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THE NUCLEUS

December 2019 *Vol. XCVIII, No. 4*

Monthly Meeting

A Medicinal Chemistry Symposium on Rare and Neglected Diseases at Sanofi in Waltham

NERM 2019 - Report from Saratoga

By Hicham Fenniri

Dorothy Phillips Honored by Vanderbilt University

Summer Scholar Report

By Shannon McCallan, Northeastern University

Report from Saratoga

Hicham Fenniri, Chair, NERM Committee, and NESACS Representative to NERACS [h.fenniri@northeastern.edu]

NERM 2019
SARATOGA - JUNE 23-26
Chemistry - the Central Science of the Northeast

NERM 2019

The Eastern New York Section of the ACS hosted the 42nd Northeast Regional Meeting (*Chemistry – the Central Science of the Northeast*) at the Saratoga Hilton and City Center in Saratoga Springs, NY, on June 23-26, 2019. The full program of the meeting is available online at <https://www.acsnerm.org/>.

The General Co-Chairs for the meeting were Lisa Coutts (SI Group Inc.) and Craig Westphal (SABIC); the Program Co-Chairs were Jan Halamek and Maksim Royzen, both from SUNY Albany. More than 600 participants registered for the three-day event, and contributed to the various scientific and professional development symposia.

The highlight of the meeting was the presentation of the plenary speaker, 2017 Chemistry Nobel Laureate Joachim Frank (Columbia University), who provided an historical perspective on single-particle cryo-electron microscopy and its application to the visualization of biological molecules in their native states. Prof. Frank began his career at the Wadsworth Center in Albany before joining the faculty of SUNY Albany, where much of his pioneering work was carried out.

2017 Nobel Laureate Joachim Frank of Columbia University was the keynote speaker in Saratoga.

In addition, Benjamin Wiley (Duke University) was this year's recipient of the Buck-Whitney Award and the keynote speaker at the symposium in his honor. Reginald Rogers (Rochester Institute of Technology) was the winner of the Stanley C. Israel Award for Advancing Diversity in the Chemical Sciences, Amy Matts (Spackenkill High School, Poughkeepsie, NY) received this year's ACS Division of Chemical Education Northeast Region Award For Excellence in High School Teaching, and Jefferson Chin (Pfizer) was presented with the E. Ann Nalley Northeast Region Award for Volunteer Service to the ACS.

Several professional development events for undergraduates, graduate students, and postdoctoral fellows were held during the conference. Notably, the leaders of the Younger Chemists Committee hosted a luncheon to discuss their mission and objectives, and the Undergraduate Program featured a session on Professional Development for Undergraduates with Yogi Surendranath (M.I.T.) as the keynote speaker on *"Using Renewable Electricity to Rearrange Chemical Bonds."* Finally, a Senior/Younger Chemists Committees joint Mentoring-Networking Breakfast was also held to promote cross-generational collaboration.

Debra Rolison (U.S. Naval Research Laboratory) was the featured speaker at the Women Chemists Committee Luncheon on Monday; the title of her talk was, "*Removing the Fake Mustache: Strategies for Success as a Women Chemist.*" Dr. Rolison is the author of over 225 articles and holds 38 patents.

Overall, the technical program had a strong focus towards bio/analytical chemistry, materials chemistry, and nanomaterials. This author was a keynote speaker in the applied nanomaterials symposium track.

Reginald E. Rogers, Jr. of Rochester Institute of Technology was the recipient of a Stanley C. Israel Award for Advancing Diversity in the Chemical Sciences at NERM. 2018 NESACS Chair, Mindy Levine, was a 2016 recipient of this award.

The Exhibition included over 30 booths, representing universities and non-profit organizations, local companies, instrumentation companies, and chemical/service companies. ◇

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Contents

Cover: *Robert Umans (center) receives his certificate honoring his 50 years of membership in the American Chemical Society from Program Chair, Anna Sromek (L) and NESACS Chair Andrew Scholte(R) at the September NESACS Meeting. (Photo by Michael Singer).*

Editorial Deadlines: *February 2020 Issue: December 22, 2019*
March 2020 Issue: January 22, 2020

THE NUCLEUS

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Dorothy Phillips Honored by Vanderbilt University

Photo Courtesy of Vanderbilt University

On October 18, 2019 Dorothy Phillips was introduced as one of five 2019 inductees into the Vanderbilt Trailblazers. Her portrait was unveiled by Interim Chancellor and Provost Susan R. Wente in the Mary McClure Taylor Lobby of Kirkland Hall along with portraits of her fellow inductees: Kate Lupton, David Williams II, K.C. Potter and Muhammad Yunus.

The Vanderbilt Trailblazers initiative recognizes individuals in the Vanderbilt community who have broken barriers and made a positive impact both at the university and in society at large. Established in 2018, the portrait series seeks to expand the visual narrative of Vanderbilt's history and create welcoming, inclusive and accessible spaces with art that recognizes, reflects and celebrates the diversity within the Vanderbilt community.

2019 Vanderbilt Trailblazers include:

- Kate Lupton (painted by John Woodrow Kelley), the first woman to graduate from Vanderbilt University.
- David Williams II (painted by Simmie Knox), Vanderbilt University's first African American vice chancellor and the first African American athletics director in the Southeastern Conference.
- K.C. Potter (painted by Jared Small), Vanderbilt dean of residential and judicial affairs, emeritus.
- Dorothy J. Wingfield Phillips (painted by Donna L. Woodley), was the first African American woman to receive an undergraduate degree from Vanderbilt.
- Muhammad Yunus (painted by Sedrick Huckaby), Vanderbilt's first Nobel Peace Prize winner

Last year, Vanderbilt honored Bishop Joseph Johnson, the Rev. James Lawson, the Rev. Walter R. Murray and Perry Wallace with portraits by renowned portrait artist Simmie Knox.

The Vanderbilt Trailblazers Committee, which includes students, faculty and staff, oversees the initiative, determines honorees and decides where the depictions of Trailblazers will be permanently housed at the university.

Boston Vanderbilt Alumni Chapter Honors Dorothy Phillips

Dorothy Phillips, BA'67, was recognized in May 2017 for her contributions to diversity in STEM education during a Boston alumni chapter event.

Vanderbilt honored Phillips by creating two Dorothy J. Wingfield Phillips Chancellor's Faculty Fellowships. The fellowships support midcareer faculty members who are leaders in diversity in STEM at Vanderbilt. Vanderbilt athletics also honored her, among other civil rights leaders, during the 2017 Equality Weekend.

Phillips received her doctorate from the University of Cincinnati in 1974. She has been an American Chemical Society member since 1973 and was elected to the society's board of directors in 2013. She worked for Dow Chemical Company in Midland, Mich., and the Waters Corporation, an analytical laboratory instrument manufacturing company in Milford, Mass., where she became board director. ◇

Monthly Meeting

The 992nd Meeting of the Northeastern Section of the American Chemical Society

"Rare and Neglected Diseases"

A Medicinal Chemistry Symposium

Thursday, December 12, 2019

Sanofi

153 Second Avenue, Waltham, Massachusetts 02451

Meeting Agenda

3:00 pm Refreshments
3:15 pm Welcome, Raj Rajur, Medicinal Chemistry Program Chair, CreaGen, Woburn, MA
3:20 pm Introductory Remarks, Paul Greenspan, Takeda, Cambridge, MA
3:30 pm Maolin Yu, Ph.D., Goldfinch Bio, Cambridge, MA
Title: *Discovery of a potent and selective small molecule TRPC5 inhibitor, efficacious in focal segmental glomerulosclerosis model*
4:15 pm Brendan Crowley, Ph.D., Merck Research Laboratories, West Point, PA
Title: *Safer Oxazoldinone-class Antibiotics for the Treatment of Tuberculosis*
5:00 pm Susan Ashwell, Ph.D., Ra Pharmaceuticals, Cambridge, MA
Title: *Macrocylic Peptide Inhibitors of Complement C5 for the Treatment of Systemic and CNS immune Disorders*
6:00 pm Social Hour
6:45 pm Dinner
7:45 pm Keynote Presentation
Sungtaek Lim, Ph.D., Sanofi, Waltham, MA
Title: *Metachromatic Leukodystrophy: Targeting the CNS with Small Molecules*

Symposium Organizing Committee: Brian Aquila, Mark Ashwell, Scott Edmondson, Dan Elbaum, Jeremy Green, Paul Greenspan, Adrian Hobson, Blaise Lippa, Lisa Marcaurelle, Andrew Scholte, Kap-Sun Yeung, Raj (SB) Rajur

NESACS Board Meeting
The Board Meeting will be 4:30-5:30 pm in the Purdue Conference Room

YOU MUST REGISTER IN ADVANCE TO ATTEND THE SYMPOSIUM
THERE IS NO REGISTRATION FEE TO ATTEND THE MEETING;
DINNER RESERVATIONS ARE REQUIRED.
THE PUBLIC IS INVITED

- Dinner reservations should be made no later than 11:30 pm, Thursday, December 5, 2019. Reservations are to be made using Eventbrite: https://nesacs-rare-and-neglected.eventbrite.com Members, $30; Non-members, $35; Retirees, $20; Students, $10.
- If you wish to join us for this meeting and not eat dinner, please register by 11:30 pm, Thursday, December 5, 2019. Reservations are to be made using Eventbrite: https://nesacs-rare-and-neglected.eventbrite.com Select "Seminar Only."
- New Members or those seeking additional information, contact the NESACS administrative coordinator, Anna Singer, via email at secretary@nesacs.org

Directions to the Sanofi R&D Waltham Site

Please use 153 Second Avenue, Waltham, MA 02451 as the destination on your mapping program. From North or South 95/Rt 128. Take Exit 27B/Winter Street. Follow the signs for Second Avenue (stay in the right lane). Take a right after the Embassy Suites Hotel onto Second Avenue. Go past Costco on the right and at Bioverativ take a right. Proceed between Bioverativ garage and Bioverativ offices. (There is a sign directing you to Sanofi). Porceed about 100 feet. Sanofi will be on the right. Please enter through the main entrance (near stairs) and present yourself. ◇

Summer Scholar Report

The Role of Leaf-associated Transcription Factors (LTFs) in the Regulation of Vindoline Biosynthesis

Shannon A. McCallan[1], Lauren F. Cole[2], Lily Ha[2], Carolyn W.T. Lee-Parsons[3,4]
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Introduction

The medicinal plant *Catharanthus roseus* produces the anticancer compounds, vinblastine (0.0002% by weight) (1) and vincristine, from the precursors, vindoline and catharanthine. The pathway for vindoline biosynthesis was recently mapped (2); starting with the compound tabersonine, the pathway consists of 7 steps: T16H2, 16OMT, T3O, T3R, NMT, D4H, and DAT (Figure 1). Unlike catharanthine, the expression of the vindoline pathway genes is found exclusively in young leaves (3) and is not present in the roots (4)(5). This localized vindoline production prevents vinblastine extraction from any other part of the plant other than the leaves. Understanding the regulation of the vindoline pathway and why it preferentially occurs in leaves may allow for transfer of vindoline production to a more industrially efficient culture system, such as cell suspensions or hairy root cultures.

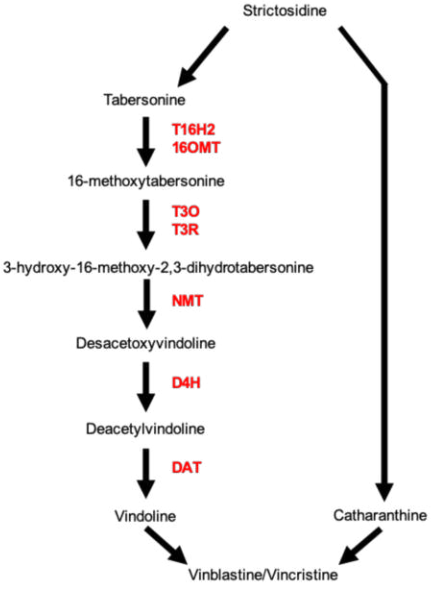

Figure 1: Strictosidine is the precursor of both vindoline and catharanthine biosynthesis. Each of the 7 vindoline pathway genes is highlighted in red. The condensation of vindoline and catharanthine leads to the production of vinblastine and vincristine.

According to a correlation analysis based on *C. roseus* RNAseq data, the expression of a class of transcription factors we dubbed LTFs (Leaf-associated Transcription Factors) was highly correlated to that of the 7 vindoline pathway genes, but not to that of the pathway leading to tabersonine, which is found throughout the plant (3) (6). We hypothesized that LTFs activate the vindoline pathway genes, causing their expression and vindoline production only in the leaves. The goal of this project was to investigate the regulation of the vindoline pathway genes by LTFs through overexpression, silencing, and transactivation experiments.

Methods:

Cloning Overexpression Plasmids

According to the correlation analysis, LTFs 8, 9, 11, and 15 were most closely correlated to the vindoline pathway genes (6). Thus, the coding sequences for each LTF were amplified from *C. roseus cDNA* and transferred to an overexpression plasmid. The expression of the LTF sequences was driven by the constitutive 2x35S promoter. The GUS reporter gene was also overexpressed using the 2x35S promoter as a control; this protein has no effect on the vindoline pathway.

Virus Induced Gene Silencing (VIGS)

VIGS is a method that relies on post-transcriptional gene silencing to decrease the expression of the gene of interest (i.e. LTF8 and LTF9). A plasmid containing the Tobacco Rattle Virus (TRV) machinery (pTRV1) and a plasmid encoding a fragment of the LTF coding sequence (pTRV2) were each transformed into *Agrobacterium tumefaciens* (GV3101). These two plasmids were then co-infiltrated into *C. roseus* to silence each LTF, as adapted from Liscombe & O'Connor (7). This viral system transcribes the LTF sequence into double-stranded DNA, which is recognized by the plant as foreign and diced into short-interfering RNA (siRNA). The siRNA is incorporated into the RNA induced silencing complex (RISC), which degrades mRNA complementary to the siRNA throughout the plant. This mechanism effectively silences the target gene (i.e. LTF8 and LTF9).

Cloning Vindoline Pathway Promoters

Modular Cloning (MoClo) is a plasmid construction system which allows various parts, such as promoters, coding sequences, and terminators, to be combined and rearranged by overlapping fusion sequences. Through the "domestication" process, the T16H2, 16OMT, T3O, T3R, NMT, D4H, and DAT promoters were first amplified in fragments from *C. roseus* genomic DNA (gDNA) to mutate their native Type-II restriction sites since these restriction enzymes are necessary for moving sequences into subsequent higher-level plasmids. The promoter fragments were then assembled in their own "Level 0" (L0) vector. Each promoter L0 vector was combined with other L0 vectors containing the firefly luciferase coding sequence (F-Luc) and the constitutive *Nos* terminator, to produce a L1 vector. This L1 vector contained a transcription unit (TU), with the pathway promoter driving F-Luc. Each L1 vector was combined with another L1 vector containing a *Renilla* luciferase (R-Luc) driven by a constitutive promoter; the R-Luc acts as a reference reporter in promoter transactivation assays. The result was 7 plasmids containing

continued on page 7

Summer Scholar Report

Continued from page 6

the pathway promoter driving F-Luc, and the reference reporter on the same plasmid, in opposite directions.

These plasmids will be used in promoter transactivation assays. Because each pathway promoter is driving F-Luc, the luciferase enzyme will be expressed if the promoter is activated by an added factor. Protein isolation and administration of substrate d-luciferin allows for luminescence, which is measured and corresponds to the level of promoter activity. Such an assay demonstrates regulation of the pathway promoters.

Transformation of Plasmids into *C. roseus* via EASI

Efficient *Agrobacterium*-mediated Seedling Infiltration (EASI) is a transient expression method in seedlings developed by the Lee-Parsons Lab (8). *Agrobacteria* were transformed with the plasmids of interest (i.e. GUS control, LTF overexpression (OE), or LTF-OE combined with the vindoline pathway promoter driving firefly luciferase gene); *C. roseus* seedlings were transformed with these *Agrobacteria* strains via vacuum infiltration. Transformed seedlings were planted in Gamborg's B5 full strength media without added sucrose in clear petri dishes and incubated in periods of light and dark to allow for transient expression of the LTFs. Cotyledons were harvested for RNA extraction and qPCR analysis. GUS staining was performed with the GUS-OE seedlings, as this enzyme converts a colorless substrate to a blue-colored compound which can be visualized and serves as an indicator that the plant transformation was successful.

RNA Extraction and qPCR

Three days after infiltration, the transformed cotyledons were flash-frozen in liquid nitrogen and stored at -80°C. Homogenization of the tissue using glass beads was followed by RNA extraction using RNAzol and column purification. RNA was reverse transcribed into cDNA using reverse transcriptase, which was used as template in a quantitative PCR reaction with SYBR green dye and designed primer pairs for each pathway gene (7). qPCR was used to measure the resultant levels of vindoline pathway expression in response to overexpression and silencing of LTFs.

Results:

LTF overexpression resulted in inconsistent effects on vindoline pathway gene expression

LTF8 and LTF9 were transiently overexpressed in *C. roseus* seedings and the effect on the expression of the vindoline pathway genes was monitored. GUS staining of the GUS transformed seedlings within the same experiment confirmed that the vacuum infiltration was successful. GUS was overexpressed throughout the seedlings in all LTF overexpression experiments.

Figure 2: GUS transformed seedlings (shown as blue stained) demonstrate successful transformation.

In the first two overexpression experiments (LTF-OE 1 and 2), LTF8 overexpression was confirmed by qPCR measurement. In comparison with the GUS overexpression control, LTF8 expression was successfully increased up to 30-fold, as seen in Figure 3.

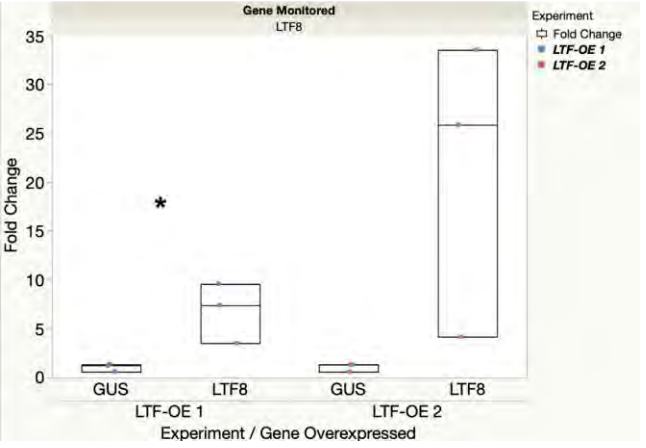

Figure 3: The expression levels of LTF8 in the LTF overexpressed condition versus the GUS control. There is significant overexpression of LTF8 (indicates P=0.040, determined by t-test).*

Given the hypothesized relationship, overexpression of LTFs in *C. roseus* should lead to an increase in transcript levels of the vindoline pathway. In Experiment 1, both D4H and NMT genes were upregulated with LTF8 overexpression (Figure 4). Similar upregulation was observed with other vindoline genes and LTFs (not shown). In Experiment 2, this upregulation was not observed (Figure 4).

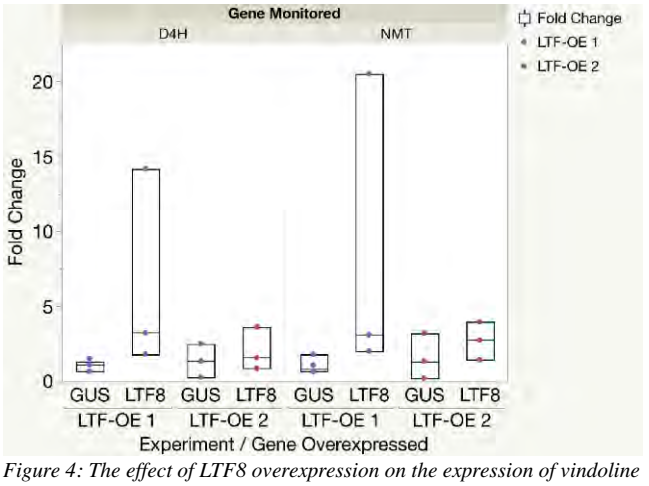

Figure 4: The effect of LTF8 overexpression on the expression of vindoline pathway genes (D4H and NMT). Upregulation of pathway expression is observed in LTF-OE 1.

Jasmonate may be necessary for the activation of the vindoline pathway genes by LTFs

The transformed seedlings were noticeably unhealthier in Experiment 1 than in Experiment 2. Jasmonate is produced in *C. roseus* during wounding, so we hypothesized that the jasmonate concentration was higher in the unhealthy seedlings

continued on page 8

Summer Scholar Report

Continued from page 7

of Experiment 1, allowing LTFs to induce the vindoline pathway. To replicate the conditions observed in Experiment 1, seedlings were vacuum-infiltrated with the plasmid-containing *Agrobacteria* followed by methyl jasmonate (100 uM MeJa) treatment. In Experiment 3**,** LTF and pathway gene expression were measured with and without MeJa. Figure 5 shows that the overexpression of LTF8 was successful.

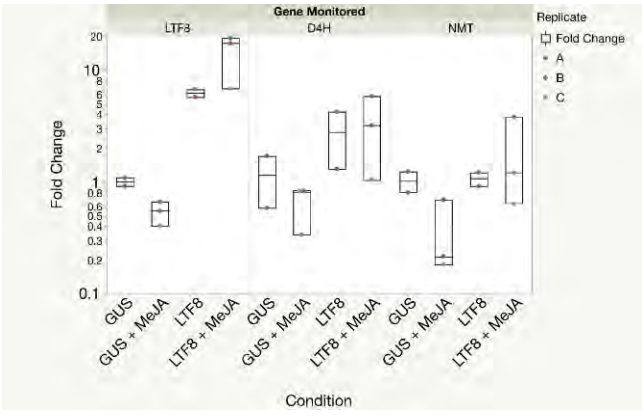

Figure 5: The effect of LTF8 overexpression on the expression of vindoline pathway genes (D4H and NMT) with and without MeJa. All values are normalized to GUS without MeJa.

Similar to Experiment 2, there was no upregulation of D4H and NMT expression with LTF8 overexpression in the absence of MeJa. However, there was an increase in D4H and NMT expression with LTF8 overexpression in the presence of MeJa. For example, D4H increased about 4-fold while NMT increased about 5-fold when LTF8 was overexpressed in the presence of MeJA. The expression of the other pathway genes also increased with LTF in the presence of MeJA (data not shown). These results support the hypothesis that LTFs positively regulate vindoline pathway gene expression. Interestingly, the addition of MeJa decreased LTF and vindoline pathway expression. It is possible that vindoline pathway gene expression decreased in the presence of MeJa due to decreased LTF expression. Overall, MeJa seemed to decrease LTF and vindoline pathway expression, but when LTF was overexpressed, an increase in pathway expression was observed.

Double silencing of LTF8 and LTF9 did not impact vindoline pathway gene expression

Given the hypothesized relationship, silencing of LTF8 and LTF9 expression in *C. roseus* by VIGS should lead to a decrease in transcript levels of the vindoline pathway. Figure 6 shows significant silencing of LTF8; LTF9 was also significantly silenced (not shown). However, under the LTF silenced condition, NMT and D4H expression showed no downregulation.

Successful cloning of the vindoline pathway promoters will aid in future experiments

The promoter of each pathway gene was amplified in parts from *C. roseus* gDNA, combined, and cloned to drive firefly luciferase (F-Luc) gene expression. Co-infiltration of the LTF overexpression plasmid and the vindoline pathway promoter driving F-Luc plasmid into seedlings would allow for studying potential transactivation of the promoter by the LTF. Luminescence would increase and indicate activation of the pathway promoter by the LTF. Given the hypothesized relationship, the overexpressed LTFs should activate the pathway promoters, either directly by binding the promoters or indirectly by interacting with a secondary factor.

This assay could not be performed within the timeframe, as problems arose in cloning the promoter. The MoClo system used to construct the promoter plasmids requires cutting via BsaI and BpiI restriction enzyme sites. Thus, native sites within the promoters must be removed via mutation and amplification in fragments. For instance, the NMT promoter was divided and amplified in 3 fragments due to 2 BsaI sites. After failed attempts to amplify the T3O promoter, we hypothesized that our genomic sequence (derived from the "Little Bright Eyes" cultivar) differed from the reference genome that was available from which the primers were designed (derived from the "Sunstorm Apricot" cultivar). However, the *C. roseus* reference transcriptome is derived from Little Bright Eyes (3), and although promoter sequences are not part of the transcriptome (as they are not transcribed), the genes immediately downstream of the promoters are. A primer was designed in this location and this fragment was successfully amplified. Difference in sequences between the two cultivars was confirmed by its sequencing, explaining our earlier failure to amplify the promoter. Using this correct sequence, primers were designed to successfully amplify the promoter. All of the pathway promoters have successfully been cloned into their respective L2 plasmids and are prepared for promoter transactivation assay. These plasmids can be used to detect activation of the pathway promoters by LTFs and other candidate transcription factors, enabling a faster screening process.

Discussion

Controlling the light environment is important when studying the vindoline pathway

The variability in light exposure could potentially affect the expression of the vindoline pathway genes and introduce variations between experiments, such as that observed between Experiment 1 and 2. To reduce these variations, we established a consistent incubation period of dark (48 hours) followed by

continued on page 9

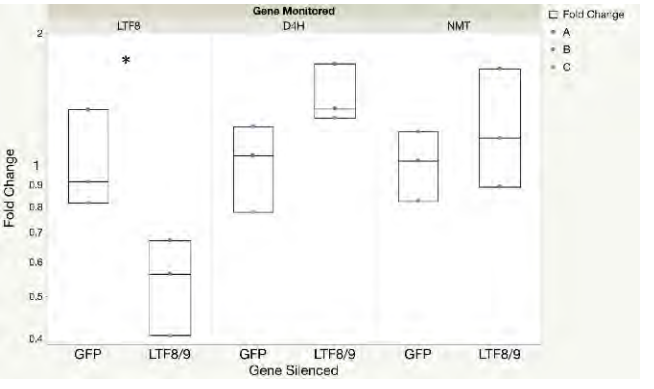

Figure 6: LTF8 was significantly silenced compared to a GFP control (indicates P=0.039, determined by t-test).*

Summer Scholar Report

Continued from page 8

a consistent period of continuous light (24 hours). Liu et al. (2019) showed that the highest expression of most of the pathway genes occurs after 24 hours of light exposure; thus, harvesting at this time point ensures that light is not limiting expression and that plants have fully recovered from the dark period (10).

Jasmonate appears to affect the activation of the vindoline pathway by LTFs

In Experiment 1, transformed seedlings appeared unhealthy (wilted and browning) and showed upregulation of the vindoline pathway genes, while in Experiment 2, seedlings were healthy and showed no upregulation. Jasmonate is produced in *C. roseus* during wounding, so we hypothesized that jasmonate was present at higher concentrations in the unhealthy seedlings of the Experiment 1. The potential effect of jasmonate on the activity of the LTFs is shown in Figure 7. MeJa degrades JAZ so that the co-activating factor (CF) can bind LTFs and therefore activate vindoline pathway expression; this model potentially explains the upregulation of Experiment 1. In Experiment 3, GUS control and LTF-OE conditions with

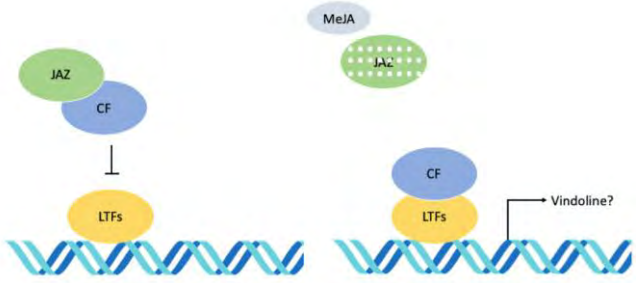

Figure 7: In the absence of MeJa, JAZ is hypothesized to bind and inhibit the CF. In the presence of MeJa, JAZ is degraded and the released CF coactivates LTFs (Produced by Lily Ha).

and without MeJa were designed to test this hypothesis. Based on the model shown in Figure 7, the addition of MeJa in LTF-OE seedlings was expected to induce pathway upregulation. When LTF8 was overexpressed in the presence of MeJA, there was an increase in vindoline pathway gene expression. Interestingly, in the GUS controls, MeJa was shown to decrease LTF and vindoline pathway expression. In Arabidopsis, a decrease in LTF expression was also observed with the addition of MeJa (11). We expected MeJa to increase vindoline pathway expression (12), as explained in Figure 7 resulting from the activation of LTF by the released CF.

LTFs are likely to be redundant

Simultaneous silencing of LTF8 and LTF9 did not lead to a decrease in pathway expression as hypothesized. This lack of decrease may be a result of LTF redundancy. Even though LTF8 and LTF9 were silenced, other LTFs could activate the vindoline pathway genes; no decrease in pathway expression would be observed if other LTFs compensated.

In summary, LTFs are promising candidates in regulating vindoline pathway expression. Additional LTFs will be studied and the experiments conducted and repeated under controlled light and MeJA environments. Promoter transactivation assays will be performed to determine direct regulation of the vindoline pathway by LTFs and other candidates.

References:

1. Noble, R. L. (1990) The discovery of the vinca alkaloids - Chemotherapeutic agents against cancer. in *Biochemistry and Cell Biology*, 10.1139/o90-197
2. Qu, Y., Easson, M. L. A. E., Froese, J., Simionescu, R., Hudlicky, T., and De Luca, V. (2015) Completion of the seven-step pathway from tabersonine to the anticancer drug precursor vindoline and its assembly in yeast. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 6224–9
3. Góngora-Castillo, E., Childs, K. L., Fedewa, G., Hamilton, J. P., Liscombe, D. K., Magallanes-Lundback, M., Mandadi, K. K., Nims, E., Runguphan, W., Vaillancourt, B., Varbanova-Herde, M., DellaPenna, D., McKnight, T. D., O'Connor, S., and Buell, C. R. (2012) Development of Transcriptomic Resources for Interrogating the Biosynthesis of Monoterpene Indole Alkaloids in Medicinal Plant Species. *PLoS One*. 10.1371/journal.pone.0052506
4. Shanks, J. V., Bhadra, R., Morgan, J., Rijhwani, S., and Vani, S. (1998) Quantification of metabolites in the indole alkaloid pathways of *Catharanthus roseus*: Implications for metabolic engineering. *Biotechnol. Bioeng.* 10.1002/(SICI)1097-0290(19980420)58:2/3<333::AID-BIT35>3.0.CO;2-A
5. Pan, Q., Mustafa, N. R., Tang, K., Choi, Y. H., and Verpoorte, R. (2016) Monoterpenoid indole alkaloids biosynthesis and its regulation in *Catharanthus roseus*: a literature review from genes to metabolites. *Phytochem. Rev.* **15**, 221–250
6. *Prepared by collaborators Jie Wang and Kevin Childs (Michigan State University).*
7. Liscombe, D. K., and O'Connor, S. E. (2011) A virus-induced gene silencing approach to understanding alkaloid metabolism in *Catharanthus roseus*. *Phytochemistry*. **72**, 1969–1977
8. Mortensen, S., Bernal-Franco, D., Cole, L. F., Sathitloetsakun, S., Cram, E. J., and Lee-Parsons, C. W. T. (2019) EASI Transformation: An Efficient Transient Expression Method for Analyzing Gene Function in *Catharanthus roseus* Seedlings. *Front. Plant Sci.* **10**, 1–17
9. Kellner, F., Kim, J., Clavijo, B. J., Hamilton, J. P., Childs, K. L., Vaillancourt, B., Cepela, J., Habermann, M., Steuernagel, B., Clissold, L., Mclay, K., Buell, C. R., and O'Connor, S. E. (2015) Genome-guided investigation of plant natural product biosynthesis. *Plant J.* **82**, 680–692
10. Liu, Y., Patra, B., Pattanaik, S., Wang, Y., and Yuan, L. (2019) GATA and Phytochrome Interacting Factor Transcription Factors Regulate Light-Induced Vindoline Biosynthesis in *Catharanthus roseus*. *Plant Physiol.* **180**, 1336–1350
11. Hickman, R., Van Verk, M. C., Van Dijken, A. J. H., Mendes, M. P., Vroegop-Vos, I. A., Caarls, L., Steenbergen, M., Van der Nagel, I., Wesselink, G. J., Jironkin, A., Talbot, A., Rhodes, J., De Vries, M., Schuurink, R. C., Denby, K., Pieterse, C. M. J., and Van Wees, S. C. M. (2017) Architecture and dynamics of the jasmonic acid gene regulatory network. *Plant Cell*. 10.1105/tpc.16.00958
12. Liscombe, D. K., Usera, A. R., and O'Connor, S. E. (2010) Homolog of tocopherol C methyltransferases catalyzes N methylation in anticancer alkaloid biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1009003107 ◇

The Commonwealth of Massachusetts

A Proclamation

Whereas, The American Chemical Society, the largest scientific society in the world, has designated the week of October 20-26 as National Chemistry Week to promote the value of chemistry in everyday life; and

Whereas, Knowledge of the chemical elements are central to improving the human condition and addressing the world's great challenges related to water, food, health, security and energy; and

Whereas, Over 5000 residents of the Commonwealth of Massachusetts are members of the Northeastern Section of the American Chemical Society and hundreds of these members participate annually in public outreach activities during National Chemistry Week; and

Whereas, The Commonwealth of Massachusetts is a national leader in bringing the benefits of science and chemical science to the world; and

Whereas, It is critical that the brightest young minds continue to be attracted to chemistry and other branches of science in order to ensure the next generation of scientists, engineers and innovators,

Now, Therefore, I, Charles D. Baker, Governor of the Commonwealth of Massachusetts, do hereby proclaim October 20 – 26, 2019, to be,

National Chemistry Week in Massachusetts

And urge all the citizens of the Commonwealth to take cognizance of this event and participate fittingly in its observance.

Given at the Executive Chamber in Boston, this first day of October, in the year two thousand and nineteen, and of the Independence of the United States of America, the two hundred and forty-third.

By His Excellency

Charles D. Baker
Governor of the Commonwealth

Karyn E. Polito
Lt. Governor of the Commonwealth

William Francis Galvin
Secretary of the Commonwealth

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Calendar

Check the NESACS home page for late Calendar additions: http://www.NESACS.org

Note also the Chemistry Department web pages for travel directions and updates.
These include:
http://www.bc.edu/schools/cas/chemistry/seminars.html
http://www.bu.edu/chemistry/seminars/
http://www.brandeis.edu/departments/chemistry/events/index.html
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http://www.uml.edu/Sciences/chemistry/Seminars-and-Colloquia.aspx
http://www.unh.edu/chemistry/events
https://www.wpi.edu/academics/departments/chemistry-biochemistry

December 3

Prof. Minjung Son (MIT)
MIT, Rm 34-401, 12:00 pm

Prof. Emily Weiss (Northwetern Univ.)
Selective Triplet-Initiated Intermolecular Cycloadditions Photocatalyzed by Quantum Dots
MIT, Rm 6-120, 4:00 pm

December 5

Prof. Mark D. Allendorf (Sandia Nat. Lab)
Nanopores, Nanoparticles, and "Molecular Hydrides" for Hydrogen Production, Transport, and Storage
Boston College, Merkert 130, Time TBA

Prof. David Powers (Texas A&M)
"Development of High Valent Aerobic Oxidation Catalysis."
Harvard, Pfizer Lecture Hall, 4:15 pm

December 6

Prof. Jarad Mason (Harvard)
UMass Lowell, Olney 218, 3:30 pm

December 9

Prof. Daniel Nomura (UC Berkeley)
MIT, Broad Inst., Rm 1154, 4:00 pm

Prof. Thomas Poulsen (Aarhus University)
"Chemistry and Biology of Freak Electrophiles and Hubrid Ionophores."
Harvard, Pfizer Lecture Hall, 4:15 pm

December 10

Prof. Kang-Kuen Ni (Harvard)
MIT, Rm 34-401, 12:00 pm

Prof. Matthias Waegele (Boston College)
MIT, Rm 6-120, 4:00 pm

December 11

Prof. Jonas C. Peters (Caltech)
Harvard, BioLabs #1080, Divinity Ave., 4:15 pm

December 20

Prof. Hosea Nelson (UCLA)
MIT, Rm 6-120 4:00 pm

Notices for The Nucleus Calendar of Seminars should be sent to: Samurdhi Wijesundera, Email: samu.amameth@gmail.com ◇