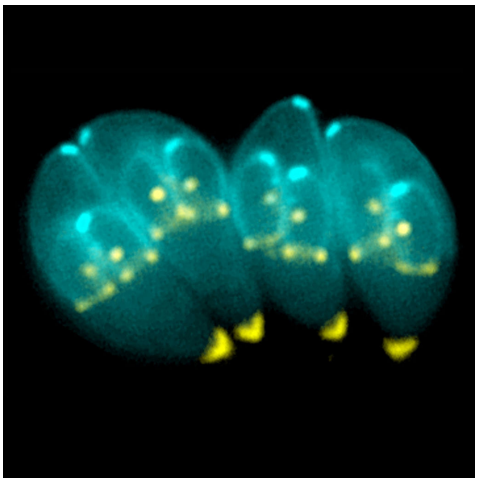
Toxoplasmosis mRNA Vaccine Proposal



Biology 10C
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1. Abstract

Toxoplasmosis is a parasitic disease affecting humans, livestock, and cats. This single known species in the genus Toxoplasma gondii is considered one of the world's most successful parasites, because of its ability to infect and persist in most warm-blooded animals. The parasite that can cause Toxoplasmosis is found in cat feces and in undercooked meat. Toxoplasmosis is a major risk for pregnant women and individuals with compromised immune systems (HIV patients), can be deadly or cause serious birth defects for a fetus and Lymphadenopathy for people with weakened immune systems. According to the CDC reports, more than 60% of the world's population have been infected with Toxoplasma gondii in 2018 and 2020. Vaccines are one of the most important tools in public health and play an important role in infectious diseases control. Over the last few years, new strategies for developing a vaccine have emerged especially due to the development of genetic-engineering technologies. RNA-based vaccines represent an irresistible and safe immunization strategy which decreases the risks of genomic integration and malignant cell transformation. The strategy in this vaccine is based on the single antigen. Based on the research that has been done the ROP17 (rhoptry protein 17) is determined as a target gene.

The structure and characteristics of mRNA vaccine include 5' cap, coding sequence, untranslated regions (5' and 3' UTR sequence), poly(A) tail which directly impacts mRNA stability and translation efficiency. In mature eukaryotic mRNA, the 5' cap is located at the beginning of the mRNA at the 5’ end. The 5’ cap that our team used for this vaccine is the m7gpppNMN. NM is the first nucleotide that regulates gene expression because the host organism is human. Therefore; for codon sequences, the original sequence from POR17 has to be changed into the codon that is more common in the human body. The modification will be to select the GC-rich codon since the GC-rich codons are more stable due to their structure. The start codon should include the Kozak sequence. mRNA also needs 5′ and 3′ UTRs around the open reading frame (ORF) to regulate the mRNA stability and mRNA vaccine expression levels. The length of poly-A tails which was used in this vaccine will be the long poly-A tail (120-150 adenosine) which is relatively longer than the median overall length of the poly-A tail (57 nt). The advantages of a long poly-A tail are higher stability of expression level, and longer half-life.Therefore, the vaccine that we designed will target ROP17 and it will successfully prohibit Toxoplasma gondii from infecting humans.

2. Background

Toxoplasmosis is a zoonotic parasitic disease that seriously endangers the health of the host. In addition to blood transfusion and organ transplantation, the infection is usually through the consumption of undercooked contaminated meat, contacting infected cats feces, or mother-to-child transmission during pregnancy. The development of effective vaccines is the main strategy to prevent toxoplasmosis. According to the Centers for Disease Control and Prevention (CDC) reports in 2018 and 2020, more than 60% of the world population have been infected with Toxoplasma gondii. The United States has over 40 million carriers and over 11% of the patients are 6 years old or above. The infections increase by more than 800,000 people per year. 750 people die from toxoplasmosis every year, of which 375 are caused by eating contaminated meat. Besides, every year there are 300-4000 cases of congenital toxoplasmosis related to mother-to-child (congenital) transmission, infecting the infants and damaging the health of the infant. The symptoms usually do not exhibit at birth but may develop later in life with potential vision loss, mental disability, and seizures. Toxoplasma can survive in humans and other animals in the life term. This symptom usually occurs in patients with lower immune

systems, in serious cases, toxoplasmosis will damage the eye, brain, and other organs. Moreover, toxoplasmosis may be related to schizophrenia because toxoplasmosis will stimulate neurological disorders and function changes by chronic infections, which disrupts normal brain growth and differentiation, even possessing the mechanisms of the host's central nervous system (CNS) (Fuglewicz, et al., 2017; Webster, et al., 2013).

Current medical technology can not eliminate parasites, the parasite can live in tissue cells with an inactive level. mRNA vaccines, compared to other treatments, have the advantage of being able to be modified. Most of the traditional vaccines use an inactivated virus or other pathogen composition. These vaccines usually need to take a longer time to produce and have a higher risk. The other bacteria vaccines produce protein composition under normal circumstances but they do not always induce a strong immune response, so scientists need to search for a suitable adjuvant, a chemical that enhances the immune response. Besides, traditional vaccines are inappropriate due to the complexity of the parasite life cycle and the ability of parasites to escape from the human immune system. mRNA vaccines are safer than DNA vaccines because mRNA vaccines do not need to be integrated into the nuclear genome of the host cell and the protein can be translated directly into the cytoplasm, preventing mutations. mRNA vaccines induce the host cell to produce their coding protein replication and trigger a stronger immune response. Once the mRNA enters the cell, it will be translated to protein, this protein will be released to activate the immune system and activate the immune responses. Although the mRNA vaccines have a fast decay period and will be attacked by the immune system, our vaccine has improved the stability and extended the decay period to solve this problem.

According to Panas, et al. (2019) research, when toxoplasma infects the cell, it will build a protective parasitophorous vacuole (PV) in its surrounding. Although the protective parasitophorous vacuole provides the protection, it is also an effector protein that affects and controls host cells. They discovered that the parasite rhoptry protein ROP 17 is necessary against intramolecular immunity. Therefore, in order to prevent the export of effector proteins, our vaccine targets rhoptry protein ROP17; We are confident that we can successfully prevent Toxoplasma gondii from infecting humans by damaging ROP 17.

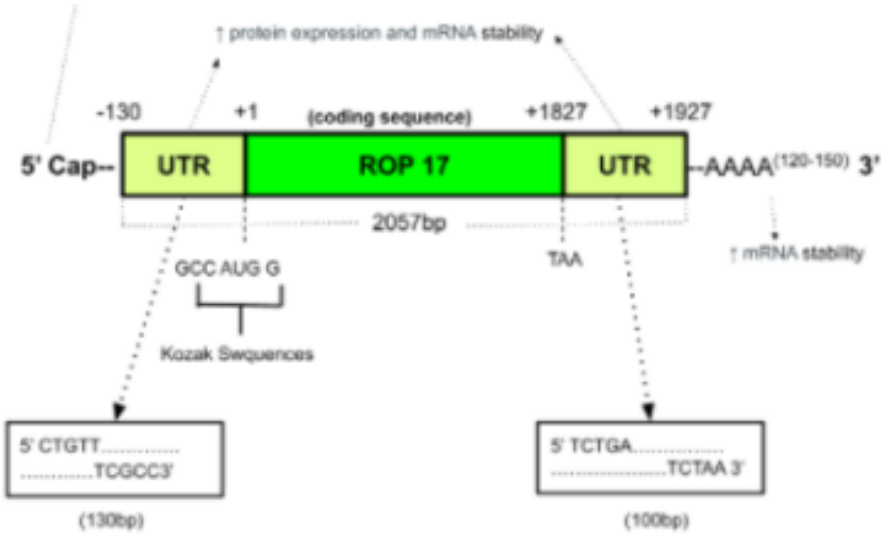
3. Strategy/Approach

The approach of the mRNA vaccine is to use the strategies to target the Rhoptry protein 17 (Figure 2), which is related to toxoplasmosis. Toxoplasmosis can infect humans with Toxoplasma gondii parasites and can result in body illness for several weeks. The kinase domain of ROP17 was chosen because the protein is essential for Toxoplasma gondii. Rhoptry protein, called ROP protein, is a kinase derived from the parasite's Rhoptry organelle, which is unique to organisms in the Apicomplexa phylum. They are injected into the host cell at the precise moment when the parasite internalizes and manipulates the host's immune response. These effects make ROP17 a key factor in regulating the virulence of Toxoplasma gondii. It shows an important factor for the virulence and survival of the parasite (Molina, 2018). Adding on to that, according to the study, the combined activity of ROP17 and ROP18 protects the parasite from being cleared by interferon-activated macrophages. In order to prove that, the researcher generated the ROP17 deletion mutant and injected 100 amounts in the mice, and results in slight attenuation of virulence relative to the parental control (Etheridge, 2014). Therefore, it can be assumed that there is some connection between the activity of toxoplasmosis in the body with the activity of ROP17. Thereby, the mRNA vaccine will choose ROP17 as the target gene to teach the body's cells to make a harmless piece of a “spike protein”, so that the immune system can recognize that it does not belong there and responds to get rid of the Toxoplasma gondii parasites.

Moreover, mRNA vaccine minor modifications also need to be considered. In our strategy due to mRNA decay easily and stabilizing mRNA may lead to high expression. There are several factors that can influence the expression and stability of mRNA vaccines. The structural features of mRNA such as 5' Cap, poly-A tails, and Untranslated region (UTR) can directly affect the stability and translation efficiency of mRNA. In addition, 5' and 3' UTR modifications can increase half-life, mRNA vaccine expression levels, and translation (Kim, et al, 2021). Therefore, in order to increase the efficiency and effectiveness of the vaccine, the structure of this mRNA vaccine is to add a 5' cap which is m7GpppNMN in the sequence which can enhance the translation efficiency of mRNA and improve the protein expression level encoded by mRNA after transfection. According to the article (Yamagishi, et al, 2011), the strongest gene expression and most stable of 5' UTR length is between 120 bp to 140 bp. Therefore, our UTR (untranslated region) decides to use 130 bp in order to have the most appropriate 5' UTR. The research (Tanguay & Gallie, 1996) have applied the different 3' UTR

lengths to increase the gene expression in CHO (Chinese hamster ovary) cells, when the bases number is 104 then the translation will be the most efficient which is increased 38 fold of CHO cells. Therefore, our 3' UTR length was decided to be 100 bases due to the translation rate. The poly-A tail length of our mRNA vaccine is 120-150 adenosine. According to the research (Versteeg, et al, 2019), the poly-A tail length of parasites is 120-150 adenosines in order to improve the protein expression, also increase the stability and translation efficiency in cells. (Figure 1)

4. Figures



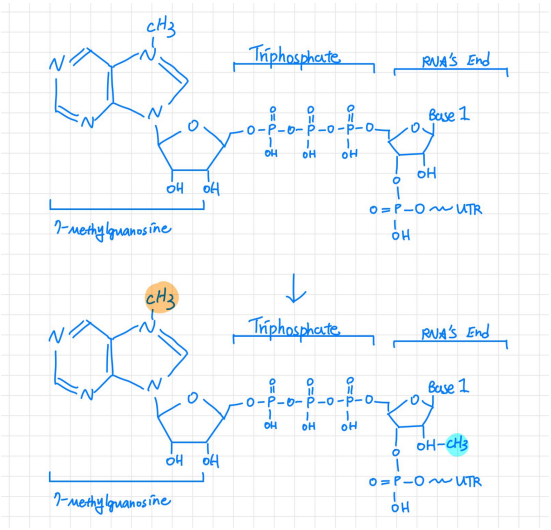


Figure.1 (Toxoplasmosis mRNA Construct)
5’ cap can enhance the translation efficiency of mRNA and improve the protein expression level encoded by mRNA after transfection and decreased mRNA immunogenicity.

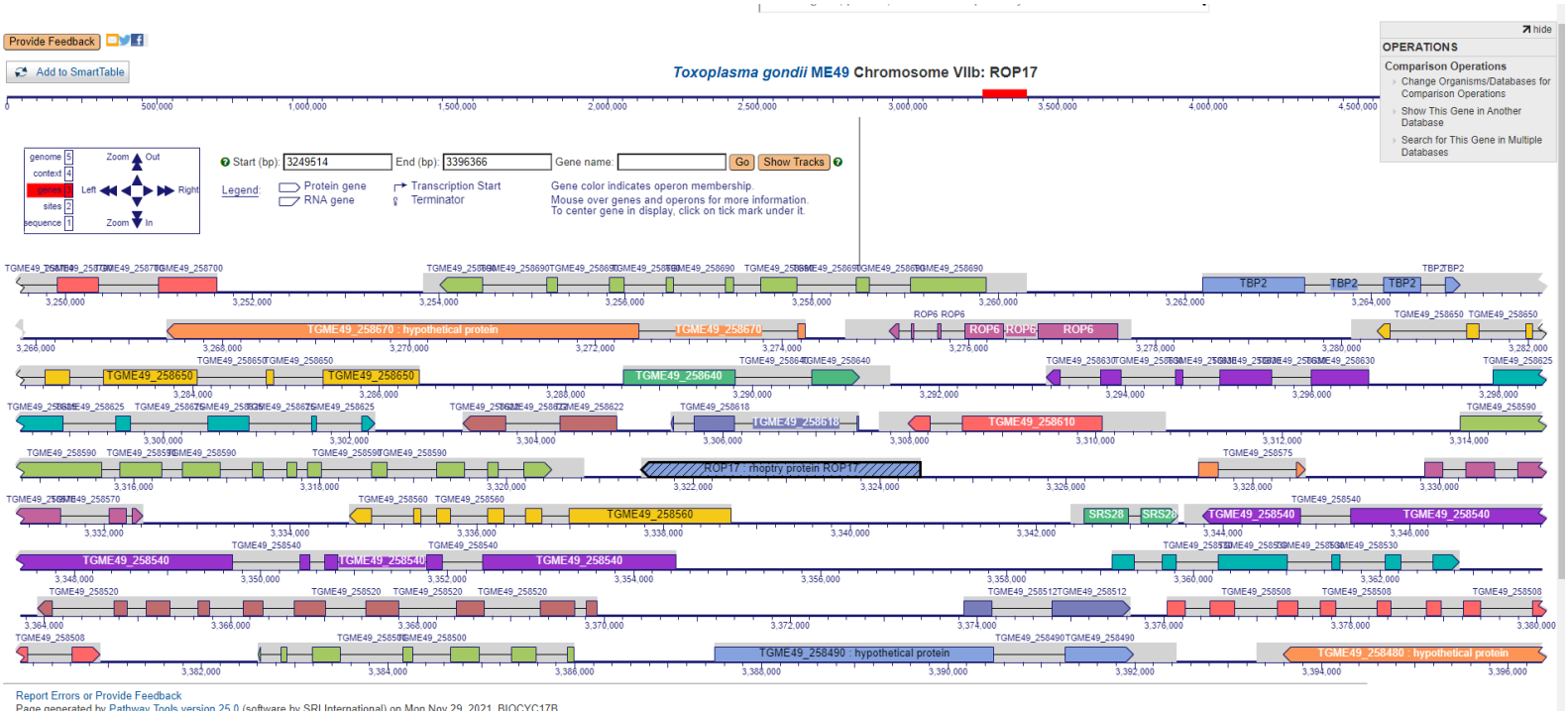


Figure.2 (ROP 17)

https://biocyc.org/ provides us with the information of the *Toxoplasma gondii ME49* from its genes, proteins, enzymes, metabolic pathways and allows visualization of the genome.

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