Narrative on progress from FAPESP-SPRINT 2015 Project “The auto-regulatory loop involving miR828, TAS4, and target MYB transcription factors: basic and applied studies” by Rock, Maia, and Domingues.

Biofuels and agriculture are areas where Brazil has excelled in recent years in terms of high-impact research as a result of sustained investment in public research universities, graduate education, and research institutes. This project is focused on functional genomics to control polyphenolic secondary metabolite production in crops. Polyphenolics are important for biofuels and human health, as they constitute the large classes of molecules that confer properties to fibers important to construction and textiles and ‘nutraceuticals’ with anti-lipidemic, anti-proliferative, and anti-oxidant salutary effects on cardiovascular function, cancer prevention, and neurological processes. *MICRORNA828* and *TRANS-ACTING SMALL-INTERFERING RNA LOCUS4* are non-coding genes that negatively regulate polyphenolic biosynthesis in plants and are subject to an autoregulatory feedback loop by the myeloblastosis proto-oncogene *MYB* transcription factors they silence (1,2). Mitochondrial uncoupling proteins (UCP) dissipate the proton electrochemical gradient for production of ATP established by the respiratory chain, thus affecting energy status and modulation of reactive oxygen species (ROS) (3). Plant polyphenolics are important anti-oxidant stress response effectors impacting pathogen resistance, biofuels (lignin in wood), and human health. For example *trans*-resveratrol, abundant in berry skins, improves mitochondrial function, is a potent inhibitor of topoisomerase (validated cancer treatment target), and extends lifespan in yeast and mice.

In the *atucp3* mutant of Arabidopsis (a model plant for molecular genetics), the knockdown of the corresponding transcript is clearly compensated by an increase in the transcript levels of both *AtUCP1* and *AtUCP2*, suggesting the existence of a coordinated auto-regulatory feedback network for the three gene isoforms (4). This is not the case for the *atucp2* mutant in which compensatory expression is only observed under abiotic stress. Nevertheless, when compared with wild-type (WT) Col-0 plants, the *atucp2* plants exhibit phenotypic perturbations such as reduced number of leaves, delayed flowering time and elevated number of sterile siliques. Intriguingly, cellular ATP content is significantly increased in the *atucp2* mutant compared with the WT, a feature that is not observed in the *atucp3* mutant. On the other hand, both mutants are found to significantly accumulate more ROS within mitochondria as compared to WT, indicating that both UCPs contribute to ROS homeostasis. Overall, these recent results from the Brazilian collaborator Ivan Maia provide novel *in planta* insights into the role of individual AtUCPs. A Ph.D. student (Fakhrul Azad) in the Rock lab has generated pairwise double mutants of the *UCP1/2/3* knockout alleles provided by the collaborator. RNA-Seq transcriptome and small RNA libraries from sucrose- and abiotic stress-induced samples are being generated and characterized to identify the cross-regulatory networks and molecular mechanisms of transcriptional and post-transcriptional control bridging primary (*UCP*) and secondary (*MICRORNA828*) metabolism. The prospects for collaborations and training going forward with

Brazilian and regional colleagues working on citrus, coffee, sugarcane, grapevine, and banana continue to develop.

1. Luo Q-J, Mittal A, Jia F, Rock CD. (2012) "An autoregulatory feedback loop involving *PAP1* and *TAS4* in response to sugars in Arabidopsis." *Plant Mol. Biol*. 80: 117-129
2. Rock, CD (2013) *Trans-acting small interfering RNA4* : key to nutraceutical synthesis in grape development? *Trends Plant Sci.* 18:601-610
3. Vercesi AE, Borecký J, Maia IG, Arruda P, Cuccovia IM, Chaimovich H. (2006) Plant uncoupling mitochondrial proteins. *Annu. Rev. Plant Biol.* 57: 383-404
4. Maia I.G., Vasconcellos A, Barreto P, Rock C, Arruda P. "Characterization of Arabidopsis insertion mutants for the mitochondrial uncoupling protein (*UCP*) genes *AtUCP2* and *AtUCP3*." https://pag.confex.com/pag/xxv/webprogram/Paper24268.html

**Papers**

1) Rock, C.D. (2017) "Phenylpropanoid metabolism." In: *eLS* (Encyclopedia of Life Science). John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0001912.pub2.
2) Sunitha S, Loyola R, Alcalde JA, Arce-Johnson O, Matus JT, Rock CD. (submitted to *Frontiers in Plant Science*) "The role of UV-B light on microRNA activity during grapevine berry development."

This project is a solicited collaboration with the PI Rock by Chilean colleagues, with potential for expansion of the research network to Brazilian scientists and stakeholders interested in cross-border cooperation. The higher education systems in Brazil and Chile share a common interest in internationalization and expanding their global reach; by building on the connections established by individual scholars, institutional partnerships that endure beyond the scientific mobility program will be fostered. For example Bayer has an ongoing partnership with TTU (Project Revolution for cotton and soybean), and with Brazilian wineries and producers http://www.foodchainpartnership.cropscience.bayer.com/en/Brochures/SearchResults/Americas/the_brazilian_wine_grape_project.aspx

**Presentations**

1) (poster, international conference) Vasconcellos A, Basu S, Azad Md.F., Harmon C, Sukumaran S, Domingues D, Maia I, Rock CD. "Effects of uncoupling protein mutants (*ucp1-3*) on regulation of microRNA828 and anthocyanin biosynthesis in Arabidopsis." 11th International Plant Molecular Biology Congress. Oct. 24-30, 2015. Iguacu Falls, Brazil

2) (seminar by C. Rock) Dept of Genetics, Botucatu Institute of Biosciences, Sao Paulo State University-Botucatu, Brazil. Nov. 4, 2015. "Plant Polyphenolics, small RNAs, and Darwin's 'Abominable Mystery.'"

3) (seminar by C. Rock) State of Sao Paulo Institute of Agronomy, Division of Citriculture, Cordeiropolis, Brazil. Nov. 6, 2015. "A Role for MICRORNA828 and downstream noncoding

RNA effector TAS4 in polyphenolic nutraceutical biosynthesis in citrus, and implications for evolution of fruit traits in banana."

4) (poster #900-022, international conference) Dinghra A, Balasubramanian V, Basu S, Mendu V, Rock C. “The *Wilty4* mutant in maize- effect on vascular bundle cell wall S/G lignin composition.” Plant Biology 2016: Annual Meeting of the American Society of Plant Biologists. July 9-13, 2016. Austin, TX.

5) (poster #1000-063, international conference) Sukumaran S, Traore S, Azad Md.F, De La Fuente L, Rock C. “Conservation of an autoregulatory feedback loop regulating anthocyanin biosynthesis in dicots.” (this presentation included material results on coffee). Plant Biology 2016: Annual Meeting of the American Society of Plant Biologists. July 9-13, 2016. Austin, TX.

6) (poster #P0263, international conference) Maia I.G., Vasconcellos A, Barreto P, Rock C, Arruda P. “Characterization of Arabidopsis insertion mutants for the mitochondrial uncoupling protein (UCP) genes *AtUCP2* and *AtUCP3*.” International Plant and Animal Genome XXV Meeting. Jan. 14-18, 2017. San Diego, CA.

7) (poster, international conference) Nair D, Rock C. “Studies on microRNAs in select species of Zingiberales, Liliales, and Arecales: monocot orders that develop fleshy fruits.” Phenome 2017: Connecting the Bioeconomy. Feb. 10-14, 2017. Tucson, AZ.

8) (oral presentation by Ph.D. student) Dinghra A, Rock CD. "Cloning and characterization of the *Wi4* locus of maize and its role in vascular development and stress adaptation.” 8th Texas Tech Annual Biological Sciences Symposium, TTABSS. April 8, 2017, Museum of Natural History, TTU, Lubbock. Won second prize for best paper in Cell and Molecular Biology division.

## Proposals

1) ORS#15-1036, 06/1/15. “Genome editing of *MICRORNA828* and target effectors in sour orange and Mexican lime as a critical test of greening disease etiology.” C. Rock, PI. USDA-National Institute of Food and Agriculture Specialty Crop Research Initiative/Citrus Disease Research and Extension Emergency Response Program. $500,000, 02/1/15- 01/31/19. Declined.

2) ORS#16-0185, 10/23/15. “Advancing understanding of *MICRORNA828* and associated small interfering RNAs in polyphenolic synthesis and regulation in citrus, blueberry, and *Curcuma longa*.” C. Rock, PI. NIH Center for Complementary and Alternative Medicine R15. $421,561, 07/01/16- 06/30/19. Declined.

3) ORS#16-0904, 05/19/16. “Evaluation of *MICRORNA828* and downstream genes as a source of resistance to HLB within citrus genome.” C. Rock, PI; V. Ancona [TAMU], O. Alabi [TX-AgriLife], A. Brown [TTU Biology], R. Kreuger [USDA Riverside CA], Co-Is. Letters of international collaboration from A. Alves de Souza, Scientific Researcher, Sylvio Moreira Center for Citriculture, Instituto Agronomico de Campinas (IAC), Cordeirópolis, Sao Paulo, Brazil and H. Della Coletta Filho, Lab Head, Centro de Citricultura Sylvio Moreira - IAC, Cordeirópolis,

Sao Paulo, Brazil. USDA-National Institute of Food and Agriculture Specialty Crop Research Initiative/Citrus Disease Research and Extension Emergency Response Program. $700,000, 02/1/17- 01/31/20. Declined.

4) ORS# 16-1210, 08/11/16. “Cloning *Wilty* mutants of maize for translational improvements to drought tolerance and biomass production.” C. Rock, PI; V. Mendu [TTU PSS] sugarcane translational component. USDA-National Institute of Food and Agriculture Foundational Program. $500,000, 07/01/17- 06/30/20. Declined.

5) ORS# 17-0993, 06/28/17. “Cloning *Wilty* mutants of maize for translational improvements to drought tolerance and biomass production.” C. Rock, PI; V. Mendu [TTU PSS], sugarcane translational component in scope for collaboration with D. Domingues [São Paulo State University-Rio Claro, Brazil] USDA-National Institute of Food and Agriculture Foundational Program. $500,000, 05/01/18- 04/30/21. Pending.

6) ORS# 17-0875, 05/05/17. “A role for phosphate homeostasis and host microRNAs in Verticillium wilt of cotton.” C. Rock, PI. Important connection to ‘blue disease/leafroll dwarf virus/atypical vein mosaic’ of cotton, a major threat in Brazil and potential subject for collaboration. Like Verticillium wilt, there is no good model for cotton host resistance genes yet miR828 is implicated in blue disease and Vert wilt by pathologic symptoms of anthocyanin/lignin accumulations. USDA Cotton Incorporated TX State Support Committee. $51,975, 01/01/18- 12/31/18. Pending.