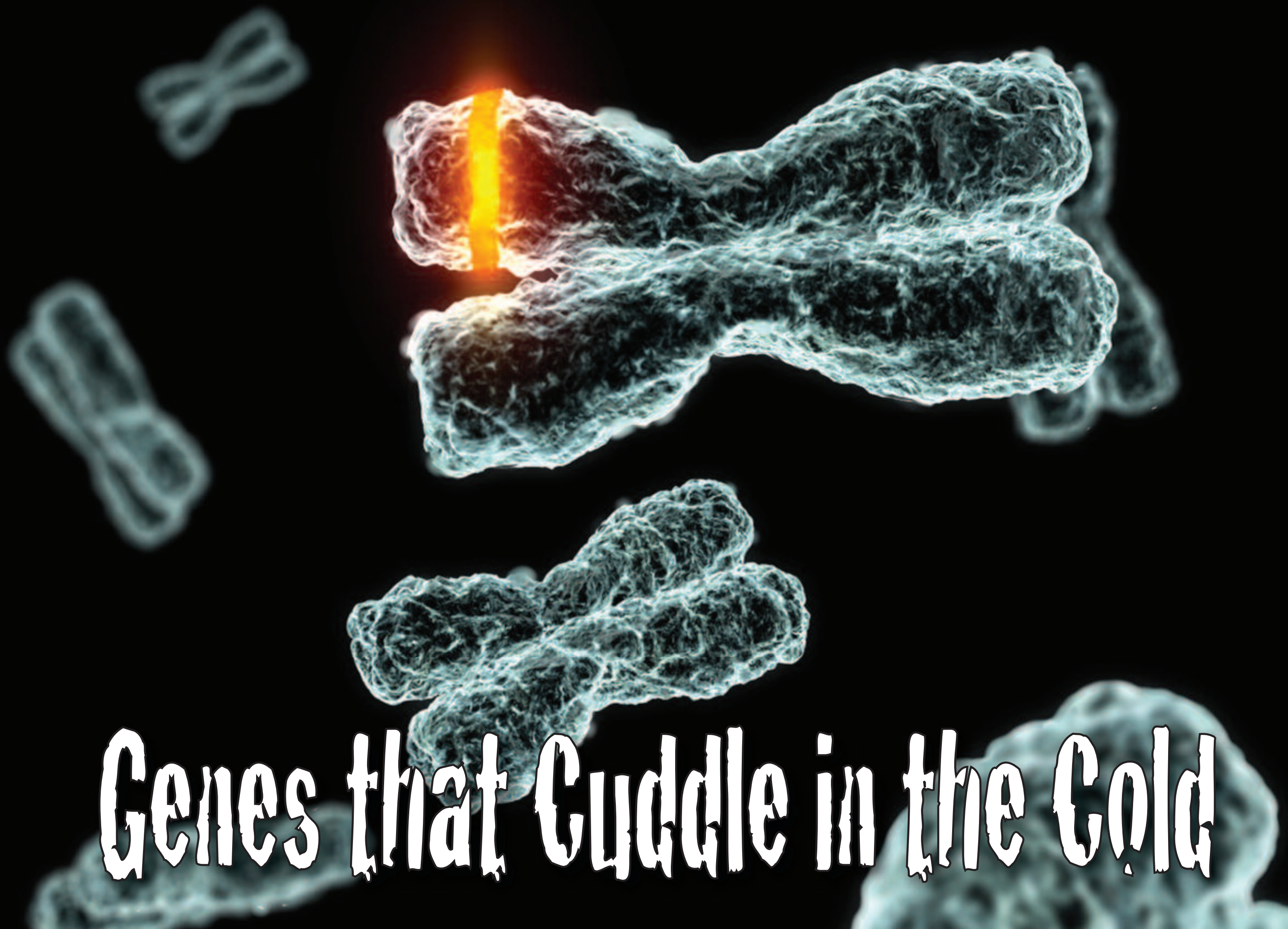


Genes that Cuddle in the Cold

JOSHUA MYLNE

An ingenious experiment has allowed scientists to observe how plant genes move around the nucleus to locations that either stop or stimulate flowering depending on temperature.

Every cell of the humble model plant *Arabidopsis* contains five chromosomes, each of which carries about 5000 genes. Each cell stashes some of its genes away and keeps some in ready reserve, while the others remain active and busily make proteins.

After decades of effort, scientists feel like they have a good understanding of how genes are regulated between these active and inactive states, but it's beginning to dawn on them just how much genes move around while this happens, and that this might be a part of the regulatory process.

I have been part of a team of plant biologists that has developed a way to watch one particular gene move about the nucleus of a cell of a living plant. As the gene responded to cold, we saw how its copies came together in one place and were inactivated, showing that physical movement is involved in its control.

How a Plant Senses and Remembers Winter

All organisms, including plants, respond to their environment by regulating genes. *FLOWERING LOCUS C* (*FLC*) is the key gene in the model plant *Arabidopsis* that controls the decision to switch from making leaves to making flowers. As long as *FLC* is active, *Arabidopsis* keeps producing leaves. But the passage of winter – or even simply putting the plants in the fridge for a month or so – inactivates *FLC* and primes the plants to make flowers when they are returned to warmth.

This process of flowering stimulated by the cold is called vernalisation, and it is a very important trait for agriculture in the Northern Hemisphere. More than 90% of cereal crops in northern Europe are varieties that need to be vernalised. This involves sowing a crop so that, after it grows a little, it spends the rest of the winter vernalising and will flower soon after the warmth of spring arrives.

In Australia, the cooler southern states also use crops needing vernalisation. Queenslanders take advantage of vernalised crops in another way. With Queensland's warm winters, fodder crops grown for livestock grazing can't vernalise, and hence they will not flower at all.

Jetting off to Ol' Blighty

In October 2001, at the beginning of the northern winter, I boarded a plane to the UK for a postdoctoral research stint in

Professor Caroline Dean's lab in the Cell & Developmental Biology Department at the John Innes Centre in Norwich. In the UK, the relevance of vernalisation struck me physically. Out there in the fields of the English countryside were plants too cold to do much growing, but they were vernalising to begin flowering in 3–4 months when spring arrived.

I used those months to get my head around all the genes involved in flowering and was given a project to find new genes involved in sensing and remembering winter.

The Birth of an Idea

After a year of coming to grips with vernalisation and *FLC*, I came across a newsgroup message from two teams in New Jersey and New York that piqued my interest. They were going to try and map the distribution of genes in a live and "resting" *Arabidopsis* nucleus.

The system they were going to use was one that took advantage of an interaction that happens in bacteria, where a DNA sequence called *lacO* is bound very strongly by a protein known as LacI. This strong *lacO*–LacI interaction can take place in any organism.

The American researchers intended to scatter long strings of the *lacO* sequence randomly around the *Arabidopsis* genome and then work out exactly where each *lacO* string had landed by adding fluorescent LacI proteins. With hundreds of different places in the genome tagged they could then put together a three-dimensional map of the physical position of all of the DNA in a nucleus.

It was an elaborate approach, and it started me thinking about how I might use the *lacO*–LacI interaction to ask a much more specific question: does the *FLC* gene physically move around in the nucleus when it is inactivated?

The American approach had some problems though. One was that the strings they were using consisted of repeated sequences of *lacO*. Organisms generally don't like repetitive DNA – it causes them all sorts of problems when they have to copy their chromosomes. As a result, long strings of repetitive DNA are often unstable. The Americans had found some workarounds, but it didn't alter the fact that the highly repetitive sequence could cause problems and misbehave in a living system.

Just as I was starting to work I found a solution close by. Professor David Sherratt from Oxford University had developed a string of *lacO*s in which he put random DNA sequences between each one to break up the repeating pattern. This made his *lacO* strings behave more naturally in living systems.

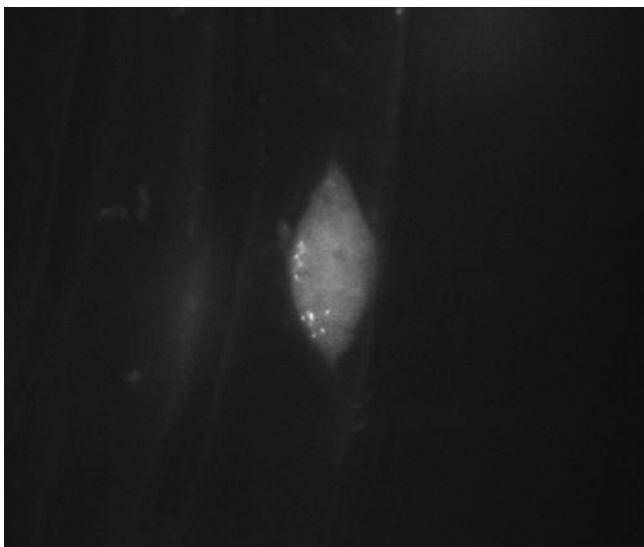
Down to Business

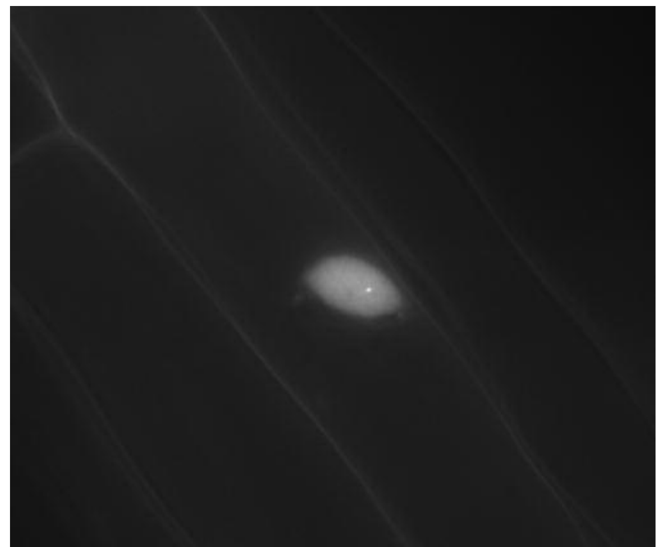
To make an *FLC* that could be tracked, I took the spaced *lacO* string from Sherratt's lab in Oxford and, working in bacteria, inserted a *lacO* string just after an *FLC* gene. Once these constructs were ready, I moved them into nature's genetic engineer, a soil bacterium called *Agrobacterium tumefaciens* that can splice constructs into the *Arabidopsis* genome.

While all this was going on I had made another transgenic plant that incorporated a yellow-fluorescing LacI protein. Once everything was ready I crossed the two lines, bringing the two different constructs together in one plant.

So, after 3 years of toil, I had a double-transgenic plant that had an *FLC* gene with a *lacO* string right beside plus a glowing LacI protein that would show me where it was.

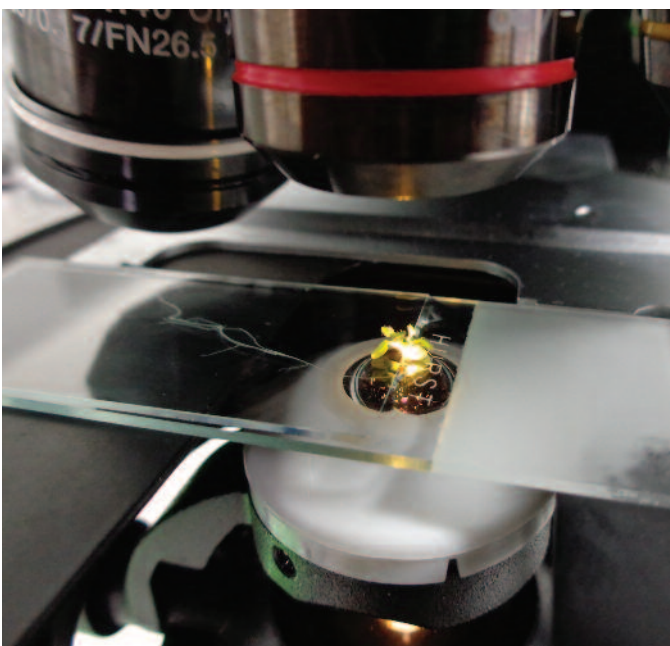
But by now I was running out of time. After 4 years in the UK, I received an offer to return to Australia that was too tempting to resist so I packed up my family and headed home.





LEFT: the nucleus in a root cell of the transgenic plant grown without any cold treatment, with its gene copies in the unclustered active state. RIGHT: the effect of 2 weeks of cold treatment on the same transgenic plant, showing a single spot where copies of the gene have clustered together and been switched off. Credit: Stefanie Rosa

Arabidopsis thaliana is a small, fast-growing and simple plant that is used in thousands of laboratories worldwide. In addition to being easy to genetically engineer, different genetic lines can be mixed by crossing, which is done here by emasculating the flower with needle-sharp forceps. The emasculated flower can then be pollinated by another plant. Credit: Julie Leroux



The transgenic system allowed the monitoring of the physical position of the *FLC* gene within the nuclei of whole and live *Arabidopsis* plants. Credit: Josh Mylne

All I managed to do before I left was show that the *FLC–lacO* DNA sequence appeared as a spot in the nucleus. So I knew the system worked, but whether FLC would move in the cold remained unresolved.

The Eureka Moment

Back in the UK, the project lay dormant. It would not be until 2 years later that Prof Dean would resurrect the project under cell biologist Stefanie Rosa. Fortunately her study coincided with a visit I made to the UK to attend a conference, so I stayed on to see the first vernalised transgenic plants come out of the cold.

The eureka moment came when Stefanie took the plants out of the fridge and put them under the microscope. Instead of dozens of tiny spots showing where the *FLC* gene was distributed, we saw only one bright spot in each cell. Plant after plant was taken out of the fridge and put on the microscope stage only to see the same thing – a single bright spot in each cell.

It couldn't be more obvious that *FLC* genes were moving to cluster together in the cold. Without cold, when *FLC* was active and blocking flowering, its copies were spread about the nucleus. After cold, when *FLC* was inactive and the plant was primed to flower, all the *FLC–lacO* genes were clustered together in one place!

Hard Graft

Far from providing the conclusion to a story, this eureka moment signalled the beginning of a long journey of hard graft to prove what we saw was real and meaningful. Scientists are a sceptical bunch, so whenever you want to tell them something that'll surprise them you've got to be sure your logic and methods are solid and you're not looking at an artefact.

It took another 5 years after that eureka moment in 2008 to gather enough evidence to convince the most sceptical scientists that what we saw was real. The paper describing the work was finally published in September 2013, almost 10 years after the very first *FLC–lacO* construct was made.

So What? And Where to from Here?

So what is this elaborate discovery going to do for the world? Knowing that genes move around won't increase crop yields, cure cancer or help replace fossil fuels. This project was about seeking knowledge – pure and simple. What this work does, in a very visual way, is show how the physical movement of a gene is intimately involved in its regulation.

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