true Kuiper-belt object, Gomes' work gives a new perspective on these bodies. If the hot Kuiper belt is really a record of the Uranus–Neptune planetesimal population, a comparison of the detailed physical properties of the hot and cold objects could provide valuable information on the way in which the temperature and chemical properties of bodies varied with increasing distance from the Sun in primordial times. As astronomers slowly unlock these, and other, mysteries, we will learn more about the early life of our planetary system. ■

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Plant biology

Mobile plastid genes

Pal Maliga

The direct demonstration that chloroplast DNA can be incorporated into the nuclear genome of plants, even though it is unlikely that such DNA would be functional, will influence thinking in plant biotechnology.

Plastids are organelles found in the cells of higher plants, the best-known form being the chloroplast, the site of photosynthesis. They arose when, way back in time, plant ancestors assimilated photosynthetic prokaryotes — unicellular organisms such as bacteria which, in contrast to eukaryotes such as plants (and ourselves), typically lack a membrane-bound nucleus. The creation of functional plastids involved the migration of huge numbers of genes from the prokaryote genome to the plant nucleus. But is transfer of plastid DNA into the nucleus of higher plants still occurring?

As they describe on page 72 of this issue[1], Timmis and colleagues conclude that it is, at a rate comparable to spontaneous mutation of the nuclear DNA. There are various implications here, not least for plant biotechnology. One proposed strategy for the future is to engineer genes for some trait or other into chloroplasts, rather than nuclei, because — in principle — those genes will affect the characteristics of the individual plant and will be transmitted to future generations by the maternal parent, but not 'escape' in pollen and spread uncontrollably.

The extent of gene migration during evolution becomes clear when we consider that the plastids of higher plants today contain only about 120 genes, compared to the 3,000–4,000 thought to have been present in the genome of the ancient photosynthetic prokaryote. Plastid function depends on some 3,000 nuclear genes, the products of which are transferred to complement those of the remaining plastid genes[2]. We have a relatively good idea about the identity and gene content of the organisms that were involved when the original eukaryotic plant ancestor took in the prokaryotic forerunner of the plastid[3]. But little is known about the evolutionary process of gene transfer from the plastid to the nucleus.

This is what Timmis and colleagues[1] set out to study by designing a smart screen to estimate the rate of plastid-to-nucleus DNA transfer in tobacco plants. Their system involved measuring the transfer rate to the plant nucleus of a gene (*neo*) — which confers resistance to the antibiotic kanamycin — that had been engineered into the tobacco plastid genome (along with a marker gene, *aadA*, of which more later). The kanamycin-resistance gene was endowed with the various signals, including a promoter sequence, required for eukaryote-type expression in the nucleus. Plastids, in general, have a prokaryotic-type gene-expression machinery[4], so the nuclear kanamycin-resistance gene was not expected to be expressed there (Fig. 1a, overleaf).

Timmis's group found that transfer of *neo* to the nucleus took place in a total of 16 heritable events in 250,000 seedlings — that is, at an incidence of around 6×10^{-5} — and was detected by testing for kanamycin resistance in the tobacco plants (Fig. 1b, c). Molecular analyses confirmed that the *neo* genes, together with flanking plastid DNA of variable size, had indeed been incorporated into the tobacco nuclear DNA at different genomic locations.

Another cellular organelle found in eukaryotes is the energy-generating mitochondrion. Previous work[5] with yeast has shown that here, too, there is transfer of organelle DNA to the nucleus, and the rate (about 2×10^{-5} per cell per generation) is comparable to that found by Timmis *et al.* in tobacco. In yeast, the fragments of mitochondrial DNA were incorporated into the chromosomes by a mechanism known as 'double-strand-break repair'[6,7], which is

100 YEARS AGO

All inquirers have perceived that great men are of two types, and it would conduce to clear thinking if we could accustom ourselves to classify them under different names… The first class, to which I should prefer to restrict the name genius, may be described primarily as men of fine, delicate, sensitive, impressionable constitution, and strong, restless innate tendencies which appear early in life, as a rule, and take their own shape. These men work energetically, often at high pressure, and in general die comparatively young… The second class I would describe as men of talent. When preeminent they exhibit striking aptitude in learning and in imitation, and develop extraordinary powers of work… In nature there is great variety, and genius, so far, is one of the varieties which often recur, but scarcely ever survive even for two generations. It is a rare and delicate thing, and the utmost we can hope for it is that endeavours may be made to collect and preserve it like some hot-house plant, in order that it may suggest combinations which men of talent may put to practical account. The position of the second type in the struggle for existence is beyond doubt. The stability of a country and its place among the nations depend upon the number and ability of men of this stamp.
From *Nature* 5 March 1903.

50 YEARS AGO

There has been a progressive increase, both absolute and relative, in the proportion of old people in the population, this increase being the result of the decline in infant and young adult mortality produced by the medical and social advances that have been made possible by the application of the scientific method during the past century. As a result of this, more old people must be supported by a diminished proportion of wage earners. There is a corresponding increase in the numbers of elderly invalids to be cared for, who are suffering from the degenerative disease of old age which medical treatment may ameliorate, but not cure. Meanwhile, the expense of medical treatment, especially hospital treatment, has risen enormously and continues to rise, and it has been suggested that the community is faced with a gloomy prospect of unlimited 'medicated survival' to be met by diminishing resources.
From *Nature* 7 March 1953.

usually involved in repairing damaged DNA. The same mechanism might be involved in the transfer of *neo*-containing plastid DNA, although an earlier study[8] of double-strand-break repair in tobacco found no evidence of plastid (or mitochondrial) DNA transfer during the process.

Given the readily detectable plastid-to-nucleus transfer of DNA, it is not surprising that the tobacco nuclear genome contains long tracts of plastid DNA. For example, a search[9] for plastid DNA fragments containing a particular gene, *rbcL*, found that there are 15 fragments of different size containing *rbcL* plastid DNA in the tobacco nuclear genome, the largest being 15.5 kilobases (kb) in size. But the amount of organellar DNA present in the nucleus seems to depend on the plant species concerned. For instance, the nuclear genome of *Arabidopsis thaliana*, the favoured model for plant biologists, contains only 17 small insertions of plastid DNA (total size 11 kb)[10], and 13 small insertions (7 kb)[10] and one large insertion (about 620 kb) of mitochondrial DNA[11]. The differences may in part be due to different organelle-to-nucleus DNA transfer rates. Indeed, 40% of double-strand-break repair in the tobacco nucleus was accompanied by insertions (although, in the specific instance, not of organellar DNA), whereas no insertions accompanied double-strand-break repair in *Arabidopsis*[8]. If the conclusions of the study by Timmis *et al.*[1] can be generalized, crops that have larger nuclear genomes are likely to have higher transfer rates between plastids and the nucleus. But the relative abundance of organellar DNA in nuclear genomes may also reflect the efficiency of mechanisms that work against 'genome obesity' by selectively eliminating nuclear sequences[12].

What about the biotechnological implications of the new research? In general, incorporation of genes for some trait or other into the plastid genome is an attractive strategy, as plastids in most crops are not transmitted by pollen. So incorporation of, for instance, genes conferring herbicide or insect resistance in the plastid genome would mean that these genes would not be transmitted by pollen to neighbouring stands of the same crop or to weedy relatives[13]. This is a common problem with transgenes incorporated in the nuclear genome[14].

It is reassuring that the marker gene (*aadA*, which confers resistance to another antibiotic, spectinomycin) that Timmis and colleagues incorporated into plastids along with the kanamycin-resistance gene, was not expressed in the nucleus: this gene had a plastid-specific promoter. But although *aadA* residing in the nucleus on a plastid genome segment is not expressed, broken plastid gene pieces, incorporated next to a nuclear promoter, could be expressed at a very low frequency[15].

The authors tested for spectinomycin resistance only in the 16 plant lines carrying the transferred *neo* gene. To estimate the transfer rate of functional *aadA*, however, a seedling population of comparable size (250,000) or larger would have to be screened for expression of spectinomycin resistance. The frequency of fortuitous transfer that results in expression of the transferred plastid gene may be 100–100,000 times lower than the rate of transfer of the nuclear *neo* gene (the values will have to be experimentally determined).

Nonetheless, incorporation of transgenes in plastids should still be effective for containment of those genes. If a transgene is incorporated in the nucleus, each pollen grain will carry the transgenic trait. By contrast, assuming that the probability of plastid gene expression from a broken fragment is only 100 times lower than the transfer rate of plastid DNA, only 1 in 1.6 million pollen grains will carry the expressed plastid gene.

It could be, however, that functioning of the transferred plastid genes in the nucleus could be prevented altogether. Examples of genes that are expressed in plastids but not in the nucleus are bacterial genes containing a high content of adenine and thymine, such as those encoding the *Bacillus thuringiensis* insecticidal protein or the *Clostridium tetani* toxin[13,16]. The *B. thuringiensis* insecticidal genes could be expressed in the plant nucleus only by modifying them to a 'eukaryotic design'[17,18]. So modifying a gene to a 'bacterial design' would be expected to facilitate its expression in plastids and prevent it in the nucleus.

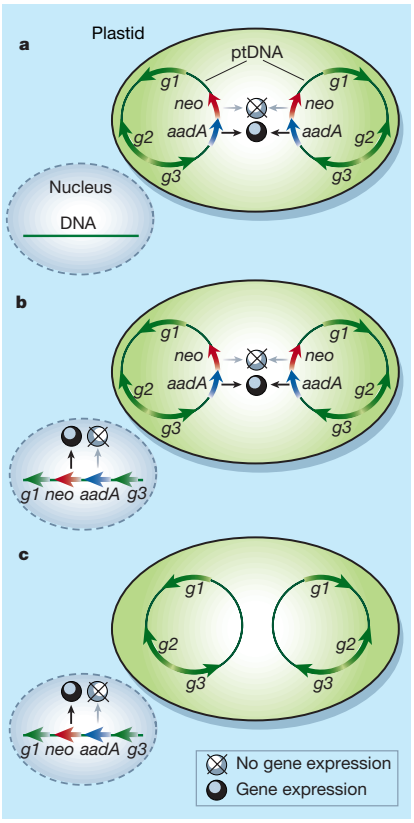

Figure 1 **Transfer of plastid DNA to the nucleus of a tobacco cell, as studied by Timmis and colleagues[1].** a, **Tobacco cell with a transformed plastid genome (ptDNA) and a wild-type, non-transformed nucleus. Three plastid genes (*g1, g2, g3*) are shown in the plastid genome, along with the kanamycin-resistance (*neo*) gene and the spectinomycin-resistance (*aadA*) gene. Crucially, *neo* has the necessary ancillary sequences for expression only in the nucleus, whereas *aadA* can be expressed only in the plastid. Only two ptDNA copies are shown out of the 1,000–10,000 copies present in a plant cell.** b, **A rare cell, in which a fragment of ptDNA has been transferred to the nucleus: note that *neo* is expressed here but *aadA* is not.** c, **Timmis *et al.* had to study the expression of plastid DNA in the nucleus in cells with wild-type ptDNA, the cells being created by pollinating non-transformed plants with pollen from plants possessing transformed plastids. One in 16,000 pollen grains carried a *neo* gene inserted in a chromosome. Because plastids are not transmitted by pollen, only the nuclear *neo* gene (and flanking ptDNA) was transmitted in the cross. Separation of plastid and nuclear transgene copies was essential as there are many more plastid *neo* and *aadA* copies than nuclear copies, and the large number (10,000 per leaf cell) of plastid copies interferes with the study of the few copies in the nucleus.**

Timmis and colleagues' demonstration[1] of the transfer of plastid DNA to the nucleus of higher plants will be the subject of much debate from both the scientific and biotechnological viewpoints. The implications in the latter respect will depend on the likelihood of plastid genes becoming functional nuclear genes, and on the success of gene designs that can prevent the expression of transferred plastid genes in the nucleus. ■

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