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Mutations in Influenza Viruses and Prevention Against Mutant Strains

Rohit Rao[1], Rajgopal Appavu[1], and Jothsna Kethar[#]

[1]Central Bucks South High School, Warrington, PA, USA
[#]Advisor

ABSTRACT

The flu originated in 1918 and started a pandemic that spread around the world, killing millions. Other than the current COVID-19 pandemic, the Flu Pandemic of the late 1910s and early 1920s has killed and infected more people than any other virus in modern world history. Since then, the flu has become a seasonal virus that has predictable times of infections. Due to modern medicine, most people are able to survive such infections, and continue on with their lives with little impact. However, as you might come to wonder either eventually or even right now, a person may be curious behind why such an "outdated virus" still continues to live on every winter season. This paper will research the mechanisms of such processes that allow these viruses to stay "alive" (because they technically are not a living thing) and prevent themselves from being eradicated by today's medical technology. We also look into common and new preventions/ treatments that is/ can be used to eventually eradicate the flu.

Introduction

The flu: It is the common winter virus that infects people year in and year out and gives people the perfect excuse to get out of school. However, science and advances in technology have been able to create devices to help fight and prevent these flu viruses. So why do people still get the flu even though they get vaccines and proper medication regularly. The cause can be attributed to mutations of the virus, which eventually develops into the strains of the virus that people get. The flu is one of the most influential (pun not intended) and recognized viruses in the *Orthomyxoviridae* family. They are spherical or filamentous [spaghetti-string like] in shape, with the spherical forms about 100 nm (nanometers), in diameter and the filamentous form more than 300 nm in length. The virus at hand, the influenza virus, is spherical in shape. Though the influenza virus carries common characteristics at hand, people still get sick with the virus even with thorough vaccination, meaning there is a change in the virus from winter to winter. By researching and understanding mutations in influenza viruses, we can begin to understand how and why just taking annual vaccines is not enough to completely eradicate influenza viruses. Understanding mutations in influenza viruses will help millions across the world that are impacted, and even save people and money from mutated strains of the virus and spending that money on making more vaccines in hope that infection numbers do not increase dramatically. Influenza viruses undergo various processes and unintentionally create viral strains of the virus.

What are the causes of these viral mistakes?

First off, to understand mutations, the definition of the mutations and the Central Dogma needs to be understood. Sanjuán and Domingo-Calap describe mutations as "the probability that a change in genetic information is passed to the next generation." (Sanjuán, R. et al 2016). In this case, a generation can be defined as a cell

infection cycle and the cells that come from each subsequent viral cell infection cycle. Some viruses have different levels and ability to mutate than other viruses do. According to The Baltimore Classification of Viruses, the reason why there are different mutation rates has to do with the different Genome size. This is evident when looking at the genome size graph in figure 1. For example, the virus labeled "RT", it is clear that the Genome size does correlate to the rate of mutations. We can also look at the other side of the spectrum when looking at the virus labeled "ds", or also known as dsDNA. dsDNA has a larger genome compared to the other types of viruses that are shown on the graph on the right. Using the trend, we saw with the "RT" virus, the "ds" virus has a lower rate of mutation. What we can gather from this is that the rate of mutations happens relative to the size of the genome. Since the genomes of influenza viruses are shorter compared to most other viruses, this means that the rate that influenza viruses take to mutate is a lot less than most other viruses. That is why we see multiple strains of the virus more often than we do most other viruses. The only exception to this rule is coronaviruses: This is a family of viruses that consist of the common virus that everybody experiences, as well as the very notorious COVID-19.

To understand the basic mechanical concepts and processes of viral mutations, we must look at the Central Dogma. According to NCBI (National Center of Biotechnology Information), the central dogma is the "coded genetic information hardwired into DNA transcribed into individual transportable cassettes, composed of messenger RNA (mRNA); each mRNA cassette contains the program for synthesis of a particular protein (or small number of proteins)" (Central Dogma of Biology: Classic View 2007). These proteins are the building blocks that help create each of these viral particles, and the process repeats itself until the host either dies or the virus is gone from the host. Viruses are made by three stages: initiation of infection, replication, and expression of the genome, and the release of mature virus particles from the infected cell. Critical to the point of this paper, the structure of Influenza Viruses help us understand the mutation processes that happen. On the outside of the cell there are protein spikes. These protein spikes are like keys: when matched up with a protein receptor on the outside of a cell, the protein spikes allow for the virus to enter the host's cells. The inside consists of the capsid, which contains all the genetic information. Though there are more specific parts, it is just important to know about the capsid, the type of genetic information stored inside of these influenza viruses (which is RNA), and the protein spikes. The Central Dogma of Biology first transcribes DNA inside of the nucleus into RNA, where it reaches ribosomes, either on the rough endoplasmic reticulum or out in the cytoplasm of the cell. From there, the mRNA gets translated into proteins using amino acids to synthesize proteins. Viruses are made by three stages: initiation of infection, replication and expression of the genome, and the release of mature virus particles from the infected cell. Viruses here, in this case, use RNA and use a process called reverse transcription, a process using an enzyme to make the one stranded RNA into the two normal two stranded DNA. It then places that DNA into a host cell's DNA and waits for transcription and translation of that DNA to take place. Transcription is one of the most important parts when trying to understand viral mutations. It is here that viral mutations occur. In the case of influenza viruses, the reverse transcriptase, the enzyme responsible for reverse transcription, creates a DNA strand for this RNA that was encased inside of the capsule to be inserted into the host cell's DNA and be replicated to make functional proteins that eventually make other viruses. There are multiple times where mutations happen in viruses. There are few checks to make sure there are little to no mutations. To better understand, take the case of Microsoft Word and its grammar check. A replication process of normal DNA would have multiple different enzymes making sure that replication goes smoothly with preciseness. In the analogy, this would be like typing on Microsoft Word and a grammar check. However, viruses do not have as many enzymes or such in place to make sure there are no mistakes when transcribing. Using the analogy, this would be like using Microsoft Word and not using grammar check and just typing everything out without checking what is being typed out.

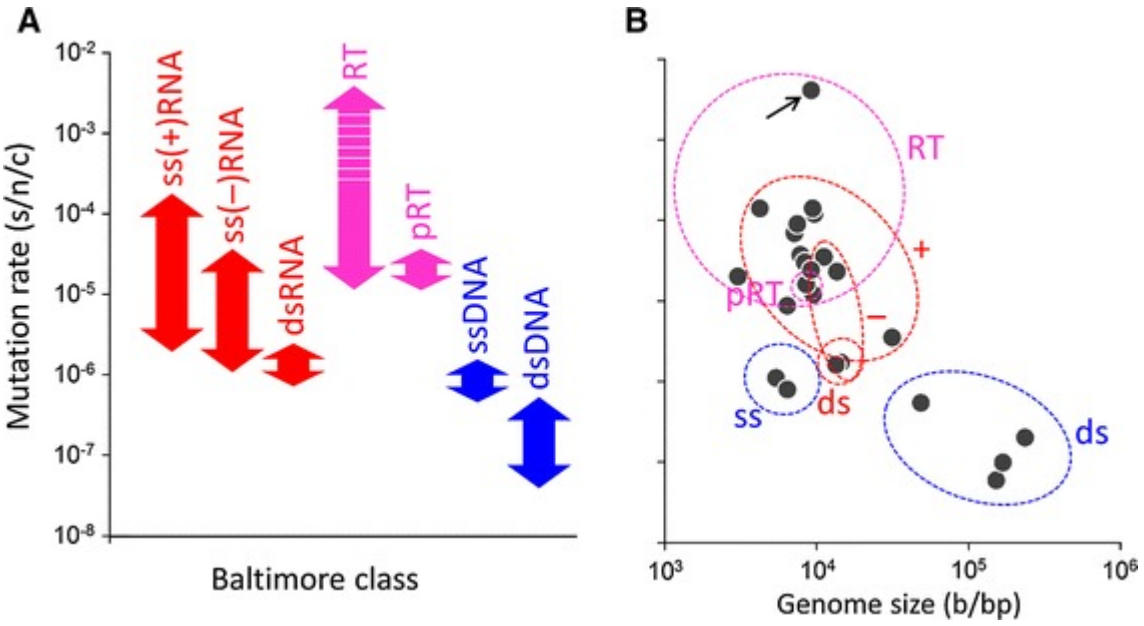

Figure 1. The graph on the left shows the mutation rates of several select viruses, and the graph on the right shows the genome size of those very several select viruses. This figure sourced from source "Sanjuán, R. et al 2016".

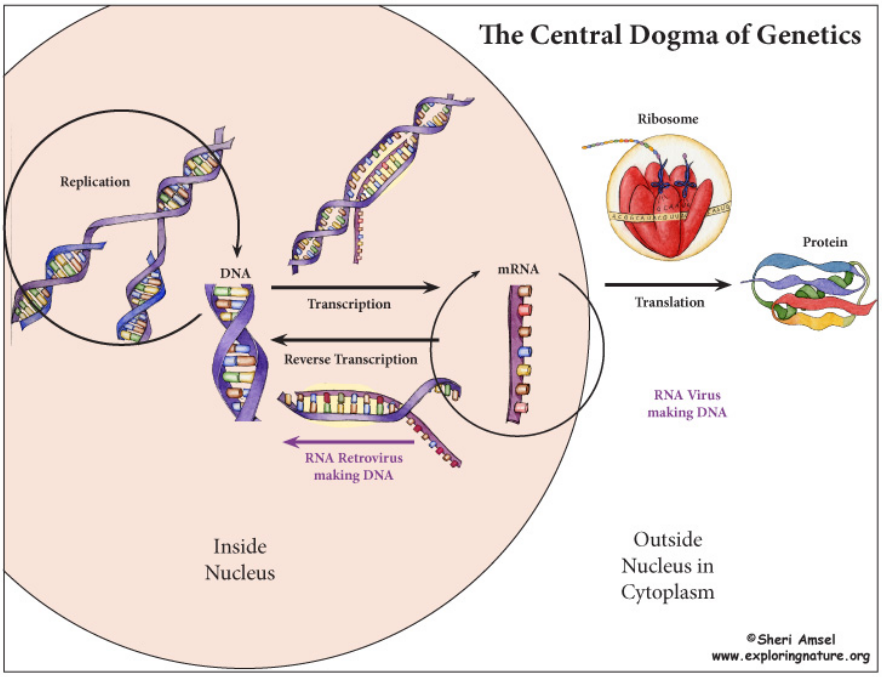

Figure 2. This is a diagram showing the steps taken in the central dogma. Viruses use the Central Dogma to replicate since viruses cannot replicate by themselves and need help from living cells to replicate. Sourced from "The Central Dogma of Genetics n.d.".

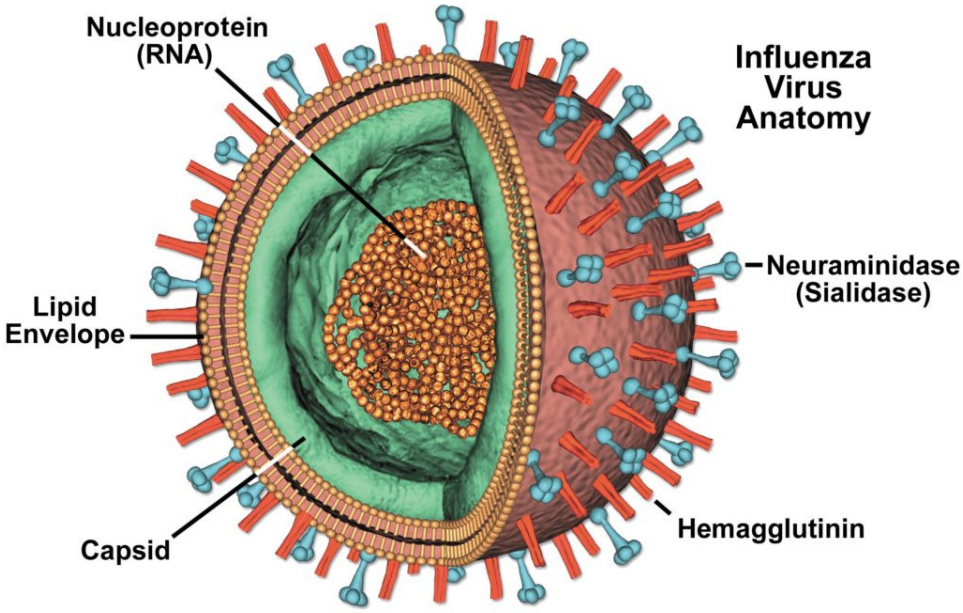

Figure 3. This is a diagram showing the various layers and components of an influenza virus, such as the different layers. Sourced from "Chandra, K.C. n.d."

Why does the mistranslation and mis transcription of these viruses make viruses more dangerous?

If viruses were not able to mutate, then the idea of having annual flu vaccine shots would not exist. Viruses would be wiped from the planet wherever they need to be. However, since it is known that it is not easy to fully eradicate a virus with a single drug, prescription, or some other medical intervention, this means that these mutations (that usually have a negative connotation to them) can be useful to these viruses. These mutations allow these viruses to escape eradication while also becoming more efficient at creating more viruses to spread to more hosts.

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each of them start to get higher and higher, even though more and more people have taken some form of intervention (such as vaccines) over these three years. This evidence shows that though the effectiveness of vaccines keeps the visits for influenza-like illnesses at bay for most of time, there is always a spike in each of the seasonal months. If influenza viruses were not as prone to mutations as other viruses, then influenza viruses would not be such a problem that it is today.

What implications can viral mutations cause: As explained, viral mutations in these viruses can be detrimental to the effort to eradicate them. Though not all mutations are helpful for the virus, some viruses are able to create viruses and promote those mutations via evolutionary theory (A theory created by Charles Darwin to explain the expansion and difference of traits amongst animals and semi-life forms). As Grubaugh, Petrone, and Holmes say, "these viruses employ an intrinsically error prone RNA polymerase for replication, their genomes will accumulate mutations during every copying cycle" (Grubaugh et al 2020). What this means is that though that there are bound to be errors by viruses that host RNA and the RNA polymerase as its only method for replication, those mutations that are promoted by evolution can become dangerous since these mutations allow the virus to either counter whatever medications or medical interventions, we have in place to stop these viruses and it can make symptoms and create problems to a more severe degree. Take, for example, the deadliest flu pandemic/epidemic: the 1918 influenza pandemic. According to the CDC (Center for Disease Control), "it is estimated that about 500 million people or one - third of the world's population became infected with this virus. The number of deaths was estimated to be at least 50 million worldwide with about 675,000 occurring in the United States." (CDC flu). Though today's society is a lot more efficient with the way that it uses its resources and the technology given to it, the flu pandemic of 1918 can be contributed to the fact that the H1N1 strain of the influenza virus was able to mutate quickly enough to keep itself from being eradicated right away, even with most of the world's resources going towards stopping the H1N1 strain. As given by the example, it is evident that one of the main factors that keeps the influenza virus stay existent and variably dangerous is due to its ability to mutate easily (but not easily to the point where it hurts the virus's ability to continue existing).

How can these viral mutations in Influenza Viruses be prevented and protected against?

There are multiple separate ways that influenza viruses can be prevented against, some that are widely used today, and some that are not as used because of their newness. It is especially important that viruses do not spread from one person to another, even if the virus itself is not impacting the overall health of the person. If a person is carrying an influenza virus and they are just carrying it around with them, then it creates a possibility for a new strain of influenza viruses to start, and that can lead to the need for more medical intervention which could mean more illnesses and money being thrown to fight something that could have been prevented.

Making new vaccines and its impact on preventing viral mutations (herd immunity): Vaccines are a big part in trying to mitigate the damage that viruses can do. Influenza Viruses are known to change rapidly and still infect people even with what is considered decent intervention. According to the CDC, "recent studies show that flu vaccination reduces the risk of flu illness by between 40 and 60 percent among the overall population during the season when most circulating flu viruses are well-matched to those used to make flu vaccines' ' (CDC). This means that flu vaccines are effective most of the time and they are helpful in at least 40 percent of the cases. Because mutations are random and influenza viruses are prone to mutating frequently, it makes sense that the vaccination number is as low as it is. However, it is the most affordable and widely used approach for people of all incomes. These vaccinations also create something called herd-immunity. Herd Immunity is the resistance of the spread of, in this case, influenza viruses within the population. The reason why some people might not get the virus is because of reasons such as fear of getting the vaccine or because their immune system is comprised (too weak) to handle the vaccine.

CRISPR technology and its impact with preventing Influenza mutations: CRISPR gene technology is a new age invention and is revolutionary in manipulating gene sequences. It has tremendous potential for mak-

ing non-heritable DNA changes that can treat or even cure some disorders and diseases such as HIV and muscular dystrophy. According to Dr. Francis Collins, "a recent animal study shows that another CRISPR system – targeting viral RNA instead of human DNA – could work as an inhaled antiviral therapeutic that can be preprogrammed to seek out and foil potentially almost any flu strain" (Collins 2021). What this means is that the CRISPR gene editing technology can target viral RNA inside of a person. By using a guide RNA guide, the guide directs a bacterial enzyme called Cas13a to the right spot in the viral genome, where it can cleave (cut) and bind the viral RNA, thus stopping viruses from replicating in lung cells. According to findings of Phillip Santangelo whose work was published in the journal, nature Biotechnology, Santagelo's team "rather than delivering the Cas13a protein itself to the lungs, the CRISPR system works by supplying a messenger RNA (mRNA) with instructions to make the antiviral Cas13a protein" (Collins 2021). According to the same study, this is the same idea that Pfizer and Moderna makes its mRNA based Covid-19 vaccines by temporarily directing muscle cells to produce the viral spike proteins needed to launch an immune response against these viruses. Though it is in the works, it can be easier (and more effective) than ever to stop mutations in Influenza Viruses when the use of CRISPR technology becomes clinically approved.

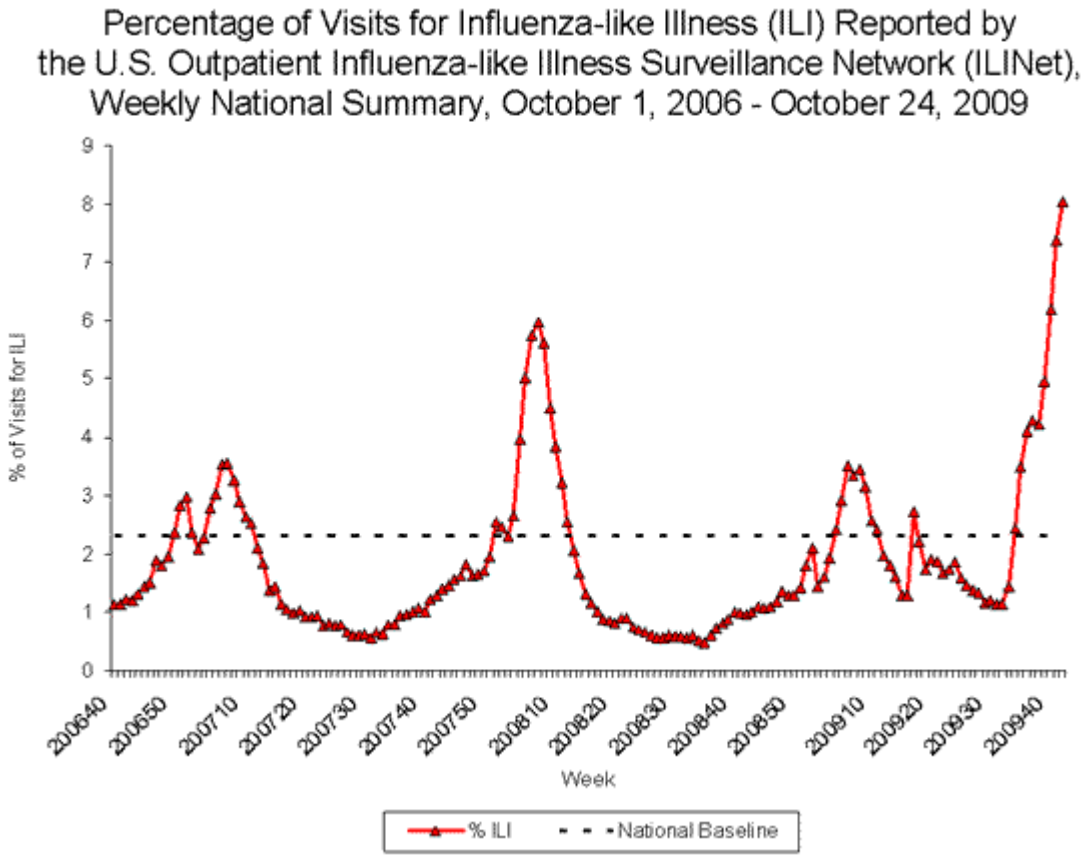


Figure 4. Discusses the visits to the doctors for symptoms representative of influenza infections. Graph sourced from "Percentage of visits for influenza – like illness (ILI) reported by the U.S. outpatient influenza-like illness surveillance network (ILINet), weekly national summary, september 28, 2008 – november 12, 2011".

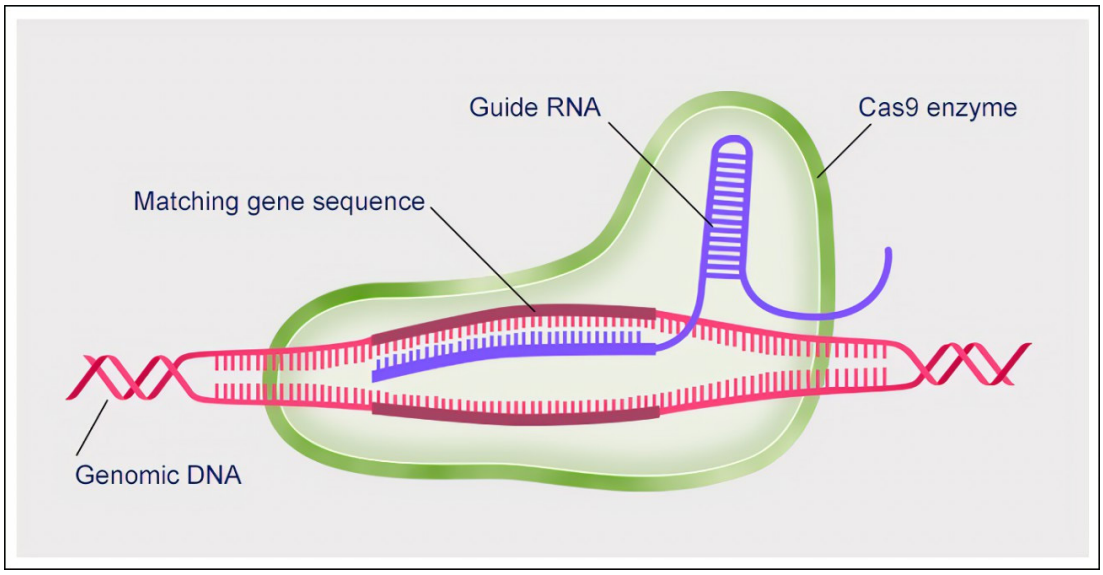
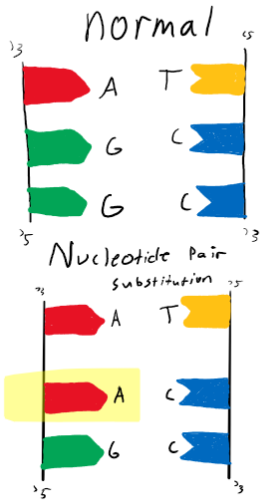


Figure 5. This shows how CRIPR technology takes place and how it uses several proteins such as the already discussed CAS9 gene. The Illustration is sourced from "What is CRISPR n..d.".

Type of Mutation	What is it	Severity of the Mutation (Scale from 1-10)	Visual Representations
Base Substitution	Base Substitution is considered the simplest of the mutations. Base substitutions are self-explanatory: One nucleotide base is switched for another nucleotide base when replication is occurring. For example, an Adenine may be substituted for a Cytosine.	Genetic coding programs for several types of amino acids, the base unit of proteins, using three different nucleotides at a time, which are also called codons. Since this is just one base pairing, there is still a possibility that the amino acid that will be produced is still the same as what the correct amino acid must have been. The severity of the mutation on the scale is 4/10.	

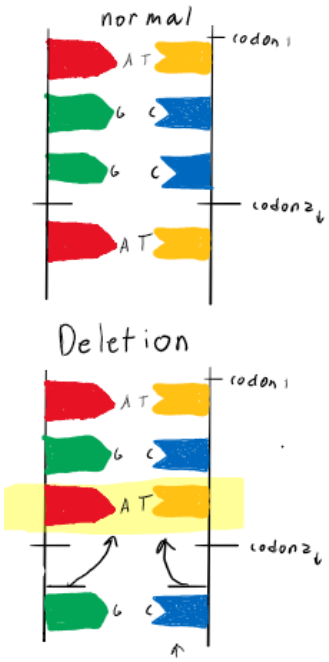
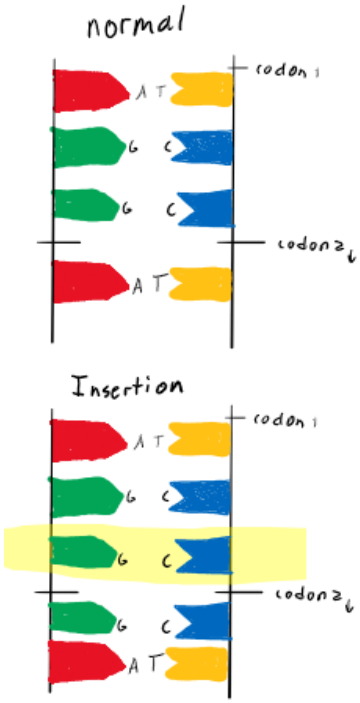
Deletion	Nucleotide deletion is a mutation that occurs during replication. This mutation deletes a nucleotide or a string of nucleotide bases. A single deletion of a nucleotide is called Point deletion. For example, a guanine and cytosine may be accidentally cut out, and the rest of the sequence gets cut short due to those missing parts of the sequencing.	Since codons are specific sequences, when one nucleotide or string of nucleotides are missing, this can start a chain reaction that can result in mutations that is far from what the normal sequencing was (when you count codon to codon). The severity of the mutation on the scale is a 8/10. These mutations can either enhance the ability of the virus to replicate and damage hosts more effectively or become incapable of doing anything due to these mutations.	
Insertion	Insertion is a mutation that happens during replication. An insertion mutation can be small, just adding in one nucleotide to each side in a single type of codon. It can also be an insertion of a group of base pairs which is just inserted into a random part of the genome sequencing.	Since the specific sequencing of each codon makes a specific amino acid, a chain effect can also be seen here as it was seen in deletion mutations. Since this can completely enhance or cripple the ability of viral particles to spread and infect	

Figure 6. This table describes three different mutation types, the severity of the mutations, and includes a graphical representation of the mutations. Each and every one of these mutations can either cripple or enhance the abilities of the virus to spread, infect, and kill hosts more effectively. The Severity Scale just gives perspective as to how impactful these mutations are when constructing new viral particles. This table was created by Rohit R.

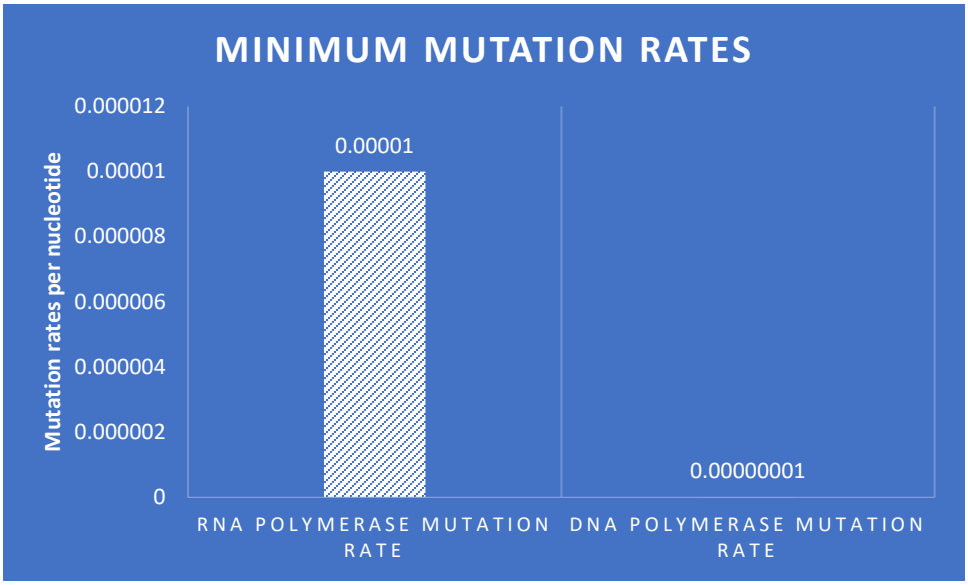


Figure 7. This graph shows the mutation rates of RNA Polymerase and DNA Polymerase, each with a mutation rate of 0.00001 (10^-6) and 0.00000001 (10^-9) per nucleotide respectively. Based off these statistics, It is evident that RNA Polymerase makes genetic mistakes more frequently than DNA Polymerase. This can also explain the high rates of mutations in Influenza Viruses since they use RNA Polymerase. Graph Created by Rohit R.
** Data was sourced from "Cann, A."

Limitations

All of the research that was conducted here in this article was done in a very specific environment, and there can be other factors that can influence such mutations to occur. There is also the possibility that cells that were used in finding out the mutation rates of influenza viruses can be impacted when the cell is already pre-diagnosed with some medical issues before getting infected by influenza virus particles. There is also a possibility that time-restraints when going through the trials may have not given enough ample time to get accurate measurements on things like mutation rates. Other factors like the overall "health" of the virus particles (impacted by mutations significantly more than agreed as normal) could also give inaccurate measurements for mutation rates per unit of genome size.

Conclusion: Mutations in Influenza Viruses and Prevention against Influenza Viruses

Influenza viruses are no exemption to viral mutations. Their "tenacity" comes from their ability to mutate more than most other viruses. Because these mutations help these viruses grow stronger and counter more medical interventions such as the vaccines and CRISPR, this makes them more dangerous and harmful to us biologically, and because of the impacts that funding research on finding new medical interventions for influenza strains can cost. Influenza viruses are made up of RNA and are predisposed to make mistakes as they do not have an enzyme that can reliably code the genome of the virus with high accuracy like does DNA polymerase (they are stuck with RNA polymerase) There are multiple different ways that society go about dealing with influenza viruses. One of the most prevalent interventions that we use as a society are vaccines. With the use of vaccines,

influenza virus illnesses have dropped significantly. It is possible that soon that there can be a transition from having CRISPR technology to start being used by clinicians and other medical physicists around the world to administer vaccines. As influenza viruses start to get more and more advanced with the eventual onsets of beneficial (to the virus as a species) mutations and because of the rapid mutations that happen to each generation of influenza viruses that get produced after each cell cycle, CRISPR technology would be able to develop effective medical interventions faster than the normal timeline would take to create a vaccine for influenza viruses. This can reduce the cost it would take to research for an effective intervention, and it can allow for greater efficiency in terms of the time and money it would take to get the intervention to people across the globe as well. With the onset of CRISPR technology, it is possible to not only eradicate viruses like influenza viruses, but also viruses like the current SARS-CoV-2.

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References

Cann, A. (2008). Replication of Viruses. *Encyclopedia of Virology*, 406–412. https://doi.org/10.1016/b978-012374410-4.00486-6

Central Dogma of Biology: Classic View. (2007). *National Center for Biotechnology Information*, 1. https://www.ncbi.nlm.nih.gov/Class/MLACourse/Modules/MolBioReview/central_dogma.html

The Central Dogma of Genetics. (n.d.). [Illustration]. Exploring Nature. https://www.exploringnature.org/graphics/genome_art/central_dogma72.jpg

Chandra, K. C. (n.d.). *Structure of influenza viruses* [Illustration]. EazzyNotes. https://i0.wp.com/eazzynotes.com/wp-content/uploads/2020/04/influenzaviruslabeled-scaled.jpg?resize=768%2C503&ssl=1

Collins, F. (2021). CRISPR-Based Anti-Viral Therapy Could One Day Foil the Flu—and COVID-19. *NIH Director's Blog*. https://directorsblog.nih.gov/2021/03/16/crispr-based-anti-viral-therapy-could-one-day-foil-the-flu-and-covid-19/

Grubaugh, N. et al. (2020). We shouldn't worry when a virus mutates during disease outbreaks. *Nature Microbiology*, *5*(4), 529–530. https://doi.org/10.1038/s41564-020-0690-4

How Flu Viruses Can Change. (2021, September 21). Centers for Disease Control and Prevention. Retrieved September 21, 2021, from https://www.cdc.gov/flu/about/viruses/change.htm

Influenza Milestones 1917 – 2009 Timeline | Pandemic Influenza (Flu) | CDC. (n.d.). Centers for Disease Control and Prevention. Retrieved March 22, 2018, from https://www.cdc.gov/flu/pandemic-resources/1918-commemoration/milestone-infographic.htm

McNeil, T. M. (2021, August 20). *How Viruses Mutate and Create New Variants*. Tufts Now. Retrieved January 8, 2022, from https://now.tufts.edu/articles/how-viruses-mutate-and-create-new-variants

Past Seasons Estimated Influenza Disease Burden | CDC. (n.d.). Centers for Disease Control and Prevention. Retrieved October 1, 2020, from https://www.cdc.gov/flu/about/burden/past-seasons.html

Percentage of visits for influenza- like illness (ILI) reported by the U.S. outpatient influenza-like illness surveillance network (ILINet), weekly national summary, september 28, 2008 - november 12, 2011). (n.d.). [Graph]. Centers for Disease Control and Prevention. https://www.cdc.gov/flu/weekly/weeklyarchives2011-2012/images/picILI45.GIF

Sanjuán, R. et al. (2016). Mechanisms of viral mutation. *Cellular and Molecular Life Sciences*, *73*(23), 4433–4448. https://doi.org/10.1007/s00018-016-2299-6

Vaccine Effectiveness: How Well Do Flu Vaccines Work? | CDC. (2021, October 25). Centers for Disease Control and Prevention. https://www.cdc.gov/flu/vaccines-work/vaccineeffect.htm

What is CRISPR? (n.d.). [Illustration]. Absolute Knowledge. https://www.absolute-knowledge.com/wp-content/uploads/2018/04/CRISPR.jpg