

Expression of *RBCS* genes in *Arabidopsis thaliana*

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week #1 (12 February)

week #2 (19 February)

The *RBCS* gene family encodes the small subunit of the enzyme RUBISCO, which is responsible for catalyzing photosynthetic carbon fixation in the Calvin cycle. It is essential for a plant to produce the right amount of RUBISCO at the right time and place in order to correctly carry out photosynthesis. One of the ways that the amount of RUBISCO production is controlled is through regulation of the amount of *RBCS* mRNA that is present.

Even without having any prior knowledge about how *RBCS* gene expression is regulated, there are a number of factors that we might reasonably expect to be involved. It might make sense to hypothesize that the *RBCS* gene family is developmentally regulated such that it is more highly expressed at certain stages of the plant's life than at others. We might also suspect that the amount of *RBCS* gene expression might differ between tissues (i.e. roots vs. seeds vs. leaves). One might also wonder whether environmental factors such as the time of day or the amount of light available could have a significant effect on *RBCS* gene expression.

Your job will be to design an experiment in which you will investigate some aspect of *RBCS* gene expression in *Arabidopsis thaliana* plants. There are a wide variety of possibilities, so choose whatever type of experiment you think will be most interesting. You might wish to find out when and where *RBCS* mRNA is most abundant. Is it present in equal amounts throughout the entire life cycle or is it present only at a certain stage? What organs or tissues have the most *RBCS* mRNA. It is also possible to investigate environmental factors that you suspect may affect *RBCS* gene expression. Plants could be subjected to drought, flooding, high or low light intensity, a lack of nutrients, etc., etc.

Logistics and timetable. You and your lab partner will plan out by your experiment during lab on **12 February**. You will need to produce a written research proposal describing what question(s) you wish to address, how you will carry out the experiments, what samples you will test, and what controls you will need. Your experiment should be set up so that it will involve isolating RNA from a total of 6 samples, including all necessary controls. Turn in your proposal by the end of lab. Detailed instructions for the format of your Research Proposal may be found on page 7. After reading over your proposal, I may make some suggestions or require some changes before approving a final draft of your proposal. Once your proposal is approved, you may begin work on your experiment.

During the **week of 19 February** you will carry out the treatment of your plants and the collection of your samples. This will involve subjecting your plants to whatever treatments are appropriate and collecting the six tissue samples that you will use for RNA isolation. When doing your sample collections, obtain 0.2 g of each sample that you wish to assay for *RBCS* mRNA. Quickly place the tissue into a 1.5 ml tube, close the lid and toss the tube into liquid nitrogen. The samples should be stored frozen until you are ready to use them. It is important that tissue samples be frozen very quickly after removal from the plant as damage to RNA may occur once tissue is wounded or damaged by removal from the plant.

During lab on 26 February you will isolate RNA from your samples. Work very carefully to make sure that you obtain a good yield of clean RNA from each of your six samples.

During the following two weeks you and your partner will determine how much *RBCS* mRNA is present in each RNA sample.

USEFUL REFERENCES

Busby JS, Yamada T, and Tobin EM (1990) A light-regulated DNA-binding activity interacts with a conserved region of a *Lemna gibba* *RBCS* promoter. *Plant Cell* 2:805-814.

Manzara T, Carrasco P, and Grussem W (1991) Developmental and organ-specific changes in promoter DNA-protein interactions in the tomato *RBCS* gene family. *Plant Cell* 3:1305-1316.

Wanner SF and Grussem W (1991) Expression dynamics of the tomato *RBCS* gene family during development. *Plant Cell* 3:1289-1303

INSTRUCTIONS FOR RESEARCH PROPOSAL PREPARATION

Your research proposal should clearly describe what question(s) you are trying to answer, why your experiment is relevant or important, and how you plan to carry out the experiment. Your research proposal should be divided into the following three sections.

INTRODUCTION

A paragraph or two briefly summarizing the current state of knowledge on the subject that your research intends to address. You might close this section with a mention of a still unresolved issue that you want to investigate.

RESEARCH OBJECTIVES

This section should state the question that you intend to answer or the hypothesis that you intend to test. Clearly describe the type of experiment(s) that you will carry out. Describe, for example, the types of treatments that you plan to subject the plants to, what samples will be collected, when they will be collected, etc. What type of control(s) will be needed for your experiment?

REQUIRED MATERIALS

Describe any chemicals, supplies or equipment you will need that are NOT mentioned in the protocols of your lab handouts. Also indicate what age of *Arabidopsis* plants you will need, and how many plants will be needed.