Gene regulation

Throwing light on plant genes

from Robert Shields

OVER the past year or so there have been several demonstrations that the Ti plasmid of *Agrobacterium tumefaciens*, the bacterium that causes crown gall tumours in plants, can be exploited to insert functional foreign genes into plant cells[1-3]. The inserted genes are incorporated into the nuclear genome of the plant cells, which, in some cases, have been regenerated into plants and the inserted genes thus transferred by meiosis to the seed[4]. Once the gene is inserted and functioning, the challenge is to determine what features of the gene are necessary for its correct regulation, for example its light response. It does not take a clairvoyant to guess that, as with other eukaryotic genes, the regulation of plant genes is governed by DNA sequences surrounding the gene itself. In two recent papers, one on page 115 of this issue of *Nature*[5] and one in *Science*[6], this has been shown to be the case, although a number of intriguing questions remain unanswered.

Both papers deal with the light induction of the genes for the small subunits of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), the chloroplast enzyme that fixes CO_2 in the photosynthetic cycle. The family of genes for the small subunits is located in the nucleus of the plant cell. Members of the family are regulated by light; individual members are expressed in a tissue-specific manner. Thus, it is found that the amounts of Rubisco are higher in the leaves, pods and peas of the pea plant than in the root and stem[7].

Broglie *et al.*[6] describe the insertion of a Rubisco small-subunit gene of the pea (which accounts for about 30 per cent of small-subunit transcripts in pea leaf[7]) into petunia tissue, where the gene becomes light-regulated. There is about 50 times as much specific transcript in illuminated tissue as in dark-held tissue, which is similar to the situation in pea leaf. The inserted gene is transcribed from the normal initiation site; the transcript appears to be correctly spliced and polyadenylated and its protein product is associated with the petunia Rubisco large subunit in the petunia chloroplast. In addition to the coding region, the inserted pea gene has over 1,000 nucleotides of upstream sequence and more than 500 nucleotides of non-coding downstream sequence and it is likely that features responsible for its light regulation lie somewhere in these sequences.

The paper in this issue of *Nature*[5] describes experiments in which 973 nucleotides lying upstream of a different Rubisco small-subunit gene of the pea (one that is expressed relatively weakly in pea leaves) have been spliced in front of the bacterial gene for chloramphenicol acetyl transferase; since the presence of chloramphenicol acetyl transferase is easy to measure, its production under the control of the Rubisco upstream sequences can be determined. Sequences downstream of the bacterial gene were provided by the nopaline synthase gene of the Ti plasmid, since these are known to function properly in plants. When the chimaeric gene was inserted via *A. tumefaciens* into the genome of tobacco tissue, the bacterial gene became light-regulated. This is strong evidence that sequences upstream of the Rubisco gene are, at least in part, responsible for its regulation by light.

While Broglie *et al.* show that the extent of regulation of the pea Rubisco genes by light is the same in petunia tissue as in pea leaf, the absolute amount of transcription is far lower. Nevertheless, the plant cell contains up to five copies of the inserted gene. So although light regulation is independent of tissue type, the absolute amount of transcription may be governed by other factors. What are they? Quite possibly, the gene promoters in the upstream sequences show species-specificity as well as tissue-specificity; pea promoters may just not take to petunia or tobacco. The position of the inserted gene in the foreign genome may also influence the level of transcription. With the availability of disarmed agrobacterium-based vectors that permit the efficient regeneration of plants from genetically transformed tissue, these questions can be approached if not answered. □

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5. Herrera-Estrella, L. *et al. Nature* **310**, 115 (1984).
6. Broglie, R. *et al. Science* **224**, 838 (1984).
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Planetary science

Source of the oldest lunar basalt

from S.R. Taylor

THE earliest history of the Moon, as determined from records of craters, basins and uplands established by the Apollo missions, is marked by two very significant events: a period of intense meteoritic or planetesimal bombardment at the close of the formation of the Solar System, and an outpouring of basalt lavas on a global scale. The former preceded the latter, but with a degree of overlap that has been the subject of much discussion. Results reported in a recent paper by L.A. Taylor *et al.* (*Earth planet. Sci. Lett.* **66**, 33; 1983) bear on this issue. Evidence from rocks from the Apollo 14 site in the Fra Mauro region has revealed that some basalt flooding occurred as early as 4.23×10^9 yr ago, far earlier than hitherto believed and well before the end of the bombardment phase.

Younger and more aluminous mare basalts, with ages ranging from 3.85 to 3.96×10^9 yr, had previously been found in the Apollo 14 breccias, indicating that some mare basalt volcanism preceded the last of the large basin-forming collisions. The breccias are located on the Fra Mauro ejecta blanket formed during the impact event responsible for excavating the Imbrium basin at 3.82×10^9 yr.

The close of the great bombardment forms a natural marker in lunar geological history. The next period was dominated by large-scale outpouring of mare basalt lavas, forming the lunar maria which cover 17 per cent of the surface. Some studies have already indicated that mare basalt volcanism began before the end of the period of the great collisions. For example, basalt sample 10003, the third sample picked up by the Apollo 11 astronauts in Mare Tranquillitatis, gives K–Ar dates of 3.86×10^9 yr, earlier than the date of the Imbrium event. Other evidence comes from the presence of dark halo craters, which were excavated in highland plain units and indicate the presence of buried basaltic lavas. All these observations are consistent with a date of $\sim 4.00 \times 10^9$ yr for the beginning of basaltic volcanic activity on the Moon. The significance of the new results is that they push back the date of eruption of olivine–ilmenite basalts to 4.23×10^9 yr.

The sample analysed bears the typical mare basalt signature of depletion in europium and, by analogy with the Apollo 12 basalts, is derived by partial melting from depths of 200–400 km. The source region of such mare basalts had itself previously undergone a differentiation. The generally accepted explanation is that these pyroxene–olivine-rich regions were formed during the differentiation which followed the large-scale melting of the Moon. This melting, which probably extended to the whole Moon, is conventionally ascribed to accretional melting and is dated at about 4.45×10^9 yr. (This sets the formation of the Moon about 10^8 yr later than the meteorites, in accordance with the Safronov–Wetherill scenario for accretion of the terrestrial planets from a suite of planetesimals.) The subsequent crystalliza-