CELL SIZE REGULATION AND THE TNY1 PROTEIN

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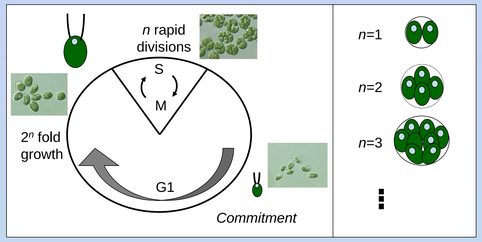
The Umen Lab in the Plant Biology Department of the Salk Institute for Biological Studies uses the algae *Chlamydomonas reinhardtii* as a model system to understand how cells control their size, and therefore, how they coordinate growth and division to ensure optimum cell size. Several characteristics such as their single-celled structure, haploid nature, sequenced genome, and the genetic and molecular tools available make "Chlamy", as they are affectionately known, ideal specimens for studying cell size control. Work in the Umen lab has shown that the Chlamydomonas Retinoblastoma tumor suppressor protein (RB) plays a key role in size homeostasis, while genetic screens have also identified other proteins involved in the RB size control pathway. A protein of interest is the RNA binding protein TNY1, that when disrupted, leads to deregulated cell division and small daughter cells.



Chlamy divide by means of a multiple fission cell cycle containing two size checkpoints that prevent cells from dividing if they are too small (commitment checkpoint) and a second checkpoint that controls the number of divisions during mitosis.

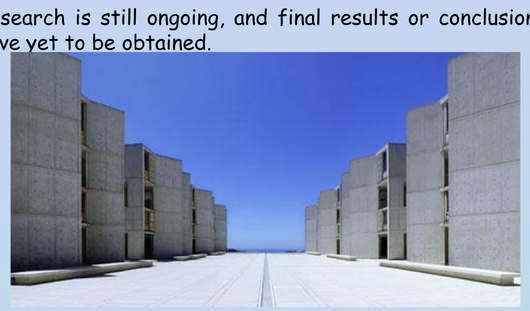
Projects for Summer 2010

Work under the guidance of post doc Cristina Lopez Paz and assist with her research involving Chlamydomonas. In the Umen lab, I will work with *tny1,* an insertional mutation that causes the cells affected to exhibit a smaller size phenotype than their wildtype counterparts. Current experiments suggest that TNY overexpression causes a large cell size phenotype, while a lack of it creates a small size.

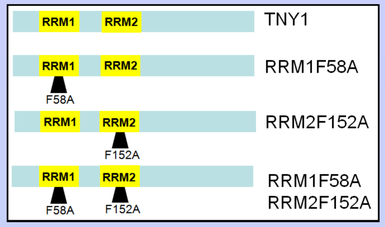
I will mainly work with the following two experiments:

1. Clone mutated versions of TNY1 into a Chlamy expression vector to overexpress and see what phenotype will result when the cells overexpress these mutations.
2. Transform Chlamy with artificial microRNAs that will hopefully silence the TNY1 gene, and create a smaller cell size.

Research is still ongoing, and final results or conclusions have yet to be obtained.



Cloning mutated versions of TNY1 for overexpression in Chlamydomonas



Work is being done to clone mutated versions of TNY1 into a Chlamydomonas expression vector. These point mutations are in the RRM motifs as shown in the above figure. *In vitro* experiments have shown that these specific amino acids are important for RNA binding. The mutations are cloned into the pEZ Chlamydomonas vector, and will be transformed into wild type cells that will overexpress the mutated forms.

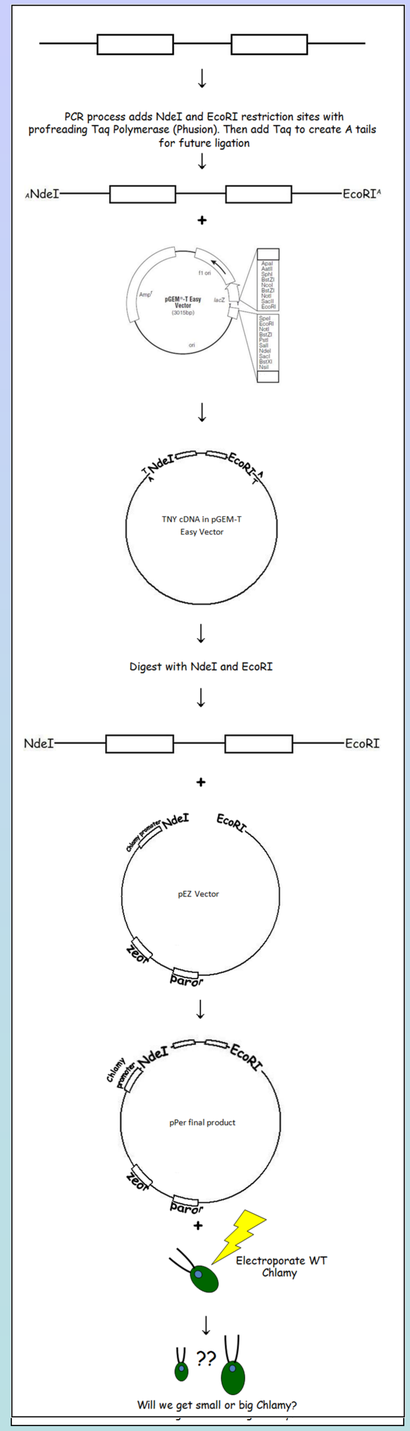
Facts:

- In wild type cells, TNY1 disruption results in a small cell size phenotype, whereas TNY overexpression produces a large cell size phenotype.

What I hope to figure out:

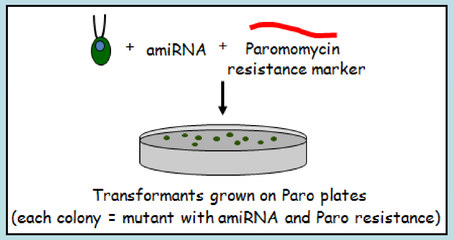
- Are TNY1 levels important in controlling cell size? By transforming Chlamydomonas with artificial microRNAs designed to knockdown TNY, we expect to obtain transgenic lines with decreased levels of TNY.
- Will the overexpression of the mutated forms of TNY1 result in a large size phenotype like the WT overexpression?

TNY1 Cloning Process



TNY1 Controlling cell size

In order to show that TNY protein levels are important for controlling cell size, artificial microRNAs (amiRNA) are genetically engineered to specifically silence TNY1. These artificial microRNA are cloned under a constitutive promoter in a plasmid that contains resistance to the antibiotic paromomycin (Paro). This acts as a method by which to select Chlamydomonas transformants, as it will ensure that those Chlamy that contain the desired amiRNA will survive in the presence of paromomycin.



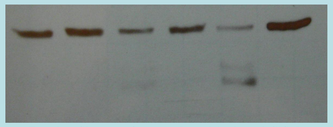
Wild Type Chlamy are transformed by means of electroporation with the plasmid containing amiRNA or a plasmid with no amiRNA as a control. Once screened on a Paro plate, the individual clones are picked and analyzed to determine if they were affected in the desired way and now exhibit the small phenotype that we expect as a result of their disrupted TNY1 gene.

Confirming a Transformation

If TNY levels are decreased due to the effect of the artificial microRNA, we will expect to see a small size phenotype. So how do we know we have this?

Coulter Counter: The Coulter Counter is a machine that detects change in electrical conductance as a means by which to measure the size of a particle. Samples of transformed Chlamy are resuspended in an isotonic solution and run through the machine so that their size can be counted. In this way, it is possible to determine if the cells are small. If they are, we will screen them further.

Western Blot: Western blots are used as a way to determine if a certain protein is present in a sample. In this case, we are looking to see if the *TNY1* protein is NOT present or levels are decreased.



In the figure above, the third and fifth samples from the left are potential candidates for decreased levels of *TNY1.*

Acknowledgements

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