

2008 Pauline and Irving Tanner Dean's Scholar Proposal

Name: Bailey Chang

Local Address: 322F Alice Cook House, Cornell University, Ithaca, NY 14853

Mobile: 678-361-2827

Email: byc6@cornell.edu

Major: Biological Sciences, Concentrating in Biochemistry

Project Advisor:

Dwight D. Bowman, M.S., Ph.D.
Professor of Parasitology
Dept. Microbiology & Immunology
College of Veterinary Medicine
Cornell University
C4-119 VMC
Tower Road
Ithaca NY, 14853-6401
Phone: 607-253-3406
Email: ddb3

Faculty Advisor:

George Hess,
Molecular Biology and Genetics
216 Biotechnology Bldg.
Phone: 607-255-4809
Email: gph2

Abstract:

Gene expression, with an emphasis on post-transcriptional regulation, is a fundamental biological process that still remains somewhat obscure in many ways. It is responsible for the generation of all proteins, and as a result, the expression of all bodily tissues. The purpose of this study is to construct a complementary DNA library (cDNA) library for the *Toxocara canis* parasitic roundworm in order to better study the process of gene expression in eukaryotic organisms. The generation of the cDNA library a fairly difficult process that is prone to error (due to the fragile nature of the mRNA), but the subsequent elucidation of all of the expressed messenger RNAs (mRNAs) will allow a much greater understanding of the workings of the *Toxocara* organism, from whence more studies can be conducted with regards to the post-transcriptional regulation of genes. By the end of my summer stay, which will last approximately seven weeks, I hope to complete a fairly complete, rudimentary cDNA library expressed in phage-transduced *E. coli* which can be used for further studies, and which I may use as a precursor to an honors thesis in Biology.

Biographical Sketch:

Ever since my mother and father moved me and my baby brother Andy in a tiny two-door Nissan Sentra from the Upper Peninsula of Michigan about 18 years ago, I have been proud to call Atlanta, Georgia, my home.

My parents both grew up surrounded by the privations of the Cultural Revolution in Shanghai, China. Naturally, to compensate for what had passed them by, they wasted no time in exposing me to the “liberal arts education” very early in my life. They heavily emphasized reading, writing, drawing, playing piano, and in general, an attitude of “try a little bit of everything and take what you really like.” This especially applied to reading, which transported me to lands and times otherwise unheard of, all from the comfort and shelter of 2875 Shurburne Drive in Atlanta.

I was especially struck with the writing of Jules Verne, after reading *Twenty Thousand Leagues Under the Sea*. The characters in the book, painted against the awesomeness of nature and the elegance of technology, exposed me to a world in which the analytical power of science was firmly allied with the creativity and wisdom of the literary world. Ever since then, I knew that my destiny would very likely take me towards the sciences.

My path towards my current concentration in biological sciences was by no means a straight shot—the sinuous paths of high school pulled me first towards mathematics, then towards creative writing, and then towards comparative government. Probably due to some combination of the characters I have encountered after one too many classical novels, and the influence of my dear parents, I hold in very high esteem the ideal of the jack of all trades. I aspire to be well-versed in multiple fields, from philosophy to driving stick-shift. Still I find myself drawing, playing the piano, and exploring diverse, remote corners of the academic and social world. At Cornell, I am involved with the Alpha Phi Omega service fraternity, the Cornell Catholic Community, and the Art Club in addition to my academic pursuits.

However after many years and many issues of *Scientific American* and *National Geographic*, and after being granted the honor of studying at Cornell, I feel as if I can safely say that I am fulfilling my aforementioned destiny by directing myself towards the natural sciences. Spurred on by the excellent faculty and staff that have given me advice during these years, I am filled with gratitude for all the wonderful individuals, the peers and the professors that have guided me and nurtured me during my intellectual and spiritual journey here. My process of self-discovery still continues of course, like the charting and exploration of an infinitely large continent, of which my past life is only a small subspace. And I hope that during the summer, in a more relaxed (and definitely more temperate) environment, I will be able to take the next steps by continuing my research with Dr. Bowman.

Project Description:

This project focuses on the gene expression of the nematode *Toxocara*, a species of nematode belonging to the family Ascaridae, which infects the gastrointestinal tract of dogs and cats. It is capable of migrating through tissue and causing visceral and ocular larval migrans in humans (Zhu et. al 1997). The purpose of this project is to construct a cDNA library of the nematode *Toxocara* and use the resulting information to study the effects of post-transcriptional gene silencing as discovered by Fire and Mello (2006). Not only will this have direct clinical relevance, in that the protein makeup of a larval parasitic roundworm can be determined, but it will also yield further insight into the workings of posttranscriptional modification in gene expression, which may be conserved across many species, including humans.

I am currently working on this project under the aegis of Dr. Dwight D. Bowman and Janice Liotta in the Veterinary School, with my lab partner Laura Chiu. With the support of the Dean's Scholar funding, I hope to continue this research into the summer.

The first step of this project involves the generation of the cDNA for the purposes of obtaining the DNA sequence. In virtually all organisms, proteins, which form the bulk of the physical makeup of the organism and the biochemical mechanisms that drive its metabolic processes, etc., are coded for by deoxyribonucleic acid (DNA). In eukaryotic organisms, genetic information is first transcribed from the genomic DNA into messenger ribonucleic acid (mRNA), which contains sequences with extraneous segments (introns) spliced out during posttranscriptional processing. Proteins are translated directly from these mRNA transcripts. Theoretically, then, it should be possible to establish the

identity of the protein by reviewing the information contained within these mRNA transcripts (Sambrook et. al 2006).

However, from a practical standpoint, mRNA is readily degraded, so it is advantageous to convert these transcripts into a complementary double-stranded DNA sequence (cDNA) which is more readily manipulated in the molecular biology setting. The derivation of the cDNA molecules is the first stage of the experiment. This is accomplished by using the reverse-transcriptase enzyme found in viruses, and in this experiment, it was done according to the protocol and using the reagents supplied with the Promega cDNA synthesis kit. *This is the current point of progress of this project, from which the summer work should theoretically start.*

The initial cDNA solution will contain a mixture of all the expressed mRNAs, so it will then be necessary to attach each individual cDNA segment to a vector and transform bacteria (or other host) with the vector, thereby creating many clones expressing this segment of cDNA (Sambrook et. al 1989). Thus, the second stage of the experiment is the transfer of the derived cDNA molecules to a reproducing host bacteria species. In this project, this will be accomplished by attaching the appropriate EcoRI adaptors to the cDNA, and then ligating these adapted cDNA molecules to bacteriophage DNA. These transformed bacteriophages will then be allowed to infect *E. coli* cells. The bacterial suspension will then be diluted to an appropriate concentration and plated out on agar. Theoretically assuming that each individual *E. coli* bacterium takes up a segment of cDNA and survives to replicate, the proportion of colonies expressing different cDNA molecules should reflect the proportions of cellular concentrations of mRNA inside the organism at the time the experiment was started.

After colonies are observed it will be possible to check for the expression of *Toxocara* proteins by the use of *Toxocara* antibodies. Each colony would then be sampled, lysed, and analyzed for its cDNA insert content. This can be accomplished by using a sequencing method such as pyrosequencing which will yield the base-pair sequence of the cDNA transcripts. It may also be possible to amplify only the insert area by using the polymerase chain reaction (PCR), using primers complementary to the EcoRI adaptor sequence which was used to ligate the cDNA products into the bacteriophage sequence. From the observed base pair sequences, it should be possible to catalogue and identify a significant portion of the mRNA synthesized by the *Toxocara* at the larval stage.

The third and final stage of the experiment is the investigation of the double-stranded RNA in interfering with the expression of homologous genes. This is significant in the context of the recently-elucidated process of RNA interference which was catalogued and mapped by Fire and Mello for the 2006 Nobel Prize in Med/Phys. The study illustrated that dsRNA can serve to suppress mRNA homologous to the dsRNA. This mechanism is precipitated when the Dicer enzyme cuts the injected dsRNA into small pieces (about 24 basepairs in length). The antisense portions of these small pieces bind to the complementary sequence on the homologous mRNA, prompting the binding of an RNA induced silencing complex (RISC) which subsequently destroys the homologous mRNA, rendering it untranslatable (Fire and Mello 2006).

If the previously described procedures proceed successfully, it should be possible to use the cDNA sequences to synthesize double-stranded RNA for the larval *Toxocara* proteins, and conduct a similar procedure to that of Fire and Mello. After injecting a certain synthesized dsRNA into a related organism such as *C. elegans*, (the reason being

that *Toxocara* itself is not culturable), some phenotype would ideally be significantly altered, indicating the suppression of the corresponding gene. This would yield a functional description of the gene and of how it works within the organism, in addition to the sequence information yielded by the previous sequencing step.

This final stage is the ultimate goal of the project, which I hope to reach (or at least draw nearer to) during the summer in Dr. Bowman's lab.

Bibliography

This bibliography is expected to grow in number as the project progresses, especially as new newer protocols and theoretical components are discovered.

Fire, Andrew Z., and Craig C. Mello. Advanced Information: RNA Interference. Nobel Foundation. Stockholm, Sweden: Nobel Foundation, 2006. 8 Feb. 2008 <http://nobelprize.org/nobel_prizes/medicine/laureates/2006/adv.html⁸>.

To whom it may concern,

I hereby waive my right to access, under the Family Educational Rights and Privacy Act of 1974, 20 U.S.C.A. Par. 1232g (a) (1), to this letter of recommendation in regard to my application for Dean's Scholar research funding for the year 2008.

Bailey Chang

A handwritten signature in black ink, appearing to read 'Bailey Chang', written in a cursive style.

Hu, Min, Xingquan Zhu, Stefan D'amelio, Lia Paggi, and Robin B. Gasser.
"Electrophoretic Detection of Population Variation Within *Contraecaecum Ogmorhini*
(Nematoda: Ascaridoidea: Anisakidae)." *Electrophoresis* 22 (2001): 1930-1934.

RNeasy Mini Handbook. 3rd ed. Valencia, CA: Qiagen, 2001.

Sambrook, J., E F. Fritsch, and T Maniatis. Molecular Cloning--a Laboratory Manual.
2nd ed. Vol. 2. Plainview: Cold Spring Harbor Laboratory P, NY. 8.3-8.81.

Technical Manual--Universal RiboClone CDNA Synthesis System. Madison, WI:
Promega Corporation, 2005.

Ying, Shao-Yao. *Generation of CDNA Libraries--Methods and Protocols*. Totowa, NJ:
Humana P, Inc., 2003. 1-11.

Zhu, Xingquan, D E. Jacobs, N B. Chilton, R A. Sani, R. A. B. Cheng, and R B. Gasser.
"Molecular Characterization of a *Toxocara* Variant From Cats in Kuala Lumpur,
Malaysia." *Parasitology* 117 (1998): 155-164.

Approximate Budget for stay (approximately from 16 May to 1 July):

Ideally, by staying in a co-op, I will be able to keep living costs down fairly low. In addition, I will be utilizing the Bowman lab resources, reagents and equipment to accomplish my project. I have not applied for nor do I expect to receive any other forms of funding for this summer stay.

\$400 lodging at 660 Stewart Cooperative (\$50 per week for non-members)

\$300 for food and toiletries

\$600 approximate roundtrip airfare from Atlanta, GA to Ithaca, NY via US Airways