Activation of a Floral Homeotic Gene in Arabidopsis

By Maximiliam A. Busch, Kirsten Bomblies, and Detlef Weigel

Presentation by Lis Garrett and Andrea Stevenson
Wild type

lfy- mutant
**LFY & AG**

- *LFY* is an upstream regulator of *AG*
- Normal *AG* expression is in the centre of the flower
- *lfy* mutations cause complex interactions on *AG* expression
  - *AG* expression similar to wild-type levels in later developing flowers
  - *AG* ectopically expressed in later-developing flowers and in stems
  - LFY has both negative and positive roles in regulation?
- *AG* is both ectopically and over-expressed in LFY:VP16 plants
  - Interaction between LFY and region-specific coregulators
Goal of Study

To understand the interaction between LFY and AG
1) LFY is a transcription factor with a DNA-binding domain that binds to promoters

2) However LFY does not contain an activation sequence for RNA-polymerase and therefore requires another transcription factor with an activation domain to bind and form a complex
When LFY binds transcription cannot occur without additional factors

Transcription can occur wherever LFY binds
**Agamous gene**

- AG promoter
- Hind III restriction sites
- intron 2
- 3kb Hind III restriction fragment

**KB9 Reporter Construct**

- “KB9” 3kb restriction fragment
- GUS gene
- minimal promoter

**GUS gene**
We know:
- That LFY is a transcription factor that regulates AG expression by interacting with enhancers
- The location and sequence of AG including restriction sites

We want to find out:
- Where in AG are the enhancers located
AGAMOUS

use known restriction sites

Reporters with different fragments of AG
KB9 Reporter
3kb restriction fragment closely matches intron 2

When transformed into the three Arabidopsis backgrounds, GUS expression matched the known AG expression pattern.
Reporter Gene Constructs Conclusion

KB9 reporter fragment must have enhancers within the 3-kb intron 2 region responsible for transcriptional activation of AG
Deletion analysis of the 3kb fragment

We know:

- That one or more enhancers which are collectively necessary and sufficient for AG expression are present in the 3kb intron 2 restriction fragment

We want to find out:

- Is there one or more than one enhancer in the 3kb region
- Where in the 3kb restriction fragment are the enhancers located
Hind III restriction sites on intron 2 of AG
Part of the 3kb Restriction fragment used

5' 3'

KB14

KB31

leafy^- wild type LFY:VP16
Part of the 3kb Restriction fragment used

5'  |
| 3'  |

KB14

KB18

leaky^-

wild type

LFY:VP16

D

E

F

J

K

L

*  

4  

*  

4  

8  

p  

*  

p
Deletion analysis of the 3kb fragment

Conclusions

There must be at least two enhancers, one in the 5' fragment, and one in the 3' fragment.

The enhancers likely interact with other unknown elements (cryptic regulatory elements) to fully explain the expression patterns seen.
Deletion analysis of the 3’ end

We know:
- That there is an enhancer in both the 3’ and 5’ fragments of the restriction fragment

We want to find out:
- The exact location of the enhancer binding sites
KB14 vs. KB31

~2,250 bp vs. ~750 bp
Part of the 3kb Restriction fragment used
KB37
No

KB24
Yes

KB28
Yes

KB26
No
Deletion analysis of the 3’ end Conclusions

The 3’ end enhancer is located in the 230bp overlapping region between KB24 and KB28
Immunoprecipitation & Electromobility Shift Assay

We know:
● LFY can bind to DNA

We want to find out:
● Can LFY bind to the 3' AG enhancer
Immunoprecipitation of DNA-protein complexes

Electromobility Shift Assay (EMSA)

- Separate protein-DNA complexes from free DNA by gel electrophoresis
- Determines relative binding affinities

http://www.nature.com/nmeth/journal/v2/n7/abs/nmeth0705-557.html

http://andrew.gibiansky.com/blog/genetics/technique-primers/images/emsa.png
bound DNA at 2 sites
bound DNA at 1 sites
free DNA
Immunoprecipitation & EMSA Conclusion

LFY binds to two sites (AG I & AG II) *in vitro* at similar affinities. These two sites are separated by 31 bp.
Sequencing

We know:
- There is a LFY binding site within the promoter of the \textit{AP1} gene
- LFY binds with similar affinity to both \textit{AG} binding sites, but more strongly to the binding site within the \textit{AP1} gene

We want to find out:
- What are the sequences of the two binding sites found in \textit{AG}?
- Is this sequence of the LFY binding sites in \textit{AG} similar to binding site found in \textit{AP1}?
Sequences Conclusion

The sequences are the two AG binding sites are similar to each other and are 31 bp apart. They are also similar to the LFY binding site of AP1.
Small Deletions in AG Enhancers

We know:
● LFY can bind to AG enhancer *in vitro*

We want to find out:
● Does LFY also bind to AG *in vivo*?
• Introduced two small deletions into 3' AG enhancer
  ○ Deletion 1 (KB45): deleted AG I site
  ○ Deletion 2 (KB46): deleted both AG I and AG II sites
Small Deletions in AG Enhancers Conclusion

There are two LFY binding sites involved with AG expression. They are similar, but only partially redundant -- both are required for *in vivo* activity of the 3’ AG enhancer.
Both binding sites are needed for \textit{in vivo} activity. There may be some kind of cooperative action between the two sites that is not seen in the \textit{in vitro} analysis.
Point Mutations

We know:

● LFY can bind to AG enhancer in vivo
● A 2-bp mutation in the LFY binding site in the AP1 promoter prevents LFY binding

We want to know:

● Was the loss of enhancer activity due to the loss of the LFY binding sites?
● Will the same 2-bp mutation in AG cause a loss of LFY binding?
Point Mutations

First mutation (m1; MX68): CC → AA
Second mutation (m2; MX100): A → G
*In vitro* results of point mutations

[Diagram showing electrophoresis results with labeled bands for bound and free DNA]
In vivo results of point mutations
Point Mutations Conclusion

Binding of LFY to AG I and AG II sites is regulating AG expression.
Overall Conclusions

Binding of LFY to the 3' enhancer sites in AG is necessary for activating transcription of AG.

*LFY* homologs in other species are likely to have the same function because only one functional copy exists per haploid genome.
Further Research

AG is only active in a subset of cells that are expressing the LFY protein, so there must be coregulators involved in AG expression.

Negative control of AG by AP1, AP2, LEUNIG, and CURLY LEAF are suggested as good starting points for further elucidation of AG regulation.
Questions
Distribution of staining strength among primary transformants

KB10
KB11
KB13
KB14
<table>
<thead>
<tr>
<th></th>
<th>activity range nwis</th>
<th>wild type</th>
<th>Ify</th>
<th>LFY:VP16</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB9</td>
<td>F</td>
<td>++</td>
<td>++</td>
<td>Y</td>
<td>24</td>
</tr>
<tr>
<td>KB9</td>
<td>R</td>
<td>+</td>
<td>n/d</td>
<td>Y</td>
<td>29</td>
</tr>
<tr>
<td>KB10</td>
<td>F</td>
<td>-</td>
<td>n/d</td>
<td>n/d</td>
<td>5</td>
</tr>
<tr>
<td>KB11</td>
<td>F</td>
<td>-</td>
<td>n/d</td>
<td>n/d</td>
<td>19</td>
</tr>
<tr>
<td>KB13</td>
<td>F</td>
<td>-</td>
<td>n/d</td>
<td>N</td>
<td>21</td>
</tr>
<tr>
<td>KB14</td>
<td>F</td>
<td>++</td>
<td>±</td>
<td>Y</td>
<td>19</td>
</tr>
<tr>
<td>KB16</td>
<td>R</td>
<td>++</td>
<td>n/d</td>
<td>Y</td>
<td>17</td>
</tr>
<tr>
<td>KB17</td>
<td>R</td>
<td>+</td>
<td>n/d</td>
<td>n/d</td>
<td>20</td>
</tr>
<tr>
<td>KB20</td>
<td>F</td>
<td>-</td>
<td>n/d</td>
<td>Y</td>
<td>16</td>
</tr>
<tr>
<td>KB21</td>
<td>R</td>
<td>++</td>
<td>+</td>
<td>Y</td>
<td>27</td>
</tr>
<tr>
<td>KB18</td>
<td>R</td>
<td>++</td>
<td>±</td>
<td>Y</td>
<td>19</td>
</tr>
<tr>
<td>KB31</td>
<td>R</td>
<td>++</td>
<td>+</td>
<td>Y</td>
<td>35</td>
</tr>
<tr>
<td>KB33</td>
<td>R</td>
<td>+</td>
<td>n/d</td>
<td>Y</td>
<td>14</td>
</tr>
<tr>
<td>KB30</td>
<td>R</td>
<td>±</td>
<td>-</td>
<td>Y</td>
<td>20</td>
</tr>
<tr>
<td>KB35</td>
<td>R</td>
<td>-</td>
<td>n/d</td>
<td>n/d</td>
<td>15</td>
</tr>
<tr>
<td>KB37</td>
<td>R</td>
<td>-</td>
<td>n/d</td>
<td>n/d</td>
<td>15</td>
</tr>
<tr>
<td>KB24</td>
<td>R</td>
<td>-</td>
<td>n/d</td>
<td>Y</td>
<td>13</td>
</tr>
<tr>
<td>KB28</td>
<td>R</td>
<td>-</td>
<td>n/d</td>
<td>Y</td>
<td>16</td>
</tr>
<tr>
<td>KB26</td>
<td>R</td>
<td>-</td>
<td>n/d</td>
<td>N</td>
<td>5</td>
</tr>
<tr>
<td>KB45</td>
<td>R</td>
<td>+</td>
<td>n/d</td>
<td>Y</td>
<td>17</td>
</tr>
<tr>
<td>KB46</td>
<td>R</td>
<td>±</td>
<td>n/d</td>
<td>N</td>
<td>15</td>
</tr>
<tr>
<td>MX68</td>
<td>R</td>
<td>±</td>
<td>n/d</td>
<td>N</td>
<td>45</td>
</tr>
<tr>
<td>MX100</td>
<td>R</td>
<td>++</td>
<td>n/d</td>
<td>n/d</td>
<td>73</td>
</tr>
</tbody>
</table>

- **Whole fragment**
- **5’ end fragments**
- **Central or both end fragments**
- **3’ end fragments**
- **3’ end fragments with deletions or mutations in the enhancer region**

- **Legend:**
  - H3: Histone 3
  - Bc: B-catenin
  - Sc: Snail
  - Sn: Snail
  - Sp: Snail
  - N: Notch
  - XB: Xenograft
  - H2: Histone 2
  - H3: Histone 3
**A**

- β
- α
- free
- LFY

**B**

- wt m1 m2
- wt m1 m2

**C**

- GGACCAGTGTCC
- AATCCATGGTTA
- TACCCAAATGTGTT
- CCANTG

<table>
<thead>
<tr>
<th>AP1</th>
<th>wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG1</td>
<td>wt</td>
</tr>
<tr>
<td>AG2</td>
<td>wt</td>
</tr>
<tr>
<td>consensus</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>m1</th>
</tr>
</thead>
<tbody>
<tr>
<td>........</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>m2</th>
</tr>
</thead>
<tbody>
<tr>
<td>........ G ........</td>
</tr>
</tbody>
</table>