The Ebola Wars
Advanced Edition

Invincibility lies in the defense; the possibility of victory in the attack.
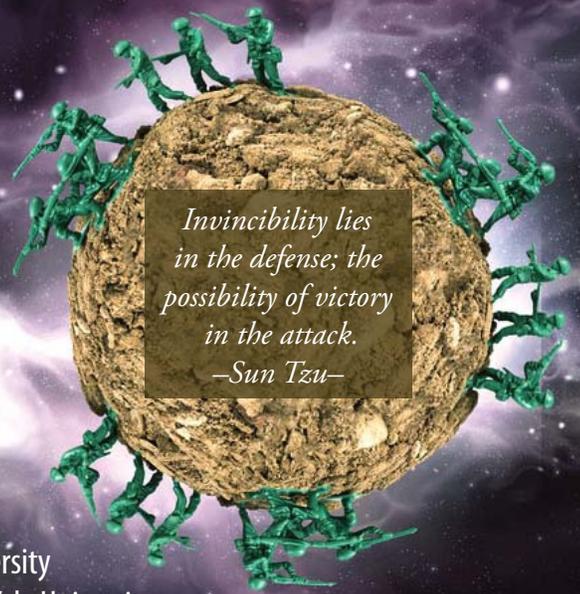*—Sun Tzu—*

by
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Part I – What Is a Virus?

"I can't believe I'm a college sophomore," thought Terry in amazement while taking the train back home for the summer. "The school year went by so fast, but I made it through even though I had to change my major."

A text popped up on Terry's smartphone, "Terry, when r u going on ur trip? Let's meet up before u go." Terry recognized that the message was from Alex, a long-time friend from high school. "Leaving in a week," Terry quickly texted back. "Never been on a trip outside of the US and can't wait to work with doctors and nurses to help sick people. Alex, let's meet up Fri."

After a week of seeing family and friends, Terry was off to the west coast of Africa for ten weeks. This would be a life-changing experience. The agency placed Terry in a rural site where residents had limited access to medical care. The locals sometimes traveled miles to be seen at their clinic. The health care professionals on the team were unbelievably positive and devoted to educating and providing patients with the best care possible. Residents came in with many types of ailments. Terry's responsibilities included greeting patients and their families, bandaging wounds, and providing additional assistance to doctors and nurses as needed.

Just like the school year, the summer experience seemed as though it was over in no time. Terry was about to return home when unexpected news came. Terry's team was contacted by the Centers for Disease Control and Prevention (CDC) and notified that they had likely been exposed to a patient infected with the Ebola virus as there was an outbreak near the medical relief site. The patient who had contracted the virus had died. Struck with fear, Terry quickly tried to determine which patient it could have been and her likelihood of being infected, but she found this nearly impossible to figure out. As a precaution, all individuals on the team returning to the US had to be screened and undergo a 21-day quarantine where they would be monitored for any Ebola-like symptoms. Not doing so could potentially place others at risk for contracting this deadly virus.

"I need to find out more about viruses so that I can understand what's going on," thought Terry as she reached for her general biology textbook. Glancing through the section on virology, she was reminded that viruses are tiny particles considered to be non-living since they cannot metabolize energy, do not create waste, do not grow, and require host cells to multiply. Indeed, in order to replicate, viruses hijack the machinery present within the cells that they infect. The additional viral particles produced inside host cells can exit and infect other cells. "These viruses seem kind of creepy," thought Terry. "They're like parasites to cells."

Terry continued reading:

Viruses have either a DNA or RNA genome, which can be single- or double-stranded. These genomes are housed in a capsid made of proteins. Viruses can be classified by their specific genomes and the unique features of their capsids, including shape (e.g., icosahedral, helical, complex) and protein constituents. Some viruses have lipid envelopes derived from host membranes that enclose the virus particle, while others do not. Surface glycoproteins on these membranes, or spike proteins protruding from the viral capsid in non-enveloped viruses, can play a role in viral attachment and entry into the host cell.

Terry spent the next few minutes summarizing the information she had just read.

Questions

1. Label and describe the function/role of the following structures on the diagram of the viral particle below.

*Figure 1. A general viral particle.*
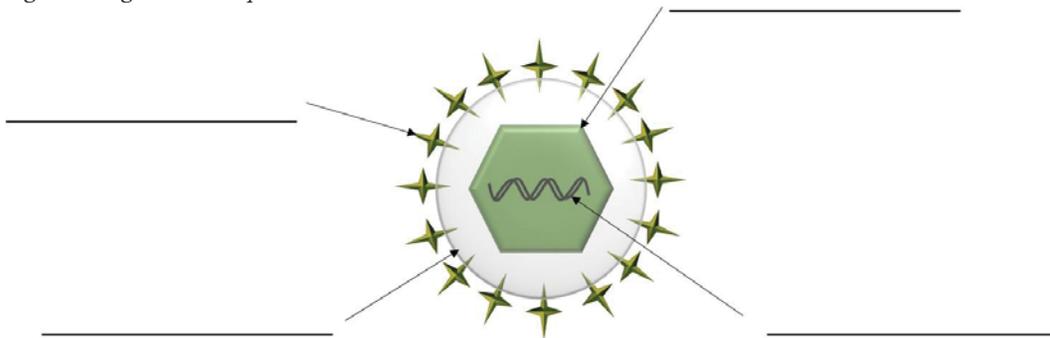
2. Design an imaginary viral particle. Create a diagram of your virus and label its major features. Your virus should have a different capsid shape than the one above and be non-enveloped.
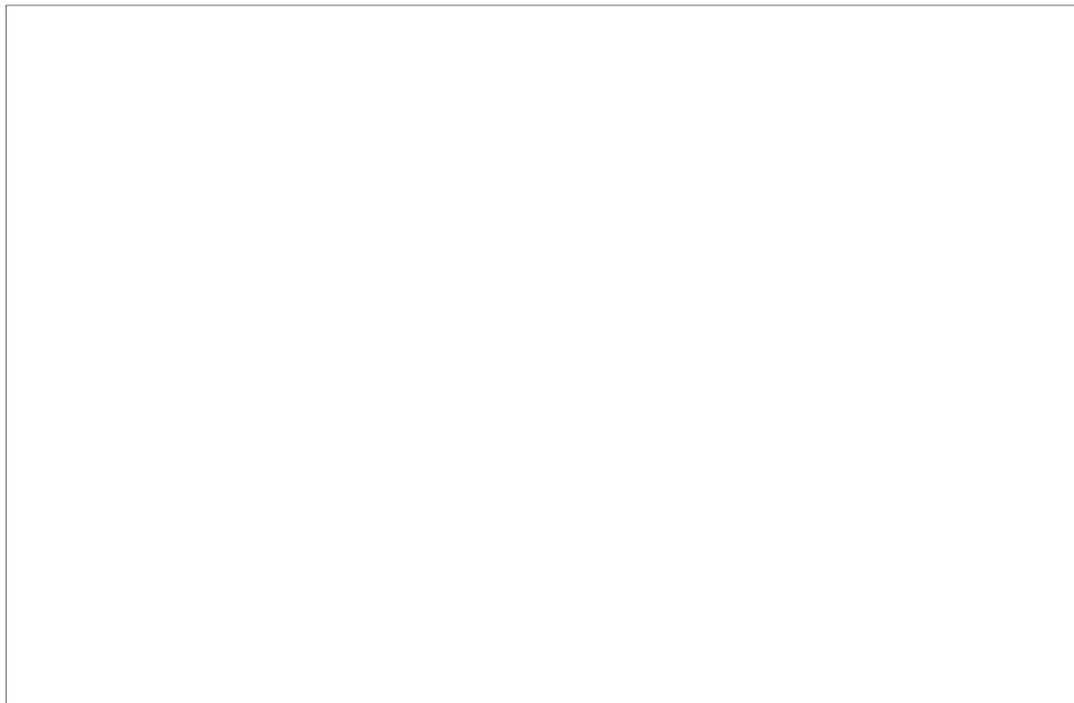
3. Examine the diagram below depicting viral infection of a typical cell.

    a. Describe what is happening in each of the five steps.

    b. What are the roles of the two cellular factors shown on the diagram?

*Figure 2. A general viral life cycle.*
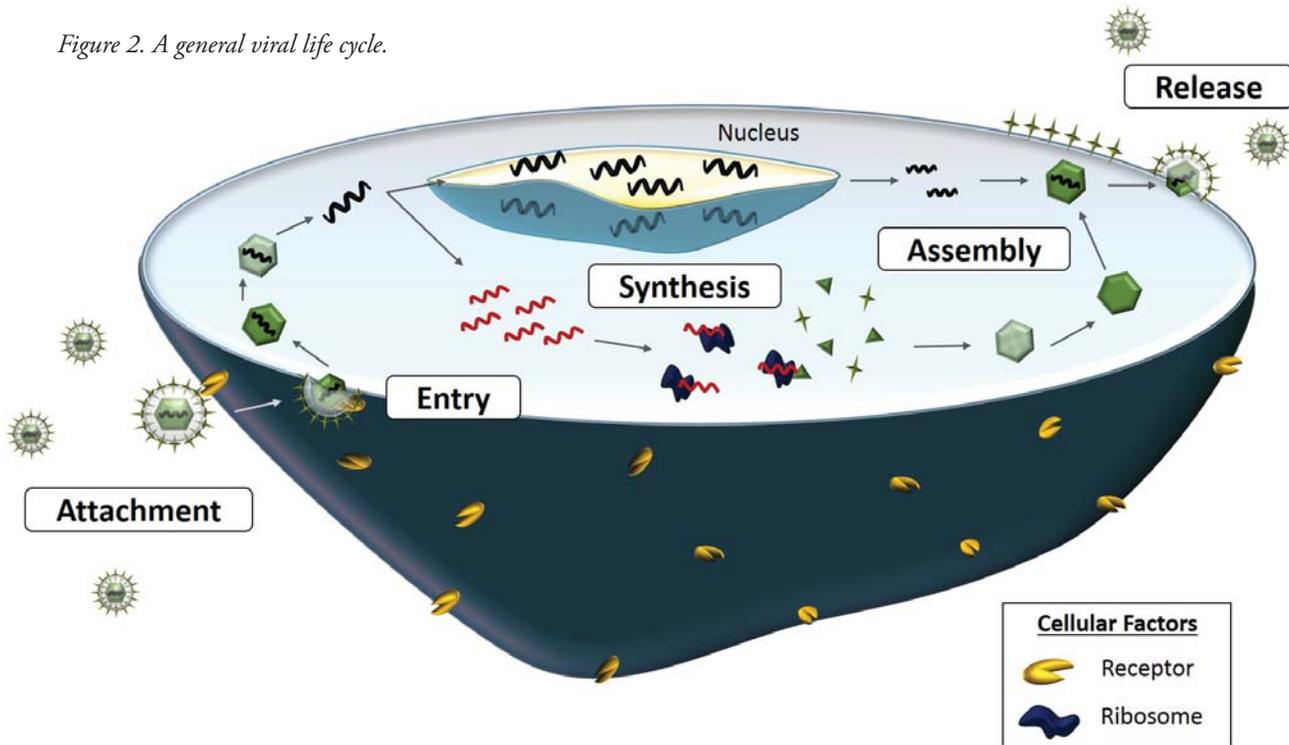
Part II – The Ebola Wars

Terry was nervous about her potential exposure to a patient infected with the Ebola virus, so she wanted to know more about Ebola specifically. She searched the internet for information using what her previous professors had told her were reliable scientific resources and found that while Ebola virus is similar in many ways to other viruses, it also has some distinct structural features and behaves differently when infecting and replicating in a host cell.

Ebola is an enveloped virus with a single negative-strand RNA genome, which belongs to the *Filoviridae* family of viruses. It has a single viral envelope glycoprotein (GP) that consists of a GP1/GP2 heterodimer grouped into trimers on the viral surface. This GP is important for attachment of the virus to the host cell and for directing the virus to gain entry into the cell. Internally, the virus particle contains VP40, a structural protein that determines the filamentous shape of the virus and interacts with VP24, the nucleoprotein (NP) and VP30 proteins that bind to the RNA genome and make up the ribonucleocapsid. The virus particle also carries a RNA-dependent RNA polymerase (L) enzyme and VP35 (a polymerase cofactor), which are important for the synthesis of new viral factors.

Figure 3. Ebola virus particle.
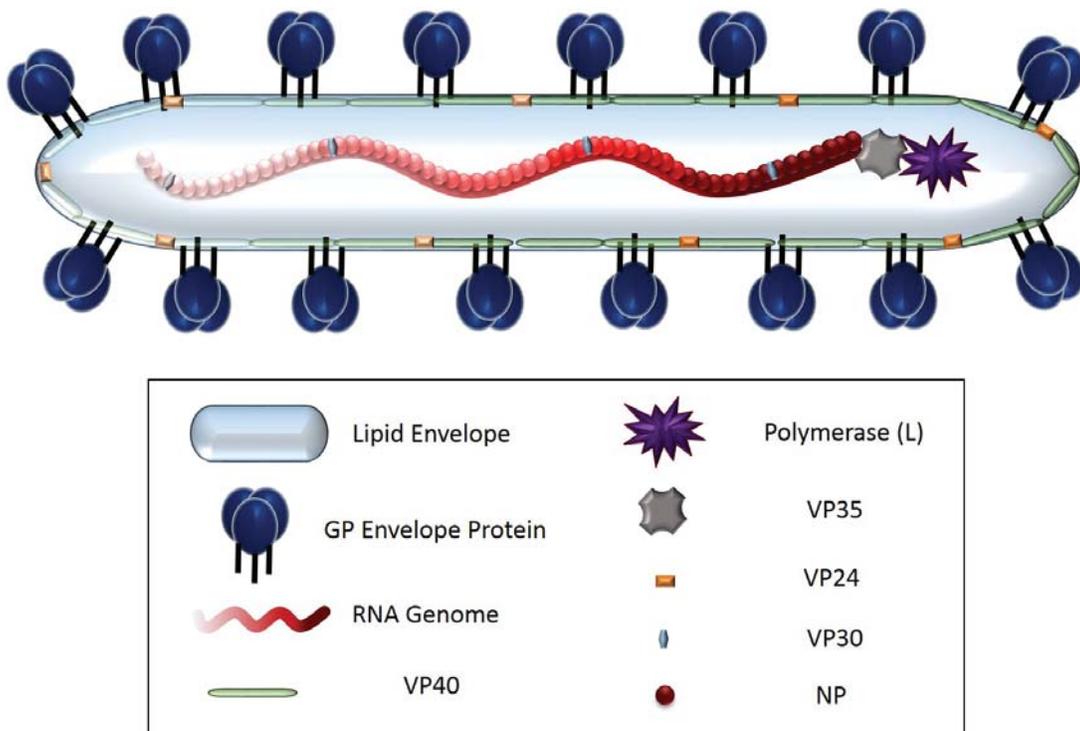
"Wow, this is a complicated system," Terry thought. I had no idea viruses relied on so many different proteins to help them infect our cells. As she investigated further, she quickly realized that the entry, replication and budding of the Ebola virus required some specific proteins and pathways that other viruses do not use when infecting a cell. "It says here that during Ebola infection the virus uses GP to attach to receptors on the cell membrane. After attachment, the virus enters into the host cell via the endocytic pathway, followed by the synthesis of viral proteins and new viral genomes utilizing both viral enzymes and the host cell machinery. After the new viral proteins are made by the host cell, assembly of the virus can occur with the help of the VP40 protein. Finally the virus exits the host cell."

Figure 4. Ebola virus life cycle.
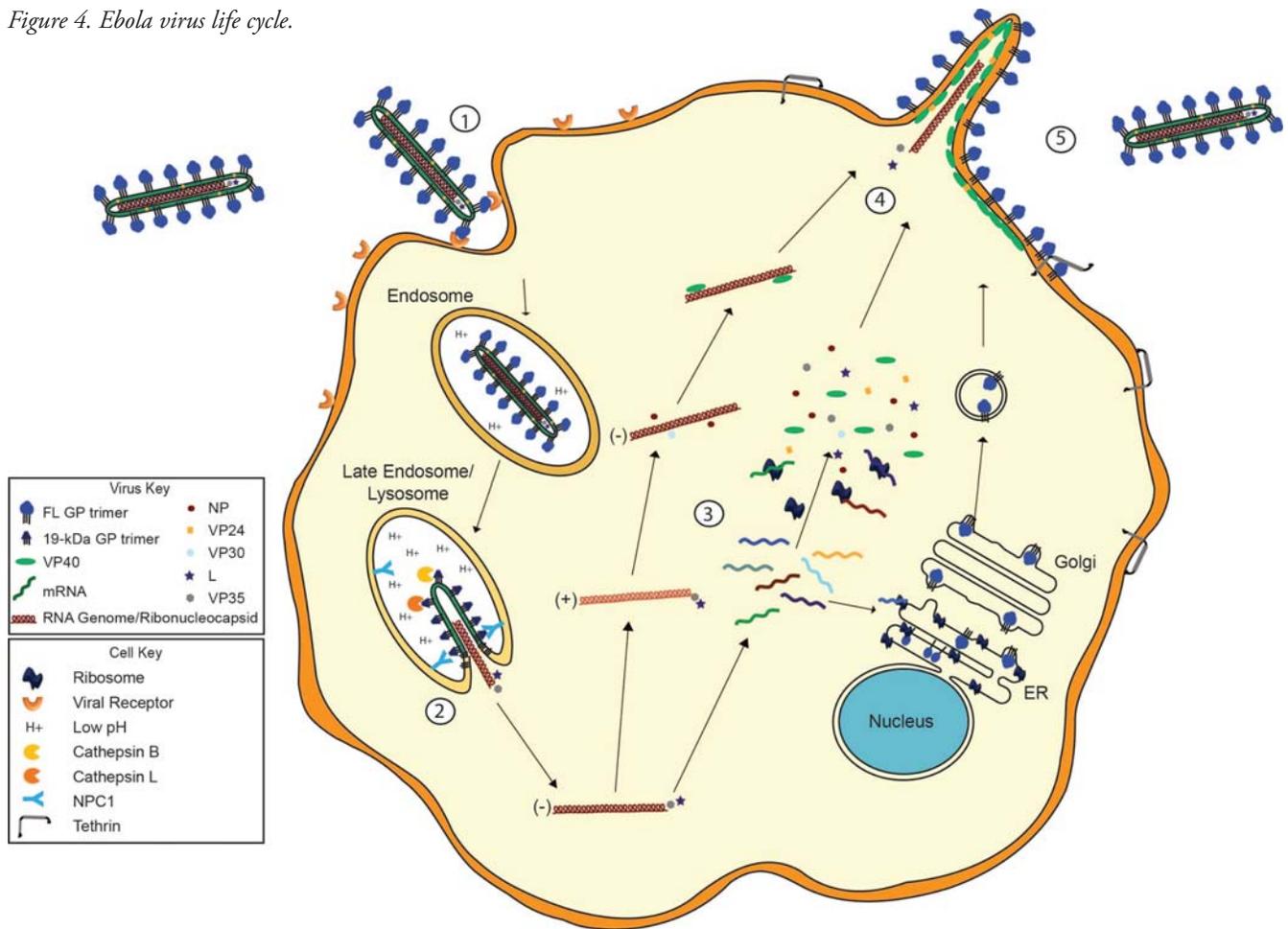
Questions

1. Which "reliable scientific resources" do you think Terry accessed for her research? Why do you consider these reputable resources?

2. Compare and contrast the structure and components of the Ebola virus with that of enveloped and non-enveloped general virus particles shown or created in Part I of the case.

3. Choose one of the five steps of the Ebola virus life cycle and explain the attributes of that step that could pose as a therapeutic target.

Part III – In Enemy Territory

Terry understood from her research that Ebola virus, like other viruses must attach to the host cell in order to get inside the cell. As she read through some journal articles she quickly realized that a functional Ebola virus receptor on the host cell still has yet to be identified. There are, however, two lectins called DC-SIGN and DC-SIGNR that bind GP on the Ebola virus and help it concentrate at the surface of target cells. After attachment, and potential interaction with an as of yet unidentified receptor, the Ebola virus is taken into the host cell, through the endocytic pathway. This starts in an early endosome with a pH of 6.0–6.5 that then matures as it progresses to the late endosome and lysosome stage, with pHs as low as 4.5–5.5. Normally, the function of the lysosome is to degrade proteins and pathogens through this low pH. However, the Ebola virus subverts this attempt of the host cell to destroy it, and in fact the low pH allows the optimal activity of host enzymes, Cathepsins B and L, to cleave Ebola GP into a 19 kD form primed for mediating membrane fusion. This 19 kD form is able to associate with the endocytic membrane-bound host protein Niemann-Pick C1 (NPC1), which seems to act as a fusion trigger. This complicated process allows for Ebola GP to mediate successful fusion of the viral membrane with the lysosomal membrane, thus releasing the viral genome tightly associated with the nucleocapsid proteins (NP and VP30), as well as additional viral enzymes, into the host cell cytosol. As Terry reflected on this information, she quickly realized viruses are highly efficient at entering a cell and setting up to replicate themselves within host cells.

Figure 5. Ebola virus entry. (An animation of this process can be found at: http://makeagif.com/i/MKqWnP.)
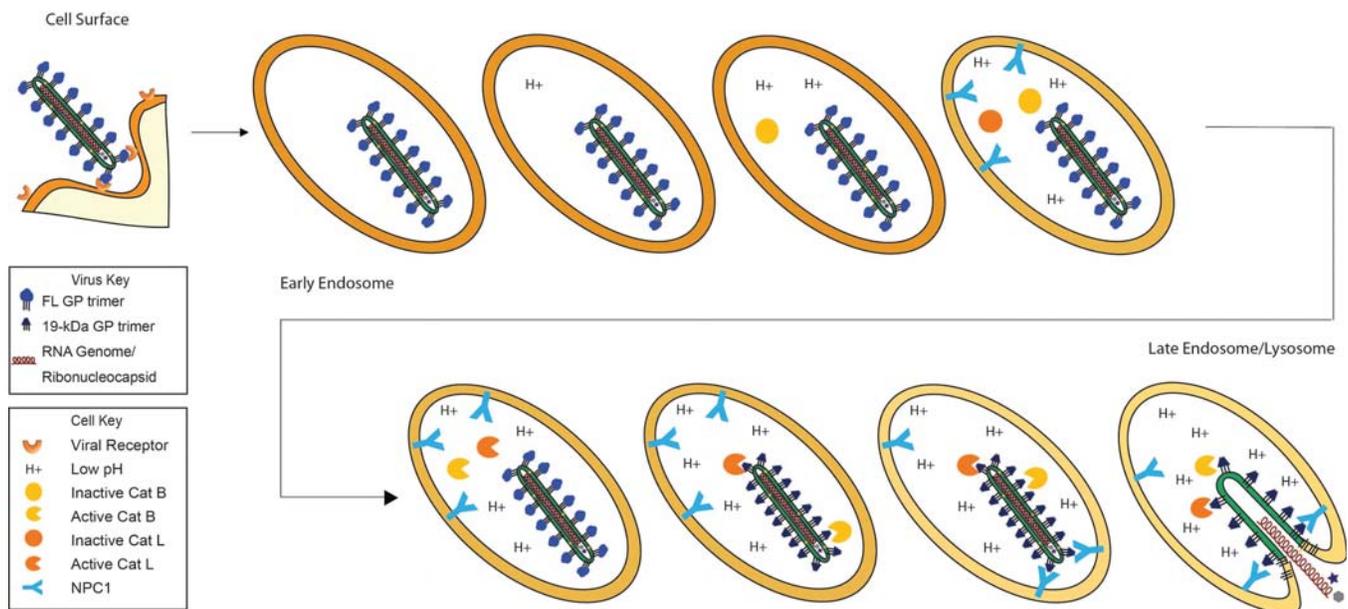
Questions

1. How does the host cell initially fight back against Ebola virus infection? Why is this attempt unsuccessful?

2. If the following host cell factors are rendered non-functional by mutation or therapeutic intervention, what would be the outcomes relative to the Ebola virus life cycle?
   a. DC-SIGN/DC-SIGNR
   b. Cathepsins B and L
   c. NPC1

Part IV – The Ebola Virus Devises Its Battle Plan

As Terry read more journal articles she came across additional information about how viruses replicate their proteins using some of the transcription and translation machinery of the host cells. Because it was such a complicated process, she started to take notes on how the Ebola virus begins to replicate itself:

> *Now that fusion has occurred, the Ebola virus enzymes VP35 and L immediately begin synthesizing mRNA from the RNA-containing ribonucleocapsid template. This template encodes eight proteins, and transcription occurs in a manner where the most 3' genes are transcribed in the highest number, and progressively less so as one moves to the 5' end of the negative-sense RNA genome. These mRNAs are then translated into proteins using the host cell ribosomes and translational machinery found within the cytoplasm for most viral proteins, or at the endoplasmic reticulum for membrane-associated GP. In addition to the full length, membrane-bound GP, there is an alternative form produced known as soluble GP (sGP), which rather than being included in the virus particle itself is secreted by the cells into the extracellular environment to act as an immune decoy.*

Figure 6. Negative-Strand RNA Genome of the Ebola Virus.

> *Since it is the most 3' gene in the Ebola virus genome, NP is transcribed first and in the largest quantity. This is important because in addition to serving as a template for mRNA transcription, the ribonucleocapsid (which primarily consists of NP surrounding the RNA genome) also serves as the template for positive-strand antigenome production and the subsequent production of additional negative-strand RNA genomes. Both of these processes are mediated by the Ebola RNA-dependent RNA polymerase (L), and the resulting RNAs are quickly encapsidated by newly formed NP and VP30 to form new ribonucleocapsids.*

Questions

1. Which genes within the Ebola virus genome encode proteins that are structural and which gene encodes a non-structural protein?

2. If the Ebola virus genome was rearranged as shown below, what would be the outcomes relative to protein production and virus particle assembly?

Figure 7. Hypothetical Rearrangement of Ebola Virus Genome.

Part V – The Ebola Virus Builds its Army and Mounts an Attack

Terry was growing more confortable with her understanding of the topic. She remembered learning about transcription and translation in class. It seemed to her that the production of viral proteins followed the same steps for transcription and translation as would any other protein that was made by the host cell. Terry thought for a minute and wondered, "What happens to all these proteins that are made for the virus?" As she continued her research, she discovered that the viral proteins are assembled to form a new virus particle that could then leave the cell and move on to infect other nearby cells in the host. She kept taking notes to be sure she understood all the information involved in virus assembly and budding. There was a lot of information about the later stages of the viral life cycle of Ebola; her notebook was getting full! Below is a page from her notes.

> With the synthesis of new viral genomes and proteins complete, the process of viral assembly can begin. GP is transported from the ER to the plasma membrane by vesicular transport. The majority of the VP40 proteins transit to the inner side of the plasma membrane where they associate with VP24 and with the cytoplasmic tails of GP. Additional VP40 proteins are thought to bind to the newly formed ribonucleocapsids and aid in their transport to the plasma membrane. VP35 helps L associate with the ribonucleocapsid during assembly. It is at the plasma membrane where the Ebola virus particle begins to form its filamentous structure, primarily due to VP40, and buds from the cell usurping a portion of the plasma membrane for its own viral lipid membrane, ready to bind to and infect additional host cells.

> However, the host cell is not simply a passive partner in this process, and acts to inhibit the budding of Ebola virus particles through the presence of a host protein called tethrin. Tethrin is a plasma membrane protein that has a transmembrane domain on one end and a GPI-anchor on the other end. This allows the protein to interact with lipid membranes at two distinct connections. When an enveloped virus attempts to bud off from the cell, the transmembrane portion of tethrin remains associated with the host cell, and the GPI-anchor is embedded within the viral lipid membrane, and thus the virus particle cannot be fully released to infect other cells. Not surprisingly, Ebola virus has a mechanism for inhibiting the action of tethrin. Although the mechanism is not fully understood, VP24 is known to inhibit the interferon signaling within the cell that stimulates the production of tethrin and GP is thought to have a direct role in preventing its action.

After completing her research, Terry sat back, sipped her coffee, and felt very nervous. She was still worried about the potential for contracting Ebola virus disease since she was potentially exposed to it during her trip. The more she thought about it, the more likely it appeared that she could be infected with the virus.

A few days later Terry began to feel gravely ill. Feverish, achy, nauseated and weak, Terry's worst fears came true—the symptoms were consistent with an Ebola virus infection. While she now knew that Ebola virus could not be transmitted through the air, she recalled that during her trip to West Africa she had a small cut on her hand when she was bandaging a patient who had high fever and hemorrhaging. The exchanged fluid may have contained Ebola virus particles that infected Terry's cells. After five days of illness including repeated vomiting and extreme pain, Terry wondered if this was the end. A decision was made to transport Terry to a special facility for treatment and isolation.

Questions

1. Design an experiment to test the impact of non-functional VP35 protein on the Ebola virus life cycle.

2. How does the host cell attempt to prevent new Ebola virus particles from exiting?

3. Propose a way to therapeutically inhibit the release of Ebola virus particles.

Part VI – Treatment Strategies and the Path to Victory

Quick action was necessary to help Terry survive the Ebola infection. At the time there were a few promising vaccines and antiviral treatments including ChAd3-ZEBOV, rVSV-ZEBOV, ZMapp™, and BCX4430. Each drug had a different known (or unknown) mechanism of action and it was up to Terry's doctor to choose one for treatment. Such a decision would require judgement on the behalf of Terry's physician because at the time none of the drugs were proven to be both safe and efficacious for Ebola therapy through human clinical trials.

Vaccines are generally given to prevent future infections by exposing the immune system to a non-pathogenic, weakened or non-living form of the infectious agent. This exposure triggers an immune response leading to the creation of memory cells that produce antibodies which aid in the elimination of the foreign invader from the body. The underlying rationale for giving someone a vaccine prior to infection is that if the individual is exposed to the actual virus or bacterium in the future, they will mount a quick immune response, minimizing or deterring a full-blown infection.

ChAd3-ZEBOV is a vaccine that was created from a virus that infects chimpanzees but does not cause disease in humans. ChAd3 stands for chimpanzee adenovirus type 3. This vaccine contains GP from two different species of Ebolavirus, Zaire and Sudan, which have been responsible for many of the infections. Another vaccine, rVSV-ZEBOV, consists of an attenuated version of the vesicular stomatitis virus which normally infects livestock (not humans). To make this vaccine Ebola specific, rather than VSV's native surface protein, the Ebola GP is expressed at the viral surface and thus exposed to the vaccinated individual's immune system.

There were also experimental drug options for treating Terry. ZMapp, classified as immunotherapy, used three unique antibodies against Ebola GP made in tobacco plants. While there had not been any large-scale human trials at the time Terry was infected, when these antibodies were injected into Ebola-infected mice and rhesus macaque primates, the animals show increased survival. As such, ZMapp was thought to be a potentially effective antiviral/immunotherapy-based treatment for Ebola infections in humans, and in a few cases was used to treat human Ebola patients during the 2014 outbreak. Although the mechanism of action for ZMapp had not been fully elucidated at the time, researchers hypothesized that since the antibodies in ZMapp bind to the glycoprotein (antigen) on the Ebola virus, it prevented viral attachment to the cells and did not allow entry or viral replication.

BCX4430 was an antiviral medication developed by BioChryst Pharmaceuticals, Inc. that inhibited viral RNA-dependent RNA polymerases critical to transcribing the viral genome. When metabolized, this drug attached to RNA polymerase and next integrated within the growing RNA chain causing premature termination. Blocking replication of the viral genome prevents the synthesis and assembly of new viral particles. BCX4430 was also shown to inhibit the polymerases of other viruses including the Marburg virus.

Ultimately, Terry's doctor chose one of the therapies described above. Luckily, Terry's symptoms cleared after two weeks of treatment. Terry was free of the virus. The battle was now over and Terry's cells won. Still, she would never know whether it was the treatment, her own immune system, or a combination of the two that won the war against Ebola. In the end, Terry survived the potentially-deadly infection and was able to return home without any long-term effects, other than a newfound understanding and appreciation of how viruses, like Ebola virus, replicate and cause disease.

Questions

1. Consider the vaccines and experimental drugs described above. Provide an argument for which seem to be the most promising for treating Terry and which do not. Use scientific reasoning to support your claims.

2. The Ebola virus outbreak of 2014 was historically the largest in scope as many people were infected in multiple countries. What does the race to provide therapies to subdue an outbreak such as this reveal about the nature of science and scientific discovery?

Extension Activities

- Locate a recent research-based article published in a reputable scientific journal on the structure or lifecycle of the Ebola virus. Summarize the major findings of the study. Critique the scientific methodology and propose at least one future experiment that could be performed furthering the outcomes of the study.

- Perform a literature search to find several reliable sources on the Marburg virus, which belongs to the same family as the Ebola virus. Compare and contrast the structure and lifecycle of the Marburg virus with that of the Ebola virus.

- Using the following Ebola database provided in the link below, construct a graph that shows the current data for Ebola infections. Your graph should include the percentages of the types of Ebola infections (New, Confirmed, Probable, Suspect) represented by each country.
  http://www.who.int/csr/don/2014_07_31_ebola/en/

In the time of darkest defeat, victory may be nearest.

—William McKinley—

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