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SPECIAL BIOSCIENCE ISSUE
ISSUE SPECIALE DE BIOSCIENCE

- PLGA Effects on Neural Stem Cells
- IMI CYP450 Synergist Lowers Toxicity
- Colletotrichum Truncatum Markers
- Activation de la Phosphatase PP2A
- Telomerase Mutations in DKC

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SABC History Timeline and Highlights

Rick Levick

Founder of the Sanofi-Aventis BioTalent Challenge

This year, the Sanofi Aventis BioTalent Challenge becomes older than most of the high school students participating in this annual science competition in biotechnology. Today, there are past participants who are now high school teachers with teams entered in SABC competitions.

1994 – The first competition, called the Connaught Student Biotechnology Exhibition, is held at the international Biotechnology Industry Association (BIO) conference in Toronto. The students' presentations of their research becomes a highlight of the conference.

1995 – The Connaught Laboratories decides to continue supporting the program in the greater Toronto area with local supporters including York University, Seneca College, Metro Toronto, the Toronto Biotechnology Initiative and the Toronto public and Catholic school boards.

1996 – The Toronto program partners with the Ontario Science Centre to include a lecture series on biotechnology for high school classes. The Biotechnology Institute in the US, the education arm of BIO, holds its own version of the competition – the BioGenius Challenge -- for students in northeastern US states. The winning team from the Toronto competition is invited to compete at the US competition, a tradition that continues to this day. Several groups from across Canada approach Connaught about bringing the program to their communities.

1997 – Programs begin in Montreal and London in partnership with local supporting organizations.

1998 – A new program is set up in Ottawa with support from the National Research Council Canada, which becomes the second national supporter of the program.

1999 – The SABC program expands into British Columbia, Saskatchewan, Nova Scotia and Newfoundland with financial support from Human Resources Skills Development Canada through its Biotechnology Human Resources Council (BHRC). CSBE wins the Michael Smith Award for Science Promotion, named after Canadian Nobel Laureate, the late Dr. Michael Smith of Vancouver. BHRC's BioMars video game and booklet on careers in biotechnology are launched at CSBE events across Canada.

2000 – Manitoba and Edmonton become program sites. The program is renamed as the Aventis Biotech Challenge (ABC) due to a change in corporate ownership of the lead supporter.

2002 – New Brunswick and Calgary, launch regional ABC programs. BIO returns to Toronto and the first place teams from the 12 regional programs are invited to the first national competition during the BIO conference.

2003 – A virtual national competition is launched using video-conferencing technology allowing the first place regional teams to present their research from their home communities to a panel of judges located at the National Research Council in Ottawa. The Canadian Institutes of Health Research becomes a national program supporter.

2004 – Genome Canada and the Canada Foundation for Innovation become national supporters. A tracking study of past ABC participants finds that most have continued their studies and careers in biosciences, the main goal of the program, and most found that the ABC experience helped them decide on a career path.

2005 – The ABC program is launched in Prince Edward Island, making the ABC truly national in scope.



A pilot program is launched in northern Manitoba to test the concept of students in rural and remote communities participating by using videoconferencing to work with mentors remotely. The ABC program becomes the Sanofi-Aventis Biotech Challenge (SABC) following a change in corporate ownership. VWR International becomes a national supporter.

2007 - The program name changes to Sanofi-Aventis BioTalent Challenge after BHRC becomes BioTalent Canada. The National first place winner goes on to take first prize at the Sanofi-Aventis International Biogenesis Challenge at BIO and at the Intel Science Competition in the US. The Canadian Louis Pasteur Foundation becomes a national supporter.

2008 – Northern Manitoba becomes the 14th SABC region based on the success of the remote mentoring pilot program. The National competition becomes a “face-to-face” event with the first place teams from each of the 14 regions traveling to Ottawa to compete at the National Research Council’s headquarters. The SABC National Awards Ceremony in Ottawa is attended by several federal Cabinet Ministers, a tradition that continues into 2009 and 2010. The National first place project on influenza prevention by an Ottawa student achieves global media coverage.

2010 – SABC receives an Honorable Mention in the Global Best Awards for science education sponsored by the Conference Board of Canada.



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Spring of Ideas and Innovation

It is so special to have a special Bioscience issue in the spring. Spring is always the beginning of something new and we are glad to be part of the spring of ideas and innovation.

This special issue was made possible due to financial support of the Canadian Institutes of Health Research through their involvement in the Sanofi Aventis BioTalent Challenge.

We would like also to acknowledge the other national supporters of students' bioscience education including Sanofi-Aventis, Sanofi Pasteur, National Research Council Canada, Genome Canada and BioTalent Canada, through the Government of Canada's Sector Council Program.

I would like to take this opportunity to introduce our newly formed International Academic Advisory Board. Prominent scientists join in an effort to review and comment on the research papers of this Bioscience issue.

Led by Dr. Dennis McCormac, they are bringing a wealth of expertise in various fields:

Dr. Dennis McCormac is the director of Platform Development with the Ontario Genomics Institute. Dr. McCormac has a wealth of relationships with opinion leaders in both industry and academia and his approach to engaging researchers in student education expands the horizons of bio education for an entire generation.

Dr. N. Bryant from the University of Glasgow uses a combination of genetics, biochemistry and cell biology to further the understanding of how eukaryotic cells control the specificity of membrane traffic. This process enables eukaryotic cells to establish and

maintain one of their distinguishing characteristics: intracellular compartmentalisation.

Dawn Thompson from the Broad Institute of MIT and Harvard is actively involved in the Broad Institute program that connects high school students and teachers in the Boston/Cambridge area with cutting-edge biomedical research. Through this program, students expand their understanding of scientific work and careers and develop their academic and career interests in science.

Dr. Bodo Stern is the director of Research Affairs of the Harvard FAS Center for Systems Biology. He was the Senior Scientific Editor at Cell for four years before joining the FAS Center for Systems Biology. He is now among those who run the Bauer Fellows program helping to grow the scientific community at the Center. Dr. Stern is focused on the emerging area of systems biology that integrates knowledge in the field of molecular biology into a description of biology as a system, with the goal of defining the organizing principles that explain how complex behaviour in cells and organisms arises from interacting components.

In this issue we are introducing QR coding to complement information about reviewers. It would allow our readers to use their smartphones to interact with the text and literally read more between the lines about the research institutes and projects that the experts from our International Academic Advisory Board are taking part in.

In the issues to come, we will be using QR coding to expand and enrich the reading experience of our audience as well as the means for authors to demonstrate and deliver their research ideas.

Editor-in-Chief Alexandre Noukhovitch, Ph.D.





The Effects of a PLGA Biomaterial on Neural Stem/Progenitor Cells

Amy-Jayne J. Hutchings

Grade 12, All Saints Catholic High School, Ottawa

Ottawa Hospital Research Institute, Ottawa

Amy-Jayne has always been enticed by any kind of challenges. She relishes in the complexity and perfection of the world that surrounds us; her sudden interest in science has motivated her to stay up late reading textbooks and journals. In the summer of Grade 10, she participated in an all day cooperative education program, which landed her a placement in Dr. Eve C. Tsai's neuroscience research laboratory at the Ottawa Hospital and she has never looked back since. AJ feels a passion towards the science, particularly medical research, as her step-mother has multiple sclerosis and her grandfather recently contracted colon cancer. She believes that medical research brings out the best characteristics in an individual. The nervous system of the human body, to Amy-Jayne Hutchings, is both mind boggling and fascinating, as it is the center of daily life; controlling everything from sleeping patterns, feeding behaviour, and breathing sensations, memories, and learning. It is also enigmatic, since science has not come close to realizing the full potential of the human nervous system. Amy-Jayne's research focuses on spinal cord injury and the pursuit of clinical

Stem cells are viewed to be the saving grace for many neurological disorders, including spinal cord injury (SCI), Parkinson's disease, and multiple sclerosis. Endogenous Neural Stem Cells (eNSPCs) are heralded for use to relieve SCI because of their favourable characteristics to combat the hostile environment that follows injury. In particular, the use of eNSPCs combined with a biodegradable hollow fiber channel to act as a guidance vehicle/factor delivery system to battle SCI is viewed as a possible clinical therapy. In this project, the response of eNSPCs to the biomaterial of Poly (lactide-co-glycolide) (PLGA) in the form of a PLGA 50/50 hollow fiber channel is investigated. Cell viability and proliferation constraints were measured to determine the cytotoxic effects of PLGA upon eNSPCs. Experimentation displayed the similar characteristics elicited from eNSPCs gathered from two locations of the central nervous system. PLGA cytotoxic effects were found to be minimal upon both sets of eNSPCs; however, more experimentation is needed to definitively confirm the effects that PLGA inflicts upon eNSPC proliferation.

Les cellules souches sont considérées à la grâce salvatrice pour de nombreux troubles neurologiques, y compris les lésions de la moelle épinière (SCI), la maladie de Parkinson et la sclérose en plaques. Endogènes cellules souches neurales (eNSPCs) sont annoncés pour une utilisation pour soulager SCI en raison de leurs caractéristiques favorables pour lutter contre l'environnement hostile qui suit blessures. En particulier, l'utilisation de eNSPCs combiné avec un canal à fibres creuses biodégradables à agir comme un véhicule d'orientation / système de distribution des facteurs de bataille SCI est considérée comme une thérapie cliniques possibles. Dans ce projet, la réponse de eNSPCs sur le biomatériau de poly (lactide-co-glycolide) (PLGA) sous la forme d'un PLGA 50/50 canaux à fibres creuses est étudiée.



therapy for those suffering from debilitating disease. She revels in the study of stem cells from different locations of an adult body, the complexity and underlying mechanisms of the nervous system. As a result of her immense interest in the sciences, she will continue her work in scientific research.

contraintes de viabilité cellulaire et la prolifération ont été mesurées afin de déterminer les effets cytotoxiques de PLGA sur eNSPCs. L'expérimentation a montré des caractéristiques similaires suscitées par eNSPCs recueillies à deux endroits du système nerveux central. Les effets cytotoxiques de PLGA solide ont été trouvés être minimes sur les deux ensembles de eNSPCs, mais plus d'expérimentation est nécessaire pour confirmer définitivement les effets que PLGA inflige à la prolifération eNSPC.

Background

The Central Nervous System (CNS), which is composed of the spinal cord and brain, contains three predominant cell types: neurons, oligodendrocytes, and astrocytes. The neurons are responsible for conducting the electrical messages throughout the body; whereas, the oligodendrocytes and astrocytes play a supporting role. Spinal Cord Injury (SCI) is a devastating physical condition that still possesses no cure to date; science is struggling to provide relief to this disorder due to the hostile environment that is left after the injury. Following SCI, a cellular debris is created by the death of neurons and oligodendrocytes, and then the astrocytes bind together to form a physical scar^[1]. In addition, CNS neurons lack the capacity to regenerate freely following the injury^[2,3].

It is widely believed that stem cells are the key to treating many neurological disorders, including SCI. In 1992, a particular kind of stem cells called Endogenous Neural Stem/Progenitor Cells (eNSPCs) were discovered by Canadians Brent Reynolds and Samuel Weiss that displayed neurogenesis within the rodent for the first time^[4]. From this paper, many of the predominant cell culture protocols have been established. These eNSPCs can be found within the adult sub-ventricular zone (SVZ)^[5] and sub-granular zone (SGZ)^[6] of the brain, and the sub-ependymal zone of the spinal cord^[7,8]. Although there are many different candidates of stem cells available (including fetal and embryonic stem cells), eNSPCs lack the ethical and practicality issues that their cousins are faced with^[9]. eNSPCs are particularly promising because of their immaturity, which leads researchers to believe that they lack the receptors for inhibitory molecules found after SCI. eNSPCs are also multipotent, which gives them the capacity to differentiate into neurons, oligodendrocytes, and astrocytes^[10,11].

It has been shown however, that when introduced following an SCI, eNSPCs disproportionately proliferate into astrocytes, creating an unwanted balance of cell types. Therefore, it is necessary for the incorporation of known factors Retinoic Acid (RA) for neurons^[12], Platelet Derived Growth Factor (PDGF) for oligodendrocytes^[13], and Bone Morphogenic Protein-4 (BMP-4) for astrocytes^[14] to encourage a stability of proliferation after a SCI and allowing the future potential of eNSPCs in the treatment of neurological disease.

If these differentiating factors are to be successfully incorporated into an injury model, a functioning delivery system is needed. Models that are currently being used, such as mini-osmotic pumps, are inaccurate and could actually worsen the injury site^[15,16]. One alternative delivery method is that of a biodegradable hollow fiber channel (HFC). The HFC is designed to encompass the injury site to act as a guidance vehicle, but to also degrade over an extended period of time to gradually release differentiation factors. A possible biomaterial to be utilised is the polymer poly (lactide-co-glycolide) 50/50 (PLGA 50/50). This biomaterial can already be found in literature acting as a matrix for neurite outgrowth^[17]. When PLGA 50/50 breaks down, the bi-products lactide and glycolide are created^[18] both of which are common molecules within the human body. It is for this reason that it is believed that PLGA 50/50 channels will exhibit a low toxicity on NSPCs whilst still maintaining the viability of the cells.

Purpose

This project was designed to test the toxic effect of PLGA 50/50 channels upon eNSPCs and to test the cell viability following exposure to PLGA 50/50 channels. eNSPCs were taken from both the Sub-Ventricular Zone and the Spinal Cord in order to compare



the resultant effects with stem cell location. The results from this project will be vital in determining PLGAs feasibility as a possible biomaterial for use in HFCs.

Hypothesis

It is hypothesised that both SVC and SC progenitor cells will exhibit low cytotoxicity when exposed to PLGA 50/50. It is also believed that both SVC and SC will maintain their viability following exposure to a PLGA 50/50 HFC.

Procedure

This study was completed by utilising an *in vitro* cell culture method. eNSPCs were harvested from the sub-ventricular zone of the brain and the subependymal zone of the spinal cord from the adult CNS. All protocols were approved by the Canadian Council on Animal Care. Following extraction, cells were placed in a culture media that maintains neural tissue. This culture media was supplemented with Epidermal Growth Factor (EGF) and basic Fibroblast Growth Factor (bFGF) to support the growth of eNSPCs. It is known a PLGA channel takes approximately 14 days to substantially degrade following implantation within the body; therefore, eNSPCs were exposed to PLGA for 16 days. The viability and proliferation were assessed every 4 days. Cell viability was quantified by using the cell marker trypan blue. Trypan blue is a dye that is normally actively transported out of healthy tissue; yet, when the cell is dead, it will be labelled with the marker. This allows one to create a ratio of cells that displays total cells: dead cells, and thus determine the level of toxicity. The extent of proliferation was quantified by measuring the diameter of the sphere and the total number of cells.

Results

Viability of Neural Progenitors

SC eNSPCs were seen to elicit an initial reduction of cell viability for both the control and PLGA group; therefore, at 4div there was seen to be no significant difference between the two groups. There was also seen to be no significant difference at 12 and 16 days in vivo (div). However, at 8div, the viability of SC PLGA cells was significantly greater ($p < 0.05$) than that of the control SC cells (see Figure 1; Appendix A lines 1-4). Due to the viability staying relatively consistent, it can be concluded that PLGA had minimal effects upon the cell viability of SC eNSPCs.

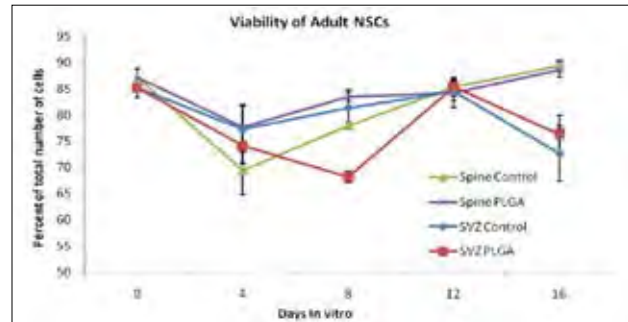


Figure 1. Trypan Blue positive cell count percentages for all four experimental groups over a period of 16 days in vivo to display cytotoxic effect of PLGA upon eNSPCs.

No significant differences were determined between the PLGA and control groups of SVC eNSPCs at 4, 12, and 16div. There was, however, a significant difference ($p < 0.05$) between these two groups at 8div, with the control SVC group having a significantly greater cell viability than that of the SVC PLGA group (see Figure 1; Appendix A lines 5-8).

It is believed that both locations of eNSPCs were reacting similarly to the cell culture protocols as no significant differences could be noted within cell viability at 4, 12, and 16div. In addition, the PLGA groups were not significantly dissimilar at 4, 12, and 16div. However, at 8div it can be noted that SVC eNSPCs in a PLGA environment had a significantly greater cell viability ($p < 0.01$) than PLGA SC eNSPCs (see Figure 1; Appendix A lines 13-16).

There were no significant changes from 0-16div for either of the two locations of eNSPCs and their respective experimental groups (see Figure 1; Appendix A lines 21-28).

Neurosphere Diameter

SC eNSPCs were found to elicit no significant differences in neurosphere diameter between the control and PLGA experimental groups at 8 and 16div. However, the neurosphere diameter of control SC eNSPCs were significantly larger ($p < 0.05$) than PLGA eNSPCs at 4 and 12div (see Figure 2; Appendix B lines 1-4).

The PLGA experimental group of SVC eNSPCs were found to have significantly larger diameters ($p < 0.05$) than control SVC eNSPCs at 8 and 16div. However, at 4div, control SVCs held significantly larger diameters ($p < 0.05$) than PLGA eNSPCs. There were no significant differences found at 12div (see Figure 2; Appendix B lines 5-8).

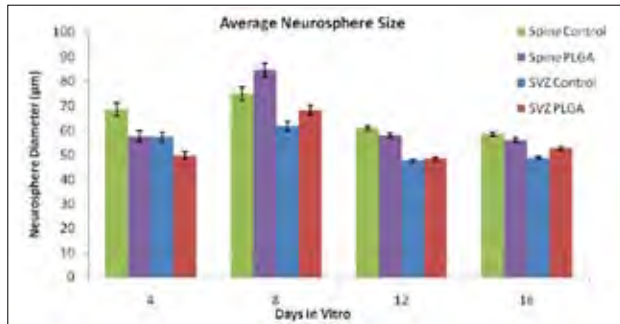


Figure 2. Neurosphere diameter measurement as a constraint for eNSPC proliferation. eNSPCs were exposed for 16 days to PLGA.

On the whole, control SC eNSPCs were found to have significantly larger diameters ($p < 0.01$) at each time point than control SVC eNSPCs. This same pattern was reflected in PLGA eNSPCs; SC cell diameters were significantly larger at all time points except 16 div (see Figure 2; Appendix B lines 13-16).

The control SC group were seen to have significant differences in neurosphere diameter between the time points of 8-12 div ($p < 0.01$) and 12-16 div ($p < 0.05$). This trend displayed a gradual decrease in diameter sizes as the preceding time points were the largest (see Figure 2; Appendix B lines 17-19). This same pattern is reflected in PLGA SC eNSPCs (see Figure 2; Appendix B lines 20-22). This gradual decrease in neurosphere size was not replicated in either SVC control or SVC PLGA experimental groups (see Figure 2; Appendix B lines 23-28).

Proliferation of Neural Progenitors

For all time points of the experiment, no significant differences could be seen between cell numbers for SC control and PLGA groups (see Figure 3; Appendix C lines 1-4). This pattern was also reflected with SVC PLGA and control experimental groups at all time points (see Figure 3; Appendix C lines 5-8).

The SC control group cell numbers were seen to increase in greater increments than that of SVC control cell numbers. There was no initial significant difference at 4 and 8 div between the two control groups; however, at 12 and 16 div SC cell numbers were significantly greater ($p < 0.01$) than cells of the SV control group (see Figure 3; Appendix C lines 9-12). A similar trend was observed with PLGA groups of SC and SVC eNSPCs; there were no significant differences in cell numbers at 4 and 8 div, yet at 12 and 16 div there was a significantly greater amount of SC

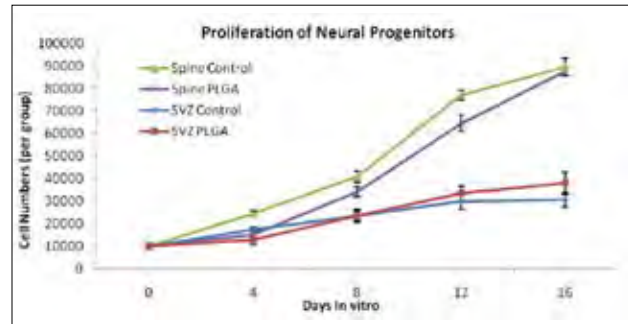


Figure 3. Cell number count following an exposure of 16 days for all four experimental groups as a constraint to demonstrate the effect on proliferation from PLGA.

eNSPCs than SVC eNSPCs ($p < 0.01$), (see Figure 3; Appendix C lines 13-16).

There was seen to be a significant ($p < 0.01$) increase in PLGA SC cell numbers across the time points 4-16 div. This gives a strong indication that successful proliferation is occurring. This same pattern was reflected with control SC cells across the 4-16 div time point ($p < 0.01$) (see Figure 3; Appendix C line 24; 19). The increase in cell numbers of SVC eNSPCs are reserved compared to those of SC eNSPCs. There was a significant increase in SVC control cells ($p = 0.025$) from 4-16 div; however, there was not a significant increase in cell numbers from 4-16 div in PLGA SVC cells (see Figure 3; Appendix C lines 29; 34).

Conclusions

The initial hypothesis that PLGA would have a minimal effect on cell viability was supported by the fact that both control and PLGA groups of SC eNSPCs responded in a similar fashion to PLGA exposure (Figure 1). This response was also reflected in SVC eNSPCs; there was only a decrease in cell viability of SVC eNSPCs at 8 div. Due to the isolated nature of this response, it can lead us to believe that this result was either an anomaly, or PLGA is not the sole cause of this decrease. Therefore, PLGA was found to be of low impact on the viability of SC and SVC eNSPCs following exposure for 16 div.

It was also hypothesised that PLGA would inflict low cytotoxicity upon SVC and SC eNSPCs; this would be seen through an increase in neurosphere diameter to demonstrate cell proliferation. It was initially seen that neurospheres from both locations were increasing; yet this was followed by



a period of decrease of sphere diameter for both SC and SVC cells. This decrease was seen in both control and PLGA experimental groups; therefore, it cannot be concluded that PLGA was the sole cause of this decrease. It can however be noted that SVC and SC cells are acting in a similar fashion following exposure to these experimental conditions; however, it was seen that SC spheres in general had a larger diameter than SVC neurospheres.

Another aspect of the hypothesis was that PLGA would not affect cell numbers; another constraint to demonstrate cell proliferation. Initially, there was no difference between SC and SVC groups in regard to cell numbers; however, after 4div, the number of SC eNSPCs increased drastically compared to SVC cells. This pattern occurred for both the control and PLGA experimental groups, making it difficult to relate this response back to a PLGA influence. This pattern also signifies a difference in behaviour between these cell types.

There must be further experimentation before a definitive answer can be found to the effects that a PLGA 50/50 HFB would inflict upon the viability and proliferation of eNSPCs from both the SVC and SC.

Future Directions

- Increase sample size to increase accuracy.
- Combine viability assessment using Trypan Blue with TUNEL and/or combination of Propidium Iodide and Hoechst.
- Utilisation of confocal microscopy to monitor change in morphology of cells.
- Testing effects of known differentiation factors upon eNSPCs (retinoic acid, platelet derived growth factor, and bone morphogenic protein-4, etc.).

Acknowledgements

I would like to offer a warm gratitude to Dr. Eve Tsai and Matthew Coyle for their support, extensive knowledge, and mentorship. This project would also not have been possible without the contributions of Ushananthini Shanmugalingam, Harrison Westwick, Nella Bianconi, and the Ottawa Hospital Research Institute.

Key Words

Neural Stem/Progenitor Cell, Differentiation, Proliferation, Cytotoxicity, Viability.

References

- [1] Meletis, K., Barnabe-Heider, F., Carlen, M., Evergren, E., Tomilin, N., Shupliakov, O., Frisen, J., 2008, Spinal Cord Injury reveals Multilineage Differentiation of Ependymal Cells, *PLOS Biology*:6:7:1494-1507.
- [2] Berry, M., Rees, L., Hall, S., Yiu, P. & Sievers, J., 1988, Optic axons regenerate into sciatic nerve isografts only in the presence of Schwann cells. *Brain Res. Bull.* 20, 223-231.
- [3] Aguayo AJ, David, S, Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. *Science.* 1981 Nov 20;214(4523):931-3.
- [4] Reynolds, B., Weiss, S., 1992, Generation of Neurons and Astrocytes from Isolated Cells of the Adult Mammalian Central Nervous System *Science, New Series, Vol. 255, No. 5052.* pp. 1707-1710.
- [5] Johansson, C.B., Svensson, M., Wallstedt, L., Janson, A.M., and Frisen, J., 1999), Neural stem cells in the adult human brain. *Exp. Cell Res.* 253, 733-736.
- [6] Bear, M.F., Connors, B.W., Paradise, M.A., 2007, *Neuroscience: Exploring the Brain* fifth edition, Lippincott Williams & Wilkins, 364-365.
- [7] Martens DJ, Seaberg RM, van der Kooy D., 2002, In vivo infusions of exogenous growth factors into the fourth ventricle of the adult mouse brain increase the proliferation of neural progenitors around the fourth ventricle and the central canal of the spinal cord. *Eur J Neurosci* 16: 1045-1057.
- [8] Okano, H., Sakaguchi M, Ohki K, Suzuki N, Sawamoto K., 2007, Regeneration of the central nervous system using endogenous repair mechanisms. *J Neurochem*: 102(5): 1459-65.
- [9] Bongoso, A., Fong, C., Gauthaman, K., 2008, Taking Stem Cells to the Clinic: Major Challenges, *J. Cellular Biochem.*, 105:6:1352-1360.
- [10] Frisen J, Johansson CB, Torok C, Risling M, Lendahl U: Rapid, widespread, and longlasting induction of nestin contributes to the generation of glial scar tissue after CNS injury. *J. Cell Biol.* 131(2), 453-464 (1995).
- [11] Teng YD, Liao WL, Choi H, Konya D, Sabharwal S, Langer R, Sidman RL, Snyder EY, Frontera WR. Physical activity-mediated functional recovery after spinal cord injury: potential roles of neural stem cells, *Regen Med.* 2006 Nov;1(6):763-76. Review
- [12] Kondo, T., et al. 2005, Sonic hedgehog and retinoic acid synergistically promote sensory fate specification from bone marrow-derived pluripotent stem cells. *PNAS*, 102: 4789.
- [13] Woodruff et al., 2004, Platelet derived growth factor regulates oligodendrocyte progenitor numbers in adult CNS and their response following CNS demyelination, *Mol. Cell. Neurosci.* 25:252.
- [14] Wen, Xuejun; Tresco, Patrick; Fabrication and characterization of permeable degradable poly(dl-lactide-co-glycolide) (PLGA) hollow fiber phase inversion membranes for use as nerve tract guidance channels; 2006; *Biomaterials*; 27 (20); 3800-3809.
- [15] Jones LL, Yamaguchi Y, Stallcup WB, et al: NG2 is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. *J Neurosci* 22:2792-2803, 2002.
- [16] Parr, A., Tator, C., 2007, Intrathecal epidermal growth factor and fibroblast growth factor-2 exacerbate meningeal proliferation lesions associated with intrathecal catheters, *Neurosurgery*:60:926.
- [17] Zheng, X., Yang, X., Liu, W., Shen, G., Pan, D., Luo, Ming., 2007, A novel method for culturing neural stem cells, *In Vitro Cell.Dev.Biol-Animal*, 43:155-158.
- [18] Nedergaard, M., Goldman, S., 1993, Carrier-mediated transport of lactic acid in cultured neurons and astrocytes, *Am J of Physiol Regulatory Integrative Comp Physiol*, 265:282-289.



Appendices

Appendix A - Summary of mean values of neural progenitor viability and ANOVA statistics					
Cell_Group_DIV		Viability (Mean ± SE %)			
Row	Between				ANOVA p-value
	1	2	1	2	
1	SC_CON_4	SC_PL_4	69.45 ± 4.74	77.66 ± 4.34	0.246
2	SC_CON_8	SC_PL_8	78.00 ± 0.73	83.50 ± 0.955	0.039
3	SC_CON_12	SC_PL_12	85.40 ± 1.18	84.17 ± 2.725	0.519
4	SC_CON_16	SC_PL_16	89.37 ± 0.995	88.63 ± 1.41	0.754
5	SVZ_CON_4	SVZ_PL_4	77.29 ± 4.37	74.17 ± 3.425	0.611
6	SVZ_CON_8	SVZ_PL_8	81.35 ± 3.475	68.15 ± 1.075	0.03
7	SVZ_con_12	SVZ_PL_12	84.425 ± 1.775	85.35 ± 1.775	0.192
8	SVZ_CON_16	SVZ_PL_16	72.68 ± 5.25	76.32 ± 3.58	0.645
9	SC_CON_4	SVZ_CON_4	69.45 ± 4.74	77.29 ± 4.37	0.346
10	SC_CON_8	SVZ_CON_8	78.00 ± 0.73	81.35 ± 3.475	0.443
11	SC_CON_12	SVZ_con_12	85.40 ± 1.18	84.425 ± 1.775	0.026
12	SC_CON_16	SVZ_CON_16	89.37 ± 0.995	72.68 ± 5.25	0.058
13	SC_PL_4	SVZ_PL_4	77.66 ± 4.34	74.17 ± 3.425	0.246
14	SC_PL_8	SVZ_PL_8	83.50 ± 0.955	68.15 ± 1.075	0.001
15	SC_PL_12	SVZ_PL_12	84.17 ± 2.725	85.35 ± 1.775	0.766
16	SC_PL_16	SVZ_PL_16	88.63 ± 1.41	76.32 ± 3.58	0.061
Within					
	1	2	1	2	
17	SC_0	SC_CON_4	87.19 ± 1.72	69.45 ± 4.74	0.016
18	SC_CON_4	SC_CON_8	69.45 ± 4.74	78.00 ± 0.73	0.207
19	SC_CON_8	SC_CON_12	78.00 ± 0.73	85.40 ± 1.18	0.007
20	SC_Con_12	SC_Con_16	85.40 ± 1.18	89.37 ± 0.995	0.112
21	SC_0	SC_PL_4	87.19 ± 1.72	77.66 ± 4.34	0.166
22	SC_PL_4	SC_PL_8	77.66 ± 4.34	83.50 ± 0.955	0.281
23	SC_PL_8	SC_PL_12	83.50 ± 0.955	84.17 ± 2.725	0.859
24	SC_PL_12	SC_PL_16	84.17 ± 2.725	88.63 ± 1.41	0.182
25	SVZ_0	SVZ_CON_4	85.20 ± 1.92	77.29 ± 4.37	0.267
26	SVZ_CON_4	SVZ_CON_8	77.29 ± 4.37	81.35 ± 3.475	0.629
27	SVZ_CON_8	SVZ_CON_12	81.35 ± 3.475	84.425 ± 1.775	0.714
28	SVZ_Con_12	SVZ_Con_16	84.425 ± 1.775	72.68 ± 5.25	0.321
29	SVZ_0	SVZ_PL_4	85.20 ± 1.92	74.17 ± 3.425	0.052
30	SVZ_PL_4	SVZ_PL_8	74.17 ± 3.425	68.15 ± 1.075	0.17
31	SVZ_PL_8	SVZ_PL_12	68.15 ± 1.075	85.35 ± 1.775	0.006
32	SVZ_PL_12	SVZ_PL_16	85.35 ± 1.775	76.32 ± 3.58	0.156



Appendix B - Summary of mean values of neurosphere diameter and ANOVA statistics

Row	Cell_Group_DIV		Neurosphere Diameter (Mean \pm SE μ m)		ANOVA (p-value)
			Between		
	1	2	1	2	
1	SC_CON_4	SC_PL_4	68.48 \pm 2.77	57.6 \pm 2.29	0.011
2	SC_CON_8	SC_PL_8	74.97 \pm 2.76	84.58 \pm 2.92	0.052
3	SC_CON_12	SC_PL_12	60.96 \pm 0.82	57.8 \pm 0.83	0.005
4	SC_CON_16	SC_PL_16	58.41 \pm 0.83	56.07 \pm 0.9	0.273
5	SVZ_CON_4	SVZ_PL_4	57.29 \pm 1.89	49.55 \pm 1.56	0.05
6	SVZ_CON_8	SVZ_PL_8	61.71 \pm 1.74	68.24 \pm 1.86	0.012
7	SVZ_con_12	SVZ_PL_12	47.52 \pm 0.63	48.35 \pm 0.7	0.331
8	SVZ_CON_16	SVZ_PL_16	48.71 \pm 0.62	52.52 \pm 0.79	0.008
9	SC_CON_4	SVZ_CON_4	68.48 \pm 2.77	57.29 \pm 1.89	0.01
10	SC_CON_8	SVZ_CON_8	74.97 \pm 2.76	61.71 \pm 1.74	<0.001
11	SC_CON_12	SVZ_CON_12	60.96 \pm 0.82	47.52 \pm 0.63	<0.001
12	SC_CON_16	SVZ_CON_16	58.41 \pm 0.83	48.71 \pm 0.62	0.003
13	SC_PL_4	SVZ_PL_4	57.6 \pm 2.29	49.55 \pm 1.56	0.024
14	SC_PL_8	SVZ_PL_8	84.58 \pm 2.92	68.24 \pm 1.86	<0.001
15	SC_PL_12	SVZ_PL_12	57.8 \pm 0.83	48.35 \pm 0.7	<0.001
16	SC_PL_16	SVZ_PL_16	56.07 \pm 0.9	52.52 \pm 0.79	0.198
			Within		
	1	2	1	2	
17	SC_CON_4	SC_CON_8	68.48 \pm 2.77	74.97 \pm 2.76	0.14
18	SC_CON_8	SC_CON_12	74.97 \pm 2.76	60.96 \pm 0.82	<0.001
19	SC_Con_12	SC_Con_16	60.96 \pm 0.82	58.41 \pm 0.83	<0.001
20	SC_PL_4	SC_PL_8	57.6 \pm 2.29	84.58 \pm 2.92	<0.001
21	SC_PL_8	SC_PL_12	84.58 \pm 2.92	57.8 \pm 0.83	<0.001
22	SC_PL_12	SC_PL_16	57.8 \pm 0.83	56.07 \pm 0.9	0.193
23	SVZ_CON_4	SVZ_CON_8	57.29 \pm 1.89	61.71 \pm 1.74	0.609
24	SVZ_CON_8	SVZ_CON_12	61.71 \pm 1.74	47.52 \pm 0.63	<0.001
25	SVZ_Con_12	SVZ_Con_16	47.52 \pm 0.63	48.71 \pm 0.62	0.74
26	SVZ_PL_4	SVZ_PL_8	49.55 \pm 1.56	68.24 \pm 1.86	<0.001
27	SVZ_PL_8	SVZ_PL_12	68.24 \pm 1.86	48.35 \pm 0.7	<0.001
28	SVZ_PL_12	SVZ_PL_16	48.35 \pm 0.7	52.52 \pm 0.79	<0.001



Appendix C - Summary of mean values of neural progenitor cell numbers and ANOVA statistics

Row	Cell_Group_DIV		Cell Numbers (Mean ± stdev)		ANOVA (p-value)
			Between		
	1	2	1	2	
1	SC_CON_4	SC_PL_4	24500 ± 3774.9	15000 ± 1290.5	0.05
2	SC_CON_8	SC_PL_8	41000 ± 5567.76	34000 ± 2581.5	0.33
3	SC_CON_12	SC_PL_12	77000 ± 7141.4	64500 ± 2217	0.093
4	SC_CON_16	SC_PL_16	89500 ± 2872.28	87500 ± 3774.5	0.63
5	SVZ_CON_4	SVZ_PL_4	17500 ± 957	13000 ± 2380.5	0.078
6	SVZ_CON_8	SVZ_PL_8	23500 ± 2986	23500 ± 2362.5	1
7	SVZ_con_12	SVZ_PL_12	30000 ± 3651	33500 ± 3095.5	0.608
8	SVZ_CON_16	SVZ_PL_16	30500 ± 3201.5	38000 ± 4760.5	0.205
9	SC_CON_4	SVZ_CON_4	15000 ± 1290.5	17500 ± 957	0.182
10	SC_CON_8	SVZ_CON_8	34000 ± 2581.5	23500 ± 2986	0.031
11	SC_CON_12	SVZ_con_12	64500 ± 2217	30000 ± 3651	0.02
12	SC_CON_16	SVZ_CON_16	87500 ± 3774.5	30500 ± 3201.5	0.002
13	SC_PL_4	SVZ_PL_4	15000 ± 1290.5	13000 ± 2380.5	0.546
14	SC_PL_8	SVZ_PL_8	34000 ± 2581.5	23500 ± 2362.5	0.098
15	SC_PL_12	SVZ_PL_12	64500 ± 2217	33500 ± 3095.5	0.009
16	SC_PL_16	SVZ_PL_16	87500 ± 3774.5	38000 ± 4760.5	0.002
			Within		
	1	2	1	2	
17	SC_CON_4	SC_CON_8	24500 ± 3774.9	41000 ± 5567.76	0.089
18	SC_CON_4	SC_CON_12	24500 ± 3774.9	77000 ± 7141.4	0.013
19	SC_CON_4	SC_Con_16	24500 ± 3774.9	89500 ± 2872.28	0.001
20	SC_CON_8	SC_CON_12	41000 ± 5567.76	77000 ± 7141.4	0.055
21	SC_Con_12	SC_Con_16	77000 ± 7141.4	89500 ± 2872.28	0.184
22	SC_PL_4	SC_PL_8	15000 ± 1290.5	34000 ± 2581.5	0.007
23	SC_PL_4	SC_PL_12	15000 ± 1290.5	64500 ± 2217	<0.001
24	SC_PL_4	SC_PL_16	15000 ± 1290.5	87500 ± 3774.5	<0.001
25	SC_PL_8	SC_PL_12	34000 ± 2581.5	64500 ± 2217	0.002
26	SC_PL_12	SC_PL_16	64500 ± 2217	87500 ± 3774.5	0.02
27	SVZ_CON_4	SVZ_CON_8	17500 ± 957	23500 ± 2986	0.069
28	SVZ_CON_4	SVZ_CON_12	17500 ± 957	30000 ± 3651	0.04
29	SVZ_CON_4	SVZ_CON_16	17500 ± 957	30500 ± 3201.5	0.025
30	SVZ_CON_8	SVZ_CON_12	23500 ± 2986	30000 ± 3651	0.135
31	SVZ_Con_12	SVZ_Con_16	30000 ± 3651	30500 ± 3201.5	0.761
32	SVZ_PL_4	SVZ_PL_8	13000 ± 2380.5	23500 ± 2362.5	0.084
33	SVZ_PL_4	SVZ_PL_12	13000 ± 2380.5	23500 ± 2362.5	0.027
34	SVZ_PL_4	SVZ_PL_16	13000 ± 2380.5	38000 ± 4760.5	0.092
35	SVZ_PL_8	SVZ_PL_12	23500 ± 2362.5	33500 ± 3095.5	0.092
36	SVZ_PL_12	SVZ_PL_16	33500 ± 3095.5	38000 ± 4760.5	0.473



Review of ***The Effects of a PLGA Biomaterial on Neural Stem/Progenitor Cells***

This project set out to investigate the effect of poly (lactide-co-glycolide) polymer channels on a class of stem cells (endogenous neural/progenitor cells; sNSPCs) with a view to using this as a delivery mechanism for factors required for specific differentiation of these cells to specific locations within the body.

The paper is well-written with the background behind the projection explained well. I really like the hypothesis driven approach used, and the way that the experiments have been designed to address the hypothesis.

Statistical analysis has been used throughout the study which is great and the future directions have been well thought through. The only thing that I could suggest to improve the rigour of the work would have been to include a substance that does have a profound cytotoxic effect on these cells (to check the assay conditions etc.). I would like to commend the author on a nicely performed, and presented piece of work.

N. Bryant, B.Sc (Hons), Ph.D., University of Glasgow





Activation de la Phosphatase PP2A: Nouveau Traitement pour la Maladie D'alzheimer

Alexandre Lemieux et Mohamed Réda Bensaidane

Cégep de Sainte-Foy, Cégep Champlain St. Lawrence,

Réda Bensaidane:

Passionné de sciences depuis un jeune âge, Reda Bensaidane a fait plusieurs projets de recherches qui l'ont permis de se rendre à l'Exposciences pancanadienne, ainsi qu'au Défi Biotalent Sanofi-Aventis. Ces expériences l'ont appris beaucoup sur le domaine scientifique et actuellement il est étudiant au Cégep Champlain St. Lawrence où il est dans sa première année en sciences de la santé. À part sa passion pour les sciences, il fait partie de l'équipe de basketball de son Cégep, et il adore voyager.

Alexandre Lemieux:

Alexandre Lemieux a toujours eu un vif intérêt pour le domaine des sciences, particulièrement le domaine de la santé humaine. Cela lui a poussé à s'impliquer dans un projet de recherche qui l'a enrichi comme un scientifique mais aussi dans un sens personnel par le biais des différentes expériences qu'il a eu à l'Expo-sciences et au Défi Biotalent Sanofi-Aventis. Maintenant il étudie en Sciences de la santé au CÉGEP de Sainte-Foy. Alexandre aussi aime jouer de la musique, comme le saxophone et la guitare, ainsi que de voyager.

La démence d'Alzheimer (DA) est une maladie neurodégénérative qui affecte environ 450 000 Canadiens et à ce jour, aucun traitement efficace n'a été développé. Il a été démontré que chez les patients atteints de la DA, la principale phosphatase de la protéine Tau, PP2A, est inactive. Il a été émis comme hypothèse que, si PP2A est activée, alors la phosphorylation de la protéine Tau pourrait être diminuée. Alors, un activateur de PP2A extrait du café, soit SIG1012, a été testé pour évaluer ses effets sur la phosphorylation de Tau. Pour appuyer l'hypothèse, les effets du composé ont été testés sur des cultures cellulaires de neuroblastomes humains (SH-SY5Y) et de neuroblastomes murins (NYA). Les protéines ont été isolées par Western Blot et les résultats ont démontrés que SIG1012 pouvait réduire la phosphorylation d'en moyenne de 39,23% lorsque comparé aux contrôles d'éthanol. SIG1012 a aussi été testé sur des cerveaux de souris C57BL/6J. La phosphorylation de Tau a été diminuée d'en moyenne 20%.

Alzheimer's disease (AD) is a neurodegenerative disorder that affects roughly 450 000 Canadians and yet no cure has been developed. It has been demonstrated that patients affected by AD have one of the principal phosphatases of Tau, PP2A, inactivated. It was hypothesized that if PP2A is activated, then, the hyperphosphorylation of Tau protein could be lowered. Therefore, a PP2A activator extracted from coffee, SIG1012, has been tested to see its effects on the phosphorylation of Tau. To support the hypothesis, the effects of this compound (SIG1012) were showed on cell cultures: human neuroblastomas (SH-SY5Y) and murin neuroblastomas (N2A). The proteins were isolated and analysed with Western Blots and the results showed that SIG1012 could lower the phosphorylation of Tau by an average of 39,23% when compared to ethanol controls. SIG1012 was also tested on C57BL/6J mice brains. Tau's phosphorylation was lowered by an average of 20% and it was confirmed throughout methylation activity that PP2A is more activated in the frontal cortex.



Introduction

La démence d'Alzheimer (DA) est une maladie neurodégénérative qui touche de plus en plus de personnes dans notre société, particulièrement la population vieillissante. Le nombre de patients atteints s'élève à environ 450 000 au Canada. Actuellement, il n'existe aucun traitement reconnu efficace pour soigner cette maladie.

Il existe de fortes corrélations entre les niveaux de phosphorylation de la protéine Tau et le développement de la DA (S. KOSIK et al, 1986). Pour les personnes en santé, Tau est une protéine neuronale dont le rôle est de solidifier les réseaux de microtubules qui forment les axones et les dendrites neuronaux, assurant ainsi la bonne propagation de l'influx nerveux. Cette interaction de Tau avec les microtubules est régulée par sa phosphorylation. Chez les patients atteints de DA, Tau est hyperphosphorylée, ce qui signifie qu'il y a trop de molécules de phosphate qui lui sont rattachées. Le résultat de l'hyperphosphorylation de Tau est son détachement des microtubules et éventuellement la formation d'enchevêtrements neurofibrillaires (NFTs) due à l'affaiblissement de la structure des axones. La présence de NFTs au niveau du tissu cérébral est un symptôme classique de DA. Cela dit, il est logique de vouloir explorer les mécanismes de régulation de la phosphorylation de Tau pour voir s'il existe une manière de contrer son hyperphosphorylation.

Les enzymes responsables d'enlever les phosphates des protéines se nomment phosphatases. Il a été démontré que la phosphatase principale de Tau est PP2A et que celle-ci est inactivée chez les patients atteints de la DA (KINS et al, 2001). Un composé extrait du café, nommé SIG1012, a récemment été développé par la compagnie pharmaceutique Signum Biosciences comme étant un potentiel activateur de PP2A.

But

Ce projet consiste en l'exploration des effets du SIG1012 sur la phosphorylation de la protéine Tau via la réactivation de la phosphatase PP2A pour en évaluer ses propriétés thérapeutiques.

Hypothèse

Il a été comme hypothèse qu'un traitement au SIG1012 permettrait de réduire la phosphorylation de la protéine Tau, puisque celui-ci est un activateur de la phosphatase PP2A.

Méthodologie

Milieu in Vitro

Traitement des Cultures et Isolation des Protéines

Des cellules neuroblastomes humains (SH-SY5Y) et neuroblastomes murins (N2A) ont été traités avec du SIG1012 et incubé à 37°C et à 30°C. À 30°C, il s'agit d'une condition hypothermique où la protéine Tau est hyperphosphorylée, reproduisant ainsi un modèle expérimental de la DA. Les contrôles ont été des traitements avec de l'éthanol 100% concentrés à 0,005µmol, aussi à 37°C et à 30°C. Après une incubation de 4 heures, les cellules ont été lavées et lysées avec du « RIPA buffer ». Cette solution hypotonique permet de faire lyser les cellules par principe d'osmose.

Préparation des Échantillons de Protéines

Les lysats de cellules ont alors été centrifugés et chauffés après y avoir ajouté du « Laemmli Buffer ». La centrifugation permettait d'isoler les protéines et l'ADN de la membrane et des déchets cellulaires, tandis que le « Laemmli Buffer », s'activant grâce à la chaleur (qui éliminait l'ADN), permettait de dénaturer et polariser les protéines.

Migration et Transfert des Protéines

Les échantillons de protéines ont ensuite été dosés et transférés dans un montage de migration de protéines qui a été programmé à 100V pendant 2 heures afin d'obtenir une migration optimale sur les gels. Ces gels, contenant des protéines ayant migré selon leur masse, ont été transférés sur des membranes de nitrocellulose à l'aide d'un montage de transfert de protéine qui a été programmé à 100V pendant 1 heure. Ces membranes ont été colorées au rouge ponceau afin d'être sur du transfert. Par la suite, les anticorps AT8 et PHF-1, dont le rôle est de cibler des sites de phosphorylation sur la protéine Tau, ont été ajoutés aux membranes et incubés. Les anticorps contrôles Tau-C et Tubuline ont aussi été ajoutés sur d'autres membranes afin de s'assurer de la quantité de protéines présentes dans les échantillons. L'anticorps secondaire porte l'enzyme HRP et il a été ajouté à chacune des membranes afin qu'il s'attache à l'un des anticorps primaires.

Détection de la Chemiluminescence

Enfin, les membranes ont été trempées dans une solution ECL (Enhanced Chemiluminescence) qui, avec une caméra dans une chambre noire, permet la libération du signal lumineux

de l'enzyme HRP. Ainsi, plus l'intensité lumineuse est forte, plus l'anticorps aura détecté de la phosphorylation.

Milieu in Vivo

Des souris C57BL/6J ont été nourries avec du SIG1012 réparti dans de la nourriture pour souris standard à une concentration de 120mg/kg (0,1% SIG1012). Pour simuler l'état d'hyperphosphorylation chez les souris, ces dernières ont été anesthésiées. Le cortex frontal de la souris a été homogénéisé et analysé par « Western », avec la même méthodologie que celle expliquée pour le milieu in vitro. Les anticorps AT8 et PHF1 ont été utilisés pour étudier le niveau de phosphorylation de la protéine Tau.

Analyses Statistiques

Tous les résultats obtenus ont été validés par le test de comparaisons-multiples de Newman-Keuls. Un t-test sur l'ensemble des données a aussi été effectué.

Résultats

Milieu in vitro

Les images de la lumière dégagée par les anticorps ont été sélectionnées et par la suite quantifiées à l'aide des logiciels Prisme et Image Gauge. Des pourcentages de phosphorylation ont ensuite été faits grâce au logiciel Microsoft Excel 2007. Ainsi, les résultats du pourcentage de phosphorylation dans les cellules neuroblastomes humaines, SH-SY5Y, et neuroblastomes murins, N2A, sont expliqués dans les Figure 1 et Figure 2. Malgré une incertitude relativement élevée, la baisse de phosphorylation la plus marquée a été dans les cellules SH-SY5Y où celle-ci a été de 82.57% ($p > 0,05$).

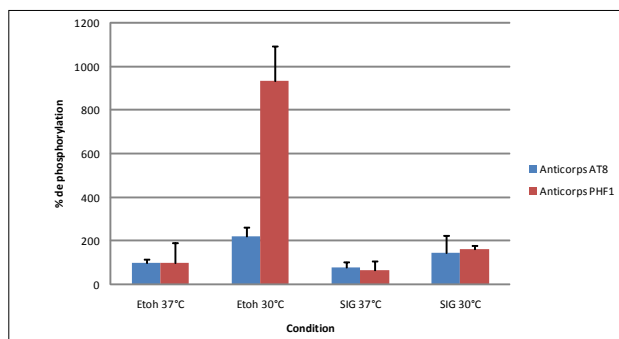


Figure 1. % de phosphorylation selon les conditions dans les cellules SH-SY5Y (n=3)

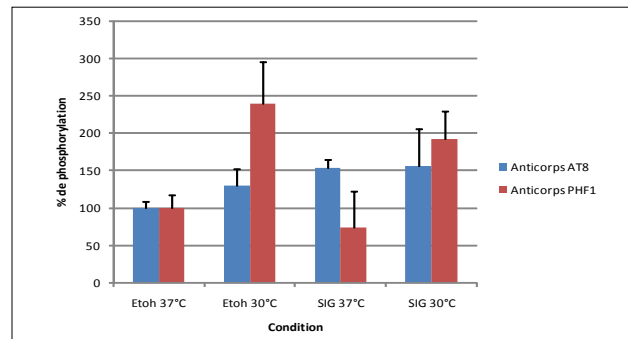


Figure 2. % de phosphorylation selon les conditions dans les cellules N2A (n=3)

Milieu IN VIVO

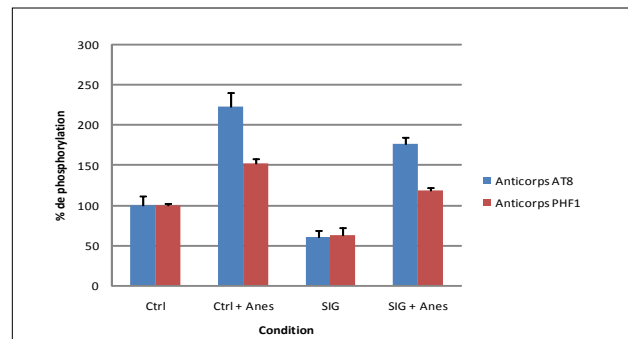


Figure 3. % de phosphorylation selon les conditions dans les cerveaux de souris C57BL/6J (n=2)

Discussion des Résultats

Les résultats démontrent tout d'abord une induction de la phosphorylation lorsque les cellules sont exposées à une température de 30°C, passant en moyenne de 100% à 383,75% (figure 1 & 2), confirmant ainsi ce qui a déjà été démontré dans le passé, soit que Tau est hyperphosphorylée en condition hypothermique. Il est possible de faire le lien avec les patients atteints du diabète de type II et leurs chances de développer la DA : les gens ayant cette maladie ont une température corporelle légèrement inférieure au 37°C physiologique normal, pouvant ainsi entraîner une hyperphosphorylation (ZETHELIUS et al, 2009).

Pour les traitements avec SIG1012, une diminution de la phosphorylation de Tau est présente dans la majorité des cas. Dans la figure 1, la baisse la plus significative a été avec l'anticorps PHF1 dans les cultures de SH-SY5Y, où la phosphorylation du contrôle à l'EtOH 30°C est passée de 934% à 163% lorsqu'il y a eu un traitement avec le SIG1012. Avec l'anticorps AT8, il y a une baisse de 221% à 144% de la phosphorylation de Tau lorsque celui-ci a été traité



au SIG1012. Chez les cellules N2A, dans la figure 2, SIG1012 démontre une baisse moins significative. L'anticorps PHF1 détecte toujours une baisse de la phosphorylation, passant de 240% à 193%.

Ces résultats démontrent une diminution moyenne de 39% ($p < 0,05$) de la phosphorylation, exposant ainsi la capacité du SIG1012 à prévenir la phosphorylation de la protéine, pouvant fort probablement retarder le développement de la maladie.

Dans les souris C57BL/6J, il y a une baisse de la phosphorylation entre les traitements avec SIG1012 et les contrôles. Contrairement aux cellules *in vitro*, dans le modèle *in vivo*, la baisse de phosphorylation est très semblable entre les deux anticorps. En moyenne, dans la figure 3, il s'agit d'une baisse de 20% ($p < 0,05$) du niveau de phosphorylation. Dans la figure 4, l'étude de la méthylation de la phosphatase PP2A démontre qu'avec un traitement au SIG1012, l'activité de la phosphatase PP2A est augmentée d'en moyenne de 32%. Ceci permet de partiellement caractériser le composé SIG1012 en tant qu'activateur de la phosphatase PP2A.

Conclusion

En conclusion, l'hypothèse initiale pourrait s'avérer vraie. Effectivement, les résultats démontrent qu'en utilisant le composé SIG1012, il est possible de diminuer la phosphorylation de la protéine Tau via la réactivation de la phosphatase PP2A. Bien que ce ne soit pas tous les résultats obtenus qui sont statistiquement significatifs, le résultat avec l'anticorps PHF1 dans les cellules SH-SY5Y (baisse de 83%) est très prometteur. Ainsi, il serait intéressant de continuer les recherches et tester d'autres concentrations de SIG1012, afin d'en trouver la concentration optimale, et l'appliquer dans d'autres tests *in vivo* et comportementaux, tel que le « Barnes's maze ». De plus, puisque SIG1012 est un extrait de café, il serait pertinent de découvrir la concentration de ce dernier dans le café et voir si le café en tant que tel peut être un agent préventif contre la DA.

Remerciements

Nous tenons à remercier le Dr. Emmanuel Panel, Françoise Morin et François Marcouiller du Centre de Recherche du Centre Hospitalier de l'Université Laval (CHUL), à Québec.

Mots-Clés

Maladie d'Alzheimer, protéine Tau, phosphorylation, SIG1012.

Références

- [1] JEFF HOFFMAN, SUSAN FROEMKE, « The Alzheimer's project : Momentum in Science », Public Affairs , 2009, 224 pages;
- [2] NIGEL. M. HOOPER, « Alzheimer's disease: methods and protocols, » Humana Press, 2000, 424 pages;
- [3] B.T KURIEN, R.H SCOFIELD, « Protein Blotting and Detection : Methods and Protocols », Humana Press, 2009, 581 pages.
- [4] K.S KOSIK, CL JOACHIM, DJ SLKOE, « Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease », <http://www.pnas.org/content/83/11/4044.abstract>, consulté en juillet 2009;
- [5] C. MOONEYHAN, « Anesthesia or Hypothermia : Warning for Alzheimer's patients », http://www.eurekalert.org/pub_releases/2009-03/foas-aoh031109.php, consulté en novembre 2009;
- [6] SIGNUM BIOSCIENCES, « PPM Pharmaceutical Development », http://www.signumbiosciences.com/ppm_pharma_dev.html, consulté en novembre 2009.
- [7] HUANG HC, JIANG ZF, « Accumulated amyloid-beta peptide and hyperphosphorylated tau protein : relationship and links in Alzheimer's disease. », <http://www.ncbi.nlm.nih.gov/pubmed/19158417>, consulté en octobre 2009;
- [8] E SONTAG, NUNBHAKDI-CRAIG V, JM SONTAG, R DIAZ-ARRASTIA, E OGRIS, S DAYAL, SR LENTZ, E ARNING, T BOTTIGLIERI, « Protein phosphatase 2A methyltransferase links homocysteine metabolism with tau and amyloid precursor protein regulation. », http://www.ncbi.nlm.nih.gov/pubmed/17360897?ordinalpos=4&itool=EntrezSystem2.PEntrez.Pubmed.Pubmed_ResultsPanel.Pubmed_DefaultReportPanel.Pubmed_RVDocSum, consulté en octobre 2009;
- [9] S KINS, A CRAMERI, D R.H. EVANS, B A. HEMMINGS, R M. NITSCH, J. GOTZ, « Reduced Protein Phosphatase 2A Activity Induces Hyperphosphorylation and Altered Compartmentalization of Tau in Transgenic Mice », <http://www.jbc.org/content/276/41/38193.abstract>, consulté en octobre 2009;
- [10] THE LOCAL, « Drinking coffee reduces Alzheimer's risk : study », <http://www.thelocal.se/16944/20090115/>, consulté en Août 2009;
- [11] SOCIÉTÉ ALZHEIMER, « Fait saillants concernant la prevalence de la maladie d'Alzheimer et les affections connexes », <http://www.alzheimer.ca/french/disease/stats-intro.htm>, consulté en Août 2009;
- [12] M GEODERT, R JAKES, VANMECHELEN E, Monoclonal antibody AT8 recognizes tau protein phosphorylated at both serine 202 and threonine 205, <http://www.ncbi.nlm.nih.gov/pubmed/7624036>, consulté en février 2010;
- [13] L OTVOS JR., L FEINER, LANG E, SZENDREI GI, GOEDERT M, LEE VM, Monoclonal antibody PHF- 1 recognizes tau protein phosphorylated at serine residues 396 and 404, <http://www.ncbi.nlm.nih.gov/pubmed/7534834>, consulté en février 2010;
- [14] ZETHELIUS B. et al. (2009), Glucose metabolism and the risk of Alzheimer's disease and dementia: a population-based 12 year follow-up study in 71-year-old men, <http://www.springerlink.com/content/g101421303752xj4/>, Uppsala, Springer-Verlag.



Commentaire sur **Activation de la Phosphatase PP2A: Nouveau Traitement pour la Maladie D'alzheimer**

Ces deux jeunes scientifiques ont choisi comme thématique de leur projet de recherche un sujet éminemment d'actualité en recherche médecine, soit le développement d'une thérapie de la maladie d'Alzheimer. Le sujet est vaste, complexe et frustrant au regard des résultats peu encourageants obtenus par les laboratoires de recherche et les entreprises pharmaceutiques qui y travaillent. La solution n'est pas pour demain.

Les auteurs se sont intéressés à une voie particulière, celle de la réduction de la phosphorylation d'une protéine par un dérivé extrait du café. L'hyperphosphorylation dénature les fonctions d'une protéine du tissu neuronal, dénommée Tau, qui participe au maintien d'une architecture optimale des prolongements axonaux et dendritiques des neurones. Dans cette étude expérimentale, sont identifiées, une protéine altérée avec la maladie soit la protéine Tau, une cible soit l'enzyme phosphatase A2 (PPA2) et enfin l'agent inhibiteur, un dérivé extrait de café soit le SIG1012.

Les méthodologies utilisées et le design expérimental sont bien présentés et l'agencement des protocoles utilisés facilite la compréhension des nombreuses étapes inhérentes à ce type de recherche en laboratoire. Il y a, à l'évidence, une très bonne compréhension de toutes ces étapes par nos deux jeunes scientifiques.

Les résultats sont sobrement présentés et faciles d'interprétation. Par contre, je n'ai pas retrouvé la figure 4 à laquelle font référence les auteurs. Les résultats obtenus suggèrent que dans certaines conditions le composé chimique, le SIG1012, modifie l'activité de la phosphatase (PPA2), confirmant l'hypothèse de départ. Par ailleurs, ce ne sont pas tous les résultats obtenus qui sont concluants tant au niveau de l'anticorps utilisé que du modèle utilisé (modèle in vitro vs modèle in vivo).

L'hypothèse est-elle vérifiée ? Oui et non auraient pu écrire les auteurs. Oui pour un type de cellules (résultats statistiquement significatifs) et pour certaines conditions expérimentales. Plus difficile à démontrer dans un autre modèle. Avec réserve, les auteurs suggèrent que cette voie, l'activation d'une phosphatase (PPA2) par un dérivé extrait du café, mérite qu'on s'y intéresse et que d'autres expériences soient menées.

Louis Dumont, Ph.D., Professeur de Pharmacologie, Directeur, projet SEUR, Université de Montréal





Novel Synthesis: Imidacloprid CYP450 Pesticide Synergist from Dill Lowers Surface Runoff Toxicity

Emma Graham

Grade 11, Lisgar Collegiate Institute, University of Ottawa

In the first year of her secondary school education, Emma Graham came across an article in a local newspaper about a pharmaceutical company's advances in chiral chromatography techniques. It prompted her to realize that scientific discovery is not only a thing of the past, when the famous scientists discovered and made their major contributions to science, but that they happen here and now. With this in mind and the encouragement of her mother, Emma decided that she wanted to do research projects of her own. Since that first moment, Emma has gone on to do work including her contribution to this journal. When she began her work she decided that she wanted to focus on environmental chemistry because of its relevance to the world and the people of today: the development of less harmful chemicals used in farms and factories during food production being one of its important applications. Having a natural curiosity for the unknown, with that question of "what if" always in the back of her mind, and knowing the sense of accomplishment that can be achieved by contributing to the scientific community, Emma will no doubt continue her work in scientific research.

Pesticide resistance involves insects developing resistance to pesticides after prolonged use, causing the farmer to use larger and larger doses to get the same effect. Therefore pesticide resistance can lead to severe environmental contamination. Insects use CYP450 to metabolize 75% of the toxins that enter their body, so if this enzyme is inhibited, the insect will not be able to metabolize the toxin. Recently, a component of the oil of Indian Dill called dillapiol has been found to be a potent CYP450 inhibitor, making it a potential pesticide synergist. The goal of this project was to find out if the structure of the natural product dillapiol could be optimized so that it is more effective at inhibiting CYP450 in the Colorado Potato Beetle (CPB) than a currently used toxic pesticide synergist called piperonyl butoxide. In part one of experimentation, a dillapiol dimer was synthesized. In part two, the dillapiol dimer was tested in vitro for its efficacy at inhibiting CYP450. In part three, Indian dill oil, dillapiol, the dillapiol dimer and piperonyl butoxide were tested for efficacy as pesticides and synergists on the CPB. In vitro, the dillapiol dimer was 95% effective at inhibiting CYP450. In vivo, the dillapiol dimer was the most effective as a synergist and the least effective as a pesticide, suggesting that its structure is effective at inhibiting CYP450 yet has limited stand-alone toxicity to the CPB.

Résistance aux pesticides consiste à développer une résistance des insectes aux pesticides après une utilisation prolongée, ce qui provoque l'agriculteur d'utiliser des doses plus en plus grandes pour obtenir le même effet. C'est pourquoi la résistance aux pesticides peut conduire à une contamination importante de l'environnement. Insectes utilisation CYP450 à métaboliser 75% des toxines qui entrent dans leur corps, si cette enzyme est inhibée, l'insecte ne sera pas capable de métaboliser la toxine. Récemment, un composant de l'huile d'Indiens à l'aneth dillapiol appelé n'a été trouvé pour être un



inhibiteur du CYP450, ce qui en fait un synergiste potentiels des pesticides. L'objectif de ce projet était de savoir si la structure de la Dillapiol produit naturel pourrait être optimisé de sorte qu'il est plus efficace pour inhiber CYP450 dans le doryphore de la pomme de terre (CPB) d'un synergiste des pesticides actuellement utilisés toxique appelé pipéronylbutoxyde. Dans la partie 1 de l'expérimentation, un dimère dillapiol a été synthétisé. Dans la deuxième partie, le dimère dillapiol a été testé in vitro pour ses efforts à l'efficacité CYP450 inhibition. Dans la troisième partie, l'essence d'aneth Indien, dillapiol, le dimère dillapiol et pipéronylbutoxyde ont été testés pour l'efficacité que les pesticides et les synergistes sur le CPB. In vitro, le dimère dillapiol a été efficace à 95% à l'inhibition du cytochrome P450. In vivo, le dimère dillapiol était le plus efficace en tant que synergiste et le moins efficace qu'un pesticide, ce qui suggère que sa structure est efficace pour inhiber CYP450 a encore limitée autonome toxicité pour le CPB.

Background

As the population of the world grows exponentially, farmers around the world have been given the difficult task of supporting this rapid population growth. Due to the limited amount of pest controlling options available to these farmers, they are being forced to use the only viable method of controlling pest populations in their crops: pesticides. However, this excessive pesticide use has caused two major problems that face our agricultural industry today: firstly, environmental contamination by pesticides and secondly, pesticide resistance.

Pesticide resistance involves insects developing resistance to pesticides after prolonged use, causing the farmer to use larger and larger doses to get the same effect^[1]. This resistance develops faster with synthetic pesticides because they are pure compounds and easier for the insect to develop resistance to. Pesticide resistance plays a large role in the degree of pesticide contamination that occurs in a given area. The more resistant the target pest is, the more pesticide necessary to control it. Therefore pesticide resistance can lead to severe environmental contamination. An example of severe environmental contamination occurs when soil reaches its saturation point and pesticide begins to flow over the land and into either bodies of water or crevices in the earth known as surface runoff.

Prime examples of an insect that can develop resistance to pesticides extremely quickly are Colorado Potato Beetles. The CPB is the most important defoliator of potatoes. Its distribution covers 8 million km² in North America, and 6 million km² in Europe and Asia^[2]. Since 1864, hundreds of compounds have been used to control the CPB. Insecticides remain the foundation for its control^[3]. The CPB has a legendary ability to develop resistance to almost any pesticide it

comes in contact with. Therefore, a pesticide formulation is needed that can effectively control the CPB.

A pesticide formulation that is effective at controlling pests and not harmful to the environment is needed to prolong the use of pesticides worldwide. This pesticide formulation should limit the resistance developed in the insect, therefore prolonging its viability as an effective pesticide and controlling the amount of toxic material applied to crops. Insects use CYP450 to metabolize 75% of the toxins that enter their body, so it is this enzyme that the pesticide synergist needs to inhibit. In this way, the pesticide synergist can prevent the insect from metabolizing the toxin, therefore ensuring that the toxin stays in the insect's system longer.

Recently, a component of the oil of Indian Dill called dillapiol has been found to be a potent CYP450 3A4 inhibitor, making it a potential pesticide synergist^[4]. Indian Dill Oil comes from a plant called Indian Dill, also known as anethum sowa. It is composed of 65% d-carvone, 25% dillapiol, and 10% other compounds.

Engineering Questions and Goals

Can the structure of the natural product dillapiol (compound in Indian Dill – anethum sowa) be optimized so that it is more effective at inhibiting the CYP450 enzyme in the Colorado Potato Beetle than a currently used toxic pesticide synergist called pipéronyl butoxide? What is the efficacy of Indian Dill oil and dillapiol at inhibiting the CYP450 enzyme?

Procedure Parts 1, 2 and 3

1. Creation of dillapiol dimer
2. In vitro bioassays for enzyme activity
3. In vivo bioassays for pesticide and synergist activity



Part 1

The variable being manipulated in these experiments is the R group attached to the alkene bond on the dillapiol structure. Three potential R groups were synthesized, however only one is presented here. Part 2 was attempted only with the dimer created by making the dillapiol compound itself the R group.

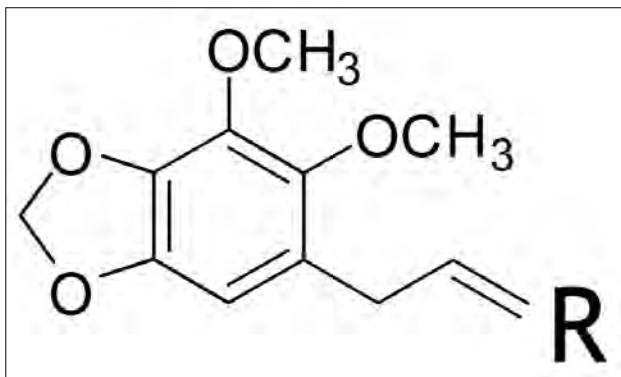


Figure 1. Dillapiol

Part 2

The cytochrome P450 inhibition properties of the compound was measured by bioassay and IC50 value.

Part 3

The effect Indian dill oil, dillapiol, the dillapiol dimer and piperonyl butoxide have on 2nd instar Colorado Potato Beetles as synergists and as pesticides was recorded.

Procedures Part 1

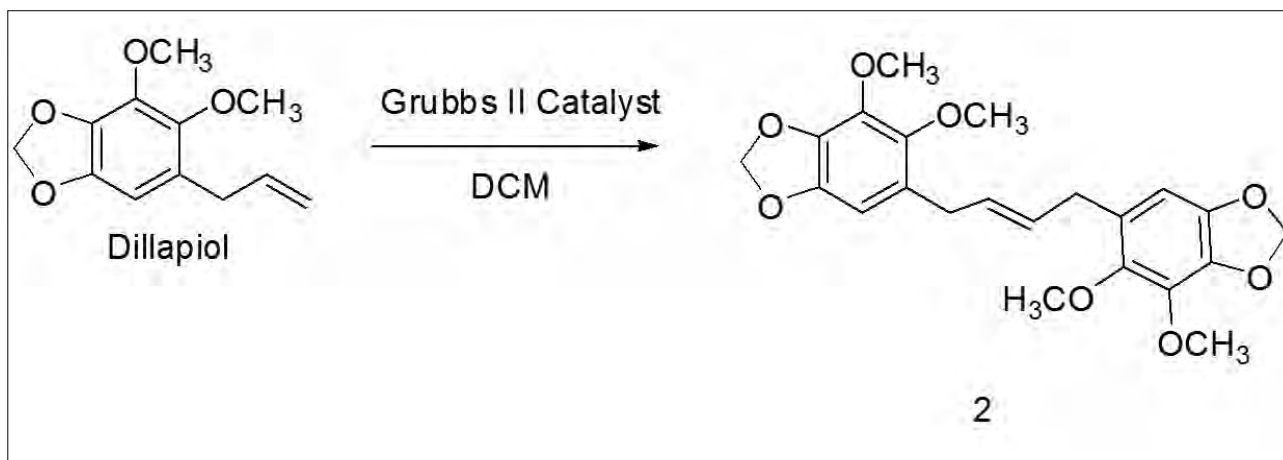


Figure 2. Dimerization of dillapiol

Method

1. Dillapiol was taken in a two-necked 10 mL round bottomed flask.
2. The Grubbs II Catalyst was added, followed by the DCM (dichloromethane).
3. A condenser was attached to the flask and fitted with a septum.
4. The system was flushed with Nitrogen, then heated to 50 deg. Celsius for 3 hours, and then stirred at room temp.

Part 2: Bioassay on Efficiency of Compound on Inhibiting Enzyme

Materials

NADPH, dibenzylfluorescein (DBF–20 μm), potassium phosphate buffer 0.5 M (pH 7.4)–KH₂PO₄-CYP3A4 + b5 (denatured and active), -ketoconazole (1.88 μM)

Method

There are 3 solutions that need to be made for the assay:

- 1) Solution A – contains NADPH and DBF
 - 2) Solution B – contains active CYP3A4
 - 3) Solution C – contains denatured CYP3A4
1. Prepare solutions A, B, and C with only water and buffer.
 2. Allocate 10 uL of extracts of interest into the wells.
 3. Turn off all the lights and add NADPH and DBF into solution A.
 4. Allocate 100 uL of solution A into all wells.
 5. Add denatured CYP3A4 to solution C and allocate 90 uL into the appropriate wells.
 6. Add active CYP3A4 to solution B and allocate 90 uL into the appropriate wells.
 7. Place the plate into the plate reader and start the automated procedures.
 8. Record the data and save the file.

Part 3: Bioassay on Efficiency of Pesticides and Synergists on the Colorado Potato Beetle

Imidacloprid is a pesticide that is currently used to control the Colorado Potato Beetle. In these assays, it was used in conjunction with dill oil, dillapiol and piperonyl butoxide to test these compounds' efficacy as pesticide synergists.

Imidacloprid, dill oil, dillapiol and piperonyl butoxide bioassays were conducted at Agriculture Canada under the supervision of Dr. Ian Scott.

Method

Potato plants were treated with Imidacloprid and Cobs were treated with, respectively: dill oil, dillapiol, the dillapiol dimer and piperonyl butoxide.

Results for Part 2

Compound	Results
Dillapiol dimer	95% Inhibition of CYP 450 3A4 enzyme compared to Ketoconazole.

Figure 3. Results table of in vitro bioassays



Figure 4. Model of how substrate (NADPH) allows the enzyme to function

	Synergism with Imidacloprid VS Imidacloprid at IC ₅₀	Individual Pesticide Activity (% dead)	Mammalian Toxicity	Comments
Indian Dill Oil	97% VS 50%	10%	None Common food additive	Promising pesticide synergist with high synergist qualities, low individual pesticide activity and no mammalian toxicity.
Dillapiol	85% VS 50%	10%	None Compound in Indian Dill	Possible pesticide synergist with medium synergist qualities, medium pesticide activity and low mammalian toxicity.
Dillapiol Dimer	100% VS 50%	0%	Untested, (novel synthesis) Derived from dillapiol, none suspected	Promising pesticide synergist with very high synergist qualities, no individual pesticide activity and no suspected mammalian toxicity makes further testing the next step.
Piperonyl Butoxide	90% VS 50%	15%	Potential carcinogen and endocrine disruptor ^[5]	Hazardous pesticide synergist with high synergist qualities, high individual pesticide activity and high mammalian toxicity.

Figure 5. Results, Observations and Comments Table for Part 3



Conclusions

While all the compounds tested in this project have synergistic properties, it was found that the most potent synergist was the dillapiol dimer. From this it can be concluded that large molecules with a dioxyphenyl group, methoxy groups and a closed double bond inhibit CYP450 3A4 with the most efficacy and have the least stand-alone toxicity. This can be used in future research to find an even more effective pesticide synergist.

The dillapiol dimer can be used as a replacement for piperonyl butoxide as it has more synergistic activity and less health concerns, as it is believed that if it has no pesticide activity, then it likely will be found to have no mammalian toxicity.

This synergist can be used to help overcome resistance in the CPB and allow for imidacloprid to be used as an effective synergist for an extended period of time.

Finally, because by using the dillapiol dimer as a pesticide synergist the rate the insects develop resistance to imidacloprid will decrease, farmers will be able to use the same amount of pesticide for a longer time. This will lessen the environmental impact of the pesticides they use.

Acknowledgements

My deepest thanks to Dr. Tony Durst, Professor Emeritus at the University of Ottawa Chemistry

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Key Words

CYP450, Dill Oil, Dillapiol, Pesticide, Pesticide synergist.

References

- [1] Zhou, S., et al., Drugs Behave as Substrates, Inhibitors, and Inducers of Human Cytochrome P450 3A4, *Current Drug Metabolism*. 2008, 9, 310-322.
- [2] Gauthier, N.L., R.N. Hofmaster, and M. Semel. 1981. History of Colorado potato beetle control. In: JH Lashomb and R Casagrande (eds). *Advances in Potato Pest Management*. Hutchinson Ross Publishing Co., Stroudsburg, PA, pp. 13-33
- [3] Alyokhin, Dr. Andrei. www.potatobeetle.org. University of Maine. Web. 1 Apr. 2010.
- [4] Majerus, S. et al., A Novel Synthesis of Dillapiol and its Derivatives and Their Role as Chemosensitizers and Insecticide Synergists, University of Ottawa Masters Program. 1997.
- [5] Hill, S. E., "Piperonyl Butoxide - Identification, Toxicity, Use, Water Pollution Potential, Ecological Toxicity and Regulatory



Review of ***Novel Synthesis: Imidacloprid CYP450 Pesticide Synergist from Dill Lowers Surface Runoff Toxicity***

This project set out to investigate if the structure of the natural product Dillapiol could be optimized to be more effective at inhibiting CYP450 in the Colorado Potato Beetle than currently used pesticides, and as such, can reduce pesticide resistance in insects.

The paper is well-written with the background behind the projection explained well. I really like the hypothesis driven approach used, and the way that the experiments have been designed to address the hypothesis. Pesticide resistance and pesticide contamination are important issues for the environment and humankind. Additionally trying to use natural products to control pests is a lucrative area of research and has been proven to be very effective at controlling agricultural pests.

The project was broken down into three parts.

Part 1

Creation of a dillapiol dimer. It has been previously shown that Dillapiol is effective at inhibiting the CYP450 enzyme, so can the compound be optimized to be more effective at being a synergist. In this experiment the researcher is trying to create a dillapiol dimer. The protocol to create the dimer is well explained, but this reviewer is unsure why the researchers took the approach to create a dimer. There was no reasoning as to why creation of a dimer over other dillapiol modifications was chosen. Had the rationale for making the dimer been explained, the research paper would be stronger.

Part 2

Assaying the efficacy of the dillapiol dimer. The results support the hypothesis that the dillapiol dimer would inhibit the CYP450 enzyme. Activity is measured over ketoconazole, but the reviewer is not sure why this compound was used as a control. Perhaps the researcher could outline why this was used as the control compound.

Part 3

Assaying the synergistic effects of dillapiol compounds on pesticide activity. Imidacloprid is a pesticide used to control the Colorado Potato Beetle and thus the synergistic effects of dillapiol and other compounds were determined. Although Figure 5 is really a Table, the results clearly illustrate that the dillapiol dimer is most effective at being synergistic to the pesticide. Statistics would make these results stronger. It will be interesting to see what the results of mammalian toxicity proves to be, as this would be a great synergist to use with the current pesticide.

Overall this was a great research project, well written and the hypothesis supported!

Dennis McCormac, Ph.D., Director, Platform Development, Ontario Genomics Institute and The Centre for Applied Genomics





Racing to Find a Marker Development of Molecular Markers for Races of *Colletotrichum truncatum*

Rui Song

Walter Murray Collegiate Institute

Rui Song has always been curious about the world around her and had participated in several school science fairs, but before the Sanofi-Aventis BioTalent Challenge, she really had no idea about what went on in the world of research. She was first introduced to the SABC in elementary school by her science teacher, Mr. Steven Vincent, and thought that it would be an exciting opportunity to work on a project with a tangible impact. Since that first introduction, Rui has participated in the Challenge for the past three years. However, her research success has not been perfect. Her first project did not have the results that she wanted, but instead of turning away from the sciences, her interest and enthusiasm for research only increased. After analyzing what might have gone wrong, she applied this knowledge into her next two projects which also focused on the same research area. Through her experience, she has seen that science and research have tremendous potential to solve some of the world's most urgent problems. One can take knowledge, facts or data and apply this through research. The nature of science is that one takes a delve into the unexplored, and this means that even if you

My research question was: What Expressed Sequence Tag (EST) based marker(s) will differentiate between the closely related Race 0 and Race 1 of *Colletotrichum truncatum* (CT), a fungal pathogen causing anthracnose on lentil? An EST-based marker that generates polymorphisms between the races is important for mapping the virulence gene of CT, and allows for monitoring of race frequencies and evolution in the fungal population, which in turn supports the strategic use of resistance genes in lentil varieties. As the races are differentiated by aggressivity and the EST library developed while the fungus was infecting lentil, I hypothesized that there would be polymorphic EST-based marker(s). Isolates of *Colletotrichum* from different taxonomic levels were tested: CT lentil isolates CT-21 (Race 1), CT-30 (Race 0) and GT-72 (sexual stage or teleomorph of CT), soybean isolate CT-55 and a *Colletotrichum graminicola* isolate. From the 2000 markers designed using the Intron Marker Pipeline (IMP), 50 EST-based markers were screened for polymorphisms using Single Strand Conformational Polymorphism (SSCP) gels and Sanger sequencing. The SSCP gels showed no polymorphisms between the races for these markers. While the samples may not be clonal, they do possess a very high level of genetic similarity. However, the intron-flanking EST markers had varied resolutions, enabling differentiation within and between *Colletotrichum* species. SSCP gels were validated as an accurate method of sequence differentiation by Sanger sequencing. The EST-based markers tested did not show polymorphisms between the races, but are valuable tools for molecular differentiation, identification and classification within the *Colletotrichum* genus.

Ma question de recherche était la suivante: Quelle séquences génomiques exprimées (SGE) marqueur à base (s) permettra de différencier entre les espèces étroitement apparentées Souche 0 et 1 de *Colletotrichum truncatum* (CT), un



start with one question or problem in mind, at the end of your scientific journey, you could end up with even more questions. For every question that we do answer, there will always be another challenge, unique problem or dilemma, ensuring that there will be no shortage of innovation and experimentation. Society grows, adapts and evolves, and our problems, knowledge and questions evolve with us. As the capacity of our knowledge grows, so does our drive to continue learning and exploring our universe. Humankind's relationship with food and its cultivation is a keystone of our society, a necessity for our survival. However, despite hundreds of years, we still haven't completely mastered the art of effective production and distribution of crops, as evidenced by the 925 million people worldwide who do not have enough food to eat. Agricultural development of crop efficiency addresses one of the most basic human needs, allowing us to feed the world, enabling all 6 billion of us to lead healthy lifestyles. Another aspect of her project, DNA molecular biology is particularly fascinating to Rui because it allows one to take an organism and boil all its functions and mechanisms down to a sequence of 4 nucleotides. It's amazing because this sequence acts as an instruction manual for building even extremely complex organisms.

Background

Colletotrichum truncatum (CT) is a fungal pathogen, which causes anthracnose on lentil. Lentil is an extremely important Canadian pulse crop; it has the second largest acreage after pea. Canada is among the world's largest producers and exporters of lentil and Saskatchewan produces 98% of this. Anthracnose, caused by CT, can decrease yield by more than 50% as well as causing seed staining, decreasing the quality and value of the seed by 5 to 10%. Anthracnose also increases production costs through fungicide usage. A former study of CT by Buchwaldt et al^[1],

champignon pathogène causant l'anthracnose de la lentille? Un marqueur SGE-qui génère des polymorphismes entre les souches est important pour la cartographie du gène de virulence de CT, et permet le suivi des fréquences des souches et l'évolution de la population de champignons, qui soutient à son tour l'utilisation stratégique des gènes de résistance dans des variétés de lentilles. Comme les souches sont différenciées par l'agressivité et la bibliothèque SGE développés tandis que le champignon infectait les lentilles, je l'hypothèse qu'il y aurait marqueur(s) polymorphe(s) EST-basé. Les isolats de *Colletotrichum* de différents niveaux taxonomiques ont été testés: lentilles isolats de CT : CT-21 (souche 1), CT-30 (Souche 0) et GT-72 (stade sexué ou téléomorphe de CT), isolat de soja CT-55 et un isolat de *Colletotrichum* gramini-cola. Des 2000 marqueurs conçus en utilisant l'Intron Marker Pipeline (IMP), 50 marqueurs SGE-basé ont été examinés pour le polymorphisme simple brin à l'aide de conformation Polymorphisme (SSCP) et gels de séquençage Sanger. Les gels SSCP n'a pas montré des polymorphismes entre les souches de ces marqueurs. Bien que les échantillons ne puissent pas être clonale, ils possèdent un très haut niveau de similarité génétique. Cependant, les marqueurs de l'intron d'accompagnement SGE avait résolutions variées, permettant la différenciation à l'intérieur et entre les espèces *Colletotrichum*. Gels SSCP ont été validés comme une méthode précise de la différenciation séquence par séquençage Sanger. Les marqueurs SGE-basé testés n'ont pas montré des polymorphismes entre les souches, mais sont des outils précieux pour la différenciation, l'identification moléculaire et la classification du genre *Colletotrichum*.

identified two races: Race 0 and Race 1. Race 0 is highly aggressive towards all lentil varieties, even those with some resistance. Race 1 is aggressive towards lentil varieties with little or no resistance but has little effect on varieties with partial resistance. An expressed sequence tag (EST) library has been developed from Race 1 while the fungus was infecting lentil leaves. EST markers have been generated from this library.

Purpose

The purpose of this project is to find polymorphic bands between the two races using SSCP gels. The



question is: "What EST marker will differentiate between the closely related Race 0 and Race 1?" A molecular method to identify races of CT is much more effective and reliable than pathogenicity tests. Such a marker would allow researchers to conduct a survey of lentil fields in Saskatchewan and inform producers about what races are present in the area. This is particularly important if Race 0 is dominant in the area as currently no lentil variety is resistant against Race 0. This would mean producers would have to be more vigilant about monitoring anthracnose in the field. Since the two races have been crossed in the laboratory, we will also be able to see how race identity is inherited by the offspring. In the future, the molecular marker will allow researchers to locate genes important for race identity on a genetic map that will be developed for CT. It will also be an effective way to monitor changes in virulence in the population of this fungus. Development of a molecular marker will also enable us to understand the co-evolution between the fungus and resistant lentil cultivars, which will be important for the development of durable resistance.

Hypothesis

I hypothesize that there will be an EST-based marker that will differentiate between Race 0 and Race 1 because the markers are closely linked to pathogenicity genes. Race 0 and Race 1 are differentiated by aggressivity, so there should be an EST-based marker that will differentiate between the races because the EST library is based on RNA isolated during the infection process.

Procedure

Five isolates of *Colletotrichum* from different taxonomic levels were tested for polymorphisms: Lentil isolates CT-21 (Race 1), CT-30 (Race 0), and GT-72 (CT teleomorph); soybean isolate CT-55, and an isolate of *Colletotrichum graminicola* (CG) (Figure 1). To extract mycelium for testing, several susceptible lentil plants were inoculated with a spore suspension and covered with a plastic sheet to increase humidity and provide an ideal growing environment for the fungus (Figure 2). The fungus was then isolated from the plants and grown on Oatmeal Agar media for 2 weeks. The mycelium was cultured in liquid GYM media and incubated on an orbit shaker for 6 days. This mycelium was then frozen and ground. Deoxyribonucleic acid (DNA) was isolated from this mycelium by adding 500 μ l of extraction buffer, and shaking the mycelium with glass beads for 7 min. The mixture

was incubated for 30 min at 37°C in a water bath. An aliquot of 500 μ l of phenol-chloroform was added, and the solution was centrifuged at 13000 RPM. The DNA concentration was quantified with a Nano Drop spectrophotometer and each DNA sample was diluted with sterile water to a concentration of 25 ng/ μ l. Using the protocol of the Intron Marker Pipeline (IMP), 2000 intron-flanking EST-based primers were selected. Of these 2000, 50 primers were selected for the PCR protocol. The PCR mastermix was prepared and a touchdown PCR protocol, developed by Don et al^[2], was used to amplify the CT genomic DNA with EST-based primers. To visualize the PCR products, both an SSCP and agarose gel were utilized. The agarose gel was 3% agar, set for 30 min. The ratio between loading dye and PCR product was 15 μ l to 3 μ l, or 5:1. The gel was run for 45-60 min in TBE buffer at 120 volts. The gel was visualized with ethidium bromide and UV light. The SSCP gel, from Orita et al^[3], was a Mutation Detection Enhancement (MDE) gel, set for 1-2 hours. The ratio of loading dye to PCR product was 16 μ l to 4 μ l, or 4:1. The DNA was denatured to allow strand separation at 95°C for 5 minutes. The SSCP gel was loaded and run at 6 watts for 15-16 hours. After allowing the samples to run, the DNA strands were fixed to the gel with Glacial Acetic Acid, stained with Silver Nitrate and developed with Sodium Carbonate. The process was stopped with glacial acetic acid, the gels were air dried and the bands were scored for polymorphism (Figure 3). To ensure accuracy of gel results, the PCR products of samples of interest were purified and sequenced using the Sanger sequencing method of the National Research Council, Plant Biotechnology Institute (NRC- PBI). Sequencher software was used to align the sequences and check for Single Nucleotide Polymorphisms (SNPs).

Results

For the 50 EST-based markers tested, the extremely sensitive SSCP gels revealed no differentiations between Race 0 and Race 1. These gels were validated by Sanger sequencing of CT-21 and CT-30 samples. These sequences confirmed that there was not even a single nucleotide difference among the races in these amplified regions. While the samples may not be clonal, this means there is a very high level of genetic similarity among the isolates (Figure 4).

Three other samples of *Colletotrichum* were also tested, CT-55, GT-72 and CG. The sensitivity of the EST markers enabled several of them to be differentiated based on species or subspecies. This is useful as a



method of identification and classification, being more time efficient than traditional morphological and pathogenicity studies. There were also several markers that distinguished CT-55 from the CT lentil isolates (CT-21, CT-30 and GT-72). This confirmed that CT-55 is indeed distinct from the lentil isolates and may represent a separate species. The main difference of CT-55 is that it causes anthracnose on soybean, as opposed to lentil. Several markers also generated different banding patterns between CG and CT (Figure 6).

Conclusions

As demonstrated by the DNA sequences and the SSCP gels, no polymorphic bands were generated by the 50 EST-based markers. This certainly proves that there is an extremely high level of genetic similarity between the races. SSCP gels are an accurate method of screening EST-based primers for polymorphic bands, as validated by the sequence analysis. They provide much more accuracy than an agarose gel by distinguishing polymorphisms based on the shape of the single strand (Figure 5). The EST-based markers have a range of resolutions, or levels of differentiation. Some can differentiate between species of *Colletotrichum*; others can distinguish between subspecies of CT. This makes them valuable tools in molecular differentiation in the *Colletotrichum* genus.

Acknowledgements

My mentor Dr. Sabine Banniza and her post doctoral fellow, Adrian Cabral, are greatly thanked for their help and guidance. Thank you to Lacey-Anne Sanderson for developing the IMP and Anthea Cabral for helping with the photos. Thank you to the University of Saskatchewan, Crop Development Centre, Genome Prairie and the Sanofi-Aventis BioTalent Challenge for giving me an opportunity to learn about biotechnology. I would also like to thank my parents for their support.

Keywords

Lentil Pathogen, Differentiation, Expressed Sequence Tag, Aggressivity

References

- [1] Buchwaldt L, Anderson KL, Morrall RA, Gossen BD, Bernier CC. Identification of Lentil Germ Plasm Resistant to *Colletotrichum truncatum* and Characterization of Two Pathogen Races. *Phytopathology*. 2004 Mar;94(3):236–43.
- [2] Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS. 'Touch-down' PCR to circumvent spurious priming during gene amplification. *Nucleic Acid Research*. 1991 Jul;25;19(14):4008.
- [3] Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics*. 1989 Nov;5(4):874–9.



Appendices

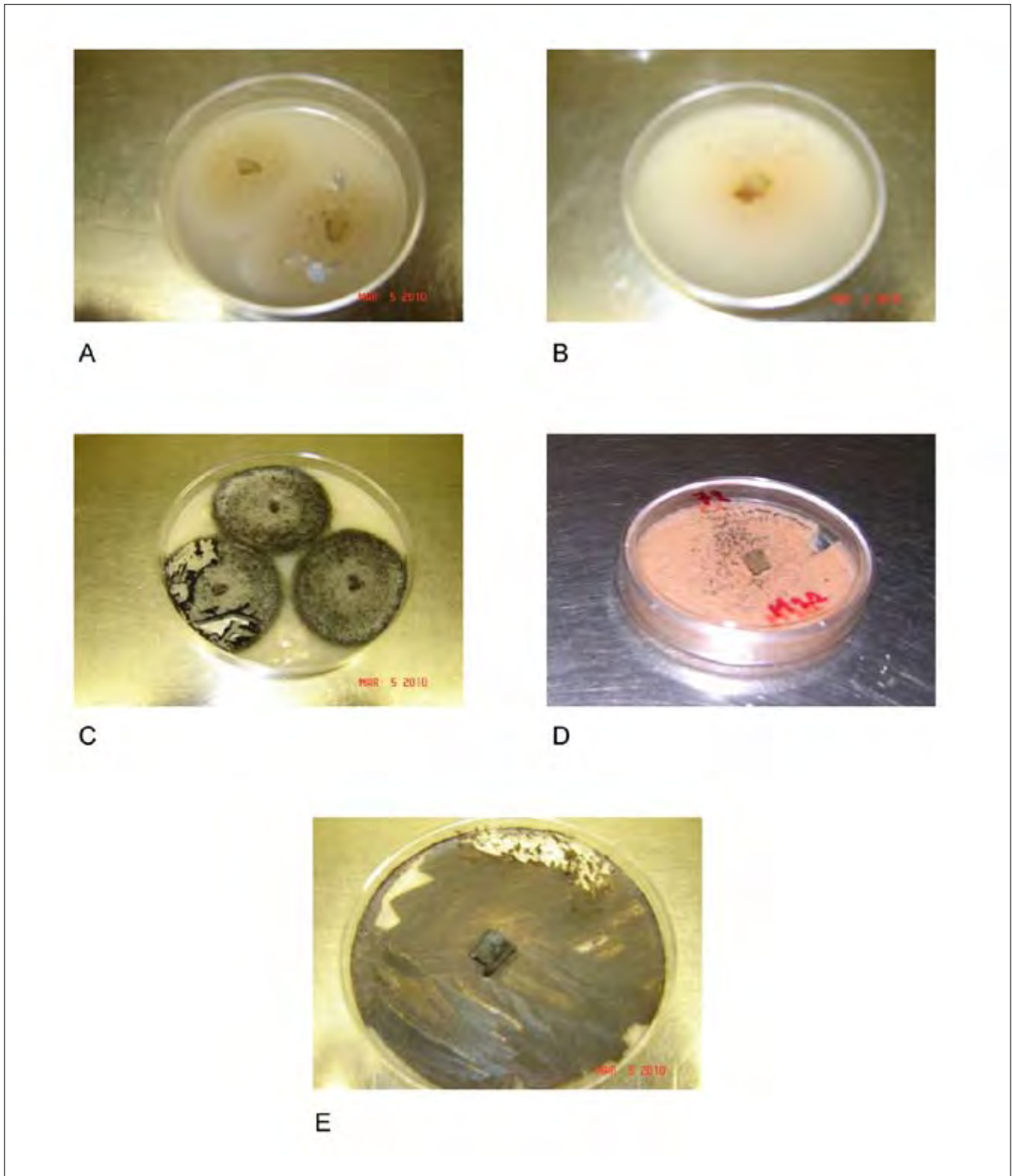


Figure 1. *Colletotrichum* isolates on Oatmeal Agar Media. A. CT-21 (Race 1). B. CT-30 (Race 0). C. *Colletotrichum graminicola*. D. GT-72 (CT Teleomorph). E. CT-55 (Soybean)

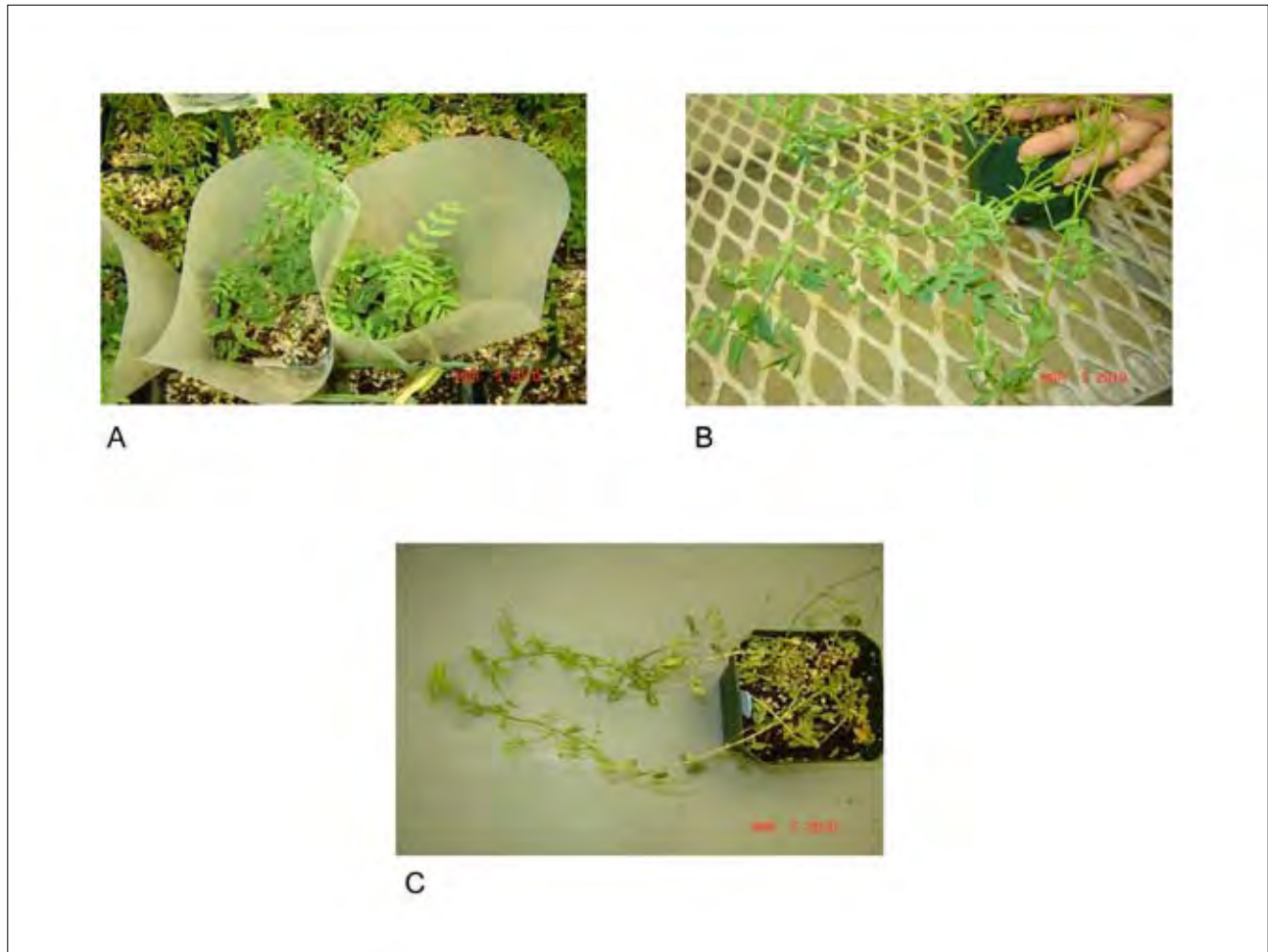


Figure 2. Inoculation of lentil plants with *Colletotrichum truncatum*.
A. Freshly inoculated plants separated by plastic covers.
B. Early disease development.
C. Final stage of disease progression.

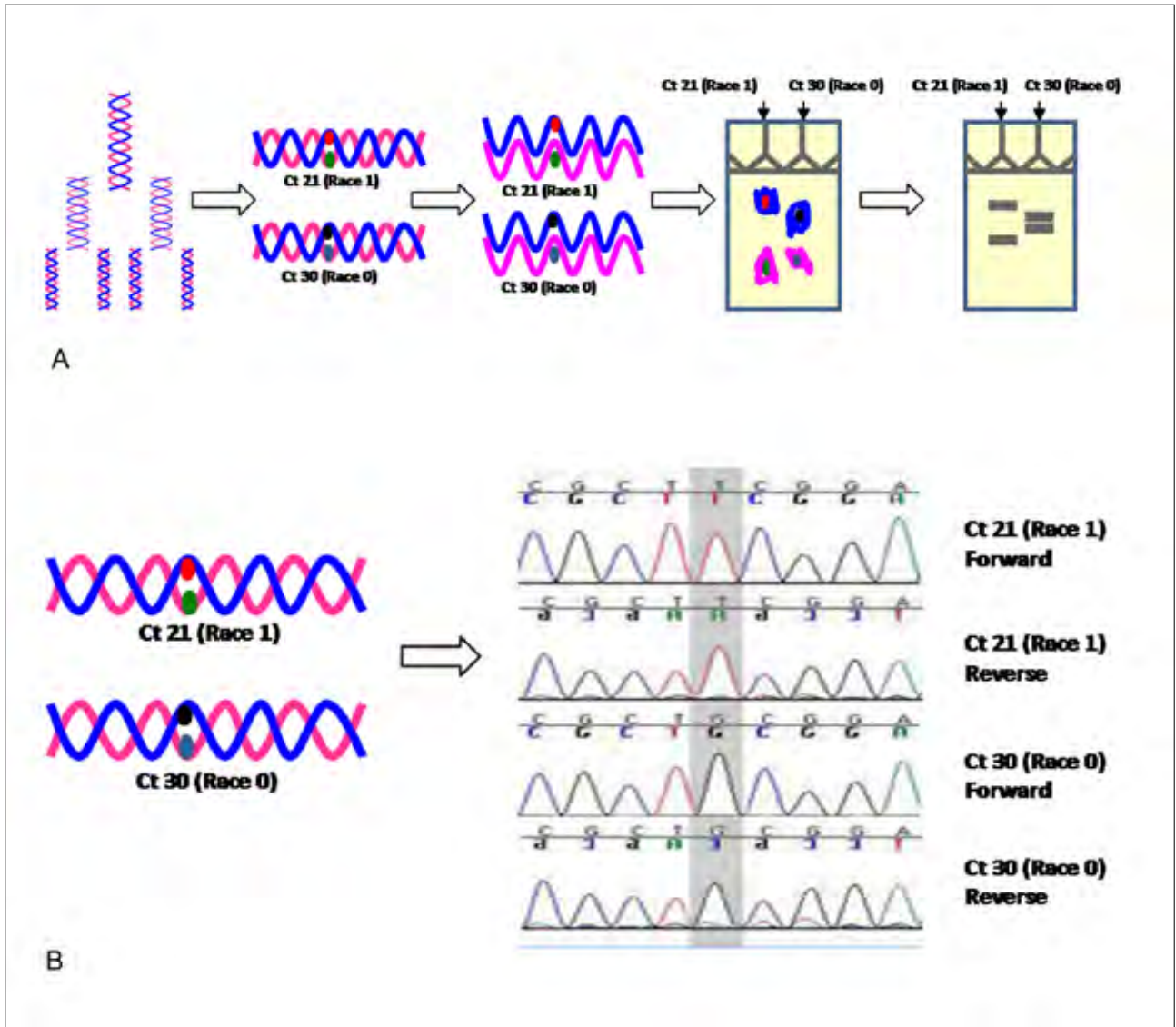


Figure 3. PCR and SSCP Gel and Sanger Sequencing Process. A. PCR and SSCP Gel Process. B. Sanger Sequencing Process.

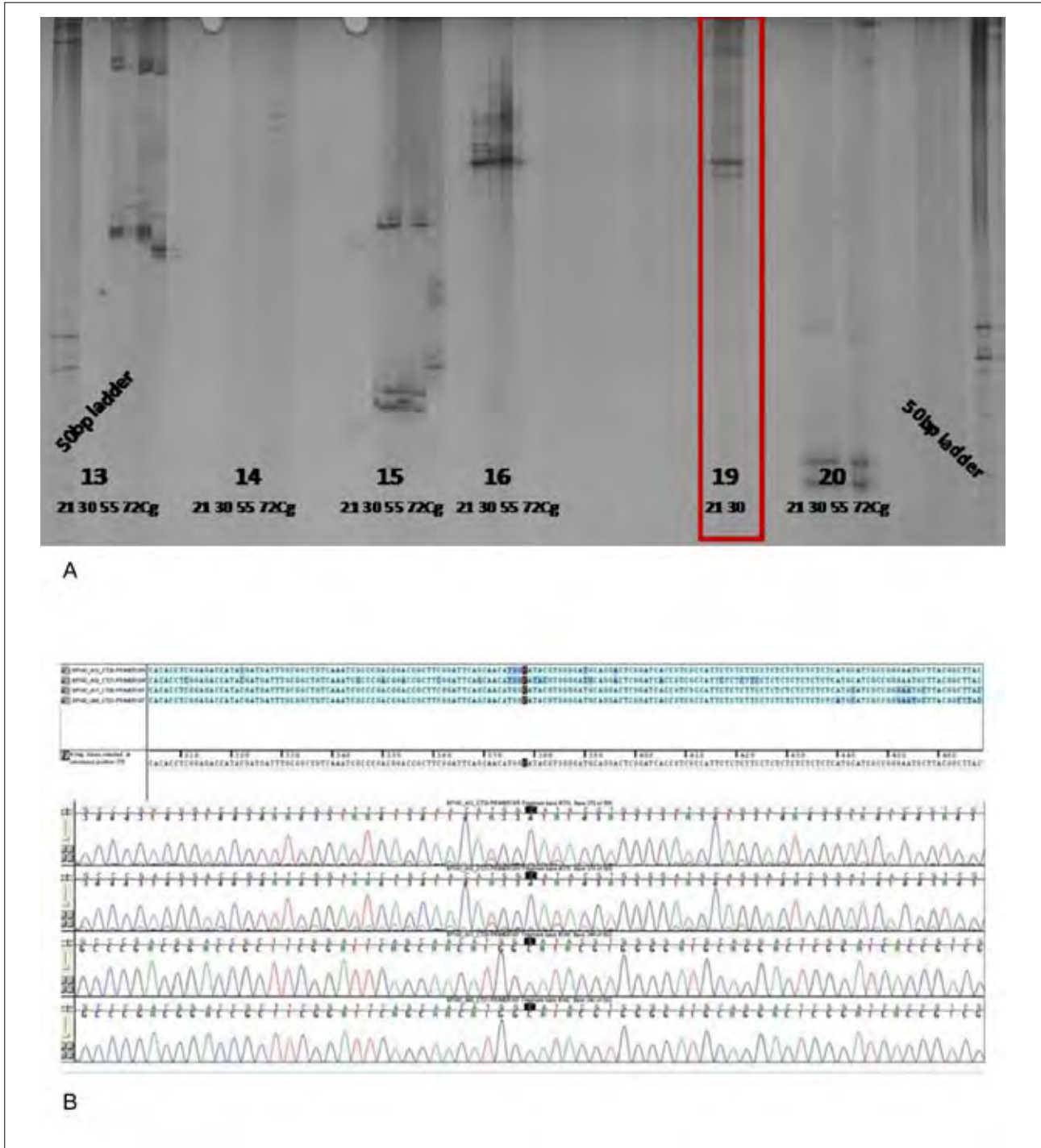


Figure 4. Validation of SSCP Gels with Sanger Sequencing. A. SSCP Gel with EST Primers 13-20 on CT-21, CT-30, CT-55, GT-72 and CG. B. Sequence Alignment and Chromatograph for Primer 19 on CT-21 and CT-30.

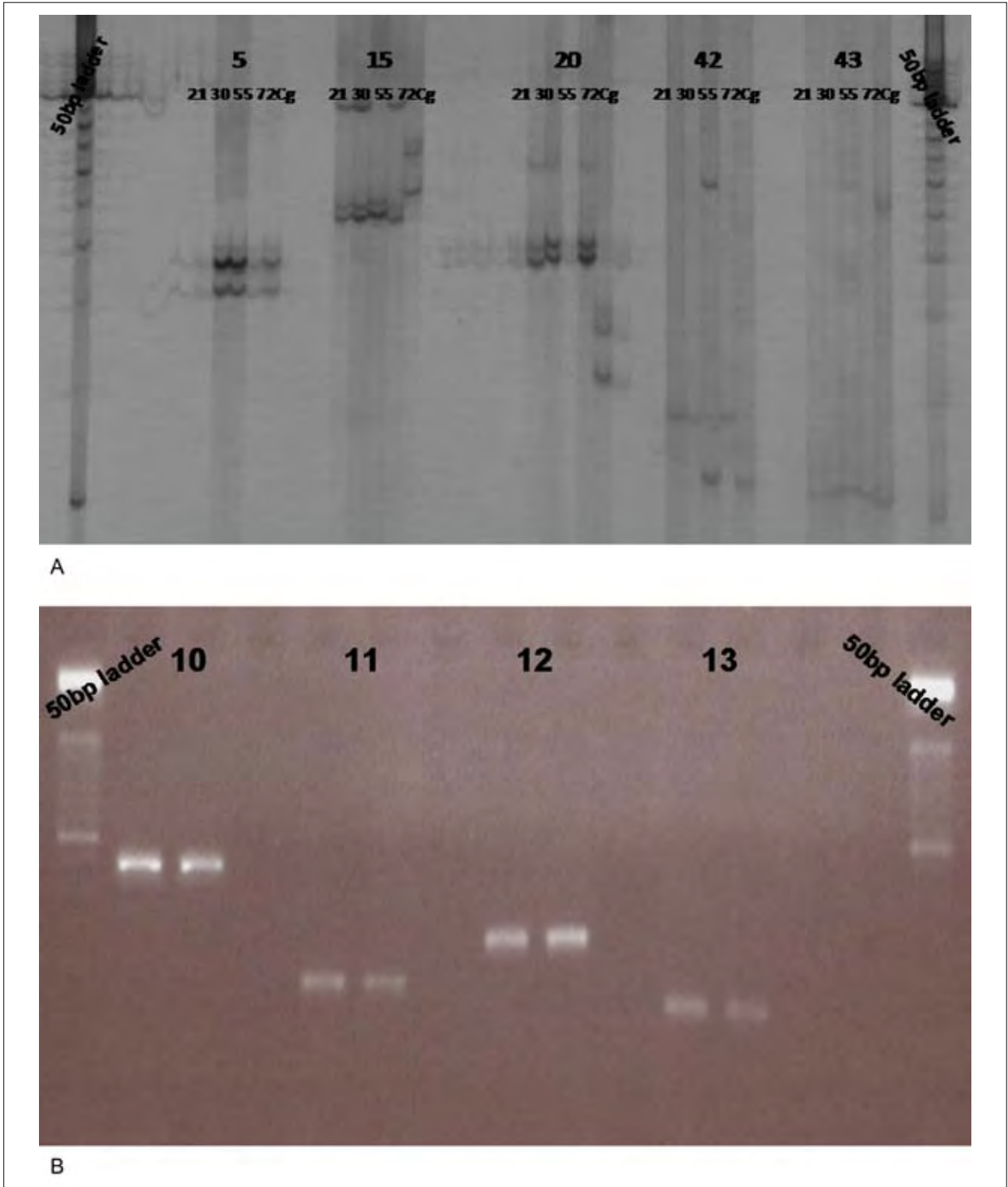
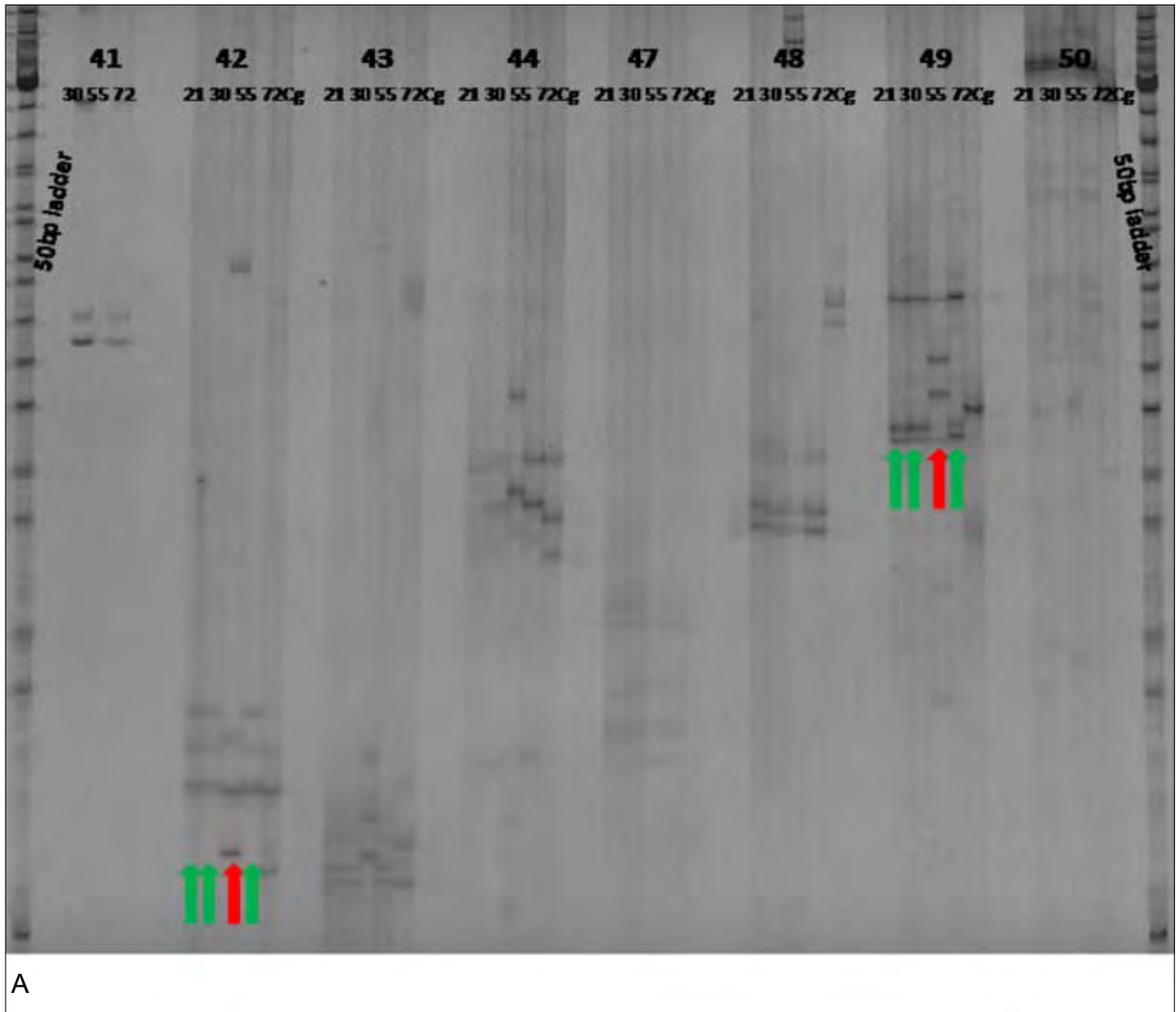


Figure 5. Comparison between the Visualization Abilities of SSCP gels and agarose gels. A. SSCP Gel with EST Primers 5, 15, 20, 42 and 43 on CT-21, CT-30, CT-55, GT-72 and CG. B. Agarose gel with EST Primers 10-13 on CT-21 (Left band) and CT-30 (Right band).



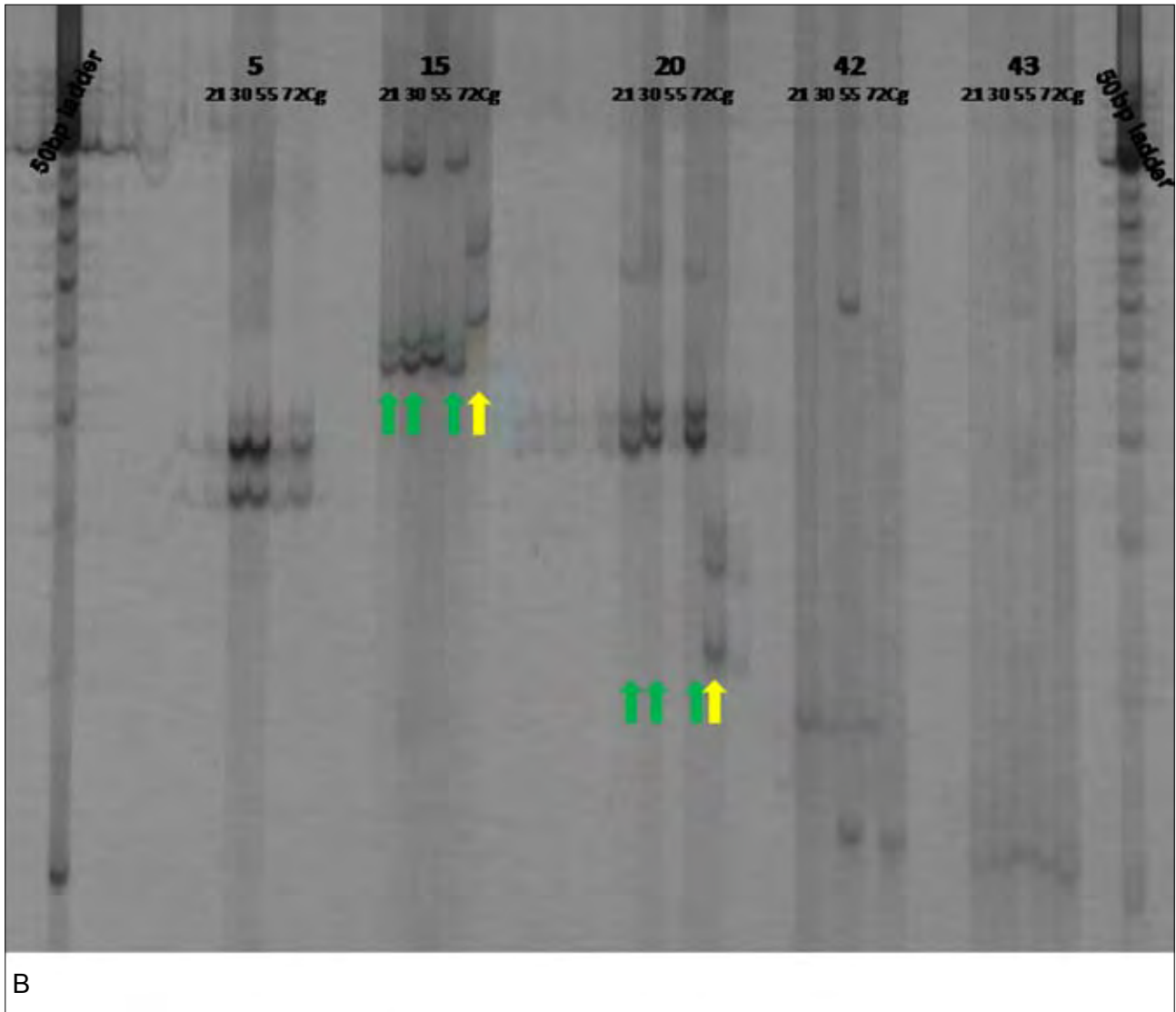


Figure 6. Varied Resolutions of EST-based markers on SSCP Gels. A. Gel indicating polymorphisms between CT-55 (Soybean) and CT Lentil isolates. B. Gel indicating polymorphisms between *Colletotrichum graminicola* (Wheat) and CT Lentil isolates.



Review of ***Racing to Find a Marker, Development of Molecular Markers for Races of Colletotrichum truncatum***

This paper describes and validates a method for identifying single nucleotide polymorphisms (SNPs) among Expressed Sequence Tag (EST) based marker(s) of *Colletotrichum truncatum* (CT), a fungal pathogen causing anthracnose on lentil. The initial goal was to differentiate between closely related Races that differ in range of pathogenicity with Race 0 being more aggressive than Race 1. The markers chosen were identical in sequence between the two races and did not support the hypothesis. However, they did detect SNPs that decisively differentiate the other CT isolates and CT and another species *Colletotrichum graminicola*. Therefore the markers described are valuable molecular tools for taxonomic identification.

This is a clearly formulated hypothesis driven study that produced easily interpretable results. The paper is well written and organized in an easily understandable fashion. The figures are high quality and well described and represent an impressive level of work for someone at the high school level.

A phylogenetic tree based on any available data depicting the degree of relatedness between the CT isolates and *Colletotrichum graminicola* would assist the reader in understanding the results as to the degree of sequence divergence and the expectation of finding SNPs.

The methods were well documented although clarification of the Intron marker pipeline (IMP) would be helpful. For example, the webpage (<http://code.google.com/p/intron-marker-pipeline/>) has the following description: "This pipeline has been developed as automated tool for design of primers which flank predicted intron location in Expressed Sequence Tags (ESTs) for species with limited to no genomic sequence data available."

The author states that the 50 markers were chosen "because the markers are closely linked to pathogenicity genes". It would be helpful to know how this linkage was assessed. Were these known pathogenic loci from the literature or from crosses where the pathogenicity phenotype was quantified as described in Figure 2?

The conclusions were clearly stated and well supported. One missing element is the discussion of future work (e.g perhaps test more candidate markers from the remaining 2000 to identify those that distinguish Race 0 from Race 1).

Dawn Thompson, Research Scientist-Regev Group, Broad Institute of MIT and Harvard





Functional Analysis of Telomerase Mutations in Dyskeratosis Congenita

Katherine Taneille Johnson and Tara L. Beattie

Southern Alberta Cancer Research Institute, University of Calgary, AB, Canada

Katherine Taneille Johnson: Attending the 2005 Canada Wide Science Fair was what it took before Johnson considered pursuing a career in science and research. She respected the young participants, who had presented work of enviable caliber, and realized that she wanted to be a part of that. The work appealed to her—from the discovery of an idea to the layout of an experiment. Not only that but, she found satisfaction in the multidisciplinary nature of her research subject, molecular biology. The fact that it has connections with other areas of science, such as biotechnology, is its appeal. With hard work and some luck, Johnson will look for her own vision for the future of molecular biology.

Telomerase is a ribonucleoprotein enzyme that functions in maintaining and elongating telomeres^[1]. Mutations in telomerase (hTERT) have been identified and linked to hematological disease states such as Aplastic anemia, Dyskeratosis congenita (DKC), and Acute myeloid leukemia^[2,3]. It is hypothesized that a decrease in telomerase activity and shortened telomeres predominantly limits the replication of specific tissue stem cells. This study investigated functional interactions within telomerase complexes expressing certain hTERT mutants in order to further understand how these mutations lead to telomere and telomerase deficiency in autosomal dominant DKC. Four naturally occurring mutants of hTERT- A279T, P721R, K902N, and R979W were studied through both in vitro and in vivo methods. These assays illustrated that the A279T, P721R, and R979W mutations do not impair telomerase activity and hTERT is still properly produced. The K902N mutant was shown to suppress a majority of telomerase activity in vitro and in vivo, with only small amounts detectable. Results were compared against a linear model of the protein and analyzed. Understanding how these mutants influence telomerase function can help determine methods of restoring enzyme activity. Further investigation into the role telomerase plays in causing the observed bone marrow failure could help elucidate treatment protocols.

La télomérase est une enzyme ribonucléoprotéique qui fonctionne dans le maintien et d'allonger les télomères^[1]. Des mutations dans la télomérase (hTERT) ont été identifiées et liées à des états pathologiques hématologiques comme l'anémie aplastique, dyskératose congénitale (DKC), et la leucémie myéloïde aiguë^[2,3]. Il est émis l'hypothèse qu'une diminution de l'activité de la télomérase et des télomères raccourcis limites principalement la réplication de certaines cellules souches tissulaires. Cette étude examinait les interactions fonctionnelles au sein de complexes exprimant la télomérase hTERT certains mutants dans le but de mieux comprendre comment ces mutations conduisent à des télomères et la télomérase carence en transmission autosomique dominante DKC. Quatre mutants naturels de hTERT-A279T, P721R, K902N, et R979W- ont été étudiés par les deux in vitro et in vivo. Ces tests ont démontré que les mutations A279T, P721R, et R979W ne nuisent pas à l'activité de la télomérase et hTERT est encore bien produit. Le mutant K902N a été montré pour supprimer la majorité de l'activité télomérase

in vitro et in vivo, avec seulement de petites quantités détectables. Les résultats ont été comparés à un modèle linéaire de la protéine et analysés. Comprendre le fonctionnement de ces mutants télomérase influence peut aider à déterminer les méthodes de rétablissement de l'activité enzymatique. Une enquête plus poussée dans le rôle que joue la télomérase en causant l'échec de moelle osseuse observée pourrait aider à élucider les protocoles de traitement.

Background

Telomeres are DNA-protein protection and maintenance structures on the ends of chromosomes that shorten during each round of cell division^[1]. The regulatory enzyme to the telomeres, telomerase, is mainly composed of an RNA template hTR and a protein reverse transcriptase component hTERT. Stem cells and germ line cells show functional telomerase activity, which allows continuous cell divisions without telomere shortening. Telomerase is hypothesized to work as a dimer, needing two copies, each consisting of hTR and hTERT for functional activity^[4].

Dyskeratosis congenita is commonly referred to as an early onset aging disorder or cancer predisposition syndrome, classified by abnormalities of the skin, nails, and mucous membranes^[5]. Eighty percent of cases are associated with bone marrow failure or dysfunction^[6]. One cause of this disease is believed to be mutations in the telomerase complex and telomere binding proteins^[7], which could be a potential treatment target. Telomerase is not working properly, and consequently these stem cells are not able to maintain continuous cell division. However exactly why these mutations manifest as a disease state in specific tissues is still unknown.

Specialized treatment models are designed specifically for each individual patient, and finding successful management pathways is difficult^[6]. Patients often do not respond to immunosuppressive therapy, chemotherapy, or radiation therapy^[8]. Genetic therapy that remedies telomerase mutations, or agonistic drugs that enhance low telomerase levels could yield important treatment models. Severe implications of defects in telomere and telomerase biology illustrate the importance of understanding telomere function in both healthy and diseased systems.

Objectives and Hypotheses

This study will investigate the functional interactions in naturally occurring heterozygous mutant of the protein component hTERT, (A279T, P721R,

K902N, and R979W) that are associated with DKC, by analyzing various biochemical properties of telomerase. These include protein stability, catalytic activity, and RNA binding. To expand on this biochemical analysis, the mutated hTERT proteins will be expressed in cells, both through stable and transient transfection, to examine different cellular consequences of mutant protein expression. The mutations that negatively influence telomerase will act dominantly, suppressing catalytic activity, causing haploinsufficiency among patients. Because the mutations are associated with hematological disorders and telomerase deficiency, it is hypothesized that they will not have a positive effect and increase enzyme activity.

Methodology

In Vitro Techniques

All techniques were optimized according to lab procedures and based on previous research^[9].

Telomerase repeat amplification protocol (TRAP assay):

All hTERT mutants mentioned above and controls (hTR only, full length wild type hTERT (FLWT hTERT)), will be prepared in rabbit reticulocyte lysate (RRL) reactions, from Promega, which serve as a system for transcription and translation. Each reaction contained hTR and was tested for functional telomerase activity through a modified TRAP assay based on Kim et al.^[10] developed by Dr. Beattie's lab. The TRAP assay uses radioactive labeled guanines to show telomeric repeats generated by functional telomerase activity. This assay also uses Polymerase Chain Reaction, and consequently results are evaluated on a comparative basis rather than numerically. Positive enzyme activity is characterized by a ladder pattern on the blot.

Western blot analysis:

hTERT protein expression was tested in RRL samples. Samples were run according to SDS-PAGE,



with an 8% polyacrylamide resolving gel, and loaded into a 5% polyacrylamide stacking gel. Gel was transferred to a polyvinylidene difluoride membrane, fixed in methanol, dried and blocked with TBS (20 mM Tris-HCL, and 150 mM NaCl) in addition to 0.5% Tween and 0.5% nonfat skim milk (1 hour) at room temperature. Blot was probed overnight at 4°C with α -hTERT 1:500, 0.1 % gelatin primary antibody (from rabbit). Blot was washed in TBS and incubated with anti-rabbit polyclonal secondary antibody (1:2500), then washed again in TBS and developed using enhanced chemiluminescence reagents. All lanes were measured against a protein ladder (Fermentas). Wild type hTERT runs at approximately 130 kDa.

Immunoprecipitations (IP):

hTERT was purified out of the RRL system through immunoprecipitation. Due to the large quantity of protein present in the RRL, all RRL samples were pre-cleared using Sepharose beads (Sigma). Following the pre-clearing, the samples were immunoprecipitated using Sigma M2 α -Flag beads for 2 hours in 150 mM salt IP buffer. In addition to the RRL samples, a beads-only control was used. Samples were centrifuged and washed once using 150 mM salt IP buffer and subsequently washed twice using 300 mM salt IP buffer, followed by a final wash with 150 mM salt IP buffer. Samples were then eluted using FLAG peptide, and analyzed by Western blot and TRAP assay.

In Vivo Techniques

Transient transfections and in vivo analysis:

Both stable and transient transfections were run using the 293T human kidney epithelial cell line. Three sets of transfections were conducted, and representative data is shown. Samples and controls (FL WT hTERT; pCI vector only) were transfected into cells twice using the FuGene (Roche) reagent to ensure DNA uptake. Cells were grown for twenty four (24) hours before the second transfection. Twenty four hours later, the cells were harvested. The 293T cells were grown in antibiotic free DMEM media, checked every 24 hours for growth, and split when at approximately 80% confluence.

IP of transient cell lysate:

The harvested 293T cell lysates were immunoprecipitated according to protocol described above. Due to the large amount of protein present in the cell

lysates, a modified Bradford/ Lowry assay (Bio-Rad) was run to determine the adjusted concentration of sample that should be added in order to achieve a 500 μ g input. The in vitro RRL samples were also immunoprecipitated as controls for the cell lysate immunoprecipitation. The pCI vector was synthesized in RRL and used as a negative control. These samples were tested by Western blot and TRAP assay following immunoprecipitation.

Stable transfections and in vivo analysis:

Stable transfections were run using 293T cells. Samples (A279T and K902N) and controls (FL WT hTERT, pBAGE (negative control)) were transfected into cells using the FuGene (Roche) transfection reagent. Only two of the mutants were run due to time restraints. All cells were transfected with GFP (green fluorescent protein). 293T cells are able to take up and integrate the plasmid naturally under correct growing conditions. Cells were harvested when at stable populations; cell lysate concentrations were checked by a modified Bradford/ Lowry assay; immunoprecipitated; and then Western blot and TRAP analyzed. The cells were grown in DMEM media for the first stage of the transfection, then transferred into DMEM + puromycin antibiotic to select for transfected cells. Each transfected plasmid has a puromycin resistance gene.

Results

In Vitro

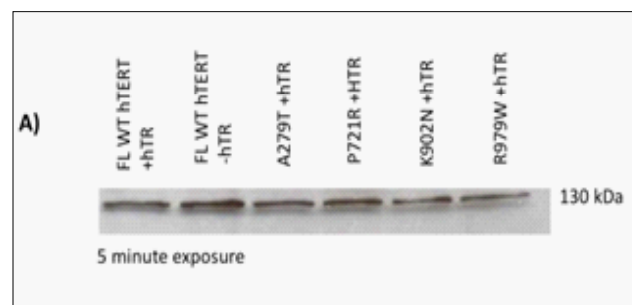


Figure A. Western blot analysis of 4 naturally occurring mutants, and controls synthesized in the RRL system. hTERT is properly produced in vitro and runs at 130 kDa. The mutants do not appear to influence translation in vitro. The presence of hTR in samples does not impact the production of the protein and is only essential for enzymatic activity. All protein is transcribed and translated properly, as shown by expected molecular weights.

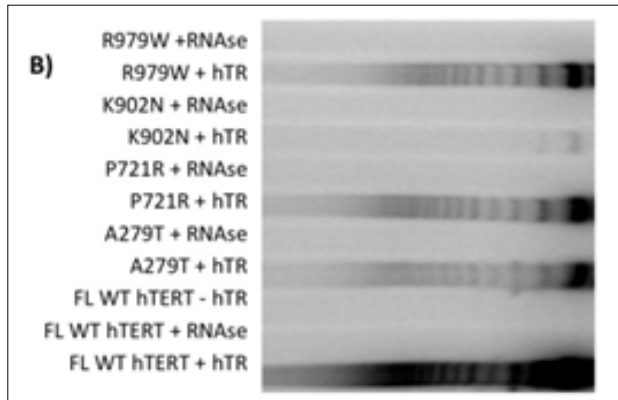


Figure B. TRAP analysis of 4 naturally occurring telomerase mutants, including RNase controls. This assay was done to further help elucidate whether the K902N has any catalytic activity present, or if low activity levels are due to external factors. The A279T, P721R, and R979W mutants all show positive telomerase activity. From comparison to the negative controls, the faint activity in the K902N appears to be due to telomerase rather than contamination or cross reactivity. In vitro analysis suggests that the A279T, P721R, and R979W mutations do not negatively influence telomerase activity. These results do not correlate with the observed disease state findings, where patients with these mutations show decreased enzyme activity. The K902N mutation could be affecting the ability of hTERT to bind to hTR properly.

In Vivo

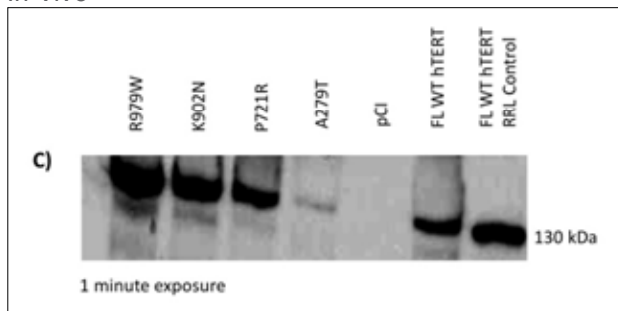


Figure C. Western blot analysis of 4 naturally occurring telomerase mutants immunoprecipitated from transiently transfected 293T cell lysates. hTERT is properly produced at 130 kDa in all lanes, however the A279T band is fainter than in other samples. This could be due to decreased levels of TERT being pulled out of solution during the immunoprecipitation. These results show that the mutations do not cause truncations or disrupt the translation process in vivo, because hTERT is generated at the correct molecular weight.

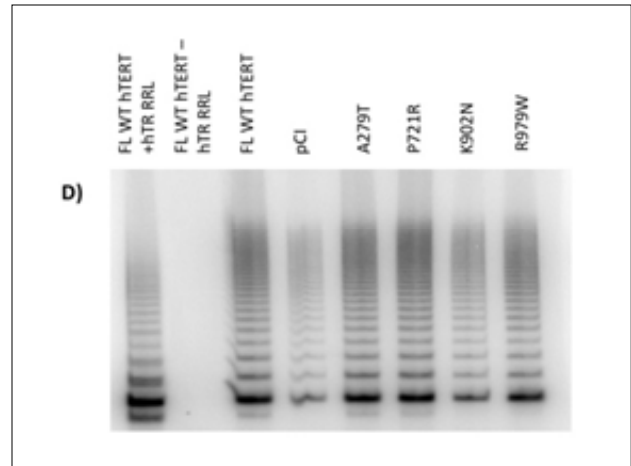


Figure D. TRAP analysis of mutants immunoprecipitated from transiently transfected 293Ts. Positive telomerase activity in the A279T, P721R, R979W, and K902N illustrates that the protein is still able to collaborate with hTR to form a structure that is catalytically active, generating telomerase repeats recognized by radioactively labeled guanines. However, positive TRAP activity in the K902N and pCI cannot be fully explained, and was not expected based on in vitro results. Negative protein in the Western blot for pCI, suggests that the positive activity in the TRAP blot could be due to factors other than telomerase activity.

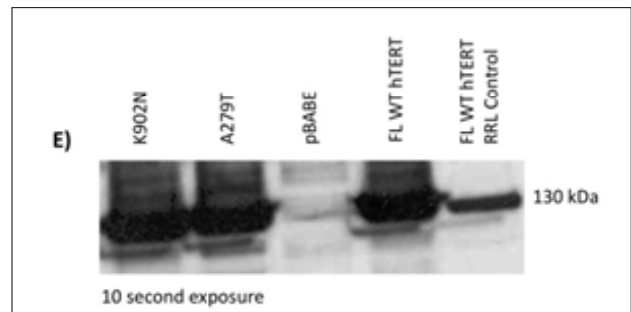


Figure E. Western blot analysis of K902N, A279T, and controls immunoprecipitated from stable transfected 293T cell lysates. Cells were harvested at mean population doubling 5. A faint ghost band appears in pBABE (negative control), but this could be due to cross reactivity or endogenous hTERT still left over from the immunoprecipitation. Beads were washed extensively, FLAG-tagged and eluted, so having endogenous hTERT present still is unlikely. The K902N and A279T both run at 130 kDa, showing that the protein was translated properly and is the correct molecular weight.

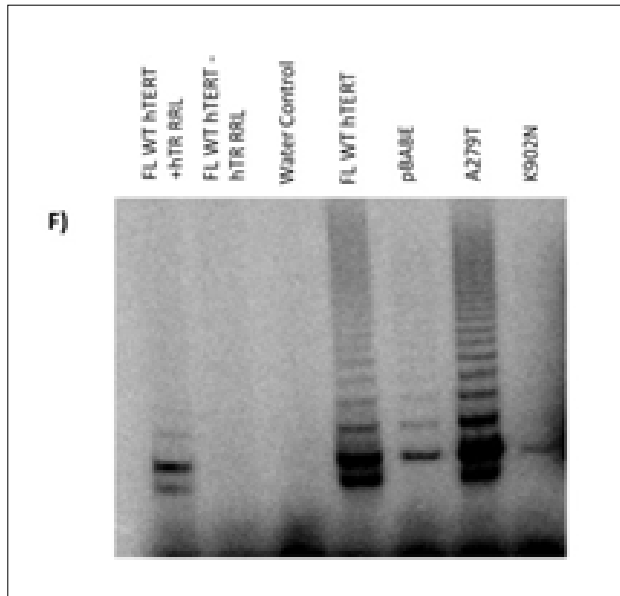


Figure F. TRAP analysis of K902N, A279T, and controls immunoprecipitated from stable transfected 293Ts. A water control was used for comparison against samples. Positive activity in pBABE is abnormal, because samples should not contain telomerase. K902N shows comparable TRAP activity to in vitro results. Positive pBABE activity could be due to cross contamination in the transfection procedures rather than in the immunoprecipitation. Experiments would be repeated for more conclusive results and to help determine how possible contamination arose.

Discussion

There are a number of naturally occurring mutations in telomerase associated with DKC. However, the hTERT protein has not been crystallized and there

fore there is no information available on how these mutations affect its structure specifically. Comparison of data gathered from in vitro and in vivo work against a proposed structure of the protein lends insight into the nature of the mutants. The mutations investigated in this study are all highlighted in Figure G.

In vitro and in vivo data from the mutants investigated illustrates that not all telomerase mutations impair or affect enzyme activity. The A279T, P721R, and R979W mutations are associated with DKC, a disease state associated with abnormally shortened telomeres. Why is telomerase activity not impaired?

The A279T mutation is present in the Linker region^[11] of the protein, a region less essential to enzyme activity. Research shows that this region is less conserved and hypervariable, therefore mutations in the Linker component would not necessarily impair telomerase activity. Healthy individuals with this mutation exhibit full functional telomerase activity^[12]. Here, we show that this mutation should be found concurrently with other telomerase mutations in order to exhibit disease state findings characterized by negative telomerase activity. Protein structure dynamics confirm in vitro and in vivo results because the A279T was not found to have an adverse effect on telomerase activity.

The P721R mutation is found in the reverse transcriptase (RT) domain, the catalytic center of telomerase^[11]. The A motif of the RT domain is conserved among all reverse transcriptases, and is essential for enzyme activity. Since this region is highly conserved, mutations in this domain should impair telomerase activity. However, in vitro and in vivo results show that the P721R mutation does not disable telomerase activity. It is hypothesized that if this mutant were

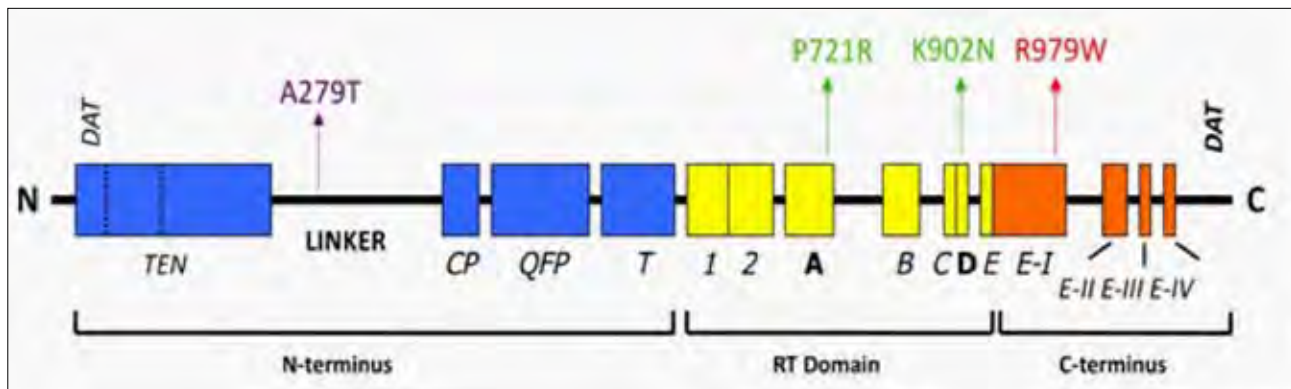


Figure G. hTERT protein layout. Mutational analysis can yield important data on the critical regions of the protein and how they influence functionality.

stable transfected it would have neutral affect on telomerase activity, which correlates with in vitro and transient transfection results. Considering the amino acid substitution though, a switch from proline to arginine makes this residue more hydrophilic, changing protein folding, which suggests that this mutation would affect telomerase activity. This result does not correlate with our data, and further investigation into the dynamics of the P721R mutation could remedy this conflict.

The R979W mutation is found in the DAT domain of the carboxyl-terminal domain^[11]. The C terminus domains are essential for telomerase activity. However, conservation of motifs in this region is moderately weak compared to the amino-terminal and RT domains. Mutations in the C terminus affect telomerase processivity- the number of telomere nucleotides synthesized^[11]. In vivo and in vitro results show that the R979W mutation does not impair telomerase activity. Therefore, it is likely far enough away from the DAT domain to not affect the recruitment of nucleotides to the telomere, which explains these findings. Mutations found in the C terminus, and especially the DAT domain should have a negative effect on telomerase activity. It is hypothesized that if this mutant were stable transfected, it would not have a negative effect on telomerase activity, which agrees with transient transfection data and in vitro results. Similarly to the P721R mutation, further investigation could help determine this mutation's specific effect on protein structure and function.

The K902N mutation is also found in the reverse transcriptase domain^[11]. In vitro and in vivo results illustrate that this mutation impairs a majority of telomerase activity. Western blot results show that the protein is still produced properly, however TRAP analysis shows very little telomerase activity. This mutation could be impairing the ability of hTR to bind to hTERT to form a functional complex. For positive telomerase activity, hTR and hTERT must come together correctly. The TRAP assay allows an indirect look at RNA binding, because if the complex is not properly assembled, then negative telomerase activity is expected. Our data showed that there are only small amounts of catalytic activity present. Patients with this mutation experience a haploinsufficiency of telomerase^[13], and as a result exhibit telomere shortening. The D motif of the RT domain where this mutation is located is highly conserved. Any changes to this area should have a negative effect on telomerase activity; protein structure dynamics confirm in vitro and in vivo results.

Conclusions

This study showed that not all mutations in the reverse transcriptase protein hTERT affect enzyme activity and contribute to the observed haploinsufficiency in DKC. Considering that these mutations are associated with the disease, some could be considered "risk factors" rather than identified as disease causing^[5]. Other mutations in hTERT in addition to the ones studied here, as well as mutations in hTR, and telomerase complex proteins such as dyskerin could also be involved. Together these mutations bring about the observed disease state. Both in vitro and in vivo results for these mutants help increase our understanding of how mutations in different areas of hTERT can affect telomerase function and activity.

The K902N mutant is of particular interest because it suppresses a majority of telomerase activity in vitro. Transient and stable transfections mostly confirm in vitro results. Further replication of these experiments and analysis would yield more conclusive data. The stable transfection TRAP blot showed inconclusive ghost bands in pBABE, which should have had negative activity. Functional analysis of these mutants was successful in in-vitro models, but could be investigated more using additional in vivo studies.

The telomerase complex is tightly regulated. Mutations in this complex could unbalance the equilibrium between telomere elongation and depletion to favor shortening. Individuals with the K902N mutation could potentially be treated with a telomerase agonist drug that could bind to the protein at some site, stabilizing it and restoring some or all of the lost activity.

Through researching mutations in the telomerase complex, treatments that can be used to prevent disorders such as DKC can hopefully be elucidated. Investigation into mutations of the telomerase complex is an important area of study, which can benefit the wider scientific community and provide hope for those suffering with telomerase related disorders.

Acknowledgements

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Keywords

Telomerase: a specialized reverse transcriptase (enzyme) responsible for telomere elongation and maintenance.

Mutation: a change that brings upon an effect, whether negative, positive or neutral.

Dyskeratosis Congenita: a hematological disorder characterized by disorders of the mucous membrane, skin and nails. Commonly presents with bone marrow failure.

References

- [1] Autexier C, Lue NF. The structure and function of telomerase reverse transcriptase. *Annu Rev Biochem.* [Review]. 2006;75:493-517.
- [2] Roth A, Vercauteren S, Sutherland HJ, Lansdorp PM. Telomerase is limiting the growth of acute myeloid leukemia cells. *Leukemia.* 2003;17(12):2410-7.
- [3] Calado RT, Young NS. Telomere maintenance and human bone marrow failure. *Blood.* [Review]. 2008 May;111(9):4446-55.
- [4] Cohen SB, Graham ME, Lovrecz GO, Bache N, Robinson PJ, Reddel RR. Protein Composition of Catalytically Active Human Telomerase from Immortal Cells. *Science.* 2007 March 30, 2007;315(5820):1850-3.
- [5] Savage SA, Alter BP. Dyskeratosis Congenita. *Hematology/Oncology Clinics of North America.* [doi: DOI: 10.1016/j.hoc.2009.01.003]. 2009;23(2):215-31.
- [6] Kirwan M, Dokal I. Dyskeratosis congenita, stem cells and telomeres. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease.* [doi: DOI: 10.1016/j.bbadis.2009.01.010]. 2009;1792(4):371-9.
- [7] Vulliamy TJ, Dokal I. Dyskeratosis congenita: The diverse clinical presentation of mutations in the telomerase complex. *Biochimie.* [Review]. 2008 Jan;90(1):122-30.
- [8] Armanios M, Chen JL, Chang YP, Brodsky RA, Hawkins A, Griffin CA, et al. Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita. *Proc Natl Acad Sci U S A.* 2005 Nov 1;102(44):15960-4.
- [9] Johnson KT. Defining Functional Interactions in Naturally Occurring Telomerase Mutants. *Canadian Young Scientist Journal.* 2010(1):41-5.
- [10] Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PLC, et al. SPECIFIC ASSOCIATION OF HUMAN TELOMERASE ACTIVITY WITH IMMORTAL CELLS AND CANCER. *Science.* [Article]. 1994 Dec;266(5193):2011-5.
- [11] Lundblad V, Blackburn E, Titia dL, editors. *Telomeres.* 2 ed: Cold Spring Harbor University Press; 2006.
- [12] Wyatt HDM. Personal conversation surrounding the affect of the A279T mutation on telomerase activity, and whether or not this mutation can be found in healthy individuals. ed2009.
- [13] Marrone A, Stevens D, Vulliamy T, Dokal I, Mason PJ. Heterozygous telomerase RNA mutations found in dyskeratosis congenita and aplastic anemia reduce telomerase activity via haploinsufficiency. *Blood.* 2004 December 15, 2004;104(13):3936-42.



Review of ***Functional Analysis of Telomerase Mutations in Dyskeratosis Congenita***

Katherine Taneille Johnson describes the functional analysis of mutations in the protein component hTERT of telomerase, the enzyme that extends the ends of chromosomes, or telomeres. Telomerase has received a lot of attention in ageing research, most recently with the spectacular finding that reactivation of telomerase in aged telomerase-deficient mice reverses ageing induced by progressively shortened telomeres (Jaskelioff, M. *et al.* Nature doi:10.1038/nature09603 (2010)).

The hTERT mutations that Katherine has studied are of particular interest since they occur in patients with Dyskeratosis congenita, a disease characterized by early onset ageing and cancer. It is important to understand how these hTERT mutations contribute to premature ageing and cancer in Dyskeratosis congenita.

I have read many undergraduate theses and can say with confidence that this paper matches the best of them in terms of setting the stage for the experiments, logical flow and description of the methodology. The results are also exciting: only one mutation –K902N - gives the “expected” result of reducing telomerase activity. Other hTERT mutants support wildtype or nearly wildtype telomerase activity *in vitro* and in cell culture assays, which begs the questions of how these mutations contribute to Dyskeratosis congenita. Maybe additional mutations need to be present to turn these telomerase mutations into risk factors, or these mutations affect a telomerase function that these assays don’t measure. Could it also be that these sites in the protein are irrelevant for telomerase activity and irrelevant for disease? All interesting questions that arise from Katherine’s experiments and that can now be addressed in future research.

If there is one aspect that could be improved in this impressive paper, it is the presentation of the results. The results are only described within the figure legend. It may have been better to include a separate results section in addition to a figure legend. The figure legend could then have given us a more detailed description of what exactly is presented in the figures. For example, the terms “FL WT hTERT”, “FL WT hTERT RRL control” in figure C and “FL WT hTERT +hTR RRL”, “FL WT hTERT -hTR RRL”, “FL WT hTERT” in figure D are not explained in the figure legend; only with some back and forth to the methodology section can the reader guess what these lane descriptions mean. The confusion is similar for “pCI” which is explained as “vector only” in the methodology section; it would be good to explain this abbreviation again in the figure legend, or even better to just say “vector only” in the figure. Also how was the FLAG tag used (this is poorly described in the methods section and the figure legend)? One of my most frequent suggestions for the improvement of figures is to make the figures as self-explanatory and independent of the text as possible, for example by using simple lane descriptors and by making sure to cover every experiment in the legend. Many reviewers and readers go first to the data and try to understand the results without even reading the text. Help them as much as you can!

There are a few results that I would have liked to read more about in the discussion: it is odd that the reduced expression levels of A279T in Figure C does not result in reduced activity in Figure D. I am also not sure I fully understand the explanation for the telomerase activities in negative control lanes of Fig. C and F. Why is cross-contamination in the transfection procedures the preferred explanation, and not, for example, spillover during gel loading?

These are minor suggestions that don’t take away from the overall conclusion that Katherine has done an impressive amount of work and made some very interesting observations in the telomerase field. Congratulations!

Bodo Stern, Ph.D., Director of Research Affairs, Harvard FAS Center for Systems Biology





Careers in Science – The new wave

Alison Symington

Ph.D., Vice President, Outreach, Ontario Genomics Institute / MaRS Centre

The fact that you are reading this journal would suggest that you are interested in science, maybe as a career. Typically, for most of us that means a professional career such as a doctor, dentist, nurse, engineer or a bench scientist that works at a university or research institution. These careers are vitally important, but reflect just a tip of the iceberg on careers that are available in science today. Science pervades all aspects of our everyday life and as science crosses those traditional boundaries, experts from many fields are needed. An example would be the Barcode of Life project. This project aims to barcode all the species of the earth using specified sequences of DNA. This project, on the face, would be a traditional science project that combines molecular biology with traditional taxonomy. However, dig deeper and it is a combination of molecular biologists and taxonomists but also bioinformaticians (math and computer scientists), educators, engineers who design laboratory equipment, accountants, project

managers and communication specialists. It even has links to video game designers using it as a basis for educational games. As the science reaches the public, the lawyers, regulatory specialists and political scientists become involved in setting policy and government regulations. More than ever, science is a “team sport”, relying on the expertise of many. So what does this mean to you? These careers are as varied as there are people. Combinations of experience are what will make the difference in the future. Science degrees with business or communication degrees will be in great demand and more likely there are combinations that have not been thought of yet. However, underpinning it all is a basic understanding of science. Our economy here in Canada and around the world will be driven by science. Not everyone will or should choose to be a bench scientist, but careers in science are as varied as the people reading this journal and you should be encouraged to find your niche.



Testimonial

Glen Kim

Science Teacher, St. Joan of Arc Catholic Secondary School, Dufferin-Peel CDSB

The Sanofi-Aventis BioTalent Challenge (SABC) is a prestigious science competition geared towards Canada's most dedicated and innovative biology students. It is no ordinary science fair, but rather an opportunity for learning and growth that far exceeds anything students might experience in the classroom. Students wishing to participate in the competition must submit original research proposals that are evaluated by a committee composed of biotechnology researchers and industry specialists. The students whose projects are accepted are then given the opportunity to conduct their experiments in a university or hospital lab. It is an outstanding opportunity for students of exceptional ability who are considering pursuing a career in science to conduct advanced scientific research in a university setting under the guidance of some of the nation's best scientists. The students are then given the opportunity to present the results of their research to a panel of Biotechnology experts; the awards given to the competition's top entries range from cash prizes and internships at Biotech companies to university scholarships, along with the opportunity to travel to Ottawa for the national competition.

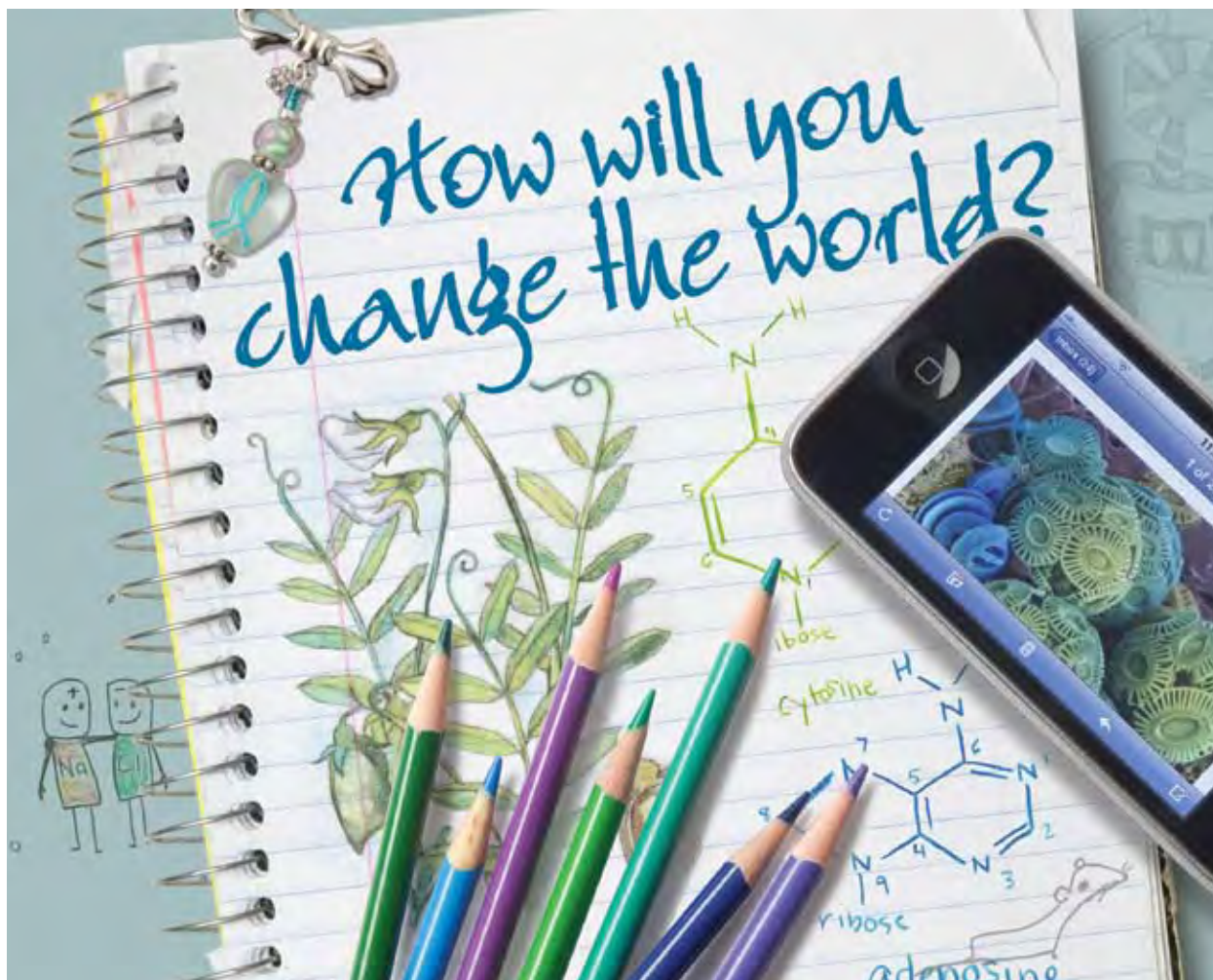
My perspective on the SABC is unique. Like many teachers in the Greater Toronto Area, I have been actively encouraging my students to avail themselves of the opportunities offered by the SABC for several years. However, my enthusiasm for this competition comes not only from the satisfaction of watching my students' skills and appreciation for science grow, but also from my own personal experience as a high school student.

Thirteen years ago, I was a competitor in the Connaught Student Biotechnology Exhibition (as the SABC was known back then). My memories of it are still quite vivid. I remember the unique challenge of designing my own experiment, my excitement at having

the chance to explore a scientific question that was of interest to me, the trepidation I felt at meeting my mentor – Dr. Verna Higgins of the University of Toronto – for the first time, my gratitude for her kind and patient guidance, and the thrill of presenting at this most prestigious (yet most accessible) of science competitions. It was one of the most seminal academic experiences of my teenage years.

When I became a teacher, I was delighted to hear that the competition had expanded in the intervening years, and I set out to ensure that my students would have access to this outstanding opportunity. When I put out my first call for proposals, I was thrilled by the response, and my students and I have been regularly taken aback by the kindness and openness of the mentors we have been so fortunate to work with. At the 2008 competition, I had the pleasure of reuniting with Mr. Gabriel Ayyavoo – my high school biology teacher, SABC teacher supervisor, and my inspiration for entering the teaching profession. It felt strange to have come full circle, but I was glad to have an opportunity to thank my former teacher in person and I was thrilled to know he was continuing to inspire young minds to explore science.

Feedback from my students and their parents has been overwhelmingly positive, and it has been a source of great pleasure for me to watch my students mature into the young scientists they have become. The technical skills, knowledge, and personal bonds that my student groups form during the course of their research projects will last a lifetime. It is worth mentioning again that the SABC is more than just a science competition – it provides a chance for young minds to learn from and be inspired by brilliant and dedicated researchers, but most importantly, it serves as proof that the future for the next generation of Canada's scientists is very bright indeed.



Enter the Sanofi-Aventis BioTalent Challenge

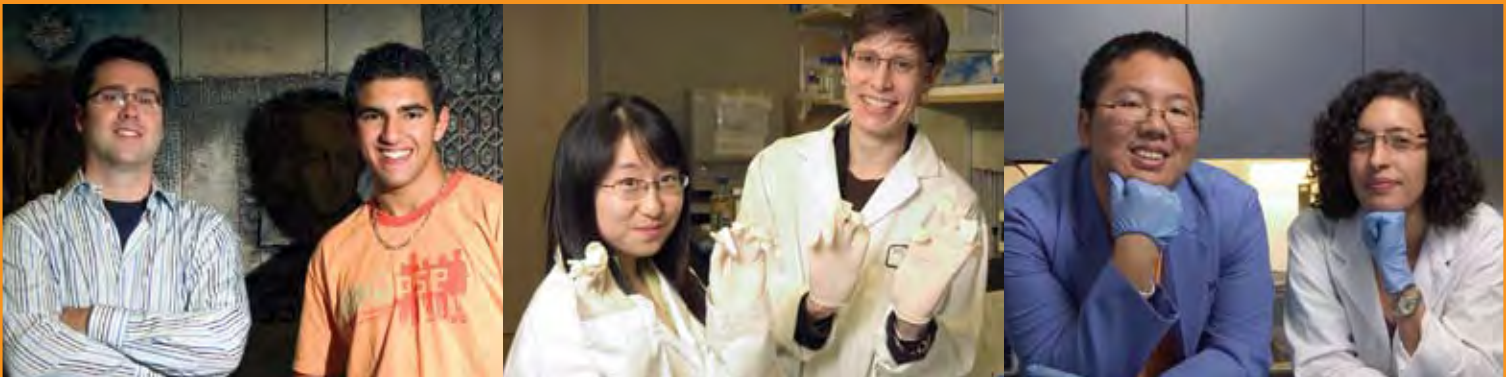
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