Plant genetics

A plant joins the pantheon at last?

from Geoffrey North

As Keith Roberts has recently remarked in *Nature*[1], plants should be wonderful systems for the study of development. Why, then, has progress in understanding cell differentiation and morphogenesis in plants been so slow? One reason, surely, has been the lack of a plant equivalent of the fruitfly *Drosophila melanogaster*: a rapidly breeding species that is easy to keep in large numbers in the laboratory, and is ideal for intensive study by the combination of molecular and genetic techniques that has proved so successful in dissecting the regulation of development in *Drosophila*[2]. But it seems plant scientists need look no further, for the indications from a recent meeting* are that the small weed *Arabidopsis thaliana* (the common wall cress; see sketch) could prove to be just the right subject. And the hope that molecular biology will transform practical plant breeding must have been boosted by reports at the same meeting of the correctly regulated expression of exogenous genes introduced into plants using the natural vector system provided by the bacterium *Agrobacterium tumefaciens*.

Although the science of genetics began with Mendel's experiments with peas, on the whole plants are far from ideal subjects for molecular geneticists. They usually require a lot of space and special conditions for their upkeep, and, worse, as a rule undergo only one or at most a few breeding cycles in a year. Furthermore, the species on which plant geneticists have concentrated most of their efforts, such as maize, tend to have enormous genomes — in the region of two orders of magnitude larger than those of *Drosophila* or yeast, which is a considerable handicap if your aim is to isolate a specific gene. These considerations led Elliot Meyerowitz (California Institute of Technology) to the temperate weed *Arabidopsis thaliana*, a member of the Cruciferae (mustards). Although the first *Arabidopsis* mutations were reported in the 1940s and it has since been used extensively in the genetic analysis of plant biochemistry (C. Sommerville, Michigan State University; see ref. 3 for a recent genetic map of *Arabidopsis*), its potential as a subject for intensive molecular genetic analysis seems hitherto to have largely been unexploited.

The features that attracted Meyerowitz to *Arabidopsis* are the ease with which it can be grown in the laboratory, its small size and extraordinarily short generation time (only 4-5 weeks), and its unusually small genome[4]. On the basis of DNA reassociation kinetics Meyerowitz estimates that the *Arabidopsis* genome is only ~ 70,000 kilobases (kb)[5], the smallest yet for any plant and comparable to the yeast, nematode and *Drosophila* genomes. For comparison, the size of the human genome is ~2,000,000 kb, and that of rye is ~7,900,000 kb. Furthermore, the DNA reassociation kinetics show that the *Arabidopsis* genome is remarkably low in repetitive sequences, which should facilitate the application of the 'chromosome-walking' technique for isolating genes that made possible the cloning of the bithorax complex of *Drosophila*. Meyerowitz hopes that the small genome of *Arabidopsis* will rapidly be covered with sites of restriction-fragment length polymorphisms — his group have already detected a few by simply using randomly selected clones from an *Arabidopsis* genome library. These can be used to map new mutations and as starting points of chromosome-walks to clone the sites of the mutations.

The economy of the *Arabidopsis* genome is also reflected at the level of specific genes; for example, where most plants have multiple copies of the genes for seed proteins, *Arabidopsis* has, according to Meyerowitz, only one. And using their cloned *Arabidopsis* seed protein gene as a probe, Meyerowitz's group have carried out the first analysis of gene expression in plants by hybridization *in situ*. In sections of seeds, they can detect seed protein messenger RNA only in embryo cells, which are known to be the sites of seed protein synthesis in *Arabidopsis*. The result in itself is to be expected; what is important is that it shows the powerful technique of hybridization *in situ* can be applied in *Arabidopsis*. It is not always easy to make it work and this has, for example, been the principal barrier to progress in research on homoeotic gene expression in vertebrates.

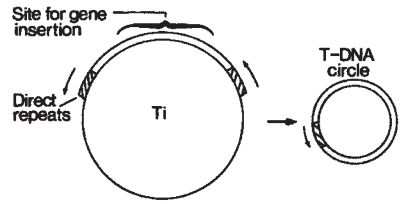
Arabidopsis seems to be as amenable to the techniques of cell culture, plant regeneration and *Agrobacterium tumefaciens*-mediated transformation[6] as plants such as tobacco (see below and box). One of the problems that has frustrated plant scientists in recent years has been the lack of any one species that is both amenable to such techniques and has been well-studied genetically. And although I. Potrykus (Friedrich-Miescher Institute) and E. Howard (CSIRO) both reported that they have been able directly to introduce DNA into protoplasts of monocotyledenous plants, the difficulties in regenerating such plants from cells in culture make it likely that *Arabidopsis* will be the first genetically well-defined plant into which exogenous genes can be introduced readily.

The most exciting prospect offered by *Arabidopsis* is in its potential for the identification, genetic mapping and cloning of those genes which, on the basis of the effects of their mutations, seem likely to be very closely involved in the regulation of

*The 1985 UCLA symposium on plant genetics was held at Keystone, Colorado, USA from April 13-19 1985.

Plant transformation

To date, the success of plant geneticists in introducing new genes into plants has depended on the exploitation of a remarkable natural system of interspecific gene transfer. The bacterium *Agrobacterium tumefaciens* can induce the formation of tumours on many plant species. This is known to involve the transfer of a fragment of DNA (the T-DNA), carried on the bacterium's tumour-inducing (Ti) plasmid, to the genome of the infected plant cell. It is the genes carried by the T-DNA that cause the transformed cells to divide to form tumours and that pervert the metabolism of the tumour cells so that they make products on which the bacterium can feed, such as nopalines.



As recently reported in *Nature*[1], circular molecules of T-DNA are induced within the bacterial cells in response to some plant-derived signal. It is presumed that these molecules repesent intermediates in the process of gene transfer — though as yet no T-DNA circles have been detected in plant cells. The junction of the T-DNA circles occurs at a precise point within the short (25 base pair) direct repeats that flank the T-DNA on the Ti plasmid, and simply by inserting a piece of DNA between these repeats it can be transferred to the genome of a plant cell. So that whole plants can be regenerated from the transformed cells, 'oncogenic' genes of the T-DNA are usually removed and replaced by a selectable marker, such as an antibiotic-resistance gene.

Unfortunately, it has so far not been possible to use this system to transform monocotyledenous plants, despite the indications that they can be infected by *Agrobacterium tumefaciens*[2,3]. One alternative approach that is being pursued at present is to see whether transposable elements could be used to introduce genes into plants, much as P-elements are used to transform *Drosophila*. As a start in this direction, R.B. Simpson (ARCO, Dublin, California) reported at the plant genetics symposium that his group has begun to explore the potential of the maize *Mu* (Robertson's *mutator*) transposable elements as vehicles for transforming monocots. **Geoffrey North**

1. Koukolikova-Nicola, Z. *et al. Nature* **313**, 191 (1985).
2. Hooykass-Van Slogteren, G.M.S., Hooykaas, P.J.J. & Schilperoort, R.A. *311*, 763 (1984).
3. Hernalsteens, J-P., Thia-Toon, L., Schell, J. & Van Montagu, M. *EMBO J* **3**, 3039 (1984).