**SYLLABUS, BIO 328D: DISCOVERY LABORATORY IN PLANT BIOLOGY**

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**Brief Overview of Experiments**

As in past years, this year's Discovery Laboratory will emphasize learning methods of experimental design, data gathering, data interpretation, and data presentation. The novel experiments you will carry out this semester will constitute an original test of a relatively new hypothesis. This hypothesis predicts that when the constitutive expression of an ectoapyrase enzyme enhances the growth and seed production of soybeans it does so at least in part by modifying soybean root architecture and the uptake of nutrients by the roots.

# General Introduction on the apyrase enzyme and on its effects on growth

Although it may seem counter-intuitive, a variety of stimuli induce plant and animal cells to release some of their cellular ATP into their extracellular matrix (ECM). Extracellular ATP (eATP) and extracellular ADP (eADP) are known to act as hormones, and they are potent regulators of cell physiology in both animal and plant cells. To limit the [eATP], and to turn off signaling pathways induced by eATP, plant and animal cells use a variety of ECM-localized phosphatase enzymes, the most important of which are ectoapyrases. Ectoapyrases can hydrolyze extracellular ATP [eATP], and thus help maintain a low [eATP]. Inhibitors of apyrases can reduce the rate of eATP destruction, and thus promote an increase in [eATP]. Clearly the activity of ectoapyrase enzymes could influence the ability of eATP to induce signaling changes in plants.

In 1999, Thomas et al. showed that the constitutive expression (= overexpression) of a pea apyrase in seedlings of the model plant Arabidopsis thaliana enhanced their uptake of phosphate and promoted their growth. Then in 2012 Liu et al. showed that the constitutive expression of Arabidopsis apyrases in Arabidopsis promoted the transport of the growth hormone, auxin. More recently scientists at UT tested whether this enzyme could affect the growth of soybean plants. They found that the constitutive expression of a pea apyrase in soybeans enhanced their growth and seed production. However, the mechanism by which this enhancement was achieved is unknown. One hypothesis is that the overexpression of apyrase in soybeans could promote auxin transport, which would be expected to increase the nutrient absorption capability of these plants by promoting certain changes in root architecture. These auxin-induced changes would include an increase in lateral root production and increased production and growth of root hairs, which, in turn, would increase nutrient absorption. The main goal of this semester’s Discovery Laboratory is to test this novel hypothesis.

**Starting Point for the Course and Subsequent Rounds of Experiments This Semester**

The experiments you will do this semester are novel, and because they have not been carried out by anyone else before, a key challenge will be to develop by trial and error the best protocols for executing them. Each lab session will begin with a lecture from Prof. Roux that will provide necessary background information for you to understand the rationale for your experiments. After the lecture on Sept. 12, you will be asked to examine the root architecture of 10 soybean seedlings growing on agar in an orientation that will allow you to easily see and evaluate their root architecture. During this lab you will learn how to take clear digital images of the roots of young soybean seedlings, and compare the architectures (i.e., lateral roots, root hairs, other root features) of the transgenic plants that are overexpressing apyrase with those of wild-type plants. You will take digital images of the growing roots, and you will be instructed on how to analyze these images to evaluate root architecture. These experiments will mark the beginning of Round 1 of experiments. The results of this test will be reported and discussed after the lecture on September 19.

Each "Round" of experiments will consist of 3 lab sessions: two sessions of comparing the root architectures and/or nutrient uptake of wild-type and transgenic seedlings, and one session of data presentation and analysis, including statistical treatment to determine the significance of any differences noted. At the end of the first week of a Round, Groups should notify the TA what their plans are for the second week before noon on the Wednesday after the lab. At the end of each Round, students will react to the results obtained by refining the experimental design to get more detailed and/or more accurate data.

**References**

**Thomas, C., K. Naus, A. Lloyd, and S. J. Roux**. 1999. Apyrase functions in phosphate transport and mobilizes phosphate from extracellular ATP. *Plant Physiol*. **119**: 543-551.

**Liu X, Wu J, Clark G, Lundy S, Lim M, Arnold D, Chan J, Tang W, Muday GK, Gardner G, Roux SJ**. 2012. Role for apyrases in polar auxin transport in Arabidopsis. *Plant Physiol*. **160**: 1985-1995.

**The lab schedule for Spring 2015 will be:**

August 29 Introduction to the course and the experimental system; microscope training

September 12 Preliminary assays of root architecture to become familiar with the system. These

preliminary assays will mark the beginning of **Round 1** of experiments. Report

preliminary imaging results on September 19. In each lab session we hope you will

improve your observation methods and record new data. After each class, you will

tabulate the results of the measurement differences in root architecture between wild-

type and transgenic plants.

19 Carry out variations of Sept. 12 experiments.

26 Meet to discuss results. Propose second round of experiments to test alternative

hypotheses that may account for results of Round 1 experiments.

October 3 Begin **Round 2** of experiments, and continue them on **October 10.**

17 Meet to discuss results. Propose third round of experiments to test alternative

hypotheses that may account for results of second round. **Half of your Final Report\***

**is due.**

24 Begin **Round 3** of Experiments [three weeks, same procedure as first two rounds]

November 14 Begin **Round 4** of experiments [3 weeks, same procedure as first 3 rounds]

December 5Open Book test**; Final Reports\* due**

**\*The instructions for the Final Report will be discussed during class. A document with a description and an example report will be posted on Canvas *by September 5.***

## INSTRUCTIONS FOR WRITTEN & ORAL REPORTS FOR BIO 328D

Each team will present one paper and one oral report for each experiment. One member will write the written report and the other member will present the oral report, so each student will do two written and two oral reports during the semester. Teams should work together when preparing reports. Written reports for each Round are due by noon of the presentation day.

**Each written Lab Report should include**:

1. A Cover page with date and names of team members (place an asterisk next to the author's name), a title for the report, and a VERY brief (less than 250 words) description of the experiment done. The description should focus on the hypothesis tested and results.

2. On p. 2 should be a bar graph to describe the results. Each bar in the graph should give the standard deviation around the mean value, with a different letter above bars that differ significantly from each other. 3. On p. 3 there should be two Tables: **Table I** should show the raw data in columns including:

The treatment being measured

The range of values measured

The mean of the values measured

The standard deviation of the values measured

The number of samples measured

**Table II** should describe the Student t-test-comparison being measured, the t-value measured for that comparison and a column stating the level of significance. Note that Level of Significance values above 0.05 are considered insignificant. Each Table and Graph should have a figure legend.

**Each Oral Report should:**

1. Be 10 min or less.
2. Include an introduction that gives some background information & specifies the hypothesis being tested.
3. Show (as Powerpoint slides) and discuss the summarized table & graph results.

Interpret the results and present what might be the next experiment.

L**AB COURSE GRADING\***

**2 ORAL REPORTS: 25**

**2 WRITTEN REPORTS: 25**

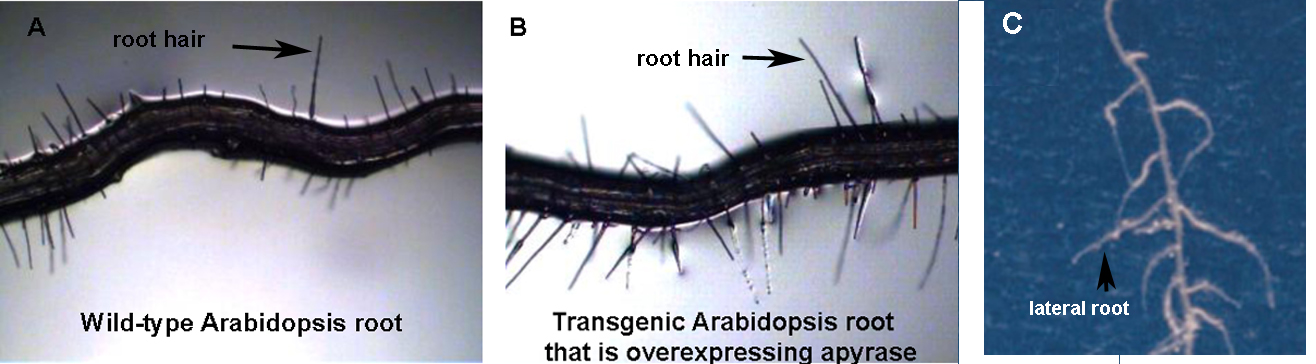
**Final exam 15**

**Final Report 20**

**Overall performance \*\* 15**

**\***Note that this course carries the Independent Inquiry flag. Independent Inquiry courses are designed to engage you in the process of inquiry over the course of a semester, providing you with the opportunity for independent investigation of a question, problem, or project related to your major. Therefore, a substantial portion of your grade will come from the independent investigation and presentation of your own work.

**\*\***The success of this laboratory experience, as is true for the success of all original research, will depend strongly on how much thought and consultation time you put into your project. Although Dr. Roux and the TA cannot predict the outcome of your experiments, we can surely help you think about your results, solve technical problems that you may encounter, and evaluate options for the new experiments you will plan. The research you will do this semester is original and could generate potentially exciting results. We ask you to take on both the joy and responsibility of discovering new insights about how plants grow and develop, and we hope you will enjoy this collaboration with us and with your student colleagues. *On-time attendance is a key factor considered in determining the Overall Performance grade. Active participation in the design and execution of your experiments and in the discussion sessions at the end of each round will enhance your Overall Performance grade; and, of course****, surfing the web, texting****, or other signs of inattention during the labs or discussion sessions will significantly hurt your performance grade.*



The image above shows features of Arabidopsis root architecture, including root hairs (A, B) and lateral roots (C)

# Evaluation of Written and Oral Reports Each report will be graded based on the following criteria:

Written Reports:

* Paragraphs are organized logically
* Checked for grammatical and spelling errors
* Figures are labeled correctly
* Sentences are clearly written and easy to understand
* Text is written in proper tense, clear and concise
* Used the layout described in Dr. Roux’s syllabus
* Cited sources using a standard format

Oral Reports:

* Slides are organized logically
* Presentation is clear and concise
* Gives a good rationale and provides evidence
* Attractive and balanced layout, legible font
* Facilitates discussion and is receptive to feedback
* Can identify what to do in the next experiment
* Cited sources using a standard format