A man who makes chromosomes

A new method of genetic engineering – constructing small, whole artificial chromosomes in plant cells – may avoid many of the drawbacks of existing methods and generate high-yielding, pest-resistant agricultural crops. **Professor James A Birchler**, of the University of Missouri, USA, is at the forefront of research bringing this technology into practice.

In a world with a rising population and ever-accelerating climatic change, agriculture is constantly playing catch-up. Degraded soils, resistant pests, unpredictable rainfall and extreme temperatures can all wreak havoc with crop yields, so new crop varieties that can withstand these pressures are now needed more than ever to meet the growing demands of the human population.

Traditional plant breeding methods, by deliberate crossing, rely on chance mutations and may take many years to develop crop varieties with the desired traits. More recently, science has turned to orthodox genetic engineering for better-targeted and faster results. This usually involves adding genes from other species ('transgenes') to a crop's existing genome.

However, transgenes are usually inserted randomly into the genome, which can cause their activity to be unpredictable. Even worse, they can land in the middle of an existing gene, disrupting its precise code with potentially deleterious consequences for plant survival.

Prof Birchler's pioneering new technique avoids these dangers by generating entire, artificial chromosomes in plant cells alongside their existing genetic material, giving scientists much more control of the genetic engineering process and its results, and providing the potential to change multiple characteristics of a crop plant in one fell swoop.

CONSTRUCTING CHROMOSOMES

Chromosomes are like the pages in the instruction book for building an organism. Each cell of an animal or plant contains several pairs of these strings of DNA, each comprising multiple genes and associated coded instructions for switching them on and off. Each chromosome also contains a specific structure used to organise it during cell division (the 'centromere') and protective caps at the ends of each string known as 'telomeres'.

Although artificial chromosomes have been produced successfully in microorganisms such as yeasts for many years, plants have proved much more complex. However, in 2007 Prof Birchler and his lab successfully ▸

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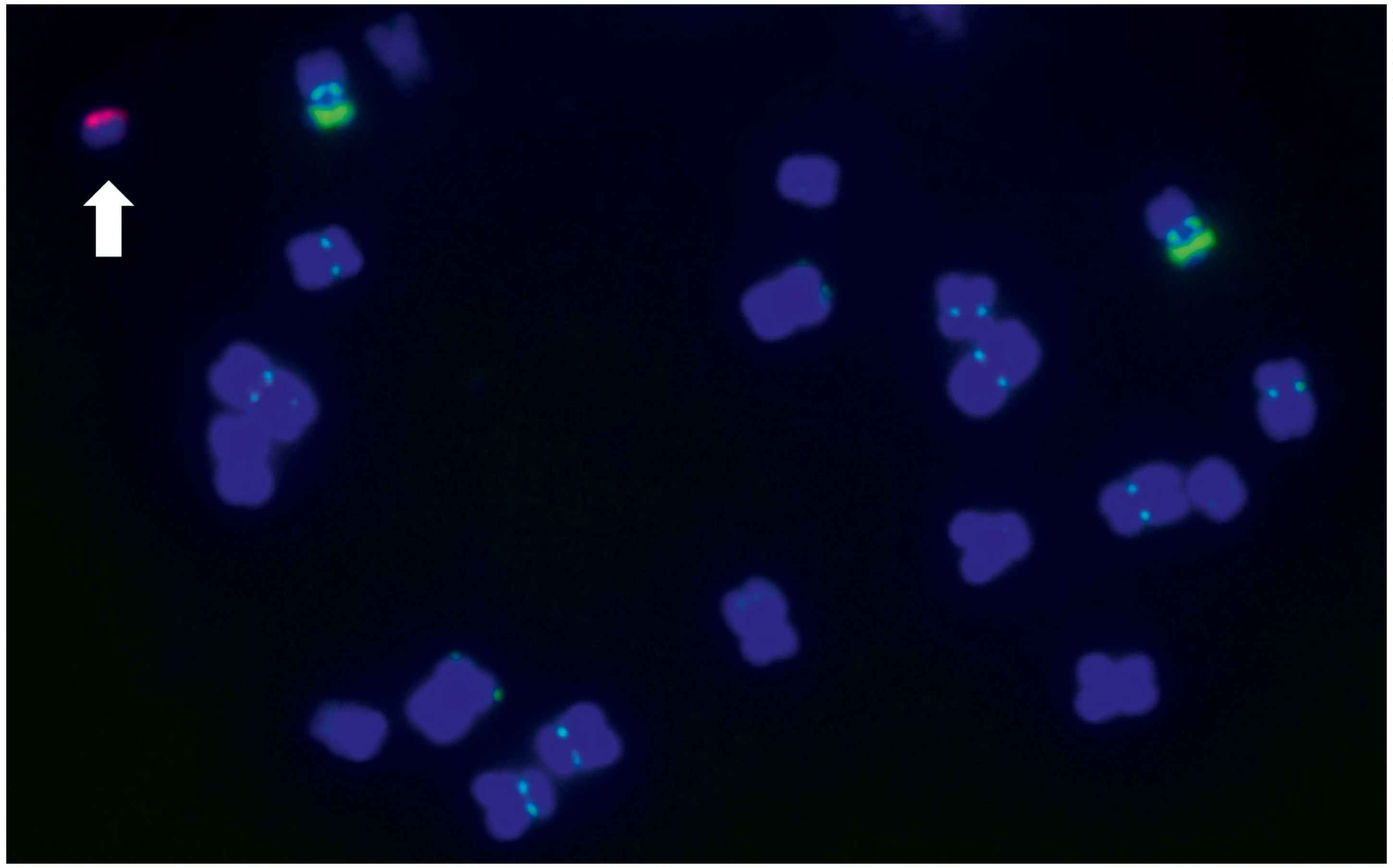

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constructed an artificial 'minichromosome' in maize. The success of the method was demonstrated by proving that the minichromosome was both genetically active within its host cell, and successfully passed on to the next generation of maize plants.

The new minichromosome was derived from a small, otherwise inactive chromosome in maize known as a 'B' chromosome. The use of B chromosomes minimises the risk that the minichromosome will interact detrimentally with the function of the plant's other, natural 'A' chromosomes. In essence, the minichromosome contains only a centromere, with the telomeres and transgenes coding for the new traits desired. It is independent of the plant's original genetic material and can be stably transmitted to the next generation: a blank slate for designing bespoke crops.

DESIGNER CROPS

The next step envisaged by Prof Birchler and his team is to start 'stacking' multiple beneficial genes upon a single minichromosome, a process that would be almost impossible to manage through traditional modification of the plant's existing chromosomes. Incorporating multiple genes in this way could introduce whole new biochemical pathways into the plant, more and more of which could be added as the needs of producers and consumers develop.

Ultimately, Prof Birchler believes we could produce crops that are resistant to multiple pests including bacteria, viruses, fungi and insect herbivores. At the same time, they could be higher-yielding, resistant to herbicides, and tolerant to stresses such as salinity, drought, or extremes of temperature. It should even be possible to introduce novel traits such as the production of therapeutic drugs, vaccines, antibodies or micronutrients.

Engineered minichromosome. A root tip metaphase karyotype of a plant carrying an engineered minichromosome in maize is shown. The arrow depicts a truncated supernumerary B chromosome that carries a transgene at the terminus. The green signals are probes to various repetitive sequence clusters that allow chromosome identification. The red signal is the B chromosome centromeric specific sequence. The B chromosome is a nonvital chromosome that is essentially inert and can serve as a foundational platform for engineered minichromosomes. The engineered minichromosome was produced by transforming a line with B chromosomes with a truncating construct containing genes and a telomere repeat at one end

Prof Birchler's current project, funded by the US National Science Foundation, explores how these ideas can become a reality, by testing methods that allow large numbers of genes to be added to a minichromosome in a single step. The methods are still under development, but emerging technologies, such as enzyme-based gene editing and haploid breeding, look likely to facilitate the process. Prof Birchler says it will also be important to explore the wide realm of non-coding DNA found in natural chromosomes, to identify sequences that are important for regulating the normal function of genes – in order for these to also be incorporated into minichromosomes. It is still likely that the activity of the transgenes supplied on a minichromosome will vary depending on the surrounding genetic environment of the host cell.

Artificial chromosome technology is one of the key 'building blocks' of the emerging field of synthetic biology

ACROSS THE PLANT KINGDOM

Artificial chromosomes have now been developed in several plant species including rice and barley, and Prof Birchler sees no reason why this method of manufacturing minichromosomes – termed 'telomere truncation' – should not be transferrable to many others. This is because the process uses a chromosome structure – the telomere – that is ubiquitous throughout the plant kingdom. In fact, Prof Birchler sees artificial chromosome technology as one of the key 'building blocks' within the emerging field of synthetic biology. Coupled with the natural variation that already exists in wild and cultivated plant populations, it should greatly expand the options available to plant breeders.

Artificial chromosomes may be particularly useful in clonally-propagated crops, both because it is in these species that the minichromosomes will be most stable, and also because these are the most difficult to improve by traditional breeding. Clonally propagated crops include some of the most important staple foods of developing countries, such as cassava, sweet potato and banana. Although, as Prof Birchler puts it, 'stacking traits for … a wide variety of characteristics [on artificial chromosomes] might be a distant goal,' it is certainly a laudable and ultimately promising one.

Q&A

Can you explain why it was so difficult initially to generate artificial chromosomes in plants compared to yeast?

The first artificial chromosomes were produced in yeast, which served as an inspiration for attempts in plants. However, the centromeres in yeast are determined by DNA sequence but functional centromeres in plants are epigenetically determined by perpetuation of chromatin state. Until this latter realisation became known, attempts at producing artificial chromosomes in plants were destined to fail (with the exception that de novo epigenetic centromeres might form on introduced DNA).

Why did you choose maize as a model species to test the artificial chromosome technology?

Our lab had worked on maize as a genetic model for several years and we were experienced with working with its chromosomes.

Can you outline the advantages of artificial chromosomes over existing methods of genetic engineering?

Artificial chromosomes are independent of the normal set and allow multiple genes to be added to them. Thus, the artificial chromosomes are separate from the other chromosomes, which allows their inheritance without linkage to them. More importantly, gene editing and insertion technology is limited in the size of the insertion; artificial chromosomes can likely accept tens of kilobases (up to ~100 kb) with continued ability for further additions – all as an independent entity.

What are the remaining barriers to using minichromosomes for breeding new varieties of crops?

Small chromosomes behave differently than normal sized chromosomes. However, one can envision straightforward ways in which normal transmission from one generation to the next can be made to be faithful. A separate issue, but one that impacts both artificial chromosome technology and gene editing, is the need for more efficient crop plant transformation.

What would be your prediction for the first crop species and/or traits to be successfully engineered and brought into agricultural production using minichromosome technology?

One never knows from whence the next new idea will emerge. The beauty of science is that it builds on the contributions of many individuals going forward.

Detail

RESEARCH OBJECTIVES

Dr Birchler's research focuses on using artificial chromosome technology in plants, and the benefits it can have on improving agricultural practices.

FUNDING

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BIO

James A. Birchler is Curators' Distinguished Professor of Biological Sciences at the University of Missouri, Columbia. After obtaining a BS degree from Eastern Illinois University in Botany and Zoology in 1972, he attended graduate school at Indiana University majoring in Genetics with a minor in Biochemistry.

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Find out more:

You can find out more about Prof Birchler's work at: https://birchler.biology.missouri.edu/maize-minichromosomes/