Genes for Flowering©

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The time at which it flowers is critical to the survival of a plant species. There must be appropriate environmental conditions such as enough warm days for seeds to mature. Plants may have to flower synchronously so that outcrossing species can pollinate. It is also critical to human survival that crops flower in a controlled manner because most of our harvested products such as grain and fruit are the results of flowering.

The transition from the vegetative to the reproductive stage in plants is an immensely interesting developmental process. Plants and animals are quite different. In animals, the germ line cells are set aside early and the nonreproductive tissue, the somatic tissue, gives rise to other somatic tissue. In contrast, in plants the growing apex gives rise first to leaves and stems and other vegetative tissue and then following floral induction switches to give rise to reproductive tissues such as petals, stamens, and ovules.

We are using molecular tools to study this switch to flowering. It has been known for a long time that plants use environmental and developmental signals as cues to initiate flowering. The most common environmental signal is day length. Long-day plants flower in the springtime when the days become long- and short-day plants flower in the autumn as the days shorten.

Another environmental signal used to initiate flowering is a long period of low temperature. This is called vernalisation and ensures that plants flower in the springtime after the long, cold winter. This is particularly true for plants from high latitudes or high altitudes, where a mechanism to prevent flowering in winter is required. Vernalisation is often coupled to long-day signalling to ensure plants flower in spring when there is enough time for seed development and maturation. While many plants require environmental signals some species flower when they reach a particular developmental stage.

Another agent that promotes flowering is the plant hormone gibberellin (GA). Whether the day length and vernalisation flowering pathways work through altering levels of GA we don't know.

In order to study the genes that control the initiation of flowering *Arabidopsis* has been used as a model. Just as there is a human genome project, there is a plant genome project and the subject of the project is *Arabidopsis*. It was not chosen because of its commercial value — it is really a small weed. But *Arabidopsis* does have a lot of advantages because it requires only a small area for growth, it can be grown in test tubes or on synthetic media. It only takes about 6 weeks for one generation and produces about 50,000 seeds per plant. Because of this short turnaround time and the fact that it is self fertile it is very easy to isolate mutants. The genome of *Arabidopsis* is now completely sequenced. So we now have a good knowledge of all the genes required for the complete functioning of a plant. We, and other labs around the world have chosen *Arabidopsis* to identify the genes involved in controlling flowering.

The approach that we, and others, have taken is to isolate mutants that are altered in flowering time. We mutagenised seeds with chemicals, x-rays, or DNA tags which insert into a gene. We grew the mutagenised seed for two generations and looked for

plants that have altered flowering time, either late or early. These mutants presumably have a gene involved in flowering that has been mutated. Once we find a mutant, because of the molecular biology tools available in *Arabidopsis*, we can go back and isolate the gene that has been mutated. We then check that this gene has a role in flowering.

Firstly, let's look at the day-length pathway to flowering. *Arabidopsis* is a long-day plant. Somehow the plant can count how many hours of day light it experiences. When the days reach the right number of hours of light there is a signal produced in the leaf that travels to the apex and induces the plant to flower. A lot of work has been done studying this signal, probably the best candidate so far is some form of GA.

Mutants have been isolated that no longer respond to long days and flower later. About 10 different mutants have been isolated which fit in this pathway. One of them is a gene called CO or CONSTANS. If this gene is mutated the plant flowers late in long days—at about the same time as it does in short days. So CO mutants have lost their ability to respond to long days. The CONSTANS gene has been isolated and shown to be a master gene that regulates other genes. Many of the genes that have been isolated from this pathway are genes for phytochrome pigments that are to do with light perception in plants and genes that regulate circadian rhythm and are concerned with counting time.

What is interesting is that in rice, which is a long way from *Arabidopsis* as evolution goes, the major gene for flowering time is the rice version of the CO gene that was isolated from *Arabidopsis*. Rice is a short-day plant and *Arabidopsis* is a long-day plant but similar genes are used to control the flowering time.

The second pathway to flowering is the vernalisation/developmental pathway. We have concentrated on this pathway and also have taken a mutant approach. We looked for mutants that were late flowering and found one that was very late. When we isolated the gene (*FLC*) we found it was also a master regulatory gene of a type that regulates other genes. In the mutant the gene is switched on to high levels. So the *FLC* gene is a repressor of flowering, it prevents flowering. It is a quantitative action, the more active the *FLC* gene is the later the flowering. We could manipulate flowering time by adding another copy of the gene and hence more *FLC* activity causing late flowering or knocking out the gene activity and causing early flowering.

To our delight we found that this *FLC* gene was switched off during vernalisation. This linked the developmental pathway with the vernalisation pathway. Treatment with the long periods of cold down regulated the *FLC* gene and flowering occurs. *FLC* seems to be the key controller of the response to vernalisation.

So far there have been about 30 different mutants isolated in *Arabidopsis* that are involved in the vernalisation/developmental pathway. We have now shown that most of them work through altering the level of activity of the *FLC* gene. So *FLC* activity is a major factor in controlling time of flowering.

We have found that in naturally occurring ecotypes of *Arabidopsis* that come from high mountains or high latitudes and require vernalisation to flower there is normally a high level of activity of the *FLC* gene. In varieties from near the equator, which don't respond to vernalisation, there is a low level of *FLC* activity. *FLC* has different activity in naturally occurring *Arabidopsis* lines and controls flowering in the wild.

We need to ask is what we find in *Arabidopsis* true in other species? We have started working on canola, a member of the genus *Brassica* and a near relative of *Arabidopsis*, to see if the same genes regulate flowering. When we introduce the

Arabidopsis FLC gene into canola, the canola flowers late. We then isolated genes similar to *FLC* from canola. We inserted them into *Arabidopsis* and flowering was delayed. This suggests that *Brassica* also uses an *FLC*-like mechanism to control its flowering. If we use a line of *Brassica* that requires vernalisation to flower, we find that the level of *FLC* activity is very high and when the *Brassica* plant is vernalised the level of *FLC* activity drops and the plant flowers early. Apparently *Brassica* uses *FLC* genes to control flowering time and respond to vernalisation. We are now in the process of looking at other plants to see if they do use *FLC* as a method of controlling flowering time.

The process of vernalisation occurs in many families of plants both monocot and dicot and has similar characteristics suggesting that it works through a similar mechanism. We need to extend our studies more widely to determine if *FLC* activity is manipulating flowering in these other species.

The ability to control flowering time may be of great significance in crop, forestry, and horticulture - either to cause flowering at an appropriate time or to prevent flowering.

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Native Grass Seed Germination©

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This presentation is a summary of the project "Native Grass Restoration in the Australian Capital Territory Water Catchment. Maximising Seed Germination". The full report can be found at <http://www.anbg.gov.au/hort.research/grass-project>. The project was funded initially by the Community Grasses Project for the Murray Darling Basin and ACTEW and later by the Natural Heritage Trust through the ACT Government.

OBJECTIVES

The objectives were to find practical, reliable, and cost-effective methods for revegetation using local provenance native perennial grasses through:

- Faster germination of seed.
- More synchronous germination of seed.
- Increasing the amount of germinable seed.
- Developing better strategies for seedling establishment.

SPECIES SELECTION, SEED QUALITY, AND SEED SUPPLY

Seven species were studied. They were all native, perennial, and deep rooted and from a range of habitats. Both summer—and winter—(C_3 and C_4) growing species were represented. The seed was collected locally.

Some species of grass have many sterile flowers, which produce no seed. The seeds on native grass heads ripen gradually so at harvest many under-ripe seed units may be collected and many ripe seeds may have already been shed. The result is a lower yield of viable seed than might be expected in some species. Seed quality is also