PHYSIOPATHOLOGIE ET TRANSMISSION DU VIRUS EBOLA :

qu'est ce qu'on sait ?
et qu'on ne sait pas encore ?

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Cellule d'Intervention Biologique d'Urgence (CIBU)

[Laboratory for Urgent response to Biological Threats]

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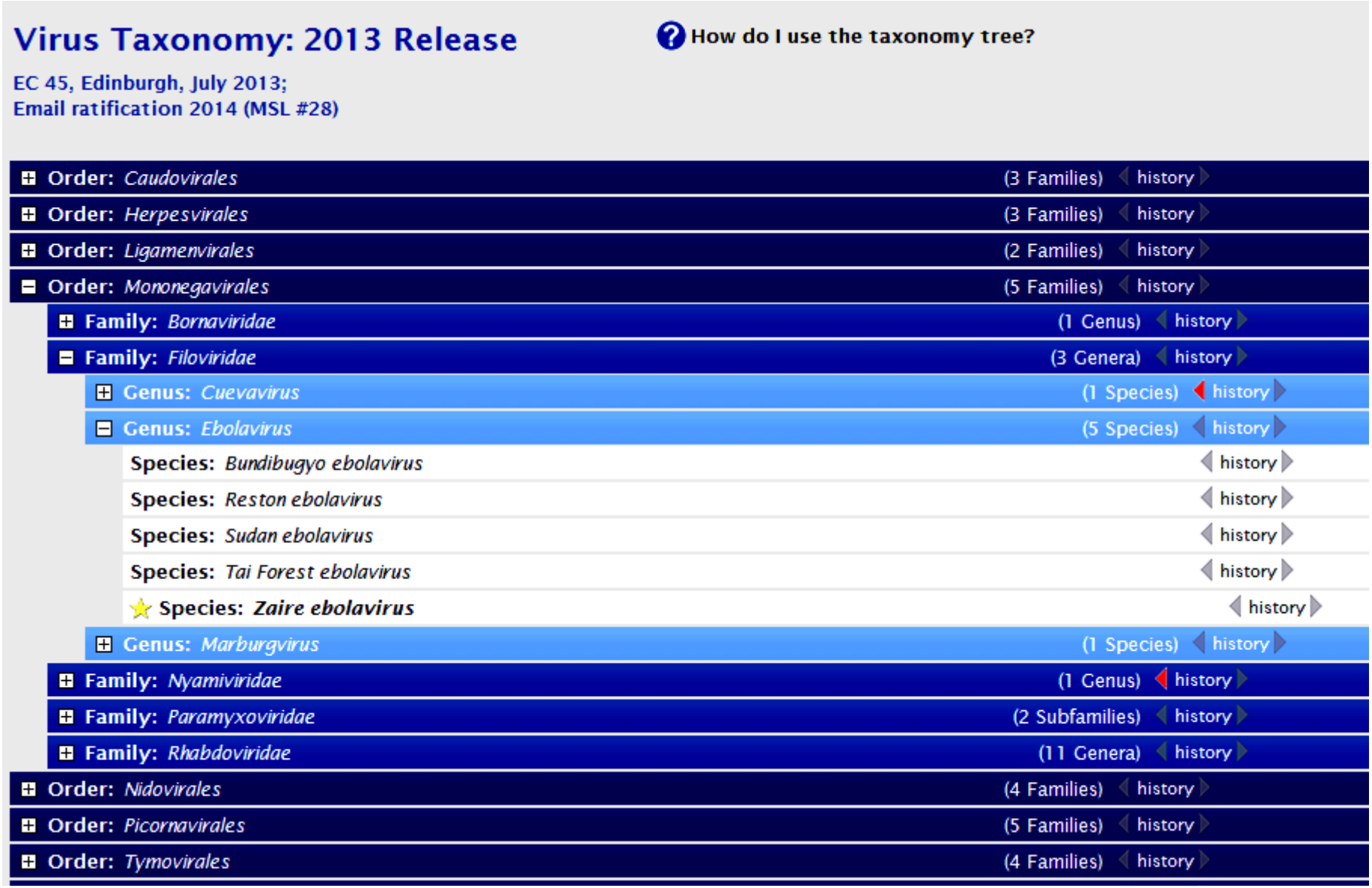
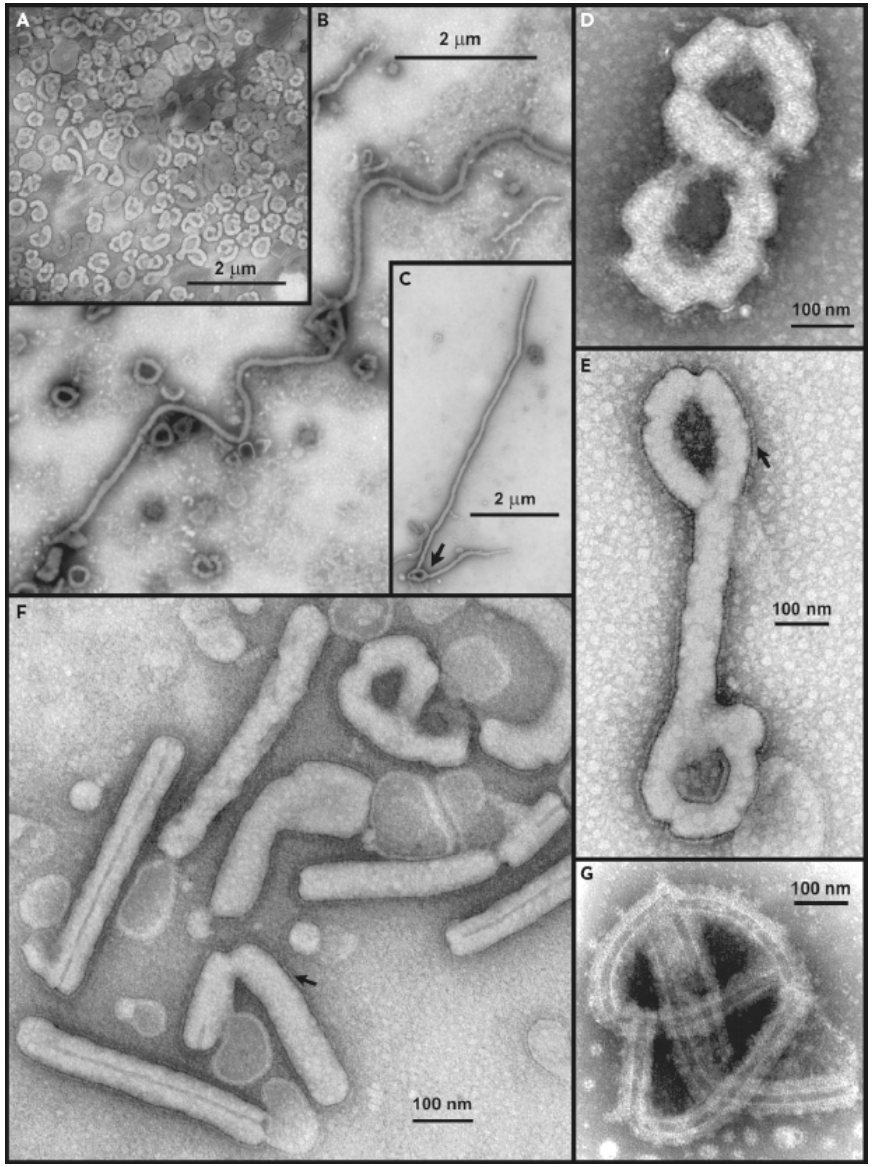
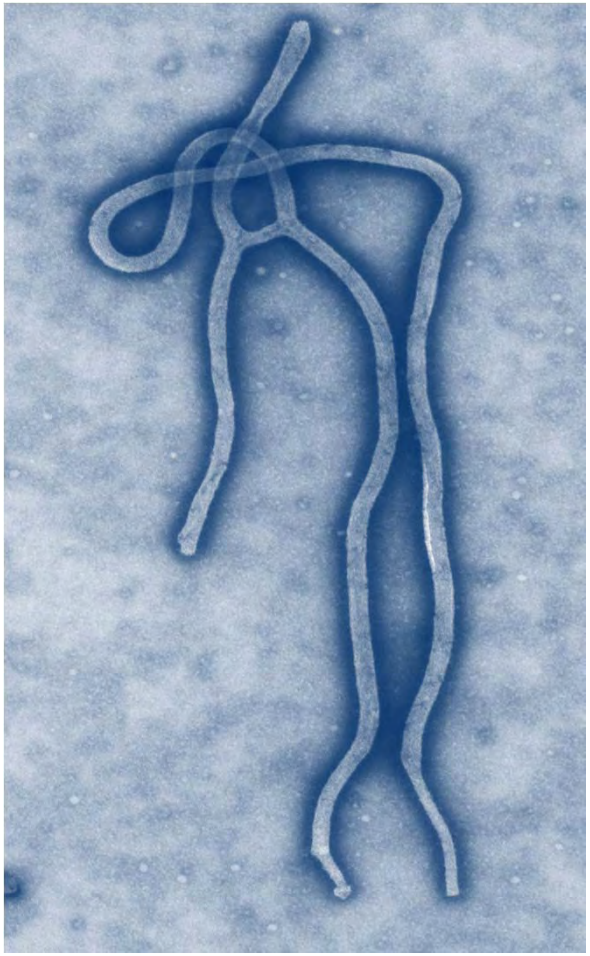
Taxonomic position of ebolaviruses



Morphostructure of ebolavirus particles + TEM



A. Sanchez and C. Humphrey, CDC, Atlanta



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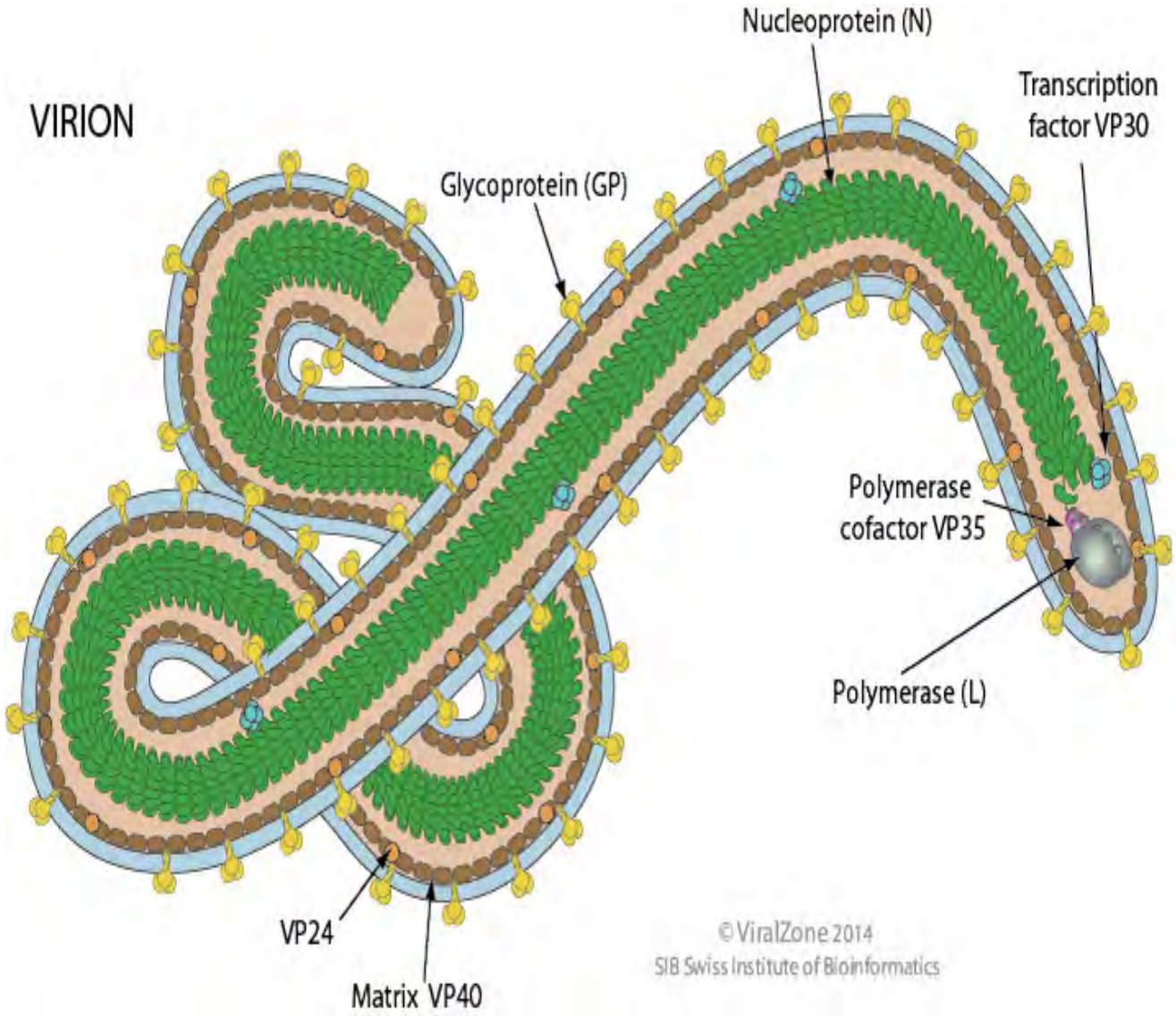
Morphostructure of ebolavirus particles + TEM



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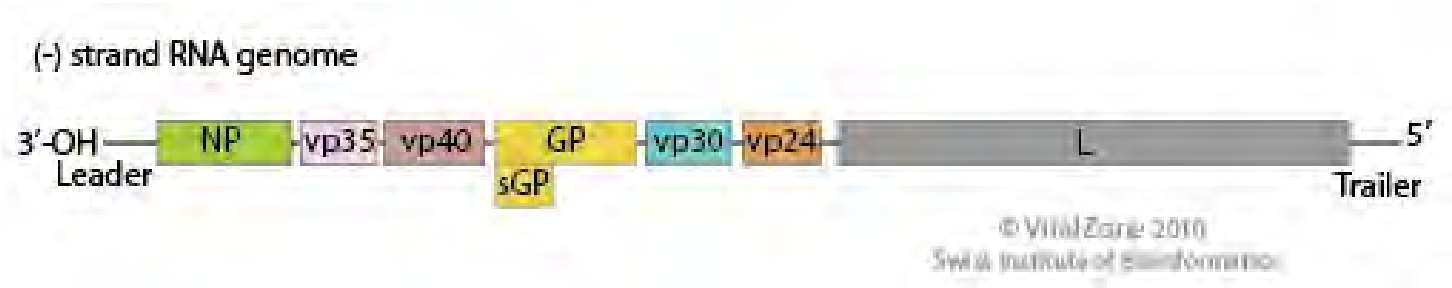
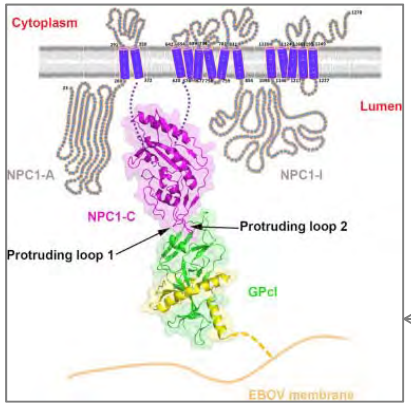
Ebolavirus genome



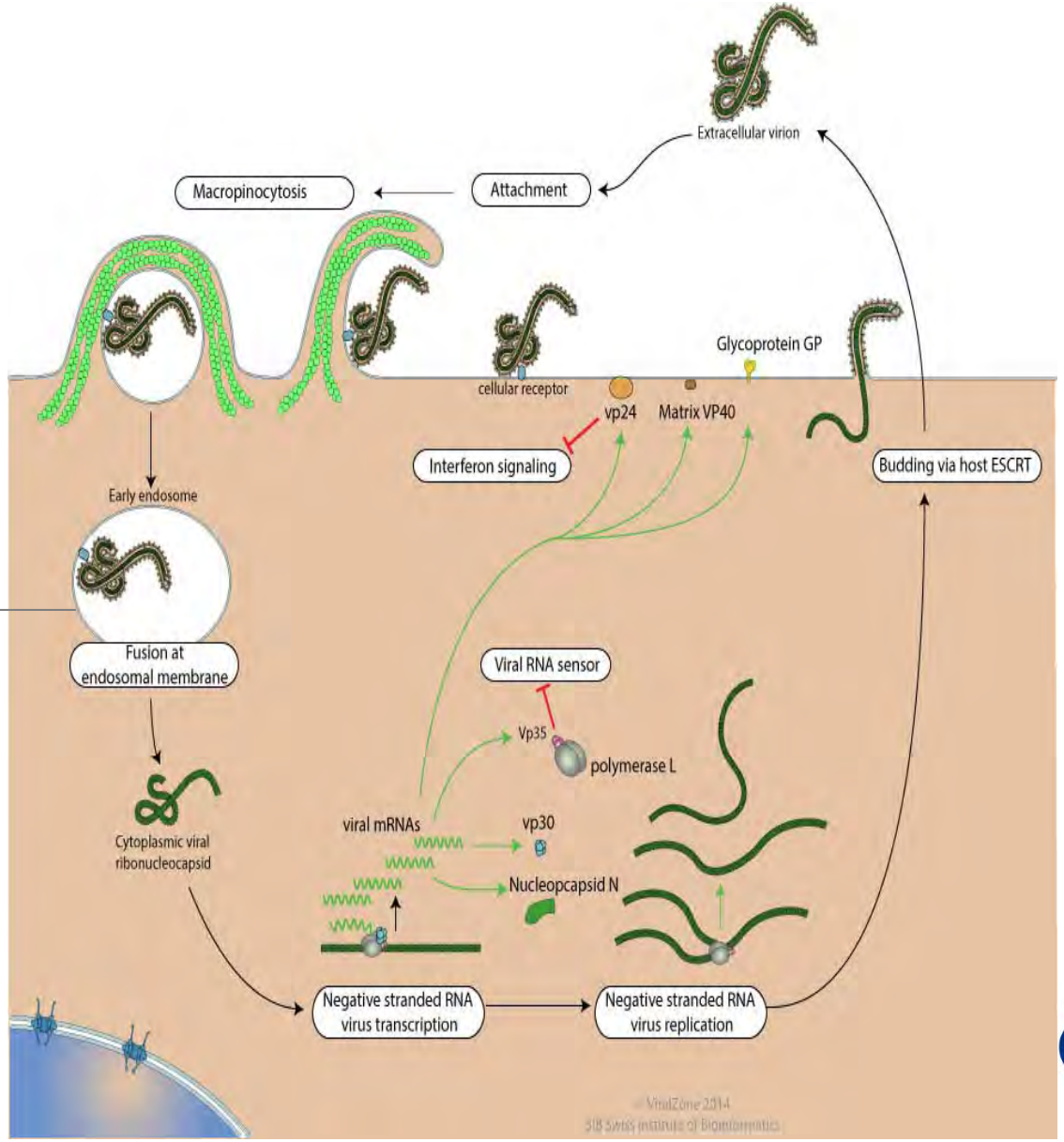
Ebolavirus replication cycle

Niemann-Pick C1*
(NPC1), identified as a necessary entry receptor



Structural basis of Ebola virus endosomal-receptor binding

- NPC1 domain C (NPC1-C) displays a helical core structure with two protruding loops
- NPC1-C binds to the primed Ebola virus GP (GPcl) protein with a low affinity
- NPC1-C utilizes two protruding loops to engage a hydrophobic cavity on head of GPcl



* Ebola Viral Glycoprotein Bound to Its Endosomal Receptor Niemann-Pick C1 Wang, Han et al. - Cell , 2016, Volume 164 , Issue 1 , 258 - 268

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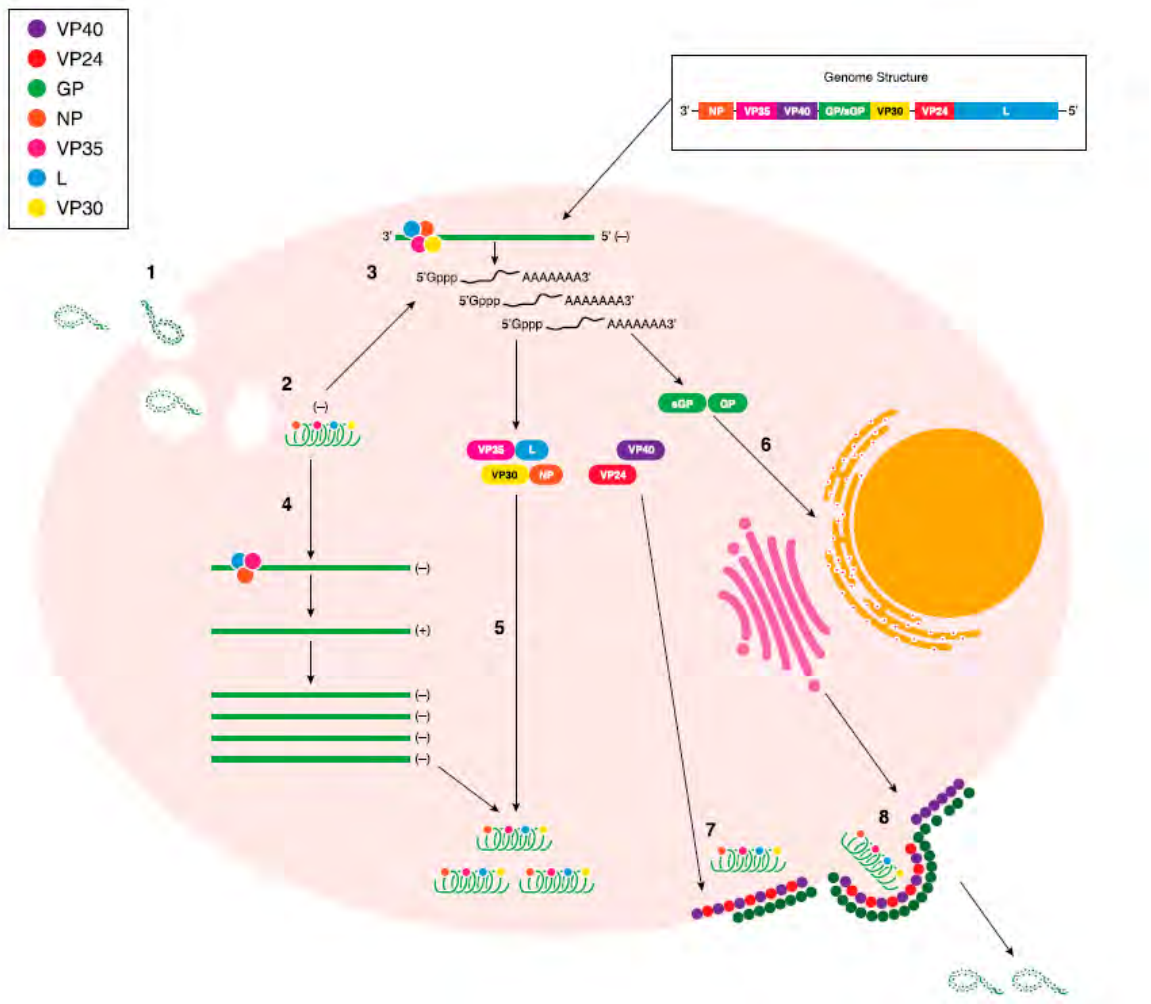
Ebolavirus replication cycle



Molecular mechanisms of Ebola pathogenesis, Andrea Rivera and Ilhem Messaoudi,J. of Leukocyte Biology Vol.100, No.5:889-904, November, 2016

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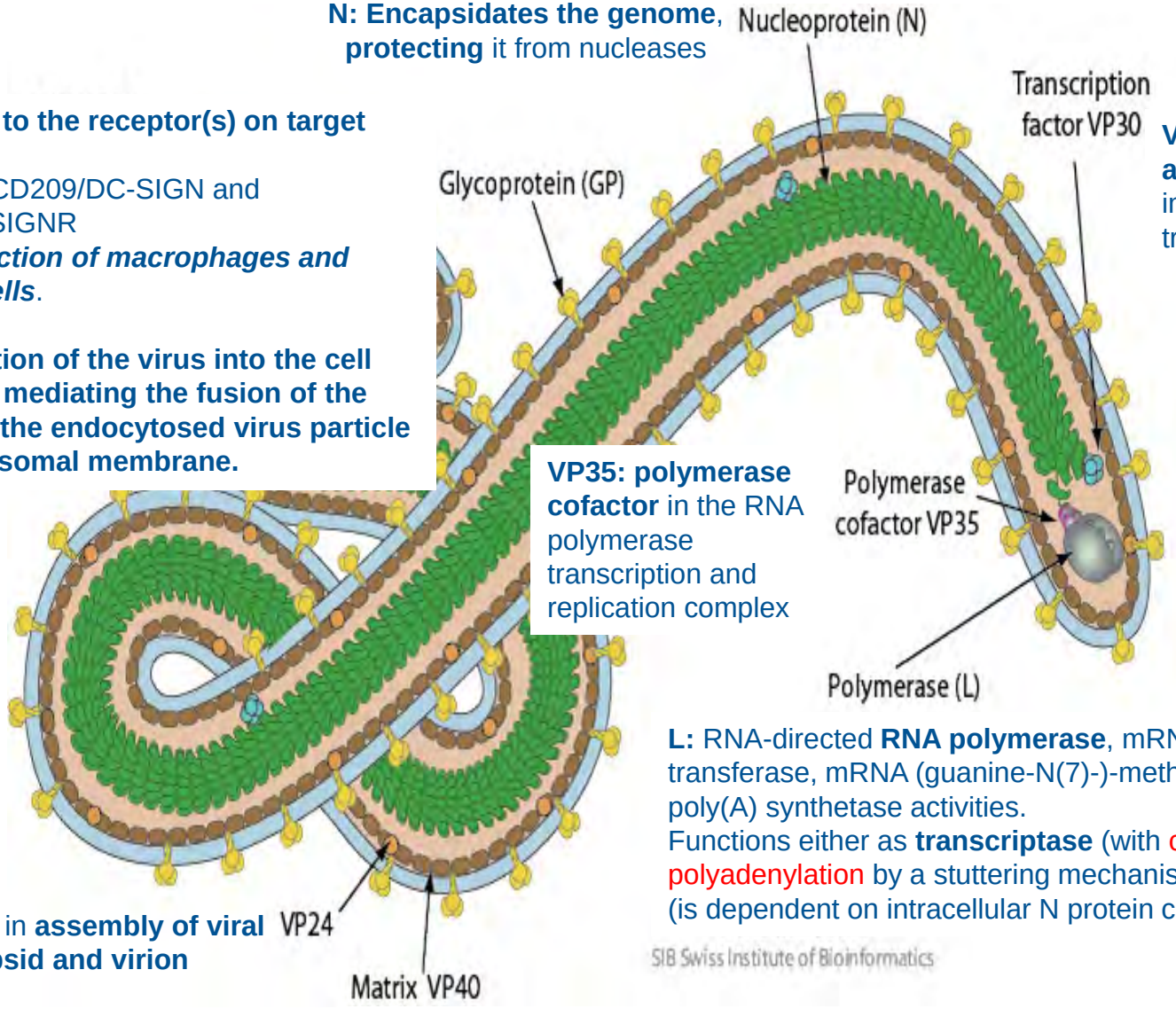
Main functions of ebolavirus proteins

N: Encapsidates the genome, **protecting** it from nucleases

GP1: Binding to the receptor(s) on target cells.
Interacts with CD209/DC-SIGN and CLEC4M/DC-SIGNR
Facilitate infection of macrophages and endothelial cells.

GP2: Penetration of the virus into the cell cytoplasm by mediating the fusion of the membrane of the endocytosed virus particle with the endosomal membrane.



VP30: Transcription anti-termination factor immediately after transcription initiation.

VP35: polymerase cofactor in the RNA polymerase transcription and replication complex

L: RNA-directed **RNA polymerase**, mRNA guanylyl transferase, mRNA (guanine-N(7)-)-methyltransferase and poly(A) synthetase activities.
Functions either as **transcriptase** (with capping and polyadenylation by a stuttering mechanism) or as **replicase** (is dependent on intracellular N protein concentration)

VP24: role in **assembly of viral nucleocapsid and virion budding**

VP40: **matrix protein** bearing the lipid bilayer

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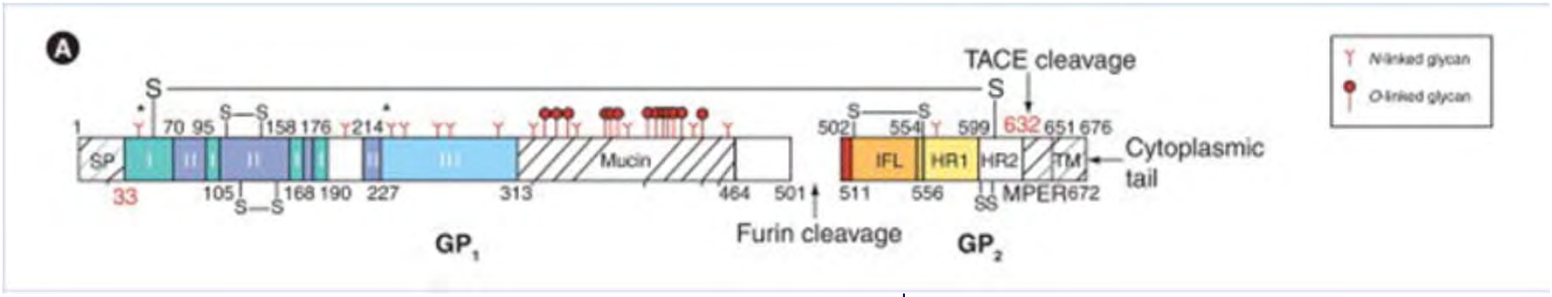
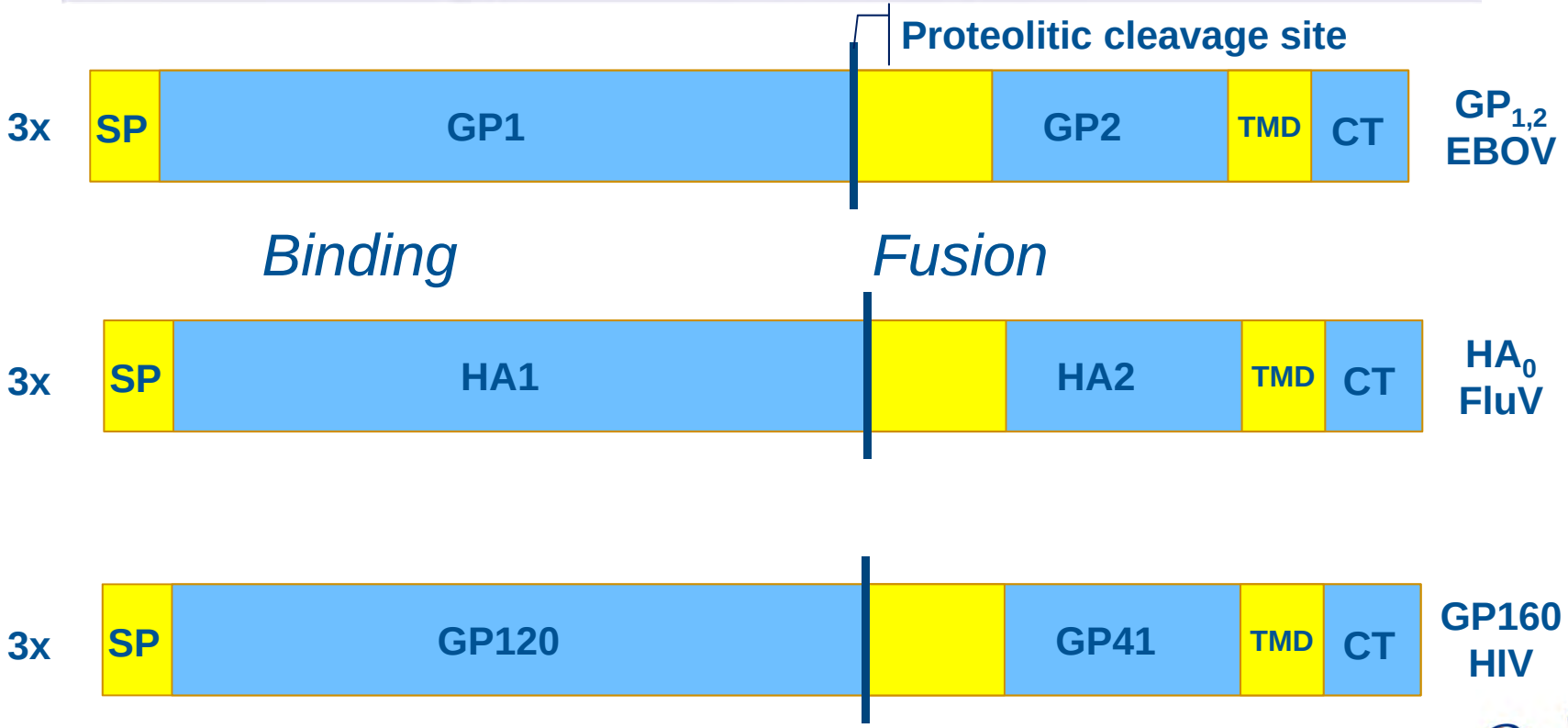
Ebolavirus, *Influenzavirus* and *Human Immunodeficiency virus* surface glycoproteins





SP: Signal Peptide; TMB: Trans Membrane Domain; CT: Cytoplasmic Tail

Future Virol. 2009; 4(6): 621–635. doi: 10.2217/fvl.09.56

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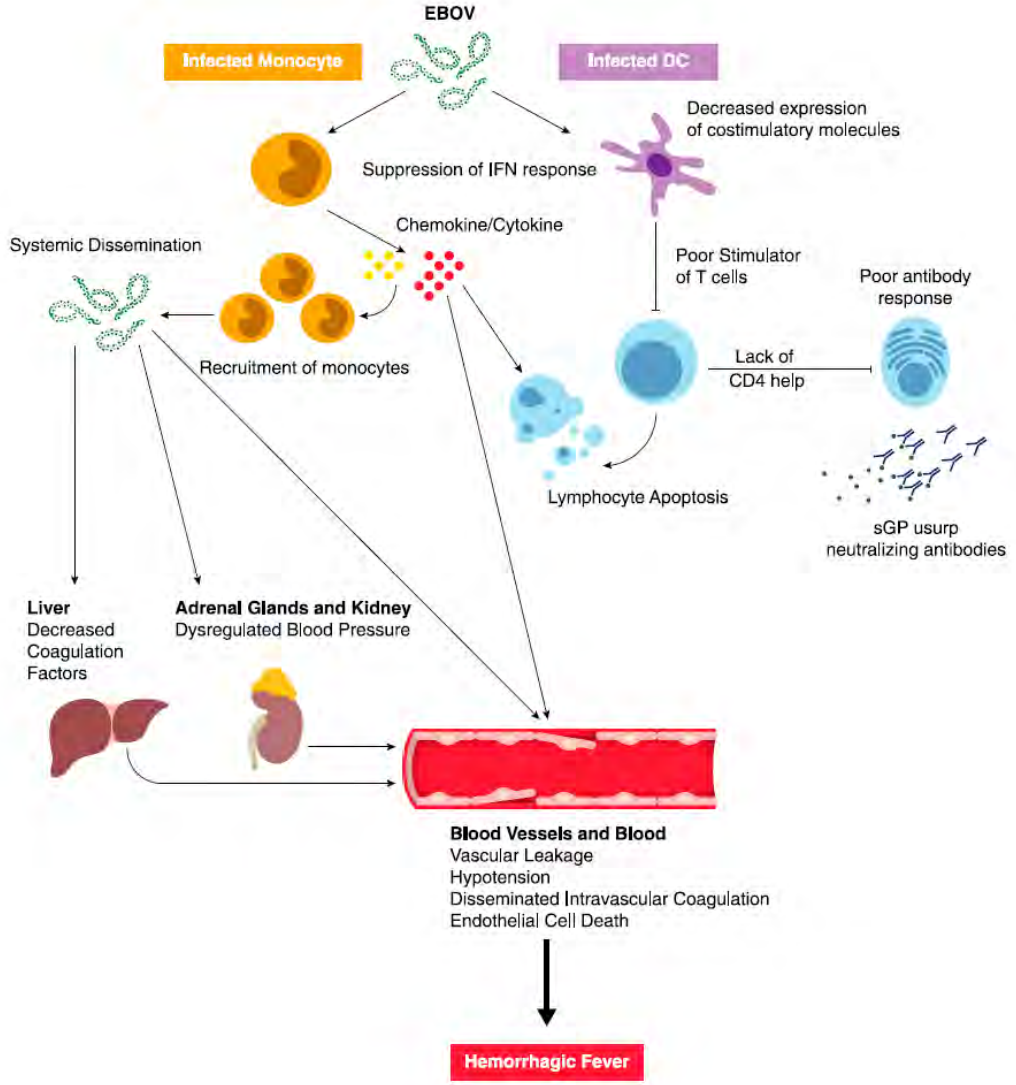
Ebola virus pathogenesis



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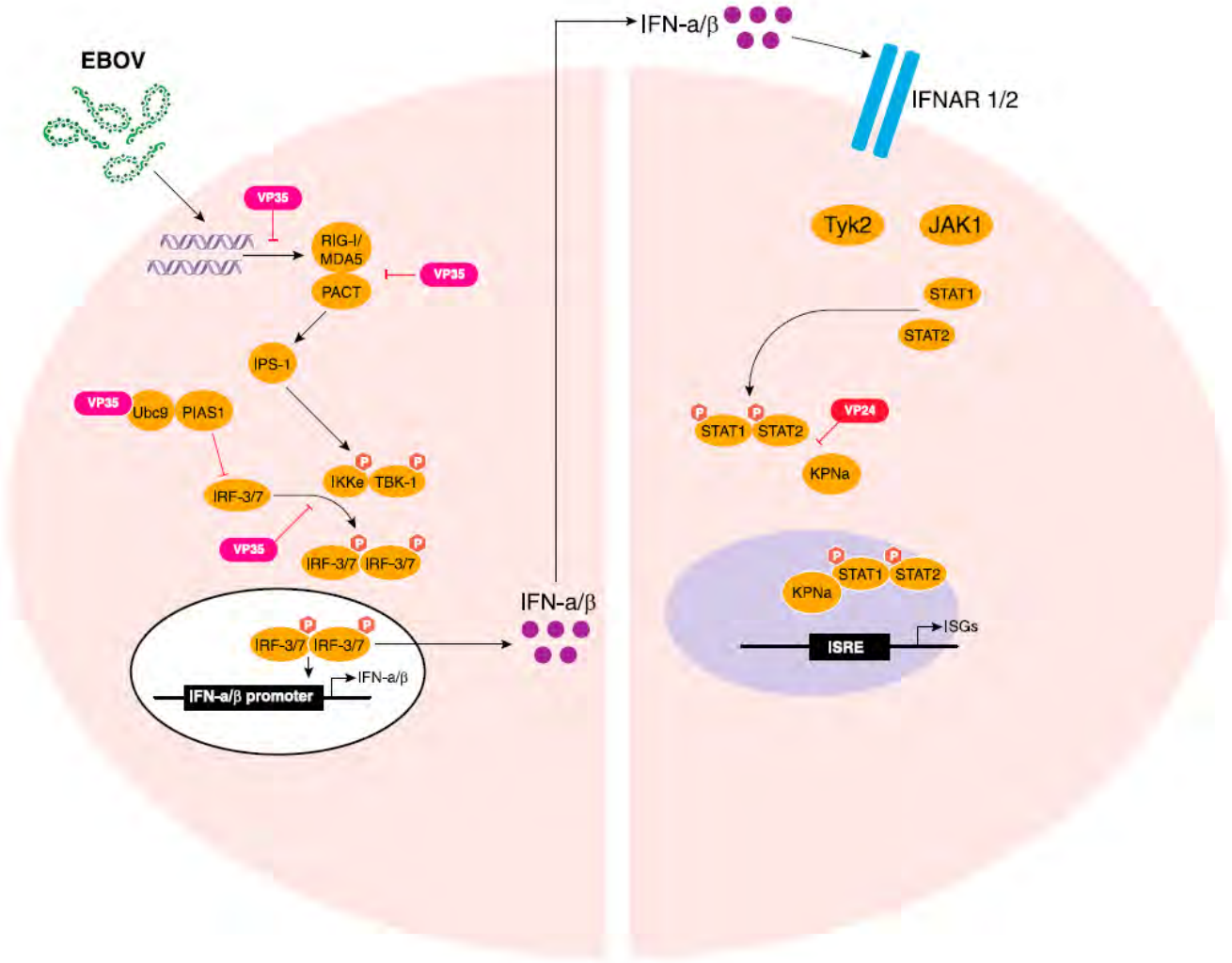
Ebola virus pathogenesis



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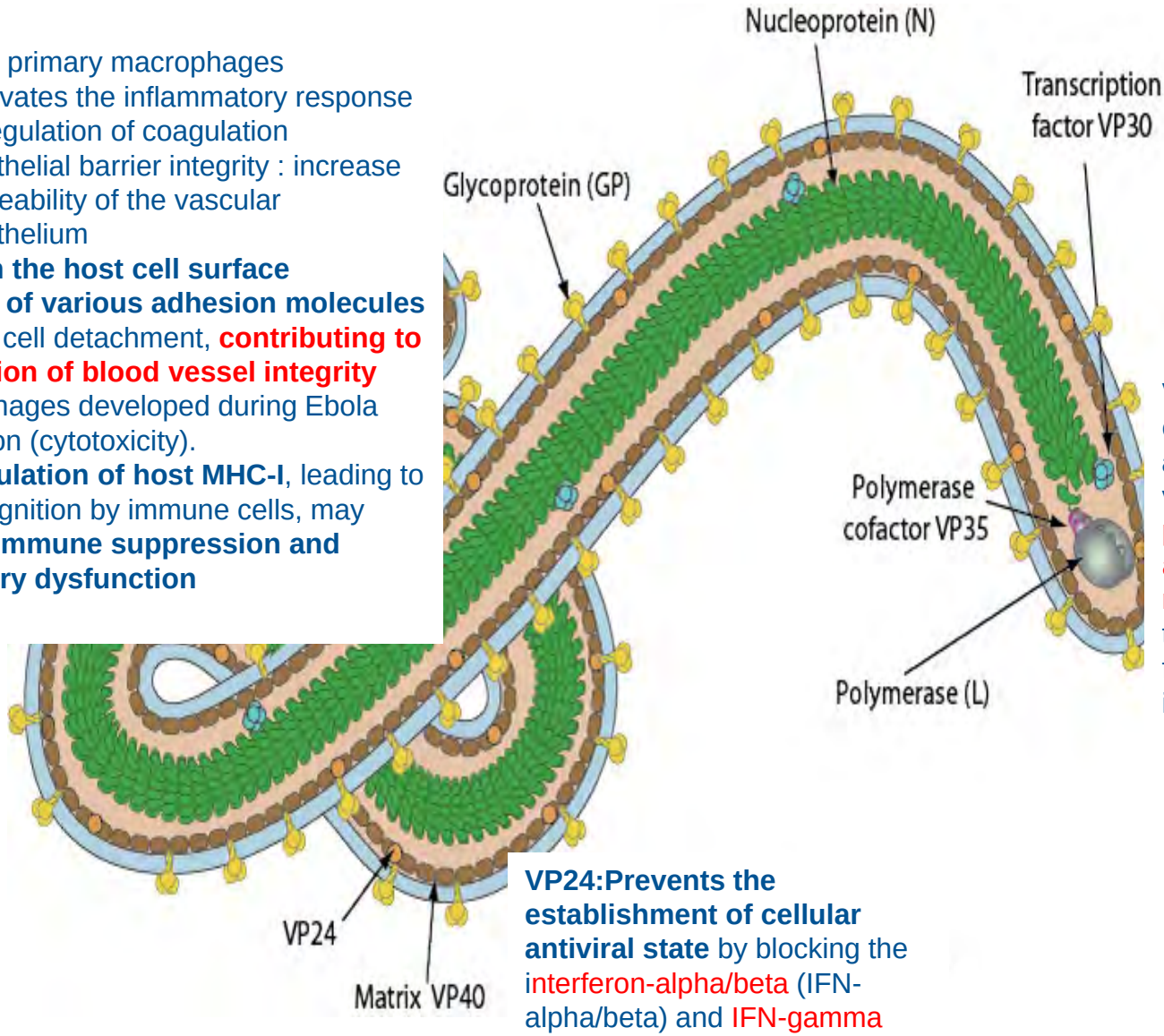
Other functions of ebolavirus proteins: virus-host interactions

GP :

- activation of primary macrophages
- strongly activates the inflammatory response
  - dysregulation of coagulation
  - endothelial barrier integrity : increase permeability of the vascular endothelium
- **decrease in the host cell surface expression of various adhesion molecules** may lead to cell detachment, **contributing to the disruption of blood vessel integrity** and hemorrhages developed during Ebola virus infection (cytotoxicity).
- **Down-modulation of host MHC-I**, leading to altered recognition by immune cells, may explain the **immune suppression and inflammatory dysfunction**



VP35: Prevents establishment of cellular antiviral state by blocking virus-induced phosphorylation & activation of interferon regulatory factor 3, a transcription factor critical for the induction of interferons alpha and beta

VP24:Prevents the establishment of cellular antiviral state by blocking the interferon-alpha/beta (IFN-alpha/beta) and IFN-gamma signaling pathways (STAT1).

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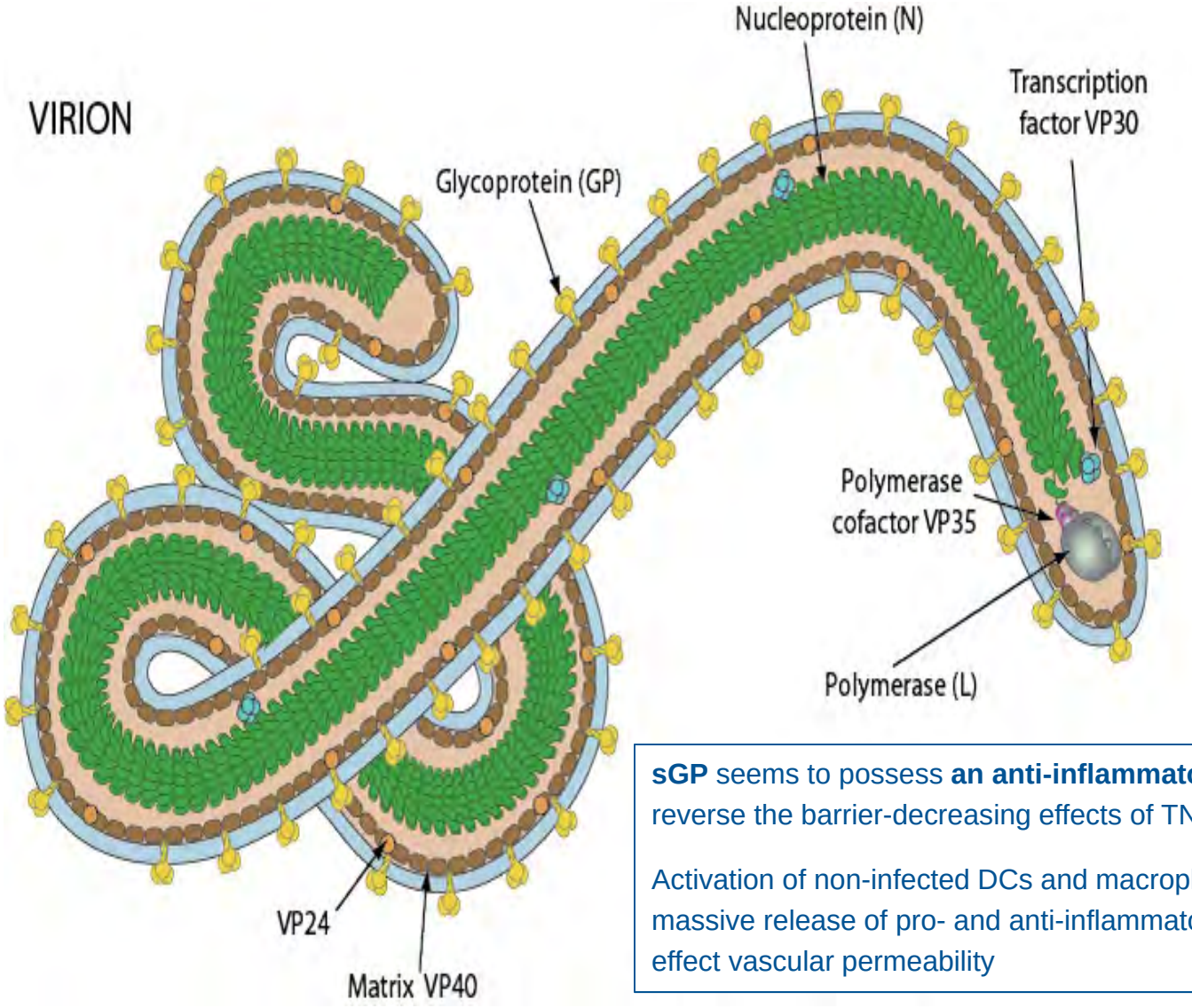
Other functions of ebolavirus proteins: virus-host interactions



sGP seems to possess **an anti-inflammatory activity** as it can reverse the barrier-decreasing effects of TNF alpha.

Activation of non-infected DCs and macrophages causing the massive release of pro- and anti-inflammatory cytokines and effect vascular permeability

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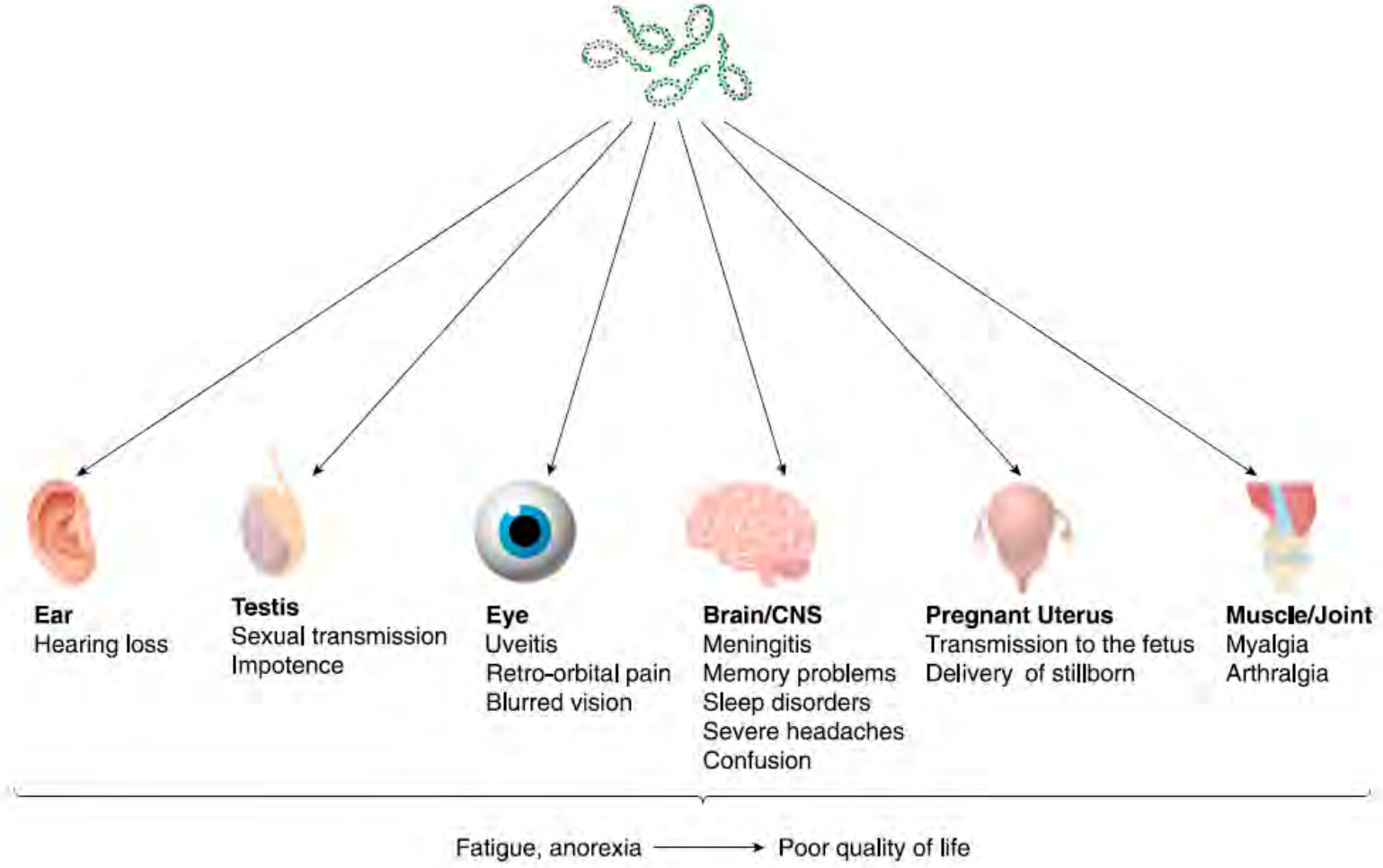
Long-term post-EBOV consequences



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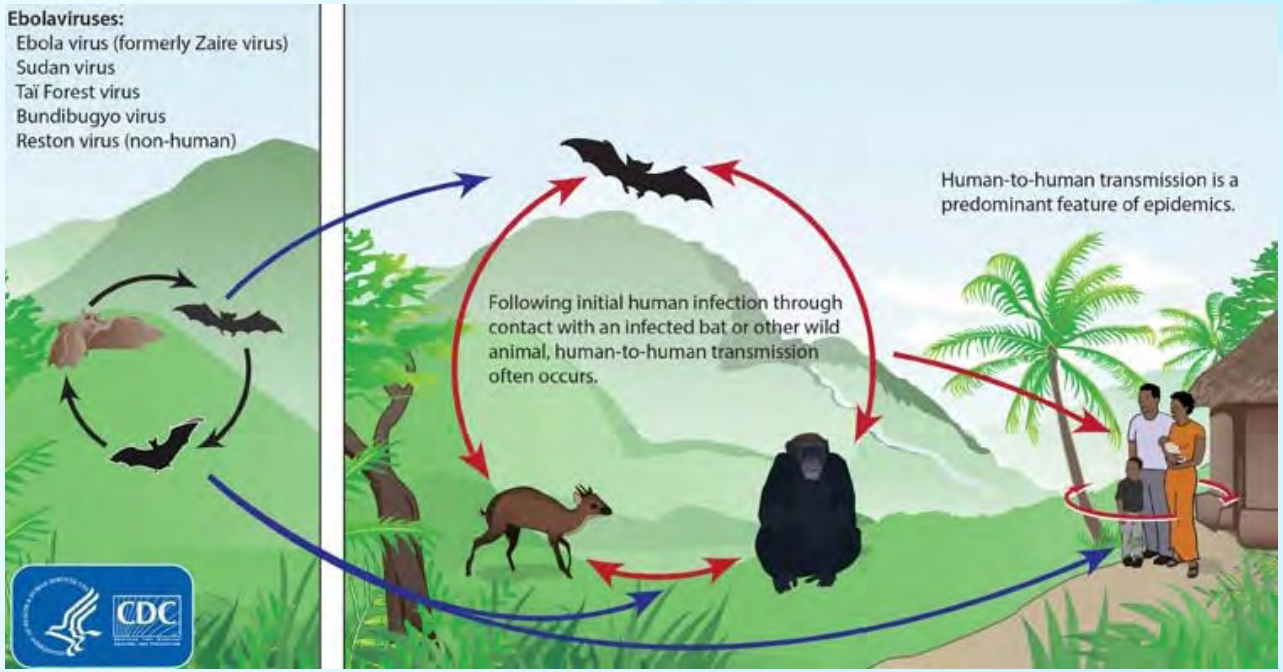
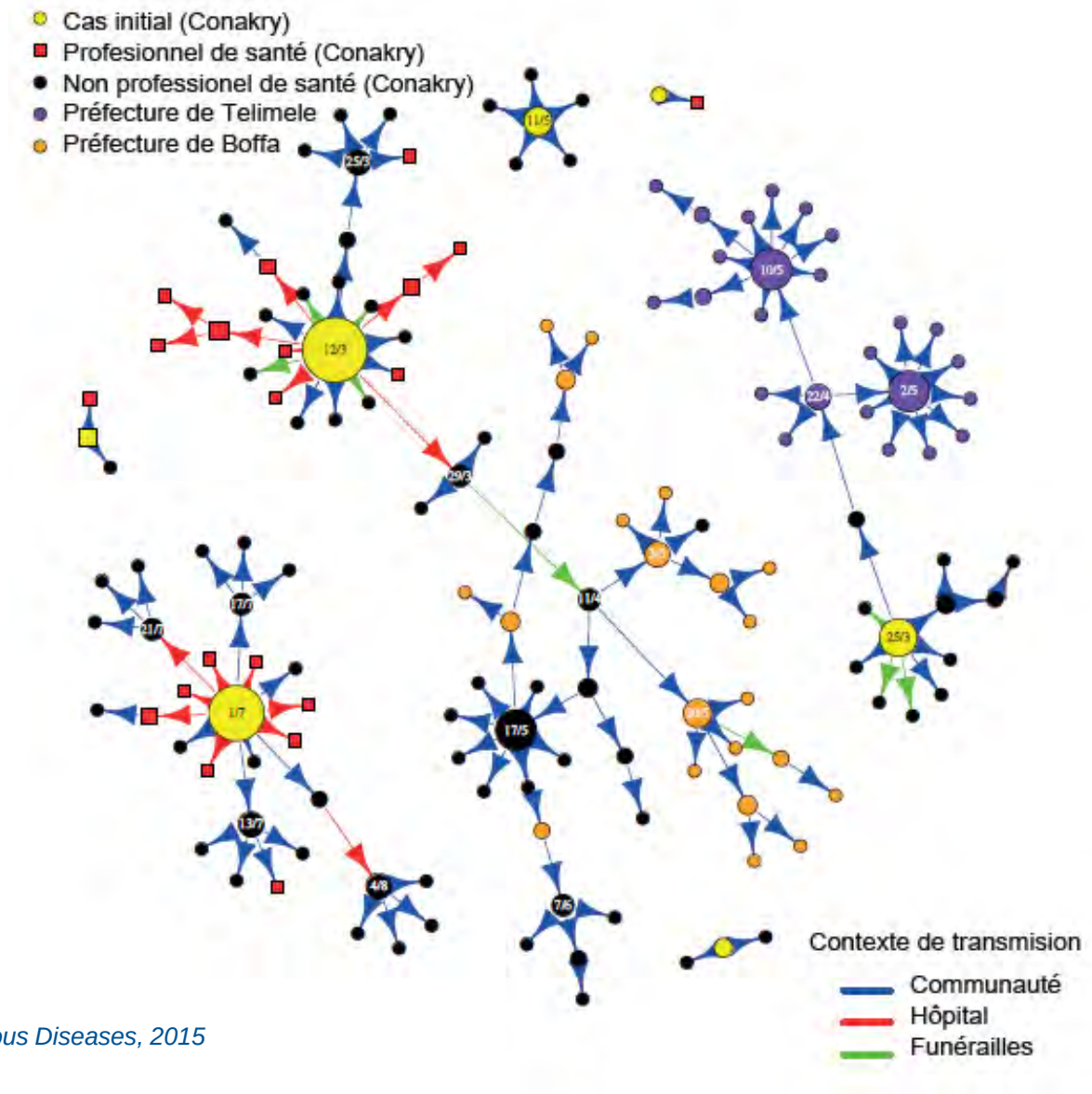
Ebola Virus

- Zoonotic virus – bats the most likely reservoir, although species unknown
- Spillover event from infected wild animals (e.g., fruit bats, monkey, duiker) to humans, followed by human-to-human transmission



Chains of transmission and control of Ebola Virus Disease in Conakry, Guinea in 2014



Faye et al., Lancet Infectious Diseases, 2015

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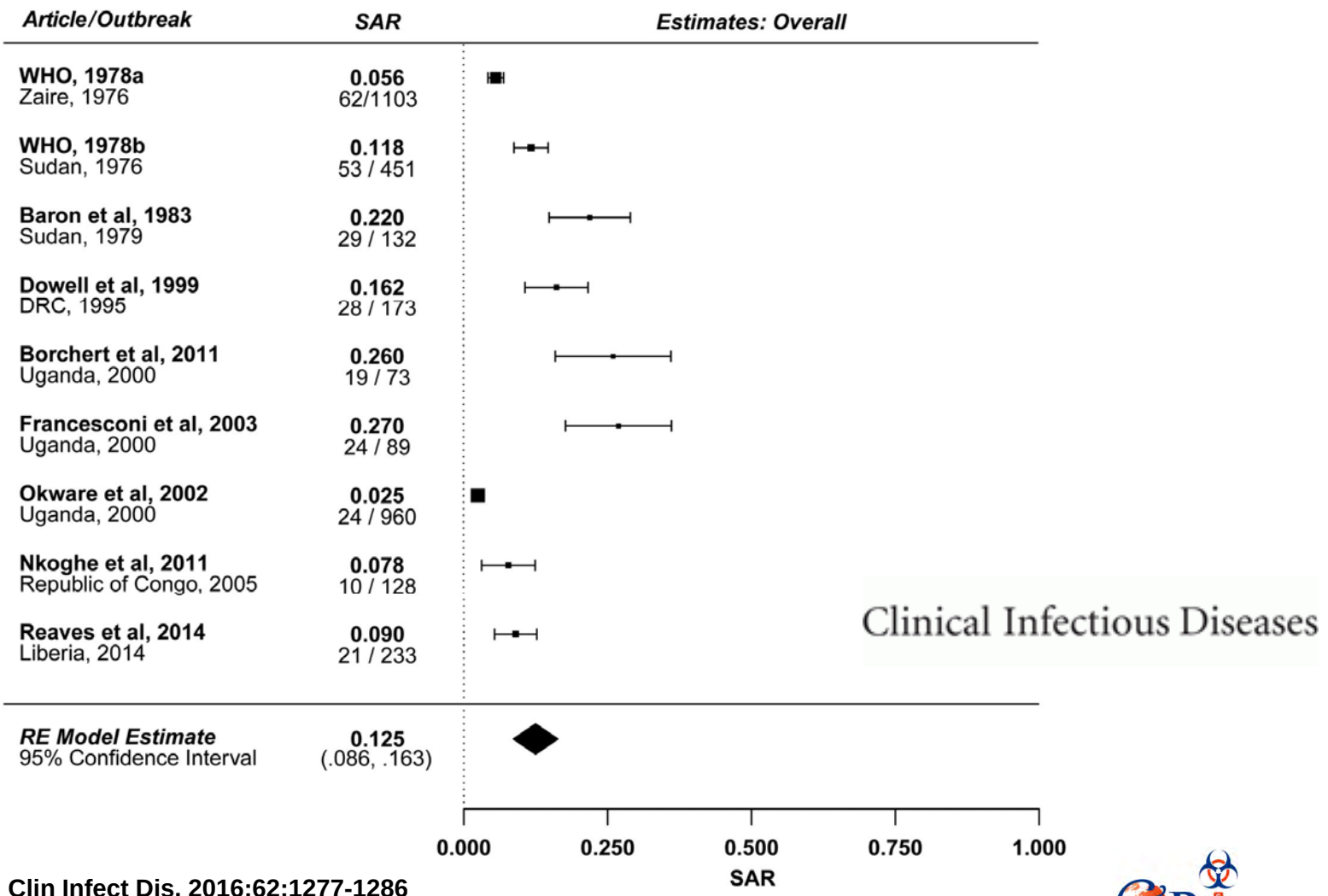
Forest plot: overall estimate



Clinical Infectious Diseases

Natalie E. Dean et al. Clin Infect Dis. 2016;62:1277-1286

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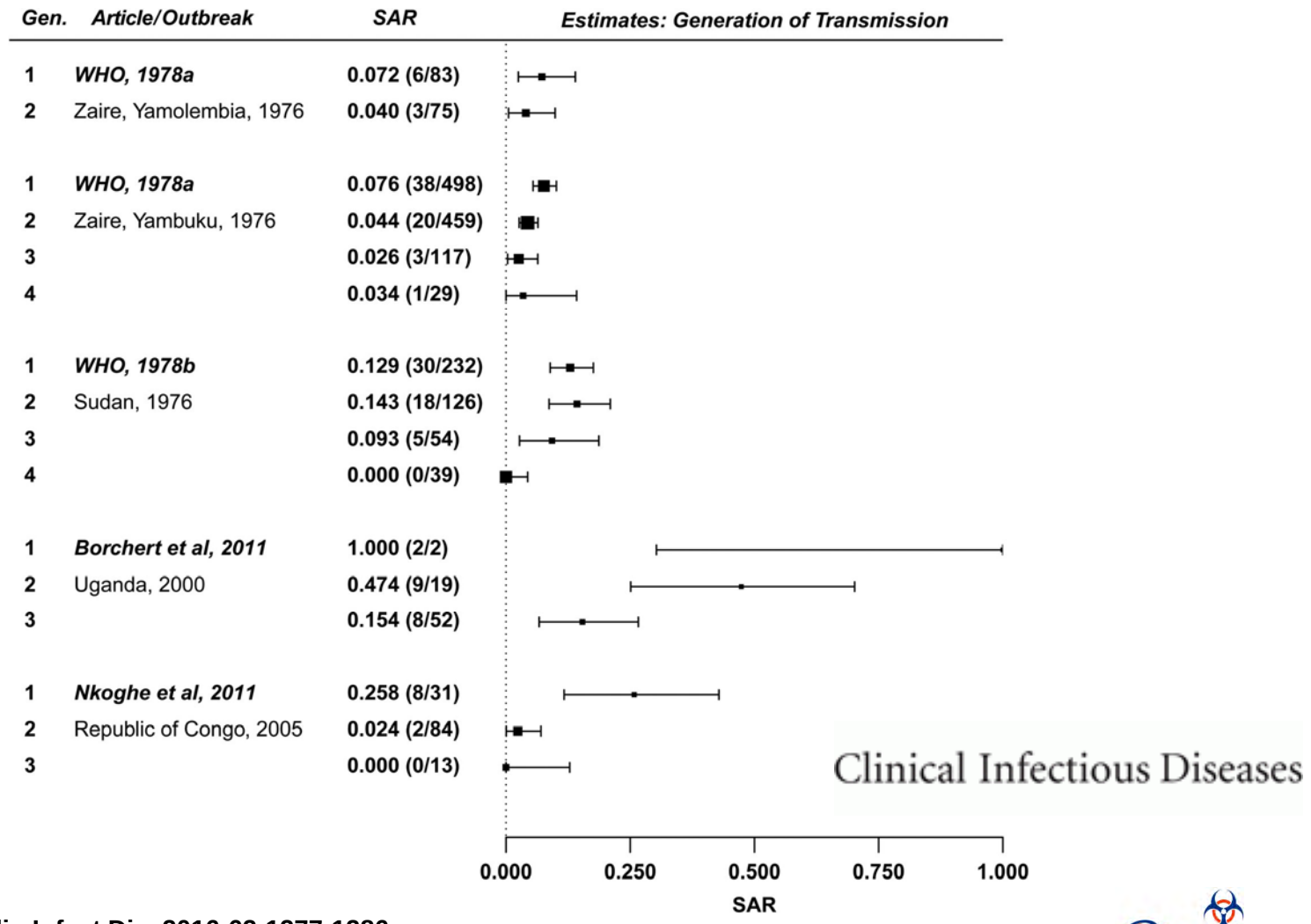
Forest plot: generation of transmission.



Natalie E. Dean et al. Clin Infect Dis. 2016;62:1277-1286

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Table 1. Maximum Interval Between Onset of Ebola Virus Disease (EVD) and Last Detection of *Ebolavirus* RNA by Reverse Transcription–Polymerase Chain Reaction (RT-PCR) and Last Detection of *Ebolavirus* by Culture, by Human Body Fluid Specimen

Specimen(s)	Last Positive RT-PCR Result, d	Last Positive Culture Result, d
Saliva	22	4
Tears/conjunctival swab	28	. . .[a]
Rectal swab/stool	29	. . .[b]
Vaginal swab	33	. . .[c]
Amniotic fluid/placenta	38	NA
Skin swab/sweat	44	NA
Urine	64	26
Aqueous humor	101	101
Cerebrospinal fluid	283	NA
Breast milk	486[d]	15
Semen	488	82

Abbreviation: NA, no report available in the literature.

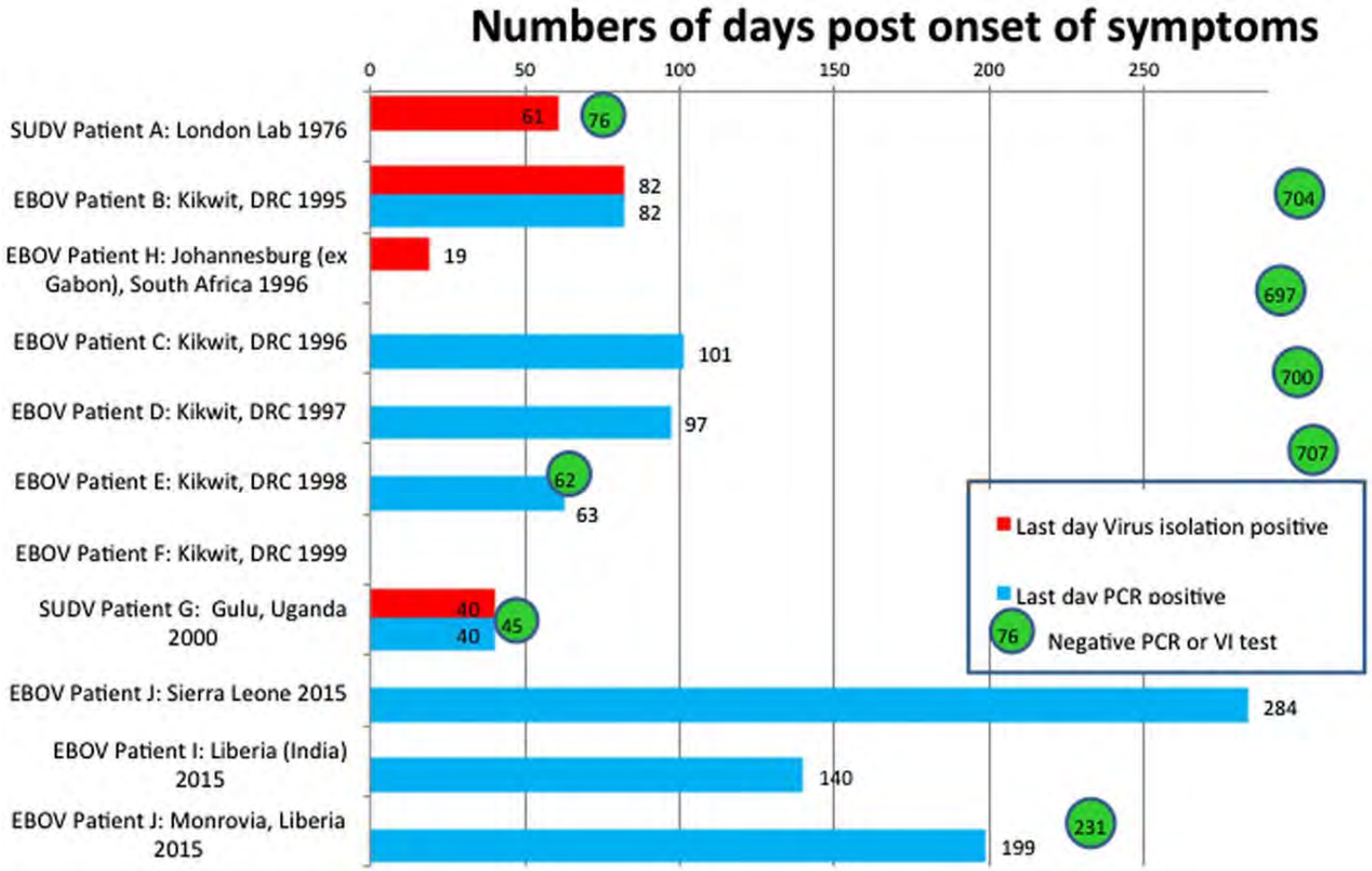
[a] No data on final positive results are available. Culture results were negative 6 days after EVD onset.

[b] No data on final positive results are available. Culture results were negative 4–12 days after EVD onset.

[c] No data on final positive results are available. Culture results were negative 22, 25, and 33 days after EVD onset.

[d] The time was specified as 16 months in the literature but is converted here to days for parallelism with units specified elsewhere in the column. This sample was *Ebolavirus* RNA positive at low levels, making interpretation difficult. Further testing is necessary to

Depiction of the duration of measured persistence of Ebola virus and Ebola virus RNA in semen (VI, virus isolation).



Thorson A, et al. BMJ Open 2016;6:e008859. doi:10.1136/bmjopen-2015-008859

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VI and RT-PCR findings in other body fluids in recovered patients

Table 1 VI and RT-PCR findings in other body fluids in recovered patients[8 9 12 13 17]

	EBOV	Faeces or rectal swabs	Throat swabs or saliva	Sweat	Urine
Patient 1, London, 1976	EBOV	–VI days 14–27	–VI days 14–27	NA	–VI days 14–27
29 Recovered patients, Kikwit, DRC, 1995	EBOV	–VI days 11–57	–VI days 11–57	NA	–VI days 11–57
8 Recovered patients, Kikwit, DRC, 1995	EBOV	–RT-PCR days 11–33 (total 18 specimens) + RT-PCR days 22 and 29 (total 2 specimens, same woman–RT-PCR days 25 and 33)	–RT-PCR days 11–33 (total 20 specimens)	NA	–RT-PCR days 11–33 (total 19 specimen)
4 Patients, Gulu, Uganda, 2000	SUDV	NA	–RT-PCR days 12–23	NA	–RT-PCR days 12–23
Patient 1, Sierra Leone, 2014	EBOV	–VI after day 17 (negative blood test day 17)	–VI after day 17 (negative blood test day 17)	–VI + RT-PCR until day 40	+ VI repeatedly until day 26 + RT-PCR until day 30

NB days post-onset of symptoms.
EBOV, Ebola virus – Zaire; NA, not applicable; RT-PCR, reverse transcriptase PCR; SUDV, Ebola virus – Sudan; VI, virus isolation.

Thorson A, et al. BMJ Open 2016;6:e008859. doi:10.1136/bmjopen-2015-008859

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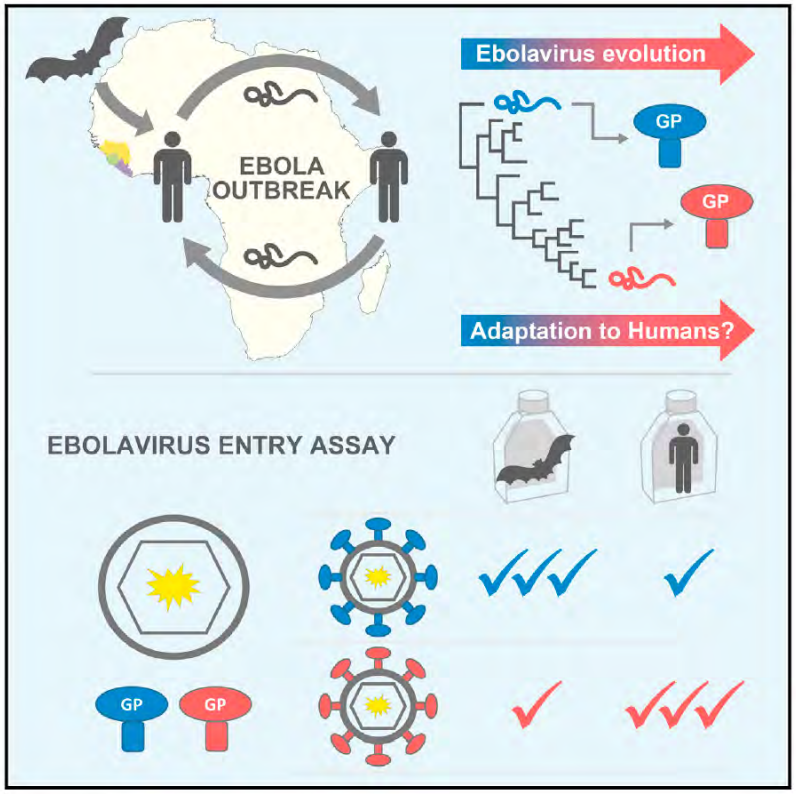
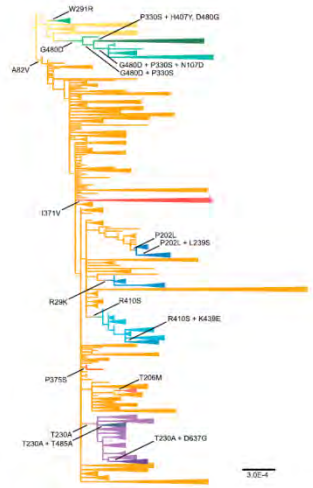
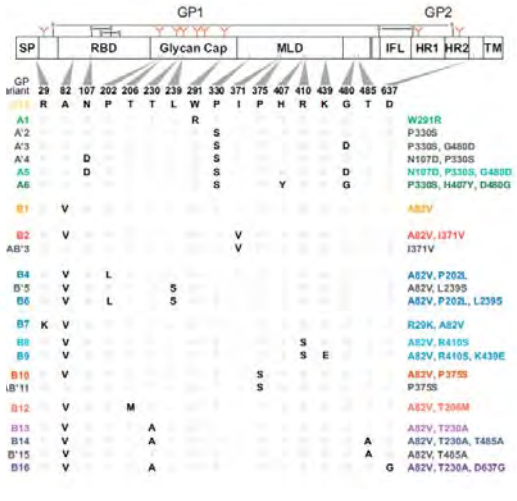
Human Adaptation of Ebola Virus during the West African Outbreak



- EBOV adapted to humans during the West African outbreak
- Amino acid substitutions in the EBOV glycoprotein increase human cell tropism
- The same glycoprotein amino acid substitutions decrease tropism for bat cells



Schematic Maximum Likelihood Phylogenetic Tree of 1,610 Complete EBOV Makona Genomes



Schematic of the 17 Lineage-Defining Amino Acid Combinations in the EBOV GP

The Ebola virus acquired amino acid substitutions in its glycoprotein that increased its tropism for human cells during the West African outbreak of 2013 – 2016.

Urbanowicz et al., 2016, Cell 167, 1079–1087 November 3, 2016

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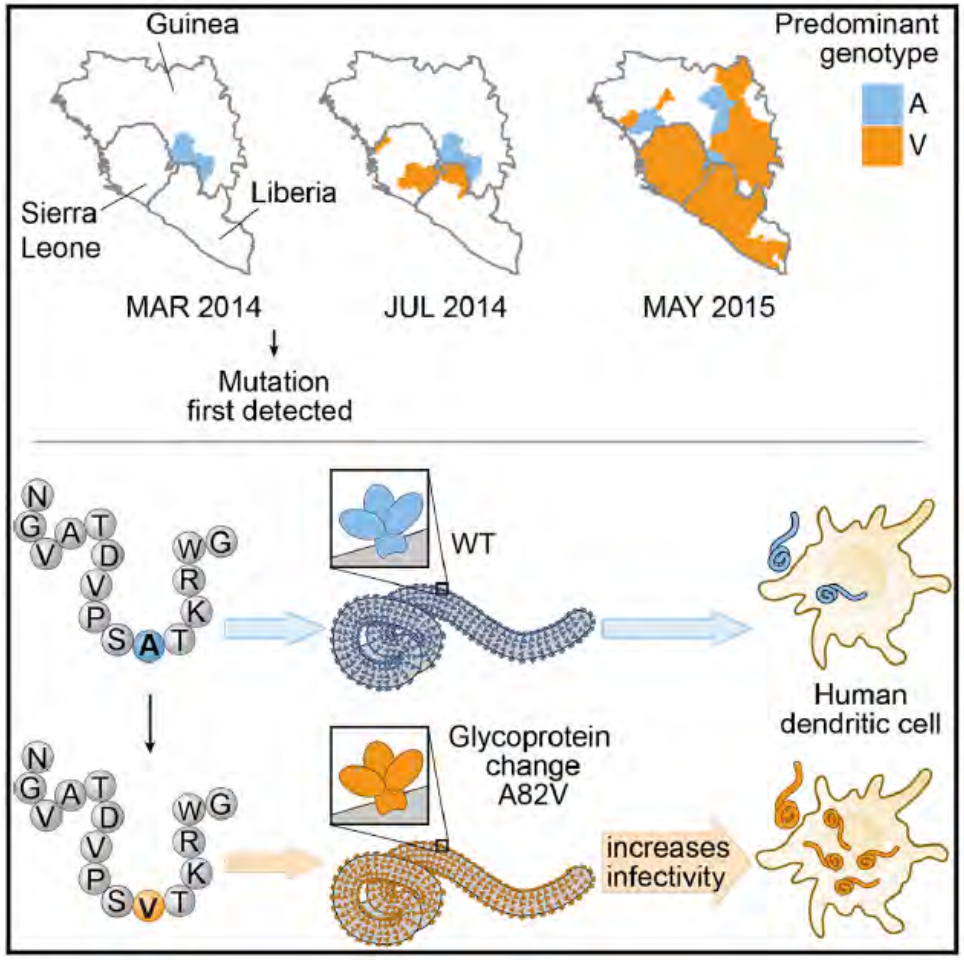
Ebola Virus Glycoprotein with Increased Infectivity Dominated the 2013–2016 Epidemic



- Ebola glycoprotein mutant GP-A82V arose early and dominated the West African epidemic
- GP-A82V infects human cells more efficiently than does the ancestral glycoprotein
- The increased infectivity of GP-A82V is specific for primate cells
- GP-A82V was weakly associated with increased mortality during the epidemic

Diehl et al., 2016, Cell 167, 1088–1098 November 3, 2016

An Ebola glycoprotein mutant that arose early during the West African epidemic increases infectivity of human cells and may have contributed to increased mortality

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Virus culture and reverse-transcription polymerase chain reaction (RT-PCR) results from 33 environmental samples

Sample	Color	Virus culture result	RT-PCR result
Outside of ward			
Changing room wall	Clear	–	–
Changing room desk	Clear	–	–
Exterior surface of door of isolation ward	Clear	–	–
Inside ward, suspected side			
Nurse's newly placed glove	Clear	–	–
Bed frame	Clear	–	–
Instrument tray for ward rounds	Clear	–	–
Inside ward, probable side			
Air (tube opened and capped, negative control 1)	Clear	–	–
Sterile swab (negative control 2)	Clear	–	–
Intravenous fluid support pole	Clear	–	–
Light switch	Clear	–	–
Floor	Clear	–	–
Handle of 0.05% bleach solution dispenser	Clear	–	–
Nurse's clean apron	Clear	–	–
Nurse's clean glove	Clear	–	–
Clean stethoscope	Clear	–	–
Stethoscope after use	Clear	–	–
Stethoscope after use and rinsing with 0.05% bleach solution	Clear	–	–
Bed frame	Clear	–	–
Bedside chair (2 different samples)	Clear	–	–
Food bowl	Clear	–	–
Spit bowl	Clear	–	–
Skin (hand) of patient attendants (3 different samples)	Clear	–	–
Clean glove of patient attendant	Clear	–	–
Corpse decontaminated with 0.5% bleach solution	Clear	–	–
Body bag decontaminated with 0.5% bleach solution (2 different samples)	Clear	–	–
Clean mattress	Clear	–	–
Intravenous tubing	Clear	–	–
Doctor's blood-stained glove (positive control 1)	Pink	–	+
Bloody intravenous insertion site (positive control 2)	Red	–	+
Total (% of all samples)	...	0 (0)	2 (7)

Bausch et al.; Bodily Fluids and Fomites in Ebola
JID 2007:196 (Suppl 2)

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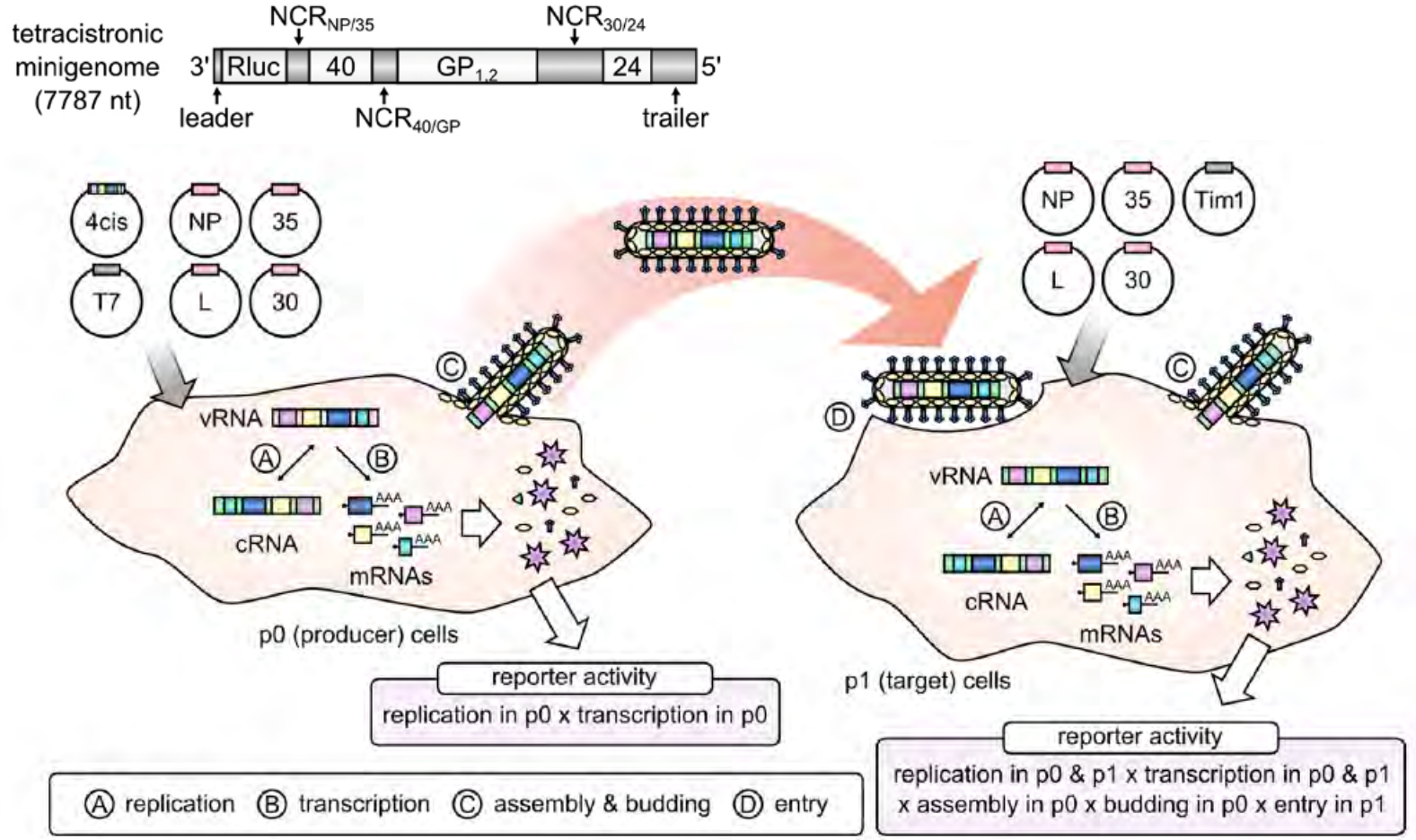
Novel Life Cycle Modeling System for Ebola Virus



Watt A et al. (2014). A Novel Life Cycle Modeling System for Ebola Virus Shows a Genome Length-Dependent Role of VP24 in Virus Infectivity. *J Virol* 88(18):10511-24

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Controlled atmosphere isolator

Temperature: 5° C – 55° C
RelativeHumidity : 20% - 98%
Use of different kinds of surfaces - Aerosols

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Summary of the current state of the field about EBOV pathogenesis and outstanding questions of basic research

Lessons learned	Outstanding questions
The virulence of Ebola virus is dependent on the species and the strain.	Which viral genes contribute to the differences in virulence? By comparing genomes of different species and different ZEBOV strains, what can we learn about the mechanisms of virulence and what contributed to an outbreak in West Africa?
Based on in vitro studies, ZEBOV infection of monocytes results in excessive inflammatory response that contributes to hemorrhagic fever, whereas infection of DCs results in suppression of DC maturation and type I IFN response. Lymphopenia and the lack of ZEBOV-specific cellular and humoral responses are hallmark characteristics of EVD.	What is the effect of ZEBOV infection on monocytes and DCs in vivo? What is the role of VP24 and VP35 in the evasion of innate immune responses in vivo? Is the subversion of the adaptive immune response determined by the outcome of ZEBOV subversion of innate immunity in vivo?
In survivors, ZEBOV persists in immune privileged sites that may contribute to the Ebola disease sequelae observed.	How does ZEBOV gain access to immune privileged sites, and what cell types could be supporting viral replication? What are the mechanisms of viral persistence? Are the Abs produced from survivors reactive across >1 strain of Ebola virus?
Clinical correlates and host genetics may determine outcome of EBOV infection.	Are there early changes in gene expression in the host after infection that are predictive of disease outcome?
Two vaccine platforms, rVSV and rAd, have been shown to be safe and efficacious in clinical trials.	What is the duration of immunity following vaccination? What are the correlates of protection compared with the surrogates of protection?
Antivirals that inhibit EBOV replication or translation or limit virus spread have entered clinical trials.	Do we need new therapies to clear EBOV from immune privileged sites? What alternative routes of administration can be used to target EBOV in immune privileged sites?

Molecular mechanisms of Ebola pathogenesis, Andrea Rivera and Ilhem Messaoudi,J. of Leukocyte Biology Vol.100, No.5:889-904, November, 2016

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Summary of the current state of the field about EBOV pathogenesis and outstanding questions of basic research

Lessons learned 1	Outstanding questions
The **virulence** of Ebola virus is dependent on the **species** and the **strain**.	**Which viral genes** contribute to the differences in virulence? By comparing genomes of different species and different ZEBOV strains, what can we learn about the **mechanisms of virulence** and **what contributed to an outbreak in West Africa**?

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Summary of the current state of the field about EBOV pathogenesis and outstanding questions of basic research

Lessons learned 2&3	Outstanding questions
Based on *in vitro* studies, ZEBOV **infection of monocytes** results in **excessive inflammatory** response that **contributes to hemorrhagic fever**, whereas **infection of DCs results in suppression of DC maturation and type I IFN response.**	**What is the effect of ZEBOV** infection on monocytes and DCs in vivo? **What is the role of VP24 and VP35** in the evasion of innate immune responses in vivo?
Lymphopenia and the lack of ZEBOV-specific cellular and humoral responses are hallmark characteristics of EVD.	Is the **subversion of the adaptive immune response determined by the outcome of ZEBOV subversion of innate immunity** *in vivo*?

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Summary of the current state of the field about EBOV pathogenesis and outstanding questions of basic research

Lessons learned 4	Outstanding questions
In survivors, **ZEBOV persists in immune privileged sites** that may contribute to the Ebola disease sequelae observed.	**How does ZEBOV gain access to immune privileged sites**, and what **cell types** could be supporting viral replication? What are the **mechanisms of viral persistence**? Are the Abs produced from survivors **reactive across** >1 strain of Ebola virus?

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Summary of the current state of the field about EBOV pathogenesis and outstanding questions of basic research

Lessons learned 5	Outstanding questions
Clinical correlates and host genetics may determine **outcome** of EBOV infection.	Are there **early changes in gene expression in the host** after infection that are **predictive** of disease outcome?

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Summary of the current state of the field about EBOV pathogenesis and outstanding questions of basic research

Lessons learned 6 & 7	Outstanding questions
Two vaccine platforms, rVSV and rAd, have been shown to be **safe and efficacious** in clinical trials.	What is the **duration of immunity** following vaccination? What are the **correlates of protection**?
Antivirals that inhibit EBOV replication or translation or limit virus spread have entered clinical trials.	Do we need new therapies to clear EBOV from immune privileged sites? What alternative routes of administration can be used to target EBOV in immune privileged sites?

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Acknowledgment

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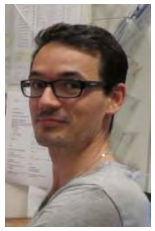

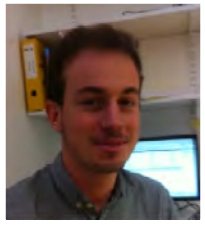

Centre for viral identification




Centre for genotyping and sequencing of pathogens



Centre for bacterial identification

Thank you for your attention

Institut Pasteur