REVIEW
DOI: https://doi.org/10.36233/0507-4088-360
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The application of pseudotyped viruses based on vesicular stomatitis virus (*Rhabdoviridae*, *Vesiculovirus*) in order to study the interaction of viruses with cells

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Abstract
Investigating the mechanisms of viral attachment and entry into cells is crucial for understanding viral pathogenesis and developing therapeutic strategies. The **aim** of this review is to characterize pseudo-typed particles based on the vesicular stomatitis virus (VSV) as a convenient and effective tool for studying viral entry into cells, based on literature data (PubMed, Scopus, and Web of Science), and to determine the prospects for combining this method with genetic and protein-based approaches.

VSV, a member of the *Rhabdoviridae* family, has a remarkable capacity for pseudotyping, which involves the replacement of its native glycoprotein (G) with envelope proteins from other viruses. This feature enables the modeling of the cell entry process without the need for wild-type viruses. The VSV genome is modified by deleting the G gene and incorporating reporter genes (e.g., GFP or luciferase), thereby facilitating the quantitative assessment of infectivity.

The methodology for generating pseudoviruses involves a two-plasmid cotransfection system in cell lines (e.g., HEK293T), with plasmids encoding the VSV structural proteins and the target viral envelope proteins. The advantages of the VSV system include high particle titers, rapid reporter signal manifestation, and the feasibility of work under Biosafety Level 2 conditions. However, limitations are associated with differences in the distribution of viral proteins on the surface of pseudoviruses compared to native virions, necessitating additional data validation.

Conclusion. Methods for analyzing virus-cell interactions were studied, such as Virus Overlay Protein Binding Assay (VOPBA), RNA interference, CRISPR/Cas9 knockout, and gene overexpression. These approaches allow for the identification of cellular receptors, investigation of specific protein functions, and assessment of the impact of mutations. Future prospects for the application of VSV pseudoviruses include screening viral entry inhibitors, analyzing antibody neutralization, and vaccine development. Despite technical limitations, pseudotyped particles remain an indispensable tool for studying highly pathogenic and fastidious viruses. For the present review, a literature search was conducted in the PubMed, Scopus, and Web of Science databases.

Keywords: *Pseudotyped viral particles; vesicular stomatitis virus; cellular receptors; viral tropism; viral entry mechanisms; HEK293T; review*

For citation: Akimov N.O., Dolgova A.S. The application of pseudotyped viruses based on vesicular stomatitis virus (*Rhabdoviridae*, *Vesiculovirus*) in order to study the interaction of viruses with cells. *Problems of Virology* (*Voprosy Virusologii*). 2026; 71(1): 13–20. DOI: https://doi.org/10.36233/0507-4088-360
EDN: https://elibrary.ru/bnqvme

Funding. This study was not supported by any external sources of funding.
Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

НАУЧНЫЙ ОБЗОР
DOI: https://doi.org/10.36233/0507-4088-360

Применение псевдотипированных частиц на основе вируса везикулярного стоматита (*Rhabdoviridae*, *Vesiculovirus*) с целью изучения взаимодействия вирусов с клетками

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Резюме
Изучение механизмов прикрепления и проникновения вирусов в клетки является критически важным для понимания их патогенеза и разработки терапевтических стратегий.
Цель обзора – на основании литературных данных (PubMed, Scopus, Web of Science) охарактеризовать псевдотипированные частицы на основе ВВС как удобный и действенный инструмент для исследования вирусного входа в клетку, а также определить перспективы сочетания этого метода с генетическими и белковыми подходами.
ВВС, относящийся к семейству *Rhabdoviridae*, обладает важной способностью к псевдотипированию – замене собственного гликопротеина (G) на поверхностные белки других вирусов. Эта особенность позволяет моделировать процесс инфицирования клеток без использования вирусов дикого типа. Геном ВВС модифицируется путем удаления гена G и введения репортерных генов (например, GFP или люциферазы), что упрощает количественную оценку инфекционности. Методология создания псевдовирусов включает двухэтапную систему котрансфекции клеточных линий (например, HEK293T) плазмидами, кодирующими структурные белки ВВС и целевые белки оболочки изучаемых вирусов. Преимущества системы ВВС включают высокий титр частиц, быстрое проявление репортерных сигналов и возможность работы в условиях биобезопасности уровня II. Однако ограничения связаны с различиями в распределении вирусных белков на поверхности псевдовирусов и нативных вирионов, что требует дополнительной валидации данных.
Заключение. Изучены методы анализа взаимодействий «вирус–клетка», такие как VOPBA, РНК-интерференция, CRISPR/Cas9-нокаут и сверхэкспрессия генов. Эти подходы позволяют идентифицировать клеточные рецепторы, изучать роль конкретных белков и оценивать влияние мутаций. Перспективы применения псевдовирусов ВВС включают скрининг ингибиторов проникновения вирусов в клетки, анализ нейтрализации антител и разработку вакцин. Несмотря на технические ограничения, псевдотипированные частицы остаются незаменимым инструментом для изучения высокопатогенных и труднокультивируемых вирусов.

Ключевые слова: *псевдотипированные вирусные частицы; вирус везикулярного стоматита; клеточные рецепторы; вирусный тропизм; механизмы проникновения вирусов; HEK293T; обзор*

Для цитирования: Акимов Н.О., Долгова А.С. Применение псевдотипированных частиц на основе вируса везикулярного стоматита (*Rhabdoviridae*, *Vesiculovirus*) с целью изучения взаимодействия вирусов с клетками. *Вопросы вирусологии*. 2026; 71(1): 13–20. DOI: https://doi.org/10.36233/0507-4088-360 EDN: https://elibrary.ru/bnqvme

Финансирование. Авторы заявляют об отсутствии внешнего финансрования при проведении исследования.
Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Introduction

Viruses are among the main human pathogens causing diseases of varying severity. The first stage of interaction between viral particles and cells is their attachment and penetration, which is facilitated by specific factors of the host cell. Several such factors are often involved in the process of virus attachment and penetration: receptors and co-receptors. Understanding the mechanisms of attachment and penetration of individual viruses is necessary to establish virus tropism, understand their pathogenesis, and could help in the search for antiviral drugs and the development of neutralizing antibodies.

Pseudotyped viral particles (pseudoviruses) can be used as models of highly pathogenic viruses, rare viruses, and viruses that are difficult to cultivate to study virus-cell interactions. Pseudoviruses can be used to model the process of cell infection without resorting to the use of wild-type viruses. Pseudotyped viral particles are non-pathogenic to humans or genetically modified enveloped viruses with envelope proteins from another virus on their surface. Examples include vesicular stomatitis virus (VSV) or mouse leukemia virus in the first case and inactivated human immunodeficiency virus-1 (HIV-1) in the second. The genes of the envelope proteins of the original viruses are usually replaced with reporter genes (e.g., genes for green fluorescent protein GFP, red fluorescent protein mCherry, luciferase), which allows the efficiency of cell infection to be assessed. Pseudoviruses are obtained by

co-transfection of plasmid vectors encoding structural and non-structural proteins of the packaging virus, as well as envelope proteins of pathogenic viruses. Pseudoviruses infect susceptible cells, but are only able to replicate for one cycle [1].

VSV is transmitted by arthropods and affects cattle, rodents, horses, and pigs. Human infection with VSV is rare, mainly affecting people who come into contact with livestock and laboratory workers who come into direct contact with VSV. VSV infection in humans is asymptomatic or causes mild flu-like symptoms.

The aim of this review is to characterize pseudo-typed particles based on the vesicular stomatitis virus (VSV) as a convenient and effective tool for studying viral entry into cells, based on literature data (PubMed, Scopus, and Web of Science), and to determine the prospects for combining this method with genetic and protein-based approaches [2].

Structure and life cycle of the vesicular stomatitis virus

VSV belongs to the family *Rhabdoviridae* and has a bullet-shaped virion with negative single-stranded RNA approximately 11 kB in size, encoding five structural proteins (**Fig. 1**) [3–5]. The N-protein forms a nucleoprotein complex with RNA and protects the viral RNA from the action of host cell nucleases [6, 7]. Protein L is an RNA-dependent RNA polymerase that mediates transcription and replication of the VSV genome [8, 9]. Protein P is an auxiliary cofactor of protein L [10]. Protein M suppresses the transcription of cellular genes and promotes the formation of the bullet-shaped virion [11, 12]. The surface glycoprotein G mediates entry into the cell via clathrin-dependent endocytosis. The receptor for attachment is the high-density lipoprotein receptor (LDLR). The widespread distribution of the LDLR receptor mediates the broad tropism of VSV [13–15]. After VSV genetic material enters the cytoplasm, further processes of replication, transcription, assembly, and release of new virions occur (**Fig. 2**).

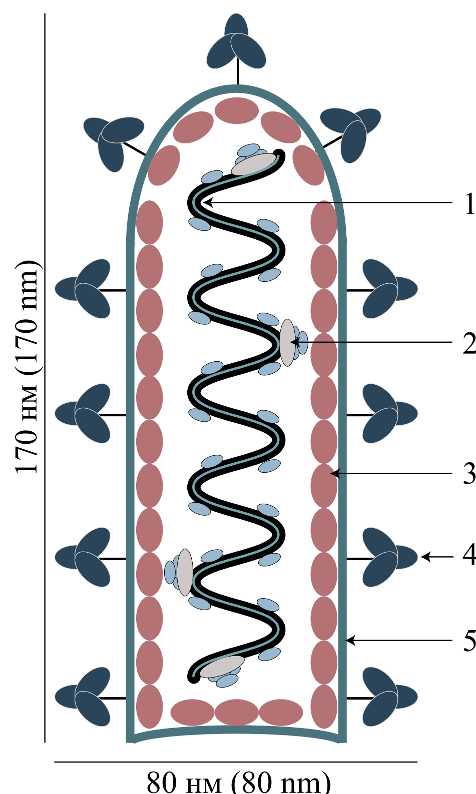

Fig. 1. Structure of vesicular stomatitis virus.

1 – nucleoprotein complex of N-protein and VSV RNA; 2 – complex of proteins L and P; 3 – protein M; 4 – glycoprotein G; 5 – lipid membrane.

Рис. 1. Строение вируса везикулярного стоматита.

1 – нуклеопротеиновый комплекс N-белка и РНК ВВС; 2 – комплекс белков L и P; 3 – белок M; 4 – гликопротеин G; 5 – липидная мембрана.

The principle of creating pseudotyped viral particles based on the vesicular stomatitis virus

VSV-based pseudoviruses are recombinant virions incapable of replication. The G protein gene has been removed from their genome and replaced with a reporter gene (e.g., GFP or luciferase) for detecting cell infection [15, 16].

A two-step generation system is usually used to produce VSV pseudoviruses.

Obtaining VSV ΔG/G reporter particles. Cells expressing bacteriophage T7 polymerase (usually HEK293T cells) are co-transfected with four plasmids carrying VSV structural protein genes and one plasmid carrying a defective VSV genome, where the G protein gene is replaced with a reporter gene: pVSV-G, pVSV-N, pVSV-P, VSV-L, VSV-dG-reporter gene. All plasmids contain the T7 promoter. T7 polymerase expression is achieved by co-infecting cells with vaccinia virus or by using a cell line that stably expresses T7 polymerase. As a result, VSV ΔG/G particles are assembled, carrying the G protein in their envelope but not containing its gene in their genome (**Fig. 3**) [1, 17–19].

Pseudotyping. Another cell line (usually HEK293T) is transfected with a plasmid expressing the envelope protein of another virus (X) and infected with the resulting ΔG/G VSV particles. During assembly and budding from the cell, new virions incorporate protein X into their envelope, forming VSV ΔG/X pseudoviruses containing the viral protein of interest [18–20]. To obtain pseudoviruses containing protein X, co-transfection with six plasmids is performed in one stage: pVSV-X (envelope protein gene of the virus of interest), pVSV-N, pVSV-P, VSV-L, VSV-ΔG-reporter, and a plasmid expressing T7 polymerase (e.g., pCAG-T7pol). This results in VSV ΔG/X particles, but their yield is usually lower than with a two-step generation system (**Fig. 4**) [17, 21].

Advantages and disadvantages of using pseudotyped particles in virus-cell interaction studies

Advantages. The application of pseudoviruses allows highly pathogenic viruses (e.g., Congo-Crimean hemorrhagic fever virus, CCHF) to be studied in biosafety level II laboratories, whereas research on wild CCHF virus requires biosafety level IV [22]. Many wild-type virus-

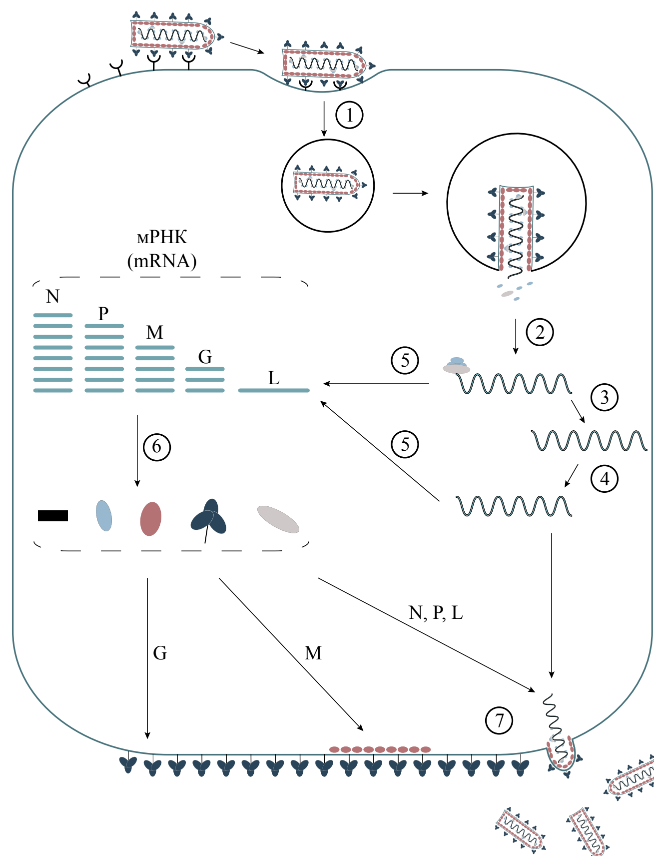

Fig. 2. Life cycle of vesicular stomatitis virus.

1 – attachment and penetration of VSV. The main receptor for VSV entry is LDLR; 2 – release of the VSV genome into the cytoplasm; 3, 4 – replication of the VSV genome; 5 – primary and secondary transcription of the genome producing mRNA for each VSV protein; 6 – translation of proteins N, P, M, L occurs in the cytoplasm; translation of G protein occurs on ER ribosomes, then glycoprotein G is processed and transported to the cell membrane; protein M is transported beneath the cell membrane; protein N forms the nucleoprotein complex with VSV RNA; proteins L and P also form complexes with each other; 7 – assembly and release of mature VSV virions.

Рис. 2. Жизненный цикл вируса везикулярного стоматита.

1 – прикрепление и проникновение ВВС. Основным рецептором входа ВВС является LDLR; 2 – выход генома ВВС в цитоплазму; 3, 4 – репликация генома ВВС; 5 – первичная и вторичная транскрипция генома с образованием мРНК каждого белка ВВС; 6 – трансляция белков N, P, M, L происходит в цитоплазме, трансляция G-белка происходит на рибосомах эндоплазматического ретикулума, затем гликопротеин G процессируется, встраивается и переносится на клеточную мембрану, белок M транспортируется под клеточную мембрану, белок N образует нуклеопротеиновый комплекс с РНК ВВС, белки L и P также образуют между собой комплексы; 7 – сборка и выход зрелых вирионов ВВС.

es are difficult or impossible to cultivate in standard cell lines [23], whereas pseudoviruses are easily produced in widely available cell lines, such as HEK293T [24–26]. Pseudoviruses are useful for studying mutations in envelope proteins of viruses with high genetic variability, such as hepatitis C virus [27]. The presence of reporter genes in the genetic material of pseudoviruses allows for simple qualitative and quantitative assessment of cell infection [28–30]. The main advantage of the VSV packaging system over packaging systems based on murine leukemia virus and HIV-1 is the higher yield of pseudoviruses. VSV also has faster genome replication, which contributes to faster detection of infection due to rapid expression of the reporter gene [31].

Disadvantages. Pseudoviruses are only suitable for studying enveloped viruses and only for the early stages of infection: attachment and penetration. Differences in the morphology of the virus of interest and the virus used for pseudotyping (VSV, HIV-1, etc.) leads to differences in the distribution of the same envelope protein on different viral particles, which can lead to false results in experiments. Therefore, it is advisable to confirm the results of experiments with pseudoviruses using authentic viruses [31, 32].

Methods for studying virus-cell interactions using pseudotyped viral particles

Interactions between proteins play a key role in understanding the mechanisms of viral infections. Protein-based methods allow the detection of protein-protein interactions between glycoproteins of the viral envelope of interest, embedded in the membrane of the packaging virus, and host cell proteins. To this end, other techniques are used in conjunction with pseudotyped viruses. One such approach used in conjunction with pseudoviruses is a method called virus overlay protein binding assays (VOPBA). This method involves the separation of cell extract (membrane protein extract can be used to exclude the interaction of the viral receptor with intracellular proteins) by electrophoresis, followed by transfer to a

DOI: https://doi.org/10.36233/0507-4088-360

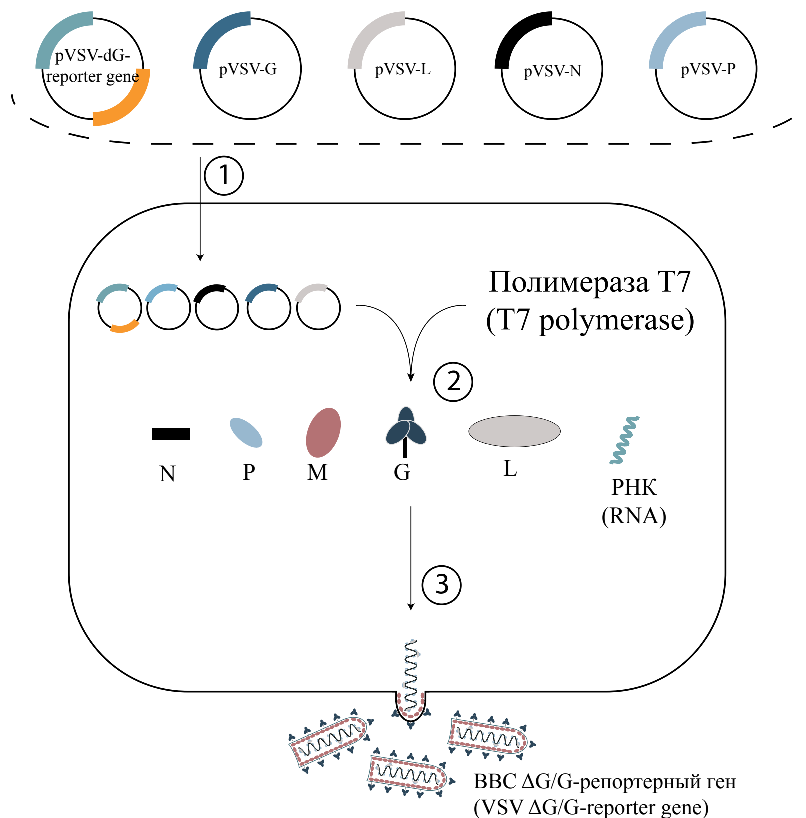

Fig. 3. Production of VSV-ΔG/G particles.

1 – cotransfection with plasmids: pVSV-G, pVSV-N, pVSV-P, VSV-L, VSV-dG-reporter gene; 2 – formation of VSV proteins; 3 – assembly of VSV ΔG/G virions.

Рис. 3. Получение частиц ВВС ΔG/G.

1 – котрансфекция плазмидами: pVSV-G, pVSV-N, pVSV-P, VSV-L, VSV-dG-репортерный ген; 2 – образование белков ВВС; 3 – сборка вирионов ВВС ΔG/G.

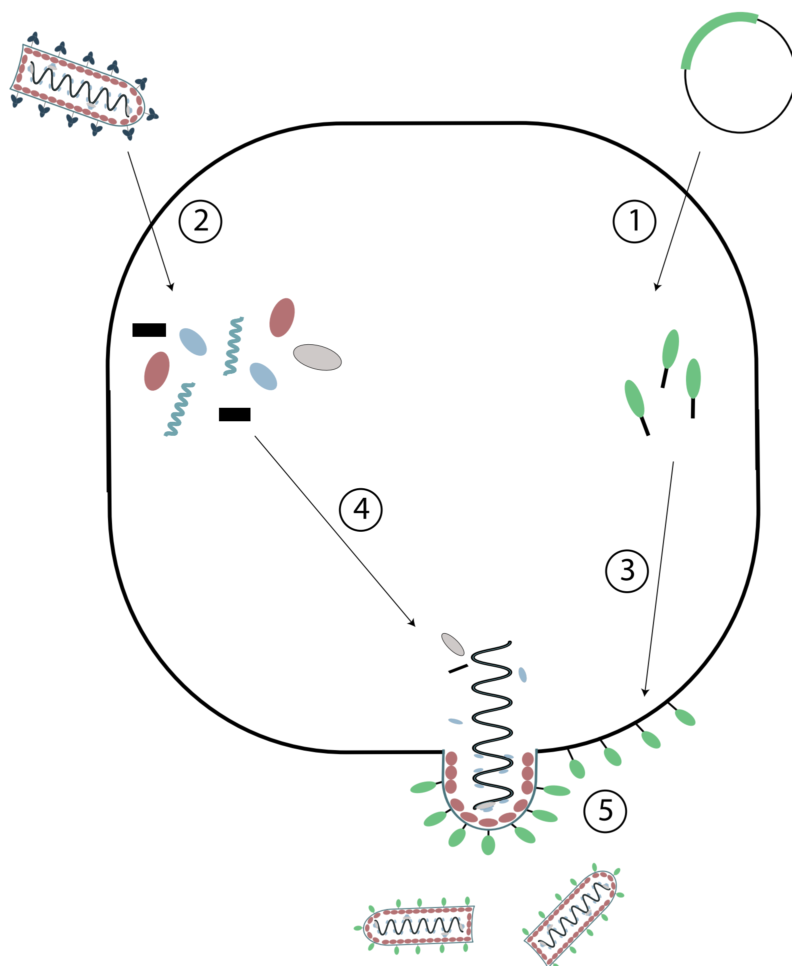

Fig. 4. Production of VSV pseudoviruses with envelope protein X of the virus of interest (VSV ΔG/X-reporter gene).

1 – transfection with plasmid expressing the envelope protein of virus of interest X; 2 – infection of VSV ΔG/G; 3 – incorporation of protein X into the cell membrane; 4 – assembly of VSV ΔG/X pseudoviruses.

Рис. 4. Получение псевдовирусов ВВС с белком оболочки X вируса интереса (ВВС ΔG/X-репортерный ген).

1 – трансфекция плазмидой, экспрессирующей оболочечный белок вируса интереса X; 2 – инфекция ВВС ΔG/G; 3 – встраивание белка X в мембрану клетки; 4 – сборка псевдовирусов ВВС ΔG/X.

membrane using Western blotting and treatment of the membrane with wild-type virus [33], inactivated virus [34], or pseudovirus particles [35]. The proteins to which the virus binds are then visualized using virus-specific antibodies. The protein itself is further identified by mass spectrometry. C.-M. Chan et al. used this method to identify CEACAM5 as an additional target of the Middle East respiratory syndrome coronavirus (MERS-CoV). In this study, HIV-1-based pseudovirus particles pseudotyped with the S protein of the MERS-CoV virus were used [35].

Genetic approaches can either weaken a cell function (to the point of loss) or strengthen it (to the point of introducing a new function that the cell did not previously possess). Both cell lines and animals can be genetically modified [36–38]. RNA interference is the temporary removal of mRNA encoding a specific protein. In mammalian cells, this effect is achieved by using small interfering RNAs (siRNAs) [39], which are introduced by direct transfection, or short hairpin RNAs (shRNAs), which are introduced by lentivirus transduction [40, 41]. The advantage of RNA interference is its simplicity, and this approach also allows the study of important genes, since, unlike genetic knockout, the gene is not completely switched off and cells can survive when the gene is switched off. To identify the role of the ZMPSTE24 protein in the process of HIV-1 infection of cells pseudotyped with the SARS-CoV-2 S protein, K. Shilagardi et al. used small interfering RNA to knock down the gene encoding this protein in HEK293T cells [42]. In a study by Y.-Q. Zhou et al., Arf6 protein knockdown was used to assess its involvement in infection caused by the VSV-SARS-CoV-2 pseudovirus [43]. In a study by M.-M. Zhao et al., cathepsin L knockdown was also used to determine its role in SARS-CoV-2 pseudovirus infection [44].

Genetic knockout is the complete shutdown of a gene. One way to knock out genes is to use CRISPR/Cas9 technology [45, 46]. This system is an adaptive immune system found in archaea and bacteria [47]. To switch off a specific gene, sgRNA is used, which is a complex of crRNA and tracrRNA. crRNA is an RNA segment consisting of 20 nucleotides complementary to the gene segment that needs to be switched off, and tracrRNA binds to crRNA and ensures recognition and binding by the Cas9 protein, which introduces a double-strand break in the gene targeted by crRNA. The resulting break is repaired by non-homologous end joining, which results in random insertions or deletions, leading to gene dysfunction [48]. In a study by Z.-S. Xu et al., the LDLR gene was knocked out to study its role in the penetration of the CCHF 36 virus. H. Tani et al. used embryonic stem cells with a knockout of the alpha-dystroglycan receptor and infected them with VSV, pseudotyped with envelope proteins of various arenaviruses [49]. K. Shilagardi et al. used HeLa and HEK293T cell lines in which the ZMPSTE24 protein gene was knocked out to identify its role in the mechanism of viral infection mediated by HIV-1, pseudotyped with the SARS-CoV-2 S protein [42].

To establish the role of a particular protein in the viral infection process, overexpression of the gene encoding that protein can be used. Temporary overexpression of the protein can be achieved by transfecting a cell line with plasmid vectors encoding these proteins. K. Shilagardi et al. transfected HEK293T cells with plasmids overexpressing ZMPSTE24 and IFITM proteins to identify their role in the infection of cells by HIV-1-based lentiviral vectors pseudotyped with the SARS-CoV-2 S protein [42], and found that overexpression of these proteins reduces cell infection. For constant overexpression of a protein, retrovirus transduction carrying the gene for that protein is used. Z.-S. Xu et al. transduced SW13 cells deficient in the *LDLR* receptor with a retrovirus carrying the LDLR gene to restore its expression [36].

Reporter systems for visualizing pseudotyped viral particles based on vesicular stomatitis virus

Fluc is one of the most common reporter genes. This gene encodes the firefly luciferase enzyme, which catalyzes the reaction of D-luciferin with adenosine triphosphate (ATP). As a result of successive reactions, oxyluciferin is formed, which is in an unstable state, and when it transitions to its ground state, a quantum of light (luminescence) is emitted [50]. This reporter system has been used to study the mechanisms of penetration of viruses belonging to the families *Arenaviridae* and *Bunyaviridae* [22, 51], Puumala and Hantaan viruses [52], and other viruses. The disadvantage of this reporter system is the relatively high cost of the luciferase substrate, luciferin, as well as the necessity for a luminometer.

Fluorescent proteins are used as alternative reporter genes, the most common of which is GFP. GFP is used to determine the cellular tropism of the Ebola virus using the pseudovirus system [13, 26], to study the functions of the E1 and E2 envelope glycoproteins of the hepatitis C virus, E1 and E2 of the Chikungunya virus [53, 54], and glycoproteins of other viruses. The advantage of GFP and other fluorescent proteins over the luciferase reporter system is the absence of an expensive substrate, but a fluorescence microscope is required to visualize the fluorescence. Other reporter genes encoding fluorescent proteins are also used, such as mCherry, RFP, YFP, etc [29, 55, 56].

Conclusion

The application of pseudotyped viral particles based on VSV is a convenient and powerful tool for studying the mechanisms of virus entry into cells. They can be used to model the processes of virus attachment and entry. By replacing the VSV glycoprotein G with the envelope proteins of viruses of interest, it is possible to study their tropism and the role of surface proteins in biosafety level II laboratories.

The main advantages of the VSV-based packaging system are packaging efficiency and ease of infectivity assessment due to the rapid expression of reporter genes (GFP, luciferase, etc.). However, the use of pseudoviruses may distort the results due to differences in the distribution of viral proteins on the surface of pseudovirus virions and wild-type viruses. Therefore, to obtain reliable results, verification using authentic viruses is recommended.

The combination of pseudoviruses with genetic approaches (knockout, knockdown, overexpression) and protein-based approaches (VOPBA) allows studying the role of cellular factors in the processes of virus attachment and penetration, identifying therapeutic targets.

The prospects for this method include the creation of platforms for screening virus entry inhibitors, studying the effect of surface virus protein mutations on virus pathogenicity, and creating vaccines based on pseudoviruses.

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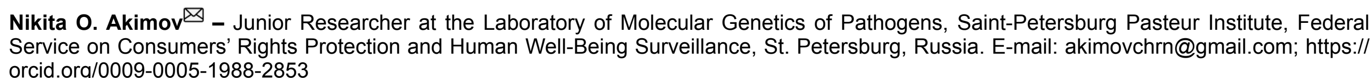

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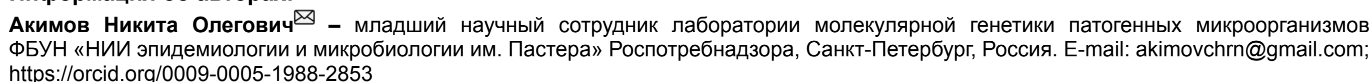
Contribution: Akimov N.O. – concept of the work, writing the text; Dolgova A.S. – editing.

Received 14 November 2025
Accepted 09 January 2026
Published 28 February 2026

Информация об авторах:



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Участие авторов: Акимов Н.О. – концепция работы, написание текста; Долгова А.С. – научное редактирование.

Поступила 14.11.2025
Принята в печать 09.01.2026
Опубликована 28.02.2026