



# Single-stranded RNA interference in gene therapy and cell-specific drug delivery systems utilizing the Tobacco Mosaic Virus

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## Introduction:

Cell specific drug delivery has been a longstanding goal of genetic and cellular engineering. With the goal of using engineered RNAi to control cancer cell growth and other diseases, Tobacco Mosaic Virus (TMV) has been chosen to deliver the fragile RNAi to targeted cells because it is the perfect protective coat for fragile, single stranded RNA. TMV is a prime vector for drug delivery because it provides a rigid protective coat to the fragile RNA, and also because its protein coat antibodies can be manipulated and designed to target specific cells. Much work has already been done to tailor the coat protein antibodies of TMV to target specific (i.e. cancer) cells with promising results. The other part of the work is in the effectiveness of RNAi once the TMV has localized to the cells. My role in this system of research was to observe the effect of RNAi using confocal fluorescence microscopy and fluorescence activated cell sorting (FACS) to see the RNAi effect on GFP expression and cell growth. I was also in charge of maintaining the HEK 293 mammalian cell line that was to be used in the research. GFP expression was chosen to be monitored because its fluorescence provides an easily quantifiable measure to monitor the effectiveness of the system.

## Project Overview:

For my internship project, I was involved in biotechnology research in the Biotechnology lab of Dr Bill Bentley in the Plant Science building on campus at University of Maryland College Park. I was working with Mr. Chiwei Hung on a project researching anti-cancer cell specific drug delivery systems. The overall goal for Mr. Hung is to research the utilization of the Tobacco Mosaic Virus (TMV) as a delivery system for single stranded RNA to be delivered to specifically targeted cells as a drug delivery system. There has already been much work done to alter the TMV coat antibodies into targeting specific cells, and Mr. Hung has been researching the effectiveness of TMV as a protective, targeting vector. My responsibilities in the lab varied over time, however the large portion of my time went to cell line maintenance and in testing the effectiveness of RNAi on inhibiting GFP expression over time.

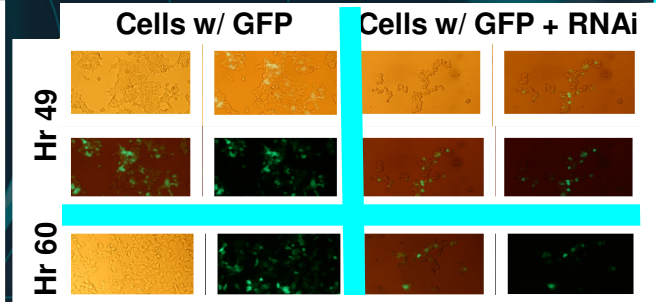
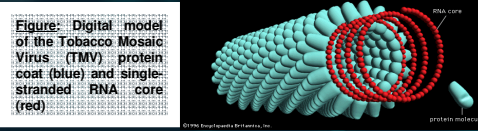


Figure: The control (Cells w/ GFP column) and variable (Cells w/ GFP + RNAi) samples at 49 and 60 hours viewed using confocal fluorescence microscopy. Note that cell growth as well as GFP expression is severely hindered in cell cultures transfected with RNAi. Visual inspections such as seen here were quantified with FACS analysis.



## Results and Discussion:

The results support the hypothesis that TMV is a suitable delivery vector for single stranded RNAi. Figures (A) and (B) show that RNAi delivery was successful in both cases; with TMV and free from TMV. However it is important to realize that when put into practice, the TMV coat is much more important in its role of protecting the RNA. The RNase sample shows that RNase is effective in digesting all types of RNA in cells, resulting in a low transcription of GFP and therefore low expression. Since RNase is fairly common in biological environments, the free RNA delivery would prove ineffective in a realistic scenario because the RNase would digest the delivered RNA and render it useless. The effectiveness of the TMV-coated sample is encouraging because the TMV coat not only provides a durable support and protective coat to the RNA, but also has the potential to be manipulated for targeted delivery in future work.



## GFP Quantification Protocol:

- 6-Well Plate seeding
  - Clear old media and trypsinize cells with 5 mL Trypsin in T-75 Culture flask for 5 mins at 37° C.
  - Add 5 mL media and pellet cells by spinning at 500 RPM for 5 mins. Resuspend in 10 mL media.
  - Use 1/4 of cells (2.5 mL) for next generation and keep the rest for plating.
  - Do a cell count to obtain cell density by volume.
  - Plate three separate 6-well plates with about .5 million cells in 2 mL media per well.
- Plasmid transfection (30 mins after plate seeding)
  - Add (in order) transfection solution dropwise to each well in control and variable plates. Solution per well: 100 µL water, 1 µL plasmid, and 7 µL Fugene.
- RNAi insertion (24 hours after plate seeding)
  - Add (in order) RNAi solution to each well in variable plate. Solution per well: 100 µL water, 3 µL RNAi, and 7 µL Fugene.
- Cell Sample Fixation Protocol
  - Fix 1 well of each plate every 15-20 hours as follows for FACS analysis.
  - Remove old media
  - Trypsinize, incubate, and resuspend cells in 1 mL PBS.
  - Add cell suspension to 3 mL 10% formaldehyde solution and let cells fix for 15 mins at RT.
  - Spin down and remove formaldehyde.
  - Resuspend in 1 mL PBS and store for analysis.
- Analyze cells using Flow Cytometry
  - Use confocal fluorescence microscopy to confirm and fluorescence activated cell sorting (FACS) to quantify GFP expression in each sample.

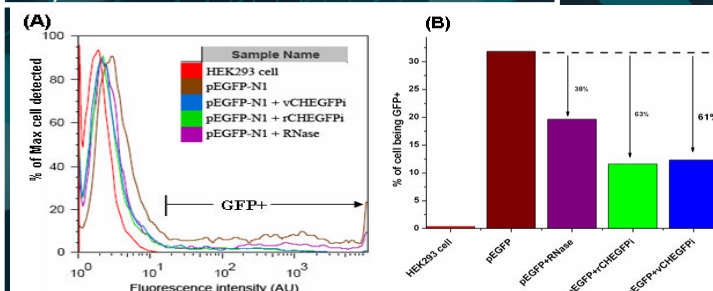


Figure (A) displays GFP expression intensity of the cell samples. Figure (B) normalizes the data from figure (A) to make the results more clear. The red sample (HEK293 cell) is the negative control, and is simply unaltered cells. The brown sample (pEGFP-N1) is the positive control where a plasmid was used to insert the GFP into the cells. The blue sample (pEGFP-N1+vCHEGFPi) utilized the TMV as the RNA delivery system. The green sample (pEGFP-N1+rCHEGFPi) utilized no TMV, and simply was RNA directly added to the cells. The purple sample (pEGFP-N1+RNase) had RNase in the sample, inhibiting expression by nonspecifically digesting all types of RNA in the cells.

## Internship Site:

Supervisors: Dr. William Bentley, Mr. Chiwei Hung.  
Biotechnology Research Lab.  
Plant Science Building, UMD College Park.

I would like to especially thank Dr. William Bentley and Mr. Chiwei Hung for the opportunity and support in this experience. Also a special thank you to Dr. Holtz, Dr. Merck, and the entirety of the College Park Scholars Staff for the privilege to be involved in the Earth, Life, and Time program.