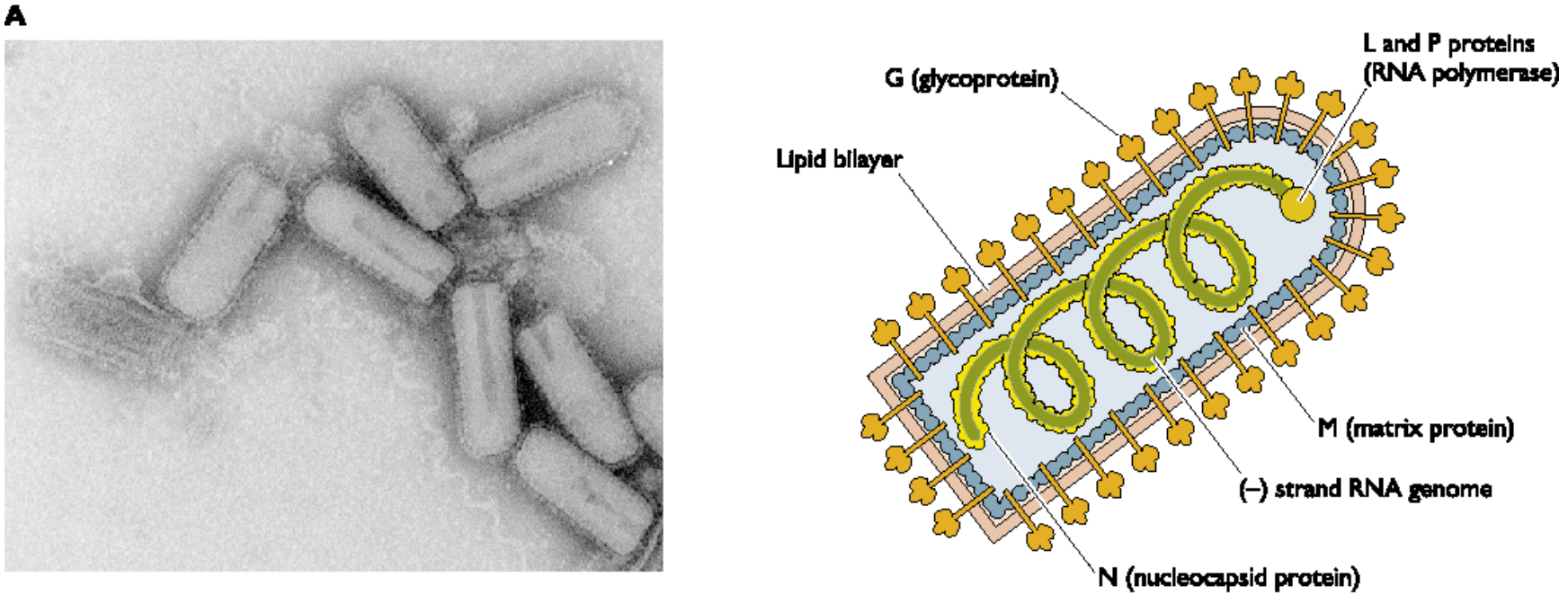
VIROLOGY

Engineering Viral Genomes: VSV Vectors

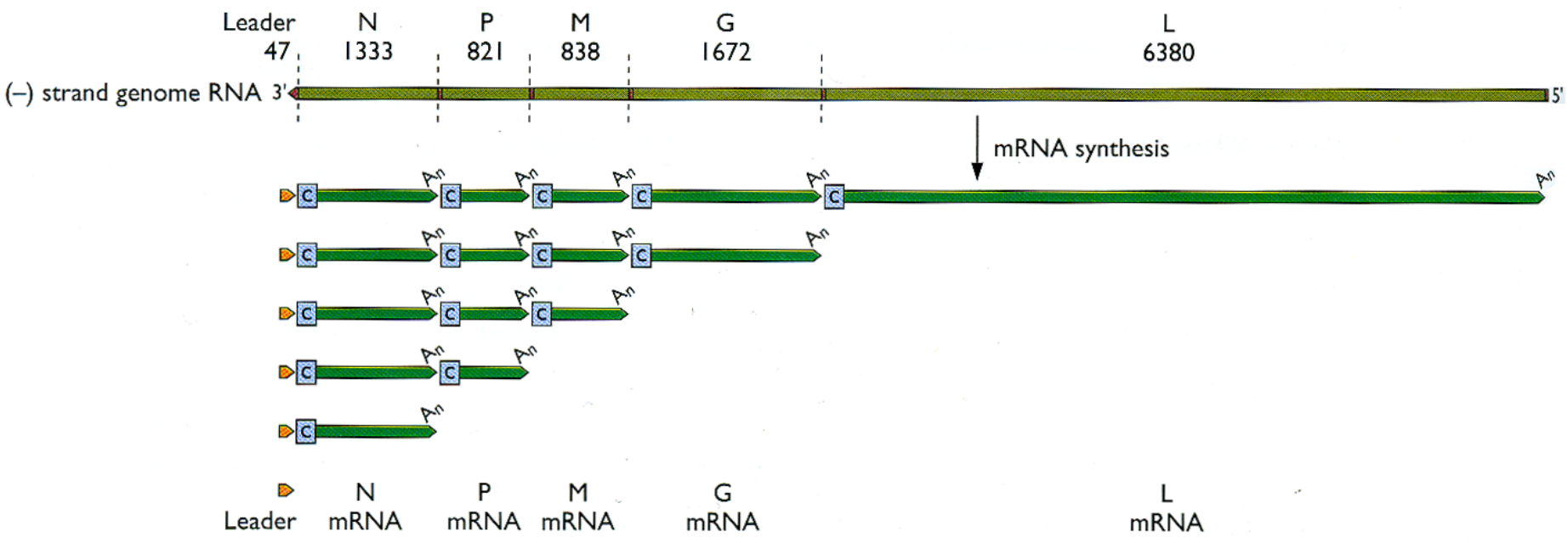
Viral vectors

Virus	Insert size	Integration	Duration of expression	Advantages	Potential disadvantages
Adeno-associated virus	~4.5–9 (?) kb	Low efficiency	Long	Nonpathogenic, episomal, infects nondividing cells	Immunogenic, toxicity, small packaging limit
Adenovirus	2–38 kb	No	Short	Efficient gene delivery, infects nondividing cells	Transient, immunogenic
Alphavirus	~5 kb	No	Short	Broad host range, high level expression	Virulence
Epstein-Barr virus	~120 kb	No; episomal	Long	High capacity, episomal, long-term expression	
Gammaretrovirus	1–7.5 kb	Yes	Shorter than formerly	Stable integration	May rearrange genome, insertional mutagenesis require cell division
Herpes simplex virus	~30 kb	No	Long in central nervous system, short elsewhere	Infects nondividing cells; neurotropic, large capacity	Virulence, persistence in neurons, immunogenic
Lentivirus	7–18 kb	Yes	Long	Stable integration; infects nondividing and terminally differentiated mammalian cells	Insertional mutagenesis
Poliovirus	~300 bp for helper-free virus; ~3 kb for defective virus	No	Short	Excellent mucosal immunity	Limited capacity; reversio to neurovirulence
Rhabdovirus	Unknown	No	Short	High-level expression, rapid cell killing	Virulence, highly cytopathic
Vaccinia virus	At least ~25 kb, probably ~75–100 kb	No	Short	Wide host range, ease of isolation, large capacity, high-level expression	Transient, immunogenic

Structure and genome organization of the **Rhabdovirus** **V**esicular **S**tomatitis **V**irus: an example of **Class V virus**



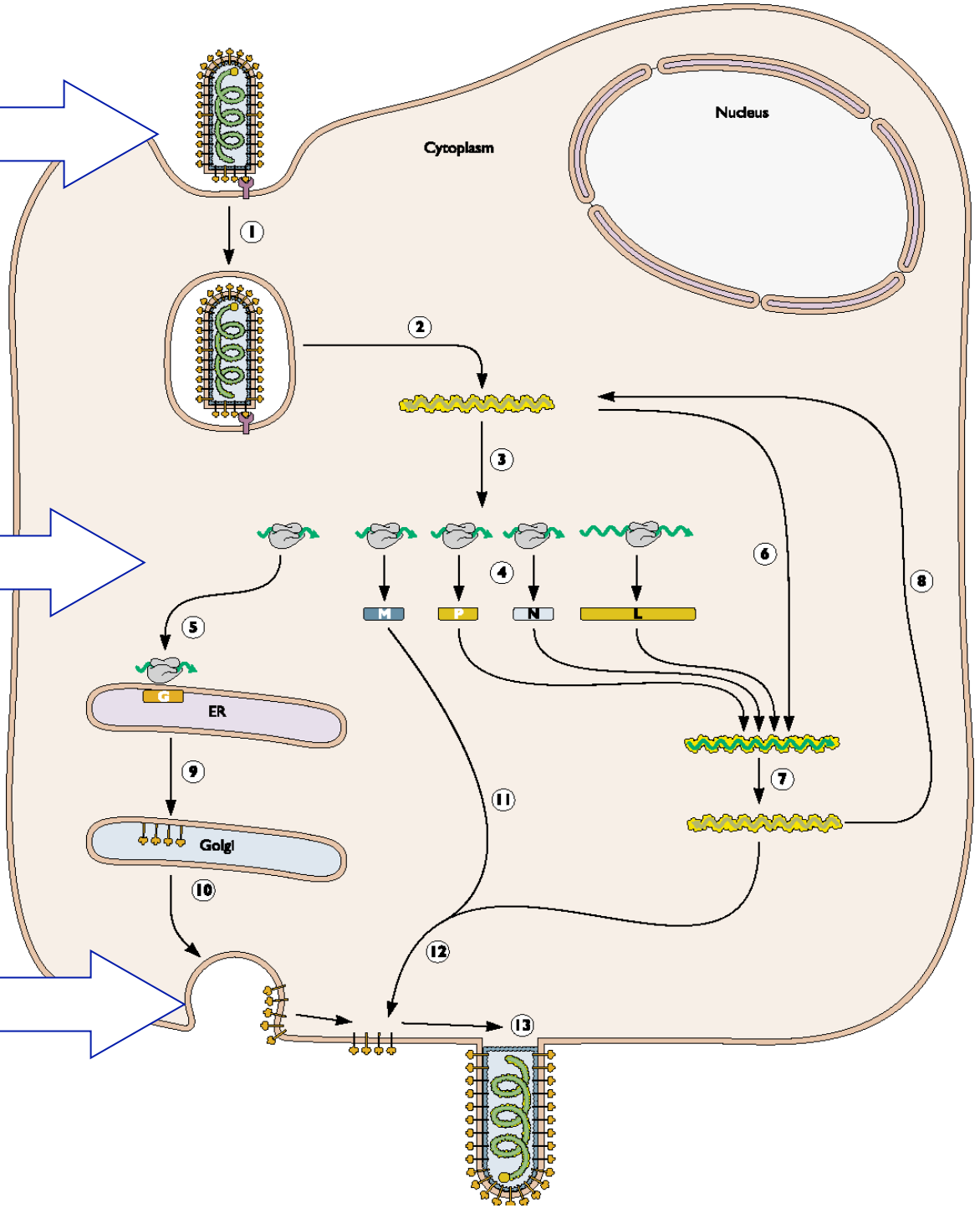
Vesicular stomatitis virus mRNA map



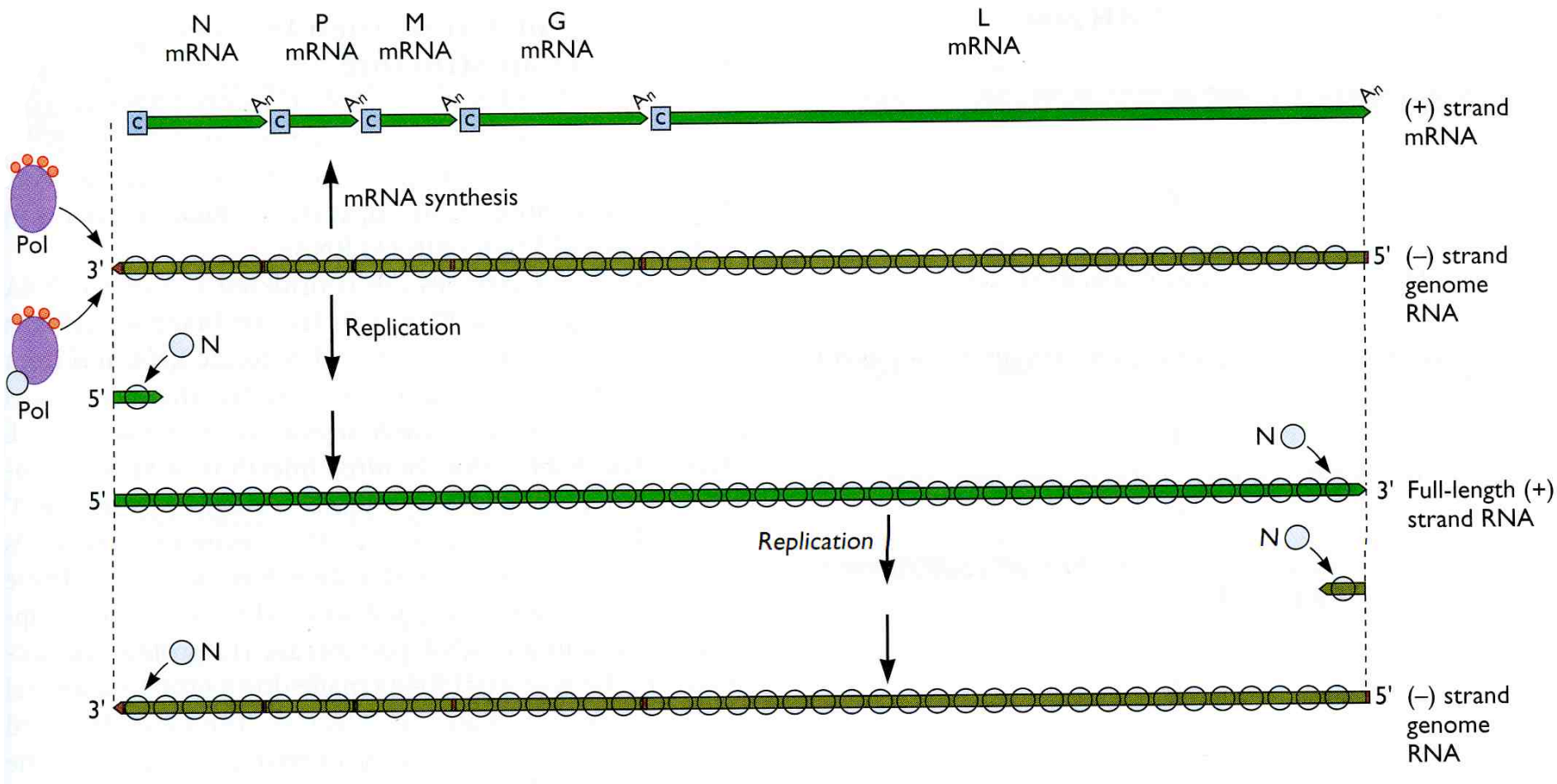
Attachment
Entry
Uncoating

Viral
Synthesis

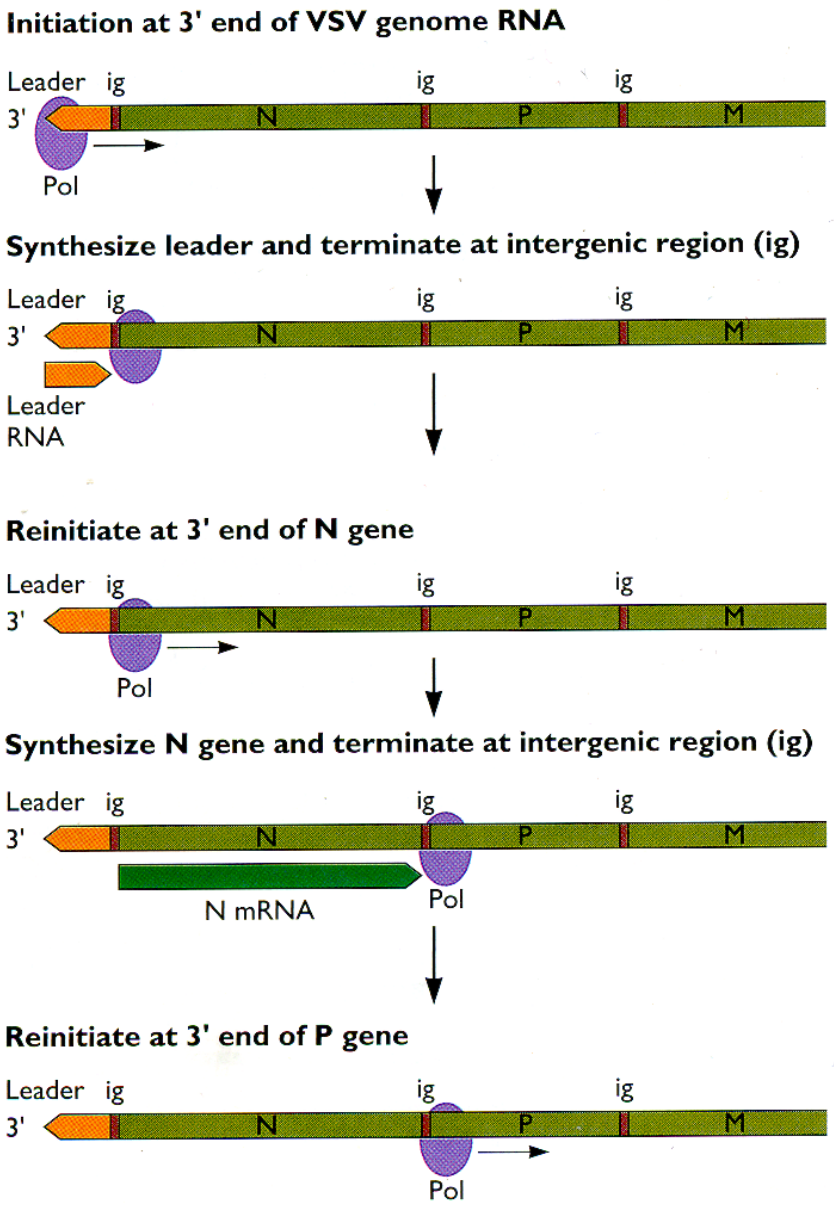
Assembly
Maturation
Exit

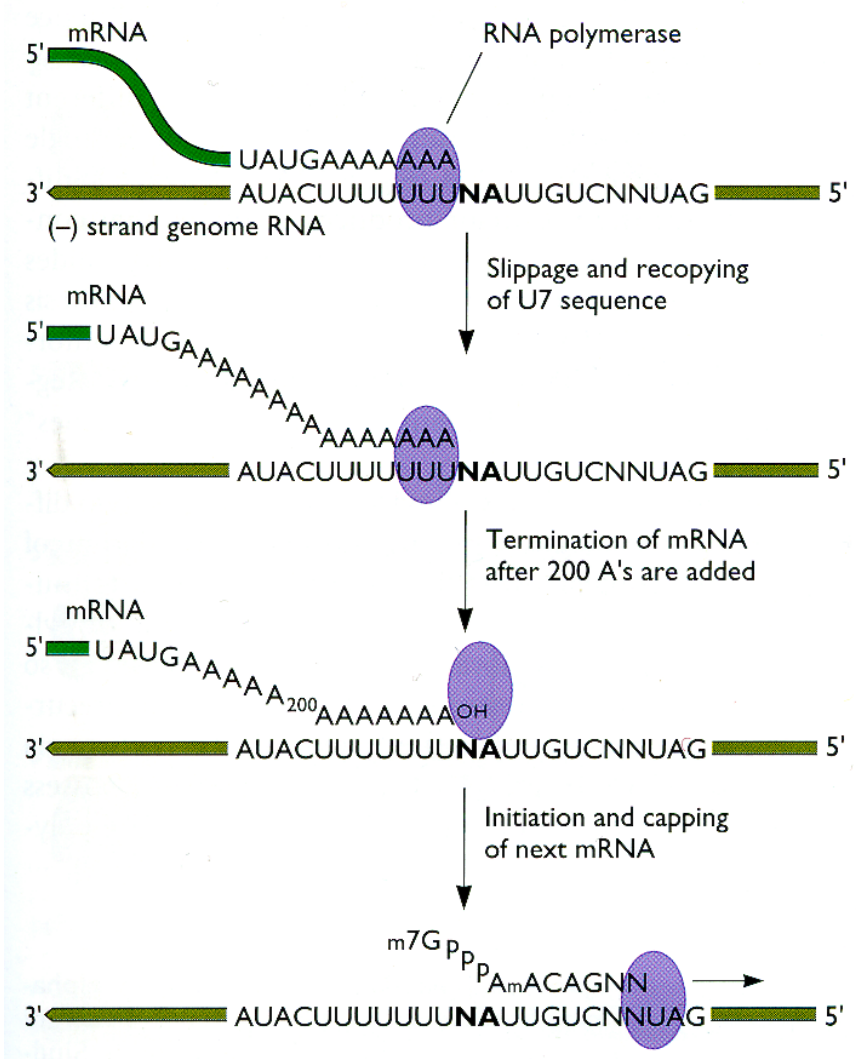


mRNA synthesis and replication of the VSV genome



VSV mRNA synthesis and function of RNA pol at an intergenic region





Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 8388–8392, August 1995
Biochemistry

Efficient recovery of infectious vesicular stomatitis virus entirely from cDNA clones

SEÁN P. J. WHELAN, L. ANDREW BALL, JOHN N. BARR, AND GAIL T. W. WERTZ*

Department of Microbiology, The Medical School, University of Alabama at Birmingham, BBRB 17 Room 366, 845 19th Street South, Birmingham, AL 35294

ABSTRACT **Infectious vesicular stomatitis virus (VSV), the prototypic nonsegmented negative-strand RNA virus, was recovered from a full-length cDNA clone of the viral genome. Bacteriophage T7 RNA polymerase expressed from a recombinant vaccinia virus was used to drive the synthesis of a genome-length positive-sense transcript of VSV from a cDNA clone in baby hamster kidney cells that were simultaneously expressing the VSV nucleocapsid protein, phosphoprotein, and polymerase from separate plasmids. Up to 10^5 infectious virus particles were obtained from transfection of 10^6 cells, as determined by plaque assays. This virus was amplified on passage, neutralized by VSV-specific antiserum, and shown to possess specific nucleotide sequence markers characteristic of the cDNA. This achievement renders the biology of VSV fully accessible to genetic manipulation of the viral genome. In contrast to the success with positive-sense RNA, attempts to recover infectious virus from negative-sense T7 transcripts were uniformly unsuccessful, because T7 RNA polymerase terminated transcription at or near the VSV intergenic junctions.**

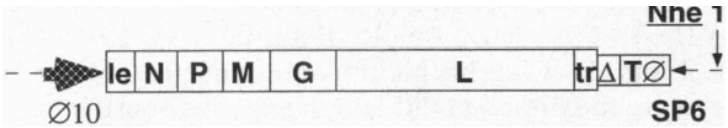


FIG. 1. The T7 transcription plasmid pVSV1(+) is illustrated, linearized at a unique *Nhe* I restriction site present within the vector. Ø10, T7 promoter; le, VSV leader gene; N, VSV nucleocapsid gene; P, VSV phosphoprotein gene; M, VSV matrix protein gene; G, VSV glycoprotein gene; L, VSV polymerase gene; tr, VSV trailer gene; Δ, HDV self-cleaving ribozyme; TØ, T7 terminator; SP6, SP6 promoter.

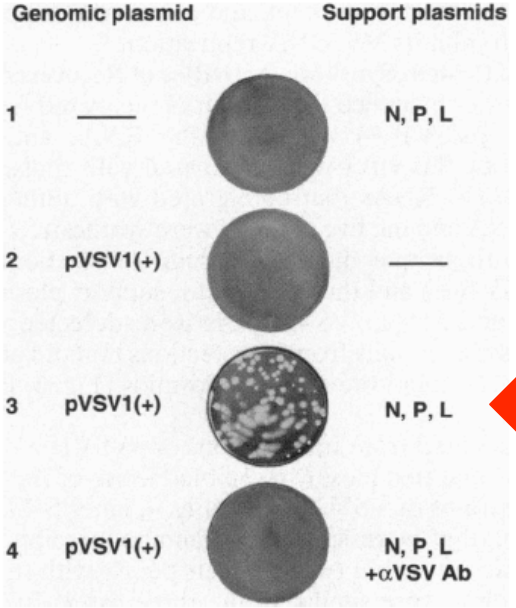


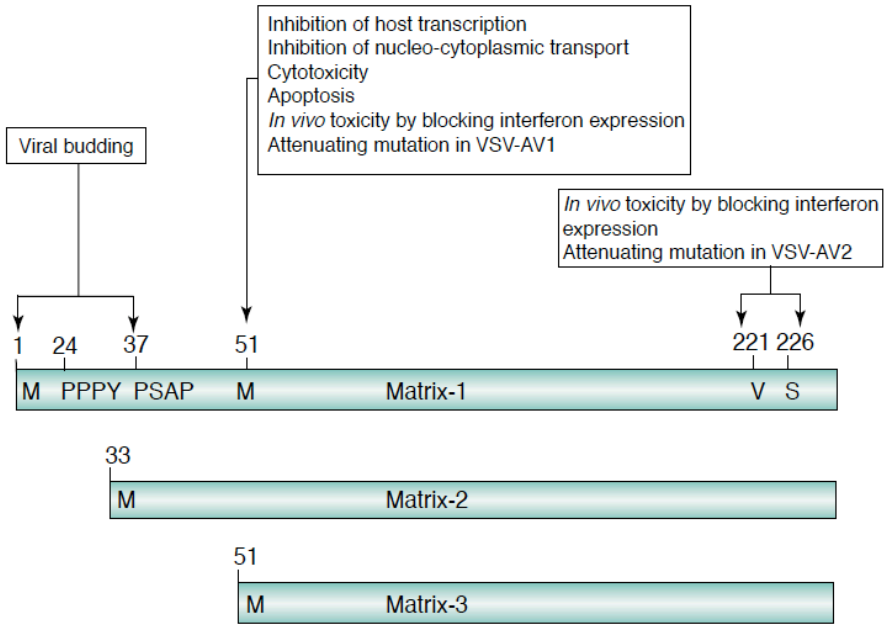
FIG. 2. Plaque assays of recovered virus. Monolayers of BHK21 cells were infected with vTF7-3 and transfected with pVSV1(+) and the N, P, and L support plasmids as indicated. After 45 hr of incubation at 37°C, the culture media were harvested and diluted 100-fold, and the infectious virus in 0.1-ml aliquots was determined by plaque assay using fresh monolayers of BSC40 cells. araC (25 μg/ml) was included in the agarose overlay to suppress the replication of VV. After 30 hr of incubation to allow VSV plaque formation, the monolayers were fixed, stained with crystal violet, and photographed. Shown are plaque assays of medium from cells that received N, P, and L support plasmids only (plate 1); pVSV1(+) only (plate 2); pVSV1(+) and N, P, and L support plasmids without (plate 3) or with (plate 4) subsequent incubation of the medium with anti-VSV antiserum (αVSV Ab).

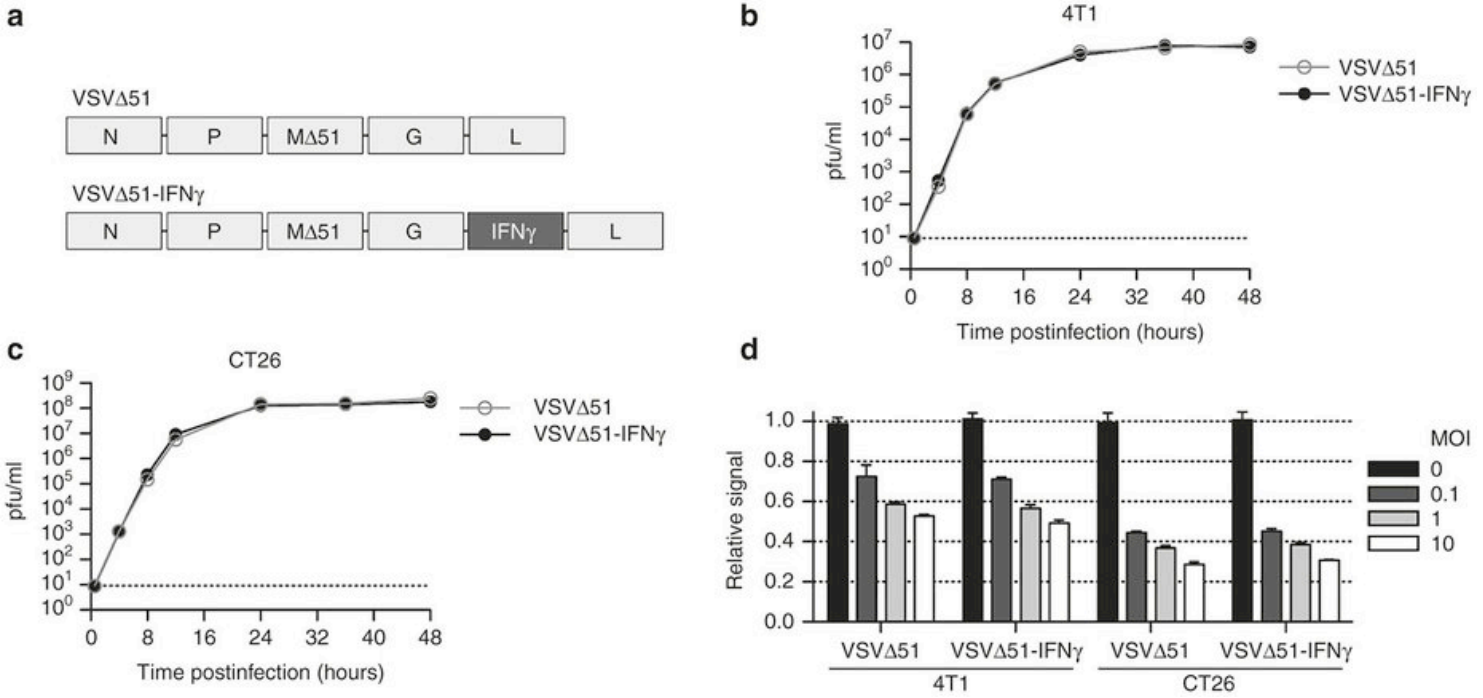
Table 1. The growing arsenal of VSV-based therapeutics for use against infectious and malignant disease[a]

VSV application	Features	Refs
Vaccine vectors		
VSV–HA	Insertion of influenza hemagglutinin gene into VSV genome; hemagglutinin antigen is expressed in VSV-infected cells and on viral surface	[27]
VSV-ΔG–HA	Improved influenza vector; attenuating deletion of VSV glycoprotein increases safety and prevents stimulation of VSV-specific humoral immunity	[4]
VSV–GagEnv	Insertion of HIV *Gag* and *Env* genes into VSV genome; VSV-infected cells express Env and Gag proteins to induce HIV-specific $CD8^+$ CTL and neutralizing antibody responses	[29,32]
VSV–MV-H	Insertion of measles virus hemagglutinin gene into VSV genome; elicits protective MV-specific neutralizing antibody despite the presence of circulating maternal antibody	[28]
VSV-ΔG–RSV-F	Insertion of respiratory syncytial virus fusion gene into VSV genome; RSV-fusion antigen is expressed in VSV-infected cells and on viral surface; attenuated by deletion of VSV-G	[31]
VSV–HCV-C/E1/E2	Insertion of Hep C gene encoding contiguous C/E1/E2 proteins; HepC antigens are expressed in VSV-infected cells	[33]
VSV-rearranged genome	Rearrangement of genes generates a stably attenuated vector	[2]
Natural oncolytic strains		
WtVSV[g]	High sensitivity to anti-viral interferons; selective replication and cytotoxicity in tumor cells exhibiting compromised interferon response	[35,37,40]
VSV-AV1 or VSV-AV2	Highly attenuated replication in normal cells but conserved tumor killing; enhanced therapeutic index	[16]
Recombinant oncolytic strains		
wtVSV–GFP	Expression of green fluorescent protein transgene	[16,39,48]
VSV–Δ51M	Deletion of Met-51 of matrix protein; highly attenuated replication in normal cells but conserved tumor killing; enhanced therapeutic index	[16]
Oncolytic VSVs expressing immunostimulatory cytokines		
VSV–IL-4	Expresses IL-4 gene; enhanced therapeutic index	[36]
VSV–IFN-β	Expresses IFN-β gene; enhanced therapeutic index	[45]
Oncolytic VSVs expressing a suicide gene		
VSV–TK	Expresses thymidine kinase gene; killing of infected and bystander cells with gancyclovir treatment	[36]
VSV–CD/UPRT	Expresses cytosine deaminase (CD)/uracil phosphoribosyltransferase gene; killing of infected and bystander cells with 5-fluorocytosine treatment	[41]
Receptor-targeted VSVs		
VSV–CD4	Expresses CD4 and can infect cells expressing HIV gp120	[43,44]
VSV–Sindbis-ZZ glycoproteins	VSV pseudotype coated with a Sindbis virus glycoprotein/protein A fusion; targeting to tumor-specific antigens when co-administered with a monoclonal antibody	[42]

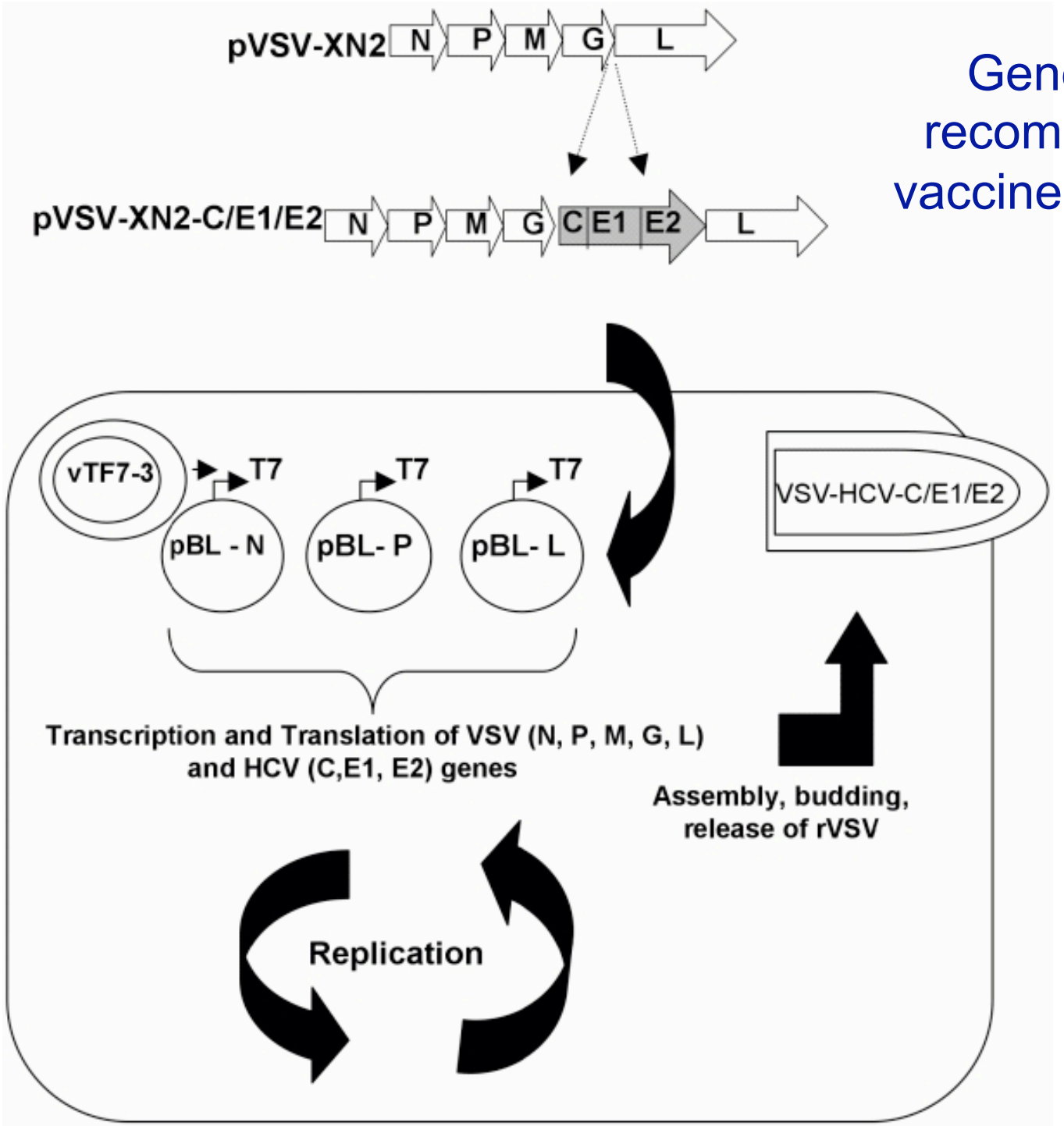
[a]Abbreviations: AV, attenuated virus; CD/UPRT, cytosine deaminase (CD)/uracil phosphoribosyltransferase; CTL, cytotoxic T lymphocyte; G, glycoprotein; HA, hemagglutinin; HCV-C/E1/E2, hepatitis C virus capsid/envelop 1/envelop 2; IFN, interferon; IL, interleukin; MV-H, measles virus hemagglutinin; RSU-F, respiratory syncytial virus fusion; TK, thymidine kinase; VSV, vesicular stomatitis virus; Wt, wild type.

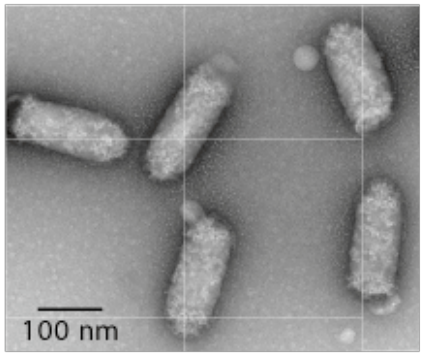
Generation of recombinant oncolytic VSV vector expressing IFN





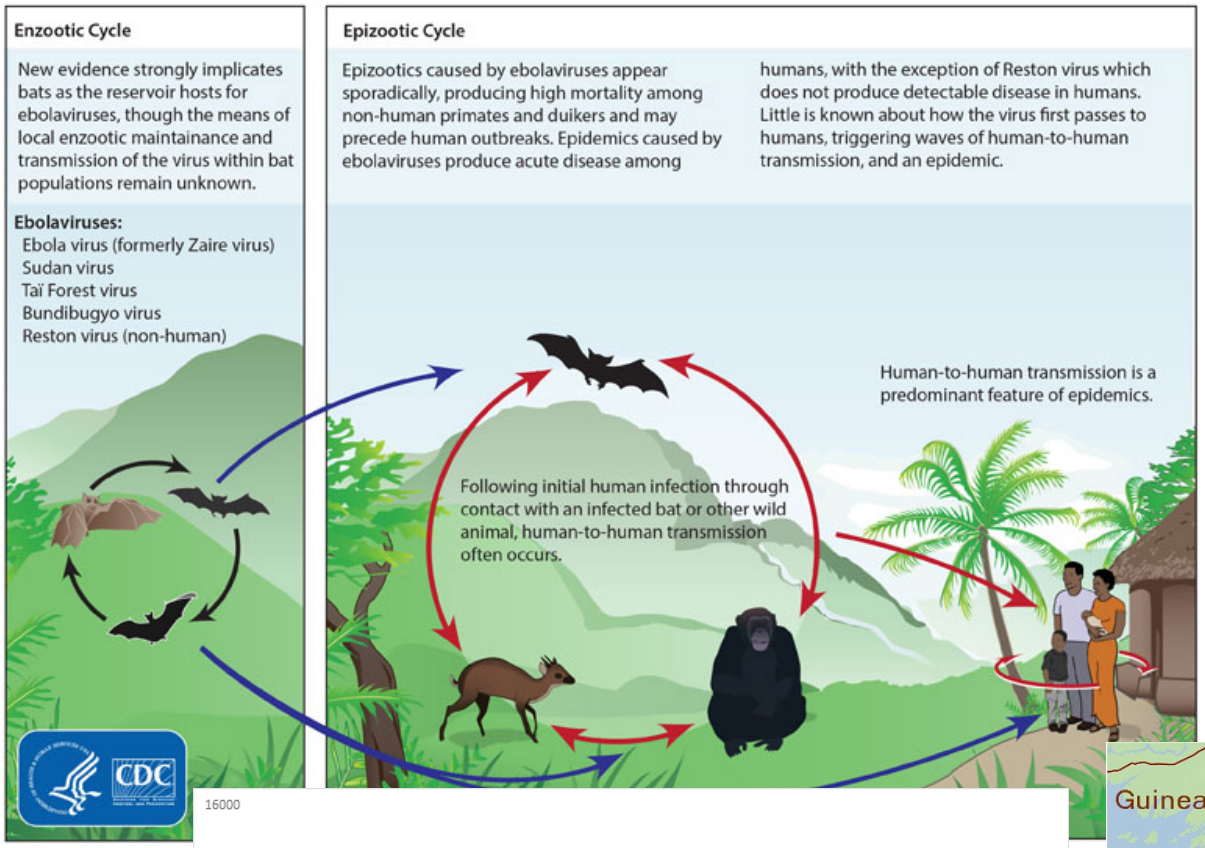
Generation of recombinant VSV vaccine against HCV

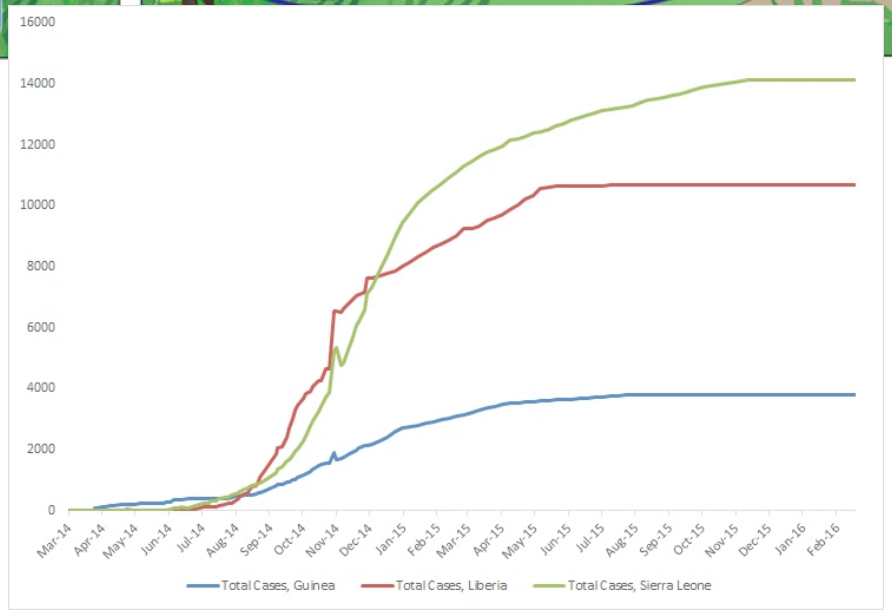


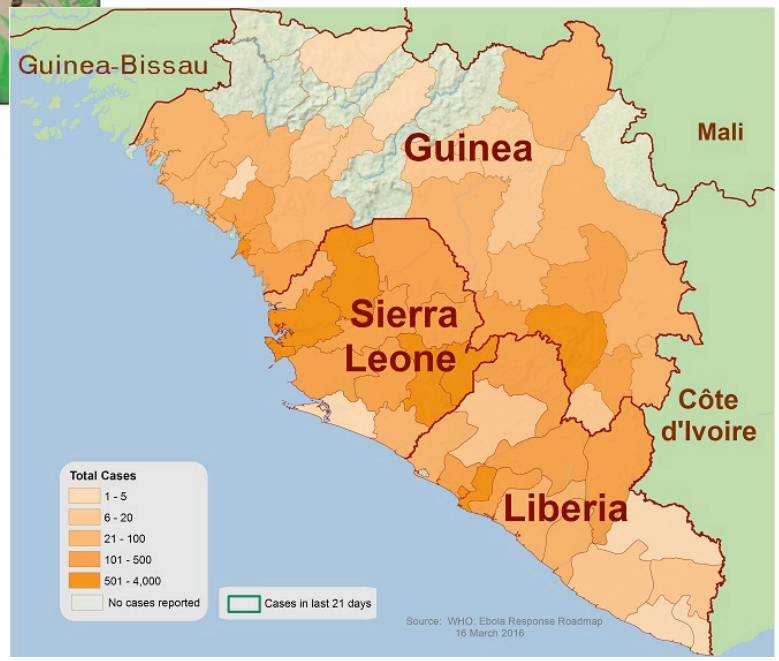


VSV-HCV-C/E1/E2

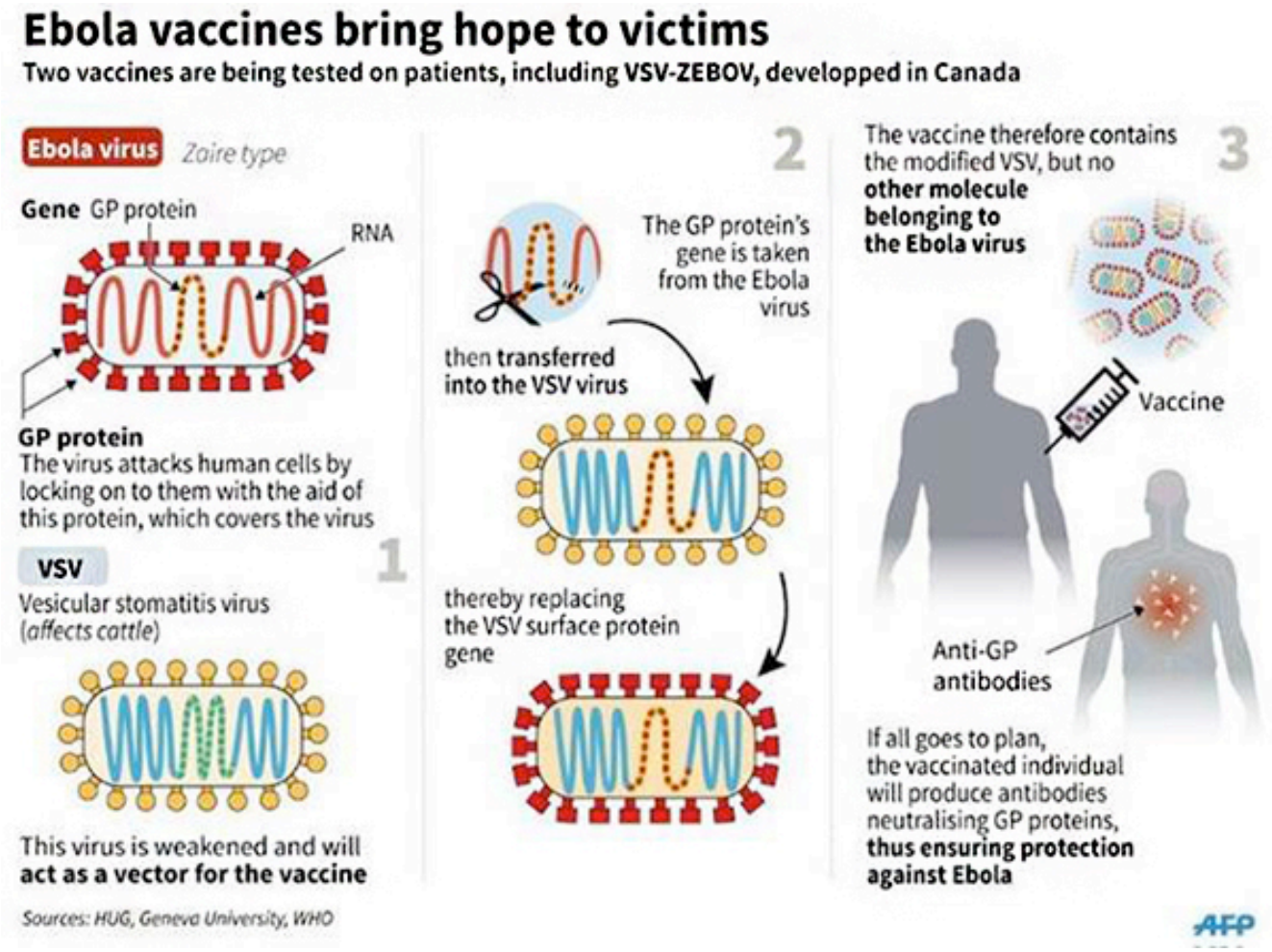
Ebola Outbreak 2014



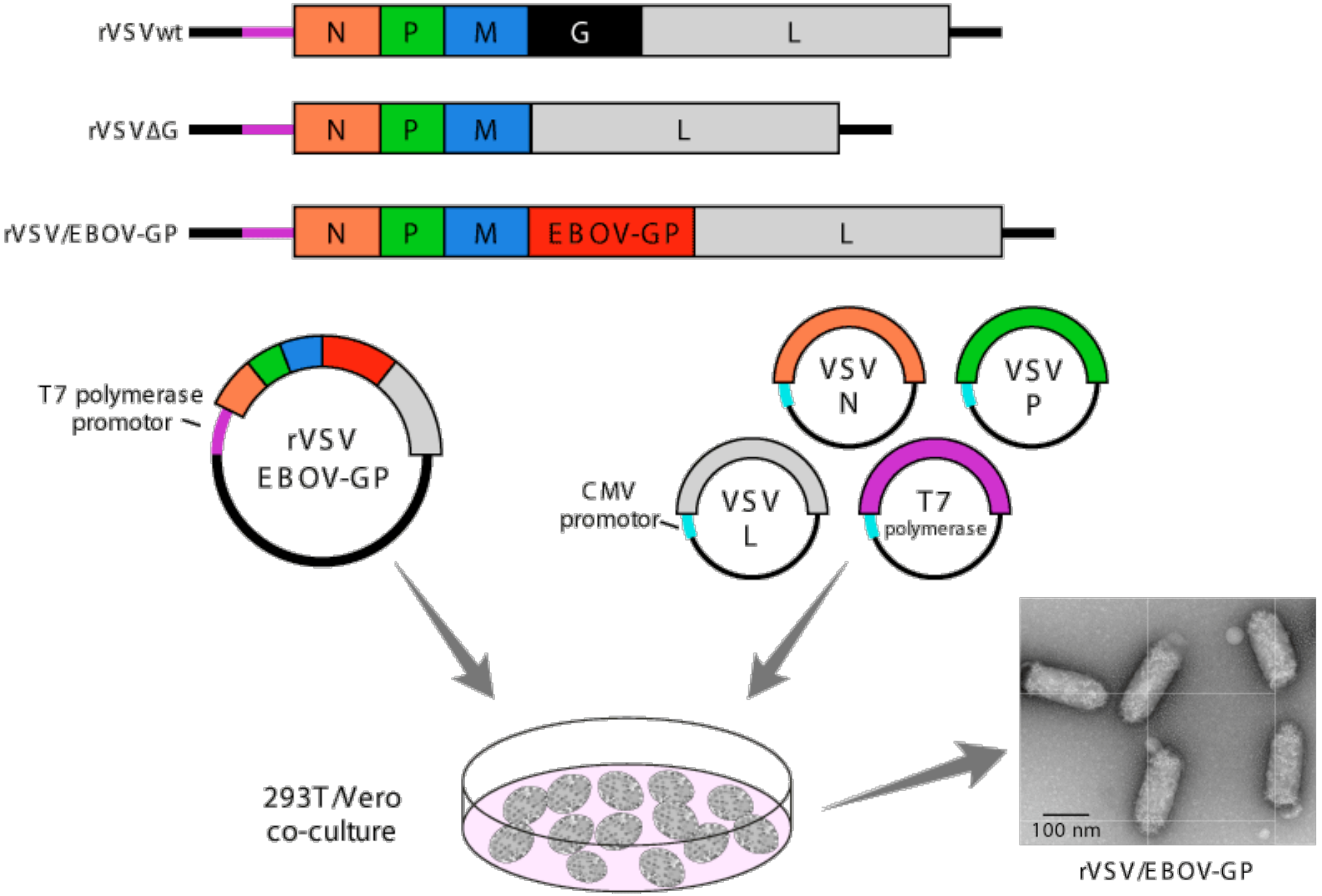




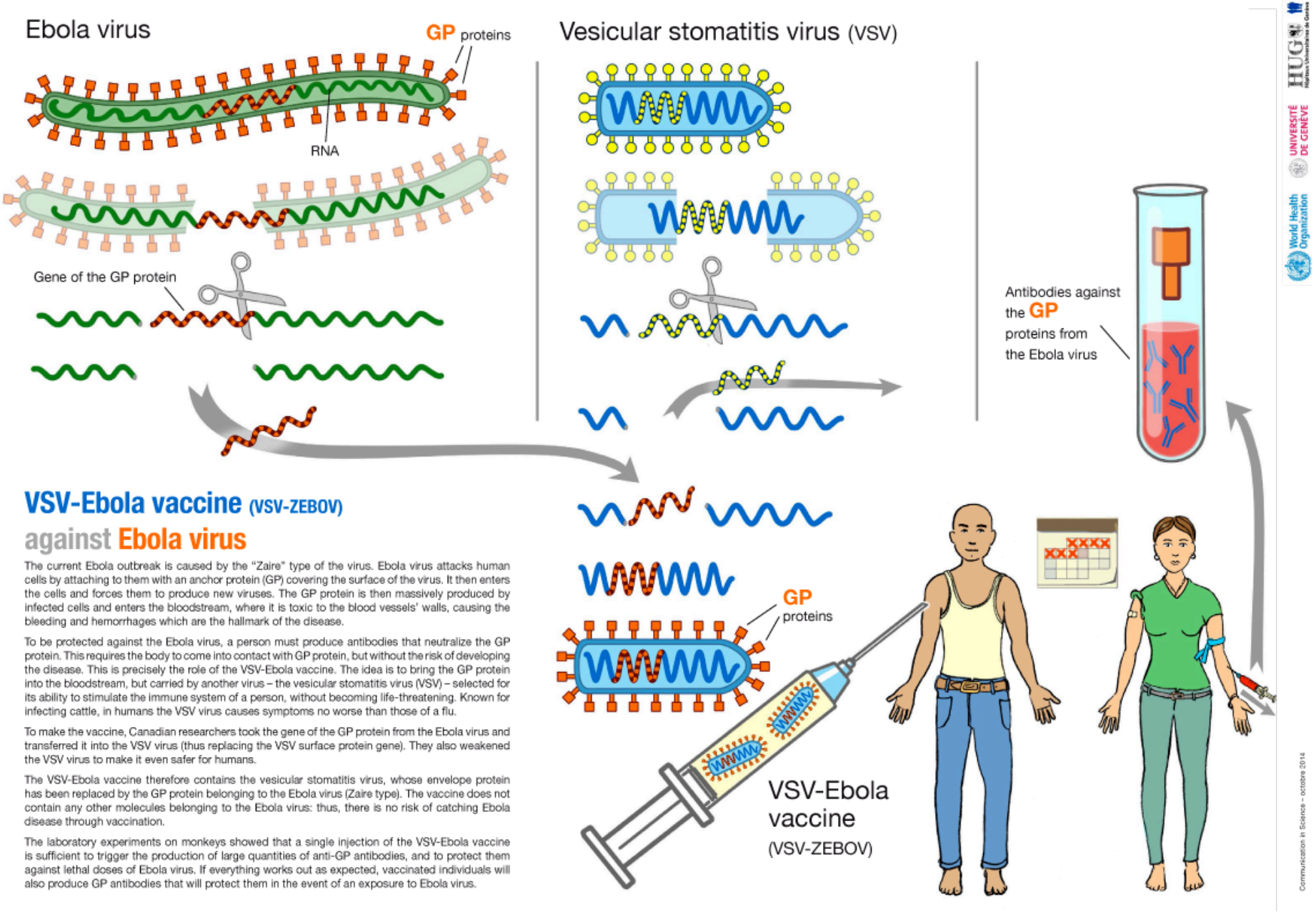
A recombinant VSV vaccine against Ebola



Generation of recombinant VSV vaccine against Ebola



A recombinant VSV vaccine against Ebola



VSV-Ebola vaccine (VSV-ZEBOV) against Ebola virus

The current Ebola outbreak is caused by the "Zaire" type of the virus. Ebola virus attacks human cells by attaching to them with an anchor protein (GP) covering the surface of the virus. It then enters the cells and forces them to produce new viruses. The GP protein is then massively produced by infected cells and enters the bloodstream, where it is toxic to the blood vessels' walls, causing the bleeding and hemorrhages which are the hallmark of the disease.

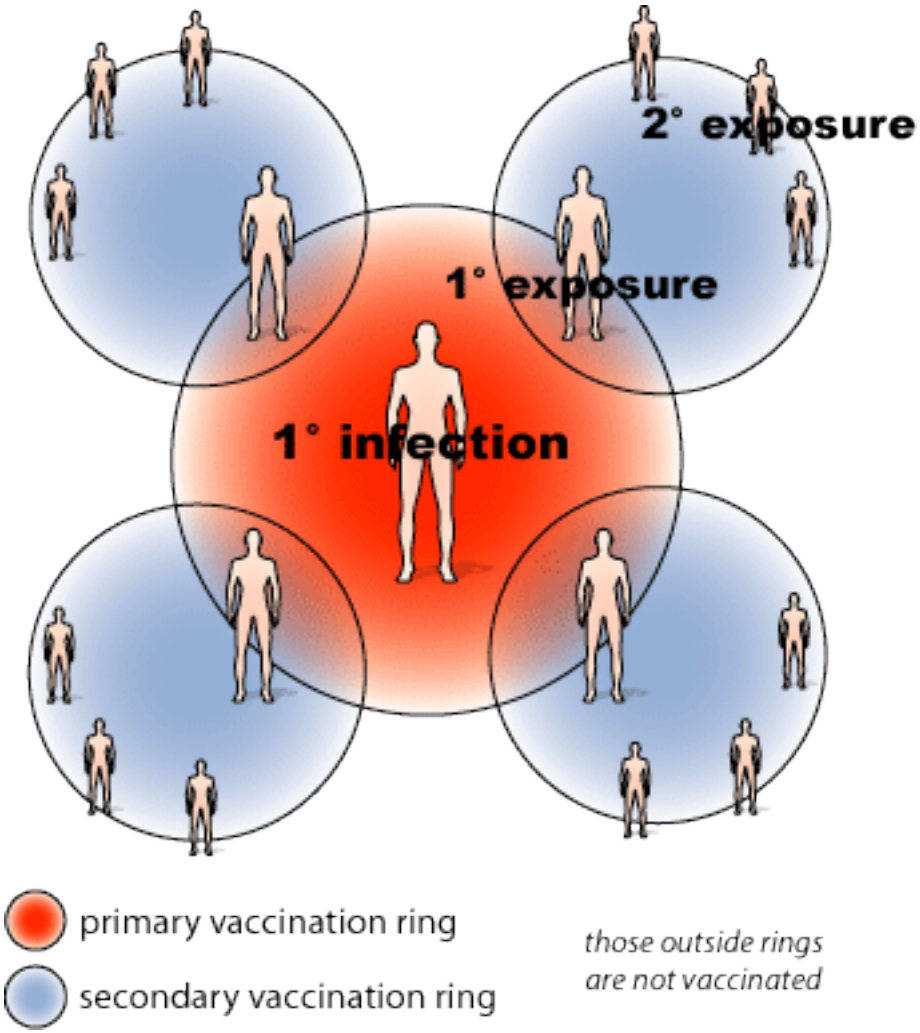
To be protected against the Ebola virus, a person must produce antibodies that neutralize the GP protein. This requires the body to come into contact with GP protein, but without the risk of developing the disease. This is precisely the role of the VSV-Ebola vaccine. The idea is to bring the GP protein into the bloodstream, but carried by another virus – the vesicular stomatitis virus (VSV) – selected for its ability to stimulate the immune system of a person, without becoming life-threatening. Known for infecting cattle, in humans the VSV virus causes symptoms no worse than those of a flu.

To make the vaccine, Canadian researchers took the gene of the GP protein from the Ebola virus and transferred it into the VSV virus (thus replacing the VSV surface protein gene). They also weakened the VSV virus to make it even safer for humans.

The VSV-Ebola vaccine therefore contains the vesicular stomatitis virus, whose envelope protein has been replaced by the GP protein belonging to the Ebola virus (Zaire type). The vaccine does not contain any other molecules belonging to the Ebola virus: thus, there is no risk of catching Ebola disease through vaccination.

The laboratory experiments on monkeys showed that a single injection of the VSV-Ebola vaccine is sufficient to trigger the production of large quantities of anti-GP antibodies, and to protect them against lethal doses of Ebola virus. If everything works out as expected, vaccinated individuals will also produce GP antibodies that will protect them in the event of an exposure to Ebola virus.


scientific
hazard
genetic
congo
microbiology
factors
outbreak
protection
toxin
harmful
science
contamination
progressing
caution
respiratory
prevention
biological
patients
deadly
terror
viral
traffic
bacteria
germs
warning
vomiting
symptoms
hemorrhaging
health
infected
people
ebola
epidemic
virus
disease
death
africa
mask
infectious
sickness
morphology
healthcare
medicine
precaution
virulence
outbreaks
fever
medical
infection
technology
effects
pathogen
microbe
illness
risk
fatal
biology
serum
hemorrhagic
mortal
human
headache
cells
danger
migraine
problem
alert
cure
threat
safety


2° exposure
1° exposure
1° infection
primary vaccination ring
secondary vaccination ring
those outside rings
are not vaccinated