarity to the herpes simplex virus ICP34.5 gene, *J. Virol.*, 1996, vol. 70, no. 12, pp. 8865–8871.
 14. Granja, A.G., Perkins, N.D., and Revilla, Y., A238L inhibits NF-ATc2, NF-kappa B, and c-Jun activation through a novel mechanism involving protein kinase C-theta-mediated up-regulation of the amino-terminal transactivation domain of p300, *J. Immunol.*, 2008, vol. 180, no. 4, pp. 2429–2442.
<https://doi.org/10.4049/jimmunol.1490049>
 15. Chapman, D.A., Tcherepanov, V., Upton, C., and Dixon, L.K., Comparison of the genome sequences of non-pathogenic and pathogenic African swine fever virus isolates, *J. Gen. Virol.*, 2008, vol. 89, no. 2, pp. 397–408.
<https://doi.org/10.1099/vir.0.83343-0>
 16. Garcia-Boronat, M., Diez-Rivero, C.M., Reinherz, E.L., and Reche, P.A., PVS: A web server for protein sequence variability analysis tuned to facilitate conserved epitope discovery, *Nucleic Acids Res.*, 2008, vol. 36, pp. 35–41.
<https://doi.org/10.1093/nar/gkn211>
 17. Nielsen, H., Predicting secretory proteins with signal P, in *Protein Function Prediction*, Kihara, D., Ed., Springer, 2017, pp. 59–73.
https://doi.org/10.1007/978-1-4939-7015-5_6
 18. Dobson, L., Remenyi, I., and Tusnady, G.E., CCTOP: A Consensus Constrained TOPology prediction web server, *Nucleic Acids Res.*, 2015, vol. 43, no. 1, pp. 408–412.
<https://doi.org/10.1093/nar/gkv451>
 19. Gallardo, C., Mwaengo, D.M., Macharia, J.M., Arias, M., Taracha, E.A., Soler, A., et al., Enhanced discrimination of African swine fever virus isolates through nucleotide sequencing of the p54, p72, and pB602L (CVR) genes, *Virus Genes*, 2009, vol. 38, no. 1, pp. 85–95.
<https://doi.org/10.1007/s11262-008-0293-2>
 20. Bastos, A.D., Penrith, M.L., Cruciere, C., Edrich, J.L., Hutchings, G., Roger, F., et al., Genotyping field strains of African swine fever virus by partial p72 gene characterization, *Arch. Virol.*, 2003, vol. 148, no. 4, pp. 693–706.
<https://doi.org/10.1007/s00705-002-0946-8>
 21. Sereda, A.D. and Balyshev, V.M., Antigenic diversity of African swine fever virus, *Vopr. Virusol.*, 2011, vol. 56, no. 4, pp. 38–42.
 22. Edgar, R.C., MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Res.*, 2004, vol. 32, no. 5, pp. 1792–1797.
<https://doi.org/10.1093/nar/gkh340>
 23. Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S., MEGA6: Molecular Evolutionary Genetics Analysis version 6.0., *Mol. Biol. Evol.*, 2013, vol. 30, no. 12, pp. 2725–2729.
<https://doi.org/10.1093/molbev/mst197>
 24. Mosavi, L.K., Cammett, T.J., Desrosiers, D.C., and Peng, Z.Y., The ankyrin repeat as molecular architecture for protein recognition, *Protein Sci.*, 2004, vol. 13, no. 6, pp. 1435–1448.
<https://doi.org/10.1110/ps.03554604>
 25. Jousse, C., Oyadomari, S., Novoa, I., Lu, P.D., Zhang, Y., Harding, H.P., et al., Inhibition of a constitutive translation initiation factor 2a phosphatase, CREP, promotes survival of stressed cells, *J. Cell Biol.*, 2003, vol. 163, pp. 767–775.
<https://doi.org/10.1083/jcb.200308075>
 26. Chapman, D.A., Darby, A.C., Da Silva, M., Upton, C., Radford, A.D., and Dixon, L.K., Genomic analysis of highly virulent Georgia 2007/1 isolate of African swine fever virus, *Emerging Infect. Dis.*, 2011, vol. 17, no. 4, pp. 599–605.
<https://doi.org/10.3201/eid1704.101283>
 27. Neilan, J.G., Lu, Z., Kutish, G.F., Zsak, L., Lewis, T.L., and Rock, D.L., A conserved African swine fever virus I kappa B homolog, Sel, is nonessential for growth in

- vitro and virulence in domestic swine, *Virology*, 1997, vol. 235, no. 2, pp. 377–385.
<https://doi.org/10.1006/viro.1997.8693>
28. Abrams, C.C., Chapman, D.A., Silk, R., Liverani, E., and Dixon, L.K., Domains involved in calcineurin phosphatase inhibition and nuclear localization in the African swine fever virus A238L protein, *Virology*, 2008, vol. 374, no. 2, pp. 477–486.
<https://doi.org/10.1371/journal.pbio.1001492>
 29. Mima, K.A., Burmakina, G.S., Titov, I.A., and Malogolovkin, A.S., African swine fever virus glycoproteins p54 and CD2v in the context of immune response modulation: Bioinformatic analysis of genetic variability and heterogeneity, *S-kh. Biol.*, 2015, vol. 50, no. 6, pp. 785–793.
<https://doi.org/10.15389/agrobiol.2015.6.785rus>
 30. Bishop, R.P., Fleischauer, C., de Villiers, E.P., Okoth, E.A., Arias, M., Gallardo, C., et al., Comparative analysis of the complete genome sequences of Kenyan African swine fever virus isolates within p72 genotypes IX and X, *Virus Genes*, 2015, vol. 50, no. 2, pp. 303–390.
<https://doi.org/10.1007/s11262-014-1156-7>
 31. Brown, J.R., Douady, C.J., Italia, M.J., Marshall, W.E., and Stanhope, M.J., Universal trees based on large combined protein sequence data sets, *Nat. Genet.*, 2001, vol. 28, pp. 281–285.
<https://doi.org/10.1038/90129>
 32. Miya, M. and Nishida, M., Use of mitogenomic information in teleostean molecular phylogenetics: A tree-based exploration under the maximum-parsimony optimality criterion, *Mol. Phylogenet. Evol.*, 2000, vol. 17, pp. 437–455.
<https://doi.org/10.1006/mpev.2000.0839>
 33. Rowlands, R.J., Duarte, M.M., Boinas, F., Hutchings, G., and Dixon, L.K., The CD2v protein enhances African swine fever virus replication in the tick vector, *Ornithodoros erraticus*, *Virology*, 2009, vol. 393, no. 2, pp. 319–328.
<https://doi.org/10.1016/j.virol.2009.07.040>
 34. Suarez, C., Gutierrez-Berzal, J., Andres, G., Salas, M.L., and Rodriguez, J.M., African swine fever virus protein p17 is essential for the progression of viral membrane precursors toward icosahedral intermediate, *J. Virol.*, 2010, vol. 84, no. 15, pp. 7484–7499.
<https://doi.org/10.1128/JVI.00600-10>