Analysis of several promoters derived from the Human Influenza A Virus by the replication of plasmids encoded with gene reporter Luciferase

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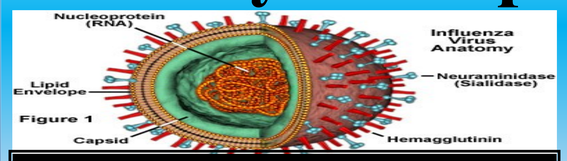

Figure 1: The influenza Virus with its respective components.

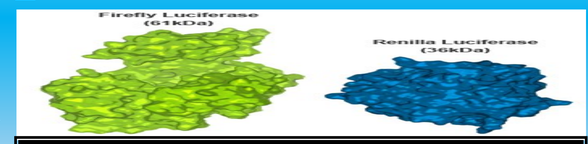

Figure 2: Luciferase and Renilla Luciferase (control) proteins.

Abstract

The UTRs (Untranslated regions) of the eight genome fragments (vRNAs) of the virus are responsible for viral mRNA synthesis and are known to target specific host pre-mRNAs. A library of mini-genomes encoding luciferase under the control of wild-type and mutated UTRs was constructed. An analysis of luciferase production under these UTRs was conducted to understand which promoter region is responsible for the synthesis of both cap-snatching and viral mRNA. Bacteria with plasmids coding for 1) the viral RNA polymerase, 2) luciferase mini-genomes and 1 subculture from each plate was placed in LB (Luria-Bertani) medium and were later purified. The presence of the plasmids were validated by gel electrophoresis. In the following days the transfection of human cells were carried out and luciferase assays were conducted.

Introduction

Yearly epidemics and a significant amount of mortality is caused by the Influenza A Virus (IAV), known to be highly pathogenic to humans. Due to its great genetic diversity, there are many limitations in therapy for the IAV. As a result it is imperative to completely understand IAV pathogenesis and the foundations of infection initiation. The synthesis of viral mRNAs is reliant on capped RNA primers which are derived from host pre-mRNAs. This process is known as "cap-snatching", where the first 10-15 nucleotides from the host pre-mRNA are cleaved, and serve as primers for the production of viral mRNA. D. Sikora and Dr. Pelchat have identified that the UTRs of the IAV eight-segmented genomic RNAs were responsible the formation of viral mRNA and the targeting of specific host pre-mRNAs. **Our hypothesis is that precise features (which incorporate the sequence and structure) on the UTRs of IAV genomic RNAs are responsible for these occurrences.** In order to elucidate these features, a library of mutated UTRs within vRNA mini-genomes encoding luciferase, a protein responsible bioluminescence activity, was constructed. Using standard molecular biology techniques, bacterial transformation, plasmid purification, cellular culture, and quantitative luciferase assay were conducted of the mutants.

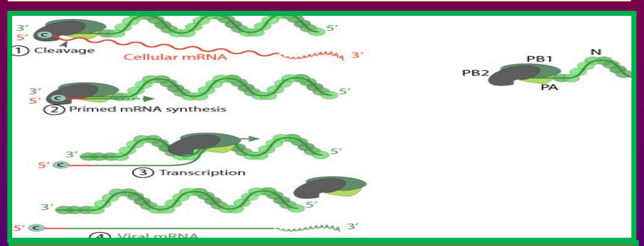

Figure 3: The phenomena of cap-snatching inside a host cell.

Objectives

The objective of this research project was to test the mutated viral RNA mini-genomes for luciferase production and to analyze the results of the luciferase assays.

Methodology & Results

1) Viral RNA polymerase which is composed of the components PA, PB2, PB1, and NP; and luciferase plasmids coding for NP/NP (positive control), NP/PB1, PB1/NP, or PB1/PB1 UTRs, and two controls (pREN and M2) were plated on agarose gel plates and were grown overnight at 37°C.

2) Tubes with 5mL LB medium containing 5µL ampicillin were prepared, and 1 colony from each plate was subcultured in the medium. The tubes were incubated overnight at 37°C.

3) The plasmids were purified the next day,. Agarose gel was made to conduct gel electrophoresis in order to confirm the presence of the purified DNA.

4) After deriving the concentrations of each plasmid DNA from a spectrometer, the plasmids were diluted to 180ng/µl.

5) One day before transfection the confluent cells (293) 1:2, were plated in a 100 µl growth medium on a 96-well plate. The cells, being 90% confluent the next day, were then transfected. 25µl of serum-free medium was added to the diluted plasmids. 0.5 µl lipofectamine 2000 transfection reagent was added with 25µl serum-free medium. The DNA and lipofectamine were combined after 5 minutes and were incubated at room temperature for about 20 minutes. 50 µl of each well was added to the epithelial cells and was mixed for 30 seconds. The cells were incubated for 2 days at 37°C.

6) The luciferase assay was conducted by removing 45µl of excess medium from each mixture. 75µl of luciferase substrate was added to each mixture and the plate was rocked for 10 minutes. The firefly luminescence was measured. Stop & Glo substrate was diltuted into a ratio of 1:100 with Stop & Glo buffer. 75µl of this reagent was added and was rocked for 10 minutes. The renilla luminescence was measured. The data obtained was analyzed.

Figure 4: The gel electrophoresis for PA, PB1, PB2, NP, NP/NP, NP/PB1, PrL, PB1/NP, M2, and PB1/PB1. M consisted 9µl of DNA ladder (molecular weight marker) and 1µl of SYBR green (a commonly used florescent dye). All the others are labelled respective to the plasmid DNA that they contain. Each contains 2µl plasmid DNA, 2µl loading buffer, 5µl H_2O,

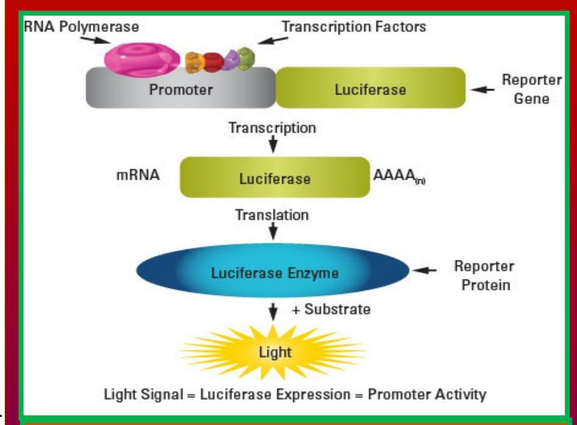

Figure 5: The function of firefly Luciferase when bound by promoter in a plasmid.

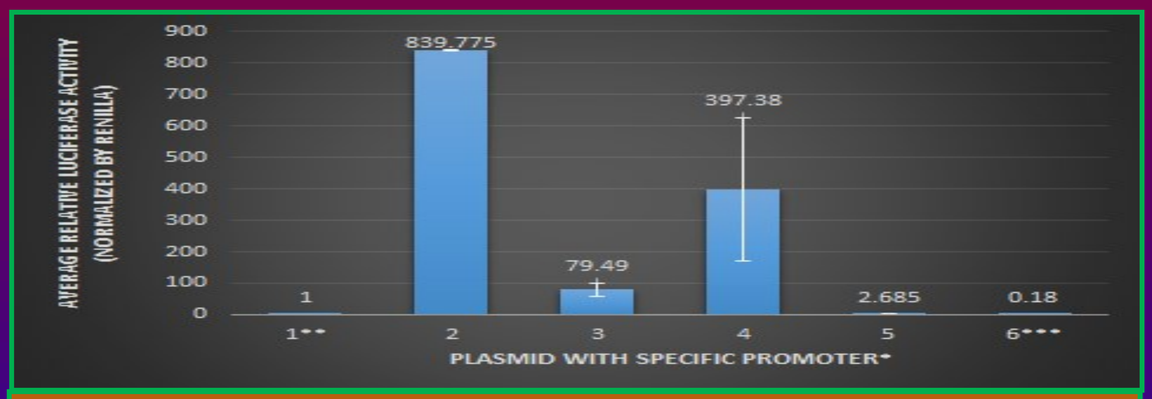

Figure 6: The average relative Luciferase activity obtained in reference to the positive control 1. Two trials of luciferase activity were conducted. *Each number represents the activity of a specific plasmid. 1 with promoter region NP/NP; 2 with promoter region NP/PB1; 3 with promoter region PB1/NP; 4 with promoter region PB1/PB1; 5 with promoter region M1; and 6 with promoter region M2. **Positive control. ***Negative control. The error values (plus or minus) for 1-6 is respectively 0, 1.21, 21.89, 227.63, 1.11, and 0.01.

Conclusion

It is evident that the plasmids are purified and the presence of DNA is verified since it can be visualized that the DNA, having a negative charge was attracted to the positive end of the gel. After the validation of the plasmid DNA the transfection was conducted, following a luciferase assay.

On the vRNA luciferase transcribed from the 3' to the 5' in order to produce the mRNA after cap-snatching. The vRNA consists of the UTR which is surrounded by the promoter regions and a pair of conserved sequences towards the 3' and the 5' end. M1 (5) does not contain any UTRs of NP/NP and contained only the conserved sequences. On the contrary, M2 (6) was missing the conserved sequence on the 3' end. All other promoters contained both the UTRs with respect to their individual promoters and these conserved regions. If light is detected, then there is luciferase production, which also indicates transcriptional and translational activity.

The most amount light produced by luciferase was detected in the NP/PB1 (2) promoter region followed by PB1/PB1 (4) and PB1/NP (3).This indicates that with the NP/PB1 promoter there is a high amount of transcriptional and translational activity of the vRNA into mRNA; almost 800 times more than our positive control NP/NP. The promoter activity of PB1/PB1 was about 400 times more than NP/NP, and with the PB1/NP there was about 80 times more activity. For M1 (5) there was about twice as much activity. In M2 (6) however, there is barely any promoter activity in comparison with our positive control. Therefore it can concluded that both the conserved regions must be present in order to have promoter activity since all the plasmids contained both the conserved sequences except for M2.

Acknowledgements

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