How do *cis*-regulatory elements evolve in plants?

Nathan Hughes

January 17, 2019

Outline

What is the project?

Main question: How do *cis*-regulatory elements evolve in plants?

- *Cis* meaning same
  - in this case elements on the same DNA strand
- i.e. how do non-coding elements on the same DNA strand affect gene expression?

What are *cis*-regulatory elements?

Cis-regulatory elements

- They are usually enhancers and promoters that control development and physiology by regulating gene expression. [1]

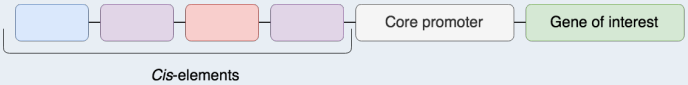

Figure 1: Example promoter containing cis-regulatory elements. Cis-elements can be repeated more than once (purple) and can recruit different transcription factors (blue/red), all driving the same gene (green)

[1] Wittkopp and Kalay (2012)

Why are they important?

Gene expression / phenotypic variation

- For most of the twentieth century, evolution of protein-coding sequences was commonly thought to be primarily (if not solely) responsible for phenotypic evolution
- Whereas more recent studies show that mutations which affect the function of these sequences contribute to phenotypic diversity within and between species
  - Many studies imply divergent *cis*-regulatory activity in phenotypic evolution [2]

[2] Carroll (2008)

How can you study the evolution of *cis*-regulatory elements, in plants?

Materials

- Start with a little known plant: *Arabidopsis thaliana*
  - Has an extremely well covered genome, as well as transcription factor database
- Will later move towards *Nicotiana benthamiana*, to evaluate how transferable work on model systems are to others'

Plants

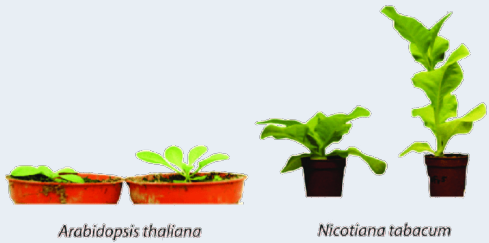

Figure 2: Example of *Arabidopsis* and *Nicotiana*

Promoter study

Procedure to evaluate ?

First choose two types of promoters to study:
The most constitutively expressed and Nitrogen-responsive
For each promoter type:

1. Identify motifs where TFs bind, check if any TFs are commonly used
2. Determine whether any patterns or similarities are present in groups/types of TFs
3. Incorporate nucleosome occupancy data (whether DNA is wrapped around a nucleosome or is open)
4. Verify hypothesis in lab

Identifying TF binding motifs of interest

Using MEME and FIMO to predict TF binding motifs

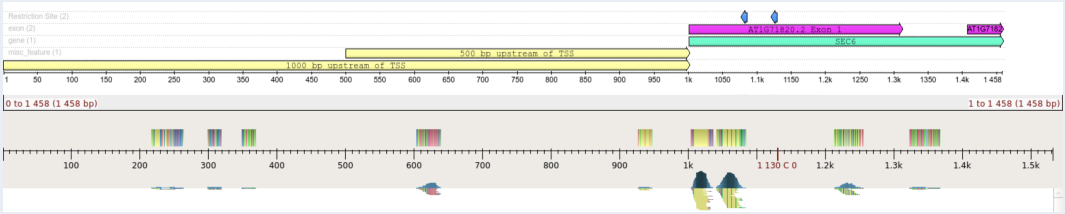

Figure 3: Example FIMO output for promoter of AT1G71820

Lab work

Progress towards testing selected regions of interest

- Designed primers in Benchling and ordered them for each of the promoters/promoter parts if mutating RE cut site
- Ran PCR with primers and At template DNA
  - didn't work, changed conditions
  - tried different DNA template - worked
- Made own At CTAB DNA template
  - PCR didnt work
- Using higher concentration of template DNA worked

References I

Patricia J. Wittkopp and Gizem Kalay. *Cis*-regulatory elements: Molecular mechanisms and evolutionary processes underlying divergence. *Nature Reviews Genetics*, 13(1): 59–69, January 2012. ISSN 1471-0064. doi: 10.1038/nrg3095.

Sean B. Carroll. Evo-Devo and an Expanding Evolutionary Synthesis: A Genetic Theory of Morphological Evolution. *Cell*, 134(1):25–36, July 2008. ISSN 0092-8674. doi: 10.1016/j.cell.2008.06.030.